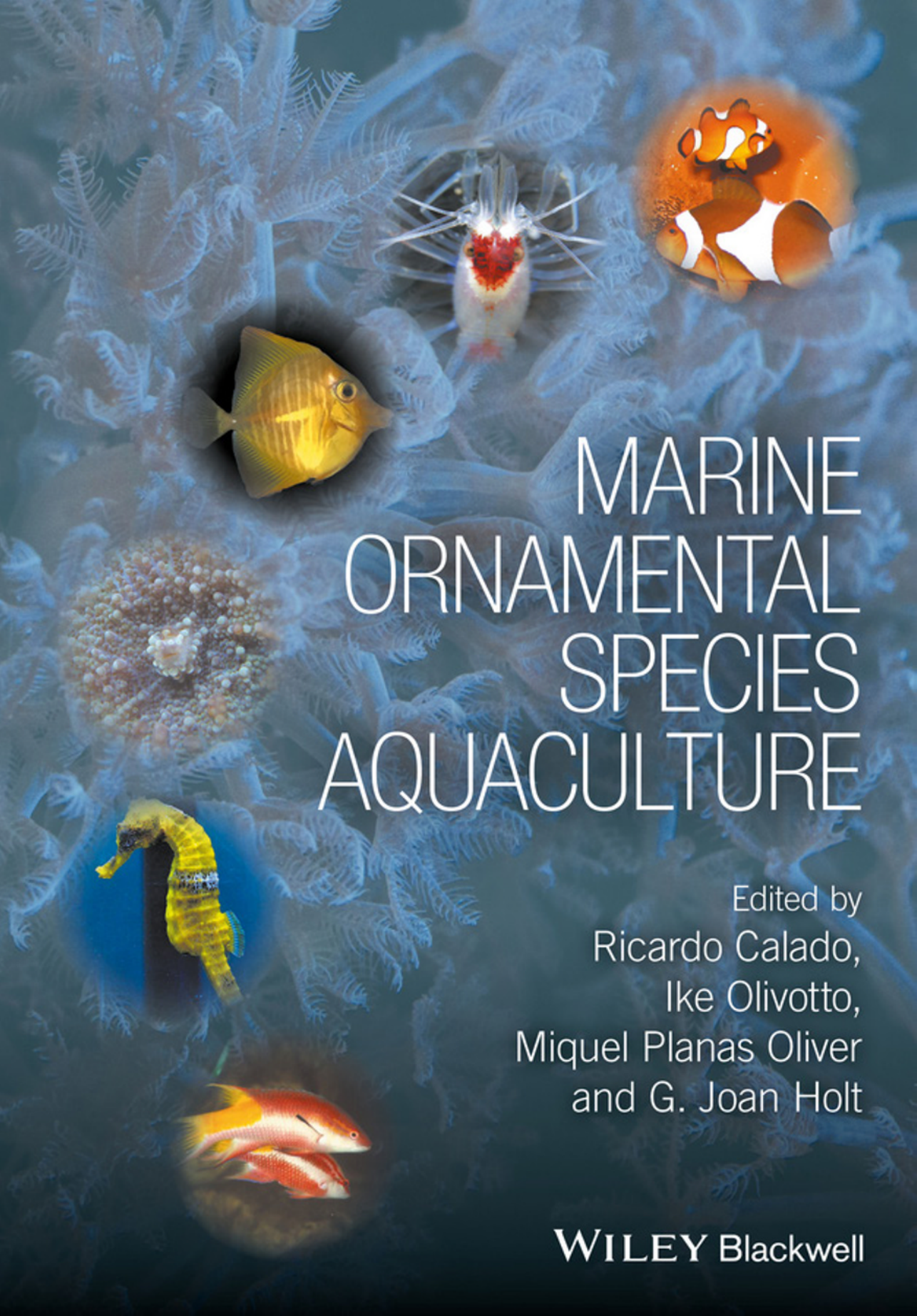


MARINE ORNAMENTAL SPECIES AQUACULTURE

Edited by
Ricardo Calado,
Ike Olivotto,
Miquel Planas Oliver
and G. Joan Holt

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Marine Ornamental Species
Aquaculture

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Dedications

To Tânia, Rodrigo and Carolina for being so understanding and for their unconditional love and support.
Ricardo Calado

To my family.
Ike Olivotto

To everyone who helped me grow as person and scientist, especially to my parents, Josep María and Cari, for always being there.
Miquel Planas Oliver

To all of my graduate students and research assistants who worked passionately for marine ornamental aquaculture success.
G. Joan Holt

To the memory of Junda Lin, colleague and friend, for his work and dedication to marine ornamental species aquaculture.
The Editors

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Foreword

The global trade of aquatic organisms for home and public aquariums, along with associated equipment and accessories, has become a multi-billion dollar industry. Although marine species made up less than 10% of the total volume of ornamental trade, the percentage in terms of value is much higher and increasing in recent years. The vast majority of marine species are collected from the Southeastern Asian countries (especially the Philippines and Indonesia) and exported to North America, Japan and Europe, and in more recent years China. For both freshwater and marine aquarium species, fish is the dominant group, although in the last 25 years, the marine aquarium trade has seen a shift in consumer preference from fish-only aquariums to miniature reef ecosystems, resulting in the trading of many invertebrate species associated with the coral reefs (e.g., anemones, jellyfish, shrimp and other decapods, starfish and urchins, and molluscs). An estimated 27 million marine ornamental fish (Townsend, 2011) from over 1800 species (Rhyne *et al*., 2012) and hundreds of coral and other invertebrate species are traded. Scuba diving opened up habitats that until then were inaccessible. Color photography made it possible to document the beauty of living animals. Interestingly, photography, the number one hobby, certainly facilitates the aquarium keeping, the second most popular hobby.

Much progress has been made in the breeding and aquaculture of marine ornamental species in the last 10 to 20 years. CORAL Magazine lists the number of captive-bred marine aquarium fish species, with more than 30 new species over the 2013 list, bringing the total to over 250 species (Sweet, 2014). However, for most of these species, it will be quite some time before they can be supplied to the aquarium trade at reasonable prices through commercial production. In contrast to the traded freshwater fish species, about 90% of which are captive bred, (much) less than 10% of the marine ornamental species traded are from aquaculture. Virtually all of the marine fishes and invertebrates marketed in the aquarium trade industry are collected from coral reef ecosystems. Extensive and destructive collection of these animals, such as use of cyanide and coral breaking and smashing, can directly deplete the target species and indirectly damage delicate coral reef ecosystems that are already under serious threats from global climate change, pollution and other anthropogenic impacts. Aquaculture of marine ornamental species, still in its infancy, is recognized as a viable alternative to wild collection as it can supplement or replace the supply of wild caught specimens and potentially help recover natural populations through restocking. However, there are still many challenges that need to be overcome. Life history and other basic scientific knowledge are unknown for

most species. Broodstock management, spawning induction, larval rearing, nutrition, live feed, diseases, systems, and cost are some of the obstacles for commercial production.

Information on aquarium animal keeping, spawning and culture, is widely distributed in trade magazines, journals, newsletters and websites (largely contributed by hobbyists and public aquariums), and increasingly, in scientific publications. Although there are a few reviews and books on aquaculture of specific groups, such as fish (e.g., Moorhead & Zeng, 2010) and shrimp (e.g., Calado, 2008), there has not been a comprehensive overview of marine ornamental aquaculture. The current book fills this critical gap and provides a valuable resource for research and development of marine ornamental aquaculture. The editors have assembled a great panel of international experts to cover a broad field of marine ornamental aquaculture and related topics, including major groups of fish and invertebrates traded in the marine aquarium industry, culture systems, nutrition, diseases, marketing, packing and shipping, and conservation. While the book is not designed as a manual for culturing specific species or groups, it does provide up-to-date information and guidance for overcoming the major bottlenecks in developing commercial production for more marine ornamental species.

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Part I

Overview of Marine Ornamental Species Aquaculture

The marine aquarium trade has a worldwide dimension and represents a multi-million dollars segment of the pet industry. This economic activity has continued to expand in recent years, revealing a voracious appetite for a remarkable range of species (Rhyne *et al.*, 2012a; Rhyne *et al.*, 2012b) that are commonly termed marine ornamentals. While at first we may think of marine ornamental species as colorful reef dwelling organisms, color is not the only feature that makes a marine organism a target of this trade. As an example, drab colored organisms that provide a service for reef aquariums, such as algae grazers and species that can control the growth of "nuisance organisms", are also in high demand and heavily collected (Rhyne *et al.*, 2009) (Chapter 1).

In the twenty-first century, the marine ornamental trade can be described in general terms as relying on the harvest of wild specimens from coral reefs (mostly in the Indo-Pacific region) and their exporting worldwide, mainly to the USA, EU countries, and Japan (Thornhill, 2012). The poor survival of collected specimens through the first links of a highly diffuse chain of custody, along with the dependence on the collection of specimens from the wild to supply a growing demand, continue to drive researchers to find solutions that can contribute to a more sustainable marine ornamental species trade.

Aquaculture is commonly considered as a potential way of alleviating fishing pressure on the wild populations of marine ornamentals, as the captive production of some of the most heavily collected species would certainly contribute to relieving the current fishing pressure on coral reefs (Tlusty, 2002). The aquaculture of marine ornamentals also provides researchers an excellent opportunity to gain knowledge on their life history (e.g., larval development, reproductive behaviour, age at maturity, fecundity, etc.) in order to improve the management of natural stocks. Additionally, cultured marine ornamentals could provide novelty to an industry that is always willing to pay higher prices for rare or unique specimens. This novelty is commonly associated with the collection and trade of rare and/or endemic species, which invariably drives a surge of fishing pressure targeting those specimens, promoting negative impacts on their natural populations. When culturing marine ornamentals, this novelty can be supplied through the breeding of unique hybrid organisms and/or new color morphotypes without impacting coral reefs. These unique specimens can be reliably traced in the chain of custody (Cohen *et al.*, 2013) and contribute to a more responsible industry (Chapter 2).

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With remarkable advances being achieved in the last decades on the aquaculture of marine ornamental species (Olivotto *et al.*, 2011; Moorhead and Zeng, 2010), some constraints continue as relevant today as they were in the early 1980s. The culture of pelagic spawners is still hampered by the lack of suitable live prey for early larval life and cues triggering maturation and metamorphosis are still poorly understood for some marine ornamental species (Chapter 3). While early culture trials were not always successful, recent breakthroughs on the husbandry and production of several species and an unprecedented number of highly motivated researchers, professional marine ornamental fish breeders and hobbyists working in this field opens a window of opportunity for advancing the state of the art of marine ornamental species aquaculture to a whole new level. The role that the leading importing countries of marine ornamental species (with emphasis on the US) can play to foster the aquaculture of these organisms is also addressed, along with potential economic and environmental risks (Chapter 4).

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1

The Marine Ornamental Species Trade

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Abstract

Marine aquarium keeping is one of the world's most popular pastimes. Aquatic life is supplied through the marine ornamental species trade which consists of businesses that collect or culture livestock, transport, maintain, and trade until it reaches the consumer. Over 46,000,000 organisms representing 2500 species are traded annually with a value exceeding US$ 300,000,000. The Philippines and Indonesia supply the majority of livestock, with most specimens being consumed by the USA, Europe, and Japan. Unfortunately, irresponsible parties have caused coral reef destruction through negligent collection practices. Trade professionals and environmental advocates have employed a variety of measures to counteract the malpractice. Production of ornamentals through aquaculture is a leading solution that could reduce destruction by decreasing the need for wild livestock and providing an alternative profession for collectors. To achieve this economic–environmental balance, aquaculture capabilities require improvement through scientific research; additionally the technology must be accessible to countries that depend on collection.

Keywords *Aquaculture; collection of marine ornamentals; environmental impact; sustainability*

1.1 Introduction

The marine ornamental species trade is a global industry that provides wild-caught and aquacultured marine life for consumers. Marine ornamental livestock is supplied to consumers through an international network of artisans, aquaculturists, entrepreneurs, and businesses. Consumer groups primarily consist of home aquarists and public aquaria who maintain marine ornamental livestock for the purposes of aesthetic display, hobby, educational purposes, and pet companionship. This chapter is an overview of the history, economics, organisms, organization, and environmental implications of the marine ornamental species trade.

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Home aquarists are the largest consumer of marine ornamental species. They are a diverse group in respect to the species and assemblages of organisms that they desire and care for. Some keep a few small fish, such as clownfish or damselfish (family Pomacentridae), in a small aquarium (e.g., 40 L). Others keep large predatory fish, such as sharks or moray eels, in enormous home aquarium (e.g., 4000 L). Many aquarists maintain a "fish only tank", which is typically adorned with a community of fish and decorative rocks. There is also the "specialty tank", aquarist who cares for an individual or group of organisms that require special conditions, such as seahorses or jellyfish. Today, the most popular version of the marine home aquarium is the "reef tank". The "reef tank" simulates a natural reef environment, typically a tropical coral reef, and features a diverse assemblage of invertebrates as well as fish.

1.2 History

Fascination with marine life and the practice of keeping aquatic life in captivity can be traced to ancient civilizations. Evidence suggests that before 2000 BC the Assyrians, Egyptians, and Chinese cared for captive fish for the purpose of maintaining a readily available food source, as well as for religious reasons. In ancient China and Egypt, captive fish began to take on an additional decorative role and likely became status symbols (Nash, 2011). Wealthy Romans continued this trend during the first century BC. They constructed elaborate sea-side pools, *vivarie piscinae*, to maintain live seafood. The pools eventually acquired the added purposes of entertainment, social status symbols, homes for aquatic pets, and served as social gathering sites (Higginbotham, 1997). The Chinese forged the path for the aquatic ornamental species trade. Historic Chinese literature suggests that they identified ornamental phenotypes of carps (*Carassius auratus*), now known as "goldfish", between 265 and 316 AD (Matsui, 1971). Goldfish were domesticated between 618 and 906 AD; culturing them became popular in Chinese society and they were also used as a tradable commodity (Fossa, 2004). In the 1500s, goldfish were exported to Japan and later to Europe in the 1700s, where they were kept in fish bowls and quickly became a ubiquitous pet (Klee, 1987).

In the 1840s British naturalists collected local marine life, such as sponges (poriferans), and maintained them in glass containers for observation (Johnston, 1842). Soon afterward, the first public marine aquarium exhibit was introduced at the Zoological Gardens of Regent's Park in London. The aquaria in this exhibit may have been the first to resemble a contemporary "reef tank", as they housed an infrastructure of rock and sand with a diverse assemblage of temperate reef dwelling organisms including fish, mollusks, crustaceans, gastropods, echinoderms, anemones, and algae among others (*The Aquatic Vivarium*). Goldfish made their way into the homes of Americans during the 1850s and articles written for home aquarists in 1857 indicated that the hobby thrived (Klee, 1987). Global expansion of the freshwater aquarium hobby stimulated demand for more and different types of exotic organisms, especially fish. During the late 1800s and early 1900s, an increasing diversity of ornamental species were collected and exported from South America and South Asia, including the chanchito (*Australoheros facetus*) and paradise fish (*Macropodus operculus*) (Smith, 1902). Fish were imported to North America and Europe by steam ship and rail and maintained in metal cans during transit (Klee, 1987). Steam ships and trains provided less than ideal accommodations to sustain sensitive species. Air travel and improved airport

infrastructure of the 1930s facilitated transport, as well as the volume and number of species available in the trade. By 1935, aquarium publications reported the prevalence of over two hundred species in the freshwater aquarium trade (Innes, 1935).

The expanding interests of home aquarists eventually encompassed the marine environment, and the marine ornamental species trade was born. Sri Lanka, already serving as a hub for freshwater ornamental exportation, became the world's first commercial site for marine ornamental species export from the 1930s through the 1950s. Collection and export locations expanded to the Philippines and Hawaii in the 1950s and 1960s. The trade was improved by the use of polyethylene bags to package live organisms for shipment, which significantly reduced the weight and cost of freight (Lim *et al.*, 2003). A handful of species, such as clownfish and damselfish, made their way into the homes of aquarists (Innes, 1966).

By the mid-1950s public aquaria were maintaining and propagating coral. Soon home aquarists were doing the same, and coral too became a staple of the trade (Delbeek, 2001). Live rock (limestone encrusted in a benthic assemblage of invertebrates) also became a popular commodity; it had the dual purpose of decoration, as well as assisting with filtration of the aquarium (Carlson, 1999). Invertebrates other than coral, such as crabs, clams, mussels, oysters, snails, and anemones, also became popular (Axelrod & Shultz, 1978). The growth of the trade in the late 1960s and 1970s was catalyzed by the commercial development of synthetic sea salt, which allowed legions of enthusiasts who did not live near the sea the opportunity to maintain a marine aquarium (Moe, 1992). Steady technological advances in filtration, lighting, temperature control, water chemistry analysis, and husbandry techniques continued to increase the accessibility and popularity of the hobby throughout the 1970s and 1980s (Carlson, 1999).

1.3 Economics of Trade

Recent studies conducted from 1997–2012 estimated that more than 2500 species comprise the marine ornamental species trade. These consist of more than 1800 fish and over 700 invertebrate species, including but not limited to cnidarians, mollusks, arthropods, echinoderms, annelids, and poriferans (Wabnitz *et al.*, 2003; Rhyne *et al.*, 2012). The most recent global estimate indicates that 46,000,000 individual organisms are collected and sold annually to approximately 2,000,000 hobbyists worldwide, with a corresponding value exceeding US$ 300,000,000 (Wabnitz *et al.*, 2003). The Philippines, Indonesia, Solomon Islands, Sri Lanka, Australia, Fiji, the Maldives, and Palau, export the overwhelming majority of livestock. The USA is the most prolific importer and consumer; other major importers include the United Kingdom, the Netherlands, France, Germany, Taiwan, Japan, China, and Italy (Wabnitz *et al.*, 2003).

1.4 Species of Trade

The marine ornamental species trade is composed of an incredible diversity of organisms, but popular taxonomic groups represent the majority of species traded. More than twenty million fish are traded annually, with pomacentrids (damselfish and clownfish) representing nearly half by volume (Rhyne *et al.*, 2012). Coral and live rock have shown the most rapid growth in popularity in recent history, with over ten million corals and

Table 1.1 Most popular marine ornamental fish and corals (after Wabnitz *et al.*, 2003; Rhyne *et al.*, 2012).

Families of fishes	Genera of stony corals	Genera of soft corals
Acanthuridae (surgeon fishes)	*Acropora* (staghorn)	*Anthelia* (waving hand polyp)
Apogonidae (cardinal fishes)	*Catalaphyllia* (elegance)	*Cespitularia* (blue xenia)
Ballistidae (trigger fishes)	*Euphyllia* (anchor)	*Cladiella* (cauliflower)
Blenniidae (blennies)	*Galaxea* (galaxy)	*Clavularia* (clove)
Callionymidae (dragonets)	*Goniopora* (flowerpot)	*Dendronephthya* (strawberry)
Chaetodontidae (butterfly fishes)	*Heliofungia* (mushroom)	*Lobophytum* (finger leather)
Gobiidae (gobies)	*Lobophyllia* (lobed brain)	*Nephthea* (broccoli)
Grammatidae (basslets)	*Plerogyra* (bubble)	*Sarcophyton* (toad stool)
Labridae (wrasses)	*Trachyphyllia* (open brain)	*Sinularia* (digitate leather)
Microdesmidae (dart fishes)	*Turbinaria* (cup)	*Xenia* (pulse)
Pomacanthidae (angel fishes)		
Pomacentridae (damsels)		
Pseudochromidae (dottybacks)		
Scorpaenidae (scorpion fishes)		
Serranidae (groupers)		

millions of kilograms of live rock being exported annually (Wabnitz *et al.*, 2003). A total of 140 species of stony corals (order Scleractinia) dominate the coral trade while approximately 400,000 individual soft corals, representing 61 species, are also traded each year (Wabnitz *et al.*, 2003) (Table 1.1). Sea fans also represent a small proportion of the trade.

About 500 species of invertebrates other than coral are popular; roughly ten million individuals are traded each year. These include mollusks (gastropods, bivalves, and cephalopods), echinoderms (starfish, urchins, and sea cucumbers), actinarians (sea anemones), crustaceans (shrimp, crabs, and lobsters), polychaetes (feather dusters and Christmas tree worms) and poriferans (sponges). Of this group, cleaner shrimp of genus *Lysmata*, boxing shrimp of genus *Stenopus*, and sea anemones of genus *Heteractis* compose about 15% of all non-coral invertebrates traded (Wabnitz *et al.*, 2003). Certain invertebrates are included in aquaria for aesthetics, however, many are chosen for the service that they provide for the aquarium. Often referred to as the "cleanup crew", these invertebrates may graze on nuisance algae, sift the substrate, filter, scavenge, prey upon nuisance species, clean parasites from cohorts, or provide habitat (Calado *et al.*, 2003; Rhyne *et al.*, 2009).

1.5 Organization of Trade

The journey of a marine ornamental from its native habitat to the home aquarium requires the services of a variety of players aligned in a complex chain. Approximately 90% of all marine ornamentals in the trade are captured from the wild, a practice that

requires the skills and experience of artisan collectors. Live rock and sessile or slow moving invertebrates are typically collected by finding and removing them from their location. The collection of fish and fast moving invertebrates requires snorkeling gear or underwater breathing devices, as well as tools such as nets, slurp guns, traps, hook and line or sedatives. Collectors either work for, or sell their catch to, a wholesaler, who stockpiles and prepares livestock for export. The wholesaler acts as an exporter or sells the livestock to one. Export normally requires government-mandated permits specific to the locale and import destination, veterinary inspection, treatment of livestock to prepare it for an international flight, and packaging (Olivier, 2003).

Exporters ship livestock by air to importers in destination countries; in some cases the exporter and importer are housed within one company. When a shipment arrives at its destination, importers unpack the livestock, inspect its health, and acclimatize it within a temporary holding facility (Wabnitz *et al.*, 2003). At this point, various importers may repackage the livestock for shipment to another destination, sell it to a wholesaler, or sell directly to a retail outlet (Olivier, 2003). Parties acting as wholesalers also acclimate the livestock and either sell it to a retail outlet or directly to home aquarists via the internet (Larkin & Degner, 2001). Public aquaria and retailers can purchase livestock directly from wholesalers and sometimes from importers. Home aquarists typically can purchase livestock only from a retailer with a physical store or an importer, wholesaler, or retailer that peddles livestock through the internet (Zajicek *et al.*, 2009).

Transshipping, which transpired in the 1970s, is an alternative pathway to trade reducing the number of middlemen involved. A transshipper amasses livestock orders from a consortium of retailers (and sometimes wholesalers) at the import destination, places the order with an exporter, and distributes the order to the consortium upon its arrival (Olivier, 2003). The purchasers of transshipped livestock are often required to pick the shipment up from the airport with no guarantee that the shipment will arrive alive. Destination site retailers and wholesalers save money by avoiding the markup that importers charge (and wholesalers), but lose the timely acclimation and husbandry benefits that they provide. While handling costs are reduced, the amount of time livestock spends packaged in transit increases, which has led to increased acclimation time, disease, and mortality (Olivier, 2003). In recent years, governments and transshipping companies have made progressive efforts to reduce the mortality rates associated with transshipping. Governments have employed quality control laws and licensing to discourage irresponsible practices. Likewise, transshipping companies have sought greater responsibility by guaranteeing the live arrival and quality of their livestock (Olivier, 2003).

1.6 Environmental Impact

The collection of wild marine ornamentals has taken a damaging toll on the environment. Destructive collection practices and nonexistent or ineffective fisheries management have led to a significant amount of coral reef habitat destruction and overfishing (McManus *et al.*, 1997). This outcome has also affected the reputations of collectors as a whole, even though the majority are conservation-minded collectors who harvest ornamentals sustainably (Dawes, 2003). A significant minority of collectors have resorted to highly damaging collection techniques that improve their catch per unit effort while imperiling the reefs that reward them with their bounty. One of the most

notorious techniques involves *in situ* sedation of fish by means of a variety of chemicals, including plant extracts, bleach, and most notably, cyanide (Bruckner, 2001). Cyanide, banned globally for this purpose, the most popular sedative is linked by abundant evidence to coral bleaching and mass mortality of non-targeted species. The effects of cyanide extend far beyond the reef; fish captured using cyanide suffer greatly increased mortality rates (Rubec, 1986). The use of cyanide fuels a sinister cycle of habitat destruction and accelerated demand to replace livestock lost due to high post-collection mortality.

Physical destruction of reefs and ill-managed removal of livestock is another hazardous result of irresponsible collection. Some collectors have dismembered corals to capture fish that have taken refuge in them (Pet-Soede & Erdmann, 1998). Fishing nets, which are typically used in an environmentally friendly manner, can become entangled in coral and lead to mortality. Another practice is to beat a coral with a blunt object to drive sheltered fish from its protection (Wood, 2001). The harvest of live rock and coral often requires the force of a hammer, chisel, crow bar, or screw driver and results in a direct loss of habitat (Bruckner, 2001). Coral and live rock harvest can be sustainable but if not managed properly, these sites may become cloaked in turf algae, eliminating much of the productivity of the once thriving ecosystem (McClanahan, 1995). Sustained removal of select species, particular sex, or life stage can compromise the reproductive capability of the species or stress non-target species causing imbalance to the local ecosystem (Sadovy & Vincent, 2006).

The majority of coral reefs are classified as threatened. The greatest threats result from coastal development, climate change, destructive food fishing methods, pollution, terrestrial runoff, and ocean acidification. The effects of the marine ornamental trade are miniscule by comparison, yet significant nonetheless (McManus *et al.*, 1997). The gravity of the situation may lead one to believe that elimination of the trade would improve the well-being of coral reefs. Trade professionals and scientists that study coral reefs and the trade, present counter arguments. They point out that public aquariums and conscientious aquarists play a pivotal role in gaining the attention of the general public and educating them about anthropogenic impacts on coral reefs (Tlusty *et al.*, 2013). Ending the trade could popularize a black market that promotes destructive collection techniques and leaves no potential for managed sustainable harvest (Shuman *et al.*, 2004). Marine ornamentals are the most valuable consumer goods that exist in a coral reef; well-managed, sustainable collection provides communities with an incentive for protecting the resource and sustainably using it (Wabnitz *et al.*, 2003). In the absence of a viable trade, many communities could turn to catastrophically destructive practices such as excavating coral reefs to produce building materials (Teitelbaum *et al.*, 2010).

The marine ornamental industry together with environmental protection advocates, have supported a variety of measures to counteract the negative impacts that negligent parties have caused. Environmental protection organizations have conducted grassroots efforts to educate collectors about the consequences of irresponsible practices and have provided instruction on how to capture and care for livestock using environmentally-friendly methods (Rubec, 1986). Several countries have employed collection management plans with varying success. These efforts have included specified collection zones, marine protected areas, no-catch zones, collection area rotations, bans, quotas, size limits, sex restrictions, access limitations, collection equipment restrictions, and exportation limits. These actions have been successful in some areas while ineffective in

others. Failure has often been due to noncompliance, coupled with the inability of governing bodies to enforce restrictions (Sadovy & Vincent, 2006). Another strategy has been the education of consumers regarding destructive fishing techniques and the corresponding environmental impacts. Organizations, such as The Marine Aquarium Council (MAC) have facilitated this effort by providing the consumer with the opportunity to promote and support sustainable practices (Shuman *et al.*, 2004). MAC certifies livestock at the retail level under the following stipulations: livestock was collected at a locale that designed and adhered to a certified management plan, and livestock was captured, maintained, and transported throughout the chain of custody using practices that minimize mortality and are deemed environmentally sustainable and humane (MAC, 2001a, b, c).

1.7 Marine Ornamentals Aquaculture

Expansion of the marine ornamental aquaculture industry can impart sustainability to the trade. Aquaculture has the potential to reduce the trade's dependence on wild collection through the sustainable production of livestock (Figures 1.1 and 1.2) (Palmtag & Holt, 2007). Reduced need of wild livestock could decrease the prevalence of destructive collection practices by incentivizing the use of sustainable collection which provide a competitive environmentally friendly product. The aquaculture industry has its detractors as some methodologies are not sustainable. Additionally, aquaculture production in import locations could reduce or eliminate the need for collectors, resulting in unemployment and reduced motivation for some local communities to protect the livestock's habitat, potentially resorting to more destructive uses of the reef

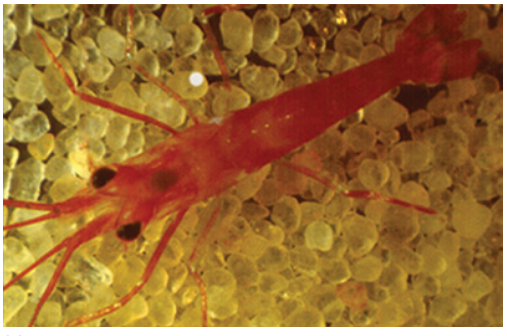


(a) (b)

Figure 1.1 Candy basslet (*Liopropoma carmabi*). (a) Adult pair of candy basslet; and (b) 58-day-old larvae raised by Todd Gardner at Atlantis Marine World in Long Island, New York in 2010. (*See insert for color representation of the figure.*)



(a)



(b)



(c)

Figure 1.2 Fire shrimp (*Lysmata debelius*) raised by Matthew R. Palmtag and Dr. Joan Holt in the late 1990s at the University of Texas Marine Science Institute in Port Aransas, Texas, USA. (a) 68-day-old post-larvae; (b) 83-day-old newly metamorphose juvenile; and (c) adult shrimp. (*See insert for color representation of the figure*.)

(Teitelbaum *et al.*, 2010). Many scientists and trade professionals argue that the industry has far more benefits than disadvantages. Sustainable aquaculture practices are well-developed and prevail in many countries throughout the world (Frankic & Hershner, 2003). It is improbable that aquaculture could fully replace the wild collection trade: under the appropriate conditions, sustainable production of marine ornamentals could be carried out simultaneously in popular collection areas (Teitelbaum *et al.*, 2010). This dynamic could provide new employment opportunities, improve awareness of sustainable practices, and enhance the economic infrastructure originally developed exclusively for wild collection (Pomeroy *et al.*, 2006). Aquacultured livestock suffer significantly less mortality than wild harvested, which reduces the need to resupply ornamentals that have succumb to stress due to wild collection. Aquaculture production

could also preserve genetic attributes of endangered populations, preserve species, and serve stock enhancement capabilities (Tlusty *et al.*, 2013).

The first cultured marine ornamentals were reared in the 1950s and 1960s, soon followed by the first documented marine ornamental aquaculture company Tropicornium, a coral aquaculture enterprise in Romulus, Michigan, USA (Innes, 1966; Delbeek, 2001). One of the defining achievements that catalyzed the development of the marine ornamental aquaculture industry was Martin Moe's success in rearing clownfish (*Amphiprion ocellaris*) in captivity in the early 1970s. Moe then spearheaded the first commercial production of marine ornamental fish with the inception of Aqualife Research Corporation in Walkers Cay, Bahamas in 1972 (Tellock, 1996). Several marine ornamental aquaculture ventures have come and gone since the 1970s, and many thrive today. One of the greatest disparities between the contemporary freshwater and marine ornamental fish trades has been the corresponding success of the aquaculture industry component. Approximately 90% of freshwater ornamental species traded are produced through aquaculture while less than 10% of marine species are cultured. The limited success of the marine ornamental aquaculture industry can be attributed to a variety of causes; two of the foremost are the complex nutrition of early life stages and the husbandry requirements of many popular species (Olivotto *et al.*, 2011). The progress that has been achieved and the obstacles that lie ahead are the focus of this book.

The collective efforts of research scientists, aquaculture professionals, professional and home aquarists over the last six decades have resulted in the successful culture of hundreds of marine ornamental species (Townsend, 2011). Many of these species have been raised with limited success; therefore the majority of them are not commercially viable. Lagging far behind the relative success of the freshwater ornamental aquaculture industry, it is fair to say that the marine component remains in its early stages (Holt, 2003). Yet, the industry thrives through a variety of means. Many proprietors culture marine ornamentals in intensive closed systems in "more economically developed countries (MEDC)". Several extensive operations also exist worldwide; these ventures utilize ponds in tropical locations to support the feeding and husbandry requirements of the livestock. Another means is to farm ornamentals extensively in their natural habitat; this is a popular and effective method often used for clams, coral, and live rock. A cottage industry exists where an individual or a family produces marine ornamentals intensively or extensively at their homes. A hybrid method also exists, where premarket size larval and juvenile livestock are collected and then raised in captivity until they reach marketable size (Lecchini *et al.*, 2006).

Some exciting new developments involving aquaculture have been introduced to the trade recently. Several aquaculture businesses produce novel phenotypes through artificial selection. This is not a new concept; the freshwater aquaculture industry has practiced this for more than a thousand years (Fosså, 2004). Production of artificially selected marine ornamentals has arisen with the relatively recent capability to culture marine ornamentals in captivity. On another front, improved accessibility and technological advances of submersibles and deep-diving, self-contained breathing devices have enabled collectors and deep water enthusiasts to introduce deep-dwelling species to the trade. The availability of these species to the aquarist are often limited and they typically command an expensive price; some consumers are willing to offer as much as US$ 30,000 for one specimen (Kaneshiro, 2012). To date, a handful of marine ornamental aquaculturists have successfully cultured several of these species (T. Gardner, pers. comm.).

1.8 Conclusions

Aquarium keeping is one of the world's most popular pastimes and it continues to grow in popularity, especially in the marine component (Livengood & Chapman, 2007). The continued viability of the marine ornamental species trade will undoubtedly depend on the ability to operate in a sustainable manner. Marine ornamental aquaculture has an opportunity to play an instrumental role in achieving a balance between economic feasibility and environmentally sound practice. Research has and will continue to play a key role in developing the capability of the industry to produce a greater number of species and improve efficiency and sustainability. This balance cannot be reached by the enterprises operating in MEDCs alone. To strike a global economic–environmental balance, effective methodologies established through sound scientific research must be published and then employed by the communities that depend on collecting wild livestock (Palmtag & Holt, 2001; Pomeroy *et al.*, 2006).

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2

The Need for Cultured Specimens

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Abstract

The aquaculture of marine ornamental species is commonly advocated as the most suitable alternative to the harvest of wild specimens from coral reefs to fulfil the demand of the marine aquarium trade. In this way, while cultured specimens are certainly needed in this industry, not all species traded should be (or are even suitable) for culture in captivity. If a fishery targeting one or more marine ornamental species is performed in a regulated way, using sustainable practices and contributing to the economic and social sustainability of local populations, the aquaculture of those species should not be promoted. Decision trees and SWOT analysis can play a key role when deciding what species to culture, where and how. The aquaculture of rare and/or endangered species should also be carefully analysed, as an unsuitable supply of cultured specimens may contribute to an increase of the fishing effort already targeting those species. The breeding of designer fish and use of new technological solutions for growing photosynthetic organisms (such as corals) open a new window of opportunity for those culturing marine ornamental species.

Keywords *Decision tree; designer fish; SWOT analysis; sustainability*

2.1 Introduction

The sustainability and environmental impact of the marine aquarium trade is commonly questioned due to its heavy dependence on the collection of specimens from the wild to supply an ever growing demand worldwide. Indeed, several studies have already highlighted how the unregulated collection of some marine ornamental fish and invertebrates may be negatively impacting their natural populations (Lunn & Moreau, 2004; Shuman *et al*., 2005). The over harvesting of certain species, as well as the use of destructive fishing practices (e.g., cyanide fishing), may negatively affect not only the species being targeted for marine aquariums, but other species as well. Nonetheless, it is important to highlight that labeling the collection of marine ornamentals from the wild to supply the aquarium trade as a predatory and destructive activity is not only erroneous but a dishonest claim as well. There are several scientific works reporting

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that well managed fisheries targeting marine ornamentals may not significantly impact natural populations and even contribute to a growing awareness of the need to preserve marine ecosystems (Williams *et al*., 2009; Dee *et al*., 2014). The positive social impacts associated with the collection of marine ornamentals must also be considered, as this activity may generate jobs and help to alleviate poverty in local communities with little or no alternative sources of income. Additionally, the collection of marine ornamentals from the wild is likely to be the economic activity able to aggregate the highest added value to most reef species, with emphasis on those that are solely used to supply this industry. Indeed, several nations already rank the collection and culture of marine ornamentals as key sectors in their economic activity, playing a growing role in the exports of these countries.

2.2 Should All Marine Ornamental Species be Cultured?

The above being said, it is legitimate to ask: is there a need for cultured specimens in the marine aquarium trade? Certainly yes! Does this mean that the collection of specimens from the wild should be banned? No; only when this collection is negatively influencing (or may negatively impact) the target species (or any other non-target species) should this practice be prohibited. Should all traded species be cultured in captivity? Not necessarily. If the collection of specimens from the wild is performed in a regulated way and that fishery can be reliably labeled as sustainable, contributing to the economic and social sustainability of local populations, than the culture of those species should not be promoted.

The social dimension of the marine aquarium trade, a feature commonly overlooked by fundamental environmentalists advocating the ban of any fishery to support this industry, also acts as a driver for local populations to oppose the culture of marine ornamentals. Those for whom the collection of marine ornamentals is the sole source of income have legitimate concerns that once the species they collect are produced elsewhere (e.g., in importing countries) their activity will cease. To prevent such scenarios it is important that those collectors are given the chance to shift their activity from harvesting to the production of the target marine ornamental species.

The work by Tlusty (2002) entitled "The benefits and risks of aquacultural production for the aquarium trade" provides an excellent starting point for those willing to determine if the culture of a given marine ornamental species is a suitable option. Responsible producers of marine ornamental species, both in exporting and importing countries, should perform a detailed risk analysis listing the strengths (S), weaknesses (W), opportunities (O) and threats (T) of their endeavor. While S refers to the characteristics of the culture of the species that may result into a competitive advantage, W refers to potential disadvantages associated with the culture of that species. Both S and W have an internal origin and refer to the culture initiative to be developed. On the other hand, O refers to external elements that the culture initiative may explore to its advantage and T to external elements that may negatively affect it. Both O and T have an external origin and refer to the economic, ecological and social environment of the project. Only after performing such exercise, commonly termed as a SWOT analysis (see Figure 2.1), should one decide to give a green or a red light to an initiative targeting the aquaculture of a marine ornamental species.

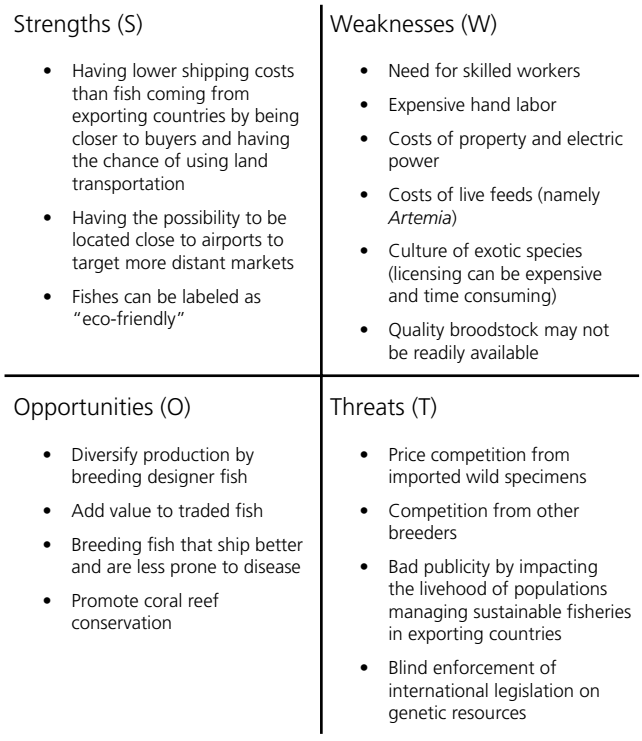


Figure 2.1 SWOT (Strengths, Weaknesses, Opportunities and Threats) analysis on the construction of a recirculated clownfish hatchery in the EU.

When supplying the marine aquarium trade with cultured specimens, it is commonly overlooked how this activity will impact the fishing pressure on wild population of the species being cultured. As already discussed by Tlusty (2002), three different scenarios may occur: (1) market demand is fulfilled by the culture of the target species, with supply being greater than demand at a given point in time; then aquaculture efforts will eventually promote a decrease in the capture of wild specimens; (2) if the supply of the cultured species largely surpasses demand, then the impact on wild populations will rapidly decrease; and (3) if demand for a given species is considerably higher than the supply of cultured specimens, then an intensification of the fishing effort targeting wild populations will occur. The last scenario described is particularly important when introducing new species to the trade, namely if they are particularly vulnerable to over harvesting (e.g., rare endemic species, species that produce a low number of offspring).

A nice example of how "good intentions can go wrong" is that of the aquaculture of the Monaco shrimp (*Lysmata seticaudata*). One of the goals of the aquaculture of this Mediterranean species, which previously had no commercial value, was to minimize the fishing effort targeting Caribbean populations of peppermint shrimp (*Lysmata* spp.) employed to control pest glass anemones *Aiptasia* spp. While cultured specimens of *Lysmata seticaudata* were highly effective in the control of these pests (Calado & Narciso, 2005) and produced by the thousands, it was only a matter of months until specimens collected from the wild entered the aquarium trade in Europe

(Calado, 2008). In certain occasions, wild specimens were even mislabeled and sold as being cultured. At present, wild populations of this species continue to be fished solely to supply the marine aquarium trade, with any negative impacts associated with this practice still being ignored.

Tlusty *et al*. (2013) provided a decision tree (Figure 2.2) that may be useful when evaluating the suitability of a given species to enter the marine aquarium trade (with emphasis on key issues such as its conservation status and potential to become invasive) and whether that species should be supplied to the industry through aquaculture or fisheries targeting wild populations. By following this decision tree, breeders of marine ornamental species will be able to make more conscientious choices at the time of selecting suitable candidate species for aquaculture and contribute to the sustainability of the industry.

2.3 Highly Demanded Marine Ornamental Species Cultured in Captivity

With the advances in the aquaculture of marine ornamentals (Moorhead & Zeng, 2010; Olivotto *et al*., 2011) a number of species cultured in captivity are now in high demand by this industry. An obvious example is that of clownfish within the genus *Amphiprion*, namely *A. ocellaris*, with a remarkable range of unique color morphotypes being supplied to the trade solely through cultured specimens. It is possible to say that we have entered the era of "designer clownfish", with commercial breeders advocating WYSIWYG (What You See Is What You Get) policies on their websites to ensure client satisfaction. New color varieties can fetch several hundreds of dollars per specimen, with breeding pairs of those varieties reaching premium values up to several thousands of dollars. Another interesting example is that of lightning maroon clownfish (*Premnas biaculeatus*). The rare phenotype exhibited by a very limited number of specimens collected from the wild, with solid white bands commonly displayed in the form of a lightning bolt, sparked the interest of breeders all over the world. Only through aquaculture could specimens with this remarkable pattern be available in the marine aquarium trade. There is no need to stress that the value these specimens continue to reach in the industry make their breeding a very appealing endeavor. It is now evident that a process of clownfish "guppification" is currently on-going in marine ornamental fish culture. This term has become popular among marine ornamental fish breeders and makes an analogy to what happened to the freshwater guppy (*Poecilia reticulata*), in the pursuit of even more extreme color and shape diversity through selective breeding. Ultimately, as in the guppy, the varieties arising in cultured specimens will attain such a level that they will vaguely resemble (if at all) the wild morphotype of the species.

The production of hybrids is also an interesting niche market for ornamental fish breeders. While hybrids of some popular marine ornamental fish species are known to occur in the wild (Pyle & Randall, 1994), hybridizing geographically isolated species produces unique specimens that may only be available to the marine aquarium trade through aquaculture. A nice example on this topic is the hybridization in captivity of two geographically isolated pygmy angelfish, *Centropyge fisheri* vs. *C. resplendens* (Baensch & Tamaru, 2009). These remarkable specimens can fetch high market prices

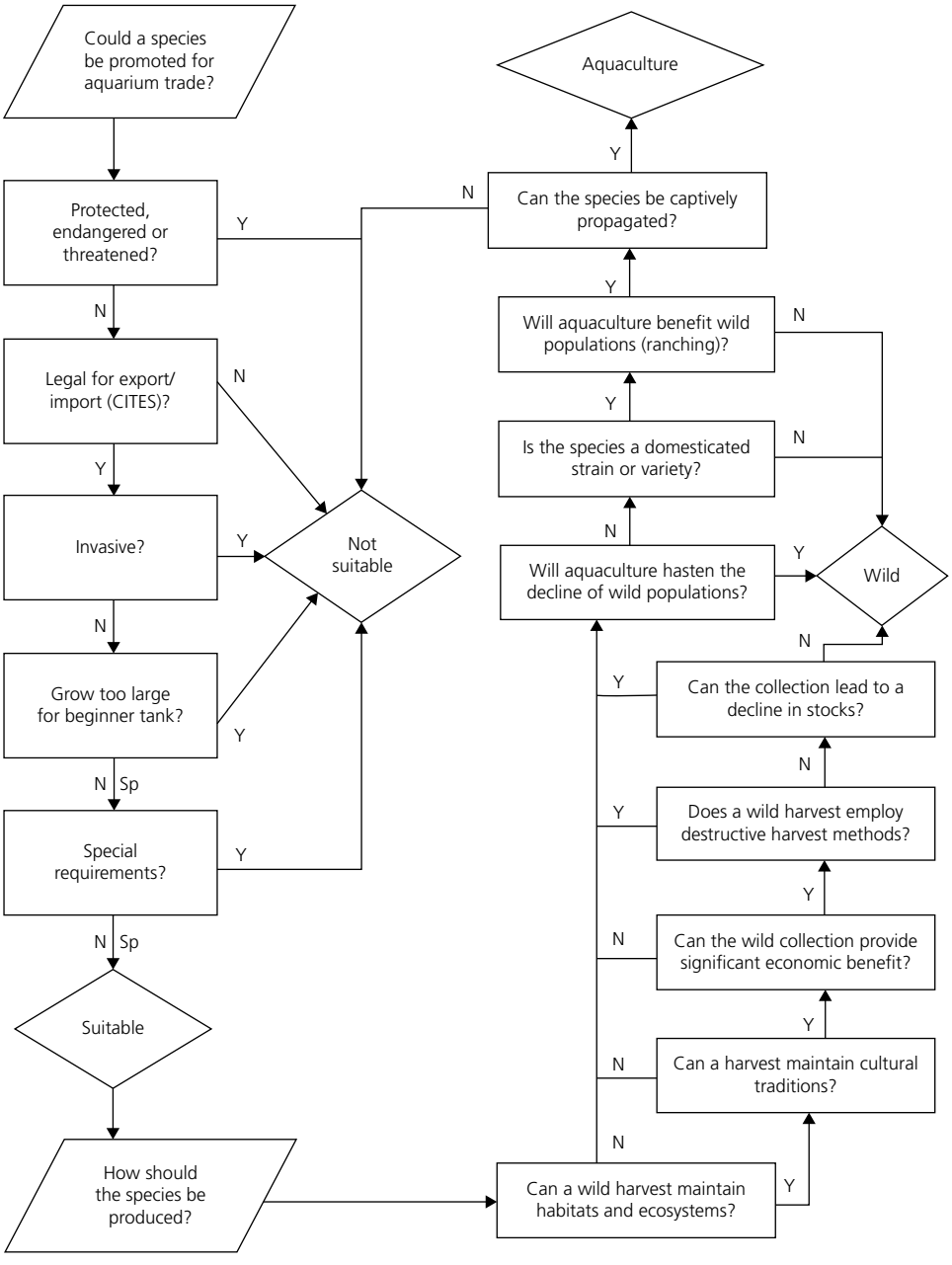


Figure 2.2 Decision tree to help with the evaluation of the suitability of a given marine species to enter the marine aquarium trade (left side) and whether traded specimens should be collected from the wild or cultured (right side). Sp on the decision arrows indicates that specialists in the hobby have the ability to care for these animals. Reproduced from Tlusty *et al*. (2013) with permission from John Wiley and Sons.

and the traceability problems so commonly affecting marine ornamentals (Cohen *et al*., 2013) are certainly not an issue for hybrid fish production.

Cultured seahorses (F2 generation or greater) are also highly demanded marine ornamentals. The shift in demand from wild to cultured seahorses was largely driven by the listing of all species within the genus *Hippocampus* on the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix II (listing species perceived as vulnerable, but less likely to be threatened with extinction; these species can be traded with an export permit as long as reliable evidence show that their harvesting is not detrimental to wild population) (Evanson *et al*., 2011). Cultured seahorses display remarkable colors and suitable sizes for stocking in small sized private aquariums, plus they do not depend on live prey to thrive in captivity as they readily accept frozen diets (some breeders even sell their "special" frozen diet to be supplied to their cultured specimens) (Koldewey & Martin-Smith, 2010).

Cultured corals are also increasingly popular in the industry, not only due to the growing awareness of the negative impacts that over-harvesting of these organisms may have on coral reefs, but also due to the remarkable colorations one may achieve when culturing these organisms *ex situ*. Recent studies have shown that contrasting light spectra from artificial light sources, such as those from T5 fluorescent lamps, light emitting diodes (LED) and light emitting plasma (LEP), may significantly affect the growth and color of hard corals (Rocha *et al*., 2013), as well as their macro and microstructure (Rocha *et al*., 2014). In the future, by fine tuning the spectra of light, coral breeders may be able to favor growth and simultaneously enhance the color of cultured specimens.

Marine ornamental species displaying short life cycles are commonly cultured to ensure that public or private aquariums can continue to display them. A nice example is that of jellyfish, which commonly have lifespans of several months to a few years (Lucas, 2001). Once solely displayed in public aquariums, the mesmerizing beauty of jellyfishes can now also be admired in private aquariums illuminated with state of the art LED technology. Given their lifespan, a permanent supply of specimens is commonly required to assure that displays are kept stocked as desired. This constraint is in fact an excellent business opportunity for some jellyfish breeders.

2.4 Conclusions

Overall, it is unquestionable that a sustainable marine aquarium trade may only be possible if the aquaculture of marine ornamental species continues to expand and a larger proportion of the wild organisms currently being traded are replaced by cultured specimens. Whether captive bred or captive raised (born from larvae spawned in captivity or in the wild, respectively), cultured marine ornamentals must be valued by hobbyists, who ultimately must be willing to pay a higher price for these specimens. If it is true that not all marine ornamentals should be cultured, hobbyists must also be conscious that preferring less expensive organisms collected from the wild using unsustainable practices may cause this industry, as we presently know it, to collapse. Restricting or even banning the harvest of some marine ornamental species from the wild to supply the marine aquarium trade is becoming a growing option when advocating reef conservation (Dee *et al*., 2014). If all those in the marine aquarium trade "look the other way" and

continue to rely on the harvesting of inexpensive specimens from the wild, it is possible that in the near future the collection of several banner species in the trade will be severely restricted, or even prohibited. This scenario is even more likely to occur for species known to display poor survival in captivity and/or along the supply chain. Wild populations of these species are probably already overfished, as more specimens than those truly needed to fulfil demand must be collected to compensate for all losses along the supply chain. These species will likely be the main targets for future aquaculture efforts, as a drop in the availability of large numbers of inexpensive wild specimens will prompt a surge on demand that can solely be fulfilled through cultured specimens.

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3

Life Cycles in Marine Ornamental Species – Fishes as a Case Study

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Abstract

Marine ornamental fish show a great variety of sexual patterns that are the result of specific evolutionary selections and environmental adaptation. Most of the species can be divided into pelagic and demersal spawners. Pelagic spawners usually release gametes in the water column at dusk while demersal spawners have adhesive embryos and parental care. These two modes of reproduction show several differences and specificities, and are fully analyzed in the present chapter. This information is fundamental to the selection of the appropriate species to culture.

Keywords *Fish sexuality; hermaphroditism; demersal spawning; pelagic spawning; embryonic development*

3.1 Introduction

Fishes constitute the largest group among vertebrates (Nelson, 2006), with more than 32,000 species known to inhabit our planet; numbers that are still increasing as stated in FishBase, a database of all extant fish species (Froese & Pauly, 2014). Fish have a wide variety of reproductive strategies and thus different expressions of their sexuality (Sadovy de Mitcheson & Liu, 2008; Erisman *et al*., 2013). This is mainly due to the great plasticity they have regarding sexual determination and sexual differentiation mechanisms (Blázquez *et al*., 1998a; Munday *et al*., 2006; Ah-King & Nylin, 2010; Kobayashi *et al*., 2013). Different sexual patterns can be found among fish including gonochorism, hermaphroditism and unisexuality. In this chapter we often refer to a comprehensive review (Devlin & Nagahama, 2002), that constitutes an up-to-date study for any researcher working on sex differentiation and fish sexuality. Since then, several advances have been made, specifically in the understanding of the molecular basis of sex determination and sex differentiation. This review also shows

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how molecular biology, and an increase in genomic resources of several species, have boosted our knowledge of these fundamental processes.

3.2 Patterns of Sexuality in Fish

3.2.1 Gonochoristic Fish

Gonochorism is the most abundant pattern and is characterized by the presence of separated sexes, i.e., individuals mature, and thus reproduce, only as males or females along their entire lifespan, regardless of their gonadal morphology. Although this pattern seems simple, it can reach some complexity giving rise to different subclasses.

3.2.1.1 Differentiated Gonochoristics

Also termed primary gonochoristics. The undifferentiated gonad develops directly into an ovary or a testis and during life they produce mature gametes of only one sex. This is the case of European sea bass, *Dicentrarchus labrax* (Blázquez *et al*., 1998b; Piferrer *et al*., 2005), tilapia, *Oreochromis niloticus* (Nakamura & Nagahama, 1985; Nakamura & Nagahama, 1989), or medaka, *Oryzias latipes* (Yamamoto, 1969; Satoh & Egami, 1972), all of them widely recognized models for the study of several aspects of fish reproductive biology, including sex determination and sex differentiation.

3.2.1.2 Undifferentiated Gonochoristics

The undifferentiated gonad develops into a rudimentary ovary to reach a point when in half of the individuals the ovarian tissue regresses and is replaced by testicular tissue, whereas in the other half the ovarian tissue continues development into a mature ovary. This is the case of zebrafish, *Danio rerio*, one of the most widely used fish model species in basic biology, for which sex differentiation in males is characterized by a juvenile ovary-to-testis transformation (Maack & Segner, 2003; Wang *et al*., 2007; Liew & Orban, 2014).

3.2.1.3 Secondary Gonochoristics

Known as non-functional hermaphrodites. Fish exhibit a period of intersexual stage with developed ovarian and testicular tissue. The difference with a true hermaphrodite depends on the fact that these fish only mature as a male or a female and thus, from a functional point of view they can only be males or females. Examples include European eel, *Anguilla anguilla* (Colombo *et al*., 1984; Beullens *et al*., 1997), and several tropical species such as Nassau grouper *Epinephelus striatus* (Sadovy & Colin, 1995) or fairy basslet *Gramma loreto* (Asoh & Shapiro, 1997).

3.2.2 Hermaphrodite Fish

Hermaphroditism has been documented in about 6% of teleost families (Sadovy de Mitcheson & Liu, 2008; Erisman *et al*., 2013), that is 27 families out of 448. Hermaphrodite fish produce mature gametes of both sexes during their lifespan and they have the ability to change sex. This sexual pattern is quite common among reef-associated and coastal marine species. Although the definition appears to be quite straightforward, there is an intrinsic difficulty to classify the hermaphrodites into

categories (Sadovy & Shapiro, 1987; Sadovy & Domeier, 2005; Sadovy de Mitcheson & Liu, 2008). Different types of hermaphroditism can be found among fish including synchronous, sequential and bidirectional; however, the exact biological mechanism that stimulates sex change is not fully understood (Munday *et al*., 2006). The size-advantage model is a widely accepted theory to explain the adaptive significance of sex change in sequential hermaphrodites (Ghiselin, 1969), although it caused some controversy in the late 1980s (Shapiro, 1988, 1989; Warner, 1989). The model considers that sex change occurs when the reproductive success of a given species increases with size or age more rapidly for one sex than for the other. In other words, when an individual reproduces most efficiently as one sex at a given age or size, but as the opposite sex when it gets older or larger.

3.2.2.1 Synchronous Hermaphrodites

Also known as simultaneous hermaphrodites, they are characterized by the presence of testicular and ovarian tissue coexisting in the same gonad. Fish produce mature gametes of both sexes, either alternating sperm and egg delivery or simultaneously, resulting in some instances in auto-fertilization. This is the case of killifish *Rivulus marmoratus*, the so far only known obligate self-fertilizing hermaphrodite among vertebrates (Harrington, 1961, 1971; Soto *et al*., 1992). Cole & Noakes (1997) demonstrated that the sequence of sex allocation in this species is similar to that of diandric protogynous species (see below) and that only in sex-changing females to males, where testicular and ovarian mature tissue coexist, can it result in self-fertilization. However, the triggering mechanisms of ovulation, auto-fertilization and spawning remain elusive (Sakakura *et al*., 2006).

3.2.2.2 Sequential Hermaphrodites

Fish start life differentiating and producing mature gametes of one sex for a given period and afterwards they change to the other sex.

Protogynous: Female-to-Male Sex Change

This is the most common form of hermaphroditism among fish (Warner, 1984; Avise & Mank, 2009; Kobayashi *et al*., 2013) and characteristic of many labrids (wrasses), scarids (parrotfishes) and other coral reef-fishes such as dottybacks and angelfishes (Figure 3.1a, b) (see Sadovy de Mitcheson & Liu, 2008 for references).

Monandry All males, also termed secondary males or terminal phase (TP) males, derive from functional females. Examples can be found in the Hawaian grouper, *Hyporthodus quernus* (DeMartini *et al*., 2010), dwarf angelfish, *Centropyge potteri* (Lutnesky, 1994) or the coral reef-fish, *Anthias squamipinnis* (Shapiro, 1981).

Diandry Two types of male are present within the population, namely initial phase (IP) males and terminal phase (TP) males. IP males, also known as primary males or sneaker males, are fish that mature first as males and remain as such throughout life. TP males, or secondary males, develop ovaries as juveniles and later during adulthood the ovary changes sex into a functional testis. IP males quite often resemble females and thus, are able to approach the harem and mate with them before the TP male can even have the chance to do so. Under appropriate conditions both females and IP males change to TP males. Female-to-TP male conversion is accomplished by a sex change, whereas IP



(a) (b)

Figure 3.1 Protogynous female-to-male sex change (a) *Pseudochromis fridmani*; (b) *Pomacanthus semicirculatus*. (*See insert for color representation of the figure.*)

male-to-TP male by a change of the reproductive status. Several examples of diandry can be found in saddle wrasse, *Thalassoma duperrey*, blue-head wrasse, *Thalassoma bifasciatum* (Warner & Robertson, 1978; Warner & Swearer, 1991), or the chocolate hind, *Cephalopholis boenak* (Chan & Sadovy, 2002; Pandian, 2012).

Protandrous: Male-to-Female Sex Change

This type is not as widely documented as in the case of protogynous species and it is more difficult to diagnose (Sadovy & Shapiro, 1987). It appears widespread among clownfishes or anemone fishes (Figure 3.2) (Chan & Yeung, 1983; Devlin & Nagahama, 2002; Sadovy de Mitcheson & Liu, 2008).

Monogyny All females in the population are termed secondary females or TP females and derive from IP males, just the reverse situation of monandry and the most abundant condition for a protandrous species.

Digyny This is a very rare condition and has been documented in *Lates calcarifer* (Moore, 1979). As shown for diandry, two types of females are present in this class, the primary females that derive from a juvenile immature fish and the secondary females that are the result of a male-to-female transition.

Bidirectional Hermaphrodites (Serial Sex-Changers)

Males and females can change sex in both directions several times during their life. First described in the goby *Trimma okinawae* under laboratory conditions (Sunobe & Nakazono, 1993), and recently confirmed in natural populations (Manabe *et al*., 2007). It is typical of several obligate coral-dwelling goby species belonging to the genera *Trimma* (Sunobe & Nakazono, 1993), *Paragobiodon* (Kuwamura *et al*., 1994; Nakashima *et al*., 1995), *Gobiodon* (Nakashima *et al*., 1996; Munday *et al*., 1998), *Lythrypnus* (Lorenzi *et al*., 2006; Rodgers *et al*., 2007). This type of sexuality has also been described in other coral-reef gobids (see Munday *et al*., 2010; Herler *et al*., 2011 for recent reviews), all of them previously classified as protogynous (Sadovy de Mitcheson & Liu, 2008).

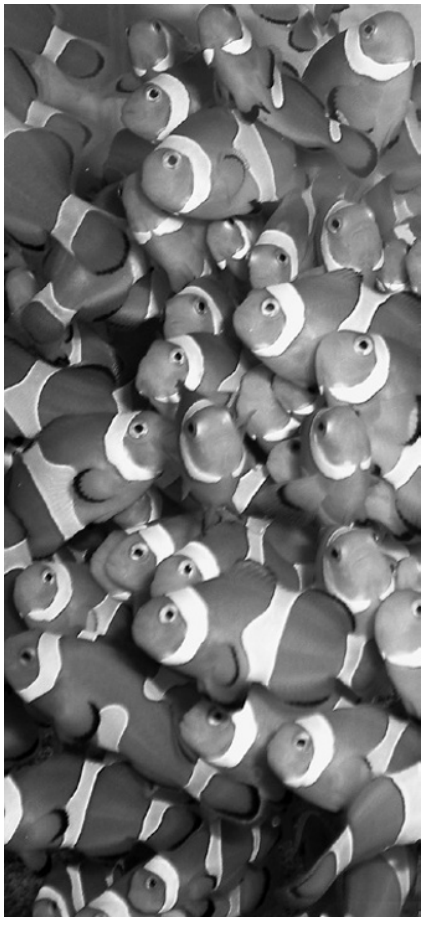


Figure 3.2 Protandrous male-to-female sex change anemonefish *Amphiprion ocellaris*.

3.2.3 Unisexual Fish

The first report of unisexuality in fish was that of Amazon molly, *Poecilia formosa* (Hubbs & Hubbs, 1932), a small all-female live-bearing species. Two sperm-induced non-meiotic types of reproduction have been found in fish, namely hybridogenesis and gynogenesis (Lampert, 2009; Lampert & Schartl, 2010; Neaves & Baumann, 2011). In hybridogenesis, oocytes are fertilized by the sperm of a closely related species, and is a hemiclonal form of reproduction. The genetic material from the male is present in the F1 generation, but it becomes excluded during the oocyte formation for the next generation and it is therefore not heritable. However, the maternal genome is transmitted along generations (Vrijenhoek, 1994; Lamatsch & Stöck, 2009), as shown in the complex *Poeciliopsis monacha* × *Poeciliopsis lucida* (see Lampert, 2009 for examples). In pure gynogenesis, sperm is only needed to activate egg division, but there is no paternal genetic contribution to the resulting offspring. Diploidy is restored by the retention of a polar body and thus, inheritance is strictly clonal. This is the case of *P. formosa*, that resuslts from a single hybridization event involving *Poecilia mexicana* (maternal) and *P. latipina* (paternal) (Avise *et al*., 1991). However, there are also ways in which

some sort of paternal DNA leakage may occur including the appearance of microchromosomes and polyploidization (Lampert, 2009; Lampert & Schartl, 2010). The addition of microchromosomes, small parts of the paternal genome that may remain in the oocyte, has been reported in *P. formosa* (Schlupp, 2005; Nanda *et al*., 2007). On the other hand, polyploidization results in an increase in the number of chromosome sets within a species (Le Comber & Smith, 2004; Piferrer *et al*., 2009) and is considered to be a significant phenomenon in fish evolution (Le Comber & Smith, 2004). It has been found in unisexual fish, and takes place after the addition of one or several haploid genomes from the sperm into the gynogen. Two different types of polyploidy exist depending on the origin of the extra chromosome sets, e.g., autopolyploidy and allopploidy. Autoploidy takes place when the multiplication in the number of chromososme sets occurs within the same species and is mainly due to failures in the normal process of meiosis (Yamazaki *et al*., 1989; Cherfas *et al*., 1991, 1995; Lampert *et al*., 2005; Lampert & Schartl, 2008). Allopolyploidy results from the multiplication of the chromosome set after intergeneric or interspecific hybridization (Lampert *et al*., 2005; Lampert & Schartl, 2008; Lamatsch & Stöck, 2009). This is the case of *Squalius alburnoides* (Alves *et al*., 1997; Matos *et al*., 2010), Prussian carp, *Carassius gibelio* and the goldfish, *Carassius auratus* (Luo *et al*., 2014).

3.3 Sex Determination and Sex Differentiation in Fish

Sex determination and sex differentiation are key processes in the field of reproductive biology. Generally speaking, sex determination is considered as the developmental process that directs the transformation of the undifferentiated gonad into an ovary or a testis, whereas sex differentiation is the actual formation of the gonads, including the appearance of both somatic and germinal tissue of each sex (Hayes, 1998; Devlin & Nagahama, 2002, Penman & Piferrer, 2008). Sex determination can be determined genetically (GSD) or environmentally (ESD), and also show transitional stages of GSD with environmental influences (Devlin & Nagahama, 2002). This is mainly due to the fact that gonadal differentiation, in the majority of fish, covers an extended period and thus, they can be exposed to different environmental factors that could interfere with the normal course of the process (Blázquez *et al*., 1998a). The most abundant type of ESD is TSD, where temperature is the external cue driving sex determination. TSD was first reported in reptiles, specifically in the agama lizard, almost 50 years ago (Charnier, 1966). Several reaction norms to temperature have been shown in reptiles whereas only one seems to be present in fish, with high temperatures resulting in an increase in the number of males of a given population (Ospina-Alvarez & Piferrer, 2008). Among all the teleosts known to react to temperature, only those belonging to the Atherinopsidae family, *Menidia menidia* and *Menidia peninsulae* (Conover & Heins, 1987; Conover, 2004) have been demonstrated to occur in natural populations. Although in the pejerrey *Odonthestes bonariensis* there is a strong conviction that TSD also applies to natural populations (Strüssmann *et al*., 2003), true evidence to prove it is still lacking (Gustavo Somoza, pers. comm). A study based on a literature review of field and laboratory data of more than 59 teleost species claimed to exhibit TSD demonstrates that in fish it is not as common as previosly presumed (Ospina-Alvarez & Piferrer, 2008). Although the influence of environmental factors, particularly temperature, in sex determination and

differentiation has been widely shown, the exact molecular mechanisms involved are still under debate (see below).

3.4 Molecular Markers of Sex Differentiation

The endocrine control of sex differentiation involves the perfect coordination of the reproductive axis and includes a fine tuned interaction among the brain, the pituitary and the gonads. Within this scenario, the brain appears to be the main sensor and integrator of internal and external cues, although its role on fish sex differentiation has not been studied in detail (Blázquez & Somoza, 2010). Yamamoto's pioneering studies with medaka were the first to show the prominent role of sex steroids in fish sex differentiation (Yamamoto, 1962, 1969). A detailed study on the endocrine control of sex differentiation is beyond the scope of this chapter, but we prompt the readers to search through several seminal studies and comprehensive reviews about this topic (Guiguen *et al*., 1999; Baroiller *et al*., 1999; Devlin & Nagahama, 2002; Nakamura *et al*., 2003; Guiguen *et al*., 2010). The development and refinement of molecular biology techniques has resulted in an increase of genomic resources to study sex differentiation (Orban *et al*., 2009). Possible molecular markers of this process include genes coding for steroidogenic enzymes, *cyp19a1a*, *cyp11b*, sex steroid receptors, several transcription factors such as *dmrt1*, *sox* genes, *sf1*, *dax1*, *foxl2*, *wnt1* and growth factors like *amh*, *igf1*. In this review we will not emphasize the role of these markers but just refer the readers to several thorough studies and reviews (Liu *et al*., 2007; Vizziano *et al*., 2007; Alam *et al*., 2008; Piferrer & Guiguen, 2008; Kobayashi & Nagahama, 2009; Blázquez *et al*., 2009; Blázquez & Somoza, 2010). Among these factors, *cyp19a1a*, the gene coding for gonadal aromatase, the enzyme responsible for the conversion of androgens into estrogens, is probably the most studied (Piferrer & Blázquez, 2005; Blázquez *et al*., 2008; Guiguen *et al*., 2010). Latest studies suggest that the expression of some genes involved in sex differentiation is epigenetically regulated, i.e., heritable changes in gene expression without involving changes in the DNA sequence (Manolakou *et al*., 2006; Matsumoto & Crews, 2012). This is the case of *cyp19a1a*, for which sex-related differences in the methylation pattern have been shown in medaka (Contractor *et al*., 2004). Moreover, temperature-driven sex differentiation in sea bass, *D. labrax*, seems to be induced by changes in the methylation pattern of *cyp19a1a* promoter (Navarro-Martín *et al*., 2011). Recently, the study of the gonadal methylome in the tongue sole, *Cynoglosus semilaevis*, showed that sex differentiation core genes, including *cyp19a1a*, *dmrt1*, *gsdf1*, and *amh* were differentially methylated as a result of temperature sex-reversal (Shao *et al*., 2014), further supporting the role of methylation in sex differentiation of some TSD species.

3.5 Transcriptomic Studies on Fish Sex Differentiation

In the past decade, a great deal of research has been focused on the study of fish transcriptomes in an attempt to reveal changes in the expression of key genes involved in sex differentiation. That is the case of zebrafish (Li *et al*., 2004; Wen *et al*., 2005; Santos *et al*., 2007; Wang *et al*., 2007; Sreenivasan *et al*., 2008; Small *et al*., 2009) and other species of commercial interest including Nile tilapia, *O. niloticus* (Ijiri *et al*., 2008; Wang

et al., 2010; Tao *et al*., 2013), Senegalese sole, *Solea senegalensis* (Tingaud-Sequeira *et al*., 2009), rainbow trout, *Oncorhynchus mykiss* (Baron *et al*., 2007, 2008; Hale *et al*., 2011), olive flounder *Paralichthys olivaceus* (Fan *et al*., 2014), Asian sea bass, *L. calcarifer* (Ravi *et al*., 2014), *Xiphophorus maculatus* (Zhang *et al*., 2011), and more primitive species such as sturgeon *Acipenser fulvescens* (Hale *et al*., 2010) and the coelacanths *Latimeria chalumnae* and *L. menadoensis* (Forconi *et al*., 2013). These studies have resulted in a new understanding of sex determination and sex differentiation and revealed new pathways, gene networks, and gene categories involved in these processes. Future directions focused on the molecular basis of sex differentiation in fish, include studies of the regulatory roles of microRNAs (miRNAs), an abundant class of small non-coding RNAs involved in post-transcriptional regulation of several biological processess (Ambros, 2004; Ivey & Srivastava, 2010; Kosik, 2010). The miRNAs have been shown to be involved in mamalian spermatogenesis (Yu *et al*., 2005, Mishima *et al*., 2008) and their potential role in ovarian and testicular differentiation has been suggested in birds (Bannister *et al*., 2009; Huang *et al*., 2010; Cutting *et al*., 2012). However, studies of their role in sex differentiation and maturation in fish are very scarce and limited to a few species including rainbow trout, *O. mykiss* (Juanchich *et al*., 2013) and Atlantic halibut, *Hippoglossus hippoglossus* (Bizuayehu *et al*., 2012). In zebrafish and medaka, miR-430 has been found to suppress PGC-specific genetic cascade in somatic cells further implicating miRNAs in gonadal formation (Mishima *et al*., 2006; Tani *et al*., 2010). Special efforts are currently being made to increase the annotation of miRNA transcripts (miRNome) in fish (Desvignes *et al*., 2014) and faccilitate the progression of functional studies to check their role in the transcription of genes involved in sex differentiation and gonadal development.

3.6 Demersal Spawners

One of the main modes of egg dispersal in fish is demersal spawning. Fish with this mode of spawning produce eggs that usually remain in direct contact with the substrate, either attached to a solid surface or spawned in small caves as gelatinous masses. In addition, monthbrooders that orally incubate their egg masses until hatching are generally included in this group (Tresher, 1984; Wittenrich, 2007) (Figure 3.3). One of the most important aspects in demersal spawning is parental care of the egg clutches, usually until they hatch.

Typical demersal spawners include clownfishes, gobies, damselfish and blennies that attach their embryos to a hard substrate; dottybacks, assessors and comets that usually spawn egg balls in small caves, and jawfishes and cardinalfishes that usually orally incubate their embryos until hatching. Some of these species are gonochorist (most of the damselfishes and gobies), namely they have a predetermined sex, while others are able to change sex during their life cycle and are thus hermaphrodites. The fish in this last group are usually sequential hermaphrodites; they can be protrandrous hermpahrodites (first males and eventually if social conditions are favorablechange sex to female) such as all clownfishes, or protogynous hermaphrodites (sex change from female to male) such as most of the dottybacks. Like most marine organisms, the life cycle of demersal spawner fish is a bipartite life cycle, which is divided into two specific phases that can be summarized in a necto-benthonic sedentary phase and a pelagic, dispersive

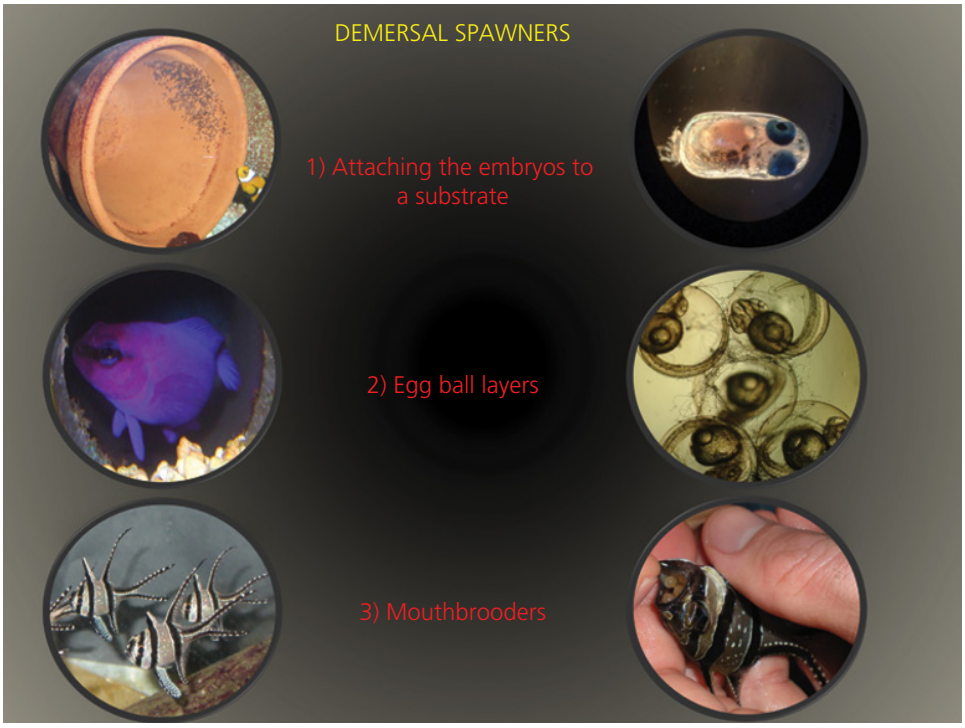


Figure 3.3 Different modes of demersal spawners: (1) clownfishes eggs are attached to a hard substrate through adhesive filaments; (2) dottybacks spawn gelatinous egg masses; (3) cardinal fish orally incubate their embryos.

planktonic phase. However, some species like the cardinal fish *Pterapogon kauderni* and the damselfish *Acanthochromis polyacanthus* completely skip the pelagic larval phase. In the first case, the embryos develop until the juvenile's stage in the male's mouth while in the second, broodstock fish display parental care until larvae reach the juvenile stage. As a consequence, dispersal and new habitat colonization are severely reduced in these species.

The life cycle of demersal spawners begins when a sexually mature male and a female start breeding. Before gamete release, demersal spawners rely on the availability of an appropriate substrate. Depending upon whether the fish are permanently territorial or not, they may possess a proper spawning site or they have to look for a suitable territory. After the appropriate substrate has been found (small caves, shells, and small pieces of coral) it is usually prepared and cleaned for spawning. Activities include fanning the substrate, biting it and removing small algae and invertebrates resulting in a very clean surface. In most cases this activity is performed by the male solely, but for example, in the case of monogamous pairs such as clownfishes and some gobies like *Gobiosoma*, both parents can be involved in nest preparation (Figure 3.4). Usually spawning activity in demersal spawners occurs during the day without a specific timing. Males and female release sperm and eggs; eggs can either be strongly attached to the substrate by adhesive filaments (like in clownfishes and damselfish) or simply spawned as a gelatinous mass (like in dottybacks and assessors) or orally incubated in mouthbrooders (cardinalfish).

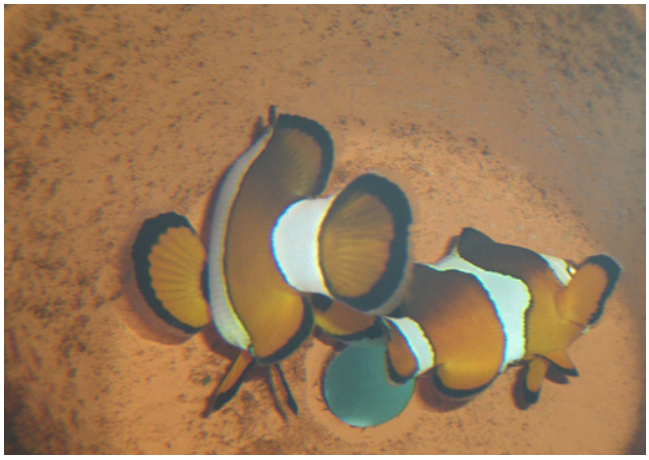


Figure 3.4 Spawning site preparation involves cleaning of the substrate. In monogamous pairs, like clownfishes, both parents are usually involved in this activity.

Once laid, demersal eggs are usually guarded and protected by the parents (typically the male) and potential predators chased. The effort put into parental care depends on the water circulation and oxygenation: the more current/oxygen present the less frequently the male has to fan the embryos. Males have often been observed mouthing the embryos, a practice that is important in removing dead embryos (Olivotto *et al*., 2011).

Demersal embryos develop in the egg until pigmented eyes and a finfold have developed, which usually occurs in several days depending on the species and water temperature. For example, at 28 °C, clownfishes usually hatch 7 days post-fertilization (pf), *Pseudochromis flavivertex* and *P. fridmani* 4 days pf and *Meiacanthus grammistes* 7 days pf (Wilkerson, 1998; Wittenrich, 2007; Olivotto *et al*., 2011). Demersal spawned eggs are usually larger than pelagic ones. Larger eggs have more yolk and usually generate larger larvae; this can be considered as a kind of maternal care since bigger larvae can feed on a greater variety of organisms and potentially increase survival.

The end of the embryo development is achieved through the breakdown of the chorionic membrane, a component of hatching. Hatching is both an enzymatic and a mechanical phenomenon determined by the synchronized action of embryonic movements, hatching enzymes, and, in demersal spawners, parental care (Olivotto *et al*., 2004). In several species, proper hatching may depend on environment light conditions, with most of demersal spawners hatching at night. The development of this photoregulated hatching is probably an ecological life strategy to reduce predation pressures on these larvae. Demersal spawned larvae hatch near the benthos which is a high predatory risk environment; however, the phototactic larvae are attracted far away from this high risk environment to the ocean's surface by the moonlight.

At hatching demersal larvae average 3–4 mm in size, and are thus bigger than larvae of pelagic spawners (Olivotto *et al*., 2011). The advantages of being a larger larva include increased mobility and the ability to feed on a great variety of potential prey organisms. Demersal spawned larvae are very competent at this stage; the yolk sac is almost exhausted and functional jaws, digestive tract and pigmented eyes are present. As soon as they enter the open ocean, where waters are clear, warm and rich in food items,

larvae undergo the transition from endogenous to exogenous feeding and start catching a variety of planktonic organisms including larvae of benthic species, protozoans and copepods. This pelagic dispersal phase is extremely important since it represents the basis of population connectivity and colonization of several coral reef areas. In previous years, when larval transport was initially studied, larvae were considered as passive animals that were transported exclusively by currents; recently researchers realized that besides this passive transport, many larvae have great motility abilities (Geoffrey *et al*., 2005). Demersal reef-fish larvae may disperse widely during their planktonic phase by becoming entrained in strong oceanic currents. For example while clownfish show the shortest larval phase in marine fish of about 10–14 dph (days post-hatch), *Pseudochromis fridmani* and *P. flavivertex* usually metamorphose in about 30–50 dph, *Meiacanthus grammistes* in 30–40 dph and *Chrysiptera parasema* in about 30 dph. While dispersing and feeding on a great variety of zooplankton, pelagic larvae usually exhibit very high growth rates, undergoing complex morphological/anatomical changes that takes them quickly from larvae to juveniles (Pérez-Dominguez & Holt, 2006). Unfortunately, the great majority of larvae die during the pelagic phase because of starvation, physical damage and numerous predators present in the ocean.

Following the pelagic larval phase, the few individuals that survive must undergo a complex process (metamorphosis) and settlement onto the reef habitat. The metamorphosis process is regulated by the hypothalamus-pituitary-thyroid axis. Metamorphosis in fish larvae is characterized by a series of processes that involve tissue differentiation, biochemical, physiological and molecular changes. In fish, prior to the maturation of the larval thyroid gland, the larval yolk sack contains significant amounts of thyroid hormones (THs) of maternal origin, of great importance for the larval early physiological regulation of growth, development and osmoregulation.

Larval fish metamorphosis is dependent upon endogenous synthesis of THs through the hypothalamus-pituitary-thyroid axis. The synthesis of THs occurs in the thyroid follicle, a single layer of epithelial cells enclosing a colloid-filled space and thyroxine (L-T_4) is the major hormone secreted. T_4 has a few roles and is thus considered as the precursor for triiodothyronine (T_3), the biologically active form of the hormone. The conversion of T_4 to T_3 occurs in the peripheral tissue of the gland by the enzymatic removal (5′-monodeiodination) of one of the iodide units of the outer ring of T_4. The biological action of the hormones is mediated by their binding to nuclear thyroid receptors (TR) (thyroid hormone receptors are members of the steroid–thyroid receptor super family) that act on target genes increasing or decreasing the expression of specific genes promoting the cellular/tissutal response to thyroid hormones inducing or deleting metamorphosis (Gilbert, 2000).

After undergoing the metamorphic process, larvae are able to settle on the reef. It has been observed that settlement activity in coral reef-fishes is sometimes related to the moon phase with settlement rates showing pulses both on full and dark moons (Irrison *et al*., 2004). Since tides are related to the moon phase, tides can facilitate reef colonization by the larvae. In addition, a larva settling with a group has better chances of surviving than a larva settling alone. To date how larvae are able to find their way to the coral reefs is still unclear. Several theories have been proposed by researchers including the attraction caused by specific sounds produced by reef's inhabitants and a possible chemical attraction caused by a different water chemistry of different areas. Since settlement usually occurs at night it has been suggested that for several larval fish species

olfaction and gustation play a pivotal role (Coppock *et al*., 2013). These olfactory cues are initially important to the larvae to detect the presence of reefs; once close to the reef larvae may use other cues to colonize appropriate areas of the reef for settlement. Several demersal spawners are well known to use olfaction to identify specific waters (Apogonidae) or island versus non-island reefs (*Amphiprion percula*) as well as host anemones (Coppock *et al*., 2013; Kohshima *et al*., 2014). In addition, clownfish recruits (*Amphiprion ocellaris*) are able to smell the difference between water containing their symbiontic anemone and water without it (Kohshima *et al*., 2014).

Even if demersal spawners protect their egg clutches through parental care, the bipartite life cycle, including a pelagic phase, causes great losses and it is estimated that only a very low percentage of the total number of larvae (1–5%) will settle on the reef and reach the juvenile stage. The juvenile stage is usually spent in areas where food is abundant and predators are few, such as lagoons and mangrove swamps. Some juveniles, like clownfish, may colonize new anemones or enter into an already existing clownfish structure in a sea anemone; others, like gobies, blennies and dottybacks will look for a territory rich in food to growth faster and reach sexual maturity, thus closing their life cycle.

3.7 Pelagic Spawners

The majority of marine fishes are pelagic spawners, which release pelagic eggs into the water column at the peak of a short spawning ascent. Out of approximately 12,000 marine teleosts, about 9000 (75%) produce pelagic eggs that are spawned, fertilized, and float, usually near the surface (Miller & Kendall, 2009). The large number of eggs that are produced and their rapid dispersal makes it impossible for the adults to show any form of parental care.

Typical fish that are pelagic spawners include snappers, grunts, goatfishes, angelfishes, butterflyfishes, surgeonfishes, jacks, lionfishes, soapfishes, tropical wrasses, parrotfishes, groupers and rockcods, that release pelagic eggs in the water column as part of the plankton. Some of these species are gonochorist (most of the snappers, grunts, goatfishes and surgeonfishes), while others are able to change sex during their life cycle and are thus hermaphrodites.

The life cycle of pelagic spawners begins when the male and female swim close together and the eggs and sperm are broadcast into the water. No parental care is involved with the eggs and the spent (spawned-out) adults may resume their prespawning activities. This is the most common spawning mode in the marine environment regardless of whether the habitat is demersal or pelagic, coastal or oceanic, tropical or boreal (Miller & Kendall, 2009). They often shed gametes in areas of strong currents that result in being carried quickly away from the reef. They spawn at times that result in the lowest risks of at least immediate predation by planktivores (at dusk and/or at the peak of the outgoing tide). In most cases spawning takes place at dusk. It can involve either individual pairs, groups spawning as a unit, or, in some species, both. For example, in the case of pair spawning in some species like the bluestreak cleaner wrasse *Labroides dimidiatus* and most angelfishes including the bluestriped angelfish *Chaetodontoplus septentrionalis*, a single male dominates a harem of two females, spawning with each at dusk, from 10 min before to 20 min after sunset.

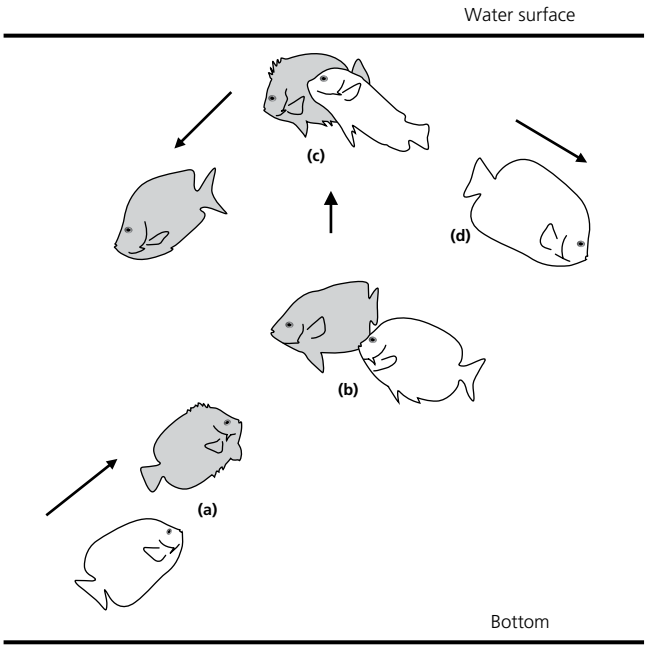


Figure 3.5 Courtship and spawning behaviour of the bluestriped angelfish *Chaetodontoplus septentrionalis* in the aquarium: (a) the male (white fish) approaches a female (gray fish) and displays his lateral body side toward the female; (b) the male nuzzles and pushes his snout against the belly of the female; (c) the pair ascend together and move forward just below the surface of water and gametes are released simultaneously; (d) after spawning, the pair returns to the bottom.

Their spawning involves soaring and nuzzling by a courting male and a slow straight swim toward the surface where gametes are shed and eggs fertilized (Leu *et al*., 2010) (Figure 3.5). The life cycle of the bluestriped angelfish is shown in Figure 3.6.

Many larger pelagic spawners, such as snappers and some surgeonfishes, are group spawners; they migrate to specific spawning sites thought to be optimal locations for offshore transport of eggs and larvae (Johannes, 1978; Robertson *et al*., 1979). A few pelagic spawners produce pelagic eggs in gelatinous masses. Lionfishes and scorpionfishes are among these. Pelagic spawning fishes in general are often larger than demersal spawning fishes. As a consequence of this general size difference, and the very small eggs they produce, pelagic spawners on average produce more eggs than demersal spawners.

Pelagic embryos develop in the egg until pectoral fin buds and the otic capsules have developed and usually this occurs in several hours depending on the species, temperature, oxygen, and salinity (Laurence & Howell, 1981; Yamagami, 1988). For example, at a 28 °C, *C. septentrionalis* usually hatch 15–18 hours pf, the sweetlips *Plectorhinchus vittatus* 17–20 hours pf at 27 °C, and the snapper *Lutjanus russellii* 27 hours pf at 24 °C (Leu *et al*., 2010, 2012; Leu & Liou, 2013). Pelagic spawned eggs are usually smaller than demersal ones. Smaller eggs have less yolk and usually generate small larval initial size; the theory being that the increased number of eggs and larvae overwhelms or saturates the predators so that at least some eggs and larvae survive.

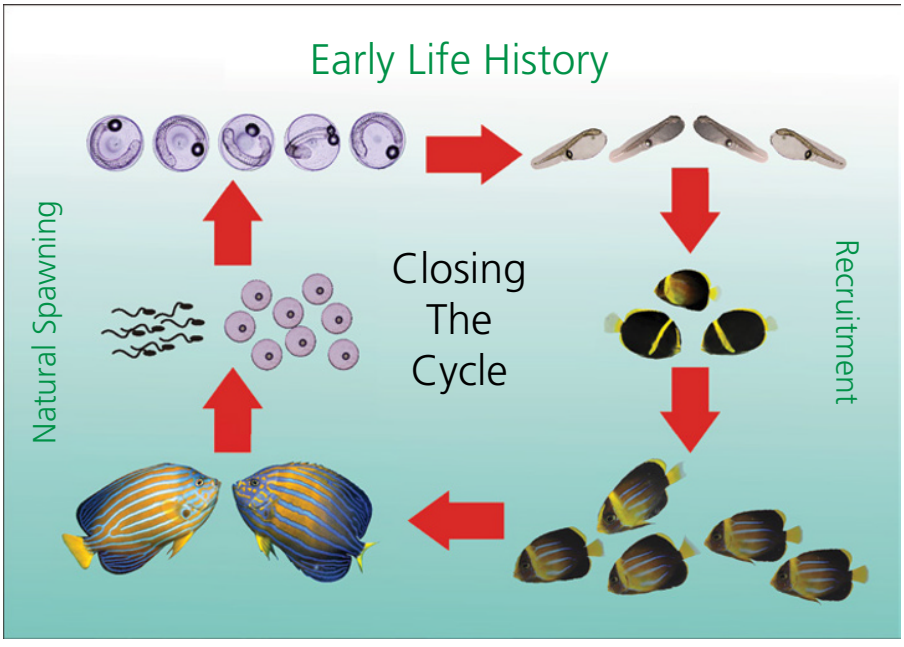


Figure 3.6 Information on the biology and life cycle of the bluestriped angelfish *Chaetodontoplus septentrionalis* in the coral reef.

Hatching occurs once the embryo has reached a certain stage of development. The mechanisms of the biochemical processes occurring during hatching are well described (Korwin-Kossakowski, 2012). Proteolytic enzymes in the perivitelline space begin to soften the envelope while enzymes produced by embryo digest proteins to form more soluble compounds. The envelope is digested from inside out, with the embryo using any products that are nutrients. As oxygen demand by the moving embryo exceeds the supply, the embryo moves more, helping it to escape the envelope. In large eggs the embryo tends to emerge tail-first; in small eggs the larva emerges head-first.

At hatching the pelagic larvae have an average size of 1–2 mm, and are thus smaller compared with those of demersal spawners (Olivotto *et al.*, 2011). The newly hatched larvae from pelagic eggs are little more than floating balls of yolk with a sliver of pro-larva attached. Such larvae are generally transparent, lack pigment in their eyes, lack differentiated fins, and are incapable of doing much more than floating. The advantages of developing during this stage outside the egg include better oxygen supply and better mechanisms to get rid of metabolites. The larvae can increase in size and develop without the restrictions of the eggs, while still getting nourishment from the yolk. They live off nourishment provided by the yolk for a few hours to a few days, depending on the species and temperature, after which they must eat to survive. During the yolk sac period, the larva develops the equipment needed to feed: the eyes become pigmented, the mouth opens and the gut forms, and locomotor capabilities increase. The period immediately after the yolk is exhausted and exogenous nutrition needs to commence is fairly brief, and may be a period of increased mortality. For example, newly hatched larvae of the grouper *Epinephelus malabaricus* are 1.93 mm long, lack functional eyes

or a mouth, are largely unpigmented, and have a prominent yolk sac that has a single oil droplet at its ventroposterior point (Leu *et al*., 2005). They are positively buoyant and are weak swimmers. By 3 dph the yolk and oil droplet disappear, the mouth and anus open, and the larva begins to feed. Laboratory experiments have determined that in some species there is a point of no return following the yolk sac period: if the larva does not eat during this period, it cannot survive, even if offered food (Houde & Schekter, 1980; MacKenzie *et al*., 1990; Canino & Bailey, 1995). At first feeding the gut of most larval fishes is a straight or coiled tube. Sections gradually develop in the gut: the foregut, midgut, and hindgut (Govoni *et al*., 1986). Prey are swallowed directly into the foregut, they are digested in the midgut, and material is absorbed in the hindgut. Larval fishes are generally visual feeders (Paul, 1983). Thus they usually feed only during the day in the upper layers of the water (Munk *et al*., 1989). Larvae eat a variety of prey organisms. The gape of the mouth determines the maximum size of the prey, which increases as the larvae grow. Energetic costs associated with eating small prey may be greater than those of eating larger prey, so even if larvae can eat small prey it may be better for them to eat larger prey. Larvae appear to be selective feeders, and often the prey in the gut is not proportional to the organisms of the same size in the water where the larvae were collected (Last, 1980). Protozoans and phytoplankton are found in the guts of some small larvae, but most commonly planktonic crustacean larvae are consumed (Stoecker & Govoni, 1984). Copepod nauplii are the most frequently found food item, and copepod eggs are another frequent prey item. For example, stomach analysis revealed that ciliates and flagellates were actively consumed by larval giant grouper *Epinephelus lanceolatus* at first feeding (Tew *et al*., 2013).

In the past years, it has become obvious that larval-fish dispersal is a biophysical process that can be strongly influenced by the behaviour of the larvae (Gallego *et al*., 2007; Leis *et al*., 2009a), and this dovetails with recent demonstrations that dispersal in coral reef-fishes can be considerably more constrained than would be predicted by physical processes alone (Jones *et al*., 2005; Almany *et al*., 2007). Larval reef-fishes have been shown to possess unexpectedly good swimming, sensory and orientation abilities that are clearly capable of strongly influencing dispersal trajectories from early in development. Coral reef-fishes provide some of the clearest evidence of constrained dispersal and self recruitment in marine systems (Swearer *et al*., 2002; Almany *et al*., 2007), and also some of the clearest evidence of strong larval swimming and orientation ability (Leis *et al*., 2009a). The non-reef species examined in that study have larval swimming abilities similar to those of reef-fishes, but reef-fish larvae have better developed orientation (Leis *et al*., 2009b). Pelagic reef-fish larvae may disperse widely during their planktonic phase by entering strong oceanic currents. For example while *Siganus canaliculatus* and *S. lineatus* show the shortest larval phase in marine fish of about 23–29 dph (May *et al*., 1974; Bryan & Madraissau, 1977), *E. malabaricus* and *L. russellii* usually metamorphose in about 30 dph (Leu *et al*., 2005; Leu & Liou, 2013), *C. septentrionalis* in 52 dph (Leu *et al*., 2015) and *Acanthurus triostegus* in about 2.5 months ph (Randall, 1961). With the transformation from the larval stage, a change in diet often occurs. This might be considered a second critical interval in the life history of fishes (Thorisson, 1994). Juveniles that settle to the bottom often start eating demersal crustaceans and infauna. With the larger body size of juveniles compared to larvae, the risk of starvation is much reduced. However, food deprivation can reduce growth rates and lead to smaller size at the end of the first growing season, which can result in increased mortality.

Feeding is only important for larvae after their yolk sac has been exhausted, but predation acts on all life-history stages. In recent years there has been much research on predation of eggs and larvae. Eggs and larvae are subject to predation from a wide variety of organisms. Major predators of pelagic eggs and larvae include chaetognaths (arrowworms), crustaceans, such as amphipods, euphausiids, copepods, and siphonophores, jellyfishes, and ctenophores. Pelagic larvae also feed on other fish larvae. Cannibalism has been found in grouper (Leu *et al*., 2005), snapper (Leu & Liou, 2013) and sweetlips (Leu *et al*., 2012). Hseu (2002) estimated that fish larvae constituted 50% of the diets of larger grouper larvae. As the abundance of these predators varies temporally and spatially, their impact on survival of young fish will vary. In fact, predation is generally thought to be the most important source of mortality in juveniles (van der Veer *et al*., 1990). Juveniles are preyed upon by a wider range of predators than are larvae. Fishes, including conspecifics, are major sources of predation as are birds, marine mammals, and a host of invertebrates (e.g., shrimp, crabs).

Juveniles are more capable of directed swimming than larvae, and thus are no longer considered planktonic. They can actively migrate to nursery areas, sometimes aided by prevailing currents. Juveniles of many species are differently colored than adults and differ from them in behaviour. Juveniles are more strongly territorial than adults, which in many cases form large feeding groups (Thresher, 1980). Color change between juveniles and adults may well reflect such striking changes in social behaviour. For example, juveniles of *P. vittatus* have three irregular, oblique, dark-brown bars on the head, trunk and tail and are territorial, whereas adults have six black longitudinal stripes on the head and trunk and are gregarious (Leu *et al*., 2012); juveniles of doctorfish *Acanthurus chirurgus* are colored much like adults, being as gregarious as well (Thresher, 1984); juveniles of several species of *Holacanthus* and *Pomacanthus* maintain regular long-term cleaning stations on and near the reef, to which larger fishes come to have external parasites removed (Brockmann & Hailman, 1976). The juvenile stage is so different from both the larval stage and the adult stage that some consider there to be two metamorphoses: one between the larval and juvenile stage, and another between the juvenile and adult stage. With such remarkable changes in structure and habitat, survival becomes problematic for reasons that are not identical to those associated with the larval stage. For example, mortality factors for juveniles can reduce the size of year-classes that had good survival as eggs and larvae (Miller & Kendall, 2009). Juveniles eat constantly and grow rapidly; they often occupy a part of the reef different from that occupied by adults. Most species reach sexual maturity in one to two years.

3.8 Conclusions

In conclusion, the present chapter gives evidence that modern molecular techniques and the study of hormones and molecules involved in reproduction carried out in previous years have been essential to elucidate the complexity of fish sexuality and reproduction. The information presented here is essential to the reader to understand the variation in fish reproduction, and for making decisions on the selection of the species to breed.

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4

Early Culture Trials and an Overview on U.S. Marine Ornamental Species Trade

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Abstract

Ornamental aquarium fishes have been traded globally for centuries. In the last few decades, the trade in marine species has expanded to over 40 countries supplying tropical and temperate marine life for both public and private aquariums. Accurate trade data covering the diversity and magnitude of species are elusive. The poor record keeping by both exporting and importing nations hinders efforts to understand the trade and its implications on marine ecosystems. We provide an overview of a multi-year data set that catalogs the imports of marine aquarium fishes and invertebrates into the United States. Further, we examine the history of marine ornamental aquaculture and assess constraints and bottlenecks to commercial production. We demonstrate that pelagic larval duration (PLD), economics, and regulatory actions are constraints to commercial production, while selective breeding and alternative species are production opportunities. Aquaculture can provide environmental benefits by reducing collecting pressure on highly traded species, but may carry economic and environmental risks, such as livelihood displacement and increasing the number of tankbusters in trade.

Keywords *Invasive marine species; larval bottlenecks; pelagic larval duration (PLD); risks and benefits of aquaculture; www.aquariumtradedata.org*

4.1 Introduction

Ornamental fishes and invertebrates have captivated humans for hundreds of years. Ornamental fishkeeping originated in the 10th century in Asia (i.e., China and Japan) followed by the western cultural centers of Turkey and the Roman Empire. The proliferation of ornamental fishkeeping is closely tied to the keeping of fishes for food. For example, brightly colored food carp were likely pulled from ponds as novelties, and became the predecessors of the modern day goldfish. By the late 16th century, ornamental fishkeeping quickly spread across the globe by sailors, who marketed goldfish in

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addition to the spices and other commodities they traded. By the early 1600s and late 1800s, ornamental fish keeping caught on in Europe and the United States, respectively (Brunner, 2005). During this time, especially in Europe, interest in the natural world (e.g., naturalist cabinets) grew substantially, and the keeping of marine organisms in aquaria became the spotlight of the naturalist movement. In London England, Phillip Henry Gosse published the handbook *The Aquarium* (Gosse, 1856), and his private interests soon culminated in the opening of several public marine aquariums, most notably the London Fish House (Brunner, 2005). Over the last few centuries, advances in filtration technology have increased the ability to keep and breed a variety of marine species, which has catalyzed not only a growing understanding of natural history and an increased popularity of fish keeping but also the potential to negatively impact aquatic ecosystems that support the rapidly growing marine ornamental hobby. In recent times, aquaculture of marine ornamental species has been touted as a solution to the sometimes environmentally destructive collection and over-harvesting of marine species for aquaria.

Robust analytical assessments of the risks and benefits of ornamental fish aquaculture and the aquarium industry are rare (see Tlusty, 2002). Any analytical study must understand the trade in aquarium fishes, its history, and the history of aquaculture. Here we provide an overview of the marine ornamental trade, a new data resource for studying the trade, and a brief history of marine ornamental fish culture. We explore life history bottlenecks, regulatory constraints, and opportunities for the aquaculture production of species, and explore the potential pitfalls of increasing the number of small juvenile stages of large reef fishes available to the trade through increased aquaculture capacity. Finally, we conclude by re-examining and updating the risks and benefits of marine ornamental aquaculture.

4.2 Import Data and the Marine Aquarium Trade

The ornamental fish hobby is extremely large, although the true magnitude of this industry is unknown. While survey data indicate that 79 million individual aquatic animals are held by hobbyists in the U.S., it is estimated that 190 million aquarium fishes, both freshwater and marine, are imported into the U.S. yearly (AVMA, 2007). Between 1.5 and 2 million people worldwide are believed to keep marine aquaria. The trade in live marine animals is a global multi-million dollar industry, worth an estimated 200–330 million USD annually, and operating throughout the tropics (Wabnitz *et al*., 2003). Estimates have placed the total number of marine fish species in the global trade between 1,471 (Global Marine Aquarium Database, GMAD) to 2,300 (Rhyne *et al*., 2012a; Rhyne *et al*., 2015) with the best estimate of annual global trade ranging between 20 and 24 million marine individuals (Wabnitz *et al*., 2003). Wood (2001) placed the total global annual marine catch ranging between 14 million to over 30 million fishes with the caveat that a number of assumptions have been made in reaching these figures, so they should be treated with caution. Rhyne & Tlusty (2012) demonstrated that both technology and health of the wider economy has the potential to greatly impact the marine aquarium trade.

Increasing the sustainability of the ornamental fish industry should be considered a primary initiative ("low hanging fruit") for retailers and the entire industry transport

chain (Tlusty *et al*., 2013; Rhyne *et al*., 2014). Increasing the sustainability of the ornamental supply chain can only come through a more thorough understanding of the magnitude of the trade (Amos & Claussen, 2009). Yet we currently lack a clear picture of the number of species and individuals in the marine aquarium trade. This is primarily a result of insufficient tracking of the global trade of marine animals, which stems from a lack of a standardized data collection format. Some countries use general categories of trade data (e.g., the Marine Aquarium Tropical Fish (MATF) designation by the U.S.), whereas other countries log the weights of shipped product (e.g., livestock and water weight by the Philippines), and yet still other countries record no data at all. A crucial first step toward a more sustainable ornamental fish industry is to assess the magnitude and biodiversity of imports for major consuming countries. The United States is a natural starting place for this analysis given that it alone is purported to account for about 50% of the trade in marine ornamental fishes (U.S. Coral Reef Task Force, 2000). Once country-specific trade assessments (both imports and exports) are completed, other germane issues including understanding and improving shipping quality and animal survival can be tackled, leading to environmental (less fishing effort as fewer fishes need to be supplied to the trade) and socio-economic benefits of this trade.

The lack of a standardized trade collection system is compounded by the fact that, even in the U.S. where multiple sources of trade data exist, trade data collection systems were not all intended for general wildlife trade monitoring. For example, endangered species are monitored by compulsory wildlife trade data maintained under federal mandates for species listed by the Convention on the International Trade in Endangered Species (CITES). Unfortunately, previous studies have demonstrated that CITES records can be inaccurate, incomplete, or insufficient (Bickford *et al*., 2011; Blundell & Mascia, 2005). Listed species (namely stony corals, giant clams, and seahorses) comprise a small proportion of the total ornamental aquatic animal trade, and only a few studies (Smith *et al*., 2008, 2009) have attempted to quantify the movement of non-CITES-listed aquarium species from source to market. Alternatively, U.S.-based studies have primarily relied on another data management system, the Law Enforcement Management Information Systems (LEMIS) as maintained by the United States Fish and Wildlife Services (USFWS). The USFWS is charged with inspection of wildlife shipments and maintains species-specific data only for CITES species per LEMIS requirements. Non-CITES-listed fish and invertebrate species are only listed with general codes (e.g., non-CITES marine fishes are coded as MATF – Marine Aquarium Tropical Fish). Thus while CITES-listed species are monitored and can be studied (Rhyne *et al*., 2012b; Foster *et al*., 2016), this general coding provides no information on volume, species diversity, or trade pathways (Smith *et al*., 2009), and hence non-CITES-listed species are not easily assessed. This dichotomy results in a data void, and there is a great need to develop a data resource that encompasses all species in the wildlife trade regardless of CITES listing status.

The lack of specific data systems for recording all species exported and imported for the wildlife trade raises two critical questions: First, how can importing and exporting governments monitor the industry effectively? Second, how should sustainability be encouraged given the paucity of data? As coastal managers scrutinize practices of the live animal trade, including efforts to reduce risks from introductions and diseases, the gap between the need for accurate accounts of trade data and the current monitoring methods are ever increasing (Bickford *et al*., 2011).

4.2.1 Reducing the Data Deficiency

To more accurately benchmark the size of the marine ornamental trade, Rhyne *et al.* (2012a) catalogued shipping invoice data for an entire year and compared the invoices to official shipment declarations for a single country of import (the U.S.). This analysis provided the first account of volume, biodiversity, and trade pathways of marine fish species beyond the limited coverage of information provided by volunteer reporting systems (i.e., Global Marine Aquarium Database, Wabnitz *et al.*, 2003).

The methods used to analyze trade invoices have been described by Rhyne *et al.* (2012a; 2015), and are briefly summarized here. To evaluate the diversity of non-CITES-listed aquarium species imported to the U.S., shipment declarations and attached commercial invoices containing MATF from four full years (2005, 2008, 2009, and 2011) were reviewed. Only fishes were recorded in 2005, and invertebrates were added to the dataset in 2008, 2009, and 2011. While 35,000 invoices contained MATF in the LEMIS database during this period, not all of these invoices were recovered and, of those recovered, not all contained aquarium species. Therefore, only about 29,000 shipment declarations and associated invoices containing MATF were recovered. Invoices were assessed at face value, as it was impossible to control incorrect information on invoices (or if the invoice was missing). Shipping information, species, and quantities from the shipping declarations and invoices were cataloged into a database. As reported by Rhyne *et al.* (2012a), two methods of data input were utilized: manual entry and automated optical character recognition (OCR). Species names were verified using World Register of Marine Species (WoRMS), FishBase, and the primary literature. Taxonomic information for species listed on invoices with junior synonymies were corrected. When species were listed under common names not isolated to a single species, or when genera- or family-level listings could not be resolved, species were listed as unknown. Taxonomic error was reduced by comparing species listed on invoices and in the database to geographic distribution records. Listings with obvious geographic mismatches were listed as "unknown".

Here we focus on major trade flows of fishes and invertebrates entering the U.S. In addition to the data presented in this chapter, we have developed an online platform where users can interact and query data (http://www.aquariumtradedata.org/). This online resource was developed to help anyone interested in the marine aquarium trade understand the trade with species-specific data from four years of import data.

4.2.2 General Trends in the Trade of Marine Aquarium Species

In 2005, 11 million marine fishes representing 1,802 species were imported into the U.S. About 5% of these fishes were unidentifiable to species level (e.g., listed as "assorted spp." on invoices, Rhyne *et al.*, 2012a). The total number of marine fishes imported into the U.S. decreased each subsequent year in the database. In 2008, 8.2 million total fishes representing 1,788 species were imported. In 2009 and 2011, 7.1 and 6.9 million marine fishes were imported, respectively. While in any given year no more than 1,802 species were imported, 2,278 different species were imported across the four years (Table 4.1).

The trade in marine invertebrates demonstrated a similar import trend as marine fishes. The number of individuals and species of invertebrates imported into the U.S. was greatest in 2008 (4.3 million individuals representing 545 species) and relatively

Table 4.1 Summary of invoice data for marine aquarium fishes and invertebrates imported into the United States across four years. No. (M) is millions of individuals imported as calculated from invoice data, ΔLEMIS is the difference (%) of between the number of fish as recorded from the USFWS LEMIS data and that of the invoice data (where a negative number indicates the LEMIS value is greater), Sp. is number of species imported, and % Sp. is percentage of individuals imported identifiable to species level.

	Fishes				Invertebrates		
Year	**No. (M)**	**ΔLEMIS**	**Sp.**	**% Sp.**	**No. (M)**	**Sp.**	**% Sp.**
2005	11.0	−30.2	1802	95.1	–	–	–
2008	8.2	−28.0	1788	97.4	4.3	545	73.4
2009	7.1	−39.7	1780	96.7	3.7	537	73.1
2011	6.9	−40.5	1798	96.7	3.7	535	72.2
Total unique			***2278***			***724***	

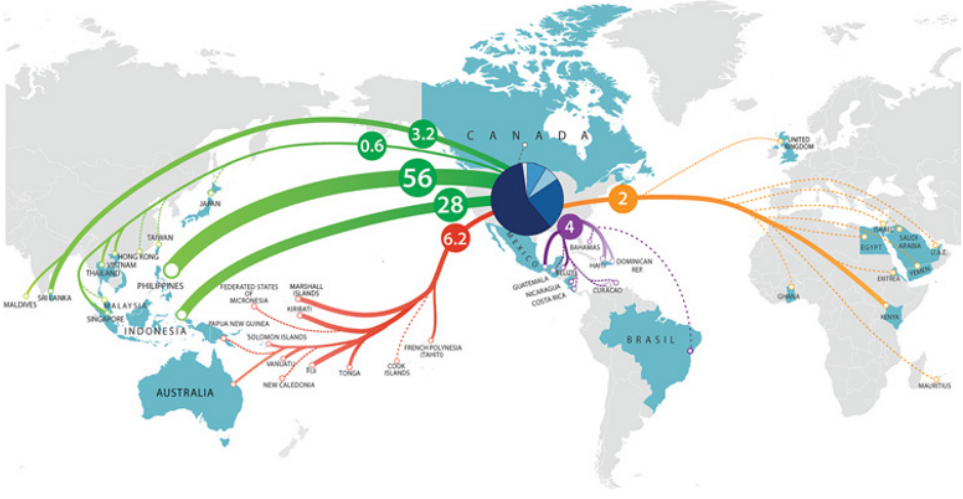


Figure 4.1 Trade flow of marine aquarium fishes from source nations to the United States. Numbers on lines represent percent total import. Pie chart within the United States represents ports of entry (with the Midwest starting at 0 degrees, and clockwise, NE, SE, SW and NW). (*See insert for color representation of the figure.*)

similar in 2009 and 2011 (3.7 million individuals and 537 and 535 species, respectively). The total number of species imported over the three years (724 species) was greater than the number of species imported in any one year (545 species). While marine invertebrate import trends were similar to those of marine fishes, relatively fewer invertebrates than fishes were identified to the species level (72.9 vs. 97.0%) (Table 4.1).

During the four years assessed, 45 countries exported marine fishes to the U.S. The Philippines exported on average about 56% of the total volume of marine fishes (Figure 4.1). The 37% decrease in the total number of fish imported by the U.S. across the years was mirrored in the number of fishes coming from the Philippines and

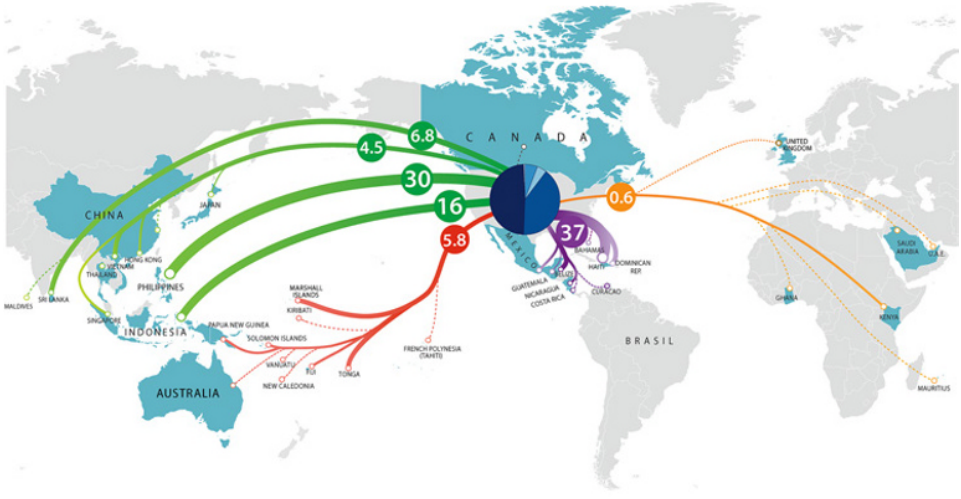


Figure 4.2 Trade flow of marine aquarium invertebrates from source nations to the United States. Numbers on lines represent percent total import. Pie chart within the United States represents ports of entry (with the Midwest starting at 0 degrees, and clockwise, NE, SE, SW and NW). (*See insert for color representation of the figure.*)

Indonesia. Sri Lanka (representing the third ranked country of export) remained consistent across the four years, whilst fourth ranked Haiti decreased exports by nearly 50% over the four assessed years, with the larger drop being a result of the global decline compounded by the 2010 earthquake.

A total of 38 countries exported invertebrates to the U.S. across the three years assessed (27 countries in 2008, 2009; 28 countries in 2011, Figure 4.2). The total number of exported individuals declined by 14% over the three years. The Philippines (3.6 million), Haiti (3.1 million), and Indonesia (1.8 million) exported the greatest number of individual invertebrates over the three years. The Philippines increased the total number of individuals exported by 24% between 2008 and 2011, while Haiti decreased exports by 48% between 2008 and 2011 (again, likely due to the massive earthquake in Haiti in 2010). The total number of individual invertebrates exported by Indonesia remained consistent across the three years; and Indonesia also exported the greatest number of species (413). The Philippines and Sri Lanka exported the second and third greatest number of species (Figure 4.2).

The top 20 species of marine fishes imported by the U.S. accounted for approximately 52% of the total volume of marine fishes imported (identified to the species level, Figure 4.3), and remained surprisingly consistent over the three years assessed. Specifically, the top 20 marine fish species list from 2008 and 2009 are identical, and, in 2011, the single difference was *Valenciennea strigata* replacing *Gramma loreto* as the 20th rank. The top seven marine fish species were consistent across all years, and represented nearly 33% of the total marine fish imports. The most popular species, *Chromis viridis* was consistently greater than 10% of total marine fish imports per year, and originated from between 13 to 16 countries yearly. The only other species of marine fish sourced from an equivalent number of countries was *Paracanthurus hepatus*, which originated from approximately 15 countries per year.

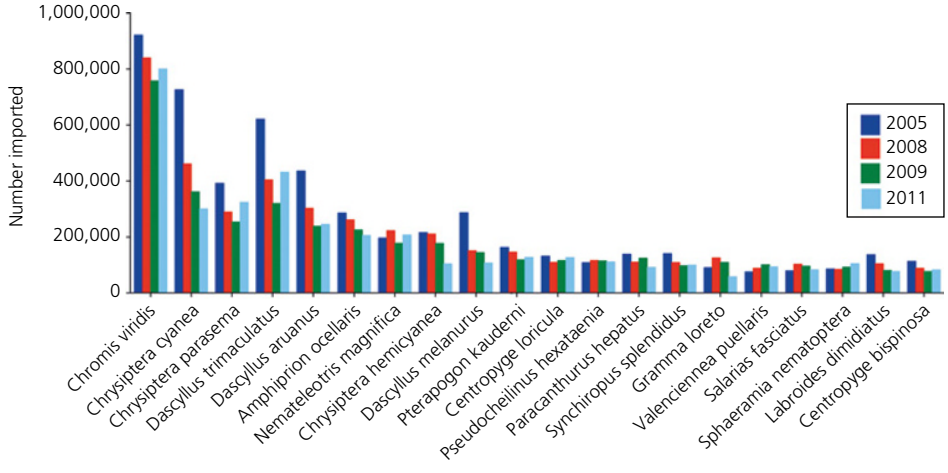


Figure 4.3 Top 20 marine aquarium fish species imported into the United States across four years. Dataset generated from www.AquariumTradeData.org.

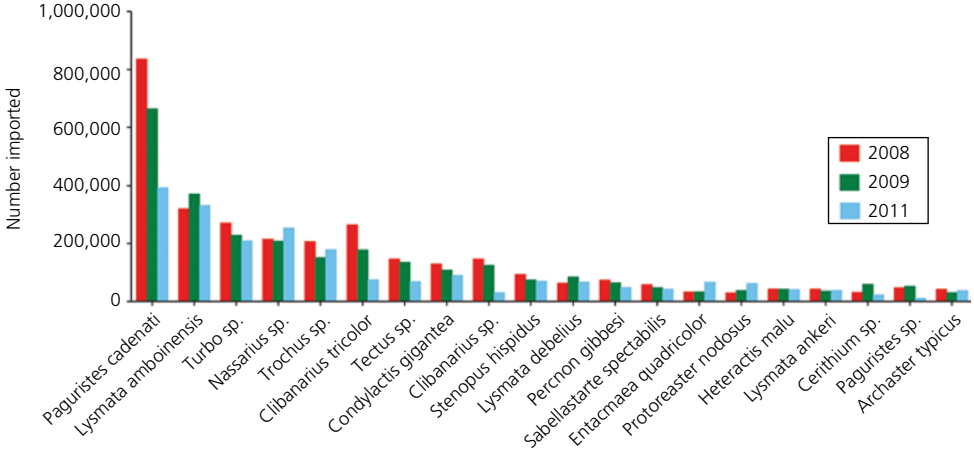


Figure 4.4 Top 20 marine aquarium invertebrate species imported into the United States across four years. Dataset generated from www.AquariumTradeData.org.

In contrast, the top 20 invertebrate species imported by the U.S. represented approximately 75% of the total volume of marine invertebrate imports (identified to the species level, Figure 4.4). Only the top two species (*Paguristes cadenati* and *Lysmata amboinensis*) were in an identically-ranked position among years. In all, 25 marine invertebrate species were represented in the top 20 list across the three years.

These data represent the volume of trade in marine ornamentals recorded as "wild capture" on import declarations. There are occasions where these self-declarations are incorrect (Rhyne *et al.*, 2012b). With the proliferation of aquaculture operations, cultured organisms can be confused with wild-captured organisms, leading to reporting inaccuracies. The most evident examples of this confusion are the stony corals (Rhyne *et al.*, 2012b) and clownfishes. It is currently impossible to accurately determine the

number of wild stony corals or clownfishes imported to the U.S. because cultured specimens are often mislabeled as wild products. A comprehensive assessment of the relative risks and benefits of both aquacultured and wild species is required to more accurately discern source trends in trade (Tlusty, 2002; Tlusty *et al*., 2013; Rhyne *et al*., 2014).

4.3 Aquaculture for the Marine Aquarium Trade: Bottlenecks and Opportunities

Brief History

Marine ornamental fish aquaculture is likely rooted in the tanks of European aquarium hobbyists, following their first successes as marine fish breeders (Schreiner, 1972; Moe, 2012). Commercial production of marine aquarium fishes in the U.S. began in the 1970s at the Aqualife Research Corporation founded by Martin Moe, followed by the Instant Ocean Hatchery founded by Frank Hoff (Moe, 1997). Moe's and Hoff's ornamental hatcheries demonstrated the feasibility of commercial production and likely served as the catalysts that prompted the prevalence of commercial ornamental aquaculture endeavors today. Moe's and Hoff's early successes were, at least in part, reliant on the growth and development of marine food fish aquaculture, as well as a deeper understanding of the life history and nutritional requirements of marine fishes in general. Perhaps the most important ingredient in the widespread successful culture of marine fishes was the discovery of a suitable live feed that could be cultured in large volumes and was accepted by marine fishes: the rotifers of the genus *Brachionus* (Ito, 1960; Lubzens *et al*., 1989). While using rotifers as a first-feed for marine fish culture greatly increased the number of species cultivated and the reliability of marine fish culture, rotifers also limited culture success to species with relatively simple larvae (i.e., consume large prey items) and nutritional requirements.

During the 1990s, commercial production of clownfishes, dottybacks, gobies, and other species with short pelagic larval phases increased, as did efforts to rear species with less developed and more demanding larvae (e.g., angelfishes from the family Pomacanthidae). Similar to ancient fish keepers in Asia, once fishes are regularly bred domestically, they can easily be bred for certain colors, shapes, and patterns. By the late 1990s, hatcheries were beginning to offer color morphs that were distinctly different from wild-type fishes, such as the pearl-eyed Clark's clownfish, *Amphiprion clarkii*, and black oynx clownfish, *A. percula*, that quickly became the first "designer" fishes in the marine ornamental fish market. The rapid diversification by selective breeding in clownfish and other marine fishes opened new opportunities for marine ornamental aquaculture (Pedersen, 2014).

Finding the appropriate foods was a critical step to the eventual success of marine fish rearing. Moe and colleagues successfully reared a few families of ornamental species in small numbers on wild plankton (i.e., copepods) (Moe, 1976, 1997; Potthoff *et al*., 1987). Moe (1997) quickly recognized the superiority of copepods over rotifers for certain species. As demand increased for seahorses and other species of marine fishes proven to be difficult to rear on traditional larval diets (i.e., rotifers and *Artemia*), researchers began developing culture methods for copepods and other live prey (Olivotto *et al*., 2011). Advancement in copepod nauplii production has provided renewed interest in marine ornamental aquaculture, and has been credited for the development and

success of seahorse aquaculture (Payne & Rippingale, 2000; Gardner, 2003) and other more demanding marine aquarium fishes (Shields *et al.*, 2005). Perhaps nowhere is this development more evident than the enormously successful *Centropyge* culture efforts of Reef Culture Technology (RCT) (Baensch, 2014). Similar to the initial successes of the first marine aquarium hatcheries, the successes of the RCT *Centropyge* breeding project has opened the door to future aquaculture possibilities, as well as a wave of challenges on the battleground where life history characteristics, culture technology, and economics converge.

4.4 Constraints and Opportunities for the Commercial Production of Marine Aquarium Species

4.4.1 Life History Characteristics as Constraints to Commercial Production

While clownfishes, cleaner gobies, dottybacks, seahorses, and a limited number of large marine angelfishes (genus *Pomacanthus*) are regularly produced at commercial levels, other fishes such as mandarin dragonets and pygmy angelfishes have not yet sustained regular commercial viability. Variations in commercial viability are usually explained by several life history attributes combined with market forces. The most consistent predictors of commercial success with respect to life history characteristics are (1) larval duration; (2) settlement size; and (3) live feed requirements. Growout duration and spawning mode are important, but minor constraints to commercial development. Most commercially available aquacultured marine aquarium species have a larval duration under 30 days (Figure 4.5). Generally, the cultured Pelagic Larval Duration (cPLD) of marine fish species is often different than the wild Pelagic Larval Duration (wPLD) of a species in its natural habitat. Much of this variation is driven by temperature, paternal identity, and larval nutrition under laboratory conditions (Searcy & Sponaugle, 2000; Green & McCormick, 2005). For example, the literature reports that angelfish species of the genus *Centropyge* have an average wPLD of about 32 days (minimum: 24 days; maximum: 39 days, Thresher & Brothers, 1985). Based on reported wPLD, *Centropyge* should be a strong candidate for commercial culture in the near future; however, the cPLD of many *Centropyge* angelfishes is substantially longer than reports of wPLD (Baensch, 2014). For example, the cPLD for *Paracentropyge venusta* is currently between 95–130 days (K. Brittain pers. comm.), in contrast to a wPLD of only 25 days (Brothers & Thresher, 1985). The discrepancy between cultured and wild PLD indicates larvae quality issues, nutrition deficiencies, or other unknown factors prevent larvae from developing at natural rates. On the other hand, clownfishes and other easily cultured species have a cPLD at or slightly shorter than published wPLD values (Figure 4.5).

Larval size at settlement is positively correlated with larval duration for most reef fishes (Wellington & Victor, 1989). A study of 100 species of damselfishes (family Pomacentridae) found larval duration ranges from 12 to 39 days with settlement size ranging from 6 to 14 mm (Wellington & Victor, 1989). Variation within and among species of the same genera has been demonstrated (Cowen, 1985; Bay *et al.*, 2006). A survey of 100 wrasse species demonstrated a high degree of variation among and within species of labrids (Victor, 1986, Figure 4.5). Reef fish species with long larval durations appear to be capable of delaying settlement and extending their wPLD (Searcy &

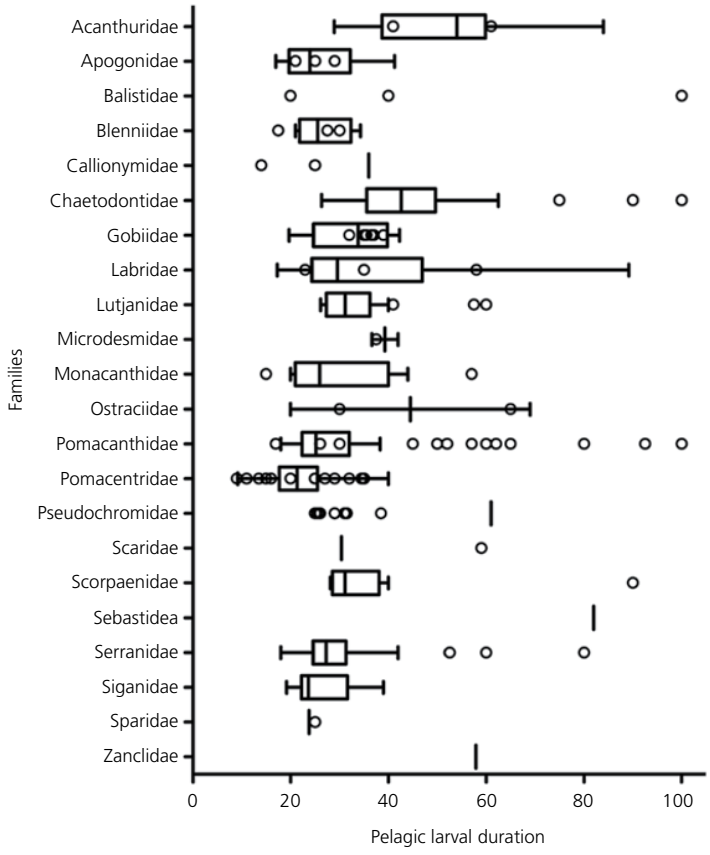


Figure 4.5 Comparison of pelagic larval duration (PLD, days) of marine aquarium fish families. Box-whisker plots represent PLD of wild specimens (wPLD) from the literature, and open circles represent PLD of cultured specimens (cPLD) from the literature and hobbyist publications. Error bars represent minimum and maximum PLD. (See Stier *et al*. and references therein.)

Sponaugle, 2000). The literature is ripe with information on the size of newly settled marine fishes and culturists should use natural history data on both settlement size and larval duration before undertaking commercial production efforts for a given family (e.g., labrids, Figure 4.5).

A current focus of the marine ornamental aquaculture industry is on tangs, most notably the yellow tang, *Zebrasoma flavescens*, and the blue hippo tang, *Paracanthurus hepatus*, and butterflyfishes such as the schooling bannerfish, *Heniochus diphreutes*, and millet seed butterflyfish, *Chaetodon miliaris* (http://www.risingtideconservation.org/). Unlike clownfishes, tangs and butterflyfishes have extended wPLD that make commercial culture challenging with current aquaculture bottlenecks. Callan (2016) reported rearing the iconic yellow tang and produced several hundred juveniles with a cPLD around 60 days, a significant improvement from previous reports (Williams, 2014 and Chapter 15 in this book). The correlation between larval size and larval duration suggests that yellow tangs likely settle at 54 days (wPLD) at around 33 mm TL (Basch *et al*., 2009). Similarly, larvae of the blue hippo tang have a reported wPLD of 37 days

and settle at around 30 mm TL (29.8 mm larvae reported by Johnson & Washington, 1987). Talbot (2016) reported the culture of the blue hippo tang in captivity for the first time by the University of Florida Tropical Aquaculture Laboratory (UF TAL) using the culture techniques Callan developed for the yellow tang. The cPLD from this cohort was 42 days (Barden, pers. comm.). While the recent developments of Callan and UF TAL are extremely promising, the life history of these species presents a significant challenge for the commercial production of tangs. Recent success by RCT in closing the life cycle of the schooling bannerfish provides additional contrast between cPLD and potential commercial production in reef fishes. Baensch (2014) reported a cPLD of 100 dph with the larvae settling at about 18 mm TL, which contrasts wPLD of 30 at 18 mm TL. While rearing marine fish species with extended PLDs is within the technical capabilities of some researchers, commercially viable production is still a challenge. Life history characteristics such as PLD and settlement size can be important predictors of commercial success for researchers and other interested parties.

4.4.2 Economic and Regulatory Constraints to Commercial Production

It has been well demonstrated that biological characteristics of marine species can hinder aquaculture production and make commercial production difficult or improbable. Likewise, economic and regulatory constraints can also inhibit the commercial success of a species or entire group of taxa. Here we provide two examples of reef fishes that are easily cultured; however, commercial success is limited due to current economic factors.

The Banggai Cardinalfish

There are few better examples of marine aquarium species that should be commercially produced via aquaculture than the Banggai cardinalfish (BCF), *Pterapogon kauderni*, a mouth brooding and geographically restricted species. Even though BCF is IUCN red listed, it is highly traded, under enormous collection pressure (Yahya *et al*., 2012), has been over-exploited, and suffers from a high level of supply chain mortality. A commercial supply of aquacultured BCF to address these issues is fleeting (see Chapter 13). The BCF quickly became a popular fish in the aquarium industry after its introduction to the trade (Allen, 1996). When first introduced it was a high valued species, retailing for over 100 USD per specimen. As seen in other aquarium species, BCF volumes increased and the retail price quickly dropped dramatically (Rhyne *et al*., 2014). Within a few years populations were being overexploited and some have yet to recover (Vagelli, 2011). A recent survey of collection sites continues to indicate evidence of over collection and population declines (Yahya *et al*., 2012). Yahya *et al*. (2012) noted that while nearly 100,000 fish a year are exported from approved locations, an additional yet unknown number is exported from other areas. While GMAD only indicates small levels of trade in 2001, our data consistently place BCF in the top 20 most imported fishes into the U.S. (Figure 4.3). These data corroborate reports (Talbot *et al*., 2013) that Indonesia's exports exceed the approved quotas put in place to protect this species. Numerous conservation organizations, scientists, and professional hobbyists have advocated for both trade protection via CITES and an increase in captive breeding efforts (Talbot *et al*., 2013). To date these efforts have been met with limited success. The key factors inhibiting the wide spread commercial production of BCF are the limited number of eggs

produced per spawn (<50) and time to market (4–5 months, Vagelli, 2011) combined with a low market price due to a high influx of low quality wild BCF (Talbot *et al*., 2013). This combination creates challenges for commercial aquaculture (Tlusty, 2002) as the efficiencies with economy of scale are difficult with pair bonded fish exhibiting low fecundity. Notwithstanding the economic challenges of commercial production of BCF, the recent regulatory environment (listed as threatened under the Endangered Species Act (ESA)) in the U.S. could prohibit the trade of this iconic species (NOAA, 2014). If an ESA listing allows trade in aquaculture fish, but limits the trade of wild caught specimens, then the regulatory environment could be a boom for aquaculture producers. Quotas on CITES-listed species have greatly increased market prices (Rhyne *et al*., 2012b) and the BCF is a likely candidate for CITES listing. It could also provide an additional challenge for unscrupulous exporters and/or importers that mis-identify wild-caught BCF. Regardless of proposed regulatory measures, a large-scale production (>120,000 fish per year or 75% of the current wild volume imported into the U.S.) of BCF within Thailand appears to have overcome the economy of scale, regardless of the low fecundity of the species (Rhyne, unpublished data), likely due to the cheaper production costs in Southeast Asia. As with other marine aquarium species, trade regulations can be a benefit or be an impediment to aquaculture production.

Mandarin Dragonets

Unlike the mouth brooding and geographically restricted Banggai cardinalfish, mandarin dragonets, *Synchiropus* spp., have a wide geographic distribution and are pelagic spawners. These species are a staple of the marine aquarium trade, consistently ranked in the top 20 marine fish species (Rhyne *et al*., 2012a, Figure 4.3). Two species represent the vast majority of trade: *Synchiropus splendidus* and *S. picturatus*. Dragonets are marketed as peaceful but delicate reef safe fish, requiring large amounts of live prey to be successfully kept in aquaria. These species suffer high collection related mortality and the fishery has been implicated in skewing population sex ratios (Sadovy *et al*., 2001). Mandarins often fail to acclimate to captivity or starve in aquariums lacking ample supplies of live feeds. As with many marine fishes in the aquarium industry, increased mortality along the supply chain increase the number of mandarins that need to be collected and sold. Mandarins have been cultured by hobbyists (Sprung, 1989); researchers (Wittenrich *et al*., 2010); and it was with great fan fair and excitement that Oceans Reefs and Aquariums, Fort Pierce Florida, U.S. produced commercial numbers of both *S. splendidus and S. picturatus* (ORA, 2010; Rhyne, 2010). Given the short larval duration (cPLD of 14–24 days) and high survival, mandarins should be an ideal species for commercial production. However, two factors have greatly impeded commercial production. First, the mandarins are extremely slow growing and require 6 months or longer to reach market size. Second, producers must compete with a large supply of cheaper wild fish. The extended growout time coupled with a large supply of cheaper wild fish yielding relative prices of 60 USD for cultured compared to 25 USD for wild) has limited the commercial success of these iconic species. Like the BCF, large-scale commercial production will likely only occur in countries with more extensive culture methods and cheaper labor forces.

CITES and ESA as Constraints and Opportunities in Aquaculture Production

The Convention on International Trade in Endangered Species of Wild Fauna and Flora is an intentional barrier to international trade, specifically targeting species that have been deemed at an extinction risk because of the trade. The Endangered Species Act (ESA) is a U.S. law that regulates listed species and can prohibit the collection, trade, and commercial sale (defined as *take*) of listed species. Both CITES and ESA provide significant regulatory hurdles for aquaculture producers, but may also provide opportunities by restricting trade to an aquaculture product or capping the trade in wild specimens. Within the marine aquarium industry the most traded CITES-listed species include stony corals (order Scleractinia), giant clams (genus *Tridacna*), and seahorses (genus *Hippocampus*). The CITES treaty member nations have specific rules to permit the commercial trade of captive bred specimens of species listed in CITES – Appendix I (https://cites.org/eng/app/appendices.php). These regulations have proven to be significant barriers to trade in stony corals. A few countries have overcome these challenges by issuing guidelines for aquaculture production (Rhyne *et al*., 2012b). For example, Indonesia has well documented guidelines (Pasaribu, 2008) for the commercial production of stony corals and supports the export of cultured corals under CITES source code F. Indonesia is actively reducing its export quota for wild corals and intends to supply the coral trade with a 100% aquacultured supply of stony corals. The Solomon Islands, on the other hand, has been producing aquacultured corals for several decades (Lal & Kinch, 2005) but has little to no government support or regulatory method for supporting CITES source code F. Thus, much of the coral shipped as wild product from the Solomon Islands is in fact aquaculture product. Production of corals in countries that have an active coral trade can provide economic and environmental benefits and should be encouraged (Rhyne *et al*., 2012b). These benefits are threatened by regulatory schemes which are designed to protect wild populations. For example, with the current listing of 20 stony corals on the ESA, the ability to import or export listed species and/or closely related species is in question. If the National Oceanic and Atmospheric Administration (NOAA) limits *take* to cultured specimens for listed species then restrictions on wild specimens could provide a market incentive to increase the value of aquaculture products and would likely increase the production of coral species via aquaculture. NOAA could however limit *take* to domestic production or prohibit the import or export of listed species. These restrictions would greatly inhibit aquaculture production. While hobbyists and researchers have little to worry about (fish tanks will not become illegal), changes in laws and trade regulations will affect commercial aquaculture vendors. The trade as a whole is entering new and uncharted waters. To develop a business, one must invest capital and time into species and, given the current uncertainties, this species may ultimately end up listed as threatened or endangered. Trade in restricted species is a difficult business model. Understanding the regulatory climate both within a country's border and within the global community is now essential to ensure commercial success.

4.5 Risks and Benefits of Aquaculture Production

The continued growth of aquaculture production offers several important benefits, both to end consumers and the reef-side communities (Tlusty, 2002; Rhyne *et al*., 2014). Capturing these benefits can be difficult and elusive; many aquaculture

initiatives are marketed as conservation efforts but fall far short of this goal. Other efforts are focused solely on the production of animals for the aquarium and food industries and have no concerns about the broader conservation issues associated with the aquarium trade. There are enormous conservation benefits that can occur with the commercial aquaculture production of aquarium species, but there are also significant risks (Tlusty, 2004). To ensure the long-term sustainability of the industry, the benefits of aquaculture must balance, if not outweigh the risks. Below we outline the most significant risks and benefits of aquaculture production for the marine aquarium.

4.5.1 Ecological Risk and Aquaculture Production as a Source of Nonindigenous and Invasive Marine Species

The introduction of the pacific lionfish, *Pterois volitans*, into the western Atlantic Ocean has caused environmental damage throughout the Caribbean and subtropical regions of the western Atlantic (Albins & Hixon, 2011). There is little doubt that the source of the founding population of lionfish was from an aquarium hobbyist or aquarium wholesaler in south Florida. The aquarium trade has been a vector for introduced marine fishes in south Florida for decades (García-Berthou, 2007). Most introductions do not rise to the level of an invasion; however, each additional species introduced into Florida or other nonindigenous location is cause for alarm. In response to the lionfish invasion, the Florida Fish and Wildlife Commission imposed a ban on the importation of all species of the genus *Pterois*. Further bans would be expected if additional species were to become established. It is therefore imperative that propagule pressure is reduced. It has been proposed that "*tankbusters*," those species that grow far too large to be housed in a home aquarium, are at the highest risk of being released by hobbyists and therefore experience a higher propagule pressure than other smaller species (Holmberg *et al*., 2015). Reef fish species often enter the aquarium trade at a small size, and become part of the food trade at a large size. Thus, aquaculture production of food fishes can generate thousands of individuals that can be sold to the aquarium trade as juveniles. Holmberg *et al*. (2015) tested the hypothesis that the desirability of marine tankbusters peaks early in life history by examining retail size and price vs. growth. Five representative internet-based marine ornamental fish vendors were surveyed, and data were gathered on retail size range, life history stage, price, origin, and maximum potential length (according to vendor) for over 700 species and compared size to data in FishBase. With an increasing volume and diversity of tropical marine fishes imported into the U.S., there is astonishing potential for the trade to influence propagule pressure. Given the risk of intentional release by consumers and the implications for propagule pressure, we posit that it is unwise to allow the sale of species that quickly and routinely outgrow their captive environment. Therefore it seems unwise to encourage or support the development of aquaculture technologies for species targeted for the aquarium trade that would easily outgrow hobbyist tanks. We propose that to increase the robustness of any preventive measures, the trade should be fully engaged in the framing of future actions. In order for this to happen, we propose the creation of a multi-stakeholder workgroup to curate a moving whitelist of species with high invasive potential, which would then be voluntarily disallowed (except for permitted activities). Until we have a better understanding of all factors involved in determining invasive

potential, we suggest that regulated quotas and caps are not sufficient given that a number of species are seldom traded yet potentially invasive (Holmberg *et al*., 2015).

4.5.2 Economic Benefits of Aquaculture in Reef Side Communities

The reef-side communities that collect marine ornamental species are fishing communities. They often have few alternate economic opportunities. While opponents of the trade have decried the collection of coral reef fishes as destructive (reviewed in Tissot *et al*., 2010), these oppositions do not address the corollary *what would be the opportunity cost of closing a fishery*? Species that are collected for a high value at a small size for the ornamental trade are still often fished for food when at a larger size, but will earn a significantly lower economic value for the fisher. Thus, ornamental fisheries need to be managed as a way to create value for coral reef socioeconomic ecosystems. For example, maintaining a relatively low biomass ornamental fishery can allow for managed no fishing zones, ecotourism, and other value-adding activities to derive the maximum benefit to these fragile ecosystems.

4.5.3 Reducing Bottlenecks and Opportunities for Growth

As the marine ornamental aquaculture industry continues to develop, it is essential that industry efforts advance the role of aquaculture as an economic and environmental tool. While innovation cannot be predicted, carefully considering new species to pursue, reducing live feed bottlenecks, and advancing culture technology and life support systems are several critical opportunities for industry growth.

Species Selection

We suggest future ornamental aquaculture efforts focus on species exhibiting high market potential and life history characteristics that lend well to aquaculture. This includes species that are already at market but have limited consumer availability (e.g., the yasha goby, *Stonogobiops yasha*), as well as species that are currently not common in the trade but exhibit strong market potential, such as the purple tailed dottyback, *Pseudochromis luteus* (Kai, 2012), or the DeJong's gramma, *Gramma dejongi* (Victor & Randall, 2010). Additionally, marine ornamental aquaculture can diversify the market for aquaculture products through the hybridization of commonly cultured species (e.g., clownfishes, Pedersen, 2014). Commercial producers should also focus on species that have similar ecological roles in aquaria as natural reefs, with life history characteristic more amendable to aquaculture production (e.g. *Mithraculus forceps* vs. *M. sculptus*, Rhyne *et al*., 2005). Similarly, researchers should invest in the foxface rabbitfish, *Siganus vulpinus*, as an alternative to tangs. Foxface rabbitfish are attractive, in high demand, and provide the same ecological services to the aquarium as tangs (i.e., algae consumption). However, the foxface rabbitfish has a more reasonable wPLD compared to the yellow tang. While "firsts" and innovations are essential aspects of industry growth, it is important to focus on species with predictive culture success based on life history characteristics and culture history and species currently threatened by the trade (e.g., unsustainable wild harvest).

Live Feed Bottlenecks

While the current culture successes of copepods prompted new waves of cultured marine fishes with more delicate and complex larval requirements, copepod culture refinements (e.g., recirculating culture systems, low-cost production) as well as the production of

alternative larval feeds (e.g., larvaceans) is likely needed before the commercial success of many current aquaculture efforts can be realized (i.e., tangs and butterflyfishes). Efforts towards commercially affordable recirculating copepod production systems will increase not only the diversity of copepods (and similar planktonic feeds) produced but also the volume of currently and newly aquacultured species that reach commercially viable scales. Many species of wrasses, tangs, triggerfishes, and other small marine larvae require foods smaller than the current copepod nauplii, and research to close the gap in the early first feeding larvae is still lagging behind efforts to culture these species. While first feeding mortality is still high, many highly desirable species exhibit dietary shifts during development (Llopiz & Cowen, 2009), often moving from copepods to larvaceans and pteropods. Research on suitable alternatives to both larvaceans and pteropods will likely increase development and survival of many coral reef fish larvae.

4.6 Conclusions

Marine ornamental aquaculture is a growing and malleable tool with implications for the sustainability and advancement of the aquarium trade as well as the ecological and socioeconomic wellbeing of coral reefs and associated reef-side communities. There is a strong need to better understand the global marine aquarium trade as a whole, as well as the risks and benefits of a larger aquaculture footprint within the trade. Concurrently, as an industry, the focus should be on the marketability of aquacultured products while decreasing production costs. To do so, collaboration across all facets of the industry is essential.

Acknowledgements

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Part II

Facilities, Culture Systems and Other Specific Requirements

The success of a marine aquaculture facility is largely dependent on a series of aspects of which the complexity of legal issues, the selection of a suitable location, the feasibility of the culture systems, and the use of high quality feed are critical issues. The adequate placement of a facility depends upon the characteristics of the surrounding media (accessibility, weather conditions, water quality), the targeted species, the culture phases to be performed and other aspects related to the final destination of the organisms produced (Chapter 5).

Economic viability and sustainability of aquaculture depend on the planning, design and operation of systems for broodstock management and reproduction of the selected species (Chapter 6). The planning of the reproduction system, the management and selection of broodstock, the knowledge of the biological, ecological and behavioural characteristics (mainly life history, reproductive strategy, spawning and breeding seasons) of the species to be cultivated play key roles in the production process. Other main aspects to consider in the development of breeding systems are location and local climate, availability of high quality seawater, tank design and dimension and technical equipment (water pumps, pipes, water filtration, treatment and disinfection systems, lighting, temperature and salinity control units and water quality monitoring).

New and more efficient technologies and equipment have been developed and introduced over the last few years in the field of marine ornamentals production (Olivotto *et al*., 2011). With those improvements, more species have been incorporated into ornamental aquaculture ventures and the resulting outputs have been improved considerably, reducing fishing pressure on wild caught ornamentals. Larviculture and production systems development are very likely the activities that have been especially enhanced with such advances (Moorehead & Zeng, 2010; Chapter 7). The application of recirculating aquaculture systems (RAS) (Tomoda *et al*., 2005) and the use of improved and novel microcosm and mesocosm rearing units have had great impact on the development of modern and new rearing procedures and hence, to the progress in production of known and new species for the aquarium trade. Recent innovations have focused on different aspects of the whole rearing system, but those related to aquaria design (e.g. Pseudo-Kreisel aquaria) (Blanco *et al*., 2014) and water quality are noteworthy. Of special interest are the improvements made to the use of mesocosm units, in which an artificial food chain is usually initiated in advance by supplementing the rearing media with nutrients and microalgae (FAO, 1985; Calado, 2008).

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The quality of live prey and formulated feeds has had great impact on the final survival and growth of raised species. The cultivation and enrichment procedures for live prey have improved considerably, especially the later. Rotifers, *Artemia* and to a lesser extend copepods continue to be the basic and almost irreplaceable live prey for cultivated species. However, new and more efficient enrichment products have been introduced into the market and also, new species of zooplankton (shrimps, amphipods) are being progressively incorporated into the food chain of ornamentals (Chapter 8).

Nutritional requirements of ornamental species are relatively unknown, even though many larval rearing improvements rely on adequate nutrition of the food organisms (Chapter 9). Lipids, particularly n-3 HUFA, are the most studied molecules but little is known of the protein, amino acids and vitamins requirements. It is generally accepted that live preys commonly used in larviculture do not meet the amino acids dietary requirements of young developmental stages. The contribution of copepods to the fulfillment of the nutritional requirements in ornamental larvae seems to be superior to that of rotifers and *Artemia*. In general, the nutritional quality of copepods satisfies most of the nutritional and energetic needs of young larvae.

Some species such as *Macrobrachium rosenbergii* have been cultivated very successfully by feeding them exclusively on formulated diets (Kovalenco *et al*., 2002). However, larvae are poorly attracted to inert microdiets and their administration often results in lower ingestion, survival and growth rates compared to live prey. The development of inert food is still limited but some improvements have been made, particularly for more advanced developmental stages. Co-feeding on live prey and formulated diets for many species has improved growth and survival compared to the use of live feed only. However, some species are unable to feed on manufactured particles even in the adult stage.

The development of optimal dietary formulations still requires specific designs for the ornamental industry since these species require special formulations for health, fin quality and coloration (Chapter 10). Ornamental fish should be eating the ideal specific food necessary for healthy growth and breeding success. Recent improvements in feeds formulation include the use of carotenoids for coloration enhancement (Gupta *et al*., 2007) and probiotics to confer benefits to a number of species including ornamentals and commercial species (Pintado *et al*., 2011, 2014).

Finally, it is important to point out that the construction, establishment and operation of a marine ornamental hatchery deserve special considerations (Chapter 11). Definitely, aspects such as market assessment, hatchery location, facility layout, water sources (seawater and freshwater), electricity planning, lighting design, tanks and aquaria, filtration systems, quarantine systems, product quality control and capabilities and training of personnel will have a great impact on the final success of the hatchery.

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5

Location

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Abstract

Selecting a suitable location for an aquaculture facility for the production of marine ornamental species is an important decision that may condition the success of the whole operation. Potential national, regional and local constraints must be carefully analysed, as well as the main target markets. The selection of an *in situ* or *ex situ* culture approach is paramount when selecting the location of the facility, as well as the use of flow-through or recirculated culture systems. Licensing and other legal issues must also be carefully analysed prior to the selection of any location, namely when production targets exotic species or marine ornamentals listed under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

Keywords In situ *aquaculture;* ex situ *aquaculture; flow-through aquaculture; recirculated aquaculture*

5.1 Introduction

As for any aquaculture venture, the selection of a suitable location for building a facility to culture marine ornamental species is a critical issue. This choice may ultimately dictate the success of any aquaculture operation and thus must be carefully considered. It is certainly unrealistic to search for "the perfect location" to establish this type of infrastructure. All sites will display advantages and disadvantages and ultimately what one needs to do is to carefully balance all favorable and less favorable features of the target location and decide if the overall combination will be suitable for developing the infrastructure. It is important to emphasize that site selection must be addressed bearing in mind other relevant details for the success of the project, such as: (1) the species to be cultured; (2) culture techniques to be employed; (3) the phases of marine ornamentals culture that will be implemented (e.g, broodstock management,

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hatchery, growout); and (4) what the target market(s) will be for the organisms being produced (e.g. local, regional, national or international).

In general, one can split the issues that may influence our decision into two broad groups: those that may act at country or regional level and those specific to the location being selected to set up the infrastructure (Wickins & Lee, 2002). If any significant limitation is detected at country or regional level to a given location it is pointless to proceed with any further analysis. If limitations at country or regional levels are not considered as bottlenecks for the development of the project, it is then crucial to consider the following features of the location being selected: cost of land and concessions, climate conditions, water supply, availability of essential inputs for production and shipping (e.g., broodstock, seedstock, feeds, goods for packing and shipping cultured specimens), reliability of electrical network and energy price, potential use of renewable energy, availability of skilled labor, proximity to target markets and/or infrastructures that allow shipping to those markets (e.g., roads, airports).

The aquaculture of marine ornamental species can be broadly classified into two main categories: *in situ* or *ex situ*. This terminology reflects the location site where the culture takes place and each presents its own set of advantages and disadvantages (Leal *et al*., 2014).

5.2 *In Situ* Culture

In situ aquaculture refers to the culture of organisms in the marine environment using natural conditions. It is most commonly employed in countries exporting marine ornamentals such as cultured live rock (Falls *et al*., 2003), corals (Shaish *et al*., 2008), tridacnid clams (Lebata-Ramos *et al*., 2010), and, less frequently fishes (e.g. seahorses) (Garcia & Hilomen-Garcia, 2009). This approach exclusively relies on available natural conditions (e.g., water physical and chemical parameters, water currents and hydrodynamics, light and nutrients) for the production of target species. The main drawback of *in situ* aquaculture is the potential exposure of cultured specimens to a range of deleterious factors, namely: extreme weather conditions (e.g., storms and hurricanes), sedimentation (an issue for sessile organisms) and the presence of pathogens, parasites, predators, and competitors that may negatively impact survival and growth (Page *et al*., 2011). While operational costs may be lower and the labor force less skilled than in *ex situ* aquaculture, the ability to control any parameter that may enhance production is very limited or even absent in *in situ* culture. It is also important to highlight that most of these culture practices require support facilities operating on land (*ex situ*), either for the production of artificial live rocks or for the attachment of coral fragments to a suitable substrate. Only after these procedures will rocks and coral fragments be ranched in the ocean until being suitable for trade. *Ex situ* facilities are also required for larviculture and growout to a minimum size of tridacnid clams and seahorses, as this procedure decreases the risks of mortality when transferring these organisms for *in situ* production. Some of the issues that need to be more carefully considered when choosing a location to perform *in situ* aquaculture are the vulnerability of that location to extreme weather conditions, its accessibility (e.g., to survey production and to ship it) and security problems related with thefts and destruction of production infrastructures.

5.3 *Ex Situ* Culture

Ex situ aquaculture is the most commonly employed approach to culture marine ornamental species, as it allows a higher degree of control over production and the fine tuning of culture conditions to maximize survival and growth. It is employed both in exporting and importing countries and requires a more skilled work force than *in situ* aquaculture. Consequently, the availability of skilled workers may condition the choice of the location for this type of facilities. Construction and operational costs are also higher in *ex situ* than in *in situ* aquaculture and are highly conditioned by the type of production system to be employed. Flow-through systems need a reliable source of high quality natural seawater, which significantly influences the location of the facility. Coastal properties in regions with good seawater quality may be too expensive and more difficult to license. One potential constraint for operating this type of facility is the higher risk of introducing exotic species into coastal waters (Semmens *et al*., 2004). Additionally, these facilities may be too far away from distribution centers (e.g., airports), which can make the shipping of produced specimens more expensive and/or technically challenging. When employing recirculated culture systems operating with synthetic seawater, it is possible to have greater flexibility in selecting the location to set up the production facility. Facilities employing these types of system can be located on less expensive properties, be closer to distribution centers, and experience fewer licensing issues (the risk of exotic species escaping to the environment and become invasive is commonly low, namely if the facility is located away from coastal waters). It is also important to refer that waste waters may need treatment prior to be discarded from the facility (the volume of the effluent, the organic load and available legislation will determine the legal complexity and financial burden of effluent discharge). While they are more capital intensive (both initially and when fully operational), *ex situ* culture using water recirculation allows a level of optimization and intensification of production that can rapidly make these facilities profitable. Another important aspect to consider is that water recirculation requires the use of a number of electrical equipment. Thus, the reliability of the electrical network is paramount when selecting a location to set up the production facility. Energy prices are crucial when searching for a suitable location to set up a recirculated facility because culture systems need to be operated non-stop. One also needs to take into account the costs associated with the use of artificial illumination when producing photosynthetic organisms (e.g., corals). The costs associated with regulating water temperature to optimal levels for the organisms being produced are also decisive when searching for a suitable location, with the use of suitable construction materials able to optimize thermal insulation also playing a key role during the implementation of this type of facility. Regions displaying extreme daily and/or seasonal temperature fluctuations should be avoided whenever possible, while tropical to warm-temperate regions are commonly favored.

5.4 Licensing and Other Legal Issues

The legal requirements associated with the licensing of a production facility for marine ornamental species may vary with location. At times, bureaucratic procedures can be so complex that they may even hinder new projects. It is common for those trying to

license this type of enterprise to interact with a large number of regulating agencies and bureaus and to face a number of "inappropriate" laws originally prepared for agriculture, fisheries or aquaculture production for human consumption. These issues can be even more complex when production targets species listed under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, also known as the Washington Convention, protecting endangered plants and animals (e.g., seahorses and hard corals)). It is therefore advisable before any investment is done, to perform a systematic survey of legislation regulating this practice at the selected location. This procedure may save months (even years) of paperwork and considerable amounts of money. Other legal issues not directly linked with the activity, such as visas, equity limits and land ownership by foreigners, should also be considered. The use of coastal properties with direct access to the sea may also be regulated by specific laws that may pose a problem for licensing. Subjects such as the use of groundwater (either fresh or marine), water discharge and use of exotic species must be licensed and comply with a number of standards.

Import and export permits are fundamental for this type of activity, in order to assure that in the selected location it will be possible to buy all materials required to assemble and operate the facility. These permits will also dictate if broodstock and seedstock can be imported and marine ornamental specimens ready for sale can be exported for more appealing markets.

5.5 Conclusions

Overall, the selection of a suitable location for culturing marine ornamentals must be seriously planned to avoid unnecessary problems and costs. Often, producers of marine ornamentals are so focused on the zootechnical challenges they need to overcome that they overlook a number of legal issues that may turn the perfect location into a constraint that will impair the whole operation. A well prepared business-plan detailing what will be produced, how, when and for whom, as well as an in depth risk analysis, are key tools for making the right choice when selecting a suitable location to culture marine ornamental species.

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6

Broodstock Systems

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Abstract

The selection and environmental conditioning of broodstock are fundamental to assure the success of marine ornamental species cultivation. In this chapter we present some important topics for the planning, design and operation of systems and infrastructures for broodstock management and reproduction, including details regarding the biological requirements of cultivated species, broodstock infrastructures and operational costs. Basic concepts adequate for the maintenance of marine ornamental organisms of different taxonomic groups are also presented. However, producers should augment the biological knowledge about the species to be cultivated, in order to adapt and develop the concepts presented here.

Keywords *Broodstock management; captive breeding; culture techniques; marine aquarium; recirculating systems*

6.1 Introduction

The cultivation of aquatic plants and animals for ornamental purposes is a growing industry in many countries. However, ornamental marine organisms continue to occupy only a small space in this industry dominated by freshwater species (Tlusty, 2002).

The low number of marine species being cultivated, and the small quantity of produced organisms, is partly due to the challenges that the cultivation of marine organisms represents compared to freshwater ones. While considerable production occurs in some countries, most of the employed technology was developed by hobbyists and commercial breeders. However, in the last few years academic researchers have become involved, which resulted in more species being successfully cultured each year (Olivotto *et al.*, 2011).

The economic viability and sustainability of aquaculture are dependent on the ability to reproduce selected species in captivity. Although some farmers may base their

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activity on collecting larvae or juveniles from the wild, or even capturing wild mature broodstock, this practice is not sustainable and may raise some ecological issues or even legal restrictions, which in the course of years can derail the aquaculture of marine ornamental species as an economic activity (Tlusty, 2002). Therefore, the selection and maintenance of healthy broodstock, kept in appropriate conditions to enable their reproduction, is fundamental to assuring the success of the cultivation of marine ornamental species (Olivotto *et al.*, 2011).

In this chapter we present some important topics and basic concepts for the planning, design and operation of systems for marine ornamental organisms' broodstock management and reproduction.

6.2 Broodstock Management and Reproduction

One of the main challenges of the aquaculturist working with ornamental species is to maintain the entire life cycle of cultured organism in captivity. In spite of the advances achieved in the previous decades, especially in the culture of corals (Rocha, 2013; Leal *et al.*, 2014), mollusks (Heslinga *et al.*, 1987), crustaceans (Calado, 2008), and some fish species (Olivotto *et al.*, 2003; Olivotto *et al.*, 2006), where the knowledge accumulated over decades of production of marine organisms for human consumption has been adapted, most of the ornamental species with breeding potential are new species for aquaculture. Therefore, the instigation and control of the reproduction of marine organisms in captivity comprise a major step in the production process.

The selection of broodstock plays a key role in the whole process. Depending on the species, the zootechnical procedures must be adapted to the management of the livestock, which requires prior knowledge of biological, physiological and ecological aspects of the species to be cultivated.

After establishing the best practices to maintain the produced organisms in captivity and close the life cycle, it is possible to improve production techniques, select the best breeders and start selective breeding programs to obtain organisms better adapted to aquaculture conditions. Furthermore, it is possible to perform some manipulation at the genetic level, which over time would allow the production of animals with certain characteristics that make them desirable for the marine aquarium hobby.

6.3 Systems Design and Planning

In many cases the aquaculturist will select the species to be reproduced and grown before selecting the location to build the facility. This may be motivated by the market interest in a particular species or because the aquaculturist has experience in the reproduction and growth of the selected species and is thus more comfortable with it. On the other hand, the potential aquaculturist may already own a piece of land or a building and desire to establish an aquaculture facility thereon. In the first scenario the aquaculturist may face the problem of having selected one or more species whose biological requirements are not easily compatible with the possibility of finding or developing appropriate facilities and systems for their reproduction. In the second scenario the

aquaculturist may be constrained in the choice of species whose reproduction and growth are feasible in the space and infrastructures available.

However, planning should be based on (i) the biological requirements of cultivated species, (ii) the broodstock infrastructures (location of the operation, broodstock tank design and technical equipment) and (iii) the operational costs and market value of cultivated species. After gathering all these elements, the aquaculturist should do a market inspection in relation to the land or buildings available and start the preparation of a business plan, aiming to ensure the feasibility and sustainability of the project.

6.3.1 Biological Requirements of Cultured Species

Knowledge of the biological requirements of the species to be cultivated is fundamental to guarantee the success of marine ornamental aquaculture (Olivotto *et al*., 2003). Before starting, in order to make a decision about the species to be reproduced, it is important to be aware of any difficulties in cultivation and reproduction of the selected species. Therefore, it is important to study the literature that may exist on the life history of the chosen species. Sometimes available information is scarce and it is important to make field observations if possible, or learn from other aquaculturists' personal experiences that often result from trial and error strategies to spawn and rear species for which the life cycle has not been controlled (Pillay & Kutty, 2005; Stickney, 2009).

Although some marine ornamental species can reproduce asexually (e.g. cnidarians – stony and soft corals, zoanthids or sea anemones) (Rocha, 2013), most species reproduce sexually (Olivotto *et al.*, 2011). There are two different sexual strategies in marine ornamental species: (1) gonochoric species, with separated sexes (male and female) that once established remain constant for life (Santangelo & Abbiati, 2001) and (2) hermaphroditic species, where the same organism is able to produce male and female gametes during the life cycle. Hermaphroditic species can be divided into two main groups: (1) simultaneous (or synchronous) hermaphrodites, organisms that have both male and female sexual organs at the same time (e.g. shrimps of genus *Lysmata* or some seaslug species), wherein two organisms are need to reproduce, since self-fertilization is usually avoided by physiological mechanisms and self-fertilization is rare in animal species (Bauer & Holt, 1998; Baeza, 2006) and (2) sequential hermaphrodites, that change sex during life. Sequential hermaphrodites first mature as males (protandrous hermaphrodites) and at some point of its life cycle reverse the sex becoming functional females (e.g. clownfish of genus *Amphiprion*) (Godwin, 1994). Other species begin as females (protogynous hermaphroditism) and later reverse the sex and became functional males (e.g. some fish species of family Labridae) (Robertson & Choat, 1974). Besides protandry and protogyny, there are some species able to perform bidirectional sex changes during the life cycle, born with female and male reproductive organs, but acting as a functional female or male during different stages in life (e.g. some fish species of family Gobiidae) (Cole & Hoese, 2001).

Because reproductive strategies in marine ornamental aquaculture species vary considerably, it is important to obtain detailed knowledge about the biology of the species of interest, to reduce the amount of trial and error required to get things right.

With regard to reproduction strategies, there are also substantial differences between marine ornamental species. There are species that broadcast their gametes into the water column and do not provide any parental care, whereas other species provide

parental care through the protection of the spawning site or even carrying the fertilized eggs (Shlesinger *et al.*, 1998). Usually the size of the eggs is related to reproductive strategies. Species that broadcast gametes in the water column tend to produce large numbers of small sized (few hundred microns) eggs, compared to species that provide parental care that produce few, relatively large eggs (Olivotto *et al.*, 2011). Planning for a broodstock facility should anticipate these issues to guarantee successful egg collection for incubation (more details are presented below in the broodstock infrastructure and technical equipment sections).

The spawning season is another important factor to control in the reproduction process. Some species have a well-defined spawning season once per year, whereas other species can have multiple spawning events throughout the year. The intervention of the aquaculturist to control spawning of the target species must go through a few steps, which again are dependent on the knowledge of the biology of the species. First, as will be explained below, it is essential to develop an infrastructure to ensure optimal conditions for broodstock reproduction; then it is essential to have enough healthy and properly nourished organisms to reproduce; finally the aquaculturist can interfere in the reproduction process through the manipulation of external factors to induce spawning.

Environmental factors such as temperature and photoperiod are usually used to induce gonad maturation and spawning (Holt & Riley, 2001; Olivotto *et al.*, 2003). For some species it is important to manipulate other factors to trigger the reproduction process, such as nutrition (e.g. the supply of live feeds to stimulate the broodstock and increase gamete quality), water chemical parameters or other particular factors (e.g. for some species the phase of the moon is also an important factor, which can be mimicked in the broodstock facilities through the simulation of moonlight during the night period) (Takemura *et al.*, 2004). The manipulation of environmental conditions and spawning induction can determine the success and viability of a marine ornamental aquaculture venture. This is very important, especially for those aiming to reproduce species that have just one spawning season during the year. In those cases, the aquaculturist should maintain at least four groups of broodstock and precede the manipulation of environmental factors to allow maturation of the gonads in a staggered manner between different broodstock groups, in order to ensure a constant production of eggs and larvae throughout the year.

Beside environmental manipulation, the spawning of some species can be induced through hormone injection, a technique developed some years ago in the aquaculture of marine species. However, hormone injections will not work if the animals are not physiologically ready for reproduction. That means hormones can be used as a trigger for gametes release, but the gonads should be well developed or under maturation, which may depend upon environmental or nutritional stimuli (Dinis *et al.*, 1999).

Many marine ornamental species may spawn spontaneously in the tank, making it necessary to collect eggs or larvae and move them to the hatchery (details will be provided in the technical equipment section); however, if there are species whose spontaneous spawning does not occur, they can be spawned by stripping, applying the techniques developed for marine fish for human consumption (Cabrita *et al.*, 2009).

After deciding which species to reproduce and assembling the tanks and life support systems, it will be time to choose the broodstock. From a biological perspective, the aquaculturist should start the broodstock with wild animals; if the choice falls on

animals reproduced in captivity, it is important to ensure some diversity so that the animals chosen to form the broodstock are not siblings, in order to maximize genetic variability (Cabrita *et al.*, 2006).

Before buying the animals it is important to make careful observations, taking into account some key factors: choose animals that are eating (more important for fishes and crustaceans) and accepting a variety of foods; animals should not be pale, nor have external injuries or lesions in the skin, fins, shell or exoskeleton or any signs of disease; animals must not show obvious signs of stress and should respond to positive stimuli (e.g. food supply).

In addition to all the above mentioned factors, the aquaculturist needs to consider some important aspects related to the biology, ecology and behaviour of the selected species. Depending on the species it may be necessary to maintain a group of animals in the same tank with a specific proportion of sex ratios, or maintain separated couples. In the case of animals which form stable pairs, whenever possible it is preferable to acquire specimens already paired. If the selected species are very aggressive, it may be necessary to apply some specific care while pairing (Calado, 2008) (e.g. the aquaculturist may need to keep animals inside the same tank with visual contact for a while, but separated by a net, to avoid aggression and prevent injuries). If the animals of selected species are hermaphrodites the aquaculturist should be aware of the developmental status and sex of the animals that will be acquired. As a practical example take the case of the protandrous hermaphrodites, clownfishes of the genus *Amphiprion,* where females are bigger and usually more aggressive; it is usually preferable to put two fishes of different sizes together to avoid the risk that both of them are in sex reversal (male to female), especially if the sizes are similar and near the medium large size of the species (Fadlallah, 1983, Olivotto *et al.*, 2003).

6.3.2 Broodstock Infrastructures

The planning of the infrastructures to maintain broodstock should consider several factors, such as the operation regime (flow-through or recirculation system), dimension and design of tanks, hydraulic components, technical equipment necessary for water treatment and filtration, photoperiod and lighting systems, and collection of eggs or larvae, depending on the cultivated species (Pillay & Kutty, 2005; Stickney, 2009).

The careful and deliberate choice of all these components will ensure effective animal welfare, and avoid stressful situations that are usually dramatic for broodstock maturation and reproduction (Pillay & Kutty, 2005; Stickney, 2009).

On the other hand, when the choice of all the necessary equipment does not meet the physiological needs of the animals, they might be subjected to stress. The most important identified stress factors are the housing of the animals in an inadequate space, and changes in water parameters (e.g. rapid fluctuations in temperature, diurnal fluctuations of pH, exposure to low dissolved oxygen). These stress factors might exacerbate the behavioural changes that naturally occur during the reproduction period (e.g. alteration of normal activity associated with the establishment of territories, which in some species may involve fighting), and the handling or maintenance procedures. In the next paragraphs we will present in some detail the components of a facility to maintain broodstock (Conte, 2004).

6.3.2.1 System Operation Regime

The choice of the operation regime of an aquaculture facility depends, among other factors, upon its location (Stickney, 2009). When it is possible to install an aquaculture facility in a place with good water quality, the aquaculturist can always choose a flow-through regime, which will reduce the implementation and operation costs of the equipment for life support systems (e.g. recirculating water pumps, protein skimmers or mechanical and biological filters). However, the operation of an aquaculture facility, especially for marine ornamental organisms, in a flow-through system has some disadvantages. If the aquaculturist has the ability to install a production facility in a tropical region, with access to good quality seawater, the flow-through operation system can be a good option. However, there is always the threat of alteration in water quality due to anthropogenic actions (such as pollution) or biological agents (parasites, pathogenic microorganisms, among others), which may put at risk the supply of seawater, and consequently the success of the production (Stickney, 2009), which may be prevented with the installation of pre-filtration systems for water treatment and disinfection.

On the other hand, if the aquaculture facility is installed in a temperate region, there is the issue of water temperature, since ornamental species are in general tropical. Thus, the decision for the system's operation regime should always include the operational costs and the risks associated with either method. Although recirculating systems are more expensive in terms of implementation, as they involve the cost of the equipment and its operation, they can be safer and more advantageous as they allow a better control of the physical, chemical and biological parameters in broodstock systems (Rocha *et al.*, 2015).

Regardless of the operating system, aquaculture of marine ornamental organisms can work with natural or synthetic seawater. The natural seawater must be mechanically filtered (e.g. 1–10 μm cartridge filter), prior to being used, to remove any particles. Additionally, natural seawater can be UV irradiated or ozonized to eliminate potential pathogens (Rocha *et al.*, 2015). Another option can be to use chlorine bleach to sterilize the water, and after 24 hours to neutralize the chlorine with sodium thiosulfate and strongly aerate the water prior to adding to broodstock systems (Stickney, 2009).

However, natural seawater may not always be available due to geographical location, and/or may be unsuitable due to pollution.

An alternative can be the use of synthetic seawater, which is expensive because it involves the purchase of salt and requires large volumes of freshwater. Nowadays synthetic seawater can be easily prepared in any geographical location using good quality salts and freshwater (deionized or purified by reverse osmosis filtration). The quality of employed salt is of paramount importance. It is fundamental to avoid opportunity businesses and buying unknown brands that may contain suboptimal levels of some trace elements, or be contaminated with undesirable chemical compounds (Rocha *et al.*, 2015).

Regardless of the constraints presented for the use of natural seawater, the aquaculturist should remember that nothing is more similar to seawater than seawater.

6.3.2.2 Broodstock Tank Design and Dimensions

The selection of tanks used to maintain the broodstock is crucial when planning an aquaculture facility. The design, size and volume are aspects to consider, since if broodstock is confined in a tank with a design or size that does not allow normal behaviour or somehow affects their physiological needs, the process of maturation, mating and

spawning can be delayed or even jeopardized due to tank associated stress (Olivotto *et al.*, 2003; Olivotto *et al.*, 2011).

The amount of control that is exerted by the aquaculturist can vary significantly according to tank size and design. Depending on the species, the tanks can be constructed from different materials (e.g. glass, plastic, acrylic, or fiberglass) (Stickney, 2009).

Glass is commonly employed to build stocking tanks for the broodstock of marine organism of relatively small size (Olivotto *et al.*, 2011; Rocha *et al.*, 2015). Glass is not a very expensive material and has the advantage of full observation of the broodstock. However, glass has a wide variability in strength and although it is weak in tension (Phillips, 1972), it is elastic up to its breaking point, and will bend to a certain point and then break. This characteristic gives some limitations related to tank size and design (e.g. cylindrical tank designs will be very difficult to build with glass). The length-to-height ratio affects the strength of the glass, therefore the thickness of the glass employed to build the tanks should be carefully calculated, with special attention to taller tanks. Additionally, the tank stand should be designed so that it fully supports the entire bottom of the tank; use a styrofoam or polystyrene pad between the tank and the stand. Despite all the pros and cons, glass remains a material of choice for construction of small and medium tanks (e.g. average volumes 50–500 L) with a reasonable price-quality relationship and allowing versatile system designs (see Figure 6.1 as an example) (Rocha *et al.*, 2015).

Acrylic can be used as an alternative to glass tanks. Acrylic is a generic term for polymethyl methacrylate (PMMA), a hard clear plastic, commonly known by brand names such as Plexiglas or Perspex. Acrylic tanks are lightweight (up to 50% lighter than glass tanks) and more crack resistant (up to 17 times stronger than glass tanks). These characteristics are an advantage for building large-sized broodstock tanks (Adey *et al.*, 2011). Additionally, the thermal forming construction process enables acrylic to take on more unusual shapes and provide unique stocking conditions for some ornamental marine organisms with specific tank design requirements (e.g. acrylic tanks are the most common choice to stock delicate jellyfish) (Lange & Kaiser, 1995).

For species that need a large space, the fiberglass composites can be the better solution. Fiberglass is a lightweight, extremely strong, robust and resistant to corrosion material. Additionally, fiberglass allows the design of a variety of tank shapes and offers the inherent strength of composite products. Fiberglass (also called glass-reinforced plastic, glass fiber-reinforced plastic, or GFRP), is a fiber reinforced polymer made of a plastic matrix reinforced by fine fibers of glass. The plastic matrix may be epoxy, a thermosetting plastic or thermoplastic. Fiberglass can also be used to coat wooden structures. After construction, the tanks should be coated both outside and inside with a fish-safe epoxy resin (Stickney, 2009).

One of the main disadvantages of fiberglass tanks is that the animals may only be viewed from above since the tank walls are opaque. To get around this, acrylic windows can be embedded in the tank walls. However, this solution will never allow an observation of animals as well as do glass or acrylic tanks.

Reinforced plastics may also be an option for building broodstock tanks, although the aquaculturist will be limited to the dimensions and shapes available in the market.

The dynamics of water in the broodstock tanks, inlet and outlet points, should be decided before the construction of the tanks. These aspects are very important. Besides

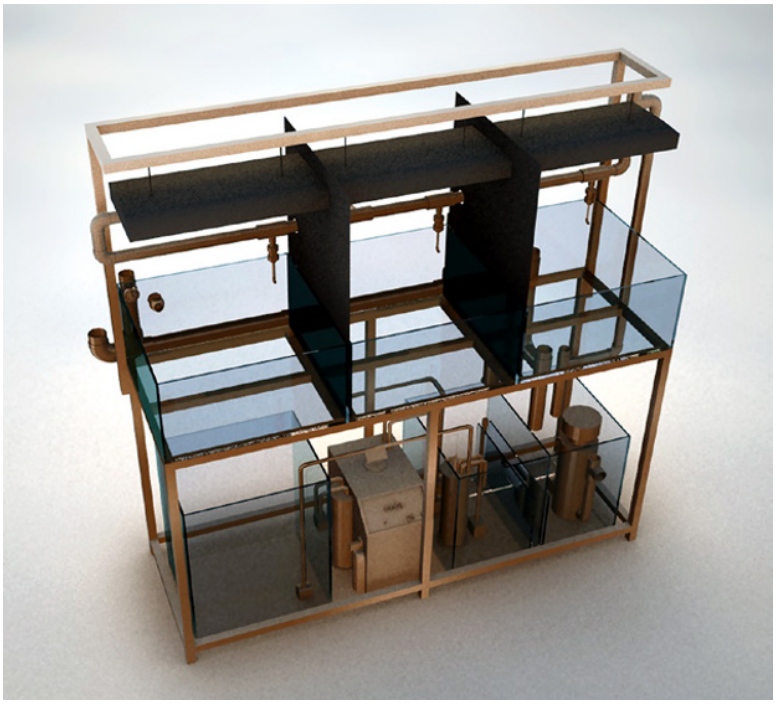


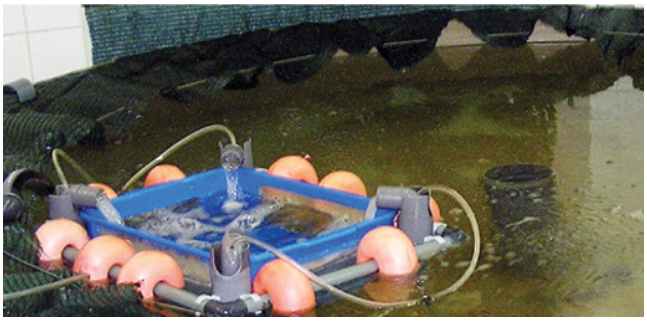
Figure 6.1 Modular culture system for marine ornamental species with: three broodstock glass tanks (ca. 90 L volume); filtration glass tank (ca. 150 L volume) equipped with 250 µm mechanical filtration bag, one protein skimmer, one fluidized sand-bed biological filter, one calcium reactor (required for scleractinian corals), one calcium hydroxide reactor, one submergible heater; glass osmosis water reservoir with a pump connected to an osmoregulator for replacing evaporated water; one water chiller; and PVC pipes, fittings, valves and accessories.

the influence they have in water renewal and organic matter removal, they might influence the way the eggs or larvae are collected (Lekang, 2008).

For species that broadcast gametes in the water column, the tank design should anticipate the installation of an egg collector, to collect the fertilized and viable eggs that are usually positively buoyant due to the lipid content, and transfer them to the incubation tanks in the hatchery (Figure 6.2). The egg collector can be placed inside the broodstock tank or outside in the outlet pipe.

Egg collectors that are placed inside the broodstock tanks, usually termed airlift collectors, consist of a screened recipient (a bucket or a box) equipped with floats and surrounded with small airlifts. The airlifts (e.g. vertical PVC pipes with an air supply connected to 90° PVC elbows directed to the egg collector) transfer the surface water of the broodstock tank into the collector and gently lift the floating eggs, by means of an air flow (Figure 6.2a), sparing them from any mechanical stress.

When broodstock tank volume or surface area doesn't allow the utilization of an internal egg collector, the solution is to use a collector placed outside the tank. The most common are the overflow collectors, which consist of a screened container (e.g. a PVC cylinder with large lateral and bottom openings screened by mesh nylon net with a size adequate to retain the eggs) that receives the water of the broodstock tank by



(a)



(b)

Figure 6.2 Egg collectors: (a) airlift collector placed inside the broodstock tank; (b) overflow collector placed outside the broodstock tank, receiving water with eggs through the overflow pipe. Photos by Aquaculture Research Group – CCMAR (www.ccmar.ualg.pt/aquagroup). (*See insert for color representation of the figure.*)

overflow (Figure 6.2b). The collector should be placed inside another container (a bucket or a plastic box). During the spawning season the broodstock tank outlet pipe should be connected with the open top of the collector, assuring that the surface water from the spawning tank flows into the egg collector. The water level inside the container where the collector is placed should be maintained by another outlet overflow



(a) (b)

Figure 6.3 Modular maturation system for ornamental crustaceans broodstock: (a) general view of the 3-level modular maturation system; (b) rectangular-base glass tanks divided with a net into two compartments to avoid the passage of broodstock, illuminated with a blue LED spot in the small compartment. (*See insert for color representation of the figure.*)

connected to the filtration system. Eggs retained in the collector by the screen should be kept floating by water flow and a gentle aeration. (See Figure 15.1 for another example of a broodstock fiberglass tank with an egg collector in the external filter box.)

For ornamental species that lay adhesive eggs on solid substrates (e.g. clownfishes of the genus *Amphiprion* or firefishes), the aquaculturist may only have to provide a substrate (e.g. a clay pot or a tile) that can be easily removed to place in the hatchery tanks (Madhu & Madhu, 2011, 2014).

The design of broodstock tanks for species that release the larvae (e.g. shrimp of the genus *Lysmata*) might require some special technical details (Figure 6.3).

The dimension of the tank will depend on the size of the broodstock. Usually a rectangular-base glass or acrylic tank is suitable. The water inlet should be placed in the top or in the bottom of the tank in an opposite wall to the outlet. The outlet of the tank should be protected with a mesh filter (to prevent the escape of the larvae to the filtration system). The rectangular-base tank should be divided with a net to avoid the passage of broodstock into both areas (approximately 80–20%); the biggest division (in the inlet wall side) is designated for the broodstock, and the small division (in the outlet wall side) should be illuminated with a blue LED spot (Figure 6.3b). The larvae, usually released during the night, are entrained by the current and simultaneously attracted to the light, remaining confined in the small compartment. Therefore, the collection of the larvae is easier and possible cannibalism by the broodstock avoided.

After setting the dimension and design of the broodstock tanks, the aquaculturist should focus on the choice of technical equipment.

6.3.2.3 Technical Equipment

The selection of technical equipment to maintain or improve the water quality in the broodstock systems should be carefully decided, in order to fulfill the specific requirements of the cultivated species.

The decision on what equipment to choose should be based of course on cost, but should also take into account the reliability of the brand and the electrical power consumption during operation, which in the medium/long term can influence the economic viability of the project. The chosen equipment must also be well-sized to the systems, in order to meet all the technical requirements, but also to avoid unnecessary expenses with oversized equipment.

Water Pumps and Pipes

There are several types of water pumps, based on different principles. The type of pump chosen depends on a number of factors, including the amount of water to be pumped and the height at which the water must be pumped (Adey & Loveland, 2011). Submerged pumps, which in general are reliable, and achieve good performances with reasonable energy consumption are most often employed in marine ornamental broodstock systems (Rocha *et al.*, 2015). However, submerged pumps with low efficiency may heat the water too much, as the energy does not dissipate and is simply transferred to the water (Adey & Loveland, 2011), which can be more dramatic in recirculating systems.

Air lift pumps are widely used in recirculating systems for aquatic organisms (Holt & Riley, 2001). The operating principle is the injection of compressed air at the bottom of a discharge pipe that should be immersed in the water. The air should be provided by a compressor or a blower. This is a very reliable and simple method with low associated electrical costs. However the flow rate and the suction capacity can be limited, which make this pumping process suitable only for systems with a low head (Loyless & Malone, 1998).

As with other equipment, the decision on the pumps to be acquired should rest with reliable brands with maximum performance and lowest power consumption. The choice of the model must take into account the needs of the system, the pump curve and the head loss (which is related to the height at which water needs to be pumped and with friction in pipe parts, fittings and accessories).

The distribution of water in the life support systems is usually made through thermoplastic pipes, namely those that can be glued: polyvinyl chloride (PVC). Pipe diameters are standardized, but there are a number of sizes available for various applications in different industries. Additionally, there are a large number of different PVC pipe fittings and accessories in the market (Rocha *et al.*, 2015).

One of the main aspects to be considered when designing the whole piping of a life support system is the tube diameter, taking into account that the diameter of the outlet tube, usually where the water falls by gravity, should be larger than the diameter of the inlet pipe, where the water flows at a higher speed because it is pumped. More details on pipe diameter and pump head loss calculations can be found in Lekang (2008).

Beside the water recirculation through the filtration systems, some filter-organisms may require extra movement inside the tanks or specific flows (laminar or turbulent). For these specific cases there are several brands of circulation pumps on the market and also the possibility of using air lift systems.

Water Filtration and Treatment

The choice of the filtering processes to be used in broodstock tanks life support systems should consider the organic load of the system, the amount and type of food and the

amount of waste produced. Filtration systems are generally divided into physical, chemical and biological processes (Stickney, 2009).

Several physical or mechanical principles and methods are used to remove particles from the water in culture systems.

Sand filters are normally used to remove particulate matter from the water. These filters can operate at normal atmospheric pressure or have an over pressure inside promoted by a water pump. When a pressurized filter starts to clog, the filter media should be backwashed; otherwise the filter will become totally blocked (Adey & Loveland, 2011).

Another option are cartridge filters that can be made of different materials, such as plastic, ceramic, spun fiber or resin bound fiber. The waste is collected either inside or outside the cartridge, depending on the way the water pass through the cartridge, and can be used for removal of small particles (e.g. for sizes below 1 μm). These filters are normally used on systems with small water volumes and with low particulate matter loads; otherwise the cost of operation becomes too high (Stickney, 2009).

Filter bags are another simple type of filter for use with small water flows. The filter bags are usually placed in the outlet of broodstock tanks (or after the egg collector in the case of species where external egg collector is installed, as explained before) and require no pumps or electrical power to operate. Particles are trapped on the surface of the bag. When no more water flows, the filter bag has to be replaced with a new or clean bag (Rocha *et al.*, 2015). These bags can be cleaned in a washing machine without detergents.

The described mechanical filters can be employed either on recirculating broodstock systems or as a pre-treatment for natural seawater, before use in recirculating or flow-through systems.

After filtration of particulate matter, the water of life support systems used to maintain broodstock in recirculated system should pass through a protein skimmer. Protein skimmers are devices used to remove organic compounds from the water before they break down into nitrogenous waste. Protein skimmers work by generating a large air/water interface, through the injection of large numbers of very small bubbles into the water column of the device. The nonpolar (hydrophobic) part of organic molecules is repelled by water molecules and attracted to air/water interface promoted by air bubbles. In the top of the protein skimmer water column the bubbles create foam that will carry the organic molecules to the skimmer waste collector. Along with proteins, there are a number of other organic and inorganic molecules that are typically removed by protein skimmers and will be exported from the system (Adey & Loveland, 2011; Rocha *et al.*, 2015).

The waste removal promoted by the protein skimmer is highly desired as it alleviates the burden of the filtration system and improves visibility and light penetration, which is very important in systems harboring photosynthetic organisms. However, the protein skimmer will also remove phytoplankton from the water (which might interfere with the feeding process of filter organisms); moreover, the use of oversized protein skimmers can remove some trace elements from the water. Therefore the protein skimmer should be carefully dimensioned to the system and adequate to the requirements of broodstock (Rocha *et al.*, 2015).

Biological filtration is another key step in recirculation systems water treatment. Biological nitrification takes place on the surface of the elements where the biofilm of nitrifying bacteria is established. Ammonium is oxidized into nitrites which are in turn oxidized into nitrates. Nitrifying bacteria needs oxygen to perform nitrification,

therefore it is important to supply enough oxygen/air in this system to provide optimal growth conditions for bacterial biofilms (Stickney, 2009).

There are three main types of biological filters, depending on the operation mode: submerged filters, trickling filters and fluidized bed filters. The media in submerged and fluidized bed filters is permanently submerged, whereas in the trickling filter, the water trickles through the filter medium where the biofilm is established (Adey & Loveland, 2011).

Different types of media can be employed on these filters. The specific surface area where nitrifying bacteria biofilm can be established (surface area per unit volume, m^2/m^3) must be ensured. However, if the stocking density or the organic load of broodstock systems is very high, sand or ceramic substrates should be avoided because even though they have a high ratio of surface area/volume, may clog easily, preventing sufficiently oxygenated water reaching deeper layers (Stickney, 2009). There is a wide variety of suitable substrates in the market, adequate for different types of biofilters.

The last step in water treatment should be the disinfection. Disinfection can be described as the reduction of micro-organisms such as bacteria, virus, fungi and parasites to a desired concentration. Note that disinfection is not the same as sterilization where all micro-organisms are eliminated.

The UVC radiation and ozone are the most commonly used disinfecting agents. These methods can be used to disinfect the water in recirculating systems and also to disinfect natural seawater before use (Hijnen *et al.*, 2006). However, UVC can be less effective against protozoa (e.g., ciliates) (Lakeh *et al.*, 2013).

UVC radiation overdose causes no major problems for systems water, but ozone is a very strong oxidizing agent (it is an allotropic form of oxygen, O_3) highly toxic to all forms of life, which should be carefully used. Due to its instability, ozone is produced "*in situ*" and whenever necessary. After being added to the water and allowing it to react for the necessary time, any residual ozone must be removed or destroyed using an activated carbon filter (Stickney, 2009).

The injection of ozone gas to broodstock systems water is usually performed in the protein skimmer chamber, to ensure good gas water mixing (Rocha *et al.*, 2015).

After all treatments mentioned the aquaculturist can also use some products that will adjust the water to the desired values. Activated carbon is one of the most important elements, which can be used in various situations, namely after a treatment with a chemical compound (antifungal, antibiotic or de-wormer) or after coral asexual reproduction through fragmentation (Rocha *et al.*, 2015).

Activated carbon is commonly placed into mesh bags through which the system water is allowed to flow; however, if the water displays too much detritus it will rapidly clog the mesh bag and decrease the filtering efficiency of activated carbon. Usually, it is commonly recommended to use about 1 g of activated carbon per liter of seawater; nonetheless this may change significantly with the type of activated carbon employed (Adey & Loveland, 2011).

Artificial Illumination

When it is not possible to rely on the use of natural illumination, artificial lighting plays a very important role in a broodstock system, especially with photosynthetic organisms.

There are some scientific and commercial concepts, associated with the illumination applied to the maintenance of aquatic organisms, which should be taken into account at the time of choosing the lighting system (Adey & Loveland, 2011).

Commercially the color temperature of a light source is often used to classify the lamps. Light color temperature is theoretically the temperature of an ideal black-body radiator that radiates light of comparable hue to that of the light source. Color temperature is conventionally stated in the unit of absolute temperature, the Kelvin, having the unit symbol K. Color temperatures over 10,000–20,000 K are called cool colors (bluish-white), while lower color temperatures (2700–3000 K) are called warm colors (yellowish-white through red) (Rocha *et al*., 2013).

The photosynthetically active radiation (PAR) intensity is measured in the spectral range (wave band) of solar radiation from 400 to 700 nanometers, within which photosynthetic organisms are able to use in the process of photosynthesis. PAR is normally quantified as μmol photons m^{-2} $second^{-1}$, which is a measure of the photosynthetic photon flux (area) density (Rocha *et al.,* 2013).

There are various types of lighting systems available in the market, which should be selected according to energetic efficiency, the cost of implementation and power consumption, and depending on the type of organism and their specific requirements (Adey & Loveland, 2011).

Fluorescent lamps or fluorescent tubes are among the most popular illumination systems used. Fluorescent tubes are gas-discharge lamps that use electricity to excite mercury vapor. The excited mercury atoms produce short-wave ultraviolet light that then causes a phosphor to fluoresce, producing visible light. Nowadays there are several color temperatures available in the market. The power and light intensity of fluorescent lamps are standardized and depend on the lamp length. Photosynthetically active radiation (PAR) can be manipulated, both by adjusting the distance between the lighting system and broodstock tanks or by increasing the number of lamps (Rocha *et al.*, 2015).

Hydrargyrum quartz iodide (HQI) – metal halide lamps, a member of the high-intensity discharge (HID) family of lamps, produce high light output for their size, making them a compact, powerful, and efficient light source. By adding rare earth metal salts to the mercury vapor lamp, improved luminous efficacy and light color is obtained. HQI lamps became very popular some years ago, due to their importance at the time for coral maintenance in aquariums (Rocha *et al.*, 2013).

The light-emitting diode (LED) is a semiconductor light source which has become popular in the aquarium industry in the last years. Introduced as a practical electronic component in 1962 early LEDs emitted low-intensity red light, but modern versions are available across the visible, ultraviolet and infrared wavelengths, with very high brightness. LEDs present many advantages over incandescent light sources including lower energy consumption, longer lifetime, improved robustness, smaller size, and faster switching.

Light emitting plasma (LEP), is begining to be applied in ornamental marine aquariums, replacing the usual fluorescent or metal halide lamps. LEP emission is characterized by a high efficient broad spectrum, with a high energetic efficiency, which makes it an interesting candidate for illumination in aquaculture facilities (Rocha *et al.*, 2013).

Temperature Control

Most marine ornamental organisms are tropical and come from ecosystems where temperature is a rather constant parameter. Therefore it is important to avoid abrupt variations, and to avoid the induction of stress or any physiologic disturbance to the broodstock. Given the volume of most broodstock systems, water temperature is maintained through the use of submergible heaters, water chillers and room temperature control (Rocha *et al.*, 2015).

In temperate countries with cold winters and hot summers, the use of both devices is recommended (heaters and chillers) to avoid temperature variations. Normally heating water is cheaper than cooling it, in terms of electrical costs, so the heat generated by pumps and lighting systems must be carefully safeguarded when planning the cooling system (Adey & Loveland, 2011).

Usually the power of the equipment to maintain the water temperature should be calculated taking into account the temperature differentials (the difference between the ambient room temperature and the temperature at which it is intended to keep the broodstock system water) (Stickney, 2009).

Water Chemistry

Monitoring and control of chemical water parameters is essential for the proper functioning of aquaculture.

Regardless of the specific requirements of the cultured organisms, there are parameters to be monitored daily (e.g., temperature, oxygen concentration, pH, and salinity). Other parameters such as concentration of nitrogenous compounds (ammonia, nitrites and nitrates) and calcium concentration should be regularly monitored more than once a week (Olivotto *et al.*, 2011; Rocha *et al.*, 2015).

Salinity is a very important water parameter which can be easily maintained by employing an osmoregulator, which automatically compensates evaporated water by dosing freshwater purified by a reverse osmosis unit (Rocha *et al.*, 2015).

Depending on the use of natural or synthetic seawater, water chemistry can be adjusted to fulfil the specific requirements of the broodstock by supplying additives. This may be particularly important in the maintenance of scleractinian corals, where there is usually the need to rely on the use of calcium reactors to restore the levels of calcium and other trace elements in the water. However, it is advisable to point out that the indiscriminate addition of additives and other chemicals can cause drastic imbalances in broodstock system water, which can bring several problems. The most natural way to replenish trace elements is through partial water changes (Rocha *et al.*, 2015).

6.3.3 Operational Costs and Market Value of Cultivated Species

Regardless of the importance of all the factors discussed above, the sustainability of the project is dependent on its economic feasibility. After gathering all the elements about species biology, implementation and operational costs, the aquaculturist should do a market survey in order to evaluate the market demand of the species intended to be produced, the possible threats and economic concurrency. In Table 6.1 we present the main topics that an aquaculturist must consider when deciding to invest in the cultivation of certain species, in order to address all the costs involved, and to assess the feasibility of the project.

Table 6.1 Costs associated with implementation and operation of an ornamental aquaculture facility.

Fixed costs	Land or building (cost of purchase or value if already owned)
	Facility construction or adaptation
	Material and installation of sewer systems
	Plumbing for fresh and salt water
	Reservoirs for fresh and salt water
	Pumps to deliver water to the broodstock systems
	Office, laboratory, storage, maintenance and reparation workshop
	Salaries
	Food storage and preparation (e.g. freezer, fridge)
Equipment	Laboratory equipment (e.g. microscope, water quality analysis equipment)
	Office equipment (e.g. computer, furniture, fax machine, copy machine)
	Maintenance and reparation workshop (e.g. drill press, mechanics' tools, carpenters' tools, cutting torch, etc.)
	Hatchery tanks and associated plumbing and aeration equipment
	Emergency equipment (back-up generator)
	Feeding systems (e.g. automatic feeders)
	Miscellaneous supplies (e.g. plumbing supplies, spare parts, buckets, fish catch nets, etc.)
Variable costs	Fringe benefits
	Animals for broodstock (purchase or production)
	Consumables (e.g. feed, salt, water analyses reagents, replacement of filtration systems components, etc.)
	Repairs and maintenance of facilities and equipment
	Energy (electricity, natural gas, petrol, diesel fuel)
	Chemicals (disease treatment drugs, supplies associated with water quality determinations)
	Interest paid
	Depreciation
	Taxes
	Insurance

6.4 Conclusions

The maintenance of broodstock in excellent conditions is a major factor for the success of marine ornamentals aquaculture. The careful planning of systems and infrastructures for broodstock management and reproduction is paramount, and must be based on a rigorous collection of information concerning the biological and ecological aspects of the species to be cultured to ensure optimal conditions and animal welfare. Finally, the planning of infrastructures, selection of technical equipment and system design should take into account the durability and reliability of selected materials and equipment, as well as implementation and operational costs.

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7

Larviculture Systems

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Abstract

Marine ornamental keeping and production is a rapidly increasing activity whose expansion has been accompanied by the development of new rearing techniques for classical and novel species. Most of the advances achieved in the last decades are related to larval rearing methodologies (zootechnics, feeding, nutrition, diseases among others) and larviculture systems in order to improve performance and efficiency. Hatchery technology for many species was rather well established in the past, but the incorporation of new species to the ornamental trade has enhanced new knowledge on biological aspects of the species and also on technical rearing methodologies. Those advances have been accompanied by the development of new and more efficient equipment and the use of small or large system volumes (microcosm and mesocosm, respectively). From an industrial and economical point of view, the development of recirculating aquaculture systems (RAS), whose technology and proficiency has been substantially improved in recent years both for small and large volume units is of special interest. The present chapter investigates the different larviculture systems, analysing the positive and negative aspects of each of them and providing essential information for successfully rearing marine ornamental larvae.

Keywords *Filtration; larval rearing; nitrogen cycle; recirculation systems; tanks*

7.1 Introduction

The development of successful rearing techniques for any species relies on several aspects that must be under a more or less strict control. One of the most important is the design, scale (size) and management of the rearing system, which will depend on both the objectives pursued and the affordability (economic cost and space availability) of the ideal system. In this regard, it is rather common to use the terms microcosm and mesocosm. Even though most people have an approximate idea of the significance of these terms, it is important to clarify that they can be defined according to two approaches. The first is related to the composition and structural organization of the

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system *per se*. The main objective is to simulate aquatic or terrestrial habitats for ecological studies (Drake *et al*., 1996; Petersen *et al*., 1999). Hence, the most important characteristic in this approach is the degree of ecological and functional complexity desired in the system. The choice of container size will interact with the ecology of the animals in the rearing system, affecting the results achieved (Wynn & Paradise, 2001). Complex designs are generally named mesocosms, whereas simplified systems are known as microcosms; the latter being a simplified version of the former. In short, an aquatic mesocosm is an artificial aquatic system that can be useful in ecological studies and in the assessment of environmental factors and biological features (Adey & Loveland, 2007). According to Odum (1984), mesocosms are limited, more or less closed experimental systems at an intermediate scale between the microcosm of the lab and the full complexity of the real world, the ecosystem.

The second approach is basically related to the size of the systems either large or small. Microcosm volumes range from a few dozen liters to 1–2 hundred cubic meters whereas mesocosm volumes are larger and intermediate between the microcosm and the real scale in natural ecosystems. The second approach will be considered in this chapter, where different larval rearing systems will be analyzed according to the size of the system rather than to complexity.

The design of a larval rearing system can vary widely, including bags, tanks and experimental basins or ponds. Microcosms are easily affordable to ornamental hobbyists whereas mesocosms are more often used in industrial hatcheries. The rearing units generally used in microcosms are rather conventional, but recent developments have increased the level of technology involved in the systems, and the design and development of new units more adapted to the needs of particular species. On the contrary, mesocosms are based on the use of large volume tanks and also basins and ponds, where a short food chain can be artificially induced.

Larviculture systems are designed and organized to maximize production and minimize space and cost. The rearing system can be open, semi open (partial water renewal) or closed, depending on the needs. Open systems are more costly but the recent development of recirculating aquaculture systems (RAS) has considerable reduced production costs and enhanced harvest with respect to the space/volume available.

7.2 Microcosms

Since larvae and prolarvae are very delicate during this early part of their life history, different larval rearing systems have been developed to mimic the open ocean conditions where food organisms are abundant and water quality is high and water is clear. Several small (<100 L) rearing systems have been developed and used in the last few years. Small tanks are particularly appreciated in laboratories where live prey production is usually a limiting factor, since they allow a large number of replicates with small numbers of larvae and low amounts of food to obtain solid statistical analysis.

Microcosms made of 20-L glass tanks in addition to cheaper solutions such as small fiber glass tanks, plastic trash cans, plastic utility sinks have been successfully used in the culture of some marine ornamentals including clownfishes (*Amphiprion* spp.), gobies (*Gobiosoma* spp.), dottybacks (*Pseudochromis* spp.) and, some blennies (*Meiacanthus* spp.) (Figures 7.1 and 7.2) (Olivotto *et al*., 2005, 2009, 2011).



Figure 7.1 Fiberglass larval rearing tanks at the Fisheries and Mariculture Laboratories, Port Aransas, TX, USA. (*See insert for color representation of the figure.*)



Figure 7.2 Small 50 L mesh baskets (rearing chambers) placed in large tanks (400–1000 L) have been used with success both for demersal and pelagic spawners. (*See insert for color representation of the figure.*)

In general the sides of the tank are dark (black) or covered with black panels to reduce light reflection and facilitate prey recognition, while the bottom is usually white to facilitate bottom cleaning. Lighting should come from the upper part of the tank solely since lateral lighting can cause larval disorientation. Usually for small tanks

(up to 50 L) an 18 W fluorescent lamp 30 cm above the water surface is more than enough. In addition to tank volume, tank shape can impact larval rearing success. Round or tanks with rounded corners are ideal since they promote uniform distribution of larvae and their prey, in addition to increased water flow and concentrating waste on the tank bottom.

Small tanks are ideal to concentrate larvae and live prey, but the major concern is maintaining water quality, particularly ammonia levels, throughout the larval period. In small water volumes nitrogen compounds can quickly rise over tolerable limits causing high mortalities. This can be avoided by changing the water either manually or through a dripping system (Olivotto *et al.*, 2003; Olivotto & Carnevali, 2004). This method is cheap, but requires a lot of maintenance and can cause stress or injury to the larvae because of rapid chemical and/or physical changes in water parameters since heating and aeration are usually performed inside the larval tank. Another method, aquaculture recirculation systems (RAS), includes centralized filtration. RAS are efficient since all the small tanks can be connected to a single central filtration system. Most marine ornamental facilities use recirculating aquaculture systems (Watson & Hill, 2006). In addition to the environmental consideration, production of ornamental fish in RAS addresses constraints such as direct access to a water source and soils suitable for facilities construction. RAS require a 10–30% of water renewal a week in addition to significantly reduced effluent volume. In addition, RAS offer a high degree of environmental control and allow year-round production (environmental factors can be manipulated on each tank system).

Recirculating water systems are well established in aquaculture both for small and large tanks. To date, engineers and scientists have succeeded in creating RAS systems capable of performing essential mechanical, chemical, and biological processes to ensure health and survival of numerous ornamental species. Successful systems must rely on the initial design being suitable for the cultured organisms, while also considering costs associated with construction and operation. Most systems are organized and designed to maximize production in a minimum amount of space at minimum cost. Major components of any system include grow-out units (i.e., tanks, aquaria), solids removal, a filtration system composed of mechanical, biological filtration, UV sterilizer and a protein skimmer, chillers and heaters.

These systems are designed to maintain nitrogen compounds near zero. In fact, most marine ornamental species have a low tolerance for ammonia or nitrite when compared to freshwater species. For example, high nitrate levels (i.e., 100 mg/L) reduced growth in the anemonefish *Amphiprion ocellaris*, and delayed larval metamorphosis with consequent decrease in growth and survival (Hoff, 1996). Thus, biological filtration must be able to maintain total ammonia nitrogen and nitrite, at levels near zero (<0.004 mg/L) for inflowing water; nitrate should be maintained below 50 mg/L. Biological filtration including bead filters, drop bead filters, and fluidized bed sand filters can be designed and sized to effectively maintain nitrogenous wastes at levels approaching zero. While stabilizing water quality the centralized filtration system significantly reduces maintenance time and stress to the larvae. In addition, heating, aeration and filtration are performed outside the rearing tanks and physical damage is thus significantly reduced.

Included in small larval tanks, small 50 L mesh baskets (rearing chambers) placed in large tanks (400–1000 L) have also been used with success both for demersal and

pelagic spawners (Figure 7.2) (Holt, 2003). The use of these rearing chambers concentrates larvae and food, increases food encounters, provides shelter, and decreases potential physical damage. Moreover, heating and aeration are carried out in the large tank and the water in the rearing chamber perfectly matches the larval requirements (Holt, 2003).

A very particular tank is the Kreisel tank that was originally designed to keep delicate jellyfish alive. The water circulation in this tank is very particular: the circular movement keeps larvae and prey in the middle of the tank reducing accidental contact with the tank walls and increasing predator–prey interactions. Pseudo-Kreisel aquaria are also used in the early rearing of many seahorse species to reduce the incidence of swim bladder hyperinflation syndrome (Blanco *et al*., 2014).

7.3 Mesocosms

Small volume systems are adequate for the maintenance or rearing of a single or, exceptionally a few species. Even though these units are very practical, they need strict attention and time for system management and parameters control. Larger volumes are more stable and predictable and hence easier to control, also offering some additional advantages. Generally, they are named mesocosms, which can be distinguished from microcosms on the basis of size, location, and complexity.

A mesocosm is a semi-intensive system allowing rearing and experimental study of aquatic organisms (Papandroulakis *et al*., 2004) in which diverse communities of organisms, supported and constrained by a given physical–chemical environment, interact to capture and process energy and nutrients in food webs (Adey & Loveland, 2007). The mesocosm approach is based on endogenous sources of food or on external supplies from monocultures of microalgae and zooplankton (Calado, 2008). The system supports the rearing of delicate larvae of invertebrates, molluscs, crustacean and fishes by bringing ecologically relevant components of the natural environment under controlled conditions.

Mesocosms are particularly adequate in studies requiring a certain degree of environmental realism in laboratory or field based experiments. Most marine aquarium hobbyists will never be interested in mesocosms, whose volumes are more suitable for professionals, industrial hatcheries or aquarium exhibitions.

Currently, there is a great variety of mesocosm units, whose type, design, construction, scale and cost depend on both the type and variety of organisms and the pursued objectives, especially in experimental studies (Figure 7.3). A huge review on aquaria design/construction, functioning and maintenance, including models of mesocosms such as coral reefs or estuaries, was published by Adey & Loveland (2007). According to these authors, glass and acrylic materials (40% the density of glass) are less convenient for large volume units (>1000 L), the later requiring more maintenance labor, especially for wall cleaning. Fiberglass and concrete units are strongly recommended for mid-size units beyond 1000 L volume.

The components integrated in a non-field mesocosm system are basically the same as in microcosms but on a larger scale. Undoubtedly, many of them are essential in the maintenance of high water quality in a recirculating system. Adey & Loveland (2007) provided useful and practical information on mesocosm modeling and pointed out the



Figure 7.3 Tanks for the rearing of seabass and seabream at Ifremer in Palavas-les-Flots (France).

importance of water quality to system function, distinguishing three approaches to water quality management:

- **Abiological treatment:** Chemical methods (ozonation, physical filtration, skimmer, and UV radiation) are used to counterbalance the effects of a poor water quality.
- **Bacteriological filtration:** In addition to abiological devices, a bacteriological or biological filter is used with the aim of trapping and breaking down organic particles, degrading excretion and waste products (urea, ammonia) and denitrifying nitrate-N to atmospheric N.
- **Wild ecosystem analogues:** Physical and chemical characteristics of the natural water, cross-boundary exchanges and food webs must be artificially simulated. In this approach, foam fractioning, filtration, and bacterial systems are not generally used in order to avoid plankton and larvae removal and imbalancing water chemistry. Fiberglass and concrete walls are not adequate generally for ecosystem mesocosms, since these materials are not transparent and the growth of microalgae and associated zooplankton is seriously hampered.

Based on the source of food provided, mesocosms for larval rearing can follow an intensive or extensive approach (Calado, 2008). The intensive approach is based on a relatively constant external supply of live food. On the contrary, the extensive (or semi-extensive) approach relies on the development and maintenance of artificial foodweb chains (endogenous blooms of zoo and phytoplankton) consisting of microalgae and live prey, commonly rotifers and copepods. The food chain can be improved by periodic external supplies of food (rotifers, copepods and different developmental stages of



Figure 7.4 Aquaria for seahorse breeding at Instituto de Investigaciones Marinas (CSIC) in Vigo (Spain). (*See insert for color representation of the figure.*)

Artemia) when symptoms of overgrazing occur, depending on both the density of larvae in the system and the availability of microalgae to prey. Foodweb chains have the advantage of providing a variety of prey in a large size range to support the feeding and nutritional demands of growing specimens.

In high volume systems, the economic cost of phytoplankton supply would be too high to permanently support a complete food chain. For that, ponds are generally used instead of indoor or semi-outdoor infrastructures (Divanach & Kentouri, 1982, 1990). The artificial food chain in the ponds usually is initiated in advance by supplementing the rearing media with a mixture of nutrients and by inoculating the microalgae (FAO, 1985). In a second step, the zooplankton is inoculated. Since growth rates of microalgae, zooplankton and targeted organisms differ substantially, a foodweb chain will be only fully operative and successful following a rigorous inoculation procedure in which temperature level and light conditions play a major role.

Generally, the density of animals in mesocosms is fairly lower than in microcosms, which implies lower final outputs, but also lesser biomass per unit volume throughout the production cycle and, consequently better water quality conditions. There are multiple designs for self-working mesocosms units including a whole water treatment system composed of mechanical water filtration, bacteriological filter, heater and cooling devices, pumps and protein skimmer. These recirculating units are easy to maintain at low biological load and only require adequate monitoring, partial water renewals and periodical cleanings of devices and tank walls (Figure 7.4).

The application of RAS systems to larviculture is more recent than to adult fish production. Ongrowing of big animals is much more expensive in terms of pumping and use of water, and solutions such as the implementation of recirculation systems were early undertaken to improve final benefits. In most cases, larviculture was based on the use of closed systems submitted to partial renewals of the culture media, which is much more cost-effective than an open-water system. However, the maintenance of water quality in closed or semi-closed systems is difficult. That is the reason why a general tendency has arisen in recent years to implement efficient RAS systems in larval rearing. One of the first RAS system descriptions available in the literature was applied to the larval rearing of the commercial fish, the red sea bream *Pagrus major*, in mid-volume tanks (Tomoda *et al*., 2005). The system was comprised of four major units: a rearing tank (4.5 m^3 volume), a reservoir tank (0.35 m^3), a foam separator unit (13.2 m^3 seawater treated per hour) and a biofilter. A mechanical prefilter was placed on the surface of the reservoir tank as a prefilter. From there, the water was collected into the reservoir tank. A portion of seawater was pumped from the reservoir tank to the skimmer, being returned to the reservoir tank. From there, the water was pumped to the biofilter and finally to the rearing tank. The biofilter consisted of a first part containing 40 Kg of porous soft ceramic beads as a substrate for nitrifying bacteria, and second part containing 60 Kg of coral sand for neutralization. About 15% of the total water of the RAS system (10 m^3) was daily renewed. The rearing was carried out in green water (1–2 10^6 cells/mL) with the daily addition of rotifers (5–10 rotifers/mL) and *Artemia* nauplii (0.1–0.2 nauplii/mL).

Mesocosms are useful for larval rearing but also for breeders, depending on the species. Due to species-specific behavioural characteristics, some species require large volumes and/or tall aquaria to breed successfully. This is the case for territorial fish that will claim their own home space and will not allow other fish to invade their territory, or many seahorse species whose breeding improves when the fish are maintained in large volume tanks at low densities. This is due to the very singular courtship displays and mating behaviour in which seahorse females transfer their eggs to the male pouch while swimming together in the water column.

Even though this chapter is dedicated to larviculture, there are some important aspects to consider in mesocosm (and microcosm) simulation that need at least a brief consideration. Feeding, behaviour and breeding of many species are controlled by tide and/or moon cycles (Takemura *et al*., 2004; Celik & Celik, 2011; Jobling *et al*., 2011). Furthermore, tide cycles and amplitude vary according to the geographical region considered. Hence, these factors should be taken into account in the rearing units when appropriate. Lighting control devices are available in the market to simulate lunar synchronization, whereas tides can be simulated by means of a trouble-free and inexpensive water-level management system based on interval timer control of stepping motors as described by Adey & Loveland (2007).

7.4 Conclusions

Larval tanks are an essential part of larviculture and the information presented in this chapter is essential for the better management of this delicate part of the fish life cycle. Additional information on larval tanks for specific marine ornamental species are provided in the following chapters.

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8

Live Prey Production Systems

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Abstract

Food is one of the most important factors in the cultivation of marine species. Unfortunately, feeding requirements of marine organisms are more restrictive than freshwater species since inert diets are generally not accepted, particularly in larval stages. Furthermore, in rearing systems it is not possible to satisfy the food requirements of ornamental species by providing the prey available in nature. Hence, live prey production based on typical zooplanktonic species commonly used in aquaculture is necessary for many stages of the cultivation process. Microalgae, rotifers, *Artemia* and copepods remain the most important live prey in aquaculture, but others are being increasingly incorporated in the rearing of marine ornamentals. A great part of the success in ornamental species cultivation relies on adequate knowledge of and good practices in the production techniques of live prey. This chapter provides basic and practical information for the production of the most commonly used live prey in the cultivation of ornamentals.

Keywords *Culture; live prey; production systems; microalgae*

8.1 Introduction

Plankton is the primary food source for many fish and their larvae as well as other organisms in the wild, and as a consequence the use of live prey in larval rearing is still a necessity in most hatcheries. In fact, the early larval stages usually do not react to formulated diets and require live prey to stimulate their predatory behaviour. The production of live feed organisms is less cost-effective and more labor intensive, requiring nutritional, zoological and microbiological knowledge.

To date most marine ornamentals are reared using rotifers (*Brachionus plicatilis* and *B. rotundiformis*) and *Artemia* nauplii as live prey because they are easy to culture at high densities. However, these live prey do not represent the natural prey of fish larvae; they can be too large for some tiny larvae and their nutritional profile does not match

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the nutritional requirements of the larvae. Copepods are the natural prey of most marine organisms during the larval stages so there has been great interest in their culture and application in laboratories and hatcheries. The present chapter focuses on the general biology, culture methodologies and nutritional characteristics of different live prey including microalgae, rotifers, brine shrimp, harpacticoid and calanoid copepods and others.

8.2 Microalgae

Microalgae (single-celled algae or phytoplankton) are photosynthetic organisms that are the primary producers in the aquatic food chain, providing many phytonutrients, including HUFAs (Coutteau, 1996). Over the last four decades, several hundred microalgae species have been tested as food, but probably less than 50 have gained widespread use in aquaculture (Guedes & Malcata, 2012). They can be free living, epibenthic, benthic or even intracellular symbionts and consist largely of single-celled forms, but some are "multicellular" in the sense that they form chains made of cells joined end to end. The size of single cells ranges from less than one micron to more than 100 μm in length. Cell shapes are round, oval and cylindrical and cell wall is made of polysaccharides, silica or calcium carbonate. Many species are flagellated and some have flagella-like structures, called haptonema, which are thought to aid in attachment, feeding, or avoidance responses. Reproduction is *via* binary fission (vegetative reproduction) yielding two daughter cells. Most species have a sexual reproduction stage in their life cycle and the release of gametes can occur when environmental conditions deteriorate (Brawley & Johnson, 1992). Diatoms represent one of the best known groups of phytoplankton. Cells are encased within a cell wall made of silica, called the frustule. Dinoflagellates possess two perpendicular flagella and their body can be covered with cellulosic plates. Prymnesiophyceae are characterized by two flagella and a thin filamentous appendage. Cells are covered by organic scales that can be calcified (coccoliths). Microalgae are used in aquaculture as live feed for all growth stages of bivalve mollusks, for the larval/juvenile stages of abalone, crustaceans and many fish species, and for zooplankton used in aquaculture food chains. Some of the species of microalgae commonly utilized are *Isochrysis galbana*, *Rhodomonas baltica*, *Chlorella* sp., *Nannochloropsis oculata*, *Skeletonema costatum* and *Tetraselmis* sp.

For maintenance of algal cultures, test tubes with screw caps or Erlenmeyer flasks with silicon plugs are sufficient and widely used (Figure. 8.1). Nevertheless, in the last decades researchers developed several cultivation technologies that are in use today to produce increased microalgal biomass: open ponds, enclosed photobioreactors (PBRs) and fermentation reactors (Olaizola, 2003). Algal growth is strictly dependent on light, pH, salinity, temperature and nutrient quantity and quality. Although the tolerated ranges are species specific, broad generalizations for the most important parameters can be made. Temperature must be between 18–24 °C and pH between 8.2–8.7. Light intensity must be increased at high cell concentrations and higher depths to penetrate the culture: 1000 lux is suitable for Erlenmeyer flasks while 5000–10,000 lux is necessary for larger volumes. Salinity of 20–30 has been found to be optimal for most microalgae. Algal cultures must be enriched with nutrients to make up for the deficiencies in the seawater and to allow algal growth and replication. Culture (or enrichment) media



Figure 8.1 Strains of microalgae (right) and rotifers *Brachionus plicatilis* (left).

are generally composed of three elements: macronutrients (phosphate, nitrate, silicate), trace metals (including zinc, cobalt, molybdenum) and vitamins (thiamin, cyanocobalamin and sometimes biotin). All three are often prepared as stock solutions at a nutrient concentration of 100 to 1000 times that required. In order to prepare the culture medium, the required amount of every component is removed aseptically and diluted in a specific volume of seawater. Several culture media have been developed and used for cultivation of microalgae. Some of them are modifications of previous recipes to meet a particular purpose and some are formulated after studies on the nutritional requirement of the organism. Walne's medium and Guillard's F/2 medium have been used extensively and are suitable for the growth of most microalgae species (Coutteau, 1996). Several specific recipes for algal culture media are described by Vonshak (1986).

In order to prevent contaminations (protozoa, bacteria, phytophagous zooplankton) in microalgal cultures, it is common practice to sterilize not only the culture vessels, but also the culture media by either physical (filtration, autoclaving, pasteurization, UV irradiation) or chemical methods (chlorination, acidification, ionization). Keller *et al.* (1998) demonstrated that the use of microwaves for sterilization of culture media and apparatus is much more rapid and energy-efficient than more conventional methods.

8.3 Rotifers

Rotifers are a group of Metazoa primarily native to freshwater or brackish water and since the 1950s have been used worldwide as the first live food for the initial larval stages of marine fishes. The epidermis is composed of keratin-like proteins and is called the lorica, and the body is differentiated into three main parts: the head which contains a ring of cilia (corona) that capture food particles and assures locomotion; the trunk

which carries the genital organs and the digestive tract and excretory system; and at the trunk's base grows a single foot, a retractable structure ending in one or more toes used for temporary anchorage. Their primary mode of reproduction is asexual, called parthenogenesis: the amictic females produce diploid eggs which hatch directly into amictic females. Sexual reproduction takes place only in response to specific environmental *stimuli*, resulting in mictic and amictic females. The former produce haploid eggs which develop and hatch into haploid males if they are unfertilized, but are also capable of becoming diploid resting eggs if they are fertilized. The resting eggs are embryos in an arrested state of development that will only develop and hatch into amictic females if exposed to particular environmental conditions (Dhert, 1996).

The reasons for the success of rotifers as first food for larviculture are multiple, including their high reproductive rate, small size (100–300 microns), planktonic nature, slow swimming rate, and tolerance to a wide range of environmental conditions. Furthermore, since they are filter feeders it is relatively easy to incorporate specific nutrients into their bodies; this process is called bioencapsulation.

Despite these astonishing characteristics, only a few species belonging to the genus *Brachionus* have been cultured as food for fish larvae. Among these, the most popular is *Brachionus plicatilis*, a species complex which includes different morphotypes. Culturists distinguish them according to lorica size as L (large), S (small) and SS (super-small) types. Based on studies of morphology and karyotypes, Segers (1995) classified L-type as *B. plicatilis* and others as *B. rotundiformis*. Recently, it has been demonstrated that S- and SS-type rotifers can be classified as different species: the former as *B. ibericus* and the latter as *B. rotundiformis* (Ciros-Pérez *et al*., 2001). L-type and S-type rotifers can be easily differentiated by their morphological characteristics: *B. plicatilis* length ranges from 130 to 340 µm, while *B. rotundiformis* length ranges from 100 to 210 µm. Moreover, *B. plicatilis* and *B. rotundiformis* differ in their optimal growth temperature: the former preferring cooler waters (18–25 °C) than the later (28–35 °C) (Dhert, 1996).

Because of the importance of rotifers as first live prey for the culture of many ornamental species, several culture techniques have been developed. In any rearing procedure parthenogenetic reproduction must be optimized and conditions which cause sexual reproduction should be avoided. The optimal conditions for parthenogenetic reproduction are: pH between 6.6 and 8.3, salinity below 35 and dissolved oxygen above $2\,mg\,L^{-1}$ (Dhert, 1996). Most of the culturing techniques can be described as batch or semi-continuous systems. In batch culture rotifers are generally reared in plastic tanks (500–2000 L) and the rotifers are harvested completely, with a part of the harvest used as an inoculum for the next culture and the remaining managed as food for fish larvae. In semi-continuous culture systems, the size of the tanks ranges from 50 to $200\,m^3$ and rotifer density is kept constant by periodic harvesting (Coves *et al*., 1990) (Figure 8.2). Japanese researchers have developed ultra-high density culture techniques which represent an effective way to produce a great number of rotifers (up to 40,000 ind/mL) in small tanks equipped with specific filtration and re-circulating units (Yoshimura *et al*., 2003).

To produce a significant biomass of rotifers a cheap food source is generally used, usually baker's yeast (0.5–1.0 g 10^6 rotifers). However, rotifers fed yeast alone lack essential fatty acids required for proper development and survival of several marine fish species (Watanabe, 1993). Rotifers can be nutritionally improved when fed with microalgae

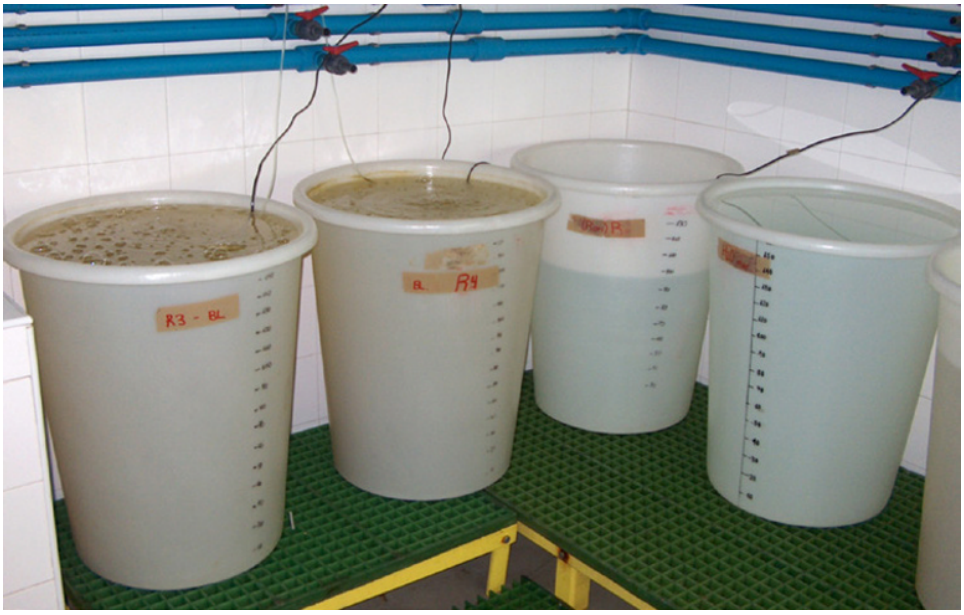


Figure 8.2 Units (130 L) for the rearing of the rotifer *Brachionus plicatilis*.

(*Chlorella* sp., *Nannochloropsis oculata*, *Tetraselmis* sp.) or with commercial diets that provide high levels of EFA and vitamins.

8.4 *Artemia*

The crustacean brine shrimp *Artemia* is the most used and essential live diet for the rearing of larval organisms, particularly fish and crustacean (Sorgeloos *et al.*, 2001). One of the most interesting characteristics of this prey is that in nature it produces cysts under some environmental conditions. When conditions in nature are not adequate (low temperature, scarcity of food, etc) mature females release brown colored cysts with a hard cover (chorion) and a dormant embryo inside. However, when exposed to oxygen, light, salinity higher than 10 and sufficient food, the embryo resumes development and emerges into the surrounding medium. Under these same optimal conditions the females produce eggs that almost immediately hatch (Lenz & Browne, 1991). The collection and further storage of these "long life" *Artemia* cysts is a great advantage in larviculture since they can be hatched and used when needed.

The cysts, conveniently stored under dry oxygen-free conditions, can remain viable for long periods of time (up to 25 years). From those cysts, the first larval stage of *Artemia* will emerge (Instar 1), with lengths usually ranging from 400 to 500 µm, depending on the *Artemia* strain. Instars I do not feed during the first 8–10 hours of life, depending exclusively on internal nutritional resources. Instar I molts into a second larval stage (Instar II), which is already prepared to ingest small particles (1–50 µm in size). After a series of molts, the larvae differentiate into the adult stage (>1 cm in length). The adult stage is reached after 8 days of development under ideal conditions

and adults are sexually differentiated, with females developing eggs (300 eggs or nauplii every 4 days) in a brood pouch (Van Stappen, 1996).

The first developmental stages of *Artemia* are also named nauplii (first instars) and metanauplii (instars that are 24 or more hours old). *Artemia* nauplii are the most widely used prey in larval rearing but their nutritional quality cannot be artificially manipulated. On the contrary, the metanauplii are generally offered to larvae after undergoing a feeding procedure (enrichment) commonly based on the use of mixtures of microalgae, oils and/or commercial emulsions or dried products. The aim of the enrichment is to improve (only to a limited extent) the nutritional quality of *Artemia* to satisfy the nutritional requirements of the target species.

Different *Artemia* strains from throughout the world are commercialized, but most are from the Great Salt Lake. The strains differ in their characteristics, especially size hatching rate (Vanhaecke & Sorgeloos, 1983) and biochemical composition (Léger *et al*., 1986). The smallest instar I nauplii are those from San Francisco Bay (about 425 µm length), which makes this strains very appropriate for larvae requiring small prey. On the contrary, China strains are among the largest ones available (about 500 µm or higher).

From an operational point of view, the production of *Artemia* nauplii or metanauplii to feed the larvae involves the following steps:

8.4.1 Cyst Disinfection

This procedure aims to reduce the bacterial load of newly hatched nauplii to minimize the risk of larval infections. Commonly, the bacterial load of the incubation media at the end of the cyst hatching procedure surpasses 10^7 CFU mL^{-1}. The disinfection technique is based on a simple treatment based on hypochlorite (Van Stappen, 1996). The Cysts (50 g cysts L^{-1}) are soaked in a 200 ppm hypochlorite solution (20 mL NaOCl in 10 L water) for 30 min. Then, the cysts are washed thoroughly with tapwater on a 125 µm screen and collected for hatching incubation.

8.4.2 Cyst Decapsulation

This is an optional procedure consisting of the removal of the hard shell of the cysts by short-term exposure to a liquid bleach solution (Bruggeman *et al*., 1980; Van Stappen, 1996). Decapsulated cysts have some advantages over non-decapsulated ones such as medium-term storage in the refrigerator of decapsulated cysts (prior dehydration), 30–40% more energy content, avoiding cyst shells in the larval rearing system (cyst might obstruct the gut of the larvae if ingested), and lower bacterial load of the emerged nauplii. Decapsulated cysts (200–250 µm in diameter) are immobile and hence visually less attractive than nauplii but can be directly used for feeding larvae in some species such as marine shrimps or carp.

8.4.3 Cyst Incubation and Hatching

This procedure can be carried out with or without prior cyst descapsulation. Nauplii hatching is optimal when cyst incubation is performed under the following conditions: temperature 25–28 °C (always <33 °C), 15–35 salinity (faster at 5), minimum pH of 8.0, near saturated oxygen levels, maximum cyst densities of 2 g/L, and strong

illumination of 2000 lux (Van Stappen, 1996). Increasing cyst density might considerably reduce hatching rates, though it depends upon the *Artemia* strain used. Cyst incubation success is enhanced when carried out in containers with translucent conical bottoms and strong aeration at cyst densities lower than 5 and 2 g L^{-1} for small (up to 20–30 L) and large volumes, respectively. An adequate technique for the production of instar I nauplii should be based on the synchrony of optimal hatching efficiencies (the number of nauplii hatched per gram of cysts) and hatching rates (the percentage of hatched cysts with respect to total number of incubated cysts). The former ensures that most newly hatched nauplii will be in a similar developmental stage with the highest possible energy content. To achieve this it is necessary to run pertinent hatching tests to establish the ideal harvesting time at which the percentage of equally developed nauplii is highest. The optimal harvesting times differ among *Artemia* strains but should not exceed 24 h in non-decapsulated cysts and more than 10 h in decapsulated ones. Hatching efficiency ranges from more than 300,000 nauplii g $cysts^{-1}$ in high quality *Artemia* to about 100,000 nauplii g $cysts^{-1}$ in lower quality cysts. The hatching rate of a high quality *Artemia* brands should not be lower than 90%.

Collection, rinsing and cleaning are very important steps in the various procedures of *Artemia* production. A considerable amount of waste (cysts) is produced during hatching that can be easily separated by 10–20 minutes of settling (with air switched off) in the hatching incubator. Empty cysts will sink to the bottom of the incubator unit whereas unhatched ones generally will float. A powerful light source will also help in the separation from the waste since the nauplii are phototactic. Further cleaning can be accomplished by placing the collected *Artemia* in a test tube filled with oversaturated seawater or tap water. A 250 µm mesh is very practical for collecting, rinsing and separating the nauplii from the cysts. Depending on the strain used, other mesh sizes could be used for separating cysts from emerged nauplii.

Practical worksheets for disinfection, decapsulation and hatching procedures are provided by Van Stappen (1996).

8.4.4 Enrichment

The nutritional quality of all *Artemia* strains is not adequate to fulfill the requirements of larvae of most species, particularly marine organisms (Sorgeloos *et al*., 2001). San Francisco Bay (SFB) is among the strains of higher quality, whereas those of Great Salt Lake (GSL) are low quality strains. In spite of such differences, enrichment in all stages of *Artemia*, adults included, is imperative in most cases for enhancing their biochemical composition. *Artemia* are deficient in phospholipids, vitamins A and C, and especially in highly unsaturated fatty acids of the *n*-3 series (*n*3-HUFA) (Bengtson *et al*., 1991; Sorgeloos *et al*., 2001). Especially important in the enrichment procedure is enhancement of the level of the fatty acids 20:5*n*-3 (EPA; eicosapentaenoic acid) and 22:6*n*-3 (DHA; docosahexaenoic acid), essential to larvae of marine crustaceans and fishes. In addition to natural enrichment sources (e.g. microalgae), numerous commercial products (dry or self-dispersing emulsions) are currently available, that provide good result in most species. Those enrichment products usually contain high lipid and essential fatty acids (EPA, DHA) levels. Some products are also enriched in protein, amino acids and vitamins, and might also contain drugs or biochemical components to help in the control of the bacterial load.

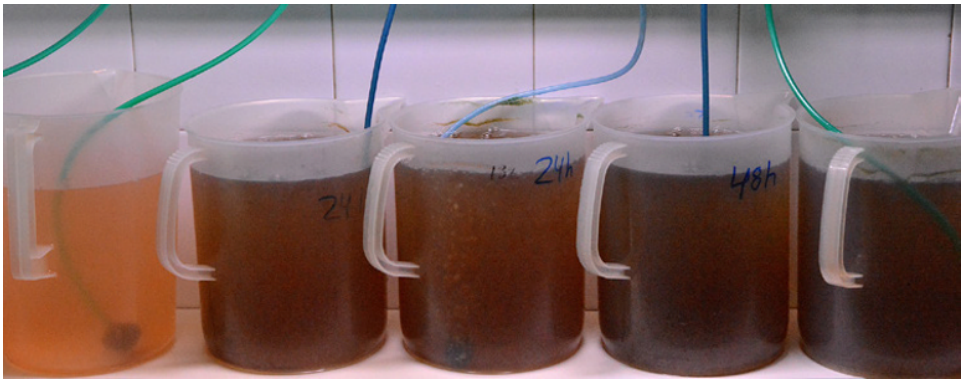


Figure 8.3 Buckets (5 L) for the enrichment of *Artemia* nauplii and metanauplii.

Commonly, *Artemia* enrichment is carried out for 24 (24 h metanauplii) and 48 (48 h metanauplii) hours at densities of 250–500 *Artemia* mL^{-1}. One of the most important factors in the enrichment is oxygen level, which should be maintained above 2 mg^{-1}. The optimal temperature for enrichments is 19–25 °C (Figure 8.3).

The use of *Artemia* in aquaculture is usually restricted to early stages (nauplii and metanauplii). However, adult *Artemia* is also an alternative for feeding large fish and crustaceans. Adult *Artemia* is commercially available in the market but can also be produced using on-growing techniques that will be determined by the quantitative needs of the producer (Dhont & Lavens, 1991).

The maintenance of *Artemia* at low temperature levels (<10 °C) will preserve their nutritional quality for some time.

8.5 Copepods

Copepods are a group of small crustaceans found in the sea and nearly every freshwater habitat. Copepods have an exoskeleton primarily composed of chitin. They represent the vital link between primary and secondary production in the oceans and are the natural food items for most marine fish larvae. Copepods are typically 1 to 2 mm long, with a cylindrical shaped body and large antennae. The head is fused with the first one or two thoracic segments, while the remainder of the thorax has three to five segments, each with limbs. The first pair of thoracic appendages are modified to form maxillipeds, which assist in feeding. Reproduction occurs sexually in copepods and spermatophores produced by males are deposited close to the genital pore of the female. The eggs may be laid directly into the water (broadcasters) or held in specialized sacs within the female until hatching occurs. At the end of embryo development nauplii are produced; they show a typical oval-unsegmented-shaped body and a naupliar eye. The nauplii molt through six stages with the last three molts characterized by the development of appendages and setae. The sixth stage molts into a copepodite that after six more molts turns into an adult copepod stage, during which reproduction can take place (Støttrup & Norsker, 1997).

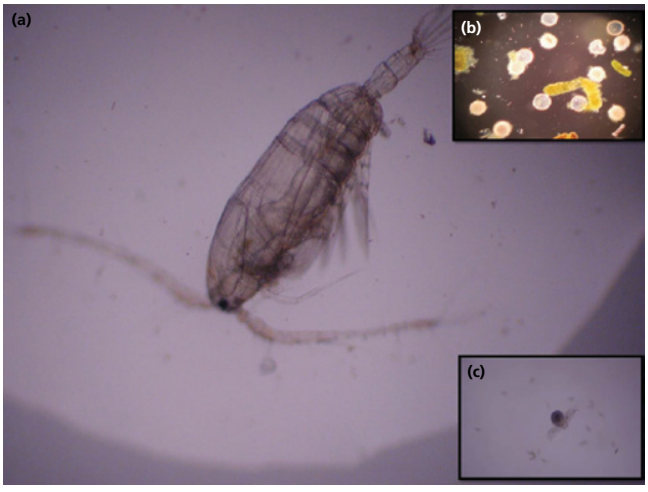


Figure 8.4 *Acartia tonsa* (a) adult; (b) embryos and (c) nauplii. (*See insert for color representation of the figure.*)

In aquaculture only a few copepod species have been cultured as prey for fish larvae. In particular the three orders Calanoidea, Harpactoidea and Cyclopoidea have been investigated since they are considered a valid food source for the culture of many larval fish. The use of cultured copepods in intensive fish larviculture has involved calanoids such as *Acartia* spp. (Schipp *et al.*, 1999), *Eurytemora* spp. (Shields *et al.*, 1999), *Parvocalanus* spp. (Olivotto *et al.*, 2006) and harpacticoid copepods such as *Euterpina acutifrons* (Kraul *et al.*, 1992), *Tisbe* spp. (Olivotto *et al.*, 2008) and *Trigriopus japonicus* (Fukusho, 1980) (Figure 8.4). Delbare *et al.* (1996) summarized the advantages of using copepods in larviculture such as the wide range of body sizes between nauplii and adults, their typical movement, and their high content of highly unsaturated fatty acids (HUFAs). These fatty acids, in particular eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3), are extremely important for larval fish survival and growth and several studies have demonstrated that they are essential in larval diets (Sargent *et al.*, 1999).

To date there are several reports of successful cultivation of copepods either in outdoor extensive basins or in indoor intensive systems. Extensive production is usually carried out in ponds or enclosed lagoons using fertilizers that promote phytoplankton production. The mixed copepod cultures usually reach 10–30 ind mL^{-1} concentration and can be harvested using plankton nets. However, these productions are highly dependent on natural meteorological cycles which limit the annual production of fish in addition to being potentially affected by parasite that use copepods as an intermediate host. Intensive production has been developed both for calanoid and harpacticoid copepods.

Different tanks have been developed for intensive indoor copepod production (i.e. larger surface area for harpacticoids; deeper tanks for calanoids) (Støttrup, 2000) (Figure 8.5). These both include 100–1000 L static tanks, with gentle aeration (these cultures are usually renewed every 3 weeks) and re-circulating systems equipped with specific filtration systems. In static tanks the copepod collection is usually carried out using different sized mesh (150–100–75–25 μm), while in re-circulating systems the copepod collection is automatic. For example at the Stazione Zoologica Anton Dohrn



Figure 8.5 Tanks (500 L) for the cultivation of the copepod *Acartia tonsa* at Instituto de Investigaciones Marinas in Vigo (Spain).

in Naples, calanoid copepods were cultured through different generations in a 500 L re-circulating system developed by INNOVAQUA, Reggio Emilia, Italy, equipped with biological/mechanical filtration. Nauplii and copepodites were attracted into a 155–300 µm mesh net size filter cage using a light, automatically collected in a 200 L tank, and finally concentrated in 15 L of seawater. This system was able to produce enough nauplii for feeding trials and may represent a starting point for the development of a mass cultivation system for calanoid copepods (Olivotto *et al.*, 2008).

In both cases copepods are usually fed different algal combinations that can include small algal species such as *Isochrysis galbana* and *Nannochloropsis oculata* and larger ones such as *Rhodomonas baltica*. The ideal concentration in the copepod tanks is 10^5 cells mL^{-1} for algae smaller than 5 µm and 10^4 cells mL^{-1} for larger algae.

Although the best results have been obtained using calanoid copepods which have a high content of HUFAs and are entirely pelagic with very small naupliar stages that are readily captured by fish larvae with small gapes at first feeding (Olivotto *et al.*, 2006), there are still several difficulties in culturing calanoid copepods on a continuous basis.

In order to produce large quantities of copepods, suitable storage techniques (i.e. cold storage) of copepod eggs are now of great interest. Both subitaneous (immediately hatching) and diapause (dormant for some time) eggs have been investigated for use in fish culture (Marcus & Murray 2001; Drillet *et al.*, 2006a, b, 2007). While it appears feasible to store diapause eggs for a long time period (up to 17 months) (Marcus & Murray, 2001) there are many difficulties regarding forced induction of hatching of these diapause eggs. For this reason subitaneous eggs may be more available and cold storage at low temperature may be an appropriate storage technique because the eggs switch to a quiescent state due to the suboptimal environmental conditions (Drillet *et al.*, 2006a, b; 2007). However, since the copepod embryo still retains physiological

activity during the cold storage period, their nutritional quality may change over this time of storage (Drillet *et al*., 2006a, b) with these changes possibly affecting both the egg's viability and marine fish larval survival and development.

8.6 Other Prey

In addition to the most commonly used live food (microalgae, rotifers, *Artemia* and copepods) there are other prey of potential interest for aquaculture. The mass production of those species has not yet been standardized, but improvements have been made in some cases. Some of the main problems related to the development of new species production techniques are: lack of information available on the know-how, long production cycles (long lifespan and low reproduction rates) and low harvesting outputs.

8.6.1 Mysid Shrimp (Mysidaceans)

Mysid shrimp are part of the natural prey of many species. Their adult size is rather large, and inadequate for the small mouth of larvae. Only the smaller early developmental stages of mysids would be appropriate for young larvae of most ornamental species. Besides this, maximum production is low because reproduction is seriously affected by high densities. Domingues *et al*. (1999) reported an average daily production of 273 hatchlings from 1000 mysids reared in a rather sophisticated static culture system. Nevertheless, mysidaceans are excellent feed for marine organisms due to their rather constant and optimal biochemical composition.

8.6.2 Grass Shrimp *Palaemonetes* spp.

Grass shrimp are also interesting species and relatively easily, to cultivate on *Artemia* nauplii and mussel meat at moderate densities in simple culture systems (Chamorro *et al*., 2010). They may be more important prey for juvenile or adult stages of marine ornamentals.

8.6.3 Trocophora Larvae

Trocophora larvae of bivalve molluscs are used by some producers for feeding marine fish larvae. Marini & Moe (2003) describe methods for rearing and collecting these bivalve larvae for feeding fish fry.

8.6.4 Caprellids (Amphipoda)

Caprellids commonly known as skeleton shrimp are among the novel and less known contributions to live prey production systems. These species are commonly encountered as part of the epifauna (many marine species depend on the availability of epifauna species). They offer some advantages compared to other prey. Caprellids can reach high densities, are rather tolerant of environmental changes and have satisfactory n-3 HUFA profiles (Woods, 2009). Most studies with caprellids have been with small-scale experimental or laboratory culture, from which the potential suitability of caprellids to larger scale culture is envisaged (Woods, 2009).

8.6.5 Other Organisms

Others including *Daphnia*, *Moina* and nematodes are freshwater prey that are potentially useful for some developmental stages of marine organisms (Delbare & Dhert, 1996).

8.7 Conclusions

This chapter underlined the importance of the transition from endogenous to exogenous feeding by the larvae. In particular, prey must have a proper size, but also a nutritional profile that matches larval requirements. For years, rotifers and *Artemia* have been widely used for the culture of many species. In some species they can guarantee optimal survival and proper larval development; however, these prey seem to be inadequate for a commercial production of most ornamental species. As a consequence, there is a strong need to identify and culture new plankton species that show a proper size, a shorter turnover time, can be cultured at high densities and that show a proper nutritional profile to sustain larval development.

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9

Larval Diets and Nutrition

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Abstract

The early development and growth of marine species largely relies on the capture, digestion and assimilation of adequate live food. The type of prey available to aquarists and professional producers is certainly limited, but there is still a range of possibilities in the selection of prey suitable for a variety of species. The most widely used preys in the rearing of larvae are rotifers and *Artemia* spp. These preys are not ideal, especially from a nutritional point of view. In spite of that, reasonably good results can be attained in the early rearing of a fairly wide variety of species when those prey are properly enriched. However, improvements can be achieved by supplying copepods as an alternative food source for the larvae. Currently, several copepod species can be successfully cultivated. Their incorporation into the feeding scheme of larvae, as unique prey or supplementary to the traditional prey, is advantageous due to their higher nutritional quality and the wider range of sizes available for different stages of larval development.

Keywords *Artemia; ciliates; copepods; fatty acids; nutrition; rotifers; zooplankton*

9.1 Larval Nutritional Requirements

The successful survival and production of marine ornamental organisms is dependent on the culture of live preys with proper size, attractive locomotory patterns and optimal nutritional profile. In the wild, pelagic larvae spend their early life in the open ocean, surrounded by plankton rich waters in which they can identify and eat small planktonic organisms that are usually selected based on their size, color, smell and movement. It is well-established that the preferred food items of marine fish larvae are copepods and that this natural diet provides the larvae with proper and well-balanced amounts of nutrients (Olivotto *et al*., 2011).

To date studies of digestion, absorption, and nutrient assimilation in marine fish larvae are still limited because most of them are small and difficult to handle, their

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mouth gape is tiny and require small feed-particle sizes, which creates difficulties for microdiet production technology. In addition, larvae are poorly attracted to inert microdiets and their administration often results in low and/or variable ingestion and survival rates in feeding experiments. The use of live food in nutritional studies may represent an alternative; however, it can create major limitations during experiments since it is not always easy to efficiently and constantly manipulate the nutritional characteristics of live preys (Holt, 2011; Hamre *et al.*, 2013).

Lipids are probably the most studied molecules during larval fish development. They are very important during the early life stages of marine fish larvae since they play pivotal physiological roles such as metabolic energy storage, are essential for cellular membrane synthesis (being an essential component of phospholipids) and are precursors of very important bioactive molecules (Sargent *et al.*, 1999). In particular, highly unsaturated fatty acids (HUFAs) such as docosahexaenoic acid (DHA, 22:6*n*-3), eicosapentaenoic acid (EPA, 20:5*n*-3) and arachidonic acid (ARA, 20:4*n*-6), have several important biological functions and are thus essential in larval fish diets. As a consequence, the amounts and ratios of these fatty acids (FA) may strongly influence essential physiological mechanisms that occur during the quick larval development (Holt, 2011). DHA has a central role as growth promoter in fish, but also in the stress response and in immune system development. While ARA benefits larval fish survival, stress resistance and pigmentation, it is usually less involved in larval fish growth regulation compared to DHA and EPA. These FA should not only be present in the diets in a certain amount but also in specific ratios: for example in order to promote proper dietary utilization of both DHA and EPA, the desirable DHA/EPA ratio is >2 (Sargent *et al.*, 1999). Since marine fish larvae have very low elongase and desaturase ability, they are not able to produce *de novo* DHA, ARA and EPA starting from their precursors (linoleic and linolenic acids; 18:2n-6 and 18:3n-3) (Sargent *et al.*, 1999). As a consequence it is clear that lipid nutrition is particularly important for marine fish (Bell *et al.*, 2003) since deficiencies in these molecules can cause a general decrease of larval health, poor growth, low feed efficiency, anemia and high mortality (Sargent et al., 1999; Hamre *et al.*, 2013).

While copepods usually match the FA requirements of the larvae, conventional live preys such as rotifers and *Artemia* do not contain proper amounts and ratios of these FA and for this reason they usually need to undergo an enrichment process (Olivotto *et al.*, 2006b). This process is achieved using commercial products that rotifers and *Artemia* can assimilate through their filtration activity. An important advantage of copepods is that they may survive for days in culture with little food or in starving conditions, maintaining almost unchanged their biochemical composition (mainly phospholipids and triglycerides) and hence their nutritional value (Fleeger, 2005).

Marine fish larvae show very high growth rates and reach such high growth rates only if sufficient quantities of proteins and micronutrients together with lipids are provided through the diet.

Despite recent progress, knowledge of protein and amino acid (AA) requirements of fish larvae is still fragmentary and often qualitative rather than quantitative (Hamre *et al.*, 2013). The major difference observed in fish larvae compared to adult fish is a poorer capacity to digest and/or absorb complex proteins. However, larvae show very high protein turnover and AA catabolism necessary for rapid larval growth supporting the hypothesis of a higher AA requirement during the larval stages. There is an evident paradox when the potential fast growth of fish larvae is considered together with the

poorly developed protein processing capacity of their digestive system. Therefore, fish larvae mainly need diets rich in soluble molecular nitrogen and that avoid complex proteins characterized by low digestibility. Larvae have efficient control of AA catabolism and use dispensable preferentially to indispensable AA as an energy substrate. The primary role of AA as building blocks for body proteins, as precursors of important biological molecules such as hormones and neurotransmitters are well-known (Harme *et al*., 2013); however, AA requirements of fish larvae are not easily determined. It is established that in fish eggs with no oil globules AA represent 40–90% of the energy substrates from first feeding, while in eggs that contain oil globules AA supply about 10% of the energy at first feeding. Unfortunately, live preys commonly used in aquaculture, such as rotifers and *Artemia*, do not meet the AA dietary requirements of larvae (Aragão *et al*., 2004). In fact, phytoplankton and zooplankton all contain significant amounts of free amino acids (FAA), however, the mass-specific content of FAA varies among the various live foods. In addition, the FAA content of certain plankton species may vary depending on the life-stage and culturing conditions. For example, some zooplankton species, like calanoid copepods, contain more than twice the amount of FAA per gram wet mass than *Artemia* (Næss et al., 1995). To date, several AA and protein enrichment products are available to meet larval fish requirements.

In addition to FA and AA, liposoluble vitamins (Vitamin A, D, E, K), water-soluble vitamins (vitamins B, C) and minerals (manganese and zinc) are essential to promote larval growth and survival and reduce skeletal deformities (Hamre *et al*., 2013). It is now well-established that copepods have optimal levels of these molecules, while rotifers and *Artemia* show several deficiencies. In fact, there are several differences in the nutritional composition between copepods, rotifers and *Artemia*. For example vitamin A, E and B may vary in rotifers depending on the culture medium, while *Artemia* has very low thiamine, iodine and zinc concentration compared to copepods. For some of these elements enrichment procedures have been optimized and can assure a proper nutritional profile for both rotifers and *Artemia*. For example due to the fact that yeast is particularly rich in B vitamins, yeast cultured rotifers show very high levels of these molecules; the low concentration of trace elements detectable in rotifers can be corrected by adding these molecules either to the culture media or to the diet; *Artemia* nauplii can be enriched with iodine and thiamine, but not with zinc that causes high mortality. In conclusion, despite considerable progress in understanding dietary requirements of marine fish larvae, many questions remain open and extra details are needed, especially for a better understanding of the nutritional quantitative requirements of larvae.

9.2 Microalgae

Microalgae (Figure 9.1) represent an important food source and feed additive in the commercial rearing of all growth stages of bivalve molluscs and penaeid shrimp larvae, and the primary diet of zooplankton cultured as food for fish larvae and juveniles. Many factors contribute to the nutritional value of a microalga, including its biochemical composition, digestibility and size and shape. Several studies indicate that, in the late-logarithmic growth phase, microalgae usually contain 30–40% protein, 10–20% lipids and 5–15% carbohydrates (Brown *et al*., 1997). However, when cultured through the

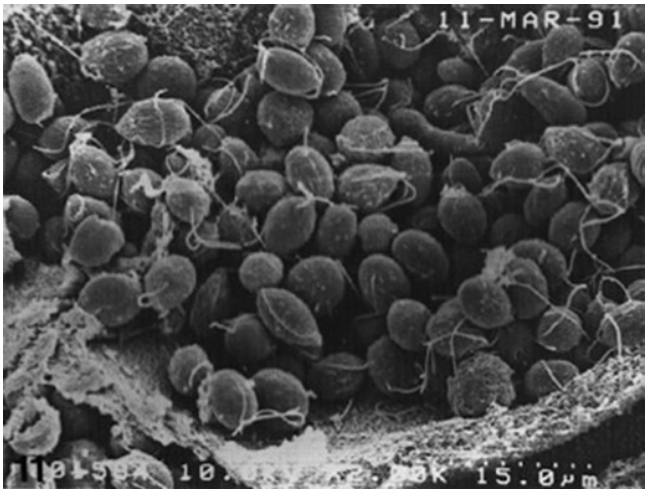


Figure 9.1 Microalgae *Dunaliella salina* in an alginate bead (Picture by Prof. M. Giordano).

stationary phase, these values may change significantly; e.g. when nitrate is limiting, carbohydrate levels almost double at the expense of protein (Brown *et al*., 1993b; Harrison *et al*., 1990). The protein quality of microalgae remains high under different culture conditions, is similar between species, and is relatively unaffected by intrinsic growth phase (Brown *et al*., 1993a) and extrinsic light conditions (Brown *et al*., 1993b). The vitamins and antioxidants content are also not uniform among microalgae; ascorbic acid shows the greatest variation (1 to 16 mg/g dry weight) (Brown & Miller, 1992).

As primary producers in the aquatic food chain, microalgae are the source of many phytonutrients, including in particular HUFAs, e.g. EPA, ARA and DHA, which are known to be essential for several marine organisms. Most algal species show moderate to high percentage of EPA (7 to 34%). Prymnesiophytes (*Pavlova* spp. and *Isochrysis* spp.) and cryptomonads are also relatively rich in DHA (0.2 to 11%), whereas eustigmatophytes (e.g. *Nannochloropsis* spp.), rhodophytes and diatoms have the highest percentages of ARA (up to 4%). Chlorophytes (*Dunaliella* spp. and *Chlorella* spp.) are deficient in both EPA and DHA, although some species have small amounts of EPA (up to 3.2%). Due to this HUFAs deficiency, chlorophytes have poor nutritional value, so they are not suitable for use as a single species diet. Prasinophytes contain significant proportions of EPA (*Tetraselmis* spp.) and DHA (*Micromonas* spp.), but rarely of both (Brown *et al*., 1997).

Even if fish larvae usually do not actively feed on microalgae, their fatty acids can be efficiently transferred to fish larvae *via* intermediary zooplankton. The most used microalgae to boost zooplankton with HUFAs are those which contain high levels of DHA (e.g. *Isochrysis* spp.). Moreover, because microalgae may be limiting in one or more of the key nutrients, a selected mixture of microalgae should be fed to fish, directly or through enrichment of zooplankton to provide a better balanced nutrition package.

A common procedure in larviculture is to add $\pm 0.2 \times 10^6$ algal cells mL^{-1} to the culture system ("green water technique") to improve larval production. Theories advanced include water quality improvement by oxygen production and pH stabilization, light attenuation which has a beneficial effect on larvae (especially on the phototactic ones), a prebiotic effect (with a consequent gut colonization by beneficial bacteria) and

maintenance of the nutritional quality of zooplankton (Brown *et al*., 1997). The most popular algae species used for green water applications are *Nannochloropsis oculata*, *Tetraselmis suecica* and *Isochrysis galbana*.

9.3 Rotifers, *Artemia* and Ciliates

The main problem in fish larviculture is the transition from endogenous to exogenous feeding by the larvae that still relies on the production of live preys. Field and laboratory studies recently demonstrated that fish larvae mainly feed on protozoans, copepods and larvae of benthic organisms (Baensch & Tamaru, 2009a, b; Olivotto *et al*., 2006a). However, there are still several difficulties in culturing these organisms on a continuous basis and no artificial feed formulation for first feeding of marine larval fish has been developed yet. For these reasons, most of the marine fish species are still reared using rotifers, *Brachionus* spp., and *Artemia* spp. nauplii. The success of rotifers (Figure 9.2) and *Artemia* as culture organisms is manifold, including their planktonic nature and small size, their filter-feeding nature that allows the inclusion into their body of specific nutrients (enrichment) and, last but not least, the possibility of rearing these animals at very high density. Aside from these apparent advantages of rotifers and *Artemia*, they are a main bottleneck in fish production. In fact, rotifers and *Artemia* are often not representative of larval fish natural prey. They can be too large for some tiny larvae (e.g. angelfishes and other pelagic spawners), and in some cases their locomotory patterns

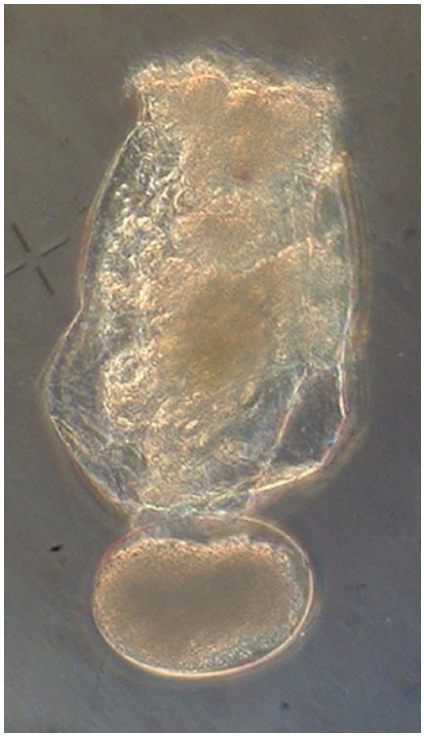


Figure 9.2 The rotifer *Brachionus plicatilis*.

may not elicit predatory activity by the larvae (Olivotto *et al*., 2011). In addition, the nutritional profile of rotifers and *Artemia* does not match larval fish requirements: they naturally lack *n*-3 HUFAs, being rich instead in linolenic acid (18:3*n*-3) and linoleic acid (18:2*n*-3). However, both rotifers and *Artemia* are non-selective feeders and can undergo an enrichment process in order to improve their nutritional profile. The enrichment process usually consists of adding a commercial *n*-3 HUFAs enrichment to the culture media, following the producer's instruction. After the enrichment period, rotifers and *Artemia* are concentrated on plankton sieves, rinsed with fresh seawater and then distributed to the larval tanks. Enrichment procedures can be performed using different methods including the administration of HUFAs-rich microalgae, fish oils, or better formulated diets that are nowadays easily available (Sargent *et al*., 1999). In rotifers, the use of formulated diets contributes to filling their gut with nutrients including lipids, vitamins and minerals (Coutteau & Sorgeloos, 1997). Although it is technically possible to produce rotifers of high nutritional value, their quality is often suboptimal because of their hygienic quality and overall condition (low swimming velocity, low reproduction rate, etc). For these reasons, the microbiological aspects involved in rotifer culture are now of primary importance (Rombaut *et al*., 1999).

In *Artemia*, the most commonly applied boosting technique is a 24-h enrichment period after hatching. However, it should be highlighted that the enrichment technique has limitations because *Artemia* nauplii have a marked propensity to retroconvert 22:6*n*-3 to 20:5*n*-3 and to catabolize some phospholipids (Sargent *et al*., 1999). Since DHA catabolism is strain-dependent, this problem could be partially overcome using strains of different geographical origin. Moreover, various enrichment emulsions have been formulated differing in the fatty acid composition of their triglycerides, and the traditional formulations rich in EPA have been replaced by new products rich in DHA and ARA. In addition, vitamin C has been incorporated in booster formulations to increase the level of AA in *Artemia* (Sorgeloos *et al*., 2001). All these changes in the formulation of enrichment diets offer more possibilities to cover the needs of different species and help to reduce problems related to diseases, stress resistance, malformation, and pigmentation in numerous fish species. The most recent developments involve the use of *Artemia* preparations other than the nauplii (e.g. juveniles and decapsulated cysts) (Lavens *et al*., 1995). In each case, it is essential to immediately administer the enriched live preys to the fish larvae since both rotifers and *Artemia* easily and quickly show an alteration in their protein/lipid balance.

To by-pass the rotifer and *Artemia* size problems, scientists also employ ciliates as live preys in fish culture. Ciliates are unicellular eukaryotic organisms and conventionally they are divided into loricate (tintinnid) and aloricate (naked) forms. Since tintinnids have often been found in the gut content of field-collected marine fish larvae, Pierce & Turner (1992) suggested their main importance is at the first-feeding stage. Naked ciliates are ubiquitous and abundant in aquaculture environments, feeding on bacteria and phytoplankton, potentially representing a food source for fish larvae. Especially for their abundance but also for their smaller size compared to rotifers and *Artemia*, ciliates may represent a valid alternative live prey in aquaculture. In fact, it is well-established that ciliates are an important food source for several zooplanktonic organisms, like shrimp larvae, because of their elevated abundance and biochemical composition, with higher amounts of nitrogen rather than carbon. They also may represent a significant source of essential nutrients like HUFAs, sterols and amino acids that are essential for

the growth of penaeid shrimp (Stoecker & Capuzzo, 1990). However, similar to rotifers and *Artemia*, their nutritional profile is strongly influenced by their diet and may vary significantly.

The use of ciliates in fish larviculture is not applied on a large scale yet and only a few studies have analyzed their potentiality as live prey for fish larvae. Moreover, in these studies ciliates were subjected to an enrichment procedure in order to improve their nutritional profile. In particular Nagano *et al*. (2000) and Thompson *et al*. (1999) showed that high ciliate densities enhanced *Paracanthurus hepatus* and *Penaeus paulensis* larval survival, respectively. More recently, Olivotto *et al*. (2005) demonstrated higher survival rates in *Gobiosoma evelynae* larvae fed on the naked ciliate *Euplotes* sp. compared to those fed on the larger *Brachionus rotundiformis* or *B. plicatilis*.

9.4 Copepods

Copepods are the feeding items preferred by most fish larvae (Delbare *et al*., 1996). They usually show a wide range of body size from nauplii to adults, making them ideal to cover the whole larval cycle; they have a typical movement that can promote the predatory activity in most fish larvae, and a nutritional profile that perfectly matches the nutritional requirements of marine fish larvae (Delbare *et al*., 1996). In particular, copepods show very high and well-balanced contents of HUFAs, high levels of carotenoids and are in general better digested by fish larvae compared to rotifers and *Artemia*. As a consequence, copepods are of great interest to investigators and several feeding studies, involving both harpacticoid and calanoid copepods, have been carried out to improve the number of reared marine ornamentals.

Harpacticoids (Figure 9.3) have been widely used in the few last years, as new potential live preys in the rearing of several species. In particular, *Tisbe* sp. harpactiocoid copepods have been used with clownfish (Olivotto *et al*., 2008b), striped blenny and long snout seahorse culture (Olivotto *et al*., 2008a; 2010a) both solely or in combination with rotifers and *Artemia*. *Euterpina acutifrons* was used during Barber goby (*Elacantinus figaro*) and bluestriped angelfish (*Chaetodontoplus septentrionalis*) culture (Cortes & Tsuzuki, 2012; Leu *et al*., 2013). Other studies demonstrated that some harpacticoid copepods like *Tisbe biminiensis* are good candidates for mass production (Ribeiro & Souza-Santos, 2011). However, most of the studies found that the advantage in the use of harpacticoid copepods can be achieved only if they are used in combination with other pelagic live preys. In fact, since the epibenthic copepods are usually found on the tank walls rather than in the water column, they are in general less available for predation by planktonic larvae. As a consequence when harpacticoid copepods are used solely, they usually cause a quick decline in larval survival. On the contrary, when used in combination with other live preys they are valuable as a source of HUFAs for the traditional diet.

Better results in larviculture are usually achieved when calanoid copepods (e.g. *Acartia* spp.) are used since they are entirely pelagic, and thus more available as prey. They have smaller naupliar stages, and thus are more available to fish larvae with small gapes, and are particularly rich in HUFAs.

Several species have been studied and tested on different marine ornamental species including demersal and pelagic spawners. In demersal spawners, such as clownfishes,



Figure 9.3 The harpacticoid copepod *Tisbe* spp.

firefish and comets, calanoid copepod administration (*Centropages typicus*, *Acartia tonsa*, *A. danae*, *C. hamatus*) was able to significantly improve both survival and growth rates compared to larvae fed on a standard diet of rotifers and *Artemia* (Holt, 2003; Olivotto *et al*., 2008a, 2012; Madhu & Madhu, 2014).

In pelagic spawners, which usually have very tiny small-gaped larvae, the administration of calanoid copepods is usually essential to stimulate first feeding, and in some cases, to reach metamorphosis. In particular, *Parvocalanus crassirostris*, *Bestiolina similis*, *A. danae*, *A. sinjiensis*, *A. tonsa* and *C. hamatus* have been cultured and tested as first food items in small pelagic spawned larvae. *Acartia tonsa* has been used for the first feeding of the Cuban hogfish *Bodianus pulchellus* and larvae survived for 25 days post-hatch (ph) (Holt, 2003) while the same copepods were not appropriate food for the small lemonpeel angel larvae. In fact, these larvae successfully started first feeding and survived for 14 dph when offered the smaller *P. crassirostris* copepods (Olivotto *et al*., 2006a). In addition, this same copepod was used to successfully rear through metamorphosis another pigmy angel fish, the flame angel fish, *Centropyge loricula* (Laidley *et al*., 2009).

Despite these great advantages in using copepods there are still several difficulties in culturing them on a continuous basis since they need to be cultured at low densities, in large tanks, and need to be fed different algal combinations (Holt, 2003). As a consequence, the technology for mass scale production of copepods is in the research stage. In order to produce large quantities of copepods, storage techniques for copepod eggs have been investigated. Marcus & Murray in 2001 developed techniques to store *Centropages hamatus* diapause eggs for long periods (up to 17 months). A short-term experiment was carried out on jackknife larvae and demonstrated that a diet of copepod diapause

eggs/nauplii was able to promote survival and growth compared to rotifers and *Artemia* spp. diet. However, only a few copepod species produce diapause eggs, and thus much research has been focused on finding suitable storage techniques for subitaneous copepod eggs. This is because subitaneous eggs may be more available and cold storage at low temperature may be an appropriate storage technique since the eggs switch to a quiescent state due to the suboptimal environmental conditions (Drillet *et al*., 2006). In particular *A. tonsa* copepods originated from 6 months cold stored eggs have been used as food during clownfish culture (Olivotto *et al*., 2012). Results showed that these copepods were suboptimal for the larvae. However, further research should focus both on the optimization of new storage techniques and on the identification of new microalgae with better fatty acid profiles in order to improve the quality of copepod eggs and nauplii.

9.5 Inert Diets (Dry Food and Preserved Copepods)

Feeding of larvae on live prey is expensive and hence efforts have been made for the development of efficient formulated diets (microbounded, microencapsulated and microcoated) for minimizing production costs and improving rearing performances (Jones, 1998; Jones *et al*., 1993). Currently, the early rearing of larvae is still mostly dependent of the production of live feeds. However, the potential use of inert diets to raise larvae of ornamental or commercial species was revealed some years ago. Jones *et al*. (1987) tested a balanced crustacean diet in penaeid larval cultures that potentially might be used to replace part or all of the live foods. Holt (1993) developed a feeding protocol for *Sciaenops ocellatus* larvae based on combining a commercial microparticulate diet with rotifers.

The effectiveness of a formulated diet depends on the properties of the diet (composition, size, shape, moisture, buoyancy, stability, palatability, and digestibility) but also on the foraging behaviour and physiological characteristics of the larvae. Most larvae are commonly attracted by prey and may not forage on motionless inert particles, especially at very early stages. The acceptance of inert diets is dependent on larval age and in some cases on the capability of larvae to modify food particles into smaller parts (e.g. caridean and stenopodidean shrimp), which also depends on the binder and extrusion methods (see review of Calado, 2008). Some species, such as seahorses, are unable to feed on inert manufactured particles even at the adult stage.

The use of solely inert formulated diets provided interesting results in the rearing of several species. Kovalenco *et al*. (2002) reported high performances of a microbound diet without supplemental live food in the larval rearing of the prawn *Macrobrachium rosenbergii*. D'Abramo *et al*. (2006) successfully cultured larvae of *Litopenaeus vannamei* fed exclusively a microbound diet. Fernández-Díaz & Yúfera (1997) detected growth in *Sparus aurata* larvae fed microcapsules. However, growth and survival improvements for many species is generally achieved by co-feeding live prey and manufactured diets compared to the use of live feed only (Holt, 1993; Abi-Ayad' & Kestemont, 1994; Rosenlund *et al*., 1997; Cañavate & Fernández-Díaz, 1999; Rhyne, 2002; Murthy *et al*., 2008). Larval digestion capabilities are not always sufficiently developed at early stages for proper ingestion and digestion of inert diets, but co-feeding may improve larval nutrition and pre-condition the larvae to more readily accept the manufactured diet for further weaning (Rosenlund *et al*., 1997).

In addition to formulated diets, preserved live prey could also be a potential food source for early larval stages. The use of preserved copepods has been assayed with low success in some marine species. Olivotto *et al*. (2010b) evaluated the potential use of preserved copepods (mixture of species) as prey in *Amphiprion clarkii* larviculture and concluded that preserved copepods may be a suitable food for marine fish larvae when used as a supplement to rotifers and *Artemia* nauplii, enhancing larval growth and survival, and shortening the larval phase. These authors also reported that preserved copepods may be easily stored for a long time at −80 °C, maintaining their fatty acid characteristics. Similar enhancements were also achieved by Piccinetti *et al*. (2014) with gilthead sea bream (*Sparus aurata*) larvae fed on a mixture of rotifers, *Artemia* nauplii and preserved copepods.

9.6 Conclusions

Even though rotifers and *Artemia* continue to be the major prey in the rearing of larvae, alternative high quality preys should be considered for future improvements in larval feeding. Currently, copepods provide the best rearing performances, but production efficiency is low and costly. Besides this, the formulation and adequacy of inert diets still needs to be improved for many species. The availability of novel and efficient formulated diets will certainly contribute to improve production efficiencies and to help decrease the dependence of rearing on traditional prey, mainly on *Artemia*. Knowledge of larval nutritional requirements and physiology are pivotal keys in the formulation of new diets.

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10

Growout and Broodstock Nutrition

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Abstract

Basic nutritional information for marine ornamentals species is lacking. Research on feeds for broodstock and growout are essential to advance the ornamental aquaculture industry. While many of the nutritional advances made in the food fish industry can be applied to ornamental fish nutrition, key differences that must be recognized when attempting to successfully transfer this knowledge into the ornamental field are discussed. Broodstock condition is essential to the survival and health of offspring so understanding broodstock nutrition is vital. Several examples of successful broodstock diets are presented. The recent use of probiotics has been shown to improve the health and wellbeing of marine ornamentals. Lactic acid bacteria are especially beneficial, positively enhancing survival and growth. Examples of successful probiotics use with ornamentals are provided.

Keywords *Proteins; lipids; fatty acids; probiotics; spawn quality; clownfish; flame angelfish; seahorses*

10.1 Introduction

Basic nutrition for tropical fish is undeniably lacking, mainly due to the concentration of research efforts on and funding for food fish species. There is a disproportionate level of research dedicated to the aquaculture production of food fish when compared to the ornamental tropical fish industry, especially when one compares the net worth of individual tropical fish to that of even the highest priced cultured food fish species (Sales & Janssens, 2003). Despite the obvious significance of such research, information concerning the nutritional requirements of both freshwater and marine ornamental fish are, at best, limited (Blom & Dabrowski, 2000; Sales & Janssens, 2003; Vijayagopal *et al*., 2008). The currently available feeds often utilized to feed marine ornamentals are certainly not designed to be species-specific

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(Chong *et al*., 2003; James & Sampath, 2004b), despite being 10–20 times more expensive than the highest priced commercial feeds developed for the aquacultured food fish industry. Current formulated diets that are commercially available for ornamental fish are generally packed in expensive, small packages, targeting the hobbyist market, making them too expensive for large-scale operations (Chong *et al*., 2003). As the marine ornamental aquaculture industry continues to grow and expand, it will be critical to direct nutritional research to alleviate the current void of information related to feeds designed specifically for ornamental fish. There is a tremendous amount of knowledge that can be transferred from the nutritional research accumulated over the past several decades for aquacultured food fish. Use of this knowledge can speed developments in ornamental fish nutrition in the coming years.

While many of the nutritional advances made in the food fish industry can be applied to ornamental fish nutrition, there are key differences that must be recognized when attempting to successfully transfer this knowledge into the ornamental field. First, there is not the critical need to optimize growth as there is in the food fish industry. Aquacultured food fish production feeds are formulated based upon costs of the ingredients and not the specific nutrient requirements of the fish for which the feeds are being deigned. While recent trends in feed formulation for food fish production are beginning to take into account factors such as water quality and digestibility, in the past, these issues have often gone ignored in the desire for producing a cost-effective feed that promotes adequate growth and health while minimizing costs of the feed through the use of least-cost formulation.

Conversely, nutrition for tropical ornamentals is not so focused upon growth rates, but rather health, coloration, digestibility and water quality. Due to the culture environment of ornamental fish tanks, there is a high level of sensitivity in terms of the production of waste from uneaten or undigested feed which can lead to water clarity issues, or worse, water quality issues related to ammonia, nitrite and nitrate buildup. Ornamental fish need diets optimized for health, fin quality and coloration, and as such should be formulated differently than a typical commercial production aquafeed for food fish production. However, despite the high monetary value of the ornamental fish trade, there simply has not been a focused effort to accurately define optimal diets for these fish species. This chapter will review basic nutritional components for fish, with an emphasis on the unique nutritional requirements of ornamental fish feed for both growout and broodstock diets and the use of probiotics to enhance growth and health of marine ornamentals.

10.2 Nutritional Components

10.2.1 Complete Feeds

Feeds that provide all the necessary nutrients in the correct combinations and concentrations are commonly called complete feeds. Over the past several decades, aquafeed formulations have moved from those manufactured utilized steam pelleting processes to that of cooking extrusion. Extrusion of aquafeeds is advantageous for several reasons. First, the cooking aspect of extrusion allows for the gelatinization of starches that are

present in the formulation. This promotes better digestibility and utilization of the carbohydrate fractions of the feed, and also allows for the production of a floating feed, which in many cases, is more desirable. Extrusion can also produce a sinking feed when the situation requires, while still promoting the gelatinization of starches. Complete feeds are formulated to provide the macro- and micronutrients required by fish. Macronutrients include protein, lipid, carbohydrates, while micronutrients include the vitamins, minerals, antioxidants.

10.2.2 Proteins

Dietary proteins are often the most expensive component of aquafeeds, and the need for high quality, highly digestible protein sources in feeds designed for ornamental fish dictate that optimal feed formulations concentrate on this macronutrient. Proteins supply amino acids to growing fish, and the inadequate level of even a single amino acid in dietary amino acid patterns, as supplied by proteins, will limit protein synthesis and ultimately inhibit the potential contribution of other amino acids to protein growth of animals (Cole & Van Lunen, 1994). Although over 200 amino acids occur in nature, only about 20 amino acids are common. Of these, 10 are essential (indispensable) amino acids that cannot be synthesized by fish, and thus must be supplied by the diet. While this is an absolute critical focal point in food fish production diets due to the emphasis on growth and feed conversion, in ornamental fish nutrition, the focus is more on high quality protein sources that possess the highest digestibility values which encourage maximal nutrient assimilation and thus significant reductions in waste products, particularly nitrogenous and fecal wastes that can detrimentally impact optimal water quality conditions.

Dietary protein sources in food fish nutrition have historically been dominated by fish meals produced from the reduction fisheries. In the past decade, there has been a dramatic shift away from fish meal-based diets towards more sustainable formulations that replace fish meal with protein sources such as poultry by-product meal, plant-based proteins such as soybean meal and soy protein concentrates, as well as novel protein sources such has mixed nut meals and even some algal meals. Additionally, cost of production feeds for commercial aquaculture is a tremendous driver of formulations due to the fact that feed costs represent over 50–60% of the cost of production for aquacultured food fish. Due to the lack of this constraint in ornamental fish, mainly because of their high selling price (Sales & Janssens, 2003), as well as the fact that ornamental fish consume much less feed than farmed fish (Santamaria & Santamaria, 2011), dietary formulations for ornamental fish can utilize the very highest quality of ingredients, many of which are simply too expensive for farmed fish production diets. The use of such ingredients, including, but not limited to high quality fish meals, fish hydrolysates, squid meals, krill meals, and algae such as *Spirulina*, can be combined to produce the highest quality feeds that provide exceptional health, coloration and digestibility for ornamental fish species.

Protein levels in feeds designed for ornamental fish generally range from 32–40% crude protein. Protein requirements usually are lower for herbivorous fish (plant eating) and omnivorous fish (plant-animal eaters) than they are for piscivorous (fish-eating) fish. Protein requirements generally are higher for smaller fish. As fish grow larger, their protein requirements usually decrease.

10.2.3 Lipids

Lipids (fats) are high-energy nutrients that can be utilized to partially spare (substitute for) protein in aquaculture production feeds. Lipids supply about twice the energy as proteins and carbohydrates. Lipids typically comprise about 10–15% of food fish production diets, supply essential fatty acids (EFA) and serve as transporters for fat-soluble vitamins.

Simple lipids include fatty acids and triacylglycerols. Fish typically require fatty acids of the *n*-3 and *n*-6 families. Fatty acids can be: (a) saturated fatty acids (no double bonds); (b) polyunsaturated fatty acids (>2 double bonds); or (c) highly unsaturated fatty acids (HUFA; >4 double bonds). Marine fish oils are naturally high (>30%) in *n*-3 HUFA, and are excellent sources of lipids for the manufacture of fish diets. Lipids from these marine oils also can have beneficial effects on human cardiovascular health.

Marine fish typically require *n*-3 HUFA for optimal growth and health, usually in quantities ranging from 0.5–2.0% of dry diet. The two major EFA of this group are eicosapentaenoic acid (EPA: 20:5*n*-3) and docosahexaenoic acid (DHA: 22:6*n*-3). Freshwater fish do not require the long chain HUFA, but often require an 18 carbon *n*-3 fatty acid, linolenic acid (18:3-*n*-3), in quantities ranging from 0.5 to 1.5% of dry diet. This fatty acid cannot be produced by freshwater fish and must be supplied in the diet. Many freshwater fish can take this fatty acid, and through enzyme systems elongate (add carbon atoms) to the hydrocarbon chain, and then further desaturate (add double bonds) to this longer hydrocarbon chain. Through these enzyme systems, freshwater fish can manufacture the longer chain n-3 HUFA, EPA and DHA, which are necessary for other metabolic functions and as cellular membrane components. Marine fish typically do not possess such elongation and desaturation enzyme systems, and require long chain *n*-3 HUFA in their diets. Other fish species, such as tilapia, require fatty acids of the *n*-6 family, while still others, such as carp or eels, require a combination of *n*-3 and *n*-6 fatty acids.

While aquafeeds designed for farmed food fish often contain a mixture of lipids from several sources, including fish oils, soybean oil, and poultry fats, these mixtures are often utilized as cost-cutting measures to reduce the cost of production feeds, and not specifically for any particular nutritional requirement. Fish oils, which contain the required *n*-3 fatty acids for marine ornamentals, as well as provide for enhanced palatability and attraction for fish that might not regularly accept prepared feeds, should dominate the lipid composition in diets designed for ornamental fish. Lipids take on an important role in feeds designed for ornamental fish due to their role in the transport of fat soluble vitamins, some antioxidants, and pigments such as carotenoids that can be utilized to enhance color in some species of ornamental fish.

Lipid levels in food fish production feeds often range from 15–32% depending upon species, often due to their potential protein-sparing effects that can reduce the cost of production feeds while promoting excellent growth and feed conversion ratio values. However, feeds designed for ornamental fish need not be so elevated, and should range from 8–12% total lipid, depending upon the species. These lower lipid diets will still satisfy EFA requirements, as well as provide a vector for the transport of fat soluble vitamins and coloration enhancers such as carotenoids, but ultimately will promote healthier fish, and most likely, longer life, due to the reduced lipid accumulation in the peritoneal cavity, and more importantly, the liver.

10.2.4 Vitamins

Vitamins are organic compounds necessary for normal fish growth and health. They often are not synthesized by fish, and therefore must be supplied in the diet. The two groups of vitamins are water-soluble and fat-soluble. Water-soluble vitamins include: the B vitamins, choline, inositol, folic acid, pantothenic acid, biotin and ascorbic acid (vitamin C). Of these, vitamin C probably is the most important because it is a powerful antioxidant and assists in the immune system in fish. Vasudhevan & James (2011) investigated the role of vitamin C in an interaction with *Spirulina* on coloration in the common goldfish (*Carrassius auratus*) and reported that combinations of *Spirulina* and vitamin C levels significantly enhanced the coloration in muscle, skin and fins of *C. auratus*. Additionally, Mustafa *et al*. (1994) observed that the co-feeding of *Spirulina* and vitamin C improved the coloration in red seabream *Pagrus major*. It appears as if vitamin C can play a major role in ornamental fish nutrition, not only as an antioxidant, but also as a coloration maintenance/promoter role as well.

The fat-soluble vitamins include the A vitamins (retinols/carotenoids; responsible for vision/antioxidants and coloration), the D vitamins (cholecaciferols; bone integrity); E vitamins (tocopherols; antioxidants), and K vitamins (menadione; blood clotting, skin integrity). Of these, vitamin E receives the most attention for its important role as an antioxidant. Deficiency of each vitamin has certain specific symptoms, but reduced growth is the most common symptom of any vitamin deficiency. In ornamental fish feeds, the A vitamins, which include the carotenoids, can be particularly important, not only from an antioxidant role (Burton, 1989), but also from a color enhancement role. This will be discussed in more detail in a later section.

Current vitamin premixes often utilized in food fish production diets are most likely adequate for ornamental fish. However, as vitamins are very expensive, and as discussed earlier, food fish production diets are least-cost formulated, with particular attention being paid to higher cost ingredients such as vitamins, new premixes which focus on antioxidants and coloration enhancers should be investigated and implemented in feeds designed for ornamental fish.

10.2.5 Minerals

Minerals are inorganic elements necessary in the diet for normal body functions. They can be divided into two groups (macro-minerals and micro-minerals) based on the quantity required in the diet and the amount present in fish. Common macro-minerals are sodium, chloride, potassium and phosphorous. These minerals regulate osmotic balance and aid in bone formation and integrity.

Micro-minerals (trace minerals) are required in small amounts as components in enzyme and hormone systems. Common trace minerals are copper, chromium, iodine, zinc and selenium. Fish can absorb many minerals directly from the water through their gills and skin, allowing them to compensate to some extent for mineral deficiencies in their diet.

Current mineral premixes utilized in food fish production diets are adequate for ornamental fish aquafeeds, although the use of chelated minerals, which promote better absorption, but are more costly, should be investigated for future formulations designed for ornamental fish.

10.2.6 Carotenoids (Coloration)

A major problem plaguing aquaculture of ornamental fish, other than technical difficulties with respect to larval rearing, relates to the challenge of producing individual animals that accurately replicate the natural colors of these fish when maintained in home aquaria. Farmed ornamental marine fish are often poor in coloration which is an important factor considered by the consumer, who places high value on this aspect of the aquarium hobby.

Fish use carotenoids, one of the most important groups of natural pigments, for pigmentation of their skin and flesh. Carotenoids commonly occurring naturally in freshwater food sources include β-carotene, lutein, taraxanthin, astaxanthin, tunaxanthin, α-, β-doradexanthins, and zeaxanthin (NRC, 1993). Various synthetic pigments (β-carotene, canthaxanthin, zeaxanthin, and astaxanthin) and natural sources have been used as dietary supplements to enhance the pigmentation of fish and crustaceans (Shahidi & Brown, 1998; Kalinowski *et al*., 2005). Natural sources of carotenoids are usually composed of several carotenoids in various forms, and these sources vary in their digestibility, making their pigmentation efficiency complicated to interpret. In contrast, studies can clearly determine the pigmentation efficiency of synthetic carotenoids (which are always a single carotenoid). There is speculation as to the efficacy of natural versus synthetic pigmentation products, but research to date has been inconclusive.

Synthetic astaxanthin and cantaxanthin have been widely used as dietary supplements for salmonids to induce the typical pink skin and flesh color, with astaxanthin being a predominant choice within the salmonid industry (Choubert & Storebakken, 1989). Other pigments have been investigated, but have had less penetration into the commercial aquaculture industry. For example, lutein, found naturally in marigold meal, is also an effective pigment for inducing the orange coloration of goldfish (Mela *et al*., 2002) and is now used commercially in ornamental fish feed.

Previous studies with clownfish (*Amphiprion ocellaris*) have demonstrated that their color can be enhanced using 25–35 mg kg^{-1} dietary astaxanthin over a 2-week period (Ako & Tamura, 1999), while more recent studies with another species, *Premnas biaculeatus* indicated that a higher concentration of astaxanthin (>200 ppm) was necessary to promote adequate coloration in this particular species (Ho *et al*., 2013). Similarly, *Spirulina* algae have also been successfully included in diets to enhance color in clownfish and the ornamental dwarf cichlid *Microgeophagus ramirezi* has been brightened using plant carotenoids (Ako *et al*., 1999).

10.2.7 *Spirulina*

Spirulina is a type of blue-green algae that is rich in protein, vitamins, minerals, carotenoids, and antioxidants that can help protect cells from damage. It contains multiple nutrients, including B complex vitamins, beta-carotene, vitamin E, manganese, zinc, copper, iron, selenium, and gamma linolenic acid (an EFA). Spirulina is available commercially for incorporation into aquafeeds, although its current price is too expensive for food fish production diets. Sun *et al*. (2012) demonstrated that growth and feed utilization parameters were not markedly improved by any pigments except *Spirulina platensis* when investigating various natural and synthetic pigments in diets for the Showa koi (*Cyprinus carpio*). This microalga has been identified as a potential protein source for animal feeds owing to its high protein content and the presence of essential

amino acids, vitamins and minerals. In addition, this type of microalga has been reported to have no cell wall, which results in improved digestion and absorption (Becker & Venkataraman, 1984). Improvements in the growth of fish by the dietary inclusion of *Spirulina* has been reported earlier in a number of studies (Nakazoe *et al*., 1986; Mustafa *et al*., 1994). Additionally, Showa koi fed various pigments showed overall better color enhancement with diets containing *Spirulina* and synthetic astaxanthin (Sun *et al*., 2012). By all indications, addition of *Spirulina* to diets of ornamentals not only increases growth, but also has positive impacts on coloration.

10.3 Broodstock Nutrition

The viability of aquaculture, as in any production-based industry is reliant on the ability to maximize efficiency of production. The focus of much of the research aimed at maximizing production efficiency in aquaculture has been centered on larval rearing and growout. Although egg quality has been examined and defined extensively (Kjorsvik *et al*., 1990, Kjorsvik, 1994, Brooks *et al*., 1997, Lubzens *et al*., 2010) factors affecting egg quality, including broodstock nutrition have been less well studied (Kjorsvik *et al*., 1990). One reason for the relative scarcity of research on this topic may be that larval diets and rearing protocols are often viewed as the primary bottleneck for bringing new species into production and for maximizing survivorship among species already being cultured (Houde, 1973). Therefore, these topics warrant most of the attention in efforts to advance aquaculture technology. There is also a common perception among aquarists and aquaculturists that if the brood fish are spawning, broodstock conditions and nutrition must be nearly perfect (Gardner, 2014). Another and perhaps simpler explanation for the disparity is that cohorts in larval and growout systems typically consist of large numbers of individuals allowing for robust sample sizes in experiments, whereas numbers of broodstock animals in most facilities are relatively small. There is a growing body of evidence supporting the importance of broodstock condition to the survival and health of eggs and offspring (Izquierdo *et al*., 2001; Watanabe *et al*., 2003) underscoring the need for more research in this area.

10.3.1 Spawn Quality

Criteria for assessing egg quality include fecundity (number of eggs produced per unit of fish mass per spawning event, or per spawning season), egg transparency, buoyancy for pelagic spawners (Brooks *et al*., 1997), although the eggs' ability to float does not correlate with viability in some species (Bromage, 1995; Gardner, 2014), morphological characteristics of larvae (Kjorsvik, 1994), cell symmetry at early stages of development (Kjorsvik *et al*., 1990; Bromage, 1995), rate of fertilization and hatching, egg size, egg composition; age of female and, survival to specific developmental stages (Kjorsvik *et al*., 1990). However, the only definition of egg quality that really matters to aquaculturists is "the egg's potential to produce viable fry" (Kjorsvik *et al*., 1990).

Factors affecting the potential for fertilized eggs to produce healthy larvae or fry include water quality, the microbial environment within which fertilization and incubation take place, the quality of the sperm cell, which is determined largely by its genetic make-up and, all of the components, genetic and nutritive, of the egg cell (oocyte).

A mature oocyte is the product of oogenesis, a complex set of biochemical interactions that cause a female germ cell to develop into a ripe egg (Lubzens *et al.*, 2010). The composition of the mature oocyte is dependent on diet and the hormonal state of the female, which may be synchronized by environmental cues such as temperature, photoperiod, lunar cycle, and other exogenous factors, but can also be affected by stress levels (Sumpter *et al.*, 1987) and attempts to induce ovulation and spawning artificially through hormone manipulation (Bromage, 1995). Oogenesis requires the deposition of yolk proteins (vitellogenesis), mRNA, vitamins, hormones, and additional proteins – all coordinated by interactions of the brain-pituitary-gonad endocrine system. Shortfalls in the broodstock diet may affect fecundity or egg viability indirectly by causing an imbalance within this system, or directly by starving it of some biochemical component necessary for egg formation (Izquierdo *et al.*, 2001). Despite the multitude of factors involved in the assembly of healthy gametes, the ultimate source for all of the raw materials required is the food consumed by the parents. Therefore broodstock diet may be the single most important determinant of a zygote's potential to survive and develop.

10.3.2 Feeding and Nutrition

Perhaps the most evident (and easily controlled) aspect of nutrition affecting fecundity and egg viability is the quantity of food offered to the broodstock. Izquierdo *et al.* (2001) cited abundant research demonstrating the negative impacts of food restriction on spawning success. A common tactic in aquaculture is to feed broodstock to satiation or even to excess. This approach necessitates frequent cleaning and optimized filtration in order to combat the deterioration of water quality and microbial blooms associated with heavy feeding and uneaten food. Feeding regimen may be a more worthy consideration than food quantity given the ecology of many of the species targeted for marine ornamental aquaculture. Most of the fish species in the aquarium trade, particularly those in culture, feed at a relatively low trophic level and eat continuously throughout the day. An ideal regimen therefore, should attempt to duplicate their feeding ecology through small frequent feeds.

Considering the broad range of species and families important to the marine ornamental fish trade, and the relative paucity of research into broodstock nutrition, it is difficult to generalize about nutritional requirements. As with all animals, proteins, carbohydrates, lipids, and vitamins are crucial to maintenance and reproduction of marine fishes. Protein, perhaps the most important of these should make up 25–50% of the diet of a typical marine fish (Wittenrich, 2007), although requirements as well as upper tolerance limits for all of these nutrients certainly varies considerably between species.

It is well established that the *n*-3 fatty acids, DHA and EPA, as well as the *n*-6 fatty acid arachidonic acid (AA), are among the most important limiting dietary components for larval marine fishes (Dhert *et al.*, 1990; Copeman *et al.* 2002) and evidence continues to accumulate for the importance of these highly unsaturated fatty acids (HUFA) in broodstock diets to egg quality and larval survivorship (Watanabe & Kiron, 1995; Furuita *et al.*, 2000, 2002; Watanabe & Vassallo-Aggius, 2003). Recent work has shown that the HUFA content of broodstock diets is correlated with larval quality at 3 weeks despite the larvae being fed live prey enriched with a fatty acid emulsion

(Fuiman & Ojanguren, 2011). These findings suggested that the effects of variations in fatty acid composition of eggs were irreversible. Other research (Fuiman & Faulk, 2013) determined that the time frame over which variations in maternal dietary intake of HUFAs translates into changes in egg HUFA content was quite short (2–6 days for red drum *Sciaenops ocellatus* broodstock). Fuiman & Faulk (2014) suggest an approach to feeding broodstock that would include three diets: (1) a non-spawning diet composed of plant-based components used outside of the spawning season; (2) a maturation diet very rich in HUFAs to fortify eggs quickly before spawning; and (3) a spawning diet with somewhat lower levels of HUFAs to maintain egg quality during the spawning season.

Raw or frozen seafood as a broodstock diet has often outperformed commercially prepared pellets (Vassallo-Agius *et al*., 1999; Watanabe & Vassallo-Agius, 2003; Callan *et al*., 2012); however there are advantages to dry pellets that should not be overlooked. Whereas raw fish products can introduce diseases and parasites into marine systems, there is virtually no risk of this when using dry feeds. Furthermore, pelleted feeds represent a greater degree of control over composition and consistency of the diet. Research into the development of pelleted broodstock diets for marine food fish in Japan has led to some encouraging developments (Watanabe & Vassallo-Agius, 2003) and may have implications for the advancement of marine ornamental fish culture. In their exhaustive review of broodstock nutrition research on finfish in Japan, Watanabe & Vassallo-Agius (2003) cite experiments demonstrating that the substitution of cuttlefish or squid meal as a protein source in place of fish meal resulted in improved egg quality. Additional enhancements to broodstock diets resulting in higher egg quality include supplementation with vitamin E at 200 mg/100 g as DL-α-tocopheryl acetate (Watanabe *et al*., 1985, 1991), astaxanthin (Watanabe *et al*., 1991, Verakunpiriya *et al*., 1997), and paprika esters (Vassallo-Agius *et al*., 2002).

10.3.3 Marine Ornamentals Examples

The following is a list of some well-known and/or relevant examples of culture successes with some information about the broodstock diet. Additional information can be garnered from any available source including hobbyist publications, public aquaria, and personal accounts of culture successes, many of which never make it into scientific literature. (Also see an example of ornamental broodstock diet used successfully at UTMSI in Chapter 11, Table 11.1 in this book.)

10.3.3.1 *Amphiprion*

One example of a commercial hatchery diet that has proven successful for anemonefishes at the Instant Ocean Hatchery (IOH) contains beef heart (12.2%), fish roe or eggs (6.1%), fish flesh (6.1%), shrimp tails (6.1%), clams (6.1%), kelp meal (6.1%), krill meal (6.1%), fish meal (6.1%), gelatin (4.9%), water (46.3%), and a vitamin/mineral supplement (0.2%) (Hoff, 1996). At C-Quest hatchery in Puerto Rico, a broodstock diet was modeled after the IOH recipe and was nearly identical except that it also contained squid, conch, lecithin powder and Zeigler Salmon StarterTM. This diet was used in the successful culture of more than 30 species of marine fishes, including dottybacks (*Pseudochromis* spp.), gobies (*Elacatinus* spp., *Tigrigobius* spp., *Gobiosoma* spp.), comets (*Calloplesiops altivelis*), mandarins (*Synchiropus splendidus*) and Banggai cardinalfish (*Pterapogon kauderni*).

10.3.3.2 *Centropyge*

Callan *et al*. (2014) investigated the effects of dietary fatty acid levels on flame angelfish (*Centropyge loricula*) broodstock performance using a formulated diet of Pollock and squid meal with a variety of vitamin, lipid, and mineral supplements. Feeds were formulated with a targeted HUFA ratio of 10:5:1 (DHA:EPA:AA) as suggested by Sargent *et al*. (1999). Three variations of the diet were used, containing total *n*-3 HUFA concentrations ranging from 1.8% to 3.6% dry weight. Angelfish offered the diet highest in total *n*-3 HUFA concentration performed the best in terms of fecundity, fertilization rate, and hatch rate. A control group offered a combination of New Life SpectrumTM pellets and a raw seafood mix, also performed very well by most measures of broodstock condition.

10.3.3.3 *Dunckerocampus*

In the only known culture success with the flame pipefish, *Dunckerocampus baldwini*, the broodstock diet was comprised of frozen *Cyclops* sp., mysid shrimps, live *Artemia* sp. enriched with Dan's FeedTM, and frozen *Spirulina*-enriched *Artemia* sp. (Welsh, 2014).

10.3.3.4 *Genicanthus*

In the only known success in the culture of the extremely valuable angelfish, *Genicanthus personatus*, the broodstock diet consisted of the frozen commercial preparations, LRS Reef Frenzy and Herbivore FrenzyTM, HikariTM *Artemia*, *Spirulina*-enriched *Artemia*, *Mysis*, and bloodworms; locally sourced clams, shrimp, and fish ovaries, dried seaweed (nori), pelleted diets from New Life SpectrumTM and TetraTM, flake foods from TetraTM, Ocean NutritionTM, and OSITM, as well as live *Artemia* and copepods (K. Brittain, pers. comm.).

10.3.3.5 *Hippocampus*

High survivorship has been attained rearing juvenile seahorses from broodstock fed on a combination of the common shore shrimp, *Palaemonetes pugio*, the freshwater mysid, *Mysis relicta*, and shaved frozen squid. Palma *et al*. (2012) investigated the effect enrichment of the broodstock diet of the long snout seahorse, *H. guttulatus*. All seahorses in their experiment were fed Atlantic ditch shrimp, *Palaemonetes varians*. Their three experimental diets consisted of shrimp fresh from the wild, shrimp that were gut-loaded with one large feed of an artificial formulated diet containing fish meal, krill meal, Cyclop-eezeTM, and other vitamin and mineral supplements, and shrimp that were fed this diet for an extended period (10 days). The seahorses that were fed gut-loaded shrimp spawned more frequently, produced more juveniles per spawn, and produced larger juveniles compared to the other two experimental groups.

The adult diet in the only known success in culturing the pygmy seahorse, *Hippocampus bargibanti*, consisted of live and frozen copepods, live *Artemia* nauplii, live and preserved rotifers, preserved eggs and ovarian tissue from oysters, mixed with several species of phytoplankton (M. Wandell, pers. comm.).

10.3.3.6 *Liopropoma*

In the only known success in the captive culture of the reef basses *Liopropoma rubre* and *L. carmabi*, the broodstock was fed a combination of soft pellets by Fauna MarinTM, frozen marine mysids, and MazuriTM gel diet supplemented with locally sourced seafood and fish roe (Gardner, 2012).

10.3.3.7 *Pseudanthias*

In the only known success in the captive culture of an Anthiine species, the broodstock *Pseudanthias squamipinnis*, housed in a 20,000 gallon reef tank at the Long Island Aquarium, was offered a diet of Cyclop-eezeTM, New Life SpectrumTM pellets, and marine mysids (N. Heinsohn, pers. comm.).

10.3.3.8 *Oxymonacanthus*

The broodstock diet in the only known successful culture of the harlequin filefish, *Oxymonacanthus longirostris* included a combination of commercial pellets, flakes, a frozen blend, as well as frozen *Artemia*, mysids, and shrimp (Pederson, 2009).

10.4 Probiotics

The term probiotic is currently used to name live microorganisms that can confer a health benefit to the host by modulating the gut microbiota. The mechanism of action of probiotics has been attributed to the delivery of proteins in the host intestine. The US Food and Drug Administration (FDA) termed the live microbes used in animal feeds "direct-fed microbials".

Most studies on the effects of probiotics have focused on species of interest for aquaculture both freshwater and saltwater species, with limited data available for marine ornamental species. In the last ten years, the introduction of direct fed microbials in aquaculture practice has become very popular (Gatesoupe, 1999; Pintado *et al*., 2011, 2014). Numerous studies have evaluated feasibility and efficacy of the lactic acid bacteria (LAB), *Bacillus* spp., or authochthonous strains (isolated from the fish) as feed additives for warm water fish (Carnevali *et al*., 2004, 2006; Dimitroglou *et al*., 2011; Tapia-Panigua *et al*., 2011) and shrimp species (Kongnum & Hongpattarakere, 2012; Dash *et al*., 2014). Most studies have been performed on larvae and juveniles (Gómez-Gil *et al*., 2011; Pintado *et al*., 2011) and it has been shown that during the initial feeding period, it is possible to establish specific bacteria strains in the fish gut conferring benefits to a number of important fish species including ornamentals and commercial species (Avella *et al*., 2010; Abid *et al*., 2013). The development, maintenance and effective functionality of the intestinal mucosa and gut associated lymphoid tissues (GALT) can in fact be regulated by the use of direct-fed microbials.

The changes in microbial populations associated with probiotic treatment induce disease resistance by stimulating the host immune system and by providing protection against pathogens in the gastrointestinal tract through production of bacteriocins (reviewed by Dimitroglou *et al*., 2011). The microbial changes may improve host digestive function *via* the delivery of exogenous digestive enzymes and vitamins, and improve feed utilization.

The administration of the probiotic *Pediococcus acidilactici* and short chain fructooligosaccharides (scFOS) to Atlantic salmon (*Salmo salar* L.) improved intestinal health, induced a decrease in the total bacterial in the anterior and posterior mucosa and posterior digesta. The microbial community diversity and richness were significantly higher in the anterior digesta of the fish treated with probiotics than the control. At the systemic level, serum lysozyme activity was significantly higher in treated fish (Abid *et al*., 2013).

Effects on gene expression, lipid metabolism (Falcinelli *et al*., 2015), larval survival (Avella *et al*., 2010), gut morphology and modulation of gut microbiota have been well-documented (Abid *et al*., 2013; Falcinelli *et al*., 2015). Probiotics have the potential to

elevate the innate defence mechanisms of fish and improve survival following exposure to a specific pathogen when treated with probiotics. In addition, recent studies have shown the probiotic potential to improve welfare and reproductive success (see review by Carnevali *et al*., 2013) and bone formation (Maradonna *et al*., 2013).

10.4.1 Application of Probiotics in Ornamental Fish

Among the lactic acid bacteria (LAB), *Lactobacillus rhamnosus*, a LAB species originally intended for human use, evidenced probiotic properties in teleost inducing clear effects on the fish immune system (Nikoskelainen *et al*., 2003).

Lactobacillus species have been demonstrated to lead to beneficial effects in juvenile fish husbandry (Balcázar *et al*., 2007a, b) as well as in intensive larval rearing, positively affecting survival and growth metabolism as has been reported for sea bass and sea bream (Carnevali *et al*., 2004, 2006). *Lactobacillus* was evaluated with a marine ornamental species, the clownfish *Amphiprion ocellaris*. The probiotic *Lactobacillus rhamnosus* was administered twice a day (at 8.00 am and 5.00 pm) *via* live prey, rotifers and *Artemia* nauplii (10 rotifers/mL and 5 nauplii/mL respectively). The live preys were incubated for 15 minutes in the probiotic re-suspended solution (10^6 CFU mL^{-1} of marine water). In one additional group probiotics were administered via live prey and via water.

The presence of *L. rhamnosus* in the larval fish digestive tract was quantified by real time PCR. The best treatment for *L. rhamnosus* retention was in the group receiving probiotic both *via* live prey and water (Avella *et al*., 2010). Probiotic treatment significantly increased survival (Table 10.1). Survival was measured over two weeks following hatching (ph). A survival of about 60% was observed in the control group. The best result was achieved in the group receiving probiotics via food and water, with a survival rate of 80 ± 3%, while in the group where the probiotic was delivered by live prey, a survival of 70 ± 4% was observed. The higher survival rate observed in the group receiving the probiotic in both water and food, was associated with reduction in mortality that usually occurs at day 3 ph supporting a possible role of the probiotic to improve larval welfare and in turn larval survival.

The larvae and juveniles reared with the probiotic had greater growth rates at 5 dph (days post-hatch) in both groups compared to the control. At 30 days, fish receiving the probiotic *via* live diet and water showed an increase of about three times; the controls did not differ from the group fed with probiotics via live diet (Table 10.1). Standard

Table 10.1 Morphometric changes in clownfish administered with probiotic (PROBIO) at 5 and 30 days post-fertilization (dpf) and survival at 15 dpf. Values are mean ± standard deviation.

Groups	Growth rate (mg) at 5 dpf	Growth rate (mg) at 30 dpf	Standard length (mm) at 5 dpf	Standard length (mm) at 30 dpf	Survival (%) at 15 dpf
Control	5.6 ± 0.1	45.5 ± 0.1	5.8 ± 0.1	11.4 ± 0.1	59 ± 3
PROBIO *via* food and water	18.4 ± 0.1	116.0 ± 0.1	5.7 ± 0.1	11.4 ± 0.1	80 ± 3
PROBIO *via* live diet and water	9.2 ± 0.1	45.6 ± 0.1	5.7 ± 0.1	12.6 ± 0.1	70 ± 4

lengths were not different at 5 dph. Juveniles reared with probiotic delivered by live diet only were significantly longer at 30 dph (Table 10.1).

Similar improvements in growth were also observed in the angelfish *Pterophyllum scalare* when administered different probiotic stains (Monroy Dosta *et al*., 2012). Three different strains of *Bacillus* were isolated from the gut of adult *Pterophyllum scalare* and named Bsp1, Bsp2 and Bsp3. The three strains were administered daily *via* live diet at the rate of 2×10^7 CFU mL^{-1} of each. The analysis of variance for total length indicated that there were significant differences between treatments ($F = 15{,}656$, $df = 4$, $P < 0.005$). The administration of Bsp3 was the most effective and the fish in this group achieved the highest total length (4.5 cm), while the control was 3 cm long. This strain also provided the greatest weight with an average of 1.90 g, compared to the control group where the weights were below 1 g.

The widths of the angel fish, administered the three different strains were not significantly different among treatments ranging from 1.10 to 1.25 cm, but were significantly larger than the control where the size averaged 0.63 cm.

10.4.2 Genes Controlling Fish Growth and Development

Fish growth is mainly linked with muscle growth, a process under the control of the IGF system that includes Insulin-like growth factor I (IGFI), and II (IGFII), and a different number of binding proteins depending on the species. These may act to enhance or attenuate IGF signaling depending on their physiological environment and the cell type. The system is down-regulated by myostatin (MSTN), a member of the transforming growth factor β (TGFβ) superfamily, regulating the inhibition of muscle cells proliferation (Duan, 2010). All these factors are involved in fish myogenesis and have been widely used as markers for muscle growth (Chauvigné *et al*., 2003). The expression of IGFI and IGFII depends on the feeding regime (Montserrat *et al*., 2007); in addition, their levels fluctuate during the organisms developmental phase (Wood *et al*., 2005).

Development is also regulated by alternative pathways involving the nuclear receptor superfamily such as peroxisome proliferator activated receptors (PPARs), Retinoic acid receptors (RARs), and Vitamin D receptors (VDRs). All these receptors are classified as natural nuclear receptors of many organic compounds and are involved in the correct development, growth and pigmentation in numerous marine and freshwater fish species (Fernández *et al*., 2008; Avella *et al*., 2012). Additional receptors including the Vitamin D receptor (VDR) and retinoic acid receptor (RARγ) are also involved in embryo and larval development; the former when bound to its ligand induces bone mineralization and organ remodeling and regulates cell growth and differentiation of numerous tissues. The RARγ is known to be involved in correct cranial development during the early larval developmental stages (Villeneuve *et al*., 2004).

10.4.3 Clownfish Example

Fish development is controlled by numerous genes and real-time PCR performed on clownfish larvae and juveniles administered with *L. rhamnosus* showed an increase of transcription of all genes mentioned above except PPAR alpha at 30 days post-hatching. This suggests a positive role for *L. rhamnosus* administration in the regulation of numerous developmental patterns involved in tissue differentiation and remodelling. Myostatin, involved in the inhibition of myoblast division, was

Table 10.2 Fold changes of gene expression in clownfish at 5 and 30 days post-fertilization (dpf).

	Gene expression fold changes						
Groups	***Igf1***	***Igf2***	***mstn***	***ppar alpha***	***ppar beta***	***vdr alpha***	***rar gamma***
Control 5 dpf	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Control 30 dpf	0.50	1.00	1.00	1.00	1.00	1.00	1.00
PROBIO *via* food and water 5 dpf	11.00	40.00	0.70	58.00	29.00	62.00	68.00
PROBIO *via* food and water 30 dpf	3.00	4.50	0.40	3.00	0.90	78.00	64.00
PROBIO *via* water 5 dpf	2.00	2.00	0.18	7.00	0.70	1.00	1.00
PROBIO *via* water 30 dpf	3.00	0.50	0.42	1.80	0.50	1.00	1.50

significantly reduced in both groups reared with probiotic while the IGF1 and 2, RARs and VDR were largely stimulated. These results support the role of these bacteria in growth rate. Table 10.2 reports the fold changes of the genes analyzed by Avella *et al*. (2010), all of which are involved in clownfish growth and differentiation. In this species, those receiving the probiotic were metamorphosed at 9 dph while in the control it generally occurs at 12 dph.

The faster growth did not alter proper skeletal development in the two groups of the clownfish reared with the probiotic. Macroscopic observations and histological analysis showed a significant reduction in skeletal deformities at 30 dph. In the controls more than 40% of the clownfish exhibited skeletal abnormalities, while in the experimental groups the deformities were reduced to less of 20% in those administered probiotic via diet and water, and about 30% in the group receiving probiotic only via the water. This shows the clear beneficial effects of *L. rhamnosus* on the reduction of skeletal deformities (Avella *et al*., 2010).

Summarizing, the morphometric and molecular results obtained in the clownfish *A. ocellaris* administered with probiotic support powerful effects of *L. rhamnosus* when administered during the early phase of the fish life cycle improving growth in both the larval and juveniles stages. The faster growth that resulted in earlier metamorphosis did not compromise proper development. Although the positive role of *L. rhamnosus* in the culture of marine fish has been proved, this study focused on the ability of the probiotic to modulate the expression of genes involved in larval and juvenile growth and cell differentiation, and provides clear evidence of the mechanistic effects of *L. rhamnosus* during clownfish development.

10.5 Conclusions

There is a great need for further research into the development of optimal dietary formulations for ornamental fish species. The nutritional library of knowledge assembled for the food fish aquaculture industry can be utilized as an excellent foundation to build new diets designed specifically for the ornamental industry. By utilizing this

knowledge and combining it with the specific needs of ornamental fish (i.e. enhanced coloration, higher digestibility for optimal water quality *etc*.), novel formulations that can assist the ornamental fish industry in its vibrancy and growth can be rapidly developed and implemented. This would provide an almost immediate relief to one of the major bottlenecks constraining the continued growth and expansion of the ornamental fish aquaculture industry.

In the absence of more direct evidence, any efforts to design an ideal broodstock diet for a given species can, and should include an examination of the feeding ecology of that species in the wild, as well as an analysis of the composition of eggs collected from the wild. Spawn quality of wild fishes is typically superior to that of captive fishes (Kjorsvik *et al*., 1990; Pavlov *et al*., 2004; F. Baensch, pers. comm.) so it stands to reason that fishes in the wild should be eating the ideal complement of foods necessary for a healthy spawn, and their egg composition should represent an optimal nutritional profile.

The practice of introducing beneficial bacteria, such as probiotics, during the last decade has helped to develop an environmentally-friendly aquaculture practice for relevant ornamental species such as clownfish and possibly in the future for other ornamental species.

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11

Considerations for Developing a Marine Ornamental Hatchery

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Abstract

Constructing and establishing a successful marine ornamental hatchery takes patience, dedication, and hard work. There are countless considerations that need to be addressed prior to starting construction, as well as during normal operation for continued success. New research findings, as well as technological advances, have led to many breakthroughs in the ornamental industry that have helped overcome some of the hurdles that once limited the successful culture of many marine ornamental species. Though there are many things to consider, this chapter focuses on several important key issues that should be considered when planning and designing an ornamental fish hatchery.

Keywords *Hatchery; location; lighting; systems; filtration; diet; marketing*

11.1 Introduction

Whether large or small, sophisticated or basic, automated or largely staffed, no two marine ornamental hatcheries are identical. It is important to remember that regardless of size, there are multiple ways to achieve success. Proper planning is needed so that the unexpected or in some cases, even the expected, does not threaten the livelihood of a facility.

As with much of the information regarding ornamental species aquaculture, many of the considerations for marine ornamental facilities are not necessarily something that are peer reviewed, but rather experienced tales of success or failure from an individual or individuals who have attempted to create a successful marine ornamental hatchery. The successful culture of ornamental species in the last decade and information has increased dramatically (Davin & Brannnet, 2009), and there is no better time than the present to plan and create a hatchery for marine ornamentals.

This chapter focuses on some of the major components that are necessary to create a successful marine ornamental hatchery. In addition to the components discussed here,

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plans for expenses such as equipment, livestock, and operating costs, as well as production, shipping, and sales expenditures, need to be incorporated.

It would be prudent for anyone planning to establish a marine ornamental hatchery to do as much research initially, as well as throughout the entire process of operating a marine ornamental hatchery, as possible. This will increase the likelihood that advances in technology, which could help increase the success of the marine ornamental facility are included.

11.2 Hatchery Location

There are many things to consider when choosing the best location for an ornamental hatchery. In general, an elevated or upstairs location should not be used. The risks associated with the weight of water, as well as extra weight from filtration and other equipment can be quite significant. If a building is selected with a multiple floor option, opt to put offices on the upper floors and culture or grow out space on solid ground. If there is any uncertainly with the robustness of a preexisting facility being converted to an ornamental hatchery, the proper inspection professional should be contacted to assess the situation.

Proximity to an airport is critical since ornamental organisms will need to be received and product shipped out. While methods for transporting organisms have improved considerably over the years, transport is still the most dangerous part of delivering product to the consumer. There are many uncertainties associated with shipping such as courier handling, weather during transit, and delays. All these unpredictable factors precede acclimation upon arrival, which is a stressful process unto itself. Close proximity to an airport reduces the time between organisms leaving the facility and arriving at the hands of the courier responsible for delivery.

11.3 Facility Layout

It is crucial to plan the layout of the facility prior to construction. This includes everything from where to house broodstock, larval rearing space, growout space, shipping and storage areas, to system filtration and water storage reservoirs. After these general areas are defined, more detailed planning will need to occur to design plumbing and filtration. There are firms that can be hired to do such planning, but it does come at an increased cost depending on the complexity of the facility. Fortunately, there are powerful design tools at reasonable costs such as AutoCAD (Autodesk Inc. 2014) and Sketchup (Trimble Navigation Ltd. 2013) that can be used for extremely detailed, digital, and three-dimensional layouts of the planned facility.

11.4 Water Sources

Perhaps the single most critical resource to consider while planning a marine ornamental hatchery is the water source. A successful facility needs a reliable source of seawater as well as freshwater, and reservoirs for each. Estimating daily and emergency water usage

can make for very smooth operations. Emergency supplies of water should be heated to the ideal temperatures of the species being culture and well aerated so that it is readily available for immediate use. It is advisable to overestimate total water usage and back-up supply if room allows. In addition, wastewater from the facility must also be planned. Special permits and licensing may be required before the facility can become operational. Local water treatment facilities and the appropriate environmental agencies must be contacted to ensure compliance with all regulations.

11.4.1 Saltwater

Depending on the proposed location of the hatchery, natural seawater may be an option. Many individuals have had abundant success bringing natural seawater into their facility; however, there are many risks associated with this method of obtaining water. Immediately following collection, organic decomposition will occur and powerful filtration such as foam fractionation or protein skimming, as well as ozone, must be in place to pull organic impurities out of the water. Ultraviolet sterilization or pasteurization may also be necessary to prepare the saltwater for use.

The threat of introducing parasites into a facility is a very real one and has caused extreme frustration among aquaculturists. This threat can be lessened by sterilizing seawater prior to use. One cost effective method is to use a pure form of 65% calcium hypochlorite or pure sodium hypochlorite and sodium thiosulfate or other chlorine neutralizer. An orthotolidine test kit may then be used to detect the presence of chlorine (Moe Jr., 2009). This method is relatively simple and cost-effective, but takes time because chlorine is not neutralized instantly. There are special considerations for using sodium thiosulfate with a municipal water supply, but that will be further discussed in the following section on freshwater. Depending on the volume of water needed at a single time, or during or emergency situations, this method may or may not be applicable for all hatchery settings. It is important to note that if this method is used to recycle seawater, it can significantly lower the pH of the water being recycled, and it will need to be buffered before use.

The alternative to using natural seawater is producing synthetic seawater. This requires a powdered or gel synthetic sea salt premix and a reliable freshwater source. While technically not identical to pure, natural seawater, the quality of synthetic sea salt has improved over the years. Synthetic seawater can sustain marine ornamentals as well if not better than natural seawater in the hatchery setting. Though this can be costly over time, mixing synthetic seawater can consistently provide conditions identical to those found on the reefs where many ornamental species thrive, because they come with essential trace elements and buffers already added in the mixture. Similar results may not be achieved using natural seawater obtained from an inshore location, where it likely has an increased organic load and the increased potential for anthropogenic pollutants.

11.4.2 Freshwater

In most cases, it is unwise to use a municipal water supply or well water as the main source of freshwater for a facility. This is primarily because the quality of these sources of freshwater can fluctuate. A municipal water source typically contains a small amount of chlorine, which can be neutralized with the dechlorination method mentioned above or a standard dechlorinator. However, times have changed, largely due to regulations by

governmental agencies such as the Environmental Protection Agency (EPA), which have called for different methods of treating municipal water prior to use (EPA, 2007). Both ammonia and chlorine, used to treat drinking water lead to the formation of chloramine. Chloramine is more toxic to marine organisms, and requires significantly more sodium thiosulfate to be neutralized (Capuzzo *et al.*, 1977). While there are other chemical methods to neutralize these toxic compounds, they are more complex, requiring more effort to make municipal water safe for aquarium use.

One way to avoid these complications and provide consistently pure water for hatchery use is to invest in a reverse osmosis and deionization unit. These units come in a variety of sizes and are relatively easy to install and maintain. Cartridges and membranes need to be replaced at regular intervals depending on use. This is a wise investment that can save frustrations down the road, and is an absolute necessity if the goal of the ornamental facility includes sensitive ornamental invertebrates, particularly corals.

11.5 Electricity

When planning electricity for a marine ornamental hatchery, it is imperative to calculate minimal energy needs, and then plan for extra to allow room for growth. Certain pieces of equipment will run constantly, and it may be necessary to hire proper licensed personnel to review the plans. All electrical outlets in "wet" space should be placed as close to the ceiling as possible, furthest away from water, and covered. Any outlets that have even the slightest possibility of coming into contact with water, humid air, or a substantial energy load should include Ground Fault Circuit Interrupters (GFCI). It is important to check on local building and electrical regulations in the event that these are required for a facility. In addition, not all GFCI switches are identical. Some may trip at the slightest power interruption and require a manual reset. Caution is needed when connecting life support systems to sensitive GFCI switches such as these, to insure that proper personnel is available to reset them at any time. Lastly, devise a schedule to regularly test GFCI switches for proper operation, and replace them at the first sign of malfunction. Proper electrical planning and use of GFCI switches can help prevent electrical fires or power losses that can be devastating to marine ornamental hatcheries.

Perhaps the most important component of the entire electrical scheme for a marine ornamental facility is having a reliable back-up power supply in the event of a power failure. This can be accomplished in a variety of ways, and will likely need to be substantial for an ornamental hatchery. The installation of a transfer switch is essential if a permanent or portable back-up generator is to be used. A transfer switch can restore power automatically or manually, and there are several options to add such a device to existing electrical systems where needed. If the planned budget does not allow for a permanent back-up generator for the entire facility, a smaller, portable generator and the appropriate transfer switch can still power the back-up of critical life support components. Modularity in terms of back-up generators can definitely help reduce costs. It is also wise to invest in an aquarium monitoring system or controller so that hatchery managers and staff can immediately respond to any power outages or emergency situations.

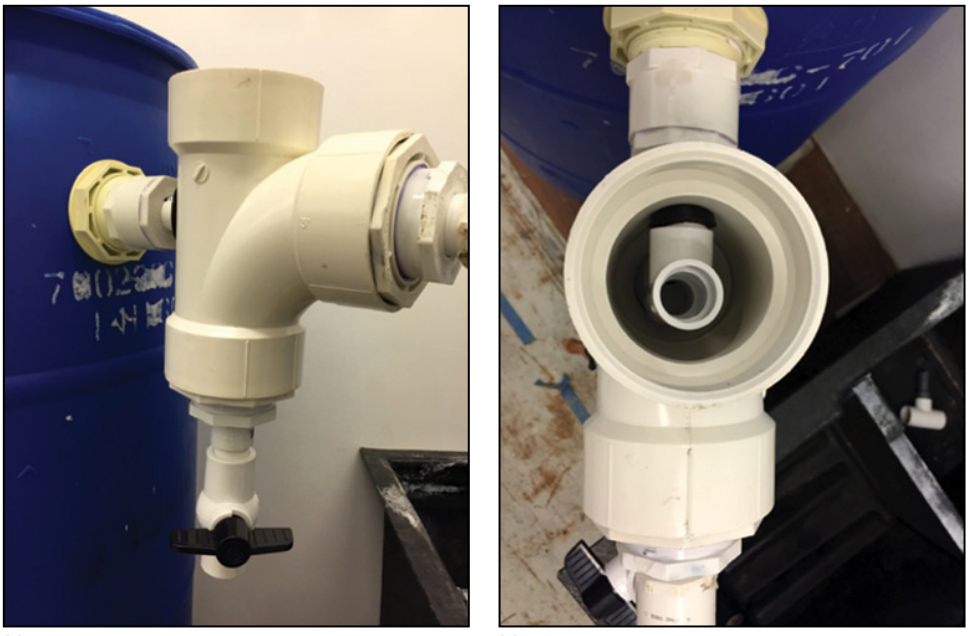
11.6 Lighting

When it comes to lighting design in the hatchery, the lighting requirement of the target ornamental species to be cultured must be taken into account. This is particularly important for sessile or primarily sessile invertebrates such as corals or tridacnid clams (Calfo, 2003). If the primary focus of the facility is marine vertebrates, then lighting the entire room is a cost saving alternative to lighting individual tanks. If the lighting is divided into different circuits, the entire room can be placed on a timer to allow for photoperiod manipulation, and a smaller circuit with a different spectrum of bulb can even provide dawn, dusk, or even lunar lighting, if necessary to induce spawning within a certain ornamental species (Takemura *et al*., 2010). With the exception of corals, traditional fluorescent type lighting is sufficient for most ornamental vertebrate and motile invertebrate species with the addition of additional bulbs of different spectrums to simulate dawn and dusk effects. Selecting the appropriate lighting for an ornamental hatchery should be an area where expenditures can be minimized, thereby saving funds for other areas of the hatchery. The most recent advancement in lighting is the use of the light-emitting diode (LED). Though they can be pricey initially, LEDs provide the best long-term lighting investment due to their low voltage, energy efficiency, longevity, and safety.

11.7 Tanks and Aquariums

There are many options for housing ornamentals within the hatchery, and how a species is housed is often dictated by any specific requirements it may have. Conventional options include glass or acrylic aquariums that work for many smaller marine ornamental species, from broodstock all the way through growout. Glass is a much cheaper and an easily obtainable alternative to acrylic aquariums though they are slightly more delicate. They can be configured in space-saving tiered or rack systems, and even drilled to share a centralized filtration system. If a large number of glass aquariums are needed, many manufactures or retailers often offer high quantities at a reduced or wholesale cost. Using standard sizes will also keep prices on the lower end. This makes working with glass aquariums much more appealing.

For larger scaled applications such as larger ornamental species, fishes that spawn in harems, or ornamentals that need greater depth for spawning such as pygmy angelfishes, wrasses, drums, and seahorses (Davin & Brannet, 2009), fiberglass, or unconventional polyethylene tanks have been successfully used to breed and rear marine ornamentals. These tanks are often modified with viewing windows for observation of the species within. Regardless of the how they are housed, some ornamentals will require special needs; modifications often need to be made to allow fry to separate from adults without being harmed or cannibalized. A fine example of a species-specific rearing system that increased survival rates and improved overall growth for ornamental decapods is seen in Calado *et al*. (2007). Figure 11.1 shows an egg collector for a system designed for pelagic spawners used at Kubecka Aquaculture that can be modified to accommodate a wide variety of ornamental pelagic spawners. Lastly, do not underestimate the ability of any marine ornamental species to propel itself from its tank to the



(a) (b)

Figure 11.1 (a) Lateral view of an egg collector designed to collect and separate eggs from ornamental pelagic spawners. (b) Dorsal view of an egg collector designed to collect and separate eggs from ornamental pelagic spawners.

floor. While this may not be the case during larval rearing, selecting an appropriate lid for all broodstock and growout systems that also allows feeding can reduce fish loss.

11.7.1 Broodstock Setup

Broodstock setups can vary tremendously between the culture of vertebrates and motile invertebrates. Specific requirements of a species need to be taken into consideration including recreating a habitat that allows the organisms to exist peacefully in captivity, without constant interruption, so that they are comfortable enough to breed. In general, a brood tank, regardless of the material, should have opaque sides (including the bottom when tanks are stacked) with the exception of one side panel or window for viewing. This will allow for regular observation, assist in tank maintenance, and assist in reducing unwanted algal growth due to overexposure to light. It also assists in creating a refuge environment for ornamental species within. In addition, the setup should be large enough for the organisms to coexist without recurrent aggression and to successfully perform spawning behaviours.

The subject of tank décor inside a brood tank varies amongst aquaculturists. For example, many species of clownfish (*Amphiprion* spp. and *Premnas biaculeatus*) do not need an anemone or substrate to thrive and reproduce in captivity. They do, however, need a suitable substrate such as ceramic tiles, terracotta pots, PVC pipe, or PVC fittings to spawn and lay eggs on (Wilkerson, 2001). Conversely, it has been proposed that the presence of an anemone is essential, claiming that success is more likely when

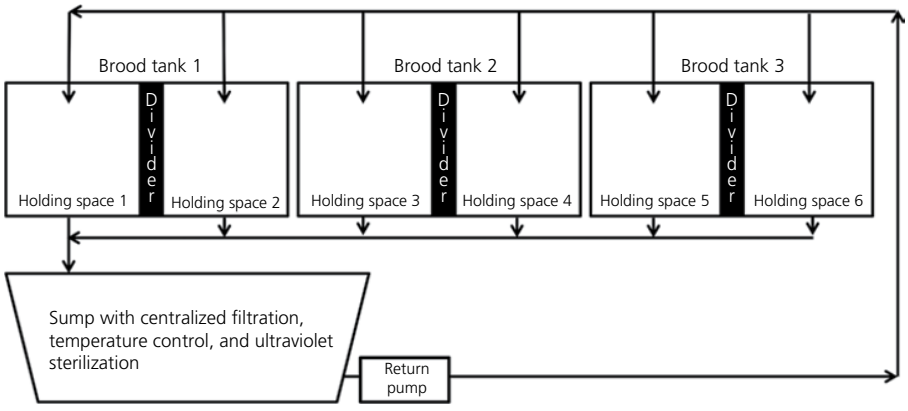


Figure 11.2 Line diagram of an ornamental recirculating system designed to accommodate increased housing capacity of broodstock ornamentals in limited space with the use dividers and shared filtration.

environments are identical to those where clownfish spawn in the wild. Given that success has been achieved under both scenarios, the decision is up to the individual, and may largely depend on budget. If a marine ornamental hatchery is diversified and successful enough to produce clownfish and invertebrates such as anemones, then this option may become more feasible. Furthermore, it may lead to an increased appeal to consumers if a hatchery can offer clownfish already paired with an anemone, thereby increasing profit. With other species, the same two ideas hold true, and minimizing décor inside the brood tank allows for a simplified maintenance routine, though it is often necessary to provide objects that are easily removed, cleaned, and replaced so that the ornamentals inside may seek out a more secluded environment when desired.

If tank space for broodstock is limited, dividing a tank into separate housing spaces is a viable option as long as the tank being divided is partitioned into appropriate sized segments and filtration is appropriate for each segment. This can sometimes be a cost-effective way to increase holding space for brood populations of ornamentals. Appropriate dividers may consist of a wide variety of materials such as acrylic, fiberglass, plastic, or even dense, rigid foam such as Poret® (EMW Filtertechnik GmbH/Swiss Tropicals, LLC© 2006–2014), and should be permeable to allow water movement across it. Choosing this method allows increased versatility and flexibility in configuring limited tank space to accommodate broodstock and is illustrated in Figure 11.2.

One last consideration for broodstock tanks is the ability to remove eggs or larvae from the tank. This is based on whether the ornamentals in culture are demersal or pelagic spawners or mouthbrooders. For demersal spawners, the broodstock setup is relatively simple and has been mastered by many. The only requirement here is that after incubation by the parents, the eggs be removed to a separate rearing chamber prior to hatching as documented in Davin & Brannet (2009). Accommodating pelagic spawners can prove to be more difficult; tanks must be designed so that eggs are easily accessible. This can be achieved by allowing the buoyant fertilized eggs to delicately flow into a collection chamber or mesh bag (Moe Jr. 2009; also see Chapter 15, Figure 15.1 in this book). In many cases, a simple surface drain that

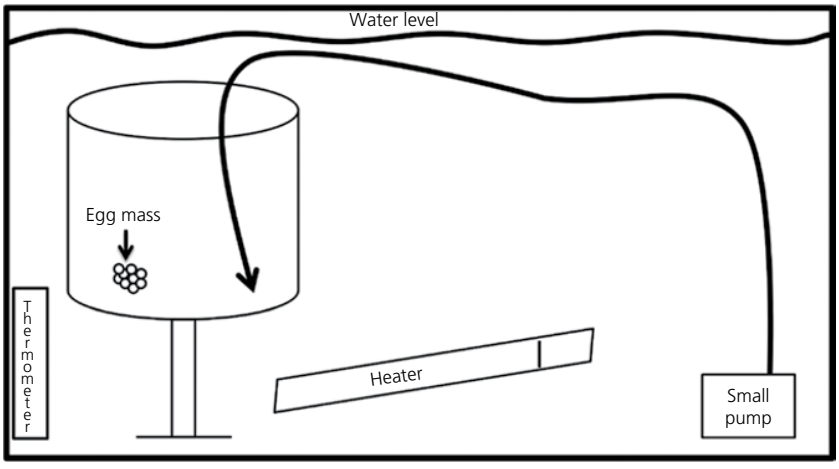


Figure 11.3 Line diagram of a tank setup used to incubate and hatch egg masses of mouth brooding ornamentals. The pump gently circulates water into the specialized container, which mimics parental care by delicately tumbling and aerating the egg mass until the eggs are ready to hatch. An appropriately sized screen divider would need to be used to protect newly hatched larvae from being sucked into the pump intake and will vary by species.

feeds into the main filtration line of the system is sufficient. The mesh bag or container may then be removed or drained to allow the eggs to be transported to a larval rearing tank. The size of the mesh bag is dependent on the size of the egg, and it is imperative that the eggs are in well-aerated water until they can be harvested.

The eggs of mouthbrooders such as fishes from the families, Apogonidae, and Opistognathidae (Thresher, 1984), may require special care if the mouth brooding parent does not hold the larvae full term. In the event that the larval egg mass is released before hatching, the eggs must gently be collected and placed in an apparatus such as a McDonald hatching jar or egg tumbler. These devices can be rather expensive, but can be constructed of low cost materials with a little planning and ingenuity. A proper hatching apparatus will mimic the parental care of the mouth brooding parent by gently tumbling and aerating the egg mass, which allows the larvae to complete incubation as shown in Figure 11.3. Juveniles should then be released from the hatching apparatus into the growout tank after all pump intakes have been blocked off or removed.

Some invertebrates, such as shrimp of the genus *Lysmata,* release live larvae at dusk or over periods of darkness. The parental *Lysmata* are often quite cannibalistic and can consume the newly hatched larvae if not removed in time. But, relocating a parent prior to hatch can be extremely detrimental to the larvae. These stress-inducing actions can cause the eggs to be released prematurely or even resorbed (Montalvo, unpubl. observ.). For situations like this, brood tank design must be such that as newly hatched larvae are released; they are gently carried by flow into a safe haven in the brood tank. This allows the aquaculturists to collect the larvae and transport them to a larval rearing chamber.

11.7.2 Growout Setup

Failing to properly plan the growout portion of a marine ornamental hatchery can be extremely detrimental to the success of the facility. A growout space that is too small can create a "bottleneck" scenario with successful and consistent spawning of

broodstock. In addition, if the growout section of a facility is initially too large, it may consume an excessive part of the initial budget that would have been better utilized elsewhere. The growout space can be estimated for different phases based on the maximum amount that the planned broodstock can produce. Those phases consist of, but are not limited to, larval rearing, juvenile to adult growout, and market-ready product. When designing systems for growout, the life stages of a species must be considered. Tank materials will vary and may be composed of similar components as the brood systems as long as they are cleaned and sterile, particularly in the case of larvae. Tanks in larval rearing systems are typically smaller. This serves to condense live food items, minimizing cost and efforts in producing first feeds and their enrichments. In addition, flow and aeration are set at a minimum or even ceased so that larvae are not damaged during the extremely delicate development preceding metamorphosis. After metamorphosis, juveniles should be transferred to holding systems where they will continue to feed and grow until they reach market size. During this portion of growout, systems should be large enough to accommodate entire spawns and allow for any aggression that may occur in individuals. Keeping spawns together and organized by age will assist in facilitating documentation and will serve to keep genetic populations distinct, which can be useful in preserving genetic diversity within a facility. If preserving genetic diversity is not a priority, then groups of similar ages may be combined to create more space as long as aggression is not an issue and the filtration capacity of the growout system is not overloaded. Consolidating specimens in this way may also simplify maintenance tasks.

11.8 Diet and Nutrition

Proper feeding and nutrition should not be overlooked when attempting to rear ornamentals. To feed only live and natural prey items at each stage of the production processes is generally impractical. There are many commercially available feeding options that may provide adequate nutrition to induce spawning in brood fish and successfully rear their offspring; however, this largely depends on the species and its specific dietary and feeding requirements. In general, most ornamental species in captivity will accept frozen natural foods that have been cultured or collected, frozen, packaged, and made readily available for aquarists and hatcheries alike. Most of these diets are available in raw forms often requiring thawing and enriching with vitamins and highly unsaturated fatty acids (HUFAs) to meet the nutritional demands of broodstock to induce spawning or maintain optimal health before feeding.

In the hatchery setting, it may be beneficial to create a custom diet for broodstock that ideally accommodates all or a majority of the species in culture by fulfilling their feeding and nutritional requirements. Though it is often more time intensive to create, this is a convenient way to include all the necessary components and feed a single nutritionally complete serving each feeding. An example of this type of diet is shown in Table 11.1.

Throughout the larval rearing process, live first feed items are needed. The type of live feed required is largely dependent on the jaw gape of the larvae, and whether it is small enough to be ingested by the larvae. Such organisms such as rotifers and *Artemia* (brine shrimp) are easily cultured at high densities in the hatchery setting, but need to be enriched

Table 11.1 An example of a successful ornamental broodstock diet used by the author at The Fisheries and Mariculture Laboratory at The University at Austin Marine Science Institute.

Ornamental feed formulation ingredient	%
Fresh Fish Fillet	33.00
Squid Muscle	7.50
Shrimp Tail Meat	33.00
NuPro	7.00
Cyclopeeze	1.50
Krill Meal	3.00
Fish Oil	1.50
Lecithin	2.00
Dextrin	2.00
Vitamin Pre-Mix	1.00
Stay-C	0.05
Astaxanthin	0.03
BioMos	0.50
Phosphotydal Choline	0.03
Gelatin	7.89
Total	100.00

or "gut loaded" prior to feeding to meet the complex nutritional requirements of developing larvae. In some cases, rotifers and newly hatched *Artemia* may still be too large for some larvae to consume. Copepod nauplii or ciliates may be required in such cases. For hatcheries in close proximity to seawater, it is often possible to collect copepods from the wild with the proper sized plankton nets. Copepod culture is far more time intensive, and copepods require the use of live plankton (microalgae) for feeding and successful culture. More recent advances in copepod culture in the ornamental industry have led to the option of purchasing starter cultures of various species of copepods. This fairly new and readily available source of copepods along with advancements in culture techniques may lead to significant advancement in the culture of marine ornamentals that were previously limited by improperly sized first food items. As larvae increase in size they can be weaned onto a readily available and nutritionally sufficient commercial pellet such as Top Dressed Otohime Chroma Boost™ (AP BREED/ Reed Mariculture, Campbell, CA, USA).

11.9 Quarantine

A successful marine ornamental hatchery is not complete without an appropriate quarantine area. This is often ignored and is quite regrettable when such areas are needed but not in place. It is unlikely that a facility will not encounter diseased

ornamentals at some point during the operation, and the best weapon against such scenarios is to be prepared to medicate and observe those affected individuals isolated from healthy individuals. A single sick ornamental specimen can devastate an entire system or wipe out entire populations during any stage of production. Within a quarantine system, it is beneficial to employ the use of UV sterilization as well as the appropriate medications to treat infected specimens (see Chapter 19).

11.10 Filtration

When it comes to filtration for the hatchery, the biggest planning hurdle is typically deciding between shared or individual filtration of tanks. Both methods have their benefits and drawbacks. Constructing tanks with individual filtration may appear to be more costly initially; however, the risk of spreading pathogens is significantly reduced, thereby reducing costs in ornamental loss as well as medications needed for treatment. An additional benefit of independent tank filtration is that the filters used in these setups are often small, modular, and easily removed. This makes regular maintenance or replacement relatively simple in the event of failure. Similar to purchasing a large quantity of aquariums at a reduced or wholesale cost, many manufacturers or retailers also offer discounts on high quantities of small and independent filters, which may make this option more appealing to some.

When constructing tanks with shared or centralized filtration, the primary benefit is increased system volume. This increase in system volume allows for a more stable environment, and may reduce costs because all components of the filtration are shared in a reservoir commonly termed "sump". Another substantial benefit is that more powerful and more efficient filtration such as a protein skimmer may be used. In comparison, providing each standalone system with a protein skimmer of its own would be very expensive. Here, a single piece of filtration, though seemingly costly, with increased capacity, can be used across all tanks in the system. The same applies for pumps, heaters, and any other pieces of filtration that are deemed necessary for the system. Furthermore, performing maintenance tasks such as water changes, adding water to compensate for evaporation, or replacing filtration media are simplified, as this only needs to be performed in one centralized location within the system. The biggest drawback when choosing this means of filtration is that when a component fails, it fails for every single tank connected to the system. In addition, replacing individual components on a centralized system may seem more costly; however, this is due to the fact that they are more powerful and suited for larger volumes. These costs may still appeal when compared to prices and effort associated with replacing multiple small filtration units in individual systems. It is also important to remember that in the event of a power loss, the potential of water to overflow due to gravity with such systems is increased. When plumbing, utilize inline check valves and ensure that the sump is large enough to accommodate the appropriate filtration as well any water that may accumulate if power is lost.

There are a variety of filtration options that can be utilized in the hatchery setting and describing all available filtration options would be a chapter in itself. It is important to research all filtration options because advances in the industry are ongoing. Regardless of the filtration methods utilized, the key to successful filtration is nutrient export. A common mistake many aquaculturists make is to place too much faith in the ability of

their filtration to take care of water quality within systems. Filtration is not a substitute for proper husbandry and together, proper husbandry along with adequately planned filtration can lead to a very successful ornamental aquaculture operation.

11.11 Market Assessment

Prior to selecting species for culture, it is essential to investigate the market for the ornamental species of interest. Completion of a Strengths, Weaknesses, Opportunities, and Threats (SWOT) analysis of the ornamental operation may provide valuable insight towards establishing a successful ornamental hatchery (Houben *et al.*, 2003). The SWOT analysis will provide information on the competition of local and regional markets, seasonal availability of a particular species, fluctuations in availability of a species, as well as what species are already being mass-produced and potentially saturating the market. Any species successfully spawned and reared in captivity typically commands a higher price as it is better suited to life in captivity. It is important not to underprice product as it can damage the market for a particular species by saturating it.

Market assessment should even include trips to local fish stores and online retailers. The odds are that a small ornamental hatchery operation will almost always be secondary or tertiary in supplying their product within the industry, but there is still a place for new marine ornamental hatcheries large and small. Careful planning, and market assessment, may only justify starting an operation on a rather small scale, and that should not be discouraging. There is always room to grow.

It is common to select species that complement each other in terms of spawning and rearing; however, it is not essential. This is the current formula for most large-scale hatcheries producing clownfish, gobies, dottybacks, etc. which produce large quantities of captive reared ornamentals. This leaves several open niches for ornamental decapods, gastropods, echinoderms, angelfish, cardinalfish, seahorses, etc. While the systems and personnel required to culture such a variety of species may be more intensive and costly, the fact that the market is not saturated with aquacultured species of these varieties may prove lucrative for those willing to do the extra research and investment.

Also important in this pre-assessment is identifying who will purchase your product, and how much can they be expected to purchase. Think about the proposed plan of production, determine whether more buyers need to be identified, and seek them out. If success is measured by profitability, as is typically the case, then retaining an abundance of captive reared ornamentals is not a good recipe for success.

Typically, large-scale hatcheries explicitly state that they only sell to business or research institution. This is advantageous for a larger sized hatchery because those institutions usually make consistently larger purchases. These facilities often establish a minimum order rule or offer certain ornamentals in "lots" or groups of a species. Requiring a customer to buy a "lot" of a particular species is often beneficial to both the producer and consumer because the hatchery may charge a slightly reduced price per unit, which is more appealing to the consumer. This is on top of prices, which are already at wholesale. This increases the odds of making the sale, and when paired with a minimum order amount, can help ensure that product is moved out consistently while increasing revenue. In addition, this reduces in-house costs in maintaining product that is market ready, which may help increase an ornamental hatchery's profit margin.

Particularly in the case of a smaller hatchery, establishing an online presence can be quite useful. The online presence is more than a basic webpage with general information, which any hatchery large or small, should have. It should also include a section dedicated to purchasing ornamental stock that is for sale directly to the end party. This is commonly referred to the: What You See Is What You Get (WYSIWYG) section. Due to the fact that most small-scale operations are not able to compete with large-scale facilities selling at wholesale prices, this is a viable option allowing for expansion outside the local market. Online sales often require much more work because they involve shipping materials and shipping costs, dealing with loss associated with shipping, as well as sales tax. Retailing of product is also an option for smaller scaled hatcheries, but may involve more space in an already size limited facility. Even a small ornamental hatchery can sell at wholesale prices. This typically involves establishing good relationships with local retailers as well as providing weekly stock lists of high quality product with hopes that they will purchase on a consistent basis. Lastly, product may be sold directly to wholesalers. This method further reduces the sale price per ornamental product, and is a good option if a hatchery is consistently producing a high number of units, and local markets become saturated. Regardless of the method or methods of sale, success will depend on the determination of the individuals associated with the ornamental operation from husbandry to growout and every step thereafter.

11.12 Quality Control

A facility should not offer poor quality ornamentals for sale. This can potentially tarnish the reputation of ornamental aquaculturists and their efforts as a whole. Culling will allow a hatchery to focus on quality and detail of each species, resulting in a higher quality and more appealing product. This extra step in quality control leads to producing an excellent product, which will often speak for itself. With the growing popularity this industry has achieved, as well as its presence in the online world, word of a high quality, captive reared organism will spread like wildfire and may all but sell itself.

The subject of quality control is a very important and controversial issue. A responsible ornamental operation should consult their respective government authority and investigate the appropriate animal welfare regulations to assure compliance.

Whether culling is needed for quality control or to avoid spreading a pathogen from an organism that has been deemed untreatable to the rest of the facility, these species should be euthanized in a humane and painless manner. Lastly, under no circumstances should an organism deemed unfit for sale be released into the wild, as this can have a detrimental impact on the local environment.

11.13 Other Considerations

There are many other considerations that simply cannot be described in any number of book chapters, research articles, or other publications. Knowledge of those considerations come from individuals who have chosen to take the risks associated with attempting to establish a successful ornamental fish hatchery. Aquaculturists who can offer “words of wisdom” should also be considered. In my personal experience in

this industry, it has become quite clear that as production becomes more intensive, reliable employees become an essential part of the plan. It is prudent to hire knowledgeable, well-trained, and experienced staff in the initial planning to assist in facilitating production when needed. Success can be limited by the size of personnel and quality of employees. This is particularly an issue when attempting to establish an ornamental hatchery in small, rural locations.

Ornamental hatcheries with small or limited personnel may consider investing in technology to automate certain processes. The use of automatic feeders, automatic top-off units, and even aquatic controllers can simplify basic, mundane tasks, and even provide real time monitoring of water parameters alerting management personnel when problems arise. Though these components may be costly, they are extremely reliable and often necessary to free up employee resources to complete other tasks that simply cannot be automated.

11.14 Conclusions

The captive rearing of marine ornamentals is often said to be more of an art than a science. As the popularity of the marine ornamental industry increases, so do advances in technology and husbandry techniques. Establishing a successful marine ornamental hatchery requires hard work and dedication from all individuals associated with it. Our abilities to successfully breed and rear marine ornamentals in the hatchery setting have never been so promising.

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Part III

Marine Ornamental Fishes Aquaculture

The marine ornamental industry represents an important economic sector that has the potential to provide invaluable economic stability for low-income coastal communities that supply the trade (Tlusty, 2002). However, ornamental fisheries face several challenges including environmental and social issues such as loss of biodiversity through over-harvesting, and a more equal sharing of benefits by coastal communities (Wabnitz *et al*., 2003). The culture of marine ornamental fishes may thus represent an alternative source to those collected from the wild and can provide useful information about their life cycles (number of spawned eggs, age at maturity, etc.) as well as important insights for better management of wild stocks (Holt, 2003). Spawning, embryonic development and the transition from endogenous to exogenous feeding by fish larvae represent the main bottlenecks of ornamental fish aquaculture. Fish spawning and embryonic development have been investigated in depth over recent years, along with environmental and nutritional factors, for both demersal and pelagic spawners. Concerning demersal spawners, great emphasis is given on those species that are more familiar to the hobbyist, such as clownfish (Chapter 12), the Banggai Cardinal (Chapter 13), as well as dottybacks, gobies and blennies (Chapter 14). Regarding pelagic spawners, advances in the rearing of several species within the genus *Centropyge*, and the more recent success on the culture of Yellow Tang *Zebrasoma flavescens*, are highlighted and discussed (Chapters 15 and 16). Results provide important information about fish sexuality, nutritional requirements and gamete quality. However, while problems related to spawning and embryonic development are mostly solved, the critical bottleneck that researchers still have to face in rearing marine ornamentals is the transition from endogenous to exogenous feeding by the larvae. It is well-known that the most commonly used live preys (rotifers and *Artemia* nauplii) are not an adequate first food for most of the marine ornamental fish larvae, because of their inappropriate size and nutritional deficiencies. As a consequence, a great deal of research has focused on culturing and testing new possible live preys such as wild plankton, ciliates, harpacticoid and calanoid copepods, dinoflagellates and other microalgae, as well as preserved copepods. New zooplankton species for better success in the cultivation of both demersal and pelagic spawners are revised and discussed in detail (Chapters 13–16). Particular attention in the selection of live preys is given not only to their size, but also to their locomotor patterns, and to their nutritional profiles. Some of those preys have provided interesting results in the culture of different ornamental fish species. In addition, other studies have also focused on the development of larval

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rearing systems that mimic the ocean's conditions, concentrating larvae and food, providing shelter and improving food encounters (Wittenrich, 2007). These systems include glass microcosms, fibreglass open or closed systems as well as large mesocosms. For example particular tanks have been developed for the culture of seahorses in order to have a more homogeneous distribution of live preys and juveniles during the rearing process (Chapter 17). The research developed in recent years, together with advances in technology, will thus enable more and more species to be cultured. In the next chapters the successes and bottlenecks of marine ornamental fish culture will be analysed in-depth providing the reader with important and updated information on this topic. The post-larval capture and culture of fish larvae may represent a valid alternative to reduce mortality during early developmental stages and contribute to increasing the supply of cultured specimens (Chapter 18). Finally welfare and health of marine ornamental fish can be influenced by several environmental, nutritional and biological factors. Keeping fish in a confined/artificial environment, whether in a farm tank or a private or public aquarium, generally results in their forced coexistence with stressful conditions that may adversely affect their wellbeing, making them prone to several diseases, primarily those due to parasitic and infectious agents. Details on the main parasitic and infecious agents will be discussed and addressed (Chaper 19).

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12

Clownfish

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Abstract

Clownfish represent one of the best examples of captive bred ornamental fish. This chapter not only considers the general biology and characteristics of this fish group, but also deals with important aspects related to their reproductive biology and rearing techniques. Details on tank design, spawning, broodstock and larval nutrition, larval rearing tanks and early feeding trials are also described in this chapter.

Keywords *Amphiprion; demersal spawners; embryo development; larval nutrition; reproduction*

12.1 Introduction

Clownfish (Perciformes: Pomacentridae: Amphiprioninae), also known as anemonefish, are among the most attractive marine ornamental fish. They flourish in the warm waters of the Indian and Pacific Ocean from the Philippines to the Great Barrier Reef and from Sumatra to Melanesia, suggesting that may be the "center of origin" of Amphiprioninae (Santini & Polacco, 2006).

To date, 30 species of clownfish have been identified (Froese & Pauly, 2014). All clownfish are members of the genus *Amphiprion* except the maroon clown *Premnas biaculeatus*, which is placed in the genus *Premnas*. A molecular systematic study of Pomacentridae initiated by Tang (2001) proposed this genus to be included in the subfamily Amphiprioninae. This monophyletic classification was recently confirmed and different authors proposed *P. biaculeatus* be reclassified within *Amphiprion* (Santini & Polacco, 2006) in a tribe named "Amphiprionini" (Cooper *et al*., 2009).

In Pomacentridae, the diversification is characterized by repeated ecological radiations and diet is the major driving force of morphological evolution in this family (Frédérich *et al*., 2013). Interestingly, evidence of a major shift in the rate of diversification is observed in the Amphiprioninae clade and most of *Amphiprion* spp.

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Figure 12.1 Symbiosis between *Amphiprion clarkii* and the sea anemone *Entacmaea quadricolor*. (*See insert for color representation of the figure.*)

(25 out of 30 species) have an age of 7 million years (Litsios *et al*., 2012). Different studies have led to the conclusion that the diversification rate in this sub-family is between 1.5 to 2.5 times higher than the background rate of diversification in all other Pomacentridae (Litsios *et al*., 2012; Frédérich *et al*., 2013). This rapid adaptive radiation might be linked to the particular life-history characteristics of clownfish, who live in close association with sea anemones. The use of novel ecological niches could be considered as a "key innovation" which enhanced rapid ecological speciation by reinforcing reproductive isolation between populations (Litsios *et al*., 2012).

Clownfish use chemical cues released by the anemone to recognize the host species and some species (e.g. *A. clarkii* and *A. polymnus*) are able to detect anemones at a distance of 8 m (Elliott *et al*., 1995). Clownfish display different degrees of choosiness concerning their host. For instance, both *A. percula* and *A. ocellaris* accept three anemone host species: *Heteractis crispa*, *H. magnifica* and *Stichodactyla gigantea* for the former and *H. magnifica*, *S. gigantea* and *S. mertensii* for the latter. Conversely, *A. clarkii* are less selective and accepts up to 10 host species (Figure 12.1): *H. aurora, H. crispa*, *H. magnifica*, *H. malu*, *Entacmaea quadricolor*, *Macrodcatyla doreensis*, *Cryptodendrum adhaesivum, S. haddoni, S. gigantea* and *S. mertensii*.

The close link that characterizes the relationship between clownfish and anemones is termed mutualism. Anemone's endosymbiotic dinoflagellates (zooxanthellae) use waste ammonia excreted by the fish, and the clownfish directly protect the anemone against predators, such as butterfly fishes (Chaetodontidae). In return, clownfishes, sheltered within anemones' tentacles that exhibit lethal nematocysts, are protected from piscivorous predators.

The ability to avoid being harmed by this venomous organelle probably comes from a protective mucous coat that prevents the discharge of the nematocysts (Mebs, 2009). This protection might explain the very low mortality rate experienced in the wild by clownfish (e.g. *A. percula*: 14% per annum) compared to other adult coral reef species (5–70% per annum) (Eckert, 1987). Some authors suggested that this protection has ultimately resulted in delayed senescence and increased longevity in clownfish capable of living up to 30 years (Buston & Garcia, 2007).

Clownfish usually live within sea anemones in social units. Typically, these units are composed of a dominant breeding pair and of zero to four nonbreeding subordinates. Within each social unit, a size-based dominance hierarchy exists; the female is the largest, the male is the second largest, and the nonbreeders get progressively smaller as the hierarchy descends. Nonbreeders do not improve reproductive success of the dominant pair and are neither involved in cooperative territorial defence; they rather form a queue for breeding position (Buston & Elith, 2011). In captivity, most of the clownfish are kept as pairs and not as units, to avoid possible competition among the fish.

Although the success of clownfish species in the aquarium trade is partly due to their incredible color diversity and symbiosis with sea anemones, they also have the advantage of being easy to rear in captivity. Olivier (2001) reported that about 70% of cultured ornamental fish species were represented by clownfish in 2001. From 1997 to 2002, *A. ocellaris* was ranked in the top ten marine ornamental fish species exported worldwide, representing 15.6% of them (Wabnitz *et al*., 2003). The popularity of this species increased following the Pixar animation movie "Finding Nemo" (2003), which also resulted in a drastic increase in the capture of wild specimens (Yong *et al*., 2011).

Due to the importance of clownfish both in the aquarium trade but also as an "easy" fish to breed and rear in captivity, the present chapter investigates not only the general biology of this fascinating fish group, but also provides the reader with important and updated advices for their culture in captivity.

12.2 Social Structure

Social interactions regulating group structure are relatively complex in clownfish. These interactions are directly involved in sex differentiation and growth rate of individuals. Social rank is determined by initial body size and well-defined size differences exist between individuals of adjacent rank. For example, in *A. percula*, a dominant fish is 1.26 times larger than its immediate subordinate (Buston & Cant, 2006). A similar ratio (1.30) is observed in different groups of *A. frenatus* (Colleye & Parmentier, 2012).

It is possible to quickly identify the dominant clownfish in forming a group by focusing on the occupation time within the anemone. Indeed, the dominant individual readily monopolize the anemone in experimental conditions by displaying aggressive behaviour: chasing or threatening the next largest sub-adult (Iwata *et al*., 2008). The energy spent by subordinates whilst escaping from attacks could also partly explain their lower growth rate.

Independent of size, sex or prior residence, a high inter-specific competition for hosts exists (Fautin, 1985). It is supposed that species that stay in close vicinity of their anemone such as *A. percula* and *A. frenatus* are generally less aggressive than species showing a high tendency to move between host anemones, such as *A. clarkii*. For instance, agonistic acts are rarely observed in *A. percula* (<1 agonistic act per hour) (Buston & Cant, 2006) and in *A. frenatus* (3.5 agonistic act per hour) (Hattori, 2005) while nonbreeders of *A. clarkii* undertake 35 agonistic acts per hour (Hattori & Yanagisawa, 1991), although this is also closely linked to colony size (Moyer & Bell, 1976). A characteristic struggle consists of rushing toward the opponent, leaning to one side while exhibiting extended dorsal and anal fins (Moyer & Bell, 1976). A threat signal

of higher intensity is a jaw-clicking and Schneider (1964) first postulated that clownfish might produce "threatening sounds" while exhibiting this behaviour. This questioning was recently investigated, thanks to the original experiments conducted by Dr Eric Parmentier and colleagues. Their work about acoustic communications in *A. frenatus* enabled scientists to detect two main categories of sound produced: agonistic and submissive sounds. They hypothesized that aggressive sounds allowed dominants to give a reminder signal and that submissive sounds, produced by subordinates, would express appeasement during interactions (Colleye & Parmentier, 2012). Interestingly, submissive sounds were always associated with a typical posture characterized by head shaking movements and the lower the rank of the individual, the shorter the pulse duration of the submissive sound (Colleye & Parmentier, 2012). In addition, researchers detected in 14 clownfish species that the size of individual was positively correlated to the frequency and pulse duration of the sound produced, despite variation in acoustic features between species (Colleye *et al.*, 2011).

12.3 Sex Reversal in Clownfish

Clownfish are protandrous sequential hermaphrodites with the sex change, from functional male to female, dependent on the social environment. This is an important aspect that each fish breeder has to consider. This sequential hermaphroditism is an important advantage for the culture of clownfish, because there is no need for sexing fish, since the biggest individual will obligatory becomes the reproductive female.

Warner (1988) proposed a size-advantage model predicting that the direction of the sex change in a species depends on its mating system. Hence, protandry would be the evolutionary stable strategy in groups of two individuals (like clownfish), because fecundity of the breeders is dependent on the female size. In clownfish, reproductive success is positively correlated to female size, while it is unrelated to male's size (Buston & Elith, 2011), supporting the size-advantage hypothesis for sex-change.

It should be noted that protandry might not be the optimal strategy for all clownfish and some individuals would rather move from their initial host to obtain larger hosts or mates, if an opportunity arises (Hattori, 2005).

12.3.1 Histological and Molecular Mechanisms of Sex Change

Sex change in clownfish depends on both the social environment and the species, and generally several weeks/months are necessary to complete this complex process (Godwin & Thomas, 1993). The sexual development process is generally characterized by six distinctive phases (Godwin, 1994):

1) Gonads of immature juveniles include both testicular and ovarian tissues, the latter being predominant and with few primary oocytes.
2) Pre-ripe male phase: sub-adult gonad include some spermatocytes and primary growth phase oocytes.
3) Ripe male phase: mature male gonads possess an ovostestis which contains mature testicular tissue (active spermatogenesis located interiorly in the testicular zone: many spermatocyte cysts at various stages of spermatogenesis, spermatids, sperms are observed) and latent or non-functioning ovarian cells (localized mainly on the

periphery of the gonad, although few are noticed intermingled within the testicular zone).
4) Transitional phase: testicular tissue degenerates while the ovarian tissue develops (full of previtellogenic oocytes) during sex change.
5) Pre-ripe female phase: premature females possess an ovarian tissue with oocytes at different stages, whereas the testicular region is small and non-functional.
6) Ripe female phase: functional females possess oocytes at all maturation stages and the testicular tissue is reduced to a narrow band of degenerated tissue surrounding the gonad.

This general pattern is slightly different for *A. clarkii* in which the gonads of all individuals first differentiate into ovaries and nonbreeders can become females without passing through a functional male stage (Hattori & Yanagisawa., 1991; Miura *et al*., 2003).

Histologically, the invagination of the male's gonad wall is interpreted as the first signal of sex change and in *A. akallopisos* it can be detected 18 days after being separated from the mate (Casadevall *et al*., 2009).

At the molecular level, the process of sexual development and maturation is regulated by several sex hormones in the hypothalamus–pituitary–gonad (HPG) axis. The recent discovery of the Kisspeptine type 1 (Kiss1), a neuropeptide thought to be at the top of the HPG axis regulating reproduction, has given new perspectives. In *A. melanopus*, Kiss appeared to stimulate gonadotropin-releasing hormone (GnRH) release as well as the production of various steroid hormones including estradiol-17β (E2) and 11-ketotestosterone (11-KT), thus playing a central role in the maturation of the clownfish (Kim *et al*., 2014). The regulation of these two hormones is also essential for the sex change process (Godwin and Thomas, 1993; Kobayashi *et al*., 2010). For example in *A. clarkii*, a clear decrease in E2 and an increase of 11-KT is observed during testicular differentiation (Miura *et al*., 2008, 2013). A key steroidogenic enzyme, the cytochrome P450 aromatase (P450arom), is involved in the estrogen biosynthesis process by catalysing aromatization of androgens. The level of gonadal aromatase (*cyp19a1a*) is significantly lower in ambisexual gonad (male) of *A. clarkii* than in females (Kobayashi *et al*., 2010). It is thought that the progressive increase of *cyp19a1a* expression, from the ambisexual gonad to the female gonad, might be essential for triggering sex change.

12.3.2 Socially-Induced Sex Differentiation

Only two breeding clownfish adults occur in one group, because they socially inhibit subordinates from breeding and chase intruders. In fact, it has been argued that the dominant female controls the sexual fate of immediate subordinates by displaying aggressive dominance. Although empirical studies tended to corroborate this hypothesis, the transducer mechanism between external signal (social) and gonadal differentiation is still elusive. In some fish species with environmental sex determination, the role of cortisol (stress hormone) has been highlighted as a possible transducer. Indeed, the cortisol inhibits the female differentiation pathway by blocking the expression of gonadal aromatase (Hattori *et al*., 2009; Yamagushi *et al*., 2010), probably via the methylation of the aromatase promoter (Navarro-Martin *et al*., 2011). Interestingly, brain aromatase production is negatively correlated to the number of agonistic acts (and possibly cortisol production) in the sex-changing bluebanded gobies, *Lythrypnus*

dalli (Black *et al*., 2005). Based on these results it is possible to hypothesized that in clownfish, aggressiveness displayed by the dominant female would lead to an increase of cortisol production in subordinate fish, followed by the blocking of E2 production (via the inhibition of the aromatase), thus blocking the female differentiation pathway. In *A. ocellaris*, plasmatic cortisol concentration was shown to be higher in the dominant (future female) compared to other fish, 10 days after group formation (Iwata *et al*., 2012). Nevertheless, plasmatic cortisol concentration tended to be lower in the dominant fish than in subordinates and inversely correlated to E2, 6 months after group formation (during the sex differentiation process) (Iwata *et al*., 2008).

12.4 Broodstock Nutrition

In the wild, clownfish food mainly consists of copepods (35%), algae (33%) and polychaetes (10%); the remaining sustenance being principally crustacean fragments, tunicates and amphipods (Allen, 1972).

Broodstock nutrition in captivity needs careful attention since proper nutrition is essential not only to fish health but also for good quality gametes. Pairs fed only on dry food usually show limited growth, irregular spawning and produce pale eggs that do not develop properly. This nutritional deficiency can be easily corrected by improving parental diet (Wilkerson, 1998).

Feeding clownfish involves less challenge compared to other marine ornamental fish, because they accept fresh, frozen and dried feeds. (e.g., frozen chopped fish and shrimp, misyd shrimp or *Artemia*). This is especially true if they are captive bred, and different food items can be provided singularly or mixed together using a blender. The best way of feeding the fish is through several small feedings during the day avoiding one or two big meals. This way of feeding is closer to their natural food consumption and allows clownfish to better digest and assimilate the small amounts of food ingested.

Aside from quantity and frequency, it is essential to feed fish with an adequate amount of nutrients including proteins, lipids, minerals and trace elements, which are important for their metabolic and reproductive activity. Proteins are essential in clownfish diets since they provide essential amino acids that the fish cannot synthetize *de novo*. Specific protein requirements for clownfish are still unknown, but food should contain about 25–50% protein. For example, Murugesan *et al*. (2011) detected that the growth rate of *A. sebae* was faster when using polychaetes (*Perenereis cultrifera*) compared to both trash fish (*Leiognathus* sp.) and clam (*Meretrix meretrix*). The reason may be the higher content of protein in *Perenereis cultrifera* with respect to *Leiognathus* sp. and *Meretrix*. Dhaneesh *et al*. (2012) evaluated four different foods for broodstock nutrition of *A. percula* and also pointed out protein content as the main factor influencing reproductive capacities.

A balanced content in lipid quantity and quality is also essential for fishes (Izquierdo *et al*., 2000; Varguese *et al*., 2009). Lipids represent efficient energy sources that contain high amounts of calories. To date, most of the data available on lipid requirements in fish are related to fish species for human consumption, and very little data are available for ornamental species. Lipids are essential for clownfish; however too much lipid in the diet can have negative effects on the fish because they will quickly satisfy their caloric needs and avoid extra food consumption. In particular, highly unsaturated fatty acids

(HUFAs) appear essential in clownfish diets. As a consequence these molecules need to be included in a 10% ratio into clownfish diets in a balanced way with respect to proteins.

Lipid rich diets are difficult to maintain throughout time, since lipids are short-lived compounds that easily undergo oxidation. As a consequence lipids can be provided to clownfish by using food naturally rich in these molecules (e.g. krill, squid, fish roe) or by adding HUFA enrichment to the food just prior to administration (i.e. fish oils; commercial HUFAs supplements).

However, it should be underlined that other molecules, usually detected at very low levels, play an extremely important role in fish nutrition. Indeed, vitamins, minerals and trace elements shouldn't be neglected when preparing the fish diet. Again, as for lipids, the stability of these elements decreases readily due to latency in production procedures and air/oxygen exposure. For these reasons it is suggested that small quantities of food are bought to be used in a short time. It has to be pinpointed that these molecules should be handled with caution since an overdose of vitamins (i.e. vitamin D and A) can cause severe injury to the fish, including bone deformities and reduced growth.

In conclusion, clownfish diet should be as diverse as possible in order to be sure to include as many nutrients as possible in their food.

12.5 Broodstock Tanks and Establishing Pairs

Due to the fact that clownfish are sequential hermaphrodites, demersal spawners and usually not stressed by captive conditions, these fish easily and regularly spawn in captivity if raised in good conditions (heavily fed, variety of food etc.). To date, clownfish are one of the best examples of commercially available marine ornamental species and captive spawning have been reported on many occasions (e.g. Bell, 1976; Ross, 1978; Wilkerson, 1998).

One of the most striking properties of tropical oceans, compared to other environments on earth, is undoubtedly their stability (McGregor & Nieuwolt, 1998). As a consequence, the physiochemical conditions in the tanks, including temperature, nitrites, nitrates, ammonia, pH and photoperiod have to be consistent over time and mimick the natural environment of tropical oceans. For optimal water quality, several filtration systems have been successfully used in clownfish culture including mechanical–biological–UV filtration, as well as very simple methods based on the use of live rocks and protein skimmers. Usually, a 10% water renewal is performed weekly in closed systems.

In the wild, clownfish spawn year round, but scientists have observed an increase in fecundity during summer, when temperatures are higher and daylight is longer. As a consequence, a photoperiod of 14 hours light and 10 hours dark, 30 salinity, pH 8.2, and 28 °C temperature are suggested for most clownfish species.

Tank lighting can be performed using natural sunlight, fluorescent bulbs, HQI lamps (especially if there are sea anemones in the tanks) and more recently light-emitting diodes (LEDs) have been recommended. In addition to daylight, all clownfish tanks should be equipped with blue nightlights that can be necessary for nest care activity performed by the male during the night.



Figure 12.2 Sea-anemones can be easily substituted by clay flowerpots. Picture shows a male *Amphiprion ocellaris* guarding the egg clutch laid in the flowerpot. (*See insert for color representation of the figure.*)

It is well-known that clownfish occupy and defend a small and stable territory near an anemone in the wild. Thus, tank habitat should be as stable as possible: moving fish to new aquaria or continuously changing the habitat by moving rocks, invertebrates etc., might destabilize the pair. Breeding tanks can either include anemones or other invertebrates such as *Rhodactis* spp., *Sarcophyton* spp. and *Cladiella* spp. that are easier to keep in a tank. However, it is suggested that surrogates such as 10–15 cm diameter flowerpots can be substituted for invertebrates. In captivity, most of the species can be spawned without a host anemone. Balamurugan *et al*. (2013) studied the impact of host anemone behaviour on the reproduction of clownfish in captivity. This study concluded that a sedentary anemone would enhance the reproduction value of the breeding fish, but anemone mobility significantly increased spawning intervals of the broodstock and affected the embryonic development of the clutches with the percentage of eggs maturing decreasing exponential with increased distance of substrate from the clutches. As a consequence, nowadays, sea anemones are usually replaced by providing clay flowerpots of an appropriate size as spawning substrate for the broodstock (Figure 12.2). Usually several flowerpots are put in the broodstock tank, so that the pair can choose the most suitable substrate for laying their eggs.

The size of the breeding tanks may change depending on the selected species: from 50 to 100 L for small species such as *A. percula* and *A. ocellaris* (Liew *et al*., 2006; Önal *et al*., 2008) to 200–300 L for bigger species such as *A. clarkii* (Ghosh *et al*., 2012).

The constitution of a healthy broodstock is probably one of the most important steps toward the successful reproduction of clownfish. Either wild caught adult fish or tank-reared fish are generally used. Nevertheless, the latter are probably better than the former in terms of reproductive and survival capacities, owing to the fact that future spawners born in captivity are already accustomed to captive conditions and pathogen free. In addition, wild caught clownfish might have suffered an important stress following both collection and transport. As a consequence, they often refuse food and spawn less readily than captive bred fish. Starting with captive reared juveniles (or pre-adults)

is thus the best way to proceed. The young fish will go through three stages of pre-courtship: the search for dominance, followed by hierarchy agreement, and finally the pair formation (Wilkerson, 1998). The only inconvenience of this method is that young fish require some time to mature; around 9 months for *A. melanopus*, 1 year for *A. clarkii* and between one and one and a half years for *A. ocellaris*. Nevertheless, the process of gonad development could be accelerated by the intramuscular administration of oestrogen and androgen every 30 days (Madhu *et al*., 2006b).

12.6 Spawning

Spawning in clownfish usually starts 1 to 3 months after the pair is established (but sometimes it takes up to one year), a period during which the female's abdomen becomes progressively swollen. Despite a report of courtship sounds in three clownfish species by Takemura (1983) (*A. ocellaris*, *A. frenatus*, *A. sandaracinos*), recent investigation failed to detect sound throughout reproduction in four other clownfish species (*A. akindynos*, *A. melanopus*, *A. percula* and *A. clarkii*) in both wild and laboratory conditions (Colleye & Parmentier, 2012). The reproduction phase in clownfish is ritualized and includes: (i) nest cleaning and courtship; (ii) spawning; and (iii) nest care.

12.6.1 Nest Cleaning and Courtship

One to three days before spawning, the male starts biting and nipping anemone tentacles, leading to their shrinking and withdrawal, resulting in exposure of the nest. If the aquaria contain artificial substrate, they also start cleaning it at this precise time. A captive pair consistently spawns at the same time of the day and this appears to be regulated with sunset or sunrise. For instance, *A. ocellaris* pairs always spawn between 4–5 p.m.; *A. melanopus* between 12–1 p.m. and *A. clarkii* between 2–3 p.m.

A few hours before spawning, the breeding pair engages in vigorous nest-cleaning activities, which are then carried out only by the female. The female may also press her belly over the spawning site during that period. A morphological indicator of the imminent spawn is the clearly visible conical ovipositor of the female.

The female clownfish lays eggs while swimming slowly in a circular motion over the nest substrate. This movement is then repeated many times while the diameter of the egg-patch progressively increases. Meanwhile, the male follows closely behind and fertilizes the eggs after each pass of the female. The whole process lasts for about one and a half hours. Clutches laid by females *A. ocellaris* and *A. melanopus* are approximately 4 cm in diameter and contain about 150–200 eggs. Larger clutches of up to 10 cm in diameter (corresponding to 300–400 eggs) have been observed for *A. clarkii*. In all of these species, spawning consists of a precise swimming procedure and the fishes often exhibit unusual postures such as an upside-down position.

12.6.2 Nest Care

Embryo development is characterized by parental care of demersal eggs. Usually, two distinct nest-caring activities are observed: egg-fanning (by pectoral and caudal fins) and mouthing (Figure 12.3). Egg-fanning, creates mild water currents over the clutch and aids in renewing oxygen (Green & McCormick, 2005) and preventing eggs from



Figure 12.3 Nest care performed by male *Amphiprion clarkii*. (*See insert for color representation of the figure.*)

rotting (Delbare *et al*., 1995). Mouthing includes activities such as removing dead, decayed or weakened eggs, as well as particles. In most clownfish species, tending is done by the male (Ross, 1978; Ignatius *et al*., 2001; Madhu & Madhu, 2006a). Nevertheless, Green and McCormick (2005) observed that 20–30% of tending was done by female *A. melanopus* and reached 56% of the total activity for a breeding pair of *A. clarkii* (Moyer & Bell, 1976). Although field observations do not report nocturnal egg-fanning in *A. clarkii* or *A. melanopus* (Moyer & Bell, 1976; Ross, 1978), a more recent laboratory experiment revealed, to the contrary, that egg-fanning peaked around midnight in *A. melanopus* (Green & McCormick, 2005). Egg-fanning also increases during embryo ontogeny (Allen, 1972; Kumar *et al*., 2010) together with embryonic oxygen consumption (Green & McCormick, 2005). The effort put into parental care depends on the water circulation in the tank; the more current, the less frequently the pair has to fan the embryos.

12.6.3 Embryo Development

The animal pole of the elliptic telolecithal clownfish egg adheres to the nest's surface by a mass of adhesive threads. Eggs are highly pigmented due to the presence of carotenoids in the yolk sac, which appear whitish-orange to purple. The size of eggs varies according to the species (between 1.5 to 3.5 mm in length and 0.8 to 1.85 mm in width) and also with broodstock nutrition (Varguese *et al*., 2009).

The development process is similar in most clownfish species investigated to date, although development depends on broodstock nutrition and incubation temperature. In general, embryos change their color dramatically during development (Wilkerson, 1998). At 3 days post-fertilization (dpf) they usually look dark grey; while between 4 and 5 dpf the embryo's eyes become pigmented (Wilkerson, 1998; Liew *et al*., 2006).

The characteristic ontogenesis of the eye, that develops a strong metallic silver color throughout time, is useful for following embryo development and predicting hatching.

Bell (1976) reported that the incubation period in *A. clarkii* varies between 6.5 and 13.5 days as a function of water temperature. Generally, incubation lasts for 6 to 8 days at water temperature of 28 °C for most clownfish species (Olivotto *et al*, 2008a,b; Dhaneesh *et al*., 2009, Kumar *et al*., 2012). Liew *et al*. (2006) and Yasir & Qin (2007) investigated the embryo development process in *A. ocellaris* at 27–28 °C and found consistent differences in the development rate. In Liew *et al*. (2006), the first cleavage appeared at 1.2 hours post-fertilization (hpf), blastula at 4 hpf, gastrula at 12 hpf, neurula at 20.5 hpf, first somites at 24 hpf and hatching at 180.5 hpf. In Yasir and Qin (2007) the first cleavage appeared at 4 hpf, blastula at 11.5 hpf, gastrula at 20 hpf, neurula at 24.5 hpf, first somites at 28.5 hpf and hatching occurred 152 hpf. Yasir and Qin (2007) found that the development rate is first slower than in Liew *et al*. (2006) but then drastically increased. At 28 °C, *A. ocellaris* pairs spawn every 9 days, *A. melanopus* every 10 days and *A. clarkii* every 8 days, with hatching taking place 7, 7 and 6 dpf, respectively (Olivotto and collaborators, unpublished).

12.7 Hatching, Larval Tanks and First Feeding

Parental care is essential for proper development of the embryos, and as a consequence it is strongly suggested that embryos are left under parental care until hatching. While the embryo is developing and growing surrounded by the chorion membrane, several different biological processes start in order to prepare the embryo for its independent life in the external environment. As a consequence, after a species-specific process of development, the embryo changes into the form that can get nutrients from external sources and on the day of hatching, the larvae already possess an alimentary tract, a liver and a pancreas with digestive capabilities (Önal *et al*., 2008). At hatching, the embryo comes into contact with the external environment by breaking the egg envelope that is a barrier between itself and independent life (Olivotto *et al*., 2004). In fish, hatching is triggered by the concomitant action of hatching enzymes (that digest the inner layer of the chorion) and larval movement (that breaks the outer chorion layer). The hatching enzymes are synthetized and stored in the unicellular holocrine glands; the hatching gland cells and their expression start in different fish species, in early embryonic stages (Inohaya *et al*., 1995). Therefore, it is suggested that differentiation of the hatching gland is programmed at a very early stage of embryogenesis, like that of the notochord.

The hatching enzyme of most teleost fish is an enzyme system composed of two distinct Zn-proteases, HCE (high choriolytic enzyme) and LCE (low choriolytic enzyme) (Yasumasu *et al*., 1992). Both of these enzymes are synthesized in the form of proenzymes in the hatching gland cells and packaged into the same secretory granules (Yasumasu *et al*., 1992). While HCE binds to the inner layer of the chorion by partially hydrolyzing it, LCE efficiently dissolves the inner layer that has been swollen by HCE (Yasumasu *et al*., 1989). Both HCE and LCE proenzymes undergo a proteolitic cleavage before their activation that is developmental stage and darkness dependent. In several demersal spawners, including clownfish, hatching usually occurs within two hours after darkness. This photo-regulated hatching process reduces predatory pressures and

darkness assists the larvae during their rise from the benthic environment to the planktonic one. These important biological processes, that are the result of millions of years of evolution, are well conserved in captive reared clownfish as well. In fact, most of the clownfish embryos hatch, at a 28 °C, 7 days post-fertilization, after the first hour of darkness.

To get the larvae from the broodstock tank into the rearing tanks, embryos can be transferred to the rearing tank before they hatch or larvae can be gently collected from the broodstock tank after hatching (Wilkerson, 1998; Olivotto *et al*., 2011a).

Regarding the first method, the substrate where embryos have been laid, has to be gently removed from the broodstock tank and put into the rearing tanks filled with water with the same chemical-physical characteristics of the parents' tank (broodstock tank water is not mandatory). This procedure should be carried out exclusively on the afternoon/evening before hatching is predicted and if performed quickly, there is no need to keep the clutch under water. After putting the substrate with the egg clutch into the rearing tank, an airstone is added exactly beneath the clutch and the airflow is adjusted in order to mimic the fanning activity of the male. Air bubbles should keep the embryos waving and provide proper oxygenation. For some species, like *A. clarkii*, egg clutches may be quite large, so two or more airstones may be necessary to properly hatch all the embryos. Delbare *et al*. (1995) proposed using a device in which weak water jets are directed toward the eggs providing oxygen renewal and eliminating metabolic wastes products. Using this method they improved the hatching rate of fish by 82% compared to a conventional method using airstones.

Once proper water flow and oxygenation are provided, embryos have to be incubated in complete darkness for about 1 hour. These conditions are essential to keep the embryos alive and activate the hatching enzymes cleavage that will start digesting the chorion membrane. This enzymatic digestion, together with embryonic movements and the airflow will result in hatching.

The second method used to obtain clownfish larvae is to let them hatch directly into the broodstock tank, capture them and finally transfer them into the rearing tank. Since larvae are very delicate during this early part of their life history, it is essential to turn off filtration systems and pumps, to avoid the use of nets and to reduce as much as possible air exposure and turbulence (Wilkerson, 1998). Since clownfish larvae are phototactic, they can be attracted in a tank corner using a flashlight. The larvae can be gently collected using a 500 mL beaker and immediately transferred into the rearing tank. This second method is more labor intensive, but guarantees a very high hatching rate because of parental care. Getting acquainted with this type of manipulation will result in a reduction of the amount of water transferred for a successful and quicker collection. On the contrary, if the personnel collecting the larvae don't have much experience, turbulence during collection and transfer might lead to very high mortality within the first 24 hours post-hatching.

After hatching, clownfish larvae are very competent: they have pigmented eyes, functional jaws and an open digestive tract; in the meantime, the yolk reserve is limited and able to sustain the larvae only for about 24–48 hours post-hatching.

At this stage larvae are extremely sensitive to any turbulence and as a consequence, different larval rearing systems, which can be successfully used to culture clownfish, have been developed. In general, all these systems mimic the open ocean's conditions

where food organisms are abundant, predators are fewer and water chemical and physical conditions are stable (Wilkerson, 1998; Olivotto *et al*., 2011a).

Since clownfish are phototactic during their early larval stages, tank walls should be dark or covered by dark panels to avoid light reflection or light entering from the sides and to create a contrast with live prey that can be more easily captured by the larvae. As a consequence, light should come exclusively from the top and the tank bottom should be clear to facilitate syphoning and cleaning. In addition, optimal water quality has to be maintained through the whole larval period, in particular ammonia and nitrites should be maintained as low as possible (close to 0 mg/L) (Olivotto *et al*., 2008a,b).

As a consequence, in static systems, daily water changes are needed to maintain optimum water quality, while in recirculating or open systems, a 24-hour dripping system will guarantee reasonable water renewal to maintain water quality.

Microcosms made of 20 L glass tanks, fiberglass tanks (50–100–150 L) and mesocosms have been successfully used for several clownfish species. Water in these tanks is filtered through specific mechanical/biological systems and renewed up to 10 times a day through a dripping system. Heating, aeration and filtration are always carried out outside of the larval tanks to avoid physical damage to the larvae.

In the wild, the clownfish larval cycle is spent in the open ocean and larvae feed on planktonic organisms like copepods, protozoans and larvae of benthic organisms. However, most ornamental species are reared using rotifers (*Brachionus plicatilis* and *B. rotundiformis*) and *Artemia* nauplii as first food. Despite their apparent advantages, these food items are not the best first feed for most marine fish larvae since they are not their natural food, are not abundant in the oceans (if present at all) and can be too large for some small fish larvae. In addition, they do not offer the proper locomotory patterns and nutritional profiles. However, clownfish are one of the few exceptions that can successfully be reared in commercial numbers using only rotifers and *Artemia* as a first food source (Wittenrich, 2007; Olivotto *et al*., 2011a) (Figure 12.4). In fact, to date there are several reports of high survival rates obtained using these live prey. Rotifers are usually administered at a concentration of 10 ind/mL from hatching until 8–9 days



Figure 12.4 Group of captive bred *Amphiprion ocellaris*. (*See insert for color representation of the figure.*)

post-hatch (dph) while *Artemia* nauplii are introduced at a concentration of 1 ind/mL at 6 dph, 2 ind/mL at 7 dph, 3 ind/mL at 8 dph and then 5 ind/mL from 9 dph until metamorphosis. Larvae are reared in green water (*Chlorella* sp., *Isochrysis galbana* or *Nannochloropsis oculata* at a concentration of 50,000 cells/mL). Metamorphosis usually occurs between 12 and 15 dph and after this time, juveniles are gradually accustomed to dry larval diets (starting with 200–400 micron particles). To ensure proper survival rates of clownfish larvae, both rotifers and *Artemia* need to be enriched by the addition of commercial products rich in fatty acids, particularly highly unsaturated fatty acids (HUFAs) including EPA (20:5*n*-3), and DHA (22:6*n*-3). Several studies have shown that the inclusion of these products in clownfish diets can improve both larval survival and growth (Sargent *et al.*, 1999; Avella *et al.*, 2007).

12.8 Early Trials

In recent years, clownfish have become excellent laboratory experimental models to be used for research (e.g. Kobayashi *et al.*, 2010; Olivotto *et al.*, 2010). They regularly spawn in laboratory conditions (Avella *et al.*, 2007), larvae can be easily fed on conventional zooplankton and show very fast growth rates (Figure 12.5) (Olivotto *et al.*, 2010), and their genome is partially available in Genbank (Avella *et al.*, 2007; Kobayashi *et al.*, 2010; Olivotto *et al.*, 2010). The above-mentioned virtues are the basis for the possible utilization of clownfish as a new laboratory model to better understand important biological processes during marine fish larval development. Indeed, they represent an excellent short larval phase experimental model to test new diets and to elucidate important biological aspects that occur during marine fish larval development. During the early larval stage, fish undergo complex morphological, physiological and anatomical changes and size progression that takes them from larvae to juveniles in a very short time (Pérez-Domínguez & Holt, 2006).

Survival during the pelagic larval phase of marine fish is highly variable and is subject to numerous factors. The most critical moment, considered as the "bottleneck" to the fish production process, is undoubtedly the first feeding stage where a sharp decline in survival usually occurs. A study by Anto & Turingan (2010) evaluated the link between

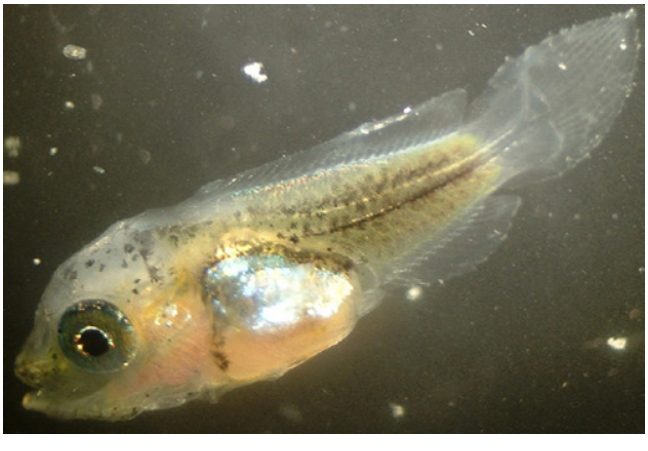


Figure 12.5 A 10 d post-hatch *Amphiprion ocellaris* larva: larvae are very well developed, competitive and close to metamorphosis at this stage.

functional morphology and prey selection to understand the relationship between morphology and mortality, using *A. frenatus* as the experimental model. Larvae reared from hatch to 14 dph on rotifers and *Artemia* showed the highest mortality from 1 to 5 dph, followed by a decrease in mortality from 6 to 14 dph. The study evidenced that larvae progressively consumed larger prey with growth and age, following ontogenic development of the gape. Mass mortality was not solely influenced by inability to establish first feeding, but also by the degree of ossification of the feeding apparatus: larvae selecting small prey items showed high mortality and few ossified elements, while consumption of large prey and cessation of mass mortality coincided with ossification of important elements of the feeding apparatus.

However, a wide range of factors are acknowledged to influence larval prey selection including prey characteristics such as size, density and motion, as well as different environmental factors such as light intensity and spectrum, water turbidity and temperature. Much literature concentrates on the relationship between prey size and mouth size, but other factors such as color, smell and movement are important attributes in prey selection as well. In particular, two studies analysed the use of both harpacticoid and calanoid copepods during the larval development of the yellowtail clownfish, *A. clarkii* (Olivotto *et al*., 2008a,b) to better understand their feeding preferences. The first study evidenced a positive effect of feeding *Tisbe* spp. copepods in *A. clarkii* larviculture as a supplemental live food to the traditional diet based on rotifers and *Artemia* nauplii. In fact, while 100% mortality was observed in larvae fed *Tisbe* spp. copepods solely, a significant increase in survival and growth was observed in larvae fed a 50% combination of *Rotifers/Artemia* and *Tisbe* sp. These data suggest that the harpacticoid copepod *Tisbe* spp. may be considered a suitable live prey for clownfish larvae only if used as a supplement to the traditional diet based on rotifers and *Artemia* nauplii. This feature might be explained by the fact that harpacticoid copepods are mostly found on the tank walls rather the in the water column, and are thus less available for predation compared to the pelagic rotifers and *Artemia*. In addition, the second study demonstrated the efficiency of the calanoid copepod *Centropages typicus* as live prey in the culture of *A. clarkii*. At the end of the feeding trial larvae fed *C. typicus* showed better survival and growth compared to those fed a standard rotifer/*Artemia* diet (90 ± 2 vs. $43 \pm 2\%$; 6.6 ± 0.2 vs. 6.0 ± 0.2 mm; 5.8 ± 0.3 vs. 4.5 ± 0.3 mg, respectively).

Copepods are thus considered an important and proper food source for clownfish and other species. However, there are several difficulties in culturing them on a continuous basis, since they only tolerate very low densities, require large tanks and need to be fed different algal combinations (Holt, 2003). As a consequence, the technology for mass scale production of copepods is still at the research stage. During the last few years, suitable storage techniques (i.e. cold storage) of copepods and copepods eggs have been studied and developed, and have been tested on some clownfish species. A first valid method may be copepod preservation, a new technology able to produce copepods that maintain their biochemical composition over time (Olivotto *et al*., 2010). A study performed on *A. clarkii* verified the possible utility of these preserved prey in clownfish larviculture. After hatching, *A. clarkii* larvae were divided in three experimental groups fed rotifers (*Brachionus plicatilis*) followed by *Artemia* nauplii; fed a mixed (50%) diet of rotifers–*Artemia* nauplii, and preserved copepods and fed preserved copepods solely. The study evidenced a positive effect of co-feeding preserved copepods together with rotifers and *Artemia* nauplii. Larvae fed the combined

diet showed the best results in terms of growth and survival compared to the other feeding groups. However, feeding preserved copepods solely caused great loss of larvae within a short time after hatching, underlying that prey motility, is an important attribute that can possibly affect larval predatory activity.

Other studies focused their attention on cold storage of copepod eggs. While it appears feasible to store diapauses eggs for long time periods (up to 17 months, Marcus & Murray, 2001) there are a lot of difficulties in the production of these diapause eggs. For this reason, copepod eggs may be more available and cold storage at low temperature may be an appropriate storage technique because the eggs switch to a quiescent state due to the suboptimal environmental conditions (Drillet *et al*., 2007). Cold stored copepod embryos still develop and retain physiological activity during the cold storage period and thus their nutritional values may change over storage time, with these changes affecting both eggs quality and larval fish survival and development. In a recent study, clownfish *A. polymnus* larvae were used to test the effects of diets based on standard rotifers and *Artemia* (control), *Acartia tonsa* copepods of a continuous culture and *A. tonsa* originated from 6 months cold stored eggs (Olivotto *et al*., 2012). This study demonstrated that after 6 months of cold storage the quality of copepods obtained from those eggs was suboptimal for *A. polymnus* larval rearing. In fact, larvae fed those copepods showed lower growth and survival performances compared to the other experimental groups, especially in relation to a significant variation and decrease in copepods fatty acid content.

The role of fatty acids in larval fish development is well-known and in particular EPA (20:5*n*-3) and DHA (22:6*n*-3), are known to be essential for larval fish survival and growth (Sargent *et al*,. 1999). Deficiencies in these fatty acids can cause a general decrease in larval health, poor growth, low feed efficiency and high mortality (Olivotto *et al*., 2011a). Clownfish have proven to be an excellent model for studying the importance of fatty acids in larval diets. A recent study by Olivotto *et al*. (2011b) evaluated the effects of a standard and a HUFAs-enriched diet on *A. ocellaris* larvae. This study evidenced that HUFAs administration in clownfish larvae improves larval development through the presence of better-structured mitochondria and a higher synthesis of energy compounds and coenzymes with a central position in metabolism. In *A. ocellaris* clowns, supplementation with HUFAs was found to be particularly important during the first week of life. Indeed, this higher energy status caused rapid growth, a shorter larval phase and lower mortality, underlying the beneficial role of HUFAs on the survival of this species.

In addition to survival, HUFAs administration can be an important method to reduce clownfish anomalous pigmentation, consisting of partial or total lack of white bands (miss-band). Since *miss-band* clownfish are sold at a lower price by the companies, there is a great interest in understanding and solving this problem. A study performed by Avella *et al*. (2007) revealed that live prey enrichment with fatty acids was able to significantly reduce the miss-band phenomenon.

Water temperature may also be an important parameter in clownfish culture since fish are poikilothermic species and their body temperature is strongly influenced by environmental conditions. Most of the literature suggests temperatures between 25 and 28 °C for their culture, however, considering the global change phenomenon, clownfish may experience higher temperatures in the wild. A study performed by Ye *et al*. (2011) tested three different temperatures (23, 26 and 29 °C) during *A. clarkii* larviculture in order to understand the physiological and ecological responses of marine fishes

to changes in water temperature. The results showed that water temperature significantly influenced the physiological performance of larvae; larvae reared at 23 °C took a longer time for post-larval development and metamorphosis compared to those reared at 26 and 29 °C; however total length and body weight for post-larval development and metamorphosis decreased with the increase in water temperature from 23 to 29 °C. The results obtained with this species are important for understanding that the decline of larval survival and growth rates, the extension of pelagic larval duration, and reduction of larval feeding at lower temperatures can have ecological impacts on larval dispersal and metamorphosis, juvenile settlement and population replenishment in the wild.

Since life cycles follow a well-defined and genetically programmed sequence in which gene expression, as well as hormone regulation, play a very important role, clinical parameters and zootechnical indexes may not be sufficient to analyse fish growth, development and reproduction. Therefore, in recent years, besides traditional markers (biochemical, histological, morphological and physiological), molecular biomarkers have gained considerable importance. Several feeding studies on clownfish include new molecular markers like genes whose expression could reasonably be modified by different feeding/culturing conditions such as insulin like growth factors (IGFs) and myostatin (MSTN) as growth factors, peroxisome proliferator activated receptors (PPARs) as lipid sensing factors, and thyroid receptors (TRs) as metamorphosis indicators (Olivotto *et al*., 2012). State-of-the-art research was also conducted on clownfish (cinnamon clownfish) to better establish the role of Kisspeptins (Kim *et al*., 2014). In addition, an important work addressing the question of sex-change was conducted by analysing, through modern techniques the expression of gonadotropin subunits and receptors as well as hormonal profiles during the life cycle of clownfish (Kim *et al*., 2010; Miura *et al*., 2008; 2013).

Finally, clownfish are also used to test new aquarium equipment such as new lights. Light emitting diodes (LEDs), a new form of lighting technology that is still being developed, can be manufactured to output specific wavelength. Narrow bandwidth light using such new technologies could provide much more efficient lighting systems than those currently used in the fish farming industry since they can be tuned to a species' environmental sensitivity by emitting narrow bandwidths. It is known that the spectral composition of incident light changes in underwater environments and that there is a rapid decrease in intensity with increasing depth; the short or blue end of the visible spectrum becomes predominant in deeper waters, whereas red light is only present in shallow waters. It has recently been demonstrated in the clownfish *A. clarkii* that certain LED spectra can induce stress response in fish, including oxidative stress (Choi *et al*., 2012). Results indicated that red light induced oxidative stress while blue and green light did not.

This chapter provides clear evidence of the detailed knowledge that we actually have about the clownfish life cycle and how such a great fish, easy to spawn, with a very short larval phase, is now becoming an interesting experimental model in many laboratories.

12.9 Conclusions

Clownfish actually represent the best example of captive cultured fish. Particularly after the release of the Disney-Pixar movie "Finding-Nemo", they became very popular in home aquaria and a lot of effort was invested by hatcheries into their captive

production. Survival rates can be as high as 90% when optimal rearing and feeding conditions are provided. In addition, several clownfish species are now used as excellent experimental laboratory models, especially because they spawn regularly in the laboratory; they require small tanks and the larval phase is extremely short (if compared to other marine fish species). Furthermore, they show all the characteristics and nutritional requirements of other marine species.

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13

Mouthbrooders – The Banggai Cardinalfish

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Abstract

The endangered Banggai cardinalfish *Pterapogon kauderni* is emblematic of the overexploitation of marine ornamentals for the aquarium trade. Advice in captive breeding is provided here and encouraged in the hope of reducing demand for wild caught specimens. *P. kauderni* reproduces all year long. It is sexually monomorphic and forms transient pairs. Females attain sexual maturity at ~6 months. The most typical courtship displays are "trembling" and "flash" by females. Males incubate the egg-clutch and fast during incubation. Embryos need ~18–20 days before hatching. Afterwards, they remain within the male's mouth for ~5 days. Juveniles begin exogenous feeding at a larger size than other marine ornamental fishes, which prevents requiring additional egg-incubating/larvae rearing tanks, and culturing small food items. However, they are highly susceptible to a deficiency in HUFAs, and prone to suffer "shock syndrome". Size of ~5.5 cm TL is attained at six months and ~ 6.5 cm TL at one year at ~26 °C. A new *Megalocytivirus* virus has been described, which is causing high mortality of imported wild caught Banggai cardinalfish specimens.

Keywords *Apogonidae; conservation; direct development; egg-clutch; mating behaviour; shock syndrome*

13.1 Introduction

It is difficult to imagine another species that better embodies the conservation value of captive breeding than *Pterapogon kauderni* (the Banggai cardinalfish). This fascinating fish has become emblematic of the overexploitation of marine ornamentals for the aquarium trade and of the difficulties of associated conservation efforts (Roberts & Hawkins, 1999; Wabnitz *et al*., 2003; Helfman *et al*., 2009; Thornhill, 2012; Vagelli, 2013).

P. kauderni presents a series of remarkable biological characteristics making it an atypical coral reef teleost of great interest in ecological/evolutionary studies. For instance, it possesses one of the most restricted geographic distributions documented

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in a marine fish (it is endemic to the Banggai Archipelago, Eastern Indonesia, where it is confined to the shallows of 34 islands, occupying just 24 km^2). It is a highly site-attached species and an obligated commensal with various invertebrates. Its reproductive biology is particularly unusual for a coral reef teleost. It includes a very low fecundity, long embryonic development, sex role reversal, and most significantly, the absence of a larval period and an advanced parental care of post-hatch embryos.

The lack of planktonic dispersal during its early ontogeny coupled with its adult sedentary behaviour precludes this species from any meaningful displacement. The resulting extreme philopatry of *P. kauderni* is reflected by the possession of the highest population structure documented for a marine fish (populations separated by a few km – even on the same islands – are genetically distinct [Bernardi & Vagelli, 2004; Hoffman *et al*., 2005; Vagelli *et al*., 2009]).

Unfortunately, the human impact on *P. kauderni* is highly significant. Since its introduction to the aquarium-hobbyist community in 1995 (Allen, 1996; Michael, 1996), it has been heavily captured for the international ornamental fish trade. By 2004, in just over a decade since its capture began, the species suffered a decline of about 90% with respect to what was considered its pre-harvest level (Vagelli, 2008). The significant population decline, a highly restricted and fragmented distribution, low productivity, and persisting high demand by the ornamental fish trade led in 2007 to its designation as an "Endangered" species by the International Union of Conservation of Nature (IUCN) (Allen & Donaldson, 2007).

The most recent comprehensive population assessment of *P. kauderni* (it covered over 90% of the species natural range), was completed by the author in early 2015. It showed the species has suffered a further significant decrease in abundance with respect to the previous surveys. For instance, 77% of all populations surveyed showed abundance conditions from "vulnerable" to "critical", and three more populations have likely become extinct. Moreover, in 2015, the mean number of groups per census site declined 27% from 2007 and the mean group size showed a 39% reduction.

In addition, *P. kauderni* is affected by habitat loss. Destructive fishing practices, including blast fishing, and cyanide use for both ornamentals and live fish-food have been, and remain widespread in the Banggai Archipelago, diminishing essential substrates for this species (Allen & Werner, 2002; Vagelli, 2011). Moreover, the latest surveys have shown a severe and widespread (79% of visited sites) decline in abundance of sea urchins, vital substrates of *P. kauderni* (Vagelli, 2015). Furthermore, a *Megalocytivirus* virus, likely present at export centers, is causing high mortality of imported specimens, thus further increasing the waste of wild-caught specimens (Weber *et al*., 2009).

The grave conservation status of wild populations encountered during the 2015 surveys in the Banggai Archipelago coupled with the lack of effective conservation actions since its inclusion in the IUCN Red List played a key role in the recent inclusion of *P. kauderni* in American Endangered Species Act (ESA) (https://federalregister.gov/a/2016-00943). Although a landmark listing (it is the first coral reef fish ever included in the ESA), the designation as a "threatened species" does not directly convey protective measures (such as an import ban). ESA will leave to its discretion whether, and to what extent, to extend in the future the protective actions that apply to (ESA) endangered species.

To date, *P. kauderni* continues to be subjected to indiscriminate collection. Despite being listed as an Endangered and Threatened species in the IUCN and ESA respectively,

CITES (the Convention on International Trade in Endangered Species) has so far declined to regulate its capture and international trade. The fact that this species can be bred and raised in captivity makes this situation even more unjustifiable.

The aim of this chapter is to provide general advice to hobbyists who have little or no prior experience in breeding and raising *P. kauderni*, thus encouraging the culture of this endangered species in small settings. A general account of the reproductive biology will be presented first. Being familiar with the basic aspects of the species' reproduction will help with the planning related to its captive breeding and with correctly interpreting the nature of observed behaviours, which will enhance the chances of success. For instance, knowing the approximate age/size at sexual maturity, length and periodicity of ovary cycle, and length of embryo development facilitates decisions regarding tanks/systems and preparation of juveniles' first food items. Knowledge of various social behaviours helps to avoid losses due to agonistic interactions and or helps to prepare for imminent mating events. The following description is based on studies and observations done by the author both in the field and under captive conditions.

13.2 The Reproductive Biology of *Pterapogon kauderni*

13.2.1 General Characteristics and Ontogenetic Terminology

Perhaps the most distinctive reproductive characteristic of *P. kauderni* is its possession of direct development and the implications it has in the culture of this species. Direct development is the development of a zygote directly into a juvenile bypassing the larval period. Fishes with direct development produce larger eggs containing high-density yolk, which after fertilization allows embryos to require little to no external nutrients to develop into the definitive phenotype (juvenile).

The embryonic period is characterized principally by endogenous feeding, i.e., from parental sources, irrespective of being inside, or out of the egg (free-embryo).

The larval period, typically present in the vast majority of coral reef teleosts starts at exogenous (orally swallowed and intestine-digested) feeding. Larvae must undergo a process of metamorphosis, a profound morphological, physiological, ethological and ecological remodeling in order to replace transient organs, structures, and behaviours specialized for separate niches (Balon, 1999; Leis & McCormick, 2002).

A juvenile is the beginning of the definitive phenotype, in which most of the embryonic or larval temporary structures have disappeared and most of the permanent adult organs are present. In juveniles, the gonads are immature; the maturation of gonads marks the beginning of the adult period.

One important consequence of possessing direct development is the avoidance of the typical high mortality associated with the larval period and metamorphic process. This is a challenge posed by the overwhelming majority of captive bred marine ornamental fishes.

In species with direct ontogeny, the ample endogenous food supply (yolk) enables embryos to form permanent organs directly without the necessity of an extensive remodeling of temporary larval structures. In this case, the exact point of transition from embryo to juvenile may be difficult to determine because the transitional forms (although frequently very short) may display mixed feeding, i.e., both endogenous

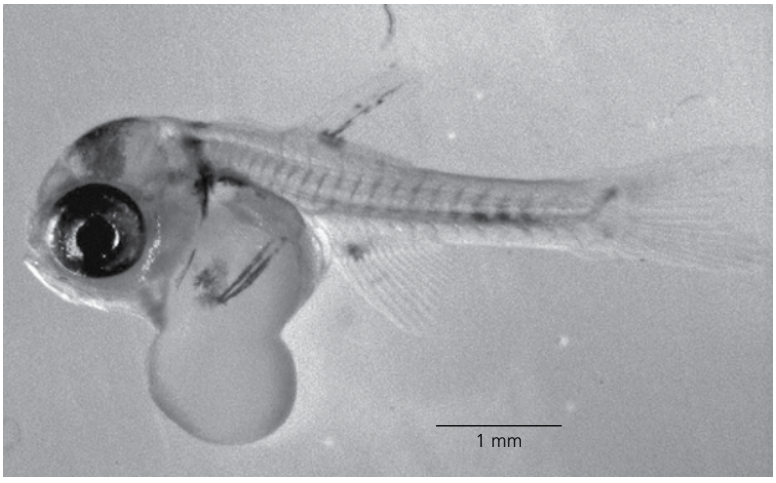


Figure 13.1 Newly hatched embryo. After hatching, embryos remain in the male's oral cavity for about seven days, while completing their development into juveniles, feeding endogenously on their large yolk reserves, before release.

(from the yolk) and exogenous (inhaled). Individuals during this transitional period are referred to as yolk-sac juveniles (Figure 13.1).

Pterapogon kauderni is a monomorphic species, i.e., does not show evident secondary sexual characters, hence, it is not possible to distinguish gender. In this species, the sex-role is reversed. Females possess a higher potential reproductive rate, whereas males are responsible for parental care (Vagelli, 2011).

P. kauderni has been wrongly described as capable of reversing its sex similar to sequential hermaphrodite fishes such as anemonefishes (Pomacentridae), and wrasses (Labridae). The fact is that *P. kauderni* is a gonochoristic species, and there exists no gonadal evidence that suggest otherwise. Thus, the gender at which the individual attains sexual maturity will not change.

In captivity (~26 °C) females attain sexual maturity (able to reproduce) at an age of approximately six months, a size of about 35 mm standard length (SL) (=excluding the caudal fin), and at about 5.5 cm total length (TL) – which is the length measured from the tip of snout to the tip of the upper lobule (the longest) of the caudal fin. In the wild, the smallest females found showing signs of gonadal development measured ~33 mm SL. Large (>2.5 mm) mature oocytes were found in females >45 mm SL (Vagelli, 2011).

In the wild, it is very rare to find brooding males <40 mm SL (~60 mm TL). The two smallest males found incubating were one of ~34 mm SL that was brooding small eggs of ~2.6 mm in diameter (indicating he mated with a small female, as small females tend to produce smaller eggs), and a male of ~32 mm SL that was brooding eggs of ~3 mm in diameter (likely produced by a significantly larger female). Thus, small males seem capable of successfully mating and brooding (Vagelli, 2011).

P. kauderni is a batch spawner. In captivity, it reproduces continuously without showing seasonality, and females require a minimum of ~20 days to complete oogenesis. The highest spawning frequency observed in captivity was one deposition every 25 days (at 26 °C). However, in the wild it appears to have a lunar reproductive

cycle, with a major spawning peak during the full moon, and smaller peaks during the last quarter and at the new moon (Vagelli & Volpedo, 2004).

There is no evident relationship between size and fecundity. Females spanning the entire size range of adults (SL ~35–60 mm) may produce clutches between ~60–70 eggs.

At spawning, the size range of oocytes, which seems to depend on the size (age) of the female, varies between 2.5 and 3.3 mm in diameter. Newly ovulated oocytes are generally spherical, bright yellow-orange, mostly filled by a dense pyriform-shaped yolk, and full of small lipid globules forming a cup-like aggregate in the upper part. An average egg-clutch of 40 ova forms a quasi-spherical mass of ~1.5 cm in diameter, and the oocytes are held together by strong chorionic filaments that originate from a circular area of ~0.5 mm in diameter on the chorion, and extend outwards entangling with filaments from other oocytes (Vagelli, 1999, 2011).

13.2.2 Courtship Behaviours and Pair Formation

Reproduction involves the formation of transient pairs and characteristic courtship displays that are generally initiated by the female. After "selecting" a potential mate, the female begins a set of displays leading to pair formation. In females, the most typical courtship displays are trembling, warping, flash swimming, and nuzzling. In males, the most common is "mouth-opening".

The process of pair formation starts when a female begins courtship displays towards a selected male, seemingly aimed to attract and isolate him from a group, simultaneously moving away from the group and creating a virtual home or reproductive site. Once the breeding pair is formed, it remains separated from other members of the group for up to a few days. During this time, the pair aggressively defends the breeding site.

One of the female's most characteristic behaviours during this phase is the "flash" behaviour, in which the female seems to seek the attention of the chosen mate. It consists of a sudden outburst of high-speed swimming, passing a few centimeters from the male (often describing a loop that almost encircles the male), and returning to her original position but at a slower pace. The flash behaviour is displayed at irregular intervals, but increases in frequency during the time leading up to the pair formation. Once the breeding pair is established, the flash behaviour is rarely displayed, but it is repeated if the male seemingly indicates a loss of interest by swimming away from the breeding site.

After pair formation, the most characteristic behaviour displayed is "side by side trembling" by the female, which can last a few hours. As the male remains almost motionless, the female, while vigorously trembling, approaches the male, generally from behind, places herself alongside the male and begins inclining her body until reaching an angle of about 30° with respect to the vertical plane. At this position the male and female's caudal and anal fins come in to contact, and simultaneously, the female pushes the male sideways while the male remains almost non-responsive to the female's display. This behaviour lasts for a few seconds, after which the female stops trembling and slowly swims away a few centimeters, only to immediately return trembling and pushing the male. After the behaviour is repeated two or three times against one side of the male, the female swims backwards until she is behind the male, and then she begins the same display but against the male's other side.

Side by side trembling may occur at different depths, but commonly takes place near the bottom of the tank, particularly when the mating is imminent. If the pair is not isolated, this pre-mating behaviour is frequently interrupted by conspecifics, which causes an immediate response, usually by the female, consisting of a rapid and aggressive chase of the approaching individual.

During side by side trembling, the only observed conspicuous responses by the male are a noticeable darkening along the lower jaw and sporadic "mouth opening" displays, which may be related to the male's efforts to enlarge the oral cavity in preparation to accommodate the egg-clutch, and/or may signal reediness to mate.

When *P. kauderni* is maintained in groups, the sporadic participation of a secondary male (SM) may be observed. In this case, courtship displays are similar to those described above, including the home site formation and its aggressive defense. However, occasionally an individual approaches the pair, but this individual (a SM), is allowed to closely approach the breading pair (within ~5 cm). The SM follows the breeding pair with a rather submissive posture, maintaining its distance if the pair swims in his direction. Interestingly, the SM participates in the defense of the pair's home site, either alone or with one member of the breeding pair. The SM usually displays trembling behaviour towards the female, a behaviour rarely observed in the primary male (PM) (the male that originally forms the pair), and also displays "mouth opening" behaviours to the female.

If the PM responds to the female's courtship displays, then the PM mates, and the SM does not intervene. However, if the PM stops demonstrating interest in the female's displays, the female will intermittently leave the PM, and begins courtship displays towards the SM, who responds by swimming to the bottom, remaining at a short distance from the female, and frequently trembling. In these instances, the SM is often who actually mates (see Vagelli, 1999 for details).

13.2.3 Mating, and Egg-Clutch Transfer

P. kauderni reproduces all year long. In captive conditions, pair formation and courtship usually begin in the morning. Mating typically occurs during early afternoon.

Ovulation, (egg-clutch release) occurs once the breeding pair is positioned a few centimeters off the bottom and the individuals are situated, either side by side or with the male situated slightly ahead of the female. The release of eggs takes about two seconds. After the clutch is released, the male immediately turns around, fertilizes the ova, and gulps the clutch, which requires pulling the egg-mass from the female, frequently causing some eggs to be cut off from the clutch. It is presumed that fertilization occurs during the first second after the oocytes are released, before the male turns to grab them. The precise moment of sperm release is difficult to determine, since neither spermatic fluid nor spasmodic contractions by the male are distinguishable during the short mating process.

13.2.4 Post-Mating Behaviours

In captive conditions, females display a few post-mating behaviours, which commonly last no longer than ~1–2 hours. They include a home site/male protection behaviour, which entails an immediate and aggressive chase of any "intruder" approaching the newly incubating male; a more relaxed (less frequent and less sustained) "side by side

trembling" towards the male; and, the confinement of the male by the female to a sheltered spot (generally by gently pushing the male against a substrate or the side of the tank, always close to the bottom).

The only post-mating behaviour observed in males relates to the clutch incubation. After the egg transfer, the male frequently opens its mouth as it accommodates and rotates the egg-clutch, partially exposing it for a brief period. This behaviour generally lasts only until the male accommodates the egg mass to its buccal volume. If the brooding male shares the tank with other individuals, this action may attract other individuals who might approach and harass the brooding male, apparently attempting to eat the eggs.

In the wild, brooding males are rarely found completely isolated, and they are often found forming part of groups of non-reproducing individuals. It seems that the breeding pair moves away from their group to mate, and they return to their original group sometime after mating has occurred.

During the entire incubation period, which lasts about 25–28 days (at 26 °C) brooding individuals do not eat. In captivity, males occasionally swallow/spit the eggs, particularly if it is a young adult brooding for the first time. Stress related to inadequate captive conditions is likely a major contributing factor, as well as the general poor health and nutritional state of the brooding male. As mentioned above, if brooding males are maintained with conspecifics without appropriate shelter, they may be harassed by other individuals leading to the loss of the egg-clutch. This occurs particularly during the first week of incubation, especially if the incubating males are young adults.

It is unknown whether or not *P. kauderni* consumes the eggs in the wild, but stomach content analysis of wild brooding specimens have never shown evidence of egg consumption. It is likely that in this species, egg consumption is not a common incidence in the wild, contrary to what has been reported on other apogonids (Vagelli, 2011).

One interesting feature of embryo incubation observed in captive specimens is that males are seemingly able to detect and expel dead (intra-egg) embryos. It is possible that once an embryo has died, the egg envelope and chorionic filaments rapidly decay. Loose eggs may indicate their inviability to the incubating male; such eggs would be easily separated from the clutch and expelled. Occasionally, males have been observed releasing malformed and slow-developing embryos. The comparatively smaller sizes of the embryos, and/or their lower activity are the suggested factors that the male would recognize. By discharging dead or dying embryos, males would avoid the spread of bacteria/fungus to healthy embryos during the long incubation period (Vagelli, 1999).

13.3 General Ecological Characteristics of *Pterapogon kauderni*

It was previously mentioned that being familiar with basic aspects of this species' reproductive biology would likely increase the chances of success at breeding it in captivity. Similarly, being familiar with basic aspects of its ecology, including habitat and microhabitats preferences, group structure and feeding habits, would facilitate making better decisions about providing appropriate environmental conditions and overall husbandry.

This, in turn, will reduce stress and its associated negative effects, reduce agonistic interactions, and minimize "aborted" incubations. Moreover, it will result in fish having a prolonged and healthy life, displaying their full splendor and interesting behaviours.

13.3.1 Habitat, Microhabitat and Community Associations

In its natural habitats, *P. kauderni* occurs in shallow seagrass beds and coral reefs. In coral reefs, it inhabits most physiographic zones, but is limited to a narrow depth range of usually <3 m. Habitats include areas of clear waters with visibilities >20 m, often coral fields of long branching *Acropora* spp., *Anacropora* spp., and *Porites* spp. with low to moderate currents; as well as areas with low visibilities (2 m or less), generally seagrass beds (*Enhalus acoroides*) in closed calm bays with silty bottoms and high levels of suspended particles; smaller bays with turbid waters, muddy bottoms with calcareous algae and low *Padina* sp. seaweed, interspersed with monticles of *Millepora* sp., and long-branching corals (Vagelli & Erdmann, 2002).

P. kauderni is a sedentary, site-attached species that avoids predation by sheltering among sea urchins' spines, anemones' tentacles, or in coral crevices. It is an obligate commensal with those three invertebrate groups. Its most common single host is the long-spine sea urchin *Diadema setosum* (Echinoidea, Diadematidae), which is found in all habitats and most sites where the species occurs. Other common hosts are the anemones, *Heteractis crispa*, *H. magnifica*, *H. aurora*, *Stichodactyla gigantea*, and *S. haddoni* (Figure 13.2). In seagrass beds, *P. kauderni* (particularly new recruits and young juveniles) frequently associates with the anemone-like corals *Heliofungia actiniformis*. Other frequent hosts are long branching corals, primarily species of *Acropora*, and *Porites*.

P. kauderni rarely occurs in sites where its preferred hosts are absent or at a low density. On such occasions, it utilizes other available suitable hosts, such as *Tripneustes* sp. sea urchins, and anemones *Entacmaea quadricolor*; *Macrodactyla doreensis* and *S. mertensii* (Vagelli, 2002, 2011).



Figure 13.2 Adult Banggai cardinal and its offspring sheltered in a sea anemone. (*See insert for color representation of the figure.*)

Regarding fish associations, *P. kauderni* forms part of different assemblages in different habitats and with different hosts. Generally, when the hosts are anemones, and it shelters in *Stichodactyla* spp. and *Heteractis* spp., it often shares the host with either *Amphiprion clarkii, A. ocellaris*, *A. percula* or *A. sandaracinos*. In addition, apogonids are often seen close to the host anemone, commonly, *Apogon hoevenii*, and *A. hartzfeldii*, and less frequently, *A. nanus*, *A. parvulus*, *Cheilodipterus quinquelineatus*, and juvenile *A. compressu*s.

When *P. kauderni* shelters in sea urchins *D. setosum*, it may share the host with one or more apogonids, and other teleosts, including *Diademichthys lineatus* (Gobiesocidae) and *Doryrhamphus dactyliophorus* (Syngnathidae). When it shelters in branching corals, it commonly forms assemblages with apogonids (e.g., *C. quinquelineatus*, *Archamia zoerostera*, *Apogon hartzfeldii*, *A. leptacanthus*, and *Sphaeramia nematoptera*).

In its natural habitat, *P. kauderni* forms groups of mixed age classes, most being large juveniles or young adults. Groups formed of only large adults are seldom seen, and usually consists of a few individuals. It is even rarer to find groups of new recruits <6 weeks old. Very often, recruits are encountered alone, or in small groups of 2–4 individuals, in anemones or *Heliofungia* corals. New recruits are only seen in higher densities when they form part of a large aggregation sheltered in large clumps of *D. setosum* (Vagelli, 2011).

13.4 Captive Breeding of *Pterapogon kauderni*

The egg incubation of commonly cultured ornamental fishes such as anemonefishes, gobies, and other demersal spawners is often complex. It requires a separated tank with an appropriate filtration and water circulation to prevent the death of developing embryos due to poor oxygen transfer caused by debris accumulation, and growth of microorganisms on the eggs' chorion. In addition, it may be required to trigger/synchronize the hatching process at a specific moment. If the parent(s) is left with the eggs, they need to be fed regularly, and the tanks meticulously cleaned. Moreover, enough (and often for an extended period) small live cultured food items such as rotifers, microalgae or copepod nauplii is required for larval feeding. Thus, the culture of ornamental species is often challenging, vastly time consuming, and may require significant prior experience in order to achieve successful outcomes.

In contrast, the parental incubation of post-hatched embryos and the comparatively large size at which *P. kauderni* begin the exogenous feeding offers the rare experience of not requiring additional egg-incubating tanks, larvae rearing tanks, nor the culture of the above mentioned food items.

Not requiring eggs/larvae dedicated systems obviously does not preclude their use, and various alternatives exist for setting up breeding and incubation systems, and for raising the juveniles. Options include artificial incubation in egg-tumblers, raising juveniles in small nursery tanks, in-tank netting, and even extensive culture in ponds (Marini, 1996; Marini & Vagelli, 2007; Ross & Pedersen; 2008; Pedersen, 2013). However, most hobbyists probably aspire to have a few *P. kauderni* as part of their permanent reef tank, as well as wanting to breed them and raise a batch of juveniles, while enjoying witnessing the reproductive process. Thus, guidance is offered for implementing a simple reproductive method requiring minimum investment, and little to no prior experience with fish reproduction.

13.4.1 Broodstock

Efforts should be made to acquire captive bred specimens. In addition to contributing to the conservation of wild populations, acquiring captive bred specimens is a better economic investment. Individuals raised in captivity typically show considerably lower mortality, which is expected since they avoid the long and damaging capture-holding-shipping process, and they are much less likely to have been exposed to diseases that can affect specimens held in export/import facilities. As with other species, individuals raised in captivity also avoid the stressful transition from a natural to an artificial environment, which even sophisticated systems and elaborately aquascaped tanks cannot fully replicate, particularly regarding the composition and stability of natural seawater. Thus, from the onset, captive bred specimens are better prepared to accept artificial food items, acclimate to less than perfect and often variable water chemistry, and to live in an unnatural social structure.

If broodstock is acquired from an aquarium store, it is likely that their origin cannot be established with certainty and it is quite possible they are wild-caught. In this case, little can be done in terms of evaluating the specimens' health status to avoid acquiring sick individuals, and some may die within days due to disease or long sustained high stress levels. Nevertheless, it can be definitively avoided to acquire specimens with evident signs of poor health/high stress, including specimens presenting a pale or darker coloration (see diseases, below), damaged fins, and individuals that do not keep their fins well erected most of the time. Moreover, any specimen that looks particularly shy, and/or is being harassed by other specimens (an indication of potential illness or long periods of stress by others) should be avoided.

Healthy individuals will remain buoyant and level with minimum movement of fins, which are intact, their eyes are clear, and the overall coloration is rich/deep black stripes on dullish silver, dappled with variable number of bright silverfish spots. A healthy fish also reacts quickly to feeding, eagerly preying on live food items. Ideally, specimens should be large juveniles or young adults of ~5 cm TL.

13.4.1.1 Gender Determination

It has been reported that the sex in *P. kauderni* can be distinguished. Commonly cited sexual differences include a larger, or more angular lower jaw and a longer second dorsal fin in males, and a more rounded overall shape in females. However, *P. kauderni* is sexually monomorphic, and sexes cannot be differentiated morphologically (at least without a very close examination). It is worth remembering that even assessing the gender of a particular individual according to wrong assumptions, there is always a 50% chance of "correctly" identifying its gender. This should be considered before acquiring or joining a "pair" that may have been selected based on traits that were thought to be sex-specific.

Sexes can easily be distinguished once a breeding pair has been established. During courtship the adult males enlarge the buccal cavity shortly before mating. However, this expansion is due to soft tissue modification in preparation to receive the egg-clutch, and not due to permanent osteological differences (Vagelli, 2011). In addition, during courtship the males usually show an increase in black pigmentation along the lower jaw, giving the impression of a larger jaw. Thus, if a pair is observed shortly before mating, these morphological characteristics can be used to identify the male. As with other

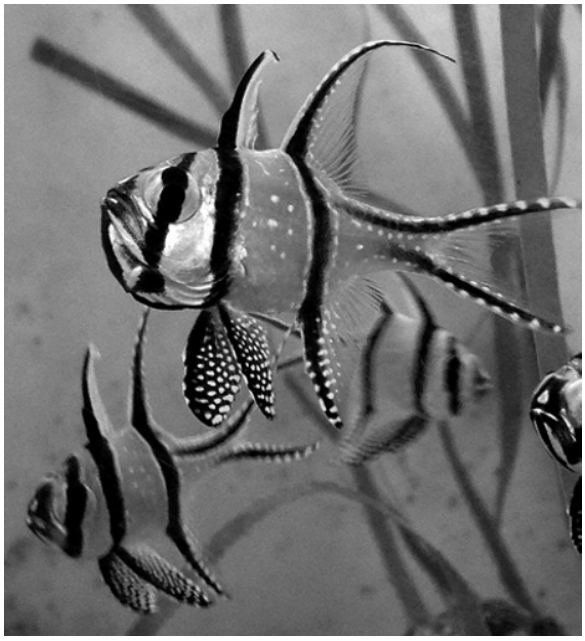


Figure 13.3 There is no conspicuous secondary sexual dimorphism in this species. However, during reproduction gender becomes quite apparent, like this brooding male. Once the pair is sexed, each fish can be permanently distinguished based on their individual-specific dot-pattern, most evident between the second and third black stripe.

species, mature females display a more rounded body and larger abdomen. However, these differences are not apparent in juveniles, nor in non-breeding adults; situations where sex differentiation is often most useful.

Fortunately, each individual possesses a unique coloration pattern based on the specific number and arrangement of silver dots located between the second and third black stripe, which allows individuals to be easily identified. Thus, once a pair is formed and sexes recognized, each individual can be permanently identified in terms of their gender (Figure 13.3).

It is unlikely that specimens from a typical store are maintained long enough and under appropriate conditions for pair formation to occur. Generally, acquiring four young adults would likely produce a breeding pair. They can be placed either in a communal tank or in a dedicated breeding tank. Depending upon their size (age) a breeding pair may be formed within a week, or it may take significant longer if they are juveniles (requiring time for attaining sexual maturation).

If the acquired specimens are placed into a dedicated tank, once a pair is formed, they can be transferred into a separate breeding tank or can be left in the same tank using a partition to isolate the pair (see below). If instead, the specimens are added to a communal reef tank, usually it is not necessary to isolate the pair since the rockwork and other substrates would allow them to create and defend a breeding territory (assuming a tank size of at least ~120 cm in length and no more than two to three other conspecifics share the tank).

In the event that new specimens are placed in a tank already containing a fish of known sex (perhaps because the other member of the pair died), observing the behaviour of the resident individual towards new specimens may be useful for

determining the gender of newly acquired fish. If the resident individual is a female, then, if a male is present among the added individuals, she will quickly attempt to form a pair. The female will try to isolate the potential mate from the new group and may begin displaying mild aggressive behaviours toward any female present. If the resident fish is a full-grown adult male, then he will likely react aggressively to the presence of a new male.

If broodstock is to be generated from juveniles raised in captivity, tanks need to be prepared to hold juveniles for about 4–5 months(depending on their age) before "good pairs" can be obtained. Although *P. kauderni* may reach sexual maturation earlier, females younger than ~6 months typically produce fewer and smaller eggs.

Similarly, males are capable of successful fertilization at an earlier age (especially if they do not share the tank with full-grown males). However, young males are relatively small, and their smaller oral volume cannot accommodate large egg clutches. In addition, younger males are more prone to terminating the incubation before embryos are completely developed.

13.4.1.2 Feeding

In addition to an adequate environment, reproductive performance is also significantly affected by the nutritional state of the broodstock. In species possessing direct development (like *P. kauderni*) it is particularly important to maintain the mating pairs in optimum nutritional condition. Fishes with direct development produce larger ova with little cytoplasm and large amounts of high-density yolk containing significant quantities of lipids, proteins and carotenoids, which after fertilization allows embryos to feed endogenously until released as young juveniles (Balon, 1999; Vagelli, 1999). Furthermore, reproduction for oral brooders (like *P. kauderni*) entails fasting during the incubation period. As described above, the incubation period in *P. kauderni* may last up to about 25 days, which for a tropical species (= with higher metabolism) of relatively small size, it likely imposes a significant energy investment. Thus, maintaining the breeding pairs in a healthy nutritional state is essential for regular production of "good quality" eggs and for avoiding aborted incubations.

P. kauderni is a planktivore–carnivore that in the wild preys mostly on microcrustaceans (quantitative analysis showed a diet composed of ~80% of copepods). However, it is a generalist–opportunist that, when available, consumes a variety of prey, principally planktonic–demersal and meiobenthic organisms (at least 30 taxa with a category of sub-order or higher of the Phyla Ciliophora, Annelida, Mollusca, Arthropoda, Chaetognatha, and Chordata). The prey size varies between 0.1 mm (calanoid copepods) to about 14 mm (megalopae and teleost larvae) (Vagelli, 2011).

Attempting to significantly modify the natural feeding habits of *P. kauderni* will likely fail. This species seems to be particularly reluctant to consume most commercially available formulated diets of flakes/pellets type. In captivity, it will eagerly feed on live zooplankton, and will always prey first on the largest items if mixed sizes are offered (for instance a mix of nauplii and adult *Artemia*). Adults will consume a variety of live crustaceans including mysids, amphipods, copepods and *Artemia*. They can easily be weaned onto a "frozen, meaty" mix of finely minced krill, clams, etc., (previously soaked in a vitamin/essential fatty acids medium, see below nutritional aspects).

13.4.2 Reproduction and Incubation

13.4.2.1 Environmental Requirements

While *P. kauderni* offers the advantage of paternal incubation, a good and stable environment is essential for avoiding premature termination of incubation.

An appropriate tank size is also necessary for pair formation, as well as for successful mating and incubation. As important as tank size is (and perhaps even more so) so too is the need to provide an adequate environment that allows the fish to reside with minimum stress.

Two principal factors will have a direct impact on the fish's stress level and consequently on the reproductive success. One is the availability of shelter-creating substrates in the breeding tank, as this fish is an obligated commensal that lives permanently associated with benthic living organisms, where it seeks protection.

The second factor is an appropriate chemical environment. This is especially important in *P. kauderni* because of the extended incubation and fasting period. In general, maintaining stable water parameters (i.e., limiting frequency of significant variations) is even more important than being slightly off from the ideal values. This will reduce the stress imposed by frequent opposing physiological adjustments required to compensate for the changes in water chemistry. For instance, adequate alkalinity should be maintained to avoid pH variations; frequent minor salinity adjustments should also be performed (rather than delaying them until the addition of enough freshwater to compensate for increases of 1 or more is required). Basic water chemistry parameters for breeding *P. kauderni* do not differ from those generally recommended for other coral reef fishes (pH: 8.0–8.2; ammonia and nitrites: 0 ppm; nitrates <20 ppm).

In the wild *P. kauderni* inhabits waters with a temperature range (spatially/seasonally) of 28–31 °C, but during incubation it is advised to maintain specimens at ~25–26 °C. Although at higher temperatures embryonic development will be shortened by a few days, under artificial conditions the higher temperature is also likely to facilitate outbreaks of diseases caused by both bacteria and protists. On the other hand, lower temperatures could significantly prolong the embryonic development, therefore imposing additional physical strain on the male.

The natural photoperiod of this species is close to 12 hours daytime (L) 12 hours nighttime (D) throughout the year, but a deviation of a few hours (e.g., 10 L–14D; 14 L–10D) does not seem to affect the reproductive cycle or behaviour.

Other stress-inducing factors which should be avoided include feeding other individuals when they are close to the brooding male, and siphoning (or other habitat disturbance) near a brooding male. In conclusion, keeping the brooding male in stable environmental conditions, and minimizing stress-inducing actions will significantly reduce the chance of males aborting the incubation.

13.4.2.2 Tank Choices

Pterapogon kauderni can survive and even breed in a tank with no substrates, and/or in small tanks. In fact, hobbyists have reported breeding them in glass tanks as small as 60 L. However, the importance for this fish is to have the ability to seek shelter (either natural or artificial substrates). Pairs or groups maintained in a more "natural" captive environment (for instance including sea urchins) even in a reduced space will show higher reproductive success. Thus, if pairs are maintained in a larger tank containing



Figure 13.4 Eight individuals are maintained in this 400 L fiberglass tank, in which the rock work/substrates are positioned in a way that facilitates the introduction of dividers that will isolate mating pairs once they are formed.

substrates, the fish will behave more naturally, their stress will be reduced, and they will have a longer "prime reproductive period", i.e., repeated mating events, high fecundity, good quality of eggs, and high rate of successful incubations.

If a breeding pair is maintained in a community reef tank, then it is likely the tank already contains substrates that provide enough hiding spaces for the fish to select its permanent territory, and eventually create a breeding site. Alternatively, if mating and incubation is required to occur in a separate dedicated breeding tank, then enough rockwork and plastic grass should be added for the pair to feel comfortable.

The tank should be prepared in such a way that will later facilitate both the brooding male's isolation with minimum disturbance, and the capture or isolation of the newly released juveniles. One useful arrangement is to construct two habitats separated as much as possible within the tank (or within each partition, if a larger tank and more than one breeding pair are held simultaneously). This will allow easier separation of the pair after mating by introducing a divider between the two habitats and gently moving the female to the opposite habitat where the male is located (Figure 13.4). It is desirable that the female is the one that remains on the side where the tank's outflow is located. This will help to prevent potential losses of newly released juveniles. Moreover, depending on the tank size, substrate position and flow, some juveniles may even become trapped in a mesh-covered outflow.

If more than one breeding pair are maintained on a regular basis, a ~180 L tank (~90 × 45 × 45 cm) may be divided into two or three partitions and will accommodate two breeding pairs comfortably. Once a male is brooding, he can be isolated in the same

tank with a new partition. A deep tank (at least 45 cm in depth) with rockwork covering the back (far from the front glass) would provide a good environment for the incubating male, even if the partitions will reduce each section length to about 20–30 cm.

Partitions should not restrict the water flow along the entire upper length of the tank. It is recommended to set partitions at ~1 cm below the water surface, to allow uninterrupted movement of the water's upper layer without the fish being able to swim over it. Partitions can be made with materials such as rigid plastic sheets or rigid mesh (both with rather small openings, ~6–8 mm). This increases the isolation of the incubating males by minimizing their sight to adjacent fish activities, such as feeding, that may elicit the termination of brooding.

Eggs should not be removed from the males in order to shorten the period between breeding events. Doing so will likely result in an unnecessary increase in stress, and even minor injuries to the male (some are quite reluctant to release the egg-clutch). In addition, it is likely that significant mortalities will occur during the artificial incubation. Nevertheless, artificial incubation ("egg-stripping") may be attempted in the case of repeated failure by the male to incubate the eggs (which most likely would be due to inappropriate holding conditions). Briefly, after capture, the brooding male is induced to release the egg-clutch. Sometimes, this is achieved just by the stress generated from netting, but in some instances, the male may be reluctant to release the clutch. In this case it may be necessary to hold the male, open its mouth, and gently shake it (always while submerged). Then, the recovered egg-clutch can be placed into an egg tumbler or even into a larger McDonald-type hatcher with a good upwelling flow of clean (filtered) water to keep the eggs in suspension. Even in this instance it is advised to leave the male incubating the eggs for the longest possible period before removing them (although difficult to accurately estimate it, an approximate time may be based on the average length of time the male kept the eggs in previous brooding events). Embryos are particularly susceptible to mechanical trauma and light exposure during the first days after fertilization. Thus, removing the eggs after a few days, and handling the egg-clutch with extreme care will help decrease the embryos' mortality during the artificial incubation.

The time required by females to produce a new batch of mature oocytes depends on several factors, including temperature, nutritional state, and stress level. In some instances, females may ovulate in less than three weeks after their last mating, but they usually need about 25–30 days to mature and produce a good quality clutch (i.e., a clutch of viable, numerous and large eggs).

13.4.3 Juvenile Rearing

At an incubation temperature of ~26 °C, embryos require about 18–20 days for completing their intra-egg development. After hatching, yolk-sac juveniles remain within the paternal oral cavity for about 5–7 days completing their development while reabsorbing the yolk sac. Hence, brooding males do not typically need to be closely observed for the first 20–22 days after incubation has begun. After that period, juveniles begin more obvious movements inside the parent's mouth, and sporadically they can be seen seemingly trying to escape. The release occurs frequently during dark hours, but it can also happen during the morning. In captive settings, juveniles are not usually released all at once. Normally the release occurs over a two to three-day period.

If the incubation is to occur in a dedicated breeding tank, the divider that separates the brooding male from the female should be replaced near the release day. A new divider should have a mesh with openings no larger than ~4 mm and it should be higher than the water level to prevent juveniles passing to the other side of the tank. Moreover, if the tank's outflow is located in the male's side, it should be covered with the mesh. In addition, it is recommended that a long spines sea urchin (*Diadema* sp.), or a dummy-urchin be added to the tank. Thus, the brooding male may remain in his tank until all juveniles are released, the male will not prey on them during the release period, and juveniles will remain closely attached to the urchin spines, or other provided shelter. When the first juveniles are released, enriched *Artemia* nauplii can be added to the tank a few times a day while the water flow is reduced (to avoid the *Artemia* from being washed out of the tank) allowing the juveniles to feed for ~15–20 minutes. Once all juveniles are released, they can easily be captured by gently moving them together with the urchin into a vinyl net (with no mesh).

If the breeding pair is to be bred in a reef community tank, then it is likely that the male will try to remain hidden during the incubation. It is ideal to avoid feeding other fishes when they are close to the brooding male, and the introduction of new specimens (including conspecifics), which may harass the brooding male. One simple method for collecting the newly released juveniles is to gently move the brooding male into a large net a few days before the estimated release date. It can be done using a squared net of about 30–40 cm with a similar depth of the tank, and containing a rock with artificial sea grass attached to provide cover to the male and juveniles. The net should be placed in a darker section of the tank, or covered to prevent the male from being exposed to bright light.

Once released, juveniles directly settle within the parental habitat without passing through a planktonic-dispersing phase. An average recovered batch of about 35 juveniles can be held together in a 50 L tank for the first 60 days. Then, they can be transferred into a ~120 L glass tank (~90 × 45 × 30 cm) with substrates (rocks, plastic grass, urchins) that will sufficiently accommodate them until reaching a selling/trade size at about 4–5 months of age. For larger production of juveniles, special grow-out systems can be designed, including semi-intensive rearing utilizing "pond culture" (Hopkins *et al*., 2005).

13.5 Nutritional Aspects and Diseases

The availability of enough small cultured food items such as rotifers, microalgae, copepod nauplii, mollusk trochophore, etc. for extended periods, is what often deters hobbyist from attempting to breed marine fishes (even those few species regularly bred in aquaculture/research facilities). The comparatively large size at which *P. kauderni* begin exogenous feeding avoids the demanding culture of those food items. However, this species is highly susceptible to a deficiency in essential fatty acids, particularly during the early stages of juvenile growth. Juveniles raised with a diet low in essential fatty acids are prone to suffer "shock syndrome", which may lead to high mortalities (Vagelli, 2004; Marini & Vagelli, 2007).

13.5.1 Shock Syndrome

Individuals suffering shock syndrome become very sensitive to external stimuli, such as feeding, sudden changes in light conditions, significant water changes, which trigger an easily identifiable set of behaviours. The most characteristic is an extremely rapid, short

and jerky burst of motion, brief upward spiral swims, and falling to the bottom of the tank with the opercula notoriously expanded, while significantly increasing the ventilation frequency. Sometimes juveniles may slowly recuperate after a period of about 5–10 minutes, however, some may die very soon after the shock. This syndrome seems to result from inadequate intake of the long chain *n*-3 and *n*-6 series of highly unsaturated fatty acids (HUFAs). HUFAs have been shown to be critical for normal development and high survival, as well as stress and infection tolerance in marine teleosts. Adequate dietary intake, particularly of docosahexaenoic, eicosapentaenoic and arachidonid acids, are thought to be necessary for normal brain and neurological tissue development, and normal behaviour (Vagelli, 2004).

The symptoms of shock syndrome do not become immediately evident in *P. kauderni* raised with a HUFA-deficient diet. They usually do not appear prior to the first week post-release. This delay is probably due to the fact that at release juveniles still possess yolk reserves, and, as shown in other species, it is likely that juveniles are able to differentially retain essential fatty acids while consuming other lipids (Vagelli, 2004).

Although newly released juveniles feed readily on *Artemia* sp., its low content of essential fatty acids is not sufficient for adequate growth. Fortunately, it is possible to avoid the shock syndrome and obtain high levels of survival by enriching the nauplii with HUFAs before feeding. A simple method for enriching *Artemia* consists of transferring newly hatched nauplii into a hatcher containing the enrichment medium and leaving the nauplii for at least 36–48 hours, where they will feed and concentrate the HUFAs into their tissues.

Newly hatched *Artemia* do not feed exogenously until they molt for first time and transform into metanauplii, which occurs, depending on the culture temperature, about 12–18 hours after hatching, and until then nauplii consume their own yolk. Hence, *Artemia* "enriched" <24 hours may not have had enough time to accumulate exogenous HUFA.

Several commercially available and easy to utilize products are good sources of HUFA. The author has successfully raised juveniles on numerous occasions by enriching *Artemia* with the following enrichment mix: Protein Selco (~300 mg/L), and spray-dried microalgae *Schizochytrium* sp. (~200 mg/L). The mix was fed to *Artemia* cultures with densities of ~ 250,000 metanauplii/L, for 24 hours at 28 °C. In fact, the author conducted a test (that included five replicas, each lasting between three and five months) using the described mix (Vagelli, 2004). The results showed that the mean mortality of juveniles (all replicates combined) fed the enrichment medium was only ~5% (in three out of the five replicates, the mortality was 0%); whereas those juveniles fed non-enriched *Artemia* suffered a mean mortality of ~81%.

Young juveniles should be fed a few times a day for a period of about 20 minutes. Enriched *Artemia* can be collected each time before feeding, or can be collected once a day and stored in a cool environment, which will reduce their metabolic activity and retain the nutritional value. After two to three weeks, juveniles can be offered a mix of *Artemia* with frozen cyclopeeze, and after ~50 days they can be fed with a mix of enriched *Artemia* and finely minced krill or clams (previously soaked in Selco). Dilute this mix in seawater and feed with a plastic pipette several times per day. At each feeding time, a few drops of the mix can be slowly squeezed over the juveniles a few times. Unavoidably some uneaten food will fall to the bottom of the tank (juveniles will not eat from the bottom), but continue the feeding (drops of mix) a few more times, and then suspend

for several hours. The young rapidly become accustomed to this feeding technique. Gently approach the tank and submerge the tip of the pipette, and the juveniles will swim up and wait for the mix.

Older juveniles and adults should be fed *Artemia* at least two to three times a week.

With an adequate diet and at about 25–26 °C, juveniles reach ~3.5 cm TL at an age of three months post-release, ~4.5 cm TL at four months, and ~5.5 TL at six months. *P. kauderni* reaches ~ 6.5 cm TL (~4.5 cm SL) at one year of age.

13.5.2 A New Viral Disease

For the first eight years or so after its appearance in the aquarium trade, *P. kauderni* was considered to be a hardy species with no particular disease problems. However, around 2002–2003, reports about the sudden death of recently purchased wild-caught specimens began to spread within the fish hobbyist community. In some cases, entire groups of ten or more newly acquired individuals were reported to display various symptoms, including reluctance to eat, unusual lethargy and conspicuous darkness in their pigmentation, and resulting in high mortality, usually 100%, within few days.

Around that time, the author was conducting breeding studies and acquired a group of *P. kauderni*. To his surprise, soon after arrival the specimens displayed the mentioned behaviours. The fish had difficulty maintaining balance and had an increased ventilation rates. They produced an unusual long, thin, white string of feces that remained attached to the fish for extended periods. Once this last symptom appeared, the fish died within a few days.

In one instance, the fish showed signs of this problem very shortly after arrival. Within two days, symptoms were evident in several specimens and one died. Others members of the group (maintained in the same tank), ate and behaved normally for a few more days before showing similar symptoms. However, the entire group of ten specimens died within 12 days. On another occasion, a group of 20 individuals followed a similar pattern, but a few did not show symptoms for almost four months (and a pair even reproduced about three months after being kept in the same system occupied by the other fish). Eventually, all developed similar symptoms and died.

Macroscopic examinations of the affected specimens showed extended internal hemorrhagic tissue in the kidneys and ovaries. Spleens were particularly swollen and hemorrhagic, and gall bladders very enlarged and filled with clear green bile. In a few specimens, the liver was very soft and displayed large clear spots, whereas in others the liver was extremely dark. The author suspected a virus infection and sent few individuals to a pathology laboratory. Eventually, a series of studies were conducted, including molecular virology, which demonstrated that a virus of the genus *Megalocytivirus*, family Iridoviridae (named "The Banggai cardinalfish iridovirus", BCIV) was the cause of the described pathology and mortality (Weber *et al*., 2009).

The genus *Megalocytivirus* is comprised of viruses associated with serious systemic infections resulting in significant mortality (up to 100%). These viruses have been reported among numerous marine and freshwater fishes including ornamental species either endemic to or exported from areas around the South China Sea, and several Southeast Asian countries (Wang *et al*., 2007).

The repeated presence in the international aquarium trade of *Megalocytivirus* virus infecting wild-caught *P. kauderni* raises the question of whether BCIV occurs among

wild populations, or if it has been the product of post-harvesting transmissions from other species at export or import facilities. It appears that the latter is a more likely scenario since no wild specimens of marine species from Southeast Asia, including Philippines and Indonesia, have been reported infected with *Megalocitivirus*. Moreover, signs of the viral infection were never detected during the author's several expeditions to the Banggai Archipelago (nor in the dissected wild-caught specimens). In addition, neither local collectors nor buyers have reported the presence of specimens presenting the described symptoms, even though fishers commonly hold hundreds or even thousands of specimens in nets from several days to a few weeks. It seems more likely that the infection occurs after capture and shipment out of the Banggai region.

Spreading diseases through the international ornamental fish trade is a well-known occurrence, including iridovirus infections due to several species of fishes (Anderson *et al*., 1993, Sudthongkong *et al*., 2002). It is likely that shipments of *P. kauderni* could be infected in some export, re-export or import centers (Vagelli, 2011).

13.6 Conclusions

It is hoped that the information provided in this chapter will help to persuade more aquarists in procuring *P. kauderni* through captive breeding and to encourage its trade within the hobbyist/aquarium community. As the availability of captive bred *P. kauderni* increases, the capture pressure on its wild populations will immediately decline. Moreover, the comparatively low mortality of captive bred specimens will lead to a significant multiplying conservation effort. By saving the Banggai cardinalfish, fish enthusiasts will set an unprecedented example in conservation for others to follow.

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14

Other Demersal Spawners and Mouthbrooders

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Abstract

Demersal spawning and mouthbrooding species comprise more than 70% of the production of marine ornamental fish. Apart from clownfish, there are several families of demersal spawners that are cultured for the ornamental trade, including gobies, blennies, dottybacks, damselfish and others. These species are characterized by producing eggs that are deposited on a substrate. The eggs are tightly packed together and may be adhered to the substrate or simply laying on it. Most ornamental demersal spawners display parental care during embryonic development and this feature is critical for successful hatching. Mouthbrooders, on the other hand, orally incubate the embryos before hatching. Newly hatched larvae of demersal spawners and mouthbrooders typically have very little yolk and are able to feed immediately after hatching. Live feeds such as rotifers and brine shrimp nauplii are frequently offered, but additional food sources including wild zooplankton, copepods and ciliates can be essential for successful mass production of some species.

Keywords *Blennies; damselfish; dottybacks; embryo development; gobies; larval rearing; mouthbrooders*

14.1 Introduction

The marine ornamental industry has grown significantly in the last decades, and as a consequence the demand for quantity and diversity of organisms has increased. Although most marine fish species are pelagic spawners, the demersal spawning species, *i.e.*, those that produce eggs that dwell on the substrate, currently represent more than 70% of all marine ornamental fish produced commercially (Hickford & Schiel, 2003; Wabnitz *et al*., 2003). This is due in part to the fact that they share several characteristics that facilitate their production in captivity, which include high resilience,

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straightforward formation of pairs or small harems, parental care and relatively large demersal eggs and larvae (Wittenrich, 2007).

Fishes belonging to the families Gobiidae, Blennidae, Pseudochromidae and Pomacentridae are very popular in the marine ornamentals market. The vast majority of the species that have been commercially produced in recent years belong to these families (Wabnitz *et al.*, 2003; Moorhead & Zeng, 2010). Despite having little data on aquaculture and low production numbers, the families Plesiopidae, Grammatidae, Microdesmidae, Apogonidae (except for the Banggai Cardinalfish) and Opistognathidae are also addressed, some of which are mouthbrooders. The following information is a compilation of information regarding the biology, care, reproduction and aquaculture of these families.

14.2 Gobies (Gobiidae)

The family Gobiidae is remarkable among most teleost families. They are significantly diverse with approximately 1700 species divided into 250 genera (Froese & Pauly, 2013). Apart from being the family with the largest number of species of marine fishes, the Gobiidae also include brackish and freshwater species (Nelson, 2006). Gobies are unmatched in their habitat plasticity, occupying several distinct marine environments such as oceanic waters, rocky and sandy substrata, mudflats, caves, tide pools and especially coral reefs and their various microhabitats, which concentrates more than half of all species (Herler *et al.*, 2011; Froese & Pauly, 2013). Coral reef gobiids typically have a short life cycle, as many species are known to live for only a year and others just for a few months (Hernaman & Munday, 2005; Depczynski *et al.*, 2007; Herler *et al.*, 2011). However, they represent a large portion of the energy flow in the reefs (Herler, 2007). The majority of gobies are small with elongated bodies and display benthic and territorial behaviour. One of the main morphological characteristics is the fusion of pelvic fins, which act as a suction-cup and allow the animals to attach to surfaces. Several features make this family quite popular, including their reduced size, vibrant colors, occasional symbiotic relationship with other organisms (e.g. alpheid shrimps) and cleaning habits (Gasparini *et al.*, 2004; Calado, 2008, Côté & Soares, 2011). Gobies are well represented in the marine ornamental fish market, totaling between 5–7% of sales (Wabnitz *et al.*, 2003). Besides the demand from the marine ornamental market, studies report the beneficial use of cleaner gobies to control parasites in the aquaculture of other species such as cobia, dusky grouper and greater amberjack (Zimmermann *et al.*, 2001; Benetti *et al.*, 2007; Souza *et al.*, 2014). The easy adaptation to captive conditions paved the way for gobies to become one of the first marine fishes to be successfully bred in captivity. The first description of reproduction and embryonic development for a gobiid species was reported in the 1970s (Valenti, 1972; Colin, 1975). Despite being one of the families with the largest number of species currently being commercially produced, scientific studies related to the culture of marine ornamental gobies are still scarce. The few studies available on ornamental goby aquaculture are related to the understanding of their reproduction, early development and larval feeding (Olivotto *et al.*, 2005; Meirelles *et al.*, 2009; Shei *et al.*, 2010, 2012; Côrtes & Tsuzuki, 2011; Pedrazzani *et al.*, 2014).

14.2.1 Reproduction

The family Gobiidae features a large variety of strategies and reproductive behaviour. Protogynous and bidirectional hermaphroditism, gonochorism and juvenile sex differentiation are found in several genera (Wittenrich, 2007; Munday *et al*., 2010; Herlen *et al*., 2011). Some species form complex social hierarchies where individuals queue for breeding rights, and growth rates are controlled by social interactions (Herler *et al*., 2011). The genera *Paragobiodon* and *Gobiodon* are typically bidirectional protogynous hermaphrodites that live in larger groups and form a hierarchical system. The top of this hierarchy includes a male and a functional female with monogamous behaviour, followed by smaller immature females that can accelerate growth when they reach reproductive condition (Kuwamura *et al*., 1994; Nakashima *et al*., 1996). There are also records of gonochorism and protogynous hermaphroditism in the same genus, such as *Elacatinus oceanops* and *Tigrigobius multifasciatus*, respectively (Robertson & Justines, 1982; De Mitcheson & Liu, 2008) (Figure 14.1).

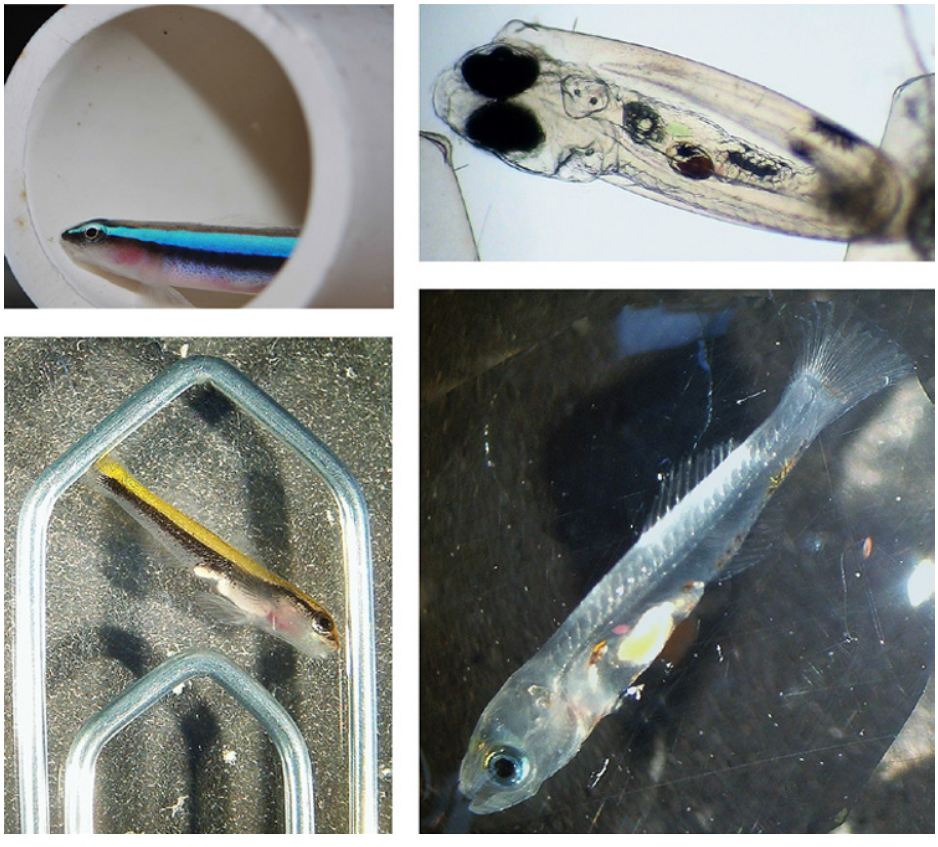


Figure 14.1 Clockwise from the top left: neon goby *Elacatinus oceanops* broodstock; pre-hatch embryo of *E. figaro* at 168 h post-fertilization; 25 days post-hatch larva of *E. evelynae*; post-metamorphosis individual displaying juvenile coloration. Photos by Marcelo Shei (top left and right) and Ike Olivotto (bottom left and right). (*See insert for color representation of the figure*.)

Generally, species that live in small groups tend to form monogamous pairs. The bond between the pairs is long lasting and is not broken even if the fishes are briefly separated, as described for *E. evelynae* (Whiteman & Côté, 2003; 2004; Harding *et al*., 2003; Herler *et al*., 2011). Most species are sexually monomorphic, but there are species in which differences can be identified through examination of the urogenital papilla. In other species, such as *E. puncticulatus*, males are slightly larger and show dichromatism during courtship and spawning. The male individuals of *Coryphopterus* spp. show the emergence of spines on the dorsal and anal fins during the reproduction period (Thresher, 1984; Wittenrich, 2007; Pedrazzani *et al*., 2014). Size can be used for sexual differentiation in many hermaphroditic species and facilitates the selection and formation of reproductive pairs (Wittenrich *et al*., 2007). In captive conditions, it is easier to observe that pre-spawning females tend to show a distended abdomen (Wittenrich *et al*. 2007; Meirelles *et al*. 2009; Shei *et al*. 2010).

14.2.2 Spawning

Pair formation must be performed carefully. Adult specimens, especially males, tend to show significant territoriality and become especially aggressive if placed in small tanks (Olivotto *et al*., 2005; Herler *et al*., 2011). Pairing of gonochorist cleaner gobies such as *Elacatinus figaro* and *E. oceanops* can be quickly attained by placing groups of three or four mature individuals in 30 L tanks with small pieces of PVC. Whenever a pair is formed, it often occurs in a matter of minutes as two individuals swim together and share one or more shelters; the outcast fishes will display submissive behaviour. This pairing process must be closely monitored and outcast individuals should be removed as soon as possible as the aggressions can cause severe injuries and even mortality (Shei *et al*., 2010). In larger tanks, the aggression is slightly less intense and can allow for smaller and immature individuals to grow and form new pairs. Hermaphroditic species such as *E. puncticulatus* and *Tigrigobius multifasciatus* are much easier to pair, as they can be kept in small groups of three or four fishes without significant aggression.

Although many species form social structures that can be simulated in captive conditions, maintaining single heterosexual pairs seems to be more appropriate as it allows the use of smaller tanks, decreases stress conditions related to defending territory and optimizes the broodstock performance (Wittenrich, 2007). Tanks of 15–40 L are adequate for most species. Broodstock should be kept in good water quality conditions, a photoperiod of 12 L:12D and fed commercial marine fish diets, brine shrimp nauplii and chopped seafood including squid, oysters and shrimp.

Spawning begins a few weeks after the pair is formed, and a few days prior the female presents a swollen abdomen while the male cleans the nest (Colin, 1975; Olivotto *et al*., 2005; Wittenrich *et al*., 2007). Spawning usually occurs in the morning, although there are reports of spawning during the afternoon (Shei *et al*., 2010). The eggs are generally elliptical in shape, adhesive and are deposited on the ceiling of the nest. The spawning process takes up to 90 minutes in the case of the barber goby *E. figaro* and the eggs measure from 1.8 to 2.1 mm in length (Meirelles *et al*., 2009, Shei *et al*., 2010). Information related to fecundity, spawning frequency and larval duration for the genera *Elacatinus*, *Gobiodon* and *Priolepis* are presented in Table 14.1.

Males display parental care and during the egg-tending period feed very little, while females begin another maturation process, which highlights the need for a

Table 14.1 Reproductive, embryonic and larval features for seven reared species of marine ornamental Gobiidae, including fecundity, incubation period, spawning frequency and larval duration. n/a = not available.

Species	Spawn size (eggs)	Incubation period (days)	Spawning frequency (days)	Larval duration (days)	Reference
Elacatinus oceanops	50–450	6–10	7–28	26–45	Valenti, 1972; Wittenrich, 2007
Elacatinus figaro	140–1020	5–7	8–10	28–44	Meirelles *et al*., 2009; Shei *et al*., 2010
Elacatinus evelynae	200–250	7	n/a	30–40	Olivotto *et al*., 2005
Elacatinus puncticulatus	200–300	6–7	7	36–42	Wittenrich, 2007; Pedrazzani *et al*., 2014
Tigrigobius multifasciatus	250	5–6	n/a	29–35	Wittenrich, 2007
Gobiodon citrinus	n/a	4	4	33–40	Wittenrich, 2007
Priolepis nocturna	268–3121	5	5–10	n/a	Wittenrich *et al*., 2007

well-balanced diet. The incubation period varies according to species and temperature, averaging 4 days for *Gobiodon* spp., 5 for *Priolepis nocturna* and 7 for *Elacatinus* spp. (Figure 14.1). Hatching of the planktonic larvae usually occurs during the first hours of darkness, but there are reports of it taking place earlier in the day (Shei *et al*., 2010). Eggs can be removed two days before the hatching for *E. figaro* and *E. puncticulatus* and that is most likely also the case for other species (Shei *et al*., 2010; Pedrazzani *et al*., 2014).

14.2.3 Larval Rearing

Adequate management and larval feeding is essential for the successful larviculture of gobies. The use of round tanks and dark walls facilitates water circulation and prey identification by the larvae. Tanks with a light-colored bottom also facilitate the removal of dead larvae and detritus. Aeration and water circulation should be moderate, but strong enough to prevent a concentration of larvae and live feed. If a static system is used, daily partial water changes should be performed in order to remove the old live feed and ensure good water quality and nutrition.

In the case of using a recirculating system, the larval tank must contain a screen allowing the passage of live feeds but not larvae. Removal of old live feed ensures that larvae will always prey on feed with high levels of nutrients, which tends to decrease over the time spent in the tank. The use of green water is common in rearing marine fish larvae and the microalga *Nannochloropsis oculata* is widely used, as it provides rotifers and larvae with essential fatty acids (Coutteau, 1996). Although it is not usual practice, sometimes the photoperiod is kept at 24 L:0D in order to allow the larvae to spend more time feeding. During the larviculture, a 200 lux illumination with 6.500 K lamps can be used (Shei *et al*., 2010).

The newly hatched larvae of *Elacatinus* spp. are large, measuring between 3.0 and 3.4 mm. They are typically transparent, apart from a few chromatophores, and have little or no trace of the yolk sac, which lasts only one day or so after hatching. The larvae are quite active and have pigmented eyes and a fully open and complete digestive tract. The mouth measures about 350 × 230 µm, allowing the larvae to feed on rotifers immediately after hatching (Olivotto *et al*., 2005; Shei *et al*., 2010; Côrtes and Tsuzuki, 2011). They are positively phototactic, which facilitates collection when hatching occurs in the broodstock tank (Wittenrich, 2007; Pedrazzani *et al*., 2014).

For gobies of the genera *Gobiodon* and *Paragobiodon*, which have smaller larvae than *Elacatinus* spp., it is necessary to supply rotifers or other zooplankton species of less than 180 µm in size in order to achieve success during the first feeding period (Wittenrich, 2007). For other species that have even smaller larvae such as *Priolepis nocturna*, there has not been success in juvenile production due to the lack of a food of adequate size and nutritional quality (Wittenrich *et al*., 2007).

The larval rearing is the most crucial process in the aquaculture of gobiids. The first feeding and the settlement period are critical and high mortality events may take place. In the first feeding period, many larvae die from starvation due to inadequately sized or enriched feed. In the settlement period, nutritional deficiencies are compounded by the large physiological effort required to complete the settlement metamorphosis.

Providing good water quality conditions and food in adequate quantities, size and nutritional quality are the key challenges to achieve success in juvenile production. Studies concerning the use of different species and sizes of zooplankton and also enrichment based on highly unsaturated fatty acids - such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) – have been conducted in order to improve results during larviculture. Olivotto *et al*. (2005) reported that the use of smaller zooplankton such as ciliates *Euplotes* sp., small strain rotifers *Brachionus rotundiformis* and enriched *Artemia* metanauplii resulted in a higher survival rate (50%) for *E. evelynae* larvae. Although likely to yield a lower survival rate, it is possible to succeed in producing juveniles of other species with the use of larger rotifers such as *B. plicatilis* and unenriched *Artemia* nauplii.

The end of the larval stage is marked by the settlement of the animals on the tanks bottom and walls. At this time, it is possible to see that the pelvic fins are already fused. In the cleaner gobies *Elacatinus* spp., this event occurs between 26 and 46 days post-hatching (dph), when the animals measure about 8.5 to 9.0 mm in total length. This time frame is dependent mainly on the species, feeding regimen and temperature. The larval duration described for the genera *Gobiodon* and *Paragobiodon* are similar. The animals develop the characteristic adult coloration a few days after settlement (Figure 14.1). Upon settlement, the juveniles accept inert diets and are gradually weaned. For *Elacatinus* spp., sexual maturity is reached at approximately 2.5 cm, when the individuals are about 90 days old and at commercial size (M. Shei, unpublished data).

14.3 Blennies (Blenniidae)

Many of the members of the family Blenniidae are also known as combtooth blennies, due to their noticeable arrangement of teeth in each jaw that resembles a comb (Thresher, 1984). Many species have rows of short canine teeth, but others have

broader teeth. Blennies are small, characterized by a blunt bulbous head, prominent eyes and lips, and no scales on the body. They generally possess an elongated body, long dorsal and anal fins and appendages called cirri on some part of the head. (Thresher, 1984; Helfman *et al*., 2009). The family has about 360 species widely distributed across the globe, from the Antarctic Peninsula to the various shallow tropical environments. Although there are representatives in fresh and brackish water, they are more abundant in marine environments, distributed in a wide range of rocky shores, tide pools and coral reefs microhabitats (Thresher, 1984; Ditty *et al*., 2005; Helfman *et al*., 2009).

A unique feature among fishes is shown in this family. Species of the genus *Meiacanthus* (including the Canary Fang Blenny *M. oualanensis*, Striped Fang Blenny *M. grammistes* and the Blackline Fang Blenny *M. nigrolineatus*) have toxic buccal glands, that are used against predation (Fishelson, 1974; Wittenrich, 2007; Helfman *et al*., 2009). The reef species, of which many are omnivorous, feed on a variety of zooplankton, small benthic invertebrates and algae. Some species such as *Aspidontus taeniatus* and *Plagiotremus rhinorhynchos* mimic the colors and swim pattern of other fishes such as the Cleaner Wrasse *Labroides dimidiatus* and attack the surrounding fishes, removing scales and skin (Thresher, 1984; Helfman *et al*., 2009; Hundt *et al*., 2014).

As well as other families discussed in this chapter, the Blenniidae family has a strong relevance in the marine ornamental fish market, listed among the 10 most traded fish families. However, studies and data regarding the aquaculture of these fish are still very scarce (Green, 2003; Wabnitz *et al*., 2003).

14.3.1 Reproduction

The blennies belong to one of the teleost families exhibiting the most diverse array of sexually dimorphic traits. Size dimorphism is common and males tend to be larger than females. During the spawning period, females are more robust. A few species show sexual dichromatism, especially males that may develop brighter or distinctive colors during the spawning period (Thresher, 1984; Giacomello & Rasotto, 2005; Wittenrich, 2007). There are also morphological differences in the anal fins. Males show flesh pads or swellings on the edge of the anal fin or on the rays of the dorsal and anal fins of several species. This is a feature seen only in males and the size of the swellings seems to change seasonally, especially during the spawning period (Zander, 1975; Thresher, 1984). However, the species belonging to the most commercially-relevant genus, *Meiacanthus*, show no confirmed sexual dimorphism (despite anecdotal observations that males may present longer rays in the caudal fin) and there is controversy on whether they are gonochorists or hermaphrodites. Further studies are urgently needed for us to better comprehend reproduction in these fishes.

In most cases, the female initiates courtship behaviour and approaches the territory guarded by the male, while occasionally displaying courtship-specific colors (Thresher, 1984; Olivotto *et al*., 2010; Moorhead & Zeng, 2011). When approached, the male also displays his courtship-specific colors, leaves his shelter hole and swims towards the female (Thresher, 1984). The male frequently moves in an up-and-down sinosoidal motion. In some species, the male poses at the shelter entrance and shakes his head vigorously as the female approaches (Thresher, 1984). Males of many species also perform an exaggerated and undulated swimming pattern while leading the female to the nest. If the female displays interest, she approaches and inspects the male's shelter

before beginning laying the eggs (Thresher, 1984). Once inside, the female and the male alternate in releasing adhesive eggs and sperm (Olivotto *et al.*, 2010).

Harems are formed instead of monogamous pairs and males will spawn with different females. Egg clutches of up to three different spawns can be deposited in the same nest and the male tends them until hatching (Wittenrich, 2007; Olivotto *et al.*, 2010; Moorhead & Zeng, 2011).

14.3.2 Spawning

To form reproductive harems, groups of adult animals can be placed in 100 to 300 L tanks with various shelters, such as 30–50 mm PVC pipes, flowerpots and small caves. Moorhead & Zeng (2011) tested different types of shelters for nesting and report that *M. atrodorsalis* prefers capped PVC pipe with a single small entry hole for spawning. Pairs can be kept in 60 L tanks (Wittenrich, 2007; Olivotto *et al.*, 2010; Moorhead & Zeng, 2011). Males are territorial towards other males, especially during the spawning period and will choose a shelter to use as a nest and territory. In conflicts between males, aggression often causes stress but very few wounds (Wittenrich, 2007; Olivotto *et al.*, 2011).

After a broodstock group is formed, the first spawn occurs within a few months. Specimens of *M. grammistes* spawned about 3 weeks after conditioning in a temperature of 28 °C, salinity of 30, pH of 8.2 and NO_2^- and $NH_3 < 0.03$ ppm. A photoperiod of 13 L:11D may be provided by two 30 W incandescent lights. For *M. atrodorsalis* spawning occurred after three months and throughout the year regardless of the season (Moorhead & Zeng, 2010). As for feeding, a mix of chopped fresh food including algae, fish, squid, prawns, mussels, seaweed and *Artemia* are described as sufficient to maintain constant spawns (Wittenrich, 2007; Olivotto *et al.*, 2010; Moorhead & Zeng, 2011).

For *M. grammistes*, spawning occurs every 8 to 10 days with a production of 150 to 200 eggs per spawn (Olivotto *et al.*, 2010). The eggs are adhesive, flattened and slightly oval-shaped, and typically measure about 1 mm in diameter (Fishelson, 1976; Olivotto *et al.*, 2010). The incubation period is reported to be 7 days at 26 °C for *M. grammistes*, 8 days at 28 °C for *Petroscirtes breviceps* and 11 days for *M. nigrolineatus* (Fishelson, 1976; Wittenrich, 2007; Olivotto *et al.*, 2011). Wittenrich (2007) describes that the nest containing the eggs can be removed for artificial incubation one day before the oldest spawn hatch. The nest may be placed in a vertical position and under low aeration to provide motion. In case where other clutches are developing after the first clutch hatched, the nest may be transferred to another tank in order to continue the artificial incubation.

14.3.3 Larval Rearing

Newly hatched larvae of *Ecsenius bicolor, Meiacanthus* spp. and *Petroscirtes breviceps* measure 3.5, 3.0 and 3.0 mm, respectively (Dotsu, 1982; Thresher, 1984; Wittenrich, 2007; Olivotto *et al.*, 2010). At this moment, larvae are very active, present little pigmentation, a finfold and a yolk sac. The mouth is already open and the digestive tract is functional. Average mouth gape height and width described for newly hatched larvae of *M. atrodorsalis* was 307.3 ± 34.7 and 263.8 ± 17.4 µm respectively. On the first

day post-hatch (dph) larvae present more developed eyes and already demonstrate predation abilities and feed on rotifers (Moorhead & Zeng, 2011).

After 10 to 15 dph, *M. atrodorsalis* larvae begin to develop caudal and pectoral fin rays and also the flexion of the notochord. The *Meiacanthus* spp. larvae begin to settle and display adult coloration at 11 to 13 mm in total length. This occurs around 26 dph for *M. nigrolineatus* and *M. grammistes* and at 35 dph for *M. atrodorsalis*. However, Wittenrich (2007) highlights that the larvae of *M. grammistes*, *M. atrodorsalis* and *P. briveceps* may have a heterogeneous size distribution and therefore higher attention to adequate feeding is demanded. Larvae can be fed rotifers *Brachionus plicatilis* until 15 dph, *Artemia* nauplii between 10 and 18 dph, enriched *Artemia* nauplii between 15 and 28 dph and a weaning diet from 22 dph onwards (Wittenrich, 2007). Olivotto *et al*. (2010) reports an improvement in growth, survival and metamorphosis rate by adding copepods for *M. grammistes* larvae.

14.4 Dottybacks (Pseudochromidae)

Dottybacks are small fishes belonging to the family Pseudochromidae, noted by their elongated body and speckles located on the dorsal region. They are found widespread in the Pacific Ocean and are endemic in specific sites in the Indian Ocean, such as the Red Sea (Lubbock, 1976; Gill & Edwards, 2004). About 150 valid species are placed in 23 different genera (Froese & Pauly, 2013), of which *Pseudochromis* is the most representative. Several new species have been described in recent years, especially in Indonesia (Gill & Allen, 2011; Gill *et al*., 2012).

Since the aquaculture of clownfish, the culture of no other group of ornamental fish has caused the commercial impact that dottybacks have (Wittenrich, 2007). The reasons that make dottybacks popular are their resilience and reduced size, apart from being docile towards other reef inhabitants and displaying a wide variety of bright colors and patterns. There are a few dozen species of Pseudochromidae that are relevant to the ornamental industry and about ten species are currently commercially mass-produced. The most relevant ones are distributed in the genera *Pseudochromis*, *Pictichromis* and *Manonichthys*.

While the aquaculture of dottybacks has been somewhat developed, it still lacks scientific data. A few bottlenecks keep it from being a worldwide activity, including pairing broodstock, successful hatching and larval mortality due to poor nutrition (Mies *et al*., 2014). These issues are critical to the commercial success of dottyback aquaculture and are addressed in the following paragraphs. However, in order to further optimize culture protocols and overcome bottlenecks, additional scientific research must be performed and more data must be produced.

14.4.1 Reproduction and Behaviour

The family Pseudochromidae is comprised of sequentially hermaphroditic species. The majority of species presents protogynous hermaphroditism, but there are some species from specific genera that present protandrous hermaphroditism (e.g. *Cypho* spp. and *Ogilbyina* spp.) (Wittenrich, 2007). In addition, some dottybacks species such as *P. fridmani* and *Pictichromis porphyrea* are known to exhibit bi-directional sex

change (Wittenrich & Munday, 2005; Kuwamura *et al*., 2015). Therefore, as protogynous species, after changing from female to male, it is possible to reverse from male to female. However, sex reversal in the opposite of the natural direction tends to take much longer and frequently over three months after pairs are placed together (Wittenrich & Munday, 2005; Wittenrich, 2007). While it has not been verified experimentally, this trait is probably found in the majority of dottybacks.

Most species of dottybacks present no significantly observable sexual dimorphism. There are only 14 species that have been described as sexually dichromatic (Wittenrich, 2007), but none of them among the species that are currently being mass-produced. There are particular species that may exhibit significant color variation. For instance, *Pseudochromis fuscus* is known to display six different color morphs (Messmer *et al*., 2005).

When a dottyback pair is placed together, aggressive behaviour is frequently observed, ranging from mild to severe (Wittenrich, 2007). Some species tend to exhibit trends in aggression. *Pseudochromis fridmani* is a species known to be docile while *Pictichromis paccagnellae* is known to be extremely aggressive towards its conspecifics (Wittenrich, 2007). Considering the lack of sexual dimorphism, same sex individuals are commonly placed together in order to form a pair, which increases aggressive behaviour (Black *et al*., 2011) and delays bonding and pair formation. Introducing individuals from different gender will generally result in earlier pair formation.

Once the pair is formed, courtship behaviour will take place and spawning will generally follow. A demersal egg mass is deposited by the female inside a shelter and tended by the male until hatching (Olivotto *et al*., 2006; Wittenrich, 2007). Unlike clownfish, the egg mass is not adhered to the substrate. The parental care displayed by the male has a pivotal role in successful hatching (Wittenrich, 2007). Under inadequate conditions and in particular situations (such as stress or low feed quality), the male may consume the egg mass in events of filial cannibalism (Mies *et al*., 2014).

14.4.2 Broodstock and Spawning

Broodstock can be kept in water conditions that simulate tropical coral reefs, at temperatures between 25–27 °C, salinity 33 and nutrient concentrations as low as possible (Olivotto *et al*., 2006; Wittenrich, 2007). Photoperiod can be set to between 10 L:14D and 12 L:12D. A broodstock pair of Pseudochromidae may be kept in a tank with a volume as low as 30 L, but a larger volume (40–60 L) is recommended. Feeding several times a day using a formulated diet with a ratio of about 3:1 (crude protein:crude lipid) is suggested (Holt, 2011; Mies *et al*., 2014), but using home-prepared fresh seafood diets are also suitable (Olivotto *et al*., 2006; Wittenrich, 2007). Individuals at an age of 6 months and with 3.5 cm in length are already sexually mature (Moe, 1997) and can be used as broodstock. However, larger individuals (between 6.0 and 9.0 cm) tend to produce a higher amount of quality eggs (M. Mies, unpublished data).

The greatest challenge in broodstock maintenance is successfully pairing the fish. Considering that dottybacks exhibit bi-directional sex change (Wittenrich & Munday, 2005; Kuwamura *et al*., 2015), in most cases, any two fish will eventually form a pair. The true challenge lies in overcoming the aggressiveness. There are many resources that can be employed to reduce aggression and effectively getting the fish to bond and tolerate each other. One of them is to introduce shelters and hiding places such as PVC

tubes and rock pieces with many crevices and orifices (Wittenrich, 2007). However, more aggressive species such as *Pictichromis* spp. and *Manonichthys* spp. display higher levels of aggression that frequently results in the death of one of the fish and must be kept separated until effectively bonded (Wittenrich, 2007). Placing the submissive fish inside a smaller tank positioned inside the larger tank has yielded good results (Mies *et al*., 2014). The internal tank is made of glass and covered with a plastic screen, therefore the fish can see each other and there is water exchange between the tanks. Every 10 to 14 days, the submissive fish can be released and exposed to the larger fish until there is no longer any life threat. In most cases, the time for the fish to bond is positively correlated to the aggression level. Another less usual but effective way of pairing dottybacks is placing a large number of juveniles within the same tank (Sayadi *et al*., 2012). A few pair formations will quickly take place and these can be removed and placed in a broodstock tank.

After the pair is formed, it usually takes about four weeks until spawning initiates (Olivotto *et al*., 2006; Wittenrich, 2007). The time between bonding and spawning is called "bonding-spawning interval". A few days prior to spawning, the female presents a swollen abdomen and eats little, until spawning takes place shortly after the pair displays courtship behaviour. Typically, the male swims towards the female and quickly returns to his shelter, wiggling his tail. After this ritual is repeated a few times, the female follows the male to his shelter and then spawns a demersal egg mass, which is promptly fertilized by the male. The spawning frequency for dottybacks varies among pairs, but it generally occurs in fixed intervals of every six to eight days (Moe, 1997; Wittenrich, 2007) and always at a specific time of day (Olivotto *et al*., 2006).

The egg mass (Figure 14.2) is oval-shaped, generally 2.5 cm in diameter and contains about 1200 embryos, each measuring 1.5 mm (Thresher, 1984). Adhesive threads hold the embryos together, similar to that described for damselfish (Mooi, 1990). After fertilization, the male tends the egg mass until hatching, guarding the shelter from intruders and vigorously oxygenating the embryos (Olivotto *et al*., 2006; Wittenrich, 2007). Time of embryo incubation is temperature dependent, but it ranges from for four to six days (Brons, 1996; Olivotto *et al*., 2006; Wittenrich, 2007) (Figure 14.2).

Hatching can be either natural or artificial. Natural hatching takes place inside the shelter guarded by the male, without human intervention. After lights out, the male aids in the hatching process by agitating the egg mass and the newly hatched larvae swim out of the shelter. The free-swimming larvae can be collected with a bowl or a pipette of large diameter. However, artificial hatching can be employed and in this case the egg mass is pulled from the shelter and placed in a tumbler already positioned in the larval rearing tank. A gentle water or airflow is provided in order to keep the egg mass in constant motion. After lights out on the hatching day, directing a light source to the egg mass will stimulate hatching. The hatch rate is generally over 90%, but in cases where this number is lower, there are techniques that can be employed to enhance hatching success. The most traditional of them is keeping the egg mass for 15 minutes in a 0.15% solution of formalin.

One of the main bottlenecks in dottyback aquaculture is successful hatching (Mies *et al*., 2014). That issue is directly related to filial cannibalism, an event where the male fish cannibalizes the egg mass before hatching (Petersen & Marchetti, 1989). This phenomenon may occur for several consecutive batches; it has been reported

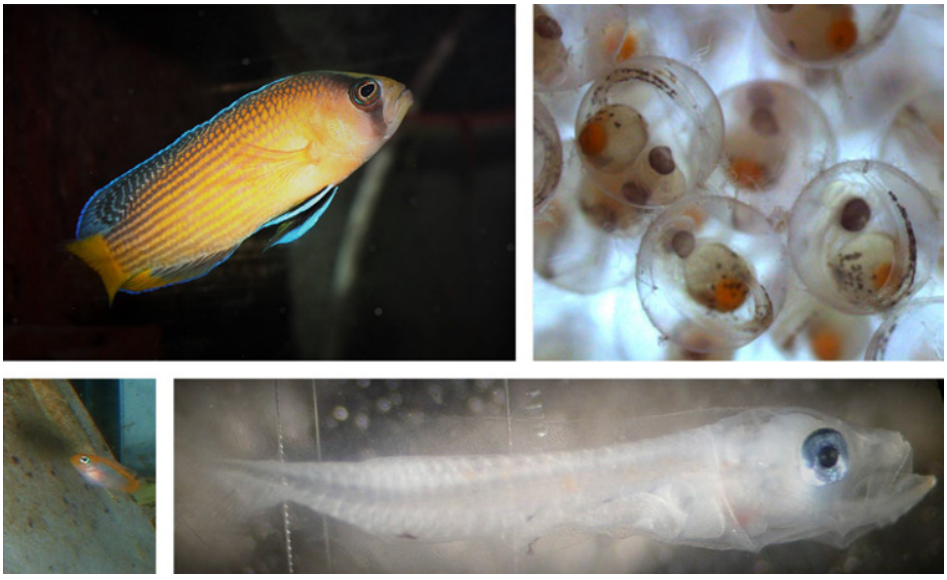


Figure 14.2 Clockwise from the top left: Splendid Dottyback *Manonichthys splendens* male broodstock; Close-up of an egg mass spawned by *Pseudochromis aldabraensis* at 96 h post-fertilization showing well-developed embryos; *P. fridmani* larvae at 7 days post-hatch and juvenile Sunrise Dottyback *P. flavivertex* two days post-settlement, showing yellow pigmentation. Photos by Marcelo Shei (top left); Arthur Z. Güth and Miguel Mies (top right) and Ike Olivotto (bottom left and right). (*See insert for color representation of the figure.*)

that a young pair of *Pseudochromis fridmani* ate its first 24 egg batches before allowing an egg mass to hatch (Mies *et al.*, 2014). Studies have linked this phenomenon to the development of hypoxic conditions within the egg mass (Payne *et al.*, 2004), which is directly related to the parental care effectiveness. However, filial cannibalism may be also related to egg quality and energy content (Sargent, 1992; Klug, 2009), indicating that broodstock may consume the offspring if its quality is low. While no practical solution has been proposed to solve this problem, acquiring large and experient broodstock, feeding adequately and offering suitable water conditions reduces the odds of cannibalism.

Therefore, when selecting dottybacks for broodstock, one must consider that pairing young fish may avoid aggressive behaviour, but it may take longer until quality eggs are spawned and filial cannibalism may occur. On the other hand, pairing larger fish may result in higher quality eggs faster, but it may take longer until bonding because of aggressive behaviour. As mentioned previously, size, sex and behaviour are significantly relevant factors that directly influence both bonding and spawning.

14.4.3 Larval Rearing

Newly hatched pseudochromid larvae typically measure about 3.5 mm in total length (Olivotto *et al.*, 2006), but this may vary among species – the larvae of *P. dilectus* are remarkably large, measuring more than 5 mm (Madhu *et al.*, 2016). They already present a complete and open digestive tract and begin feeding immediately. The mouth

apparatus of dottyback larvae is less developed than that of clownfish (Wittenrich & Turingan, 2011), but they actively hunt and are fully capable of preying on smaller organisms. Larvae of some species (especially *Pictichromis* spp.) have pigmentation and a large number of chromatophores that are rich in carotenoids (Goda *et al*., 2011). Particularly during the first week of development, larvae exhibit strong positive phototaxis and tend to swim in short bursts and slanting upwards.

Larval rearing of dottybacks presents a high level of mortality (Wittenrich & Turingan, 2011), mainly associated with issues related to nutrition and physiological changes (Olivotto *et al*., 2006; Wittenrich, 2007; Holt, 2011). Most larvae are lost near metamorphosis due to poor nutrition (Wittenrich, 2007). Dottyback larvae are extremely fragile and sensitive when compared to the larvae of closer families such as Pomacentridae (clownfish and damselfish) and Gobiidae (gobies). The highest survival rate reported is of 39%, using very low larval stocking densities and heavily employing the use of live feed enrichment (Olivotto *et al*., 2006).

The larval culture system must be designed to minimize stress. Water parameters must be kept stable and in the same conditions as to those described for broodstock. A black round tub of at least 60 L with a central standpipe covered with a 500-μm mesh is recommended. A large diameter tub is suitable and reduces overcrowding, since larvae swim towards light and tend to occupy the same depth zone. Dripping water input slowly changes the water daily and keeps the mesh from clogging. It is wise to place a heater inside the tub, over an air stone at a moderate airflow.

Larvae can be stocked in densities of about 20 individuals L^{-1} and should be fed with live prey organisms such as rotifers and *Artemia* nauplii. The greenwater technique is highly recommended, as well as enriching the live feed (Olivotto *et al*., 2006). Both techniques enhance the DHA and EPA fatty acids content in the live prey and are crucial to increasing larval survival and growth (Olivotto *et al*., 2003). The enrichment of live prey can be performed with products such as Algamac (Aquafauna BioMarine, Inc.) and Selco (INVE Aquaculture).

Three distinct dottyback larval rearing protocols are shown in Table 14.2, all based on the offering of enriched rotifers and *Artemia* nauplii. Rotifers must be offered first

Table 14.2 Three distinct larval rearing protocols (Olivotto *et al*. 2006; Wittenrich 2007; M. Mies, unpublished data) used in dottyback aquaculture, including the amount and time frame related to the offering of enriched and un-enriched live feeds. n/a = not available.

Feed	Olivotto *et al*. (2006)	Wittenrich (2007)	M. Mies, unpublished data	Recommended feed density
Greenwater	n/a	n/a	Throughout larval development	10^4 cells mL^{-1}
Rotifers	–	–	–	–
Enriched rotifers	Days 1–12	Days 1–17	Days 1–12	10–25 mL^{-1}
Artemia nauplii	–	Days 8–24	Days 9–11	4–8 mL^{-1}
Enriched *Artemia* nauplii	Day 9 to metamorphosis	Day 14 to metamorphosis	Day 12 to metamorphosis	4–8 mL^{-1}
Weaning diets	n/a	Day 26 onwards	Day 20 onwards	–

considering their small size and the fact that *Artemia* nauplii are too large for the mouth gape of newly hatched dottyback larvae. After nine days post-hatch, nauplii are introduced at lower densities and are then daily increased until it reaches a density of *ca*. 6 nauplii mL^{-1}, as the rotifer density is slowly decreased. If available, the addition of small copepods is welcome at any stage of the larval development (Wittenrich, 2007). Shortly after settlement, it is interesting to begin offering weaning diets with suitable content of fatty acids. If the larvae capture formulated diets early, the probability that they will survive through metamorphosis increases significantly. Under suitable conditions, larvae attain metamorphosis between days 23 and 35 post-hatch (Olivotto *et al*., 2006, 2009).

One month after the majority of the larvae have completed metamorphosis and are fully color and measure about 2.0 cm, they must be transferred to grow-out tanks. Rectangular glass tanks are suitable and must have lids in order to prevent the fish from jumping. Juveniles may display aggressiveness towards each other, especially if there is competition for territory or hiding places. The best approach to solve this problem is to provide shelter for each individual or no shelter at all. A typical stocking density of one juvenile for every 1.5 L is adequate. Combining that with feeding several times a day and keeping the tanks clean from detritus produces individuals of 4.5–5.0 cm in four months of grow-out.

14.5 Damselfishes (Pomacentridae)

Pomacentridae is one of the largest families among marine teleosts, comprised of 387 valid species (Froese & Pauly, 2013). This section addresses the subfamily Pomacentrinae, commonly referred to as "damselfishes", which accounts for 357 valid species. The remnant species belong to the subfamily Amphiprioninae and are addressed in Chapter 12. The damselfishes are placed in 27 different genera, of which *Pomacentrus*, *Stegastes* and *Chromis* are the most representative.

The family Pomacentridae is probably the most representative of all coral reef families, comprising a significant portion of the fish biomass in coral reefs (Williams & Hatcher, 1983). The fishes from this family are keystone species in coral reef ecology and are frequently used as model organisms in several studies regarding coral reef fish behaviour and feeding ecology. Typically omnivores, damselfishes are known to consume zooplankton, small benthic invertebrates, algae and detritus (Feitosa *et al*., 2012). Damselfishes are also known to be site-attached, living in somewhat permanent locations and population groups (Coates, 1980; Fishelson, 1998). Despite being typical coral reef inhabitants, there are four species of damselfishes known to occur in brackish or freshwater, such as *Neopomacentrus aquadulcis* (Jenkins & Allen, 2002). Adaptability and resilience are remarkable features among this family, as many species reportedly show increased resistance to pathogens (Wittenrich, 2007) and can also adapt to thermal fluctuations related to global warming (Grenchik *et al*., 2013).

14.5.1 Reproduction and Behaviour

Damselfishes display a wide variety of sexual patterns, but are typically protogynous hermaphrodites. However, gonochorism has been reported for several species, mainly in the genera *Abudefduf*, *Chromis* and *Pomacentrus* (Fishelson, 1998). Through

histological examination of the gonads, the genus *Dascyllus* has been shown to present both hermaphroditic and gonochoristic species (Schwarz & Smith, 1990; Asoh & Yoshikawa, 2003). In the typical protogynous species of damselfishes, juveniles tend to become females as they mature and social units are dominated by one or more males (Wittenrich, 2007).

Sexual dimorphism is almost absent in most damselfishes; thorough examination of the genital papilla (Emel'yanova *et al*., 2009a;b) may be the only effective method to verify the gender in species that show no significant dimorphism. Some species do present drastic sexual dichromatism such as *Chrysiptera taupou* and *C. cyanea*, which display a yellow caudal peduncle in females. There is also an occasional temporary dichromatism, in which individuals may display color changes during spawning events. This phenomenon has been described for *C. biocellata* (Thresher & Moyer, 1983) and especially for *Chromis viridis*, where males exhibit blackened dorsal fins near spawning (Wittenrich, 2007).

Damselfishes may be mature at an early age. *Acanthochromis polyacanthus* has been shown to have fully matured at 9 months of life (Kavanagh, 2000). Gonadal development in damselfishes is usually synchronous within a population and controlled by the endocrine system (Barnett & Pankhurst, 1994). Vitellogenesis is mediated by melatonin and dopamine and significantly affected by environmental cues such as photoperiod length (Badruzzaman *et al*., 2013). Artificial stimulation to maturation and spawning has been reported for *Dascyllus trimaculatus* and *Abudefduf sexfasciatus* (Emel'yanova *et al*., 2009a;b). Two injections ($5 + 15\,\mu g\,kg^{-1}$) of luteinizing hormone-releasing hormone analogue (LHRHa) may be applied under the pectoral fin and into the body cavity (Emel'yanova *et al*., 2009a). However, due to the small size of the fish and the aggressiveness of the procedure, broodstock are generally left to mature naturally under adequate water conditions and feeding regimens.

Most of the members of the family Pomacentridae are polygamous and promiscuous spawners. Females often spawn with several males consecutively on a single day and leave eggs with each one of them (Fishelson, 1970; Goulet, 1997). *In situ* observations show that females choose to mate preferably with fitter males that are already guarding egg nests (Manica, 2010). Males also mate with several females, sometimes simultaneously, as reported for *Amblyglyphidodon leucogaster* (Goulet, 1995). *Acanthochromis polyacanthus* is the most obvious exception to promiscuity, as this species is monogamous (Hilder & Pankhurst, 2003).

Compared to dottybacks, damselfishes show much less intraspecific and pairing-related aggressive behaviour. While they do vigorously defend their territory from intruders, mainly due to the responsibilities in spawning and egg-tending (Barnett & Pankhurst, 1996; Osório *et al*., 2006), death of a conspecific as a consequence of violent behaviour is very rare.

14.5.2 Broodstock and Spawning

Considering the lack of sexual dimorphism, the most effective way of pairing damselfishes is placing small groups of six to eight fishes in a 120 L tank (Wittenrich, 2007; Olivotto *et al*., 2009). After visually ascertaining that two fishes are bonding and swimming together, the pair can be transferred to broodstock tanks with a volume of at least 50 L. It is important not to underestimate the broodstock tank volume as confined

and stressed fish may have reproduction inhibited by an increase in plasma cortisol levels and subsequent reduction of androgen and estrogen steroids (Pankhurst, 2001). Having PVC tubes or ceramic pieces in the tank is also useful as it generates hiding places and allows for easy egg collection. The water conditions and photoperiod in the broodstock system can be kept in the same values as those described for dottybacks. Broodstock must be fed several times per day as some damselfishes are known to consume as much as 5% of their body weight (equivalent to 8000 copepods) every day (Pinnegar *et al*., 2007). Having large and well-fed damselfish broodstock will render higher quality eggs (Kerrigan, 1997). Broodstock diet may be based on formulated diets or home-prepared seafood, but preferably with a slightly higher content of algal ingredients, given that this is a relevant dietary item for damselfishes (Letourneur *et al.*, 1997).

Prior to spawning, broodstock pairs of damselfishes will engage in courtship behaviour. Pomacentridae typically have an elaborated courtship behaviour that includes particular swimming patterns (Fishelson, 1970) and sound production, including chirps and grunts (Mann & Lobel, 1997). The complexity of this behaviour is higher in non-dichromatic species (Thresher & Moyer, 1983), but the males of most species will at least swim around the female and entice her to his shelter (Wittenrich, 2007). A known exception is the White Belly Damsel (*Amblyglyphidodon leucogaster*), which presents no courtship behaviour (Goulet, 1995). Not long before spawning, males prepare the nesting site by removing debris from the substrate. After the courtship, the female presses her abdomen against the substrate and lays an egg mass that adheres to the substrate. It has been reported that some damselfishes only spawn well-developed and mature eggs, while pre-vitellogenic eggs are kept in the gonads (Emel'yanova *et al.* 2009a). Spawning activity in damselfishes generally takes place in a fixed time of the day, around dawn (Thresher, 1984) and there is no evidence that there is any relation to lunar cycles (Goulet, 1995). The frequency of spawning is finely mediated by temperature (Hilder & Pankhurst, 2003), but it is generally recorded every seven days, depending on the species (Asoh & Yoshikawa, 2002; Olivotto *et al*., 2003). Females may occasionally spawn on two consecutive days (Goulet, 1997; Asoh & Yoshikawa, 2002).

Damselfish egg clutches are very similar to those of other demersal spawners like clownfish and gobies, but slightly different from those of dottybacks. Damselfish egg clutches are a single layer of eggs adhered to the substrate and not a loose and spherical egg mass. Egg clutches can contain several hundreds to a few thousand elliptical eggs of about 1.25 mm in length, depending on the species (Olivotto *et al.*, 2003; Wittenrich, 2007; Setu & Ajithkumar, 2010). Embryos have many chromatophores and the eggs may have several oil droplets that help them to orientate upwards (Kingsford, 1985). The male tends the egg clutch until hatching, mainly by oxygenating and chasing intruders (Asoh & Yoshikawa, 2002). A male can hold several different egg clutches simultaneously if more than one spawning female is present (Goulet, 1997; Wittenrich, 2007). Time of parental incubation and embryonic development are also regulated by temperature, but typically last for five days (Alshuth *et al.*, 1998; Olivotto *et al*., 2003). Hatching takes place a few hours after lights out and hatch rates are frequently over 90% (Olivotto *et al*., 2003; Wittenrich, 2007). Hatched larvae can be collected with a bowl and transferred to the larval rearing tank. Another strategy is to collect the piece of rock or pipe in which the eggs are adhered and transfer them to the larval rearing tank a few hours prior to hatching. Embryos not ready to hatch at one particular night will only hatch at the next night and not during the day (Doherty, 1983; Asoh & Yoshikawa,

2002). Filial cannibalism events may take place during the embryonic development in events of small or low quality batches (Sargent, 1992), but it is much less frequent than the case for dottybacks.

14.5.3 Larval Rearing

Pomacentrid larvae are planktonic and frequently migrate from one reef to another, representing the dispersive life stage in these organisms. Apart from the genera *Altrichthys* and *Acanthochromis*, which present bi-parental larval caring (Nakazono, 1993), all damselfishes have a dispersive pelagic larva. As a consequence, damselfish larvae can comprise an important part of the ichthyoplankton in coral reefs (Kingsford, 1985), even though less than 1% successfully settles and undergoes metamorphosis (Wellington & Victor, 1989). The larval duration for one hundred species of damselfishes has been documented and typically varies between 12 and 39 days, with *Stegastes* and *Chromis* the two genera presenting the longer lasting larval development (Wellington & Victor, 1989).

Newly hatched larvae average 2.0 mm in length (Wittenrich, 2007) and are positively phototactic, swimming upwards immediately after hatching (Kingsford, 1985). The larval hearing system is well developed and may be used for localization (Leis & Lockett, 2005). Larvae are distributed in the upper layer of the water column and swim at a speed of 10 cm s^{-1} (Leis & Carson-Ewart, 2002). Growth is sigmoidal and larvae are larger than 3.0 mm at already one week post-hatch (Thorrold & Milicich, 1990; Wittenrich, 2007). Notochord flexion takes place at *ca*. 10 days and metamorphosis at *ca*. 30 days at 15 to 20 mm in length (Alshuth *et al*., 1998; Kavanagh, 2000). The completion of metamorphosis is marked by squamation, drastic pigmentation and notochord post-flexion. Unlike many other families, most damselfish larvae are unable to delay metamorphosis and perish if conditions are inadequate to do so (Wellington & Victor, 1989).

The larval system can be kept in the same water conditions as those described for damselfish broodstock and dottybacks. Wittenrich *et al*. (2012) recommends the use of larger black round tubs, with a volume higher than 100 L, as this has proven critical to the successful aquaculture of *Abudefduf saxatilis*. Olivotto *et al*. (2003) reports that employing a 24 L:0D photoperiod is fundamental to culturing *Chrysiptera parasema* larvae. However, while very useful, this technique must be employed with care given that some species may suffer from slow growth and low survival (M. Mies, unpublished data). The aquaculture of damselfish larvae has had variable survival rates. A bit more sensitive than clownfish, survival rates for damselfish larval rearing have been reported of up to 40%, especially when in lower stocking densities (Danilowicz & Brown, 1992). Densities between 7 and 10 larvae L^{-1} have yielded good results (Emel'yanova *et al*., 2009b; Wittenrich *et al*., 2012). Early starvation is a significant cause for early mortality as yolk and oil reserves are fully consumed at 4 days post-hatch (Alshuth *et al*., 1998). Therefore, feeding immediately after hatching is essential and well-fed larvae have been shown to have more stamina, swim and grow faster and have a higher chance of survival (Wilson & Meekan, 2002; Leis & Clark, 2005).

Feeding different sources of live food significantly improves larval survival rates. Apart from the traditional offering of rotifers and brine shrimp nauplii, the addition of greenwater, copepods and ciliates has proven very successful (Setu *et al*., 2010). Employing the greenwater technique is critical for culturing damselfishes such as

Dascyllus spp. and *Neopomacentrus cyanomos* (Danilowicz & Brown, 1992; Setu *et al*., 2010). *Isochrysis galbana*, *Nannochloropsis* spp. and other green algae or diatoms at 10^6 cells mL^{-1} are recommended (Gopakumar & Santhosi, 2009). Small-sized strains of rotifers can be offered until the later stages of larval development (around day 20), preferably enriched and at densities of 20 rotifers mL^{-1} (Olivotto *et al*., 2003; Wittenrich, 2007). Offering enriched *Artemia* nauplii after days 12–15 is crucial (Southgate & Kavanagh, 1999), at densities of 4–5 individuals mL^{-1} (Olivotto *et al*., 2003). For some species (e.g. *Chrysiptera cyanea*) additional food sources such as copepods and wild zooplankton seem to be the difference between successful larval development and no survival (Gopakumar *et al*., 2009; Wittenrich *et al*., 2012). As larvae approach settlement, they tend to swim to deeper areas of the culture tank. The presence of live colonies of acroporid corals appears to stimulate metamorphosis in some species (Danilowicz & Brown, 1992) and may be added if necessary. Such colonies are also useful in grow-out systems keeping a high density of juveniles.

14.6 Other Demersal Spawning Families

Apart from the demersal spawning families mentioned previously in this chapter, there are a few others worthy of note. While very little information has been produced about their reproduction and aquaculture, the families Grammatidae, Plesiopidae and Microdesmidae have been successfully cultured and are occasionally commercially available.

14.6.1 Grammatidae

The family Grammatidae is comprised of 13 species placed in the genera *Gramma* and *Lipogramma* (Froese & Pauly, 2013) and all of them are restricted to the Western Tropical Atlantic. Along with Plesiopidae, this family is very closely related to Pseudochromidae (Mok *et al*., 1990) and they share several morphological characteristics. The most famous representative in Grammatidae is *Gramma loreto*, also called Royal Gramma. The vast majority of the available information on Grammatidae aquaculture was produced for *G. loreto*. The Royal Gramma is gonochorist, but juveniles are known to differentiate their sex when reaching adulthood (Asoh & Shapiro, 1997). Sexual dimorphism is absent, but males tend to grow larger (Wittenrich, 2007). Pairing is achieved by placing several individuals in tanks larger than 100 L, until two bonding fish are spotted and then transferred to a tank of 80 L. Pairs should be heavily fed with crustacean meat and lighting should be kept dim (Wittenrich, 2007). PVC shelters should be provided in a similar fashion than that for dottybacks. Males have a complex behaviour of activities related to egg guarding and nest site maintenance, including bringing algae such as *Caulerpa* spp. into their shelter (Asoh & Yoshikawa, 1996).

Small batches of typically 25–40 eggs are produced and spawning takes place at night, sometimes for several consecutive days (Wittenrich, 2007). Therefore, eggs inside the shelter will be in different stages of development and the larvae will hatch on different nights. After 7–11 days of incubation, 3.5 mm long larvae will hatch and can be collected with bowls (Asoh & Yoshikawa, 1996; Wittenrich 2007). Rotifers at 10 individuals mL^{-1} can

be offered immediately after hatching and newly hatched brine shrimp after 10 days. Enriched brine shrimp can be offered after day 20 and weaning diets after day 26 (Wittenrich, 2007). Metamorphosis takes place after approximately 26 days and it is important to provide individual shelter for the juveniles, as aggression is significantly intensified (Wittenrich, 2007).

14.6.2 Plesiopidae

The Plesiopidae comprise 49 species distributed among 12 genera (Froese & Pauly, 2013). Commercially aquacultured species in this family include the Marine Betta *Calloplesiops altivelis*, the Blue Devilfish and the Yellow Devilfish, *Assessor macneilli* and *A. flavissimus*. However, Assessors are mouthbrooders and their culture is detailed in Paragraph 14.7.2 of this chapter. The Marine Betta, on the other hand, is a demersal spawner that comes from the Indo-Pacific and reaches a larger size of 20 cm. This species is a protogynous hermaphrodite (Olivotto *et al*., 2009) and presents no sexual dimorphism. Pairs can be kept in large tanks of 200 L and in dim lighting (Wittenrich, 2007). Courtship behaviour can be observed when the male remains motionless before the female prior to retreating to his cave (Wittenrich, 2007). Spawning frequency is very irregular, but tends to take place every 12 days (Olivotto *et al*., 2009) when the female deposits an adhesive egg clutch of 300–500 eggs (Wittenrich, 2007; Olivotto *et al*. 2009). Hatching takes place on day 6 and newly hatched larvae measure 3.0–4.0 mm (Wittenrich, 2007; Olivotto *et al*., 2009).

Larvae can be fed with rotifers immediately after hatching, brine shrimp nauplii on day 9 and enriched brine shrimp after day 15 (Wittenrich, 2007). Metamorphosis takes place between 40–60 days and offering wild zooplankton is recommended (Olivotto *et al*., 2009). While attractive, the Marine Betta often comes with a high price tag due to their slow juvenile growth rates.

14.6.3 Microdesmidae

The Microdesmidae is a family of gobioid fish whose members often do not have any ornamental value. The exceptions are a few species in the genera *Nemateleotris* and *Ptereleotris*. These organisms are called "Dartfishes" or "Hovering Gobies" due to the fact that they frequently hover over burrows on sandy substrate. While these species are very often found in commercial establishments, their aquaculture is still taking its first steps. The only technical or scientific document on their culture comes from Madhu & Madhu (2014), for *N. decora*. The authors of the article placed several individuals together and pairs formed and spawned after 6 months. The males were slightly larger and the females deposit egg clutches similar to those of gobies and clownfish. Spawning took place between 5 and 8 a.m., with 400–500 eggs per clutch. Incubation period was of 96 h at 29 °C and parental care seems critical for successful hatching. Larvae measured 2.0 mm and were stocked in 250 L tanks at a density of 1.0 larvae L^{-1}. The larval mouth gape is small (100 µm), therefore the addition of ciliates (*Euplotes* sp.) and small strains of enriched rotifers at a rate of 20 individuals mL^{-1} each was crucial to survival. Greenwater, copepod nauplii and high densities of brine shrimp nauplii (6 mL^{-1}) also seemed to perform an important role and the overall metamorphosis rate was a remarkable 66% after 35–40 days.

14.7 Other Mouthbrooders

The ecology and aquaculture techniques for the most emblematic mouthbrooding species featured in marine aquariums, the Banggai Cardinalfish (*Pterapogon kauderni*) were addressed in detail in Chapter 13; the next sections will briefly address other species of mouthbrooders, belonging to the families Apogonidae, Plesiopidae and Opistognathidae. Overall, the broodstock husbandry techniques for these species are very similar to the ones for the Banggai Cardinalfish (see Chapter 13), but the great difference is that they orally incubate only the embryonic stage, and the development is indirect. Therefore, they do have a planktonic larval stage that must be reared in similar conditions to those described for the demersal spawning species previously mentioned. While very little information is available on the aquaculture of these species, the following section gives an overview of their basic reproductive aspects and larval rearing.

14.7.1 Apogonidae

The cardinalfishes belong to the Apogonidae family, that contains 348 species (Froese & Pauly, 2013), most of which are nocturnal and do not grow larger than 10 cm. Besides *Pterapogon kauderni* (Banggai Cardinalfish), popular cardinalfish species include *Sphaeramia nematoptera*, *Apogon maculatus* and *Zoramia leptacantha*. Cardinalfishes are gonochorists and the male orally broods the embryos until hatching. As is usual for mouthbrooding species, it is important to adequately feed the broodstock, not only in order to produce quality eggs, but to also make sure that the male has energy reserves when brooding the eggs. Otherwise, eggs may be discarded or consumed by the male. Cardinalfish egg clutch size varies significantly between species and typically ranges from a few to several hundreds. The eggs are typically incubated for 7–8 days (Wittenrich, 2007) before the larvae hatch, when rotifers should then be offered. Newly hatched *Artemia* nauplii may be offered at around day 11 and enriched nauplii at around day 19 (Wittenrich, 2007). Metamorphosis usually takes place at 25–30 days after hatching.

14.7.2 Plesiopidae

The Plesiopidae family has both demersal spawning and mouthbrooding fish, as mentioned earlier. The ornamental and mouthbrooding representatives of the Plesiopidae are the Devilfish (genus *Assessor*). While three species have been described, the most popular ones are the Blue Devilfish (*Assessor macneilli*) and the Yellow Devilfish (*A. flavissimus*), which slightly resemble dottybacks. There have been no scientific experiments verifying whether these fish are gonochorists or hermaphrodites, but it has been long known that they are mouthbrooders (Wittenrich, 2007). Given the absence of sexual dimorphism, it is useful to keep several broodstock individuals together in a large tank. During spawning, females become slightly more rotund before the eggs are transferred to the mouth of the male. The eggs are then incubated for two weeks before hatching and the larval rearing protocol is very similar to those for *Pseudochromis* spp. (Wittenrich, 2007).

14.7.3 Opistognathidae

The Jawfish (family Opistognathidae) are very hardy and conspicuous fish. Despite having approximately 80 species, very few are commercially attractive. The most popular species are the Yellowhead Jawfish *Opistognathus aurifrons* and the Blue-spotted Jawfish *O. rosenblatti*. The former can be easily found hovering above burrows in the Western Atlantic (Ho *et al*., 2012). Because of that, it is important to have 10–15 cm deep sand burrows or caves when keeping these species. The Jawfish are gonochorists and mouthbrooding males can be distinguished from the females by the larger size of their jaw. Spawning pairs may produce several hundred eggs once or twice a month and incubate them for about 8 days (Wittenrich, 2007). Newly hatched larvae are initially fed with rotifers and *Artemia* nauplii may be introduced on the eighth day. Metamorphosis takes place at 15–20 days after hatching (Wittenrich, 2007). For grow-out, it is important to have several PVC tubes mimicking caves and lids on the tanks to avoid jumping.

14.8 Conclusions

This chapter contains relevant information regarding the culture, general biology, care, reproduction and larval rearing of the main families of demersal spawners and mouth-brooders. Many obstacles have been surpassed in the culture of these families especially because of the application of new technologies and the availability of scientific data. However, there is still need to increase the amount and variety of commercially cultured species. Currently, the initial feeding and lack of variety in live prey items represent the main obstacles in the production of demersal spawners, while successful egg incubation may be the main barrier in the case of mouthbrooders.

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15

Large Angelfish and Other Pelagic Spawners

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Abstract

Most marine ornamental fishes including butterflyfishes, angelfishes, and wrasses are pelagic spawners. Many are hermaphroditic, either acting both as male and female in one individual or changing sex as social structure changes and/or with growth; others spawn in pairs or large aggregations. Appropriate nutrition needed for the broodstock to produce viable eggs and healthy larvae is poorly known. Pelagic spawning fish produce tiny free-floating eggs (≤1 mm), the adults do not provide parental care, and the newly hatched larvae are planktonic and quite small (≤2.5 mm); they begin feeding 2 to 3 days after hatching. Traditional prey for rearing marine fish larvae (rotifers and brine shrimp nauplii) are generally not accepted and may not be nutritionally adequate. These issues have proven to be major obstacles to large scale production of coral reef fishes. Despite these obstacles there have been some notable successes in recent years. This chapter discusses the different spawning strategies and rearing methods for many species that have been spawned and/or reared in captivity.

Keywords *Butterflyfishes; tangs; wrasses; hermaphroditism; broodstock; spawning; larviculture; larval food; juvenile diet*

15.1 Introduction

Teleost fishes are remarkable in comparison to other vertebrates due to their high diversity and plasticity in reproductive development, sexual strategies, and spawning behaviour (Devlin & Nagahama, 2002; Sadovy de Mitcheson & Liu, 2008; Erisman *et al*., 2013), and much of this diversity is encapsulated by marine ornamental fishes with pelagic spawning. Moreover, patterns of sexuality and mating behaviour are linked

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to and can change rapidly as changes occur in social and environmental conditions, such as the size or size structure of the social group, the density of adults within the group, or the relative number of males to females (Avise, 2011). Given such flexibility, understanding these dynamics is vital for successful breeding of marine ornamental fishes in captivity.

15.1.1 Sexual Patterns

Pelagic spawning fishes exhibit a wide diversity of sexual patterns. The vast majority of species follow a gonochoric (e.g., separate sexes or dioecy) pattern in which individuals mature and reproduce as either males or females. For example, all species of snappers (Lutjanidae), grunts (Haemulidae), croakers and drums (Sciaenidae), butterflyfishes (Chaetodontidae), dragonets (Callionymidae), spadefishes (Ephippidae), and surgeonfishes (Acanthuridae) are thought to be gonochores. However, sexual patterns in certain species of seabasses (Serranidae and Epinephelidae), angelfishes (Pomacanthidae), and wrasses (Labridae) are much more complex. Hermaphroditism, or the presence of male and female reproductive functions in the same individual during its lifetime, is common within these families and can take on a variety of forms (Sadovy de Mitcheson & Liu, 2008). Many small serranid fishes (e.g. *Hypoplectrus* spp., most *Serranus* spp.) are simultaneous hermaphrodites, whereby individuals possess functional male and female gametic tissue simultaneously and thus can reproduce as either sex during the same reproductive period (Fischer, 1980; Petersen, 2006). Other fishes such as many wrasses, seabasses, and groupers are protogynous hermaphrodites that mature first to reproduce as females and later change sex to function as males (Erisman *et al*., 2013). A much less common form of sex change is protandry, in which individuals reproduce first as males and later in life as females. While this form of hermaphroditism does not occur in the groups discussed in this chapter, protandry has been documented in clownfishes, sparids, gobies, and a few other groups. A few species of wrasses (e.g. *Labroides dimidiatus*) and gobies have been shown to be bi-directional sex changers that can change sex more than once in their lifetime (Kuwamura *et al*., 2002). Interestingly, two species of serranids (*Serranus baldwini* and *S. psittacinus*) develop and reproduce first as simultaneous hermaphrodite but resorb their female tissues at larger sizes to function only as males (e.g., androdioecious; Petersen *et al*., 2006).

15.1.2 Size Matters

Many of the pelagic spawning fishes discussed in this chapter are characterized by environmental sex determination (ESD), including most or all hermaphroditic species (Avise, 2011). The environmental cues that elicit individuals to develop and function as males or females (or both) are associated with socio-demographic factors such as population density, the relative size of the individual and its position in the social hierarchy, or the structure of the mating or social system (Munday *et al*., 2006). Moreover, evolutionary theories of sex allocation provide explanations for how these fishes allocate resources to male and female functions (Charnov, 1982). The Size-Advantage Model (SAM) is a well-supported theory on the adaptive significance and evolution of hermaphroditism in fishes and proposes that individuals should change sex when the reproductive value of functioning as the opposite sex exceeds that of the current sex (Warner, 1988). According to the model, protogyny and androdioecy should occur in

polygynous mating systems in which large males tend to monopolize matings and realize a reproductive advantage relative to females, hermaphrodites, and small males. Conversely, protandry should occur in mating systems in which mating consists of random pairing within aggregations, or monogamy in which sexual partners are not matched by size, thereby taking advantage of the high egg capacity in larger females. Separate sexes should be favored when the reproductive expectations of males and females increase in a similar manner with changes in size or age. This can occur in mass matings in which multiple males surround a female during a spawning rush and compete for fertilizations when she releases her eggs into the water column. Under this scenario, the density and abundance of small males makes it difficult for large territorial males to monopolize matings, which reduces the benefits of sex change. Finally, simultaneous hermaphroditism is highly adaptive in fishes that mate in pairs. Since sperm is cheap and eggs are expensive, the pair can achieve a greater total reproductive potential than a dioecious pair if both individuals allocate most of their energy to producing eggs and a small amount of energy to produce enough sperm to fertilize all the eggs (Charnov, 1982). However, the system can be unstable and vulnerable to cheating if there are opportunities for extra-pair matings by individuals that expend more on male function. Thus, simultaneous hermaphroditism is rare and tends to be found only in those species existing at very low densities or in those with behavioural mechanisms (e.g., egg-trading) in place that limit cheating (Petersen, 2006).

15.1.3 Conformity

For the most part, the social and mating systems of the marine ornamental fishes described in this chapter follow the prevailing theories of sex allocation. Spawning in hamlets and small seabasses that are simultaneous hermaphrodites occurs in pairs characterized by egg trading, wherein individuals parcel their daily clutches and require reciprocal egg release from a partner to continue spawning (Petersen, 2006). Many species of protogynous angelfishes, seabasses, groupers, and wrasses form harems that consist of a single, large male that monopolizes matings with several females (Erisman *et al*., 2013). A dominance hierarchy of females that is usually based on size determines breeding access to the male; death or emigration of the male will elicit the dominant female to change sex to become the male in the social group (Warner, 1984). Schooling species and those that form large spawning aggregations such as most snappers, grunts, spadefishes, surgeonfishes, and some wrasses and seabasses are usually gonochoric. For these fishes, spawning occurs in groups of several males that surround a single gravid female; males attempt to maximize their reproductive fitness by competing with rival males for the closest position to the female and by producing and releasing large amounts of sperm during spawning rushes (e.g., sperm competition) (Erisman *et al*., 2009).

15.1.4 Exceptions

Examples of species with enigmatic mating patterns that seem to counter sex allocation theory include the peppermint bass (*Liopropoma rubre*) and studied species of Callionymids. Peppermint bass occur in very low densities and spawn in pairs, yet unlike other species of basses with this mating system, they show no evidence of hermaphroditism (DeLoach, 1999). Mandarinfish and dragonets are also gonochoric

despite having polygynous mating systems similar to protogynous wrasses and angelfishes where male mating opportunities are controlled by a dominance hierarchy related to body size and rival males engage in intense aggressive bouts (Rasotto *et al*., 2010; Wittenrich & Ho, 2013). It is possible that other aspects of their life history or their surrounding environment make hermaphroditism unfavorable, and future investigations that rear these fishes under captive conditions may provide answers to such questions about the evolution of fishes.

15.1.5 What can go Wrong?

The sensitivity of pelagic spawning fishes to variations in social and environmental conditions is important to consider when attempting to rear or spawn fish in captivity. Small changes in size structure of fish within a tank, the sex ratio of the broodstock, or even the total number of adults may greatly alter reproductive activity. For many angelfishes, seabasses, and wrasses, the sex ratio may change (via sex change) within a few weeks of being placed in captivity based on the relative size of fish placed in the tank or even the overall ratio of males to females (Munday *et al*., 2006). For wrasses, the mating and social behaviour may completely change based on the density of fish in the tank, such that mass spawning and no sex change will occur in high density treatments and paired spawning within male-dominated harems may form in low density treatments (Warner, 1984). Size distributions of males and females will also have significant effects on mate choice, fertilization rates, or whether fish release gametes or how much (Petersen & Warner, 1998). Finally, in species of snappers, surgeonfishes, and other fishes that naturally mate within large groups with spawning aggregations, a high abundance or density of fish may be necessary to stimulate the physiological and behavioural changes necessary for spawning activity to occur (e.g., Allee effects). Thus successful breeding of fish in captivity depends upon understanding the reproductive behaviour of natural populations and applying that knowledge. Numbers and sizes of broodstock, tank size and environmental conditions all play a role in successfully producing marine ornamental species in captivity.

15.2 Serranidae

Many serranid fishes, commonly called sea basses, are brightly colored and distributed over reefs in tropical and subtropical waters. The family includes many modestly sized, colorful reef fishes that are most suitable for the aquarium.

15.2.1 Reef Basslets (*Liopropoma* spp.)

The sandy (*L. carmabi*) and Swissguard basslet (*L. rubre*) were raised by Gardner (2012a). These brightly colored fish are found in the deeper reefs of the tropical Atlantic.

15.2.1.1 Broodstock and Spawning

Three pairs of each species were maintained in six 110 L tanks, painted black on three sides. The tanks had a common sump, UV sterilizer, and a biofilter of fluidized bio balls. Each tank had a single PVC-T fitting for shelter. Broodstock were fed a gel-based diet

containing a variety of seafood, frozen mysid shrimp, and commercial soft shrimp pellet. Spawning occurred in the early evening. Approximately 50–100 pelagic eggs were collected on a 500 µm mesh screen. Maximum egg production and viability was between July and November.

15.2.1.2 Larval Rearing

Eggs were hatched in 2 L glass cylinders with light aeration. Newly hatched larvae were moved to 208 L cylindrical, black polyethylene rearing tank with UV-sterilized water. A combination of commercially available copepods (*Acartia tonsa*, *Pseudodiaptomus pelagicus*, and *Parvocalanus crassirostris*) was added to the tank after two to three days along with a 50–50 mixture of the algae *Isochrysis* sp. and *Nannochloropsis* sp. Water quality was maintained by dripping fresh algae and clean water into the tanks; larvae were moved to new clean-water tanks at 30 and again at 50–60 days. See 58 d candy basslet in Chapter 1, Figure 1.1 of this book. Candy basslets settled between days 69 and 85, while Swissguard basslets settled much later between 126–143 days.

15.2.1.3 Additional Information

Gardner (2012b) also successfully raised the bicolor basslet (*L. klayi*) using similar techniques to the above but the fish produced only a few eggs. The larval period was about one month.

15.2.2 Hamlets (*Hypoplectrus* spp.)

Hamlets occur in tropical waters of the western Atlantic. Blue (*H. gemma*), butter (*H. unicolor*) and shy (*H. guttavarius*) hamlets were raised by Domeier (1994) as part of his study to determine if different color morphs of *Hypoplectrus* were separate species. He collected adults in the early afternoon during spawning season and placed them in 380 L (about 6 m diameter) tanks and the fish spawned at dusk. Larvae were reared in similar tanks at 28 °C, and fed wild-caught plankton and rotifers in green-water using *Isochrysis* sp. followed by brine shrimp and finally dry feed after settlement. Some larvae reached the juvenile stage; the butter and shy hamlets were reared until adult coloration was obtained. He concluded that different color morphs warrant full species rank.

15.2.3 Harlequin Bass (*Serranus tigrinus*)

Distributed in the tropical western Atlantic, harlequin bass reach ~10 cm feeding mainly on crustacean. They are simultaneous hermaphrodites.

15.2.3.1 Broodstock and Spawning

Pairs of adult fishes were maintained in 300 L tanks with external biological filters (Figure 15.1). Rocks covered with algae and invertebrates (live rock) were stacked in the tanks to simulate the reef habitat. Oxygen was kept near 6.0 ppm with air-stones in the filter box and air-wands in the tank. Salinity was maintained at 32 to 36 and pH at 8.0–8.3. Spawning tanks were equipped with overhead and internal lights on separate timers for seasonal cycling, and 15 min dawn and dusk; room temperatures matched the season (Holt & Riley, 2001). Fish were fed twice daily with commercial flake food and raw shrimp or fish. Harlequin bass began to spawn in the afternoon under spring conditions. The pair implemented "egg-trading" in which they reproduced as both male

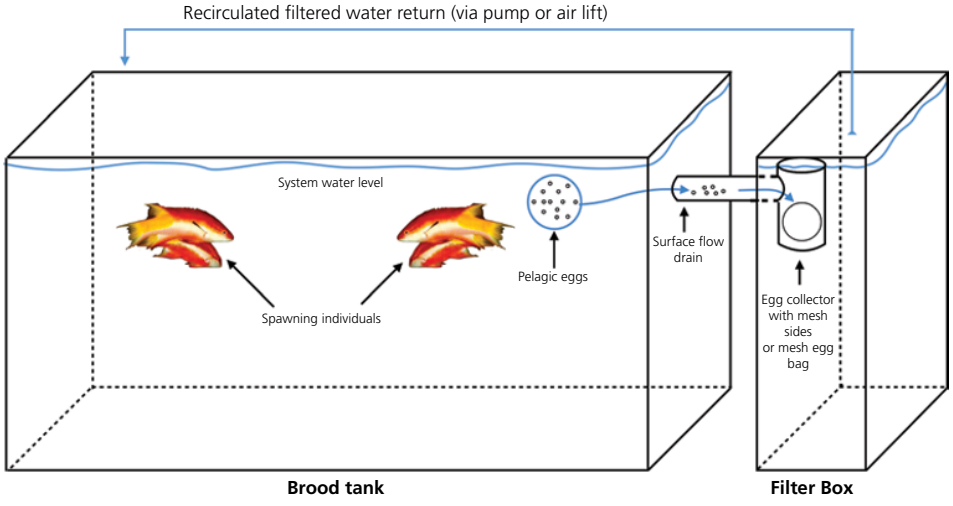


Figure 15.1 Broodstock fiberglass tank (90 w × 60 d × 60 h cm). Effluent water from the tank was airlifted into an external filter box with vertical plates covered with polyester filter material for nitrifying bacteria. Water returned from the filter box at a rate of 2.1 L/min. Eggs were collected in 150 μm Nitex mesh bags submerged in the filter box.

and female in separate spawning events. The spherical, pelagic eggs were 0.75 mm diameter. Mean fecundity was 1100 eggs per spawn with 28% fertilized.

15.2.3.2 Larval Rearing

Eggs were placed in 1 L hatching containers and then transferred to rearing tanks with salinity and temperature matching spawning conditions. Larvae hatched in 24 h (2.0 mm) and developed quickly with yolk absorption and jaw development by 48–72 hours post-hatch. Larvae were offered wild-caught plankton (30–90 μm), algae (*Isochrysis* sp.), and rotifers (5 mL^{-1}) but no food was taken. They lived 7 days at which point starvation appears to be the primary cause of mortality. Several similar species have been reared on wild plankton but difference among sites for collecting plankton (e.g. inshore Gulf of Mexico vs. tropical Atlantic or Pacific near Hawaii) may explain the lack of success here.

15.2.4 Yellow Anthias (*Odontanthias fuscipinnis*)

This species is found only in the Hawaii Islands and Johnston Island in moderately deep reefs. Baensch (2013a) reared the yellow anthias from a small number of eggs collected in waters off Oahu in December, 2012 as part of his Hawaii Larval Fish Project "to study the reproductive patterns, culture requirements, and larval development of Hawaii marine fish". Larvae were reared in 50 L recirculating culture tanks with larvae of other species, and fed wild-caught copepod nauplii. The larvae developed an elongate second dorsal spine, long pelvic fin rays, head spination and red pigment on the body. They reached the juvenile stage in about 80 days.

15.3 Lutjanidae

Snappers occur mainly in tropical and subtropical seas in a variety of habitats where they feed on crustaceans and fish. Generally considered as food fish, they do well in aquaria but require large tanks.

15.3.1 Bluestripe Snapper (*Lutjanus kasmira*)

Baensch (2013b) collected eggs (pelagic, 0.8 mm with a single oil droplet) of the bluestripe snapper from the coastal waters surrounding the island of Oahu, Hawaii. It is an Indo-pacific fish that was stocked for sport fishing in Hawaii in the 1950s. Larvae were raised in 50 L round black tubs and fed a combination of wild copepods (*Parvocalanus*, *Oithona*, *Bestiolina* and *Apocyclops*) and cultured copepods (*Parvocalanus*, *Bestiolina*). Post-flexion larvae settled at about 60 days in a mixed species, recirculating system. The early larvae are typical of many marine species, but develop an elongate second dorsal spine, pelvic spines, and visible teeth in pre-flexion. Black pigment on the dorsal fin develops in post-flexion. Survival rates were not reported except that juveniles were susceptible to lordosis under conditions of too much light.

15.3.2 Five-lined Snapper (*Lutjanus quinquelineatus*)

This species occurs in the tropical Indo-West Pacific. They are popular food fish but can also be found in the aquarium trade.

15.3.2.1 Broodstock and Spawning

Twenty-two fish were maintained in a 40 m^3 fiberglass tank (8 l × 5 w × 1 h m) at the National Museum of Marine Biology and Aquarium (NMMBA), Taiwan. The tank was connected in parallel to a recirculation system composed of a 10,000 L sump equipped with two fiberglass side-mount sand filters, a wet–dry filter with bioballs, a protein skimmer and three 40 W UV sterilizers. Dead rocks and porous limestones were stacked in the tank to provide shelter. Salinity and water temperature were maintained between 30–33 and 26–30 °C. Five-lined snapper (25–32 cm) spawned under spring, summer and autumn conditions at 26–29 °C averaging 256,000 eggs per tank with 60% fertilization. Eggs (0.75 mm diameter) were collected from the spawning tank with a dip net (100 μm mesh size).

15.3.2.2 Larval Rearing

Eggs were placed in 500 L fiberglass conical-shaped tanks. Water was kept at 27–29 °C, 33–35 salinity, with 12L:12D photoperiod. Larvae hatched within 24 h at an average size of 2.07 mm. Larvae developed quickly reaching ~6 mm in 20 d and 13 mm at one month. Larvae were initially fed trochophore oyster (*Crassostrea angulata*) larvae (30–60/mL) twice daily from 2 to 10 d, followed by enriched s-type rotifers (*Brachionus ibericus*, 10–20 L^{-1}) from 10 until 30 d, brine shrimp (*Artemia* sp.) nauplii (2–5 mL^{-1}) from 20 to 40 d and finally weaned onto dry diets at about 30 days. Survival rates to the juvenile stage at ~30 d averaged 10.5% (Leu *et al*., 2007).



Figure 15.2 A 22-day-old yellow-tail snapper *(Ocyurus chrysusrus)* larva raised at UTMSI.

15.3.3 Yellowtail Snapper (*Ocyurus chrysurus*)

Yellowtail snappers occur in the tropical western Atlantic. They feed on fish and crustaceans and are popular food fish but the beautiful juveniles do well in large reef tanks.

15.3.3.1 Broodstock and Spawning

Twenty-five fish were held in a recirculating system comprised of a 16 m^3 circular fiberglass tank, a biological filter, supplemental aeration and air lifts to circulate the water, and were subjected to seasonal shifts in day length and temperatures for one year similar to the protocol developed for red drum (Arnold, 1988). Yellow-tail snapper (27–30 cm) spawned under spring and summer conditions at 26–28 °C, averaging 300,000 eggs per tank with 40% fertilization (Turano *et al*., 2000). Eggs (0.96 mm diameter) were collected in a submerged 300 µm mesh bag tied onto the intake pipe into the filter box.

15.3.3.2 Larval Rearing

Eggs were placed in 300 and 600 L fiberglass tanks with internal biofilters. Water was 27–28 °C, 33–38 salinity, with 12L:12D photoperiod. Larvae hatched within 24 h at an average size of 2.3 mm. Larvae developed quickly reaching ~5 mm in 22 d (Figure 15.2) and 15 mm at one month (Riley *et al*., 1995). Larvae were fed enriched rotifers (5 mL^{-1}) twice daily for 5 days, followed by brine shrimp (3 mL^{-1}) until they were weaned to dry diets at about 20 days. Growth rate and survival were increased when fed prey that had been enriched with highly unsaturated fatty acids (HUFA) (Faulk *et al*., 2005). Survival rates to the juvenile stage at ~45 d averaged 2.8%.

15.4 Haemulidae

Grunts and sweetlips are typically inactive during the day near reefs and disperse to feed on benthic invertebrates in the evening. Many species are fished for food and some are popular in the aquarium trade.

15.4.1 French Grunt (*Haemulon flavolineatum*)

Species native to the western Atlantic. Researchers at The University of Florida's Tropical Aquaculture Lab successfully reared five species of grunts, including the French Grunt (Wittenrich *et al*., 2012).

15.4.1.1 Broodstock and Spawning

Broodstock were maintained in groups of eight (~10 cm fork length) in 660 L recirculating tanks. Natural spawning occurred within three weeks of acclimation under 12L:12D photoperiod at 26 °C. Eggs were collected "semi-dry" on top of polyester floss within the tank effluent.

15.4.1.2 Larval Rearing

Embryos were stocked into rearing tanks at approximately 15–30 per liter. First feeding occurred at 2–3 d when the larvae reached 2.8 mm in length. Juveniles were successfully produced using rotifers as primary prey, utilizing a standard "green-water" approach. However, additional HUFA enrichment was required. Enriched *Artemia* and dry feed were offered from 20 d until 31 d, where after only dry feed was offered. Survival to the juvenile stage (40 d) was excellent, ranging from 43–85% indicating the strong suitability of this species for culture.

15.4.2 Indian Ocean Oriental Sweetlips (*Plectorhinchus vittatus*)

Inhabits the tropical Indo-West Pacific. They feed on fish and crustaceans and are excellent food fish. They are often displayed in large aquariums because of their size and colors.

15.4.2.1 Broodstock and Spawning

Six adults were maintained in a 30 m^3 fiberglass tank at NMMBA, Taiwan; the same tank as described for the five-lined snapper above (Paragraph 15.3.2.1). Salinity and water temperature were maintained between 30–33 salinity and 20–31 °C. Photoperiod during the preconditioning was 11L:13D and for the spawning period was 13L:11D. Indian Ocean oriental sweetlips (34–36 cm) spawned under spring and summer conditions with a maximum daily spawn of 7000 eggs at 26–29 °C. Over a 73-d period, the female produced 49 spawns, 75% of which resulted in fertile eggs (Leu *et al*., 2012). Eggs (0.80 mm diameter) were collected from the spawning tank with a dip net (100 μm mesh size).

15.4.2.2 Larval Rearing

Eggs were placed in 500 L fiberglass conical-shaped tanks. Water was 23–29 °C, 33–35 salinity, with 12L:12D photoperiod. Larvae hatched within 20 h at an average size of 1.9 mm. Larvae were fed initially on copepod (*Oithona* sp.) nauplii (5 mL^{-1}) and enriched s-type rotifers (5 mL^{-1}) twice daily from 1 until 10 d, followed by copepodites and adult copepods (3 mL^{-1}) and S-type rotifers (8/mL) from 11 to 20 d, copepod adults (1 mL^{-1}) and brine shrimp nauplii (1 mL^{-1}) from 21 to 30 d and finally weaned onto dry diets at about 30 d. Larvae developed quickly reaching ~6 mm in 15 d and 10 mm at 25 d. From about 17 d, a few of the larger larvae (over 7 mm) began to exhibit agonistic behaviour. Survival rates to the juvenile stage at ~30 d averaged 13.9%. The best survival of

sweetlips juveniles was achieved by feeding a combined diet consisting of rotifers, copepods and *Artemia*. It may be attributed to the compensatory nutrition value of three types of food (Leu *et al*., 2012).

15.5 Sciaenidae

Reef drum are small sciaenids found in the tropical western Atlantic that are occasionally collected and sold in the aquarium trade.

15.5.1 Jackknife Fish (*Equatus lanceolatus*)

The species occurs in 10–40 m deep coral reefs but juveniles can be found in much shallower waters near shore. Jackknife fish were spawned and reared at the University of Texas Marine Science Institute (Holt, unpubl. data).

15.5.1.1 Broodstock and Spawning

From 3–5 adults were maintained in 9 m^3 fiberglass rectangular tanks (3.9 w × 2.0 l × 1.5 h m) connected to a filter box (1.2 w × 6.0 l × 8.0 h m) with polyester fiber covered vertical plates as substrate for nitrifying bacteria. Effluent water from the tank was airlifted into the filter box, oxygenated and recirculated to the spawning tank. Temperature and photoperiod were manipulated to simulate natural seasonal cycles using light timers and heat pumps. Salinity was 30–36 and pH 7.9–8.3. Broodstock were fed daily a mixture of fish, shrimp, squid, vitamins and carotenoids. Natural spawning occurred in spring and summer conditions (24–28 °C and 12–15 h L). Eggs were collected in a submerged mesh (150 µm) bag attached to the intake of the filter box. Eggs were 1 mm diameter and hatched in 20–24 h.

15.5.1.2 Larval Rearing

Jackknife were raised from eggs to the juvenile stage in recirculating larval rearing system consisted of 18 L cylindrical rearing chambers within 400-L round fiberglass tank with an external biofilter (see Paragraph 15.5.3.2). Temperature ranged from 24–28 °C, pH 7.9–8.3, and salinity 34–38. Larvae were fed HUFA enriched rotifers (3–5 mL^{-1}) plus copepod (*Centropages hamatus*) nauplii (1–3 mL^{-1}) daily, from 2–9 days post-hatch (dph). From 9–19 d larvae were fed HUFA enriched brine shrimp nauplii, and from 19 d on a commercial microdiet (400–700 µm). Jackknife reached juvenile stage by 35 d with survival as high as 20% and growth rate near 1 mm/d (Figure 15.3) (Holt, unpubl. data).

15.5.1.3 Additional Information

Tucker (1998) reported Forest Young raised jackknife fish on cultured zooplankton, switching to chopped seafood when they were 3 weeks old.

15.5.2 High-Hat (*Pareques acuminatus*)

Adult fish were held in 680 L tanks at the Baltimore Aquarium. They were induced to spawn with hormones and the larvae were reared on rotifers and brine shrimp in a 760 L system (Harris *et al*., 1994). Larvae hatched at 2.29 mL and reached the juvenile stage at 31 days (see Holt & Riley, 1999 for a description of larval development).

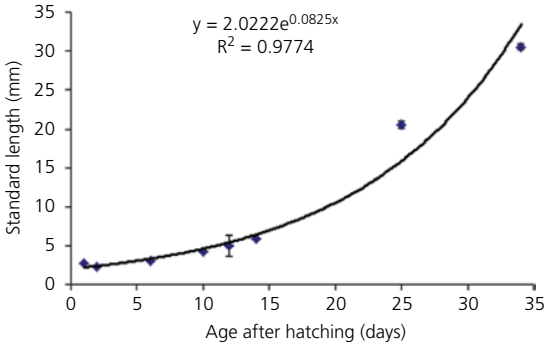


Figure 15.3 Growth rate of jackknife fish (*Equatus lanceolatus*) from 1–35 days.

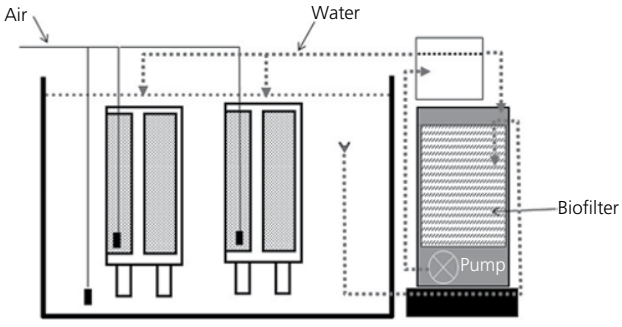


Figure 15.4 Recirculation system for rearing larvae showing two rearing chambers (18 L) with Nitex mesh on sides, airstones and waterflow (arrows and dotted lines) to and from a separate biofilter with header tank. Flows back to the tank are regulated with valves and are increased as the larvae grow.

15.5.3 Cubbyu (*Pareques umbrosus*)

This species is often associated with rocky substrates and artificial structures. Fish were collected as sub-adults from oil platforms in the Gulf of Mexico (Holt & Riley, 1999).

15.5.3.1 Broodstock and Spawning

Spawning tanks and conditions were the same as for the jackknife fish above. Cubbyu spawned best under conditions of 25–26 °C, salinity 35, and 15:9 h L/D cycle. Fish spawned from 3–5 times a week for several years averaging 2000 eggs per spawn. Eggs were clear, spherical, pelagic and 1.24 mm diameter (Holt & Riley, 1999).

15.5.3.2 Larval Rearing

Best larval survival was in a recirculation system (Figure 15.4). The rearing system concentrated larvae and food, thus increasing food encounter rates. It also provided shelter for the larvae, and decreased the possibility of physical damage from aeration, water exchange, and heating elements. Water quality parameters were pH 7.9–8.3, salinity 34–38 and temperature 25–28 °C. Larvae were fed algae-cultured rotifers ($5\,mL^{-1}$) from 3 to 10 d after hatching and then brine shrimp nauplii (1/mL) until metamorphosis at 22–28 d (Holt & Riley, 1999). Juveniles were fed pureed fish and shrimp or commercial dry diets. Survival to the juvenile stage was 18 to 25%.

15.6 Chaetodontidae

Butterflyfish occur in tropical and subtropical waters around coral reefs feeding on polyps, worms, zooplankton and algae. They are unique in having a tholichthys stage larva, wherein the head of the postlarva is covered in large, bony plates that are lost in the juvenile stage.

15.6.1 Millet (Milletseed) Butterflyfish (*Chaetodon miliaris*)

15.6.1.1 Broodstock and Spawning

Milletseed butterflyfish broodstock were maintained at the University of Florida's Tropical Aquaculture Lab in spawning aggregations consisting of a pair (1:1) or groups (3 M:8 F) and (10 M:11 F). Small spawns were recorded a few weeks after stocking from the largest group, although most of the eggs were not viable (Degidio, 2013). After several months, multiple spawns occurred, up to twice weekly, from the largest group of broodstock.

15.6.1.2 Larval Rearing

Eggs hatched approximately 24 h after fertilization and larvae began feeding at 3 d. Copepods (*Parvocalanus crassirostris*) were utilized as the first feed and early rearing trials yielded larvae to 19 d (Degidio, 2014). The addition of algae (*Isochrysis* sp.) to the tank increased the incidence of feeding. Subsequent rearing efforts confirmed that copepods can be utilized by the larvae throughout development, with the larvae surviving to 44 d. Large mortality events were documented at 11, 20, 27 and 35 d that may correspond to particular developmental milestones. For example, swim bladder inflation appears to occur at 11 d and flexion from 27–30 d (Cassiano *et al*., 2014).

15.6.2 Schooling Bannerfish (*Heniochus diphreutes*)

The species has been reared by two research groups, utilizing different approaches (Wittenrich & Cassiano, 2011; Baensch, 2014). At the University of Florida's Tropical Aquaculture Lab, eggs were obtained from captive broodstock originating at the Columbus Zoo & Aquarium. The embryos were stocked along with other "unknown" species and later identified using DNA barcoding. Copepod nauplii were used as the primary feed, with successful prey capture confirmed through larval sampling (Wittenrich & Cassiano, 2011). The larvae were reared to 41 d, and exhibited characteristic "sinking" behaviour, and increased mortality, associated with the tholichthys stage prior to settlement. Baensch (2014) was successful in rearing this species to the juvenile stage utilizing eggs collected from the wild. During that rearing attempt, the larvae were reared utilizing copepods in a protocol similar to that used by the author for dwarf angelfish. The larval period was greater than 100 days, and increased fragility of the larvae was reported in the later larval stages. The results of these discrete efforts indicate that the captive production of butterfly fish species is not only feasible, but very likely in the near future (Pedersen, 2014a).

15.7 Pomacanthidae

Angelfish are colorful inhabitants of coral reefs in the tropical Atlantic, Indian and Western Pacific oceans. Most are protogynous hermaphrodites.

15.7.1 Bandit Angel (*Apolemichthys arcuatus*)

Spawned and reared by Frank Baensch (2013c) at the Reef cultures technology lab. Eggs (0.85 mm) were obtained from a mated pair on two occasions. Larvae hatched at 1.4 mm and fed on cultured copepod nauplii at 3 days post-hatch (dph), and reached settlement near 40 dph. Baensch cautioned that larvae require high quality copepods and a stable rearing environment for survival.

15.7.2 Bluestriped Angelfish (*Chaetodontoplus septentrionalis*)

It is widely distributed throughout the Western Pacific from the Malay Peninsula, northward to southern Japan. They feed on sponges and tunicates, rarely exported through the aquarium trade. They are protogynous hermaphrodites.

15.7.2.1 Broodstock and Spawning

Five adults were maintained in a 30 m^3 fiberglass tank, the same as described for the five-lined snapper above (Paragraph 15.3.2.1). The broodfish were fed a combination of Antarctic krill (*Eupfausia superba*), squid (*Loligo chinensis*), various frozen raw fishes, such as carangid and scombrid species, as well as marine algae (*Acanthophora spicifera*, *Spyridia filamentosa* and *Chnoospora implexa*) once daily at a rate of approximately 3–5% of their body weight. Salinity and water temperature were maintained between 30–34 and 26–30 °C, respectively. Photoperiods during the preconditioning and spawning period were ambient. A single male (21.7 cm) dominated a harem of two females (18.4 and 19.5 cm), spawning with each at dusk, from 10 min before to 20 min after sunset. Each female averaged 119,000 eggs over the spawning period. Bluestriped angelfish spawned under spring and summer conditions with a maximum daily spawn of 16,300 eggs at 26–30 °C. Over a 54-d period, the female produced 34 spawns, 69% of which resulted in fertile eggs (Leu *et al*., 2010). Eggs (0.83 mm diameter) were collected from the spawning tank with a dip net (100 μm mesh size).

15.7.2.2 Larval Rearing

Eggs were placed in 500 L fiberglass conical-shaped tanks. Water was 26–28 °C, 33–35 salinity, with 12–12 h L/D. Larvae hatched within 18 h at an average size of 2.11 mm. Larvae developed quickly reaching ~7 mm in 20 d and 16 mm at 52 d. Larvae were fed initially on ciliates (*Euplotes* sp., 10/mL) twice daily from 2 until 7 d, followed by enriched s-type rotifers and copepods (*Euterpina acutifrons* and *Eucalanus* sp.) nauplii (10–15 mL^{-1}) from 2 until 20 d, copepodites and adult copepods (5 mL^{-1}) from 10 to 50 d, brine shrimp nauplii (5 mL^{-1}) from 20 to 60 d and finally weaned onto dry diets at about 60 d. Larvae survival up to the mouth opening (3 d) was 47.3%; to 10 d, 15.6%; to 30 d, 8.9%. Then 5.5% of 52 d juveniles survived but only 0.18% of 137 d juveniles. A total of 70 juveniles were reared to 5 cm (market size) or larger. The duration of the larval period for bluestriped angelfish was similar to those of other pomacanthid species that have been cultured (Baensch, 2002, 2003; Baensch & Tamaru 2009; Chapter 16 in this book). Longer larval durations of angelfish may show that the species are more difficult to rear but may also indicate that the nutrition and/or environmental conditions were suboptimal (Leu *et al*., 2015).

15.7.2.3 Additional Information

Moe (1997) successfully raised gray (*Pomacanthus arcuatus*) and French angelfish (*P. paru*), as well as crosses between the species, on wild zooplankton with added streptomycin in large (4000 L) static tanks outdoors.

15.7.3 Semicircle Angelfish (*Pomacanthus semicirculatus*)

Distributed in the tropical Indo-West Pacific. They feed on sponges, tunicates and algae and are highly prized of the coral reef fish. Close similarities in documented pomacanthid reproductive biology and in phylogenetic relationships have led some authors to assume protogynous hermaphroditism to be universal in the family (Neudecker & Lobel, 1982; Moyer, 1987). However, no direct proof of hermaphroditism has been obtained in this species.

15.7.3.1 Broodstock and Spawning

Pairs (33–35 cm) were maintained in a 30 m^3 fiberglass tank (as described for the five-lined snapper above in Paragraph 15.3.2.1). Salinity and water temperature were maintained between 33–34 and 27–28 °C, respectively. Photoperiods during the preconditioning and spawning period were ambient (Leu *et al*., 2009). Successful natural spawning of the semicircle angelfish in captivity, without the use of hormones or other treatments, occurred from February 24 to November 18, 2013 (Wang *et al*., 2014). Over a 267-d period, the female produced 28 spawns with a maximum daily spawn of 24,000 eggs, only 9% of which were fertile. Eggs (0.90 mm diameter) were collected from the spawning tank with a dip net (335 μm mesh size).

15.7.3.2 Larval Rearing

Eggs were placed in 7 m^3 fiberglass tanks. Water was 25–28 °C, 33–34 salinity, with 12–12 h L/D. Larvae hatched within 17 h at an average size of 1.73 mm. Larvae developed quickly reaching ~10 mm in 20 d and 18 mm at 36 d. Larvae were fed initially on ciliates (*Euplotes* sp., 10 mL^{-1}) twice daily from 2 to 7 d, followed by enriched S-type rotifers and copepods (*Euterpina acutifrons* and *Eucalanus* sp.) nauplii (10–15 mL^{-1}) from 5 until 20 d, copepodites and adult copepods (5 mL^{-1}) from 10 to 40 d and finally weaned onto dry diets at about 40 days. Larvae survival up to the mouth opening (3 d) was 65.8%; to 10 d, 24.1%; to 36 d, 17.9%; to 50 d, 11.5% juveniles survived and to 232 d, 8.7% of the juveniles survived. A total of 242 juveniles were reared to 6 cm (market size) or larger (Wang *et al*., 2014). To our knowledge, this is the first report on the opportunity to bring these delicate fish larvae through transformation to the juvenile stage (Figure 15.5).

15.8 Labridae

The large and diverse labrid family occurs worldwide in temperate and tropical oceans. They are among the most high-valued pelagic spawners in the aquarium trade (Moorehead & Zeng, 2010). Many are protogynous hermaphrodites with large distinctly-colored dominant males. There has been limited success with captive rearing of wrasses.

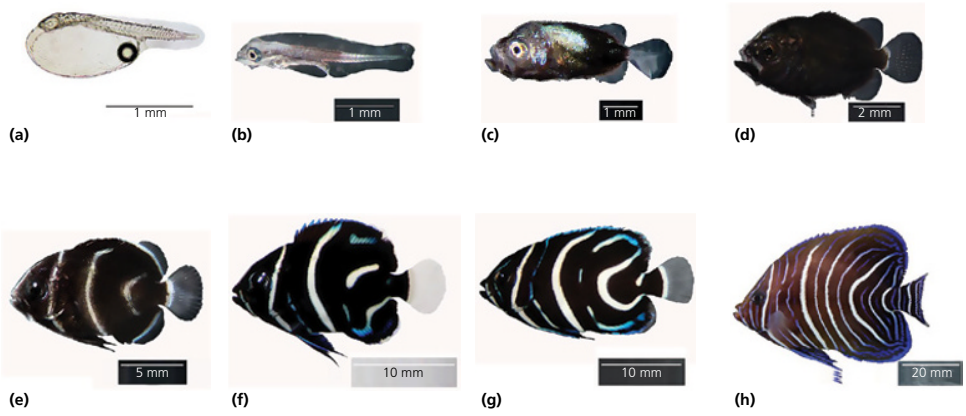


Figure 15.5 Photomicrographs of semicircle angelfish, *Pomacanthus semicirculatus*, larvae and juveniles reared at National Museum of Marine Biology and Aquarium. a = 0 day post-hatch (dph), b = 2 dph, c = 10 dph, d = 15 dph, e = 25 dph, f = 36 dph, g = 60 dph and h = 213 dph. Scale bar of a–c = 1 mm, scale bar of d = 2 mm, scale bar of e = 5 mm, scale bar of f–g = 10 mm and scale bar of h = 20 mm. Scale bars = 1 mm. (*See insert for color representation of the figure.*)

15.8.1 Spotfin (Cuban) Hogfish (*Bodianus pulchellus*)

This colorful fish is distributed in the tropical western Atlantic. It occurs on coral and rocky reefs and other hard surfaces such as wrecks and oil platforms. Juveniles act as cleaners, picking parasites from other fishes.

15.8.1.1 Broodstock and Spawning

Fish, hand collected on oil platforms in the Gulf of Mexico or reefs near Veracruz, Mexico were kept in 900 L (2.5 w × 6 d × 6 h m) fiberglass spawning tank with live rocks as already described above (Figure 15.1). Oxygen was near 6.0 ppm and salinity 32 to 36. Spawning tanks were equipped with overhead and internal lights on separate timers for seasonal cycling and 0.25 hours dawn and dusk; temperature was matched to season ranging from 20–26 °C (Holt & Riley, 2001). Broodstock were fed a soft diet containing fish, shrimp, vitamins and carotenoids. Spawning occurred in the late afternoon following courtship display by the male, and a final rush toward the surface by the pair with release of the gametes into the water. Eggs averaged 0.85 mm diameter; production was as high as 4000 eggs per spawn with larger females producing more eggs. Some fish spawned every day for a month or more.

15.8.1.2 Larval Rearing

Eggs hatched in 20–24 h and were placed in 18 L rearing tanks in a recirculating system described above (Figure 15.4). Algae (*Isochrysis* sp.) was added to the system daily. Larvae were fed nauplii of the copepod *Acartia tonsa* (2 mL^{-1}) plus enriched rotifers (5 mL^{-1}) twice daily from age 3–13 d. Enriched brine shrimp nauplii were added to the mixture from 14–24 d. Newly-hatched larvae were 1.8 mm and by 3 days the yolk was

absorbed, the eyes pigmented and mouth open. Larvae grew to 6 mm in 22–24 d, developing a red pigmented protuberance on the lower jaw and splotchy red pigment on the body. All the fins were developing and the tail was flexed but fish did not reach the juvenile stage (Holt, unpub. data).

15.8.2 Clown Wrasse (*Halichores maculipinna*)

This species is abundant on reef flats and shallow rocky area in the tropical western Atlantic.

15.8.2.1 Broodstock and Spawning

Pairs were kept in 300 L (6 h × 9 w × 6 d m) fiberglass spawning tanks with an external biolfilter, as already described above (Figure 15.2). Rocks covered with algae and invertebrates were stacked in the tanks to simulate reef habitat. Clown wrasse spawned under summer conditions (12–13 h light; 27–28 °C) in the early afternoon after a courtship display by the male and a final ascent toward the surface by the pair releasing the gametes. They produced an average of 144 eggs per spawn with maximum spawning at 13 h light and 27 °C. Eggs averaged 0.59 mm and hatched in 18-24 h (Holt & Riley, 2001).

15.8.2.2 Larval Rearing

Newly-hatched larvae (1.5 mm) were placed in 300 L round blue tanks at 27 °C and 35 salinity. A dense culture of mixed-species green algae was added along with wild-caught plankton (size sorted to 30–93 μm) from 2–8 d. Larvae did not feed and none survived past 8 days. Finding food for the very small larvae is a challenge. Riley & Holt (1993) reported that larval fish less than 0.3 mm collected at Enmedio Reef, Mexico had predominately tintinnids (8–78 μm) and dinoflagellates (5–60 μm) in their guts. The only labrids collected were larger than 5 mm; dinoflagellates (15–60 μm) were the predominant food in their guts.

15.8.3 Bluestreak Cleaner Wrasse (*Labroides dimidiatus*)

This species is distributed in the tropical Indo-West Pacific. The wrasse maintains stations where other fish come to be cleaned, feeding on their crustacean ectoparasites and mucus. They are regarded as a beneficial addition to the marine hobbyist's aquarium. They are protogynous hermaphrodites.

15.8.3.1 Broodstock and Spawning

Adults (7–10 cm) were maintained in a 30 m^3 fiberglass tank at NMMBA, Taiwan. Spawning tank and conditions were the same as for the five-lined snapper above (see 15.3.2.1). Salinity and water temperature were maintained between 32–35 and 24–29 °C. Photoperiods during the preconditioning and spawning period were ambient. Successful natural spawning of bluestreak cleaner wrasse in captivity from 1 May 2011 to 30 April 2012 was described for the first time (Hsu *et al*., 2013). Four broodfish (1 M: 3 F) laid 647,900 eggs during the spawning period. The average fertilization rate was 96.9 %, and

the hatching rate varied from 2.0 to 100.0 %. This data may prove that bluestreak cleaner wrasse is a year-round spawning fish. Eggs (0.65 mm diameter) were collected from the spawning tank with a dip net (335 µm mesh size).

15.8.3.2 Larval Rearing

Eggs were placed in 2000 L PVC tanks. Water was 26–28 °C, 33–34 salinity, with 12–12 h L/D. Larvae hatched within 19 h at an average size of 1.65 mm. Larvae developed slowly reaching ~7.8 mm in 25 d and 13 mm at 50 d. Larvae were cultured using the inorganic fertilization method described by Tew *et al.* (2013). The tanks were fertilized each week to restore the inorganic nitrogen to 700 µg N/L and the inorganic phosphate concentration to 100 µg P/L, followed by enriched S-type rotifers and copepods (*Euterpina acutifrons* and *Eucalanus* sp.) nauplii (10–15 mL^{-1}) from 9 until 30 d, copepodites and adult copepods (5 mL^{-1}) from 20 to 50 d. The inorganic fertilization method provides high densities of suitable-sized prey for larvae at the onset of exogenous feeding before they are able to consume larger, commercially available rotifers and copepods. Stomach analysis revealed that ciliates and flagellates were actively consumed by larval fish. A total of 68 juveniles were reared to 5 cm (market size) or larger by 270 d (Hsu *et al.*, 2013).

15.8.4 Bluehead Wrasse (*Thalassoma bifasciatum*)

This species occurs from Florida to Brazil in seagrass and shallow reefs. They are protogynous hermaphrodites and form harems dominated by a large male with a distinctive blue head and a group of yellow females and smaller males.

15.8.4.1 Broodstock and Spawning

One male and 2–3 females were maintained in 300 L fiberglass tank with an external filter box as described above for the clown wrasse. Spawning occurred at midday in spring and summer conditions with a maximum daily spawn of 1000 eggs. Highest fecundity occurred in the largest females and at the warmest temperatures. Eggs averaged 0.56 mm (Holt & Riley, 2001). When the dominant male bluehead died, the largest female changed color and behaviour very quickly and began spawning with the remaining small female after one month (Holt, unpub. data). Color change in a female following male removal can occur within 4 days and mature sperm produced in as little as 8 days (Warner & Swearer, 1991).

15.8.4.2 Larval Rearing

Newly hatched larvae (1.4 mm) were placed in variously sized containers from 16 to 300 L with salinity and temperature matching spawning conditions, 12 h light and static conditions. Larvae were 1.94 mm at 3 d with pigmented eyes ready to feed. Many types of prey were offered in different studies including wild-caught plankton (30–93 µm), mixtures of green algae and plankton or small rotifers, ciliates and oyster trocophores. The larvae did not eat any of the offered prey and all died by 6–7 d.

15.8.4.3 Additional Information

Jones (1993) reared bluehead wrasse for 6 days, from eggs collected in the waters around Puerto Rico and described early larval development. Tucker (1998) noted that several labrids have been lab reared on wild zooplankton including hogfish and multicolorfin wrasse.

15.9 Callionymidae

There are 191 species that mainly occur in the tropical Indo-west Pacific. These fishes, popularly known as dragonets and mandarins, are found on sandy bottoms and feed on small benthic invertebrates. They have two distinct sexes; the first dorsal fin of the males is usually enlarged with a colorful pattern.

15.9.1 Lancer Dragonet (*Callionymus bairdi*)

Researchers at the University of Florida's Tropical Aquaculture Lab (TAL) raised this dragonet. Eight fish (a dominate male, two subordinate males and five females) were stocked into a 114 L cube tank. Spawning occurred just at dusk. The dominant male spawned with all receptive females producing chains of pelagic eggs that floated to the surface. The 0.5 mm eggs were collected with a plastic water bottle and moved to the TAL.

15.9.1.1 Larval Rearing

Larvae were less than 1 mm at hatching and began feeding 3 days later. They were fed wild-caught plankton in a green-water static system. The larvae settled between 49 and 70 days. At least 25 reached the juvenile stage (Wittenrich, 2011).

15.9.1.2 Additional Information

Several species of dragonets have been lab-reared from wild-collected eggs, *Callionymus* (*Ropomucenus*) *benitiguri* on small s-type rotifers and brine shrimp (Eda *et al*., 1994), and *Callionymus decorates* on wild-caught copepods (Baensch, 2013d).

15.9.2 Mandarin Fish (*Synchiropus splendidus*)

These richly colored small fishes, perch on rocks and sand, feeding on small crustaceans and other invertebrates. Wittenrich (2009) spawned and reared the green mandarin.

15.9.2.1 Broodstock and Spawning

Well-fed heterosexual pairs were maintained with 12–14 h L and an hour of dusk and dawn. Spawning occurred in the evening just at dusk. The spawning pair rose to the surface with release of the buoyant eggs. The 0.8 mm diameter eggs hatched in 13 h.

15.9.2.2 Larval Rearing

Newly-hatched larvae were moved to a black rearing tub. On the fourth day after hatching the 2 mm long, bright yellow larvae were ready to feed. They were offered rotifers and wild harvested zooplankton in "green-water" culture. Larvae settled at 4 mm on day 25; they were tan and mottled color with well-developed pelvic fins. They took on an intricate pattern of green and brown by 50 d and were weaned to high-protein pellets. They acquired adult coloration at three months with 182 surviving from two spawns.

15.9.2.3 Additional Information

Wittenrich (2010) reported that Bill Addison raised green mandarins at his C-Quest facilities in Puerto Rico using a small strain of rotifer; Yvonne Sadovy raised mandarins using copepod dominated wild plankton; and Julian Sprung used infusoria culture from seaweed, mostly copepods, but some protozoans. Oceans Reefs and Aquariums (ORA) advertise captive-bred green, red and spotted mandarins for sale.

15.10 Ephippidae

15.10.1 Spadefish (*Chaetodipterus faber*)

The spawning and rearing of spadefish has been documented by Walker (1991) and Wittenrich & Cassiano (2012c). In both occurrences, the eggs were obtained from groups of broodstock in large, public aquarium exhibits. The eggs hatched approximately 24 h after fertilization and the larvae began to feed on day 2. Walker (1991) utilized rotifers enriched with *Chlorella* sp. as the primary feed, while Wittenrich & Cassiano (2012c) used a mixture of rotifers and copepods. Both feeding regimes yielded similar results with the larvae growing rapidly, accepting brine shrimp by 9 d, and reaching metamorphosis in just 15 days. Late-stage larvae feed on smaller fish larvae and were observed to also graze benthic hydroids in the rearing tanks (Wittenrich & Cassiano, 2012d). Post-larvae can be transferred to grow-out tanks by 25 d and by 30 d they are approximately the size of a quarter.

15.10.2 Orbiculate batfish (*Platax orbicularis*)

Eggs were obtained from the Shedd Aquarium and transferred to the University of Florida's Tropical Aquaculture Lab in Florida. The eggs were rather large for coral reef species (1.3 mm) and produced very large (3.2 mm at hatch) and easily distinguishable larvae. The larvae were reared using a combination of rotifers and cultured copepod nauplii as prey and incorporated live algae (*Nannochloropsis* sp.) as background color. Feeding began on day 3, when the larvae had fully pigmented eyes and large pectoral fins. Growth and development were rapid, with metamorphosis by 23 d. No major bottlenecks were observed during the larval period. This species also proved easy to wean to dry diets and began accepting pelleted feed by 19 d (Wittenrich & Cassiano, 2012a, b).

15.10.3 Longfin Batfish (*Platax teira*)

This species is distributed in the tropical Indo-West Pacific. It feeds on algae and sessile benthic invertebrates and is of potential commercial value as ornamental fish, as food fish and as tourist attraction.

15.10.3.1 Broodstock and Spawning

One hundred and twenty adults (50–65 cm) were maintained in a coral reef tank (total volumes 5,678,100 L) at Coral Kingdom Pavilion, NMMBA, Taiwan. Salinity and water temperature were maintained between 32–34 and 26–29 °C. Photoperiods during the preconditioning and spawning period were 12–12 h L/D. Longfin batfish spawned under summer, autumn and winter conditions. The fertilization varied from 84.6% to

100% with an average of 94.3% (Tai *et al*., 2008). Eggs (1.29 mm diameter) were collected from the spawning tank with a dip net (150 µm mesh size).

15.10.3.2 Larval rearing

Eggs were placed in 500 L fiberglass conical-shaped tanks. Water was 26–28 °C, 32–34 salinity, with 12–12 h L/D. Larvae hatched within 24 h at an average size of 2.81 mm. Larvae developed quickly reaching ~7 mm in 15 d and 24 mm at 25 d. Larvae were fed initially on enriched rotifers (*Brachionus plicatilis*, 10 mL^{-1}) twice daily from 2 to 10 d, followed by brine shrimp nauplii (5 mL^{-1}) from 7 until 25 d and finally weaned onto dry diets at about 25 days. Larvae survival up to the mouth opening (3 d) was 74.6%; to 15 d, 53.2%; to 25 d, 35.7% juveniles survived; to 35 d, 24.9% juveniles survived and to 85 d, 15.4% of the juveniles survived. From about 9 d, a few of the larger larvae (over 5 mm) began to exhibit agonistic behaviour. The rate of agonistics was reduced by strong aeration of the water. A total of 625 juveniles were reared to 10 cm (market size) or larger (Tai *et al*., 2008).

15.11 Acanthuridae

This family, also known as surgeonfishes, tangs or doctorfishes, include some of the most popular marine aquarium fishes. They are diurnal herbivores or planktivores.

15.11.1 Palette Surgeonfish (*Paracanthurus hepatus*)

This is a targeted aquarium fish because of its brilliant and unusual color pattern. It is distributed in the tropical Indo-Pacific. Juveniles feed primarily on zooplankton and adults additionally graze on benthic algae. Palette surgeonfish is the 8th most traded species in the aquarium trade worldwide (Mcllwain *et al*., 2012).

15.11.1.1 Broodstock and Spawning

Nine fish (18–23 cm) were maintained in a 30 m^3 fiberglass tank at NMMBA, Taiwan. Spawning tank and conditions were the same as for the five-lined snapper above (Paragraph 15.3.2.1). Salinity and water temperature were maintained between 32–34 and 26–30 °C. Photoperiods during the preconditioning and spawning period were ambient. Successful natural spawning of palette surgeonfish in captivity from 1 June 2011 to 31 May 2012 was described for the first time by Chen *et al*. (2013). The average fecundity per spawn was 5300 eggs (range 126–22,328 eggs). Nine brood fish (sex ratio unknown) produced 826,000 eggs during the spawning period. The average of the fertilization rate was 96.3%, and the hatch rate varied from 12.5 to 96.5%. These data may prove palette surgeonfish is a year-round spawning fish and exhibits lunar-related spawning behaviour. Eggs (0.69 mm diameter) were collected from the spawning tank with a dip net (335 µm mesh size).

15.11.1.2 Larval Rearing

Eggs were placed in 2000 L PVC tanks. Water was 25–28 °C, 32–34 salinity, with 12–12 h L/D. Larvae hatched within 24 h at an average size of 1.55 mm. Larvae developed slowly reaching ~5.2 mm in 20 d, 6.8 mm in 30 d and 12.6 mm at 51 d. Larvae were cultured with the inorganic fertilization method described by Tew *et al*. (2013). The tanks were fertilized each week to restore the inorganic nitrogen to 700 µg N/L and the inorganic phosphate concentration to 100 µg P/L, followed by enriched S-type rotifers

and copepods (*Euterpina acutifrons* and *Eucalanus* sp.) nauplii (10–15 mL^{-1}) from 9 until 30 d, copepodites and adult copepods (5 mL^{-1}) from 20 to 50 d. Stomach analysis revealed that ciliates and flagellates were actively consumed by larval fish. Only one fish reached the juvenile stage at 51 days (Chen *et al*., 2013).

15.11.2 Yellow Tang (*Zebrasoma flavescens*)

Arguably one of the most iconic fish in the aquarium trade, Yellow Tang has been the subject of on-going culture research since 2000 (Laidley *et al*., 2001, 2004; Ostrowski & Laidley, 2001). Early efforts to spawn and rear these fish in captivity were met with tremendous challenges including limited numbers of viable eggs to work with, and incredibly small (~1.5 mm) larvae (Laidley *et al*., 2004). Despite these challenges, pioneering efforts by Syd Kraul yielded larvae out to 40 d in 2004, and provided hope that cultured yellow tang might yet be a reality (Pedersen, 2014b). However, despite this early success, limited progress has been made with the advancement of culturing Yellow Tang up until recently.

15.11.2.1 Broodstock and Spawning

In the wild, Yellow Tang spawn in groups and closely follow a lunar cycle, with peak spawning near the full moon (Bushnell *et al*., 2010). This same pattern has been observed in captivity, where tens to hundreds of thousands of eggs can be obtained from small groups of broodstock (Laidley *at al*., 2011; Cassiano *et al*., 2013). At Oceanic Institute of Hawaii Pacific University (OI), Yellow Tang broodstock are maintained in groups in 5000 L and 30,000 L fiberglass tanks that are approximately 1 m deep. Both pair spawning and group spawning has been observed in these tank systems.

15.11.2.2 Larval Rearing

Eggs hatch in approximately 20 hours at 27 °C into very primitive larvae that initiate feeding at 3 d. The larvae have been reared on copepod nauplii as a first food, however high mortality is still observed several days after first feeding, despite the larvae feeding well on the copepods (Callan *et al*., 2013). In 2014, work at OI resulted in the larvae making it past this initial first-feeding bottleneck (Callan, 2014a) and documented the larval development out to 83 d (Callan, 2014b). Larvae form large dorsal and ventral spines by 14 d, which persist through the flexion stage at 26 d and only begin to recede by 80 d (Figure 15.6). Recently (late 2015), the team at OI was finally successful in getting the larvae to settle and nearly 1000 juveniles were produced from three batches of eggs.

The first breakthrough appeared to be the result of a new approach to rearing the larvae, utilizing relatively high water turn-over to maintain excellent water quality and to flush any uneaten copepods from the rearing tank before they could grow to later (too large to be consumed) stages. Although this conclusion still needs to be verified (Callan, 2014b), it provided further insights into the larval rearing requirements for this species. One somewhat unanticipated observation was the apparent inability and inefficiency of the later-stage larvae to feed on *Artemia* sp. The larvae could also not effectively consume later stages of copepods, resulting in the need to further investigate alternative prey items for this species. While very little is known about what the larvae from family Ancanthuridae consume in the wild, Sampey *et al*. (2007) report that the few late-stage specimens collected and examined all were primarily feeding on appendicularians, and not crustacean zooplankton. The apparent lack of suitable prey likely resulted in the observed slower growth, protracted development and impaired settlement.

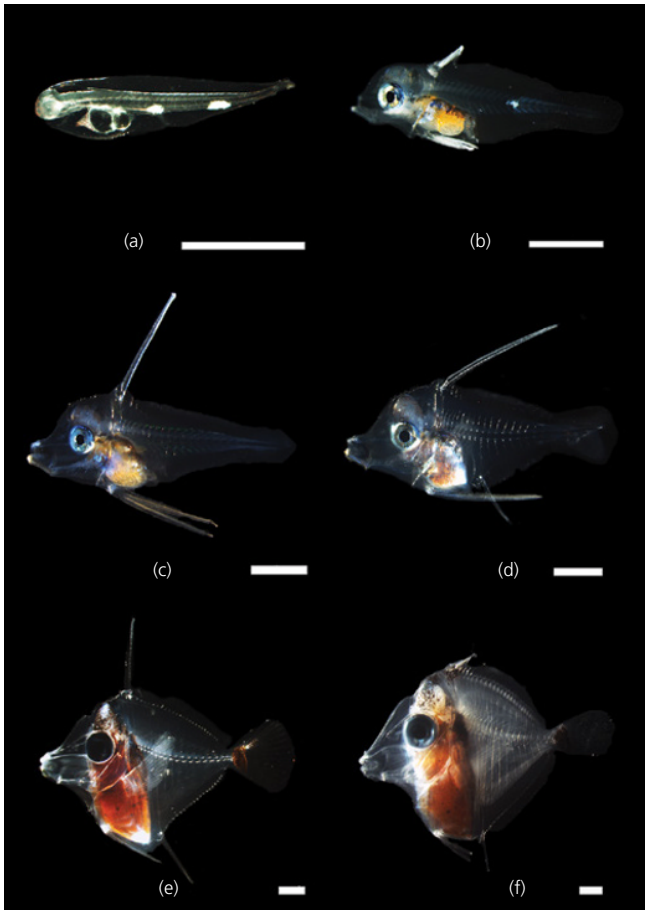


Figure 15.6 Photomicrographs of Yellow Tang larvae reared at Oceanic Institute. a = 1 day post-hatch (dph) b = 12 dph, c = 18 dph, d = 24 dph, e = 45 dph, f = 83 dph. Scale bars = 1 mm. Image credit David Hoy. (*See insert for color representation of the figure.*)

After observing these feeding challenges, subsequent batches of larvae were reared in 1000 L fiberglass tanks using broadly overlapping feed types (copepod nauplii, enriched rotifers, dry feed and *Artemia* sp.) and the results were dramatic. By 40 d, the fish were notably larger and far more developed than prior cohorts were at 60 d (Figure 15.7). It is was observed that the larvae were able to accept dry feed (<250 µm) much earlier than expected (before 21 d) and the fish also eagerly consumed enriched *Artemia* sp. (which had not been offered previously). This combination of multiple prey types in combination with dry feed was utilized throughout the entire 60-day larval period. Fish began to exhibit juvenile-type behaviours (schooling and association with benthic habitat structure around 50 d. The first signs of juvenile (yellow) coloration occurred on 55 d and by 65 d most of the fish had attained full juvenile coloration at a size of about 3–4 cm (Figure 15.7). Interestingly, this is right on schedule with the data from the wild (Claisse *et al*., 2009) where it was reported that yellow tang have an estimated larval duration of 55 days and settle at approximately 3–5 cm.

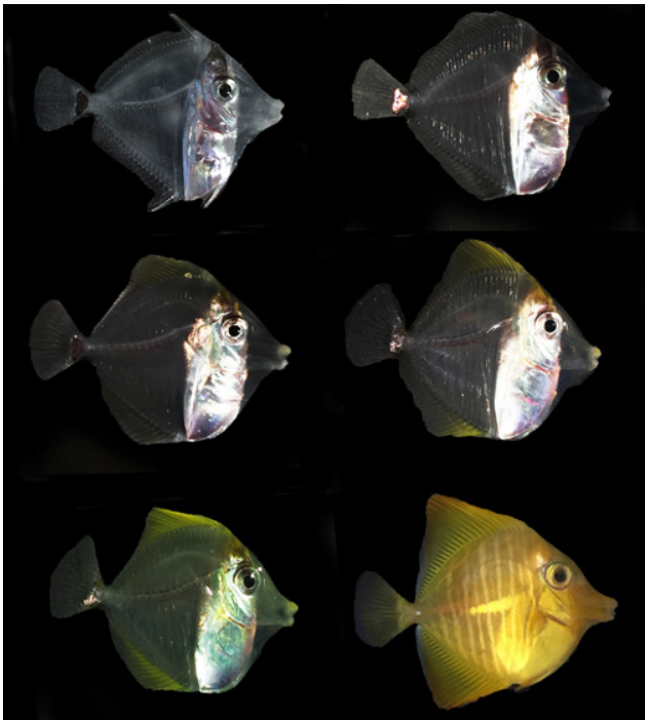


Figure 15.7 Photographs of Yellow Tang larvae from day post-hatch 40 to 65 showing the transition from a pelagic larva to a settled juvenile. Image credit Emma Forbes. (*See insert for color representation of the figure.*)

15.12 Conclusions

There are many challenges with breeding pelagic spawning fishes. Maintaining broodstock in excellent health for prolonged periods continues to be an issue for many species. Egg and larval quality depend on the health and nutrition of the parents. Generally the small larvae require large amounts of appropriate small zooplankton in very clean water systems. Recent breakthroughs reported in this book including the inorganic fertilization method for producing high densities of small ciliates and flagellates for first feeding larvae with small gape size; and the availability of commercially produced live calanoid copepods should improve prospects for successfully rearing more of these delicate larvae. Food for weaning and cues to metamorphosis or settlement continue to be a challenge in many species. Research is specifically needed in: nutritional requirements of broodstock, larval fish rearing system design and physical parameters of the culture environment, larval feeds and nutrition, weaning diets and settlement cues and metamorphosis.

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16

Dwarf Angelfish

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Abstract

The small and brilliantly colored dwarf angelfish are the most popular marine angelfish in the aquarium hobby. Farming these valuable fishes has proven to be technically demanding due to complicated requirements and long rearing phase of the larvae. Several research groups have made progress in their larviculture since 2001 using copepods as live prey. Thirteen dwarf angelfish species and two artificial hybrids have been captive-bred to date. Yet production numbers have remained in the hundreds of juveniles per trial, and the majority of dwarf angelfish entering the trade are still wild-caught. This chapter will provide a brief history of dwarf angelfish aquaculture and summarize the reproductive biology and spawning methods of adults, as well as the early life history and culture methods of their larvae and juveniles.

Keywords Centropyge; *copepods; hybrids;* Paracentropyge

16.1 Introduction

The *Centropyge* are diminutive and brightly-colored marine angelfish that inhabit reefs in all tropical and subtropical oceans, except for the Eastern Pacific and Western Atlantic. The genus is the largest within the family Pomacanthidae, comprising a total of 28 described species. A second group of smaller angelfish is currently classed in genus *Paracentropyge* (formerly *Centropyge*), made up of only three species (*P. boylei, P. multifasciata, P. venusta*). Together, genus *Centropyge* and *Paracentropyge* are commonly referred to as dwarf or pygmy angelfishes.

Dwarf angelfishes are the most heavily traded marine angelfish in the aquarium hobby. Dwarf angelfish made up approximately 77% of all marine angelfish imported into the USA in 2011 (Andrew Rhyne, pers. com.). Nine *Centropyge* species, totaling close to 330,000 individuals, accounted for the bulk of those imports (Figure 16.1). The two most popular *Centropyge* species, *C. loricula* and *C. bispinosa*, were the 14th and 16th most heavily imported marine aquarium fish to the USA in 2005 (Rhyne *et al*.,

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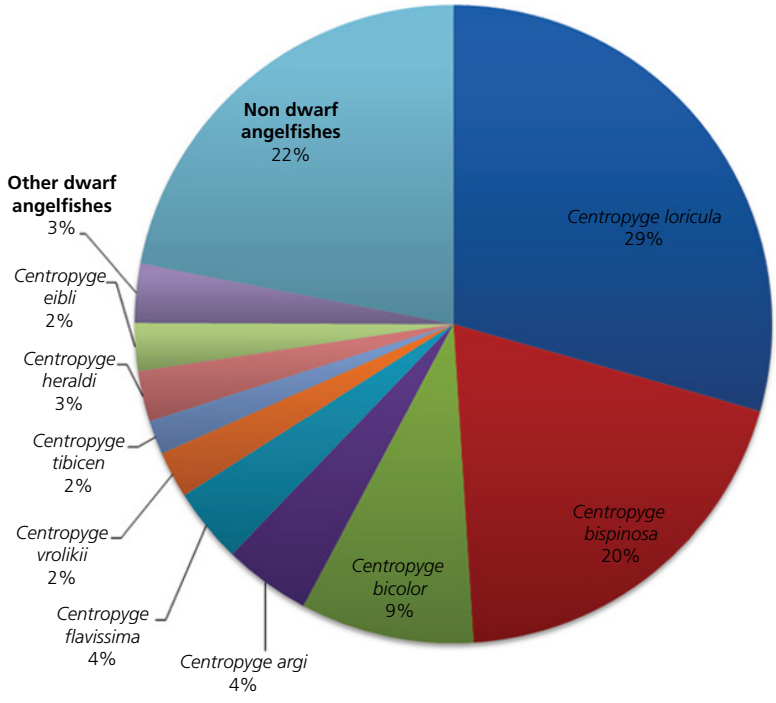


Figure 16.1 Marine angelfish US Imports in 2011 (data courtesy of Andrew Rhyne).

2012). These species are popular due to their brilliant colors, small size, ease of husbandry and relatively low price (US$20–US$40). Single individuals can be maintained for long periods in small aquariums on conventional foods. A number of attractive and rarely collected dwarf angelfish species (e.g., *C. debelius, C. resplendens, C. joculator*, and *P. boylei*) are significantly more expensive (from US$500 to US$12,000).

Dwarf angelfish that adapt well to captivity commonly spawn. Spawning methods and reproductive output have been documented for numerous species (Bauer & Bauer, 1981; Hioki & Suzuki, 1987; Hioki *et al*., 1990; Holt & Riley, 2001; Baensch, 2002, 2003; Olivotto *et al*., 2006; Baensch & Tamaru, 2009a) and one artificial hybrid (Baensch & Tamaru, 2009b). Several studies have investigated the effects of diet and environmental factors on reproductive performance (Holt & Riley, 2001; Callan *et al*., 2012; Callan & Laidley, 2010).

A series of bottlenecks have hindered the aquaculture of dwarf angelfish due to the complicated biology of their larvae. Endeavors to rear these fish in the 1980s and 1990s failed because available first food organisms (such as trochophores, diatoms, rotifers, and euplotes) could not be adapted to rearing their small-sized larvae. This bottleneck was resolved using small copepod nauplii in 2001 and subsequently resulted in the first cultured dwarf angelfish juveniles (Baensch, 2002, 2003; Laidley *et al*., 2008). Successful rearing of dwarf angelfishes is limited to five independently working aquaculture groups in Hawaii.

Reef Culture Technologies developed techniques to culture rare dwarf angelfish using wild-collected copepods from 2001–2012. The company intermittently produced

C. colini, C. debelius, *C. interrupta, C. resplendens*, and two artificial hybrids *C. fisheri* × *C. replendens* and *C. resplendens* × *C. argi* for the aquarium trade, achieving an average survival of up to 5% (up to 240 juveniles) per larval run. *Centropyge argi, C. acanthops*, *C. fisheri, C. flavissima, C. joculator*, *C. loricula, C. multicolor* and *P. multifasciatus* juveniles were also experimentally reared. The major production challenges were: (1) an inconsistent, year round supply of quality wild-caught copepods for feeding the larvae; (2) delayed metamorphosis of the larvae; (3) aggression between juveniles; (4) high cost (labor and time) of the rearing technique to produce juveniles; (5) and competition from wild-caught individuals.

The Oceanic Institute (Waimanalo, Hawaii, USA), resolved a number of key bottlenecks in the captive breeding of the flame angelfish (*C. loricula*) between 2001–2008 (Laidley *et al*., 2008). Year round broodstock spawning of 18 pairs that produced up to 45,000 eggs/day was finally achieved. The researchers discovered that the small calanoid copepod, *Parvocalanus crassirostris*, produced the suitable nauplii to culture first feeding *C. loricula* larvae and developed a technique to mass culture this copepod species. Finally, they succeeded in producing thousands of *C. loricula* larvae through the early stages (to day 14) in 1000 L tanks and reared several dozen juveniles to market size. Swim bladder hyperinflation (presumably due to microbiological issues) and the inability to provide sufficient numbers of copepodites during the later larval stages were the major challenges reported in raising more juveniles. Pale coloration in the juveniles was also reported (Laidley *et al*., 2008).

In 2003, Black Pearl Inc. (Kona, Hawaii) reported raising close to 200 *C. loricula* juveniles using the cultured copepod *P. crassirostris* (Robbins, 2003). No further efforts to raise the species were apparently made.

Between 2004 and 2014, Syd Kraul of Pacific Planktonics (Kona, Hawaii) intermittently produced *C. loricula* juveniles for the aquarium trade. The company reported achieving survival of several hundred individuals per run in 725 L larval rearing tanks using cultured *P. crassirostris*. Mass production challenges have mainly been attributed to microbial issues during the postflexion larval stages (Syd Kraul, pers. com.).

In 2013, Karen Brittain (working for the Rising Tide Initiative on Oahu, Hawaii, Kuali System 31497) raised 25 *Paracentropgye venusta* juveniles and one *C. acanthops* juvenile using cultured and wild-collected copepods from a limited supply of eggs. Five percent survival of *P. venusta* larvae was reported (Karen Brittain, pers. com.).

Few publications on the larval rearing and juvenile growout methods used to rear dwarf angelfishes exist. Olivotto *et al*. (2006) described the early larval rearing of *C. flavissima* using copepod nauplii but the larvae were not raised to the juvenile stage. Laidley *et al*. (2008) described the challenges, accomplishments and some methodology of the aquaculture project addressing *C. loricula* at the Oceanic Institute. Baensch & Tamaru (2009 a, b) described some of the rearing methods used to rear *C. debelius* larvae and *C. resplendens* × *C. fisheri* larvae at Reef Culture Technologies.

The complicated requirements of late stage larvae and the long duration of the settlement phase are still the key challenges being faced by those culturing dwarf angelfish 13 years after the first juveniles were captive bred. The following account provides an overview of important aspects of the biology and captive-breeding methods of these popular and important aquarium fishes.

16.2 Broodstock

Dwarf angels are small, highly fecund reef fishes with relatively small territories that will spawn naturally in captivity when provided the proper conditions. The reproductive strategy is to produce a moderate number of small pelagic eggs, daily, near dusk, during the breeding season or continuously throughout the year, depending on latitude (Bauer & Bauer, 1981). All examined species are protogynous hermaphrodite and have a very similar reproductive biology (Moyer & Nakazono, 1978; Lobel, 1978; Bauer & Bauer, 1981; Thresher, 1982; Moyer *et al*., 1983; Moyer & Zaiser, 1984; Aldenhoven, 1986; Hioki & Suzuki, 1987; Lutnesky, 1988, 1989, 1991; Hioki *et al*., 1990).

In captivity, females can produce anything from a few hundred to a few thousand eggs every night. The pelagic eggs are spherical, nearly transparent and contain one oil droplet that makes them positively buoyant. The eggs range from 0.65 to 0.75 mm in diameter, depending on the species. *Centropyge colini, C. joculator* and *C. flavissima* eggs have a yellow oil droplet, which makes it easy to distinguish them from most other species which have a clear oil droplet.

The rate of egg and larval development is temperature-dependent (Baensch & Tamaru, 2009a). Time to hatching occurs at 16 to 18 h at 26–28 °C, depending on the species (Bauer & Bauer, 1981; Holt & Riley, 2001; unpublished data). Broodstock tanks with surface outflows that spill into a collecting tank are ideal for collecting pelagic eggs. In such systems the eggs can easily be concentrated into screened containers overnight and stocked into the larval rearing tank the following morning.

Egg production and quality in dwarf angelfish are regulated by diet and environmental parameters. Correct social organization, nutrition, tank parameters (size, height and shelter), water quality, light period and water temperature are important to obtain a consistent production of high-quality eggs.

16.2.1 Social Organization

The predominant social organization in genus *Centropyge* is the male dominated harem (Moyer & Nakazono, 1978; Moyer & Zaiser, 1984; Thresher, 1982; Michael, 2004). Every evening, at the onset of the crepuscular period, dwarf angelfish males undergo a lengthy and intense mating ritual with each mature female in their harem. Harems have a size-based hierarchical structure that usually consists of one large male, derived from sex change, one to four smaller mature females and up to nine immature females (Aldenhoven, 1986). Sex change is a socially controlled phenomenon, inhibited by the presence of the dominant male (Moyer & Nakazono, 1978; Thresher, 1982). Commonly, in the absence of a dominant male, the highest-ranking female will change sex, engaging in masculine behaviour within seven days thus becoming fully functional within about 20 days as observed by Michael (2004). All females spawn almost exclusively with the dominant male with the mating order-determined by size.

In captivity, dwarf angelfish are extremely belligerent towards members of their own species that do not fit into their size-based hierarchical structure. Dominant males will likely kill subordinate males or females attempting to change sex. Similar-sized females will fight until a higher rank is established by death. A large male or female and a smaller female are usually selected to form a dwarf angelfish pair.

Natural hybridization is widespread among coral reef fish, including dwarf angelfish. Eight presumed or probable naturally occurring hybrids between various dwarf angelfish species have already been reported (Pyle & Randall, 1994). In captivity, interspecific spawning has been documented for members of the *Xiphipops* subgenus. *Centropyge fisheri* × *C. resplendens* and *C. resplendens* × *C. argi* displayed reproductive behaviour within a few days after the two fish were placed together (Baensch, 2009a; unpub. data). These rare, artificial, captive-bred hybrids have commanded top dollar in the aquarium trade, retailing from US$600–US$1000 each.

16.2.2 Diet

Like most marine fish broodstock, dwarf angelfish egg fecundity and fertility is affected by the type and quality of their diet. Most species are omnivorous and have a non-specialized diet; primarily feeding on algae, detritus, interstitial fauna and sessile invertebrates. Other species are believed to feed primarily on sponges and tunicates in the wild and have proven more difficult to spawn in captivity (e.g., *C. colini, P. boylei* and *P. multifasciata*) (Michael, 2004).

Three daily feedings of a diverse seafood diet (e.g., clams, shrimp, fish, scallops, and fish roe), in addition to algae have resulted in good reproductive performance of *C. fisheri, C. flavissima, C. loricula* and *C. multicolor* over two years (Baensch, 2002, 2003). Encouraging natural algae growth, such as golden diatoms, for the fish to graze on, further supports broodstock health (Bauer & Bauer, 1981; Michael, 2004; pers. obs.).

Long-chain highly unsaturated fatty acids (HUFA) are essential fatty acids for marine fish due to their inability to synthesize them through elongation and desaturation of shorter chain fatty acids (Tocher, 2010). Callan *et al*. (2012) found that changes in dietary HUFA levels have a noticeable effect on the reproductive performance of *C. loricula*, as well as in egg and larval quality. The study found that fecundity, fertilization rate and embryo viability were significantly greater for pairs receiving a 3.6% *n*-3 HUFA diet versus 1.8%, with egg quality improving in as little as two weeks.

16.2.3 Tank Parameters (Size, Height and Shelter)

Adult dwarf angelfish average only about 10 cm in size (ranging from 6 to 25 cm). They typically inhabit reef or rubble areas that provide ample food and shelter and where the dominant male maintains a territory primarily for reproductive function during the courtship ritual.

In captivity, dwarf angelfish are naturally aggressive, given their dominating and territorial tendencies. Aggressiveness is a function of the species in question, as well as the sex ratio, the size of the aquarium and the amount of shelter available. Average sized dwarf angelfish pairs will spawn adequately over many years in 200–300 L tanks with a height of 60–90 cm (Bauer & Bauer, 1981; Olivotto *et al*., 2006; Holt & Riley, 2001; Baensch, 2002, 2003). Larger and higher tanks can increase reproductive performance significantly. Laidley *et al*. (2008) observed greater egg output with increasing tank size and obtained up to 1800 eggs/female (65% fertility) for *C. loricula* pairs held in 1000 L tanks (180 cm in height) (Table 16.1).

Shelter in the broodstock tanks is necessary to calm the fish and minimize aggression. The use of PVC pipes, clay pots and/or live rock provides adequate shelter. The shelter

Table 16.1 Summary of tank environment and reproductive performance of various dwarf angelfish species in captivity.

Species	Tank volume (L)	Tank height (cm)	Light (h)	Temperature (°C)	Average # eggs	# Fertile spawns	Fertility (%)	Reference
C. argi	180	n.d.	14–16	20–24	119	44	80	Bauer & Bauer, 1981
C. flavissima	180	n.d.	14	24–28	56	44	20	Bauer & Bauer, 1981
C. fisheri	180	n.d.	14	24–28	93	44	3	Bauer & Bauer, 1981
C. argi	300	60	11	24.5	100	478	87	Holt & Riley, 2001
C. flavissima	300	60	13	28–30	200	n.d.	90	Olivotto *et al.*, 2006
C. loricula	750	180	Natural	Natural	1200	2 years+	65	Callan & Laidley, 2010
C. debelius	500	90	11–14	23–24	230	56	20	Baensch & Tamaru, 2009a
C. fisheri	480	60	15.5	25–27	400	n.d.	90	Tamaru & Baensch, 2001
C loricula	200	60	14	26–28	435	10	80	Baensch, 1996
C. fisheri	200	60	14	26–28	977	10	60	Baensch, 1996
C. fisheri	200	60	14	26–28	367	15	95	Baensch, 1996
C. fisheri × *C. resplendens*	215	82	14	26.5	730	57	22	Baensch & Tamaru, 2009b

n.d. = no data.

structures should not interfere with the spawning rise. Baensch (2003) observed an increase in egg fertility when dwarf angelfish are given sufficient room (50 cm or more) during the final rise that culminates in spawning.

16.2.4 Water Quality

Dwarf angelfish require good water quality to remain in healthy spawning condition. Good spawning performance over long periods has been obtained in recirculating, temperature-controlled systems with established biological filtration (Bauer & Bauer, 1981; Holt & Riley, 2001; Baensch, 2003; Olivotto *et al*., 2006; Laidley *et al*., 2008). Flow-through systems using filtered, sterilized natural seawater have also given good results but recirculation systems have a lower risk of disease introduction and a tighter control of temperature and salinity (Laidley *et al*., 2008).

Live rock and algae growth (such as golden diatoms) in the tank allow the fish to graze, further helping to maintain water quality, and noticeably improving long-term reproductive performance (Bauer & Bauer, 1981; unp. data).

Callan & Laidley (2010) found that water chemistry is a major factor affecting the health, reproductive performance and egg quality of *C. loricula* broodstock. Fecundity, egg fertilization rates and egg viability improved when pairs were held in sterilized (chlorinated and de-chlorinated) ocean water compared with pairs held in treated or un-treated well water (which has lower pH and higher gas pressure than natural sea water).

16.2.5 Lighting and Water Temperature

It is well documented that temperature and photoperiod are important environmental cues that control reproductive cycles in reef fish (Holt & Riley, 2001). Warm water temperatures and long days induce gonadal maturation and reproductive behaviour in many marine fish species. Sub-tropical dwarf angelfish species, such as *C. interruptus* and *C. potteri*, are known to have a distinct spawning season in the wild (Lobel, 1978; Hioki & Suzuki, 1987; Lutnetsky, 1991) while tropical species experiencing little temperature variation usually spawn year round (Moyer & Nakazono, 1978; Bauer & Bauer, 1981).

In captivity, spawning will be continuous over several years, if the light period and the water temperature reflect that of the species' natural spawning season. Holt & Riley (2001) reported that *C. argi* spawned continuously over a broad range of day-length and temperature conditions (10–13 h light and 21–27 °C). Daily fecundity was strongly influenced by photoperiod and temperature with the highest fecundity occurring at 11–13 h light and 24–25 °C, while the lowest fecundity being recorded at 10 h light and 21 C. Spawning ceased when water temperature was increased to 28 °C or decreased below 20 °C (Holt & Riley, 2001).

16.2.6 Broodstock Summary

Culture advantages of broodstock: popularity in the aquarium industry; ease of husbandry of most species (small size and non-specialized diet); sexual dimorphism; early onset of sexual maturity; daily egg production over many years; and common species are easy to source.

Culture disadvantages of broodstock: small spawning numbers may require multiple pairs to obtain sufficient eggs for rearing; and poor reproductive performance occurs when conditions (especially diet) are inadequate.

16.3 Larval Rearing

The long and complex larval phase has kept many pelagic spawners, like dwarf angelfish, from being commercially cultured. Compared to the larvae of commercially propagated species (clownfishes, dottybacks and most gobies), dwarf angelfish larvae demand a smaller, more nutritious and easily digested food source at first feeding. Moreover, they are also more sensitive to environmental changes (water quality, lighting, temperature) and require optimal nutrition throughout development. Indeed, dwarf angelfish larvae take much longer to both reach and complete metamorphosis.

Reef Culture Technologies conducted a five-year study (2001–2006) to determine suitability of dwarf angelfishes for aquaculture. The study involved nine species and one artificial hybrid: *C. colini, C. debelius, C. fisheri, C. interrupta, C. loricula, C. flavissima, C. multicolor, C. resplendens, P. multifasciatus* and *C. fisheri* × *C. resplendens*. A total of 128 larval runs were conducted; 31 of these were conducted with single species for data collection. The remaining 97 runs consisted of two or more species at some time during the larval run to maximize tank space and live food resources. The findings of this research, partly published by Baensch (2002, 2003, 2006) and Baensch & Tamaru (2009a, b), are largely summarized from here on (due to the lack of additional published information on dwarf angelfish larval rearing and juvenile growout).

16.3.1 Rearing Systems

Dwarf angelfish larvae at our laboratory were reared in, small, static systems; 40-L rectangular glass aquariums were used up to settlement (3–40 days post hatching, dph) (Figure 16.2). At this time the larvae were moved into 80-L rectangular glass aquariums until settlement was complete. The tanks had painted black sides and painted off-white bottoms. Small tanks were used because they allow for more replicas and greater control of algae, live food levels and water quality. Trials using small tanks resulted in a higher percent survival than larger tanks (80–160 L). Stocking densities ranged between 100 and 400 fertile eggs (range: 2–10 eggs L^{-1}).

16.3.2 Water Temperature

Water temperature significantly affects developmental rates of fish embryos, larvae and young juveniles (Le Ye *et al.*, 2011). The water temperature of the larval tanks was regulated by means of a temperature controlled water bath. Water temperatures were maintained near the high end of the water temperature range (25–28 °C) the species experience in their natural habitat. Extreme (outside of the species natural range) or rapid changes in water temperature caused health issues (e.g., swim bladder hyperinflation, microbial infections) leading to high mortality events.

16.3.3 Live Feeds

The larviculture of dwarf angelfishes has only been accomplished using marine copepods according to all published reports. Copepods are the dominant natural prey item of marine fish larvae and are considered nutritionally optimal largely due to their high protein and lipid levels (namely *n*-3 HUFA). Rearing success to date at our laboratory has largely been attributed to the proximity and abundance of appropriate copepod species caught from the wild in local waters surrounding the coast of Oahu.

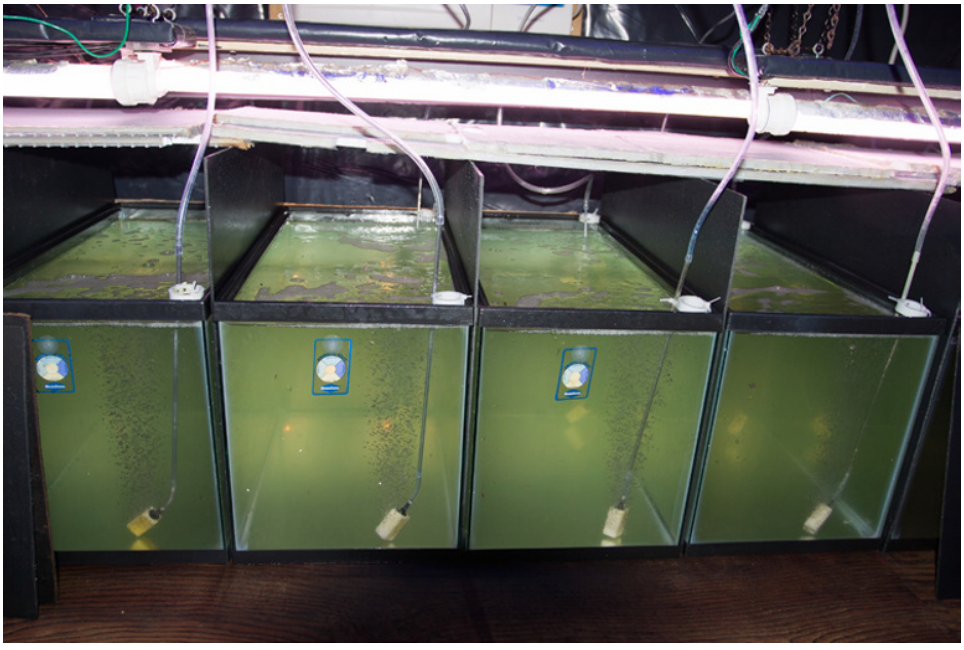


Figure 16.2 Experimental rearing systems used to culture dwarf angelfish larvae to settlement.

All angelfish larvae we have raised to date appear to have similar nutritional and environmental requirements. The main copepod species being preyed upon belong to the genus *Parvocalanus, Bestiolina* and *Oithona*. These copepod species produce life stages with the suitable prey characteristics (primarily size, nutrition and palatability) to culture the delicate larvae until they are able to accept, newly hatched *Artemia*.

In all rearing trials the larvae were fed size-strained wild copepods collected from local waters (Kaneohe Bay, Oahu, Hawaii) until settlement and newly hatched *Artemia* nauplii from settlement through the early juveniles stage (160 dph). The food types and feeding regime are summarized in Table 16.2. Survival was improved by: (1) frequently replacing old copepods with newly collected ones; (2) maintaining a high density of copepod nauplii and a low density of copepodite and adults in the tanks; and shifting *Artemia* from the Great Salt Lake strain (enriched and non-enriched) to a smaller sized, high HUFA strain from Vietnam (non-enriched).

16.3.4 Microalgae

The use of "green water", through the addition of microalgae, to encourage the initiation of first feeding by marine fish larvae is a common practice in the aquaculture industry. The use of background algae in the rearing tanks can improve the visual foraging ability of fish and contribute to maintain good water quality. The algae may also prime the larva's digestive system in readiness for exogenous nutrition and the fish may also derive direct nutritional benefit from the algae.

In all runs the microalgae *Nannochloropsis oculata* was maintained at cell densities near 1×10^6 cells L^{-1} in the rearing tank from first feeding up to the beginning of

Table 16.2 Larvae and juvenile feeding regime (*Centropyge debelius*).

Food type	Food size range	Stocking density	Approximate age (day) fed	Approximate fish size range
No food	–	–	Age 0–3	1.6–2.6 mm
Copepod nauplii	60–80 µm	10 mL^{-1}	Age 4–20	2.6–3.8 mm
Copepodites	100–200 µm	2 mL^{-1}	Age 15–30	3.7–6.0 mm
Copepod adults	300–500 µm	1 mL^{-1}	Age 25–65	4.7–13 mm
Artemia	400–500 µm	0.2–0.4 mL^{-1}	Age 35–160	7.5–33 mm
Grated gel diet, Cyclopeeze, flakes, pellets	700–1200 µm	–	Age 100–299	30–58 mm
Gel diet, flakes, pellets	1200 µm+	–	Age 300–500	58–70 mm

settlement. When algae densities were not maintained significant mortality was observed, most notably in early larval stages (through flexion). During the period when larvae began to settle microalgae density was maintained between 1×10^2 and 1×10^4 cells mL^{-1}, which could be beneficial by providing a calmer rearing environment than clear water.

16.3.5 Lighting

First feeding dwarf angelfish larvae prey more effectively on copepod nauplii under intense light but become increasingly sensitive to light in later stages. One dimmable VHO fluorescent bulb (110 watts) suspended 20–120 cm above the tank provided lighting. The light bulb was raised as the larvae grew and became more sensitive to light. All larvae were provided with a 14L:10D photoperiod cycle. The lights on was set at 6 a.m. and lights off was at 10 p.m. with a one hour dimming period in the morning and evening. The dimming period proved crucial during later stages of larval development when a higher sensitivity to light was recorded. Abrupt increases in light caused the larvae to skip on the surface frantically often resulting in mortality. Direct, intense light also prolonged the settlement period.

16.3.6 Circulation

Dwarf angelfish yolk-sac larvae are sensitive to turbulence. Preflexion larvae feed in upwelling currents created by an air stone. In initial trials, a single diffuser (1.3–5 cm) located in the corner of the rearing tank continuously provided aeration. In later trials, a second diffuser was added in the far corner opposite to the first diffuser, to create more uniform circulation within the tank. Aeration was maintained at 1–3 bubbles/sec through first feeding and then gradually increased as the larvae grew. High mortality was observed when aeration was too high during the early stages or not increased sufficiently during the later larval stages.

16.3.7 Water Quality and Maintenance

Dwarf angelfish larvae are sensitive to microbial pathogens, old/poor quality live prey and poor water quality. Copepodites, adult copepods and/or *Artemia* were replaced with fresh, appropriately sized prey every one or two days by carefully filtering the tank

water through a 50 µm screen. Water quality was maintained by water changes and bottom siphoning, live food filtering and by transferring the larvae to new tanks. The main water quality indicator used for all rearing trials was unionized or free ammonia (NH_3). New tanks were set up with 50–75% fresh seawater when free ammonia levels exceeded 0.25 mg/L, which occurred every 7–20 days. The larvae were transferred using a small glass beaker (80–120 mL) to new tanks at a maximum stocking density of one to three larvae/L. Lower feeding incidence, emaciation, rapid respiration, erratic swimming behaviour and hyperinflation of the swim bladder and mortality were observed when conditions were allowed to deteriorate. The transfer of larvae to new tanks and the replacement of live prey proved especially important to minimize mortality events in later stage dwarf angelfish larvae. A similar scenario has also been observed in yellowtail amberjack (Stuart *et al*., 2012)

16.3.8 Larval Biology

Dwarf angelfish larvae measure about 1.6 mm at hatching (0 dph) (Figure 16.3 a). At this stage they are positively buoyant, have a large yolk-sac that extends past the head,

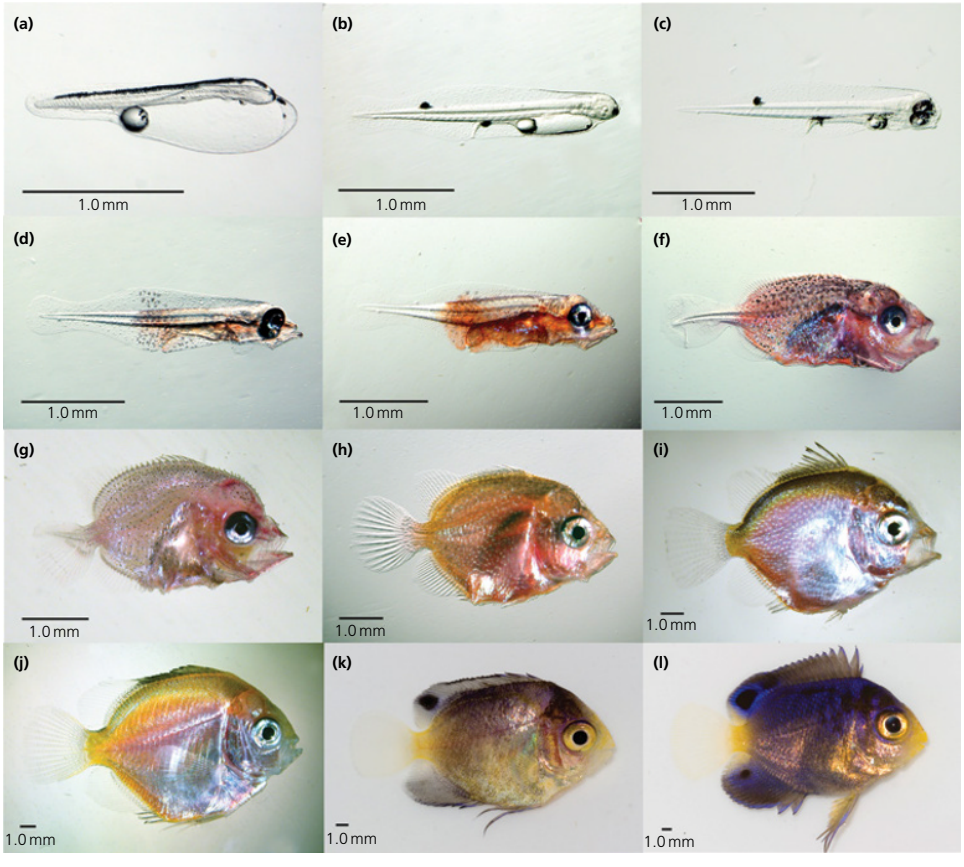


Figure 16.3 Photomicrographs and photographs of the various stages of development of a typical dwarf angelfish (e.g. *Centropyge debelius*). (*See insert for color representation of the figure.*)

unpigmented eye spots without lenses, and no discernible mouth or gut. Strong contracted melanophore pigmentation is present on the dorsal body axis and the oil globule. The larvae are only capable of single short swimming bursts.

Yolk-sac stage or hatching to complete absorption of yolk sac (Figure 16.3 b–c) occurs at 1–3 dph. As the yolk-sac is depleted dwarf angelfish larvae rapidly develop functionality. The pectoral fins begin to appear. Near 3 dph, complete eye pigmentation (when eyes become completely black) and yolk-sac regression occurs. Also at this time the mouth and anus open and jaws begin to form. The larvae are capable of short swimming bursts and begin learning how to feed. No significant differences in growth are observed up to this point. The larvae begin to feed on 60–70 µm copepod nauplii over the next 24 h.

Preflexion begins at 3–4 dph (Figure 16.3 d). Early preflexion larvae have several branched melanophores concentrated in the central zone of the larval body. The gut is coiled and the formation of blood vessels is noticeable. Small teeth and head spination also become apparent. Ontogeny and growth is very similar among *Centropyge* species up to this stage in development. Properly nourished larvae will undergo noticeable vascularization (blood vessel formation) during the first two weeks of development. Middle to late preflexion stage dwarf angelfish larvae are heavily vascularized and have a visible air bladder that is located anteriorly above the gut. Air bladder inflation occurs between day 7–9 (Figure 16.3 e). From this stage forward, significant variation in body size within each cohort can be observed.

Flexion begins at 14–18 dph (Figure 16.3 f). Dwarf angelfish undergo flexion in two to four days, depending on the species. During this stage the body becomes noticeably deeper and laterally compressed and the long narrow notochord tip bends upward. Melanophores increasingly cover the body and the larvae exhibit a red color in the water column. *Centropyge resplendens* and *C. multicolor* flexion larvae are, on average, smaller compared to other *Centropyge* species. Subtle differences in the rate of growth between species first appear near day 10. Mortality levels fluctuate between species and rearing trials but are highest through flexion. The larvae begin to feed on copepodites at this stage.

Postflexion begins at 16–26 dph (Figure 16.3 g). On average, the larvae complete flexion near 20 dph. The body of postflexion dwarf angelfish larvae takes on a silver coloration below the dorsal area and becomes increasingly laterally compressed. Size differences between species become more apparent despite significant size variation within each larval cohort. By 30 dph, each species develops pigmentation on the dorsal area. The dorsal hue of *C. colini* is a bluish-yellow, *C. fisheri* is brown, *C. flavissima* is bright yellow, *C. interrupta* is yellow-orange, *C. loricula* is red, *C. multicolor* is yellow, *C. resplendens* is gold and *P. multifasciatus* is a grey-blue. During this stage *C. colini* develops long ray extensions on each pelvic fin. Significant differences in the growth rate within cohorts are observed as the larvae complete flexion. Postflexion larvae gradually feed more on adult copepods, beginning with *Oithona* sp.

Settlement begins at 30–50 dph (Figure 16.3 k), depending on the species. Settling larvae range between 10 to 14 mm in total length (TL). A darkening of the soft dorsal and anal fins associated with substrate on the bottom of the tank marks the beginning of settlement. Swimming behaviour is erratic when disturbed. *Artemia* sp. and adult copepods are accepted at this time.

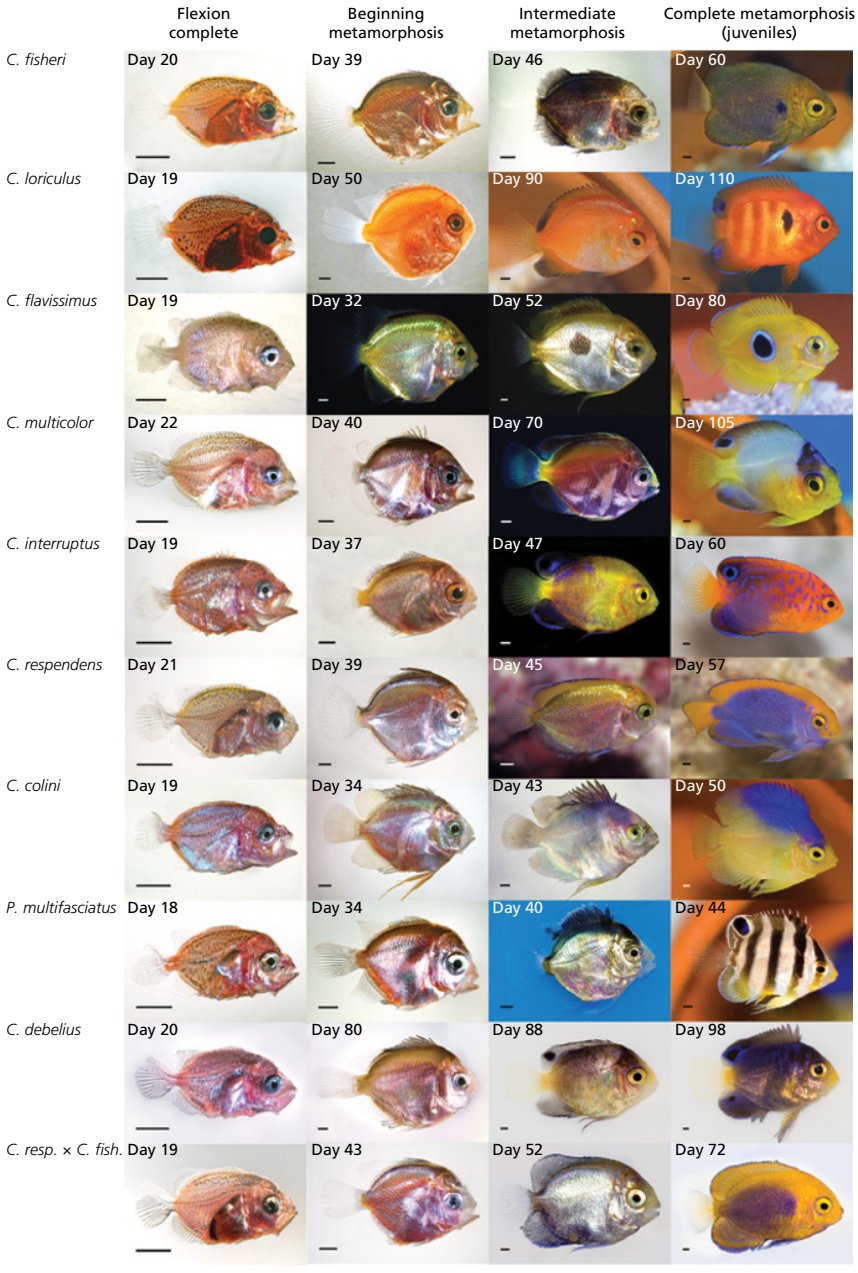


Figure 16.4 Late larval and juvenile development of nine dwarf angelfish species and one hybrid. (*See insert for color representation of the figure.*)

Juvenile stage begins at 48–109 dph (Figure 16.3 m), depending on the species. Dwarf angelfishes undergo a long settlement period. As juvenile coloration gradually fills in, the larvae become more stationary and swim less erratically. At this time they are transferred to a growout tank (Figure 16.4).

Table 16.3 Settlement times and percent survival of various dwarf angelfish species and hybrids.

Species	Years of trials	Settlement beginning (dph)	Settlement end (dph)	Survival (%)	Average # of juveniles per trial	# single species trials
C. fisheri	2001	41	55	4.3	13	4
C. flavissima	2002	32	65	3.7	9	2
C. loricula	2002	50	91	0.8	5	2
C. multicolor	2002	41	99	1.3	4	2
C. interrupta	2002–2006	38	62	6.0*	16	8
C. resplendens	2004	42	63	9.2*	46	4
C. colini	2005	37	57	10.0*	33	2
P. multifasciata	2005	36	48	6.3*	7	2
C. debelius	2005	80	95	0.7**	2	4
C. resplendens × *C. fisheri*	2006	43	70	16*	92	1
Average (Total)	–	44	70.5	5.8%	23	31

* While the basic rearing method and equipment remained the same for all species in the trials over the 5-year study several changes were made in an attempt to improve the technique and increase survival. These include: (1) changing to a Vietnam strain *Artemia* from GSL strain (smaller and higher HUFA); (2) eliminating *Artemia* enrichment media; (3) increasing the stocking density of copepod nauplii; (4) decreasing the stocking density of adult copepods; (5) improving hygiene; (6) increasing experience level.
** Low survival can be attributed to single mass mortality event that occurred on 71 dph (31 December 2005) from a *Vibrio* infection.

16.3.9 Study Results

The developmental stages of the nine species and one hybrid dwarf angelfish investigated by the author were found to be very similar up to metamorphosis. However, larval survival and duration of the larval period did vary for each species (Table 16.3). Mortality fluctuated (amongst species and trials) but decreased once the larvae completed flexion (16–26 dph). Survival averaged 5.9% for the 31 larval trials being highest for the hybrid, *C. colini* and *C. resplendens* and lowest for *C. debelius, C. loricula* and *C. multicolor*. All species started to settle between 30 and 50 dph, with the exception of *C. debelius* which only started to settle at day 80. Overall, amongst the species studied, complete juvenile transformation occurs between 58 and 99 dph (Table 16.3).

16.3.10 Larvae Summary

Culture advantages of larvae: high survival through hatching; first feeding larvae are able to feed on small copepod nauplii; high survival through early stages; high survival on *Artemia* from about 30 dph; multiple species can be cultured together due to minimal late stage interspecific aggression; larvae do well in small tanks; and larvae can be raised on both cultured and wild copepods.

Culture disadvantages of larvae: first feeding larvae cannot be reared on rotifers (unlike the most commonly mass cultured marine ornamentals); larvae have only

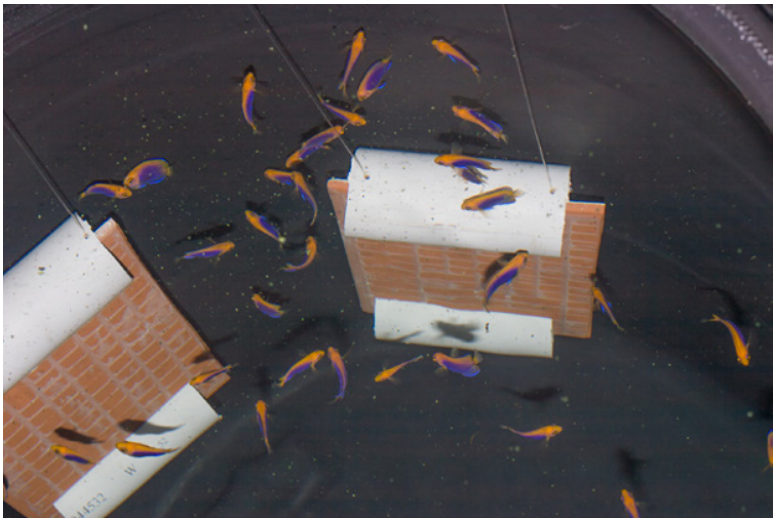


Figure 16.5 Juvenile dwarf angelfish (*Centropyge resplendens* × *Centropyge fisheri*) in 200 L tank (barrel).

been reared on specific copepod species; late stage larvae may be sensitive to bacterial problems, light and temperature changes, and disturbances (such as maintenance); larvae may delay metamorphosis, especially under crowded conditions; present culture techniques are costly and result in less than a few hundred individuals per run; and aquaculture production cannot compete with prices of common wild caught dwarf angelfish.

16.4 Juvenile Growout

Newly transformed captive-bred dwarf angelfish juveniles are well adapted to captivity and appear to have no special growout requirements. The fish were grown out in 200 L barrels or 80 L glass aquaria growout tanks, connected to recirculation systems. The filtration of each system consisted of a 10 µm filter bag, a protein skimmer, a UV filter and wet/dry filter or deep-sand beds in the sump. Juvenile aggression and susceptibility to constipation and "head and lateral line erosion" (HLLE) are main problems observed during growout (Figure 16.5).

Newly settled juvenile dwarf angels (15–20 mm TL) usually become increasingly aggressive towards conspecifics as they attempt to establish a social hierarchy. Aggressive, dominating behaviour can inhibit settlement and affect the health of smaller, weaker siblings. Dwarf angelfishes should therefore be transferred out of the larval rearing tank to growout tanks when they become bottom oriented and display juvenile behaviour. Aggression in the juvenile tank can be minimized by: maintaining relatively low stocking densities (about one fish per 4 L); separating dominating individuals; and providing ample hiding infrastructure (Figure 16.6).

As referred above, juvenile dwarf angelfishes are susceptible to constipation and HLLE. Feeding moist, low-fat foods rich in protein and algae can minimize

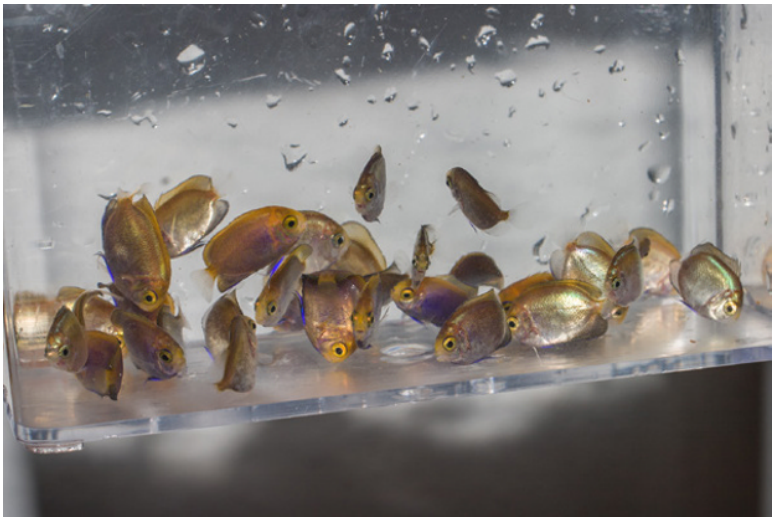


Figure 16.6 Newly settled dwarf angelfish (*Centropyge resplendens* × *Centropyge fisheri*) being transferred to juvenile growout.

constipation, which is apparently caused by the overfeeding of fat-rich (Cyclop-eeze) and dry foods (flaked foods, pellets). HLLE is a condition widely found among surgeonfishes and angelfishes kept in aquariums (Blasiola, 1990; Hemdal, 2003). No definitive cause for this condition has been determined but speculative theories include dietary deficiencies, poor water quality, activated carbon in the water, exposure to copper, stray voltage, lack of natural sunlight, tumors, viruses, parasites, bacterial, chronic stress and autoimmune disease. In our systems at Reef Culture Technologies LLC, HLLE was virtually eliminated when the in-sump biological filtration method was changed from wet/dry BioBall® towers to natural submerged substrate (live rock or deep sand beds). Laidley *et al.* (2008) observed coloration problems in juvenile *C. loricula*, which may have been related to HLLE.

16.4.1 Growout Conditions

The following growout conditions, methods and equipment resulted in high survival of healthy, salable, 3–4 cm juveniles with good coloration within one to two months following transfer from the larval tank: (1) a maximum stocking density of one fish for every for 4 L; (2) the provision of ample hiding infrastructure (such as PVC pipes, clay pots); (3) water temperature in the species natural range (maintained by a chiller); (4) a 14-h light period and sufficient lighting to stimulate growth of diatoms (provided by one 30–40 watt fluorescent bulb suspended above the tanks); (5) a moist, low fat, high protein diet fed five to six times per day, including live *Artemia* (enriched with *Pavlova* and *Isochrysis* paste) and using a frozen gel diet (home-made), as well as pre-soaked 100% spirulina flake; and (6) maintaining water quality by daily siphoning the waste from the bottom of the tanks and performing bi-monthly 50% water changes (Figure 16.7).



Figure 16.7 Four-centimeter dwarf angelfish juveniles (*Centropyge colini*) ready for sale. (*See insert for color representation of the figure.*)

16.4.2 Juvenile Summary

The culture advantages of juveniles are: reaching market size in 120–150 dph; no special growout requirements (grown out on conventional aquarium foods in small, shallow tanks); robust (ship well); and minimal interspecific aggression.

Culture disadvantages of juveniles: aggressive towards conspecifics; susceptible to bloat and HLLE; and poor coloration.

16.5 Conclusions

Progress in bringing high numbers of dwarf angelfish larvae through settlement has been slow, with few researchers and farmers working on mass culturing the larvae. The late larval stages (postflexion) continue to be the major aquaculture bottleneck thirteen years after the first juveniles were captive-bred. While *C. loricula* breeding in large larval tanks (800–1000 L) using cultured *P. crassirostris* has allowed bringing thousands of larvae through the early stages (flexion), erratic mortality due to nutritional and microbial issues results in low, fluctuating yields of no more than a few hundred juveniles per tank. On the other hand, rearing techniques employing smaller, static larval systems (40–80 L) and wild copepods (collected from Kaneohe Bay, Oahu, Hawaii) have resulted in lower mortality through settlement but are labor intensive, impractical to scale up and unreliable during periods of low copepod quantity and quality (occur during the winter months in Hawaii).

Improving dwarf angelfish survival through settlement is likely primarily a dietary issue and secondarily a matter of providing the appropriate environment (particularly light intensity, space, water quality and substrate type). Successful mass culture will no doubt involve rearing the larvae in large tanks on cultured copepods. The nauplii of

cultured *Parvocalanus* sp. have proven to be an ideal prey item for the early larval stages of *C. loricula* and will likely be suitable too for the mass rearing of other dwarf angelfish species as well. However, a diet solely providing *Parvocalanus* sp. has yielded inconsistent results for late stage larvae, which may require the qualities (size, palatability and nutritional value) of additional copepod species to survive. Large calanoid species, such as those in genus *Pseudodiaptomus* or *Acartia* spp., are readily mass cultured and produce nauplii and copepodite stages with these qualities.

The following recommendations can be made for culturists that have the desire to raise dwarf angelfish larvae: (1) acquire experience in keeping adults and establish healthy pairs (through proper selection, quarantine and pairing) by conditioning adults using the proper diet, light period, temperature and system design; (2) acquire experience in culturing microalgae, such as *Isochrysis*, to culture copepods and for using as background algae in larval rearing tanks; (3) acquire experience in culturing calanoid copepods; such *as Parvocalanus* sp.; and (4) acquire experience in raising small larvae with long larval stages. Dwarf angelfish can be reared in simple, small, static systems. The larvae do not require large tanks or technically complex larval systems to survive. Familiarity with larval biology, proper maintenance, and the correct light intensity, tank circulation, microalgae and copepod size, and stocking density changes as the larvae grow are important to achieve suitable survival.

The biology of dwarf angelfish larvae may never be suitable for mass farming. However, with so many unanswered questions, few publications and few people working on the subject, more research is needed before this question can be answered. The incentives are obvious: lessening the collection pressure on wild specimens and the strong demand for these high value products.

Acknowledgements

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17

Seahorses and Pipefish

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Abstract

Syngnathids, particularly seahorses, have long fascinated humans. Surprisingly, their biology and ecology is still poorly known. That is probably one of the reasons why reared fishes of these species have only recently been introduced into the aquarium trade. Currently, rearing techniques are available for a dozen seahorse species and for a few ornamental pipefishes. Even though the morphology of all these species is rather similar, their biology and rearing requirements largely differ among species. As a result, the methodological procedures for cultivation must be studied and assessed explicitly for each species. Noteworthy survivals have been achieved for some species but there is a need for optimization of rearing techniques, and increased knowledge in many aspects including breeding, nutritional requirements and diseases. This chapter is focused on general aspects of the biology of seahorses and pipefishes and on current knowledge of their rearing techniques. Data sheets on the rearing requirements for selected seahorse and pipefish species are also provided, as well as for seadragons.

Keywords *Breeding; culture; diseases; husbandry; pipefishes; rearing; seahorses; syngnathids*

17.1 Introduction

Pipefish and especially seahorses are phylogenetically recent but unusual fishes that can be considered a flagship species (Planas, 2014). They belong to the family Syngnathidae, which is characterized by their fused-jaws and elongated bodies. They range in size from the largest pipefish known (65 cm in length), to the smallest pigmy seahorse (about 1 cm in length). Some pipefishes inhabit freshwater areas but most species are adapted to tropical and sub-temperate shallow marine waters in association with seagrass and reefs, occurring almost throughout the world but mainly in the Indo-Pacific. All seahorse

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species (about 50) are included in genus *Hippocampus*, whereas pipefish species are distributed in more than 50 genera and about 200 species (Kuiter, 2000).

The rearing of these species is a relatively recent activity (Koldewey & Martin-Smith, 2010) with increasing demand and high market prices. Although available rearing technology already promotes acceptable survival rates for some species, more studies are still necessary to further optimize culture protocols, especially addressing nutritional and microbiological aspects (Olivotto *et al.*, 2011). Understanding the feeding and nutritional requirements is of paramount importance for rearing success. The expansion of knowledge on the biochemical composition of natural prey and embryos/larvae will significantly enhance our understanding of the nutritional requirements for breeding and rearing these highly popular marine ornamental fishes. In spite of some attempts to reduce live feed, the absence of adequate artificial or inert diets for juveniles and adults is one of the main bottlenecks in the cultivation of these fishes, and the production of live food is costly (Woods, 2003a).

Considering that many syngnathids, particularly seahorses, are submitted to conservation plans in many countries and to trade regulations, the interest in developing successful rearing procedures is obvious. The production of reared seahorses and their introduction into the international aquarium trade would certainly contribute to a reduction in fishing pressure on these amazing fishes and consequently to the conservation of the natural biodiversity in our oceans and seas. Relevant topics for the successful culture of syngnathids are expounded in this chapter.

17.2 Anatomy and General Biology

Syngnathids are true bony fish (Actinopterygii) but have some characteristics that clearly differentiate them from most other fishes, such as the large tubular snout for suction feeding, prehensile tail found in seahorses and some pipefish, and the exclusive biological trait where males take care of early embryos in a more or less developed pouch. One of the most characteristic features of these fishes is their highly modified skeleton formed into bony plating, several rings and longitudinal ridges (species specific) and, the lack of scales.

Seahorses are highly modified pipefish. The typical upright posture of seahorses is not present in pipefishes, which look like straight-bodied seahorses with small mouths. Three parts of the body can be differentiated: head, trunk and tail. The head supports a long and thin tubular snout with a small mouth (very thin in pipefishes), gill openings (near the upper posterior angle of the gill-cover) and some spines depending on the species. Some species of seahorses have cirri in the area of the facial spines and trunk ridges. Most organs, including heart, liver, swim bladder, intestine, urinary bladder and reproductive organs are located in the trunk. They possess an inefficient digestive system (without stomach) and hence they need to eat frequently. Pectoral fins are present in seahorses (to assist in navigation) but not in all pipefish. The ventral fins are always absent. The movement is controlled totally or partially by the dorsal fin. Unlike seahorses, most pipefishes have a caudal fin.

These fishes but especially seahorses have a more or less sedentary lifestyle with an efficient and very maneuverable swimming capacity, but at very low speeds. Seahorses excel at camouflage and changing color, which together with the ability to grow skin

filaments, enables them to blend in with their surroundings and to avoid their predators, mainly crabs and large piscivorous fish. Short-term color changes may also occur during courtships and daily greetings that they display before mating. Nearly all species are visual opportunistic predators feeding upon small crustaceans such as amphipods, copepods and mysids, although they can feed on other prey items occasionally. Smaller species apparently tend to have shorter longevity, whilst larger species has been regularly recorded living up to 5–6 years in captivity.

All seahorses and pipefishes are gonochoristic and viviparous. Eggs are deposited by females in different structure types of males dependent on species. When mature, male seahorses develop a brood pouch at the base of the tail, just below the last trunk ring. The pouch acts like a womb, providing nutrients and oxygen to the developing embryos, while removing wastes. The pouch also provides gradual and slow osmotic adaptation from internal body fluids to external surrounding seawater, reducing the stress of the offspring at birth. In some pipefish species, the eggs are deposited and adhered to a spongy skin along the underside of the body or at the base of the tail. In other pipefishes, there is a more or less developed pouch. Generally seahorses are monogamous (pair bonding), but many pipefishes are polyandrous (a female mates more than one male). Female fecundity, gestation period and number of newborn vary depending on the species.

Newborns receive no further parental care, are free swimming with little or no yolk sac, and start to feed immediately. Newborn seahorses are miniatures sets of parents. The duration of the pregnancy is inversely related to temperature, ranging from about two weeks to not much more than a month. The sizes of newborns of several known species vary between 6 and 16 mm (Silveira & Fontoura, 2010). Most newborns tend to have poor swimming capacity and normally spend their first few days drifting away, going through a pelagic phase where they do not use their prehensile tail to hold on to a surface. Other species are very site or host-specific and tend to have young that readily hold on to any surface as soon as they hatch. Some young have been spotted attached to floating *Sargassum*, which certainly increases their dispersal potential. Differences in this behaviour should be taken into consideration in captive breeding programs.

17.3 Reproduction and Mating System

Different types of mating patterns and brooding structures are present in syngnathids. Females transfer unfertilized eggs to the male's ventral surface or to an elaborated brood pouch with a small opening through which eggs are deposited and then fertilized. Brood structures vary considerably among species and provide different levels of physical protection, from both potential predators and external weather conditions, for the developing embryos (Dawson, 1985; Herald, 1959). There are various morphologies to brood the eggs, with pipefish having eggs below the tail in semi-enclosed to completely enclosed but unsealed pouches. In seahorses, eggs are deposited in the brood pouch through a small opening. Pouch enclosures are intermediate in some species with two ventral flaps meeting midline and sealing during brooding (Ripley & Foran, 2006). A placental fluid removes waste products and supplies the eggs with oxygen, osmoregulation and some nourishment.

The observation of the embryonic and larval development of *S. abaster* reinforces the notion that the presence of a marsupium (pouch) contributes to a higher degree of newborn development, when compared to a marsupium-lacking pipefish (Monteiro *et al*., 2003). Species with a fully enclosed pouch are able to carry an additional number of eggs, therefore are able to produce a greater number of offspring per pregnancy. There is increased protection to the developing eggs from the surrounding environment, as well as increased development of the eggs, while in the pouch.

Other than the existence of the brood pouch and longer tails, seahorses tend to be the least sexually dimorphic of all the syngnathids. Concerning pipefish species, many are dimorphic, with the female being much larger and more brightly colored than the male. In both seahorses and pipefish there are secondary sex characteristics such as iridescent bars on sexually mature specimens. In captivity several species of seahorse become sexually active in only 3 to 6 months. Seahorses frequently maintain long-term pair bonds in a breeding season, display daily greetings and are genetically monogamous in a single breeding cycle. Polygamy has been reported in some species such as *H. abdominalis* (Wilson & Martin-Smith, 2007) but is much more frequent in pipefishes (Berglund *et al*., 1989; Vincent *et. al*., 1995). Polygamous pipefishes are highly dimorphic in color, ornamentation or size. Most seahorse species are generally monogamous during a breeding cycle. In some pipefishes, quivering by the female during courtship is followed by egg transfer to the male, and a subsequent embrace by the pair (Silva *et al*., 2006a). There can be a range of display behaviours, with competition and mate selection being observed in both females and males. Some species of syngnathids have juveniles that disperse, while in other species the adults may migrate (Silva *et al*., 2006b).

Some syngnathid species have elaborate courtship displays, dancing, weaving, rolling and rising vertically in the water column. Other species are not as active. Seahorse males often display for females and follow the female closely. The males open their pouch and try to position themselves for mating. Pipefish females are often the sexual aggressor. In some species it is the female that is more brightly colored and tries to initiate mating by displaying.

Seahorse egg numbers vary according to adult size and age, and range from 10–3000. In pipefish, the number of eggs can vary from 20–200 (Browne *et al*., 2008), and eggs tend to be larger than those of seahorses. In pipefishes, the males of some species substantially contribute to egg development, through mechanisms, such as osmoregulation and gaseous exchange functions (Carpucino *et al*., 2002). The hatching period of syngnathids is between 14 to 40 days, and may be shortened at higher temperatures (4 days per 1 °C in the temperate seahorse *Hippocampus guttulatus*) (Planas *et al*., 2010). Many syngnathids will brood multiple batches of eggs during most seasons or when held at constant temperatures and photoperiods in captivity. The breeding season in captivity conditions has been successfully shifted by artificial manipulation of both temperature and photoperiod (Planas *et al*., 2013).

For syngnathid embryos a standardized classification, has been proposed considering four developmental periods (early embryogenesis, eye development, snout formation, and juvenile) which are further divided into 11 stages (Sommer *et al*., 2012).

Newborns begin feeding almost immediately after release (Figure 17.1). Young consume an enormous amount of prey. They are pelagic for some time, or hold onto



Figure 17.1 Newborn pipefish.

floating debris at the surface with their tail. Others, usually those born larger, immediately drop to the substrate and adopt the behaviour and habitat of their parents (Silva *et al*., 2006b). There is no parental care or recognition, and parents may even ingest their own hatchlings (Silva *et al*., 2006a, b).

17.4 Husbandry

Seahorses and pipefish are quite sensitive to changes in the water chemistry. It is very important to provide aquarium water parameters that mimic where they are found in the wild. Syngnathids do not adapt well to changes in water quality. Whether your system is open, closed, or semi-open, it is highly recommended to have the seahorse tank recirculating from a sump with additional filtration. This filtration should include a UV sterilizer, a particulate filter, and a protein skimmer.

Daily maintenance of the tank will require feeding, siphoning, specimen observations, and sump and filter corrections. These tasks are quite simple to perform, but are a must. The tank must be closely monitored for signs of pest growth (anemones, hydra, crabs). These tiny pests can wreak havoc on seahorses. It is advisable to test the water quality parameters daily until a set pattern is developed. Water changes too, should be performed daily until the water parameter pattern is established.

17.4.1 Habitat

Seahorses and pipefish utilize most temperate and tropical habitats. They can be found on muddy, sandy, and rubble bottom habitats. They are often found living on and around sessile bottom invertebrates. The key to these environments is that they allow plenty of areas in which the seahorse or pipefish can hide and use their natural cryptic ability to avoid predators. These habitats are areas that promote the growth of amphipods and other crustaceans, important food sources.

17.4.2 Tank Mates

Although seahorses and pipefishes are very secretive and prefer to be hidden in artificial habitats that closely simulate their natural environment, they are often maintained in community tanks. Neither competes well with the more agile finned fishes; pipefish often out competes seahorses. It is wise to only place seahorses and pipefish in with smaller, less aggressive, tank mates. It is important to watch closely to be sure they don't have difficulty competing for food.

Some species of pipefish are very territorial towards each other. In the wild, they live in male–female pairs and stake out a cleaning station or hunting ground, and chase off any competitors. In captivity, they will defend their territory until one or both are dead, even different species are intolerant of related species or similar looking species. This is often the case with the flagtail pipefish, genera *Doryrhamphus*. However, there are many species in this genus that do well in small groups.

17.4.3 Feeding

Syngnathids are mainly visual carnivorous predators that, due to their limited swimming capacity, rely more on their cryptic color and texture camouflage to ambush their prey. This creates problems in captivity as most wild fishes will not readily accept *Artemia* or artificial and frozen foods (Woods, 2003a). They need to feed frequently due to the simplicity of their digestive system. Studies of seahorse diet in the wild indicate that amphipods, together with caprellids, copepods and mysids are the main prey items (Teixeira & Musik, 2001; Woods, 2002; Storero & González, 2009; Gurkan *et al*., 2011; Valladares *et al.*, 2016). Syngnathids use their elongate tubular snouts to consume small animals, mainly planktonic and nektonic crustaceans, as well as their larvae. Species with short snouts feed on invertebrates in vegetation or among detritus on the seafloor, while those with longer snouts target prey such as mysid crustaceans and other invertebrates in the water column (Bergert & Wainright, 2004; Kendrick & Hyndes, 2003.

Artemia is the most commonly used live prey in ornamentals rearing but its nutritional quality does not seem to be the most adequate to fulfill the needs of breeders. Growth is very well supported in adult seahorses fed on *Artemia* (Planas *et al*., 2008; Otero-Ferrer *et al*., 2012) but breeding success is affected. Otero-Ferrer *et al*. (2012) demonstrated that a diet exclusively based on the mysidacea (*Leptomysis* sp.) enhanced reproductive success (spawning events, brood size and newborn length) and reduced abnormalities in newborn batches of *Hippocampus* when compared to breeders fed enriched adult *Artemia* (Planas *et al*., 2009a). Similar improvements were observed in *Hippocampus guttulatus* co-feeding on a diet consisting of a mixture of *Artemia* and Mysidacea (unpub. data). These findings demonstrate the high potential of the later as live prey for seahorses. Several species and sizes of frozen Mysidacea are frequently available at reasonable prices in the specialized market. When unavailable, an adequate enrichment of adult *Artemia* is imperative to increase breeding success and the quality of newborns (Planas *et al*., 2009b). A mixture of *Artemia* and mysidacea is also an affordable and efficient diet.

Several authors have recently pointed towards the potential of amphipods as live-prey in aquaculture (Baeza-Rojano *et al*., 2014; Woods, 2009). Interesting results were obtained by Murugan *et al*. (2009) for *H. trimaculatus* broodstock performance when fed with the amphipod *Eiopsia* sp., and by Storero & González (2009) that observed a

significantly greater consumption of amphipods (*Melita palmata* and *Ampithoe valida*) by *H. patagonicus* in captivity when presented with the choice of an alternative wild shrimp prey item. Recent experiments with *H. erectus* juveniles resulted in significant increase in survival and growth when fed either 100% live amphipod (*Parhyale hawaiensis*) diet or 50–50% amphipod-*Artemia* diet (Cruz, 2014). These findings, together with the exceptional nutrient profile of wild and cultured amphipods, provide a promising alternative to frozen mysids.

A cause for concern when collecting saltwater species (copepods, seed shrimps, water fleas and plankton) to feed syngnathids is the possibility of introducing fish parasites such as fish lice (*Calgus* and *Argulus* spp.) into the aquarium. However the value of nutritional variety, especially for such a sensitive animal as the seahorse, overrides the danger presented by parasites.

Syngnathids have a very simple digestive system and do not have a true stomach, so food is not retained for any length of time in the body. Therefore theses fishes need to feed throughout the day. If fed solely on dead food they will need to be provided with food three to four times a day. An adult seahorse can consume 50 to 200 food items a day, depending on the temperature and the seahorse size, whereas growing young may require over 3000 food items.

Proper nutrition is critical to fry's survival. The early rearing of juveniles is commonly based on the administration of two of the most commonly used live prey in larviculture: rotifers and *Artemia*. Depending on the mouth size, newborns of some species need rotifers at first feeding (e.g., *H. ingens*, and many pipefishes) whereas others (e.g., *H. abdominalis*, *H. reidi*, *H. guttulatus* and *H. hippocampus*) can be initially fed *Artemia* nauplii, replaced later with enriched *Artemia* metanauplii. One of the major problems with some syngnathids is related to their low digestion capabilities, but there are significant differences among species. The newborn of some species are capable of digesting *Artemia* nauplii and metanauplii, others are not. In the latter case, *Artemia* items are poorly digested and excreted alive. Under these circumstances, there is no assimilation of the food and consequently the juveniles are progressively submitted to a negative energetic balance (Blanco *et al*., 2011). This constraint reverses to a more or less extent with the improvement of digestive enzymatic capacity with age (Blanco, 2014).

Artemia enrichments are available to improve the quality of *Artemia* metanauplii but the results vary considerably among species and the type of enrichment applied (Wong & Benzie, 2003; Woods, 2003b; Palma *et al*., 2011; Vite-García *et al*., 2014). There are many additives now available to enhance the nutritional value of prey. These additives can be very effective for increasing the survival rate of the young. This is especially true for those species that are tiny at birth. Even though the nutritional requirements of syngnathid juveniles are unknown, a huge improvement in initial rearing has been reported for many species when juveniles are fed copepods (Olivotto *et al*., 2011; Blanco, 2010). Indeed, copepods have superior nutritional quality over *Artemia* but also provide a wider size range to fulfil the feeding needs of juvenile fishes.

Many species can be trained to eat frozen foods, but smaller specimens don't seem to feed as well when provided chopped up food. Thus it is important to provide a variety of smaller crustaceans, especially for specimens with smaller mouths. Even though seahorses and pipefish like room to move about their environment, maintaining them in a smaller tank is required to keep sufficient densities of food so it can be easily available in suitable amounts. This is especially important for the smaller genera *Corythoichthys*,

Doryrhamphus, *Dunckerocampus* and *Halicampus*. Acclimatizing syngnathids to frozen foods is not easy to achieve (especially with wild fishes) but can be accomplished by mixing increasing portions of frozen feed and live prey and offering the frozen food alone in the first daily dose of feed (Woods & Valentino, 2003; Palma *et al*., 2008). This procedure requires previous experimental test to establish the most appropriate fish size for which the change from live to frozen feed can be made. One of the main constrains related to on-growing is the lack of an inert diet to support the growth of syngnathidae (Woods, 2003a). However, Woods (2003a) reported that co-feeding with live *Artemia* seems to enhance the capture of inert particles.

17.4.4 Breeding

It is extremely important to start off with good broodstock. Seahorses will naturally spawn in captivity if the correct conditions are provided. When possible, males should be larger than females as frequently males mate with females of similar or smaller size than themselves. There is greater potential for excess eggs to be dropped if females are significantly larger. It is not always possible to obtain mated pairs (known to have previously mated). Proper selection of healthy individuals, paired or that can be paired, is the first important step to breeding marine ornamentals. Mates can be paired based on sexual differences, such as color, size and external morphology and behaviour. Multiple pairs may be kept together to increase opportunity for mating. It is extremely important to be able to follow the lineage of our captive bred seahorses to avoid interbreeding and to identify individual breeders. In seahorses, successful identification has been performed by comparing the crowns of individuals and by noting differentiation of spots and lines on the seahorse's snout, or by tying a numbered bead around the neck (Planas *et al*., 2008) or tagging with visible implant elastomers (Woods & Martin-Smith, 2004). Monitoring spots and lines on the body are the only usable indicators for pipefish identification.

After they are released from the male's brooding structure, newborns approach the water surface to gulp air and inflate their swim bladder (Figure 17.2). Weak or low quality newborns may remain at the water surface gulping air in excess resulting in swim bladder hyperinflation. That syndrome may cause high mortalities of young juveniles during the first days of development. The use of pseudo-kreisel aquaria and adequate water circulation will generally reduce such mortalities to a large extend (Blanco *et al*., 2014).

Juveniles are generally stocked at very low densities (1–2 juveniles per L) since increasing densities might result in reduced growth and survival, due to the greater occurrence of juveniles grasping and wrestling each other with their prehensile tails (Woods, 2003c). Among the physical factors, temperature is significant, affecting growth and survival (Planas *et al*., 2012).

17.4.5 Color

Syngnathids, particularly seahorses, are known to change color very easily for camouflage, sexual displays or stress (Martínez-Cárdenas & Purser, 2007; Rosenqvist *et al*., 2011). Since color is an important attribute in the ornamental trade industry, with bright and colorful animals commanding higher prices (up to 500 US$ or higher), several attempts to control this variable have been addressed. Most strategies adopt red,



Figure 17.2 Six-day-old seahorse (*Hippocampus guttulatus*).

orange and yellow artificial algae or branch-like structures for the animals to hold to. The color of the aquarium walls may also be modified. However, most animals will change their color during transport to the retail markets and the color they will ultimately adopt in the final consumer aquarium is impossible to guarantee.

17.5 Rearing Systems

Broodstock systems can be single or multiple tank set ups (Figure 17.3). They must be designed to allow for adequate space, habitat, and water quality. Good health of the adults is imperative to successful spawning. A quality multiple tank system can be made up of an appropriately sized aquarium connected with a good central filtration unit. These are best located indoors where lighting and temperature can be controlled. Each tank should have aeration and water flow-through and set up with a bare bottom with only the necessary hiding and spawning structures.

Substrates adequate for most seahorses and pipefish are crushed coral, seashells, dolomite, coarse sand and pea gravel. Very fine substrates should be avoided as they can clog the filter.

Newborns need to be moved into a separate holding tank from their parents, preferably one of the kreisel or pseudo-kreisel formats (Burhans & Melechinsky, 2000; Blanco *et al.*, 2014). It is important not to overcrowd them. The appropriate sized feeds must be provided in an appropriate concentration with mandatory daily siphoning, water changes, and water quality testing. With the high biodensity in these tanks prophylactic disease treatments should begin around week two. Once the juveniles have settled out

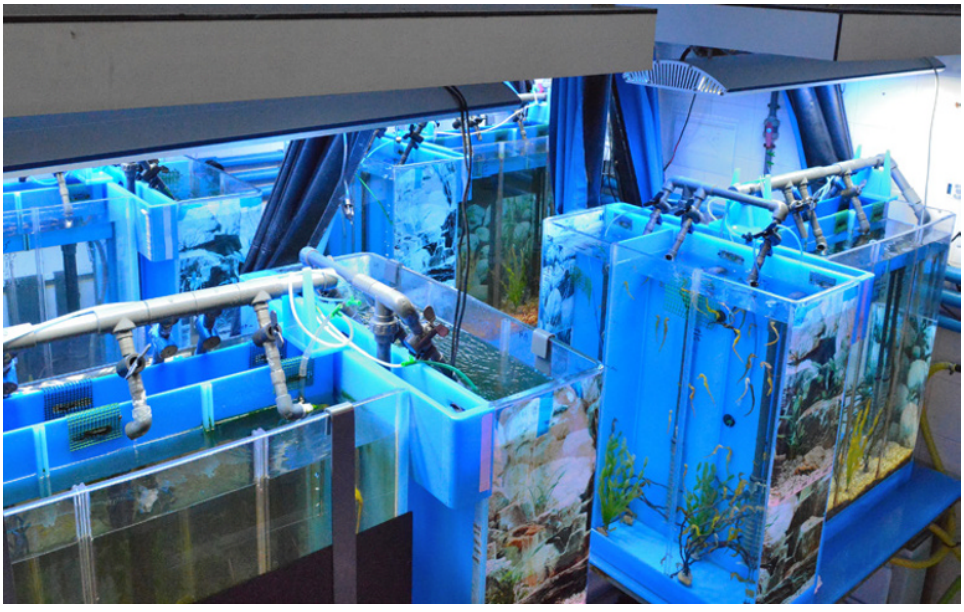


Figure 17.3 Seahorse breeding aquaria at IIM (CSIC) (description in Planas *et al.*, 2008). (*See insert for color representation of the figure.*)

they can be moved from the pseudo-kreisel to a more standard grow out tank. A tall water column (1 m) allows seahorse females to transfer their eggs to males during the vertical rising stage of mating.

Most seahorse and pipefish fry are planktonic and require special attention, for the first two to four weeks. A small grow out tank (to concentrate the food source) is sufficient for those species, such as *H. erectus* or *H. abdominalis*, that deliver fry large enough to settle out immediately after birth.

There are many types, shapes, and sizes of grow-out tanks to choose from to raise seahorse and pipefish fry. Whether it is a small scale home aquarist or the large scale aquaculturist, all must consider the same life support parameters. When raising seahorse fry we are trying to simulate the planktonic environment, but unlike in their natural environment, we have to worry about water quality. It is very important to be able to place the batch of fry into a tank that will not be over crowded. This means adding the correct number of larval fry with the correct density of their planktonic prey.

The first thing to consider when setting up a rearing tank is the size of the potential batch of offspring and their feeding requirements. This varies greatly between seahorse and pipefish species. Smaller species like the dwarf seahorse, *H. zosterae*, give birth to only 10–20 fry and the young are large enough to feed on *Artemia*. Larger species like the Pacific seahorse, *H. ingens*, may produce up to 1500 fry (Lourie *et al.*, 2004) that are extremely small and require small zooplankton as a first feed. The size of the rearing tank is obviously related to the size and potential number of fry.

No matter what size and shape the rearing tank is it must have adequate filtration and/or water exchange. This can be done with open system water exchange, mechanical filters, or by manually changing the water by hand. As with all marine fish, the seahorse

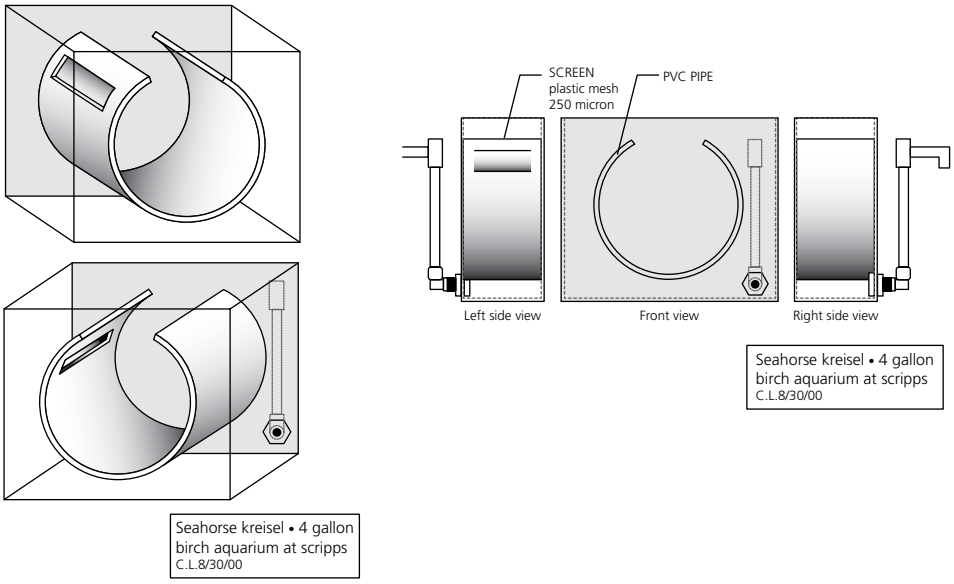


Figure 17.4 Basic pseudo-kreisel aquaria (Burhans & Melechinsky, 2000).

does not adjust well to water quality changes. Particular attention must be paid to salinity, temperature, ammonia, nitrites, and pH. Each system needs an adequate mechanical filter, UV sterilizer, and protein skimmer.

The kreisel aquarium was developed by researchers to study planktonic life while on shipboard. Water flows are generated from a pump that brings ocean water, to the tank, through a spray bar that directs the flow down past a micron mesh covered drain, follows the rounded contour of the tank, and comes up the opposite side forming the circular flow. This can easily be modified, to a closed system, by using an aquarium bio-filter unit. Since most species of syngnathids have an initial planktonic period, the kreisel makes the ideal rearing tank. The appropriately sized kreisel is dependent on the size and number of fry and the amount of live feed required to be added. Unfortunately, these tanks are very expensive to have custom made.

A pseudo-kreisel (Figure 17.4) is the economic aquarist's version of the kreisel. It is made by inserting a rounded acrylic sheet into a square or rectangular tank to enhance vertical water motion. This motion gently rotates water from the bottom of the tank to the top. This can be performed with a water flow created from the filter return, a small pump located in the outer chamber, or an airline placed directly in the middle of the inner chamber. The benefits of using the airline method are that it is more easily controlled and produces two smaller circular flows. Like in the kreisel, circular flows move both the fry and their food sources randomly about, thus giving the fry multiple opportunities to find enough food. On one side of the circular inner chamber is a water exit. This exit must be covered in a fine micron screen to prevent the loss of fry. It should also be large enough to reduce the water pressure so as not to cause the fry to be caught on it. An overflow drain is placed low, in the back wall, in the outer chamber. A stand pipe goes into this to control the height of the water in the system.

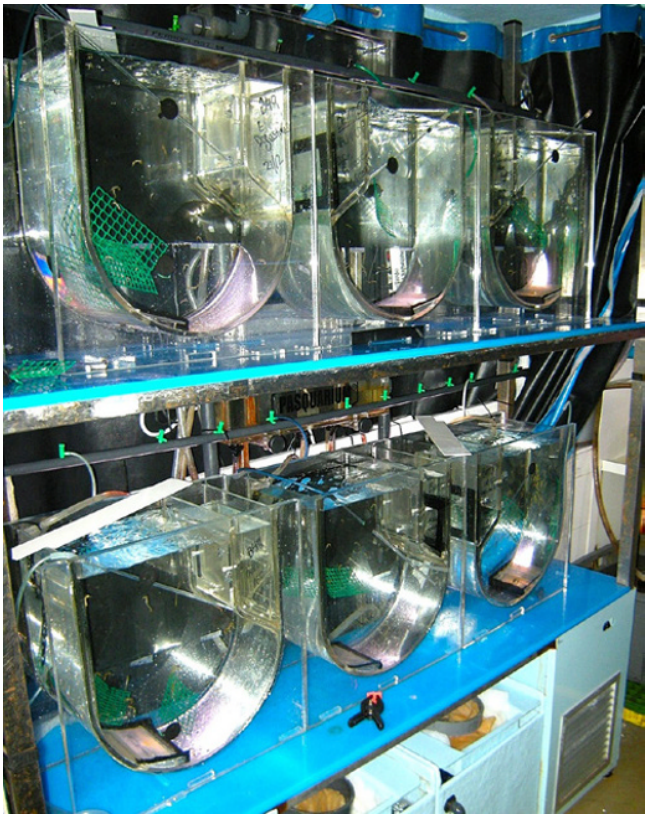


Figure 17.5 Self-working unit with six pseudo-kreisel aquaria at the facilities of the Instituto de Investigacones Marinas (IIM, CSIC) in Vigo (Spain) (description in Blanco *et al.*, 2014). (*See insert for color representation of the figure*.)

The pseudo-kreisel was initially designed to operate on an open system, but can be easily plumbed to operate as a closed system. Running a single pseudo-kreisel as a closed system, especially a smaller pseudo-kreisel, can lead to water quality problems. Open system pseudo-kreisels can be as small as 25–40 L due to the high water quality maintained in open systems. Culling may be necessary when working with species that have a large number of fry to avoid overcrowding the tank. Larger facilities will set up a multiple pseudo-kreisel system (Figure 17.5) to allow the grow-out of the entire batch of fry.

Concerning the basic aquarium set up, the tanks employed are usually 20–75 L. Filtration is performed with a sponge or carbon filter. Water motion is provided by the air driving the filter. The larger the tank size and volume, the better the ability to maintain the needed water quality. The tank size is limited by the need to maintain a concentration of feeds and the amount of live food available. One must resist the tendency to add more juveniles than the tank size can handle. If the juvenile population is culled down and feeding levels appropriately maintained, care of the juveniles is much easier.

Many seahorse fry are lost when gulping air at the surface to fill their air bladders. This is very common when rearing takes place in a square or rectangular tank. An extremely clever adaptation is to cultivate the fry in a submerged acrylic gold fish bowl placed in the larger tank. The top of the goldfish bowl should be above the water line. A micron mesh covered overflow must be cut into one side. A small power-head runs

water from the main tank to the goldfish bowl. The outer tank can then be hooked up to additional filtration.

The basic aquarium set up can be easily made into a multi tank system with the addition of a quality biological wet/dry filter. Along with this filter should be a UV filter and a particulate filter bag. The overflows need to be modified to prevent juveniles from entering the filtration, usually done with micron mesh screening.

Care must be taken not to allow excess wastes to accumulate in the grow-out tank. They can easily pollute the tank and lead to toxic tank syndrome, and total tank failure. There should be two to three scheduled feedings per day, depending on the size of the young. Between all feedings the bottom of the tank should be siphoned to prevent a build-up of waste materials. It is suggested that chopped pieces of mysis shrimp be added to the grow-out tank each day to stimulate the animals' production of enzymes needed to digest this prey later in life.

17.6 Diseases

Being that seahorses live primarily on and near bottom substrates, they are commonly found with ectoparasites (e.g., trematodes, isopods, copepods and protozoans) and endoparasites (e.g., cestodes, nematodes and protozoans). One cannot stress enough the need for a rigidly followed acclimation/treatment program, for newly acquired specimens. A three to four week quarantine (Table 17.1), is recommended, to allow time to observe the specimen(s) closely, provide prophylactic treatments and make sure the specimen(s) is eating well.

When abnormal behaviour is observed it is very important to perform a complete physical exam. The posture and buoyancy of the seahorse should be closely scrutinized. Buoyant animals are often struggling in an attempt to reach a holdfast. They should be checked for air trapped in the brood pouch (males) or hyperinflated swim bladders. If the tail appears to be swollen examine it closely for gas bubbles (subcutaneous emphysema). The seahorse's tail and anal region should be examined for redness, swelling or lesions. If a seahorse is lying horizontally, on the tank bottom, it may be in a weakened state. Prod it to make it move. If it swims that is great, if not it will require special treatment. Even if the fish swims it is a good idea to evaluate its feeding response. If it does not take live food then it is considered to be acting abnormally and

Table 17.1 Quarantine treatments for new specimens.

Day	Treatment	Dose	Comments
On arrival	Formalin bath 36–40%	0.05 mL/L/6 h.	Increase aeration
2–6	Oxytetracycline	50 mg/L	50% water change daily/re-dose
7–8	Observation		
9–14	Metronidazole	5 ppm	50% water change daily/re-dose
15–21	Observation		Observe closely for any sign of abnormality

Table 17.2 Common seahorse maladies and their treatments.

	Treatment	Dose	Comments
Bacterial infection on body	Broad spectrum antibiotic bath (Baytril, Gentamycin, oxytetracycline)	Dose from manufacturer	Tip: double the first dose to immediately knock down the bacteria.
Pouch emphesema	Bleed gas from pouch; flush pouch with antibiotic and seawater	Dose from manufacturer	May need to be repeated.
Subcutaneous bubbles	Decompression followed by an antibiotic bath with acetazolamide	Acetazolamide-inject 2–3 mg/kg interdermally at 22 mg/kg, bath (both every 5–7 days)	Decompression is best done in a non-inhabited tank.
Epidermal wounds	Nitrofuracin green	1.25 g/100 L for 5 days	Change 50% of water at the end of every day. Re-dose fully after change.
Fungus	Copper sulfate	0.01 ppm for 1 h.	Copper may inhibit seahorse feeding
Uronema	Formalin, malachite green, or formaldehyde/malachite green combination drugs Chloroquine phosphate Nitrofurazone Neomysin		Needs to be maintained for at least 21 days

may require nutritional additives and additional feedings. The specimens breathing should also be evaluated. Rapid or labored breathing may be a sign of poor water quality, gill disease, or parasitic infestation. The entire body surface including the fins should be examined for any sign of abnormalities. The tube snout is very delicate and easily injured. It should be observed closely for any sign of damage and that it is functioning properly. Common seahorse diseases and their treatments are summarized in Table 17.2.

Ectoparasites affect the skin and gill tissues whereas endoparasites (Table 17.3) attach along the digestive tract or liver of the host animal. Left unchecked, all of these parasites can slowly consume the host animal. In the wild, seahorses are usually able to survive having parasites, in low numbers. In captivity the parasites can multiply out of control very quickly. This may be due to the increased stresses associated to overcrowding and less than optimum water quality. It is very important to perform regular observations and prophylactic treatments to keep the parasites numbers in check. Regularly scheduled freshwater and formalin baths can go a long way to maintain seahorse health. This is particularly true when raising the fry of seahorses. Symptoms of poor health include cloudy eyes, erratic swimming, flashing (scraping on the rocks, decorations, or substrate), rapid breathing, weight loss, and listlessness.

Table 17.3 Parasitic treatments.

Problem	Treatment	Dose	Comments
Ectoparasites	Formalin bath 36–40%	0.5 mL/gal/L h	Increase aeration
Trematodes	Freshwater baths	2–5 minutes	Be sure the temperature and pH are equal
Crustaceans	Methylene blue	5 ppm	Will stain
Protozoans	Malachite green	5 ppm	Will stain
Endoparasites			
Flukes	Freshwater baths	2–5 minutes	Be sure the temperature and pH are equal
Protozoans	Metronidazole (Flagyl)	2 ppm	Bath or in feed
Round worms	Praziquantel		

Ectoparasites can cause damage to the skin of the seahorse and lead to bacterial infections. A seahorse must be treated immediately at the first sign of a bacterial infection. Bacterial infections are usually noted first on the snout or tail, but can actually start anywhere on the body. *Vibrio* strains are the most common cause. The inflicted specimen should be moved to a quarantine tank and treated with Nitrofuracin for any wounds and a broad spectrum antibiotic (e.g., Gentamycin, Batril or oxytetracycline) to alleviate the bacterial infection. It is important to start the treatment with a double dose to get ahead of the infection. The likelihood of a successful treatment is increased by early recognition of a problem. Ectoparasites, like *Uronema*, can quickly attack the wounded area and can be very difficult to overcome.

Pouch emphysema may result from decaying matter in the male's pouch, usually remnants of birthing, producing gases and causing the pouch to bloat. To remove the gas bubbles one can gently rub/squeeze the pouch with your fingers in an upward motion angling the pouch toward the surface. If the tissue around the pouch opening has swollen, a hematocrit tube can be gently pressed into the opening to allow release of the gas bubbles. For bacterial infections it is important to remove the bubbles and to flush the pouch to remove any remaining decaying matter. The next step would be to flush the pouch with an antibiotic solution (e.g., Neomycin, Baytril or Metronidazole).

Seahorses are very prone to a supersaturation in nitrogen of the system water, usually caused by pump cavitation, leak in the high pressure piping, or improper heat exchanger use. This issue manifest in bubbles being trapped inside the body. The solution to this is repairing the system. In extreme cases one can decompress the afflicted specimen(s) by lowering them (in a flow-through container) into a deeper tank for several hours to days. It is important to be able to get food to these specimens. The antibiotic acetazolamide has been tried for this issue with varying results.

It is extremely important to be proactive with your prophylactic treatment program for juvenile seahorses. It is better to lose specimens to the treatment, than to lose them to a parasitic infestation. Recommended prophylactic treatments for juveniles are provided in Table 17.4.

Table 17.4 Prophylactic treatments for fry.

Date	Treatment	Dose	Comments
Day 14	Formalin bath 36–40%	0.13 mL/L/30 min	Treat in an alternate container and thoroughly clean grow-out tank
Day 21	Copper sulfate	2.5 mL/100 L/1 h	50% water change after
Day 28	Formalin bath 36–40%	0.13 mL/L/L h	Treat in an alternate container and thoroughly clean grow-out tank

17.7 Rearing of Selected Seahorse Species

All seahorse species belong to the genus *Hippocampus*. The International Union for Conservation of Nature recognizes 38 species (IUCN, 2013), which are distributed worldwide. The most significant species for trade are the following (see also Tables 17.5a and b): *H. abdominalis* (big-belly seahorse), *H. capensis* (knysna seahorse), *H. comes* (tiger-tail seahorse), *H. erectus* (lined seahorse), *H. guttulatus* (European long-snouted seahorse), *H. hippocampus* (European short-snouted seahorse), *H. ingens* (Pacific seahorse), *H. kuda* (spotted seahorse), *H. reidi* (longsnout seahorse), *H. trimaculatus* (longnose seahorse), *H. whitei* (White's seahorse) and *H. zosterae* (dwarf seahorse).

The general characteristics and rearing conditions of the above species are the following:

17.7.1 Pacific Seahorse *Hippocampus ingens*

Size: Up to 26 cm.
Range: Catalina Island, California to Peru.
Habitat: Sandy bottom in or near grasses, kelp forest, and rocky bottoms holding on to sponges, tunicates, or coral. Found from shallow to 30 meters.
Husbandry: This is a temperate to tropical species. They are less prone to diseases and parasites when maintained at lower temperatures. Mating takes place at temperatures above 20 °C. It is important to maintain a consistent temperature. Prefer live mysis, but can be easily trained to accept frozen prey with nutritional additives.
Breeding: Readily mate when aquarium parameters are optimum. Larger specimens require a deeper tank to allow mating dance. Health and size of fry is directly related to adult's health and nutritional feeds. Gestation of 6–8 weeks with mating often occurring within a day of birthing. Young size is about 5 mm at birth.
IUCN Status: Vulnerable.

17.7.2 Lined Seahorse *Hippocampus erectus*

Size: Up to 19 cm.
Range: Most of the temperate and tropical west-Atlantic, from Canada to Argentina. It may be confused with *H. reidi* and specimens from Brazil and Argentina may represent another species (Luzzato *et al*., 2012; Boehm *et al*., 2013). DNA analysis showed two separate breeding populations. The northern population has a more thin and elongated body. Fry from this population are born smaller.

Table 17.5a Aquarium parameters for seahorses.

	H. ingens	*H. erectus*	*H. reidi*
Tank size	Minimum 150 L. Height of tank for larger specimens >75 cm.	Minimum 90 L with 70 cm water column height	60 cm height or less with live sand or bare bottom tanks
Circulation	Weak current promotes swimming about the tank	Weak to moderate currents	6 L min^{-1} recommended
Aeration	Aerate with large bubbles (fine bubbles may lead to subcutaneous bubbles)	Aeration off-tank, otherwise large bubbles	additional aeration not used in adult tank
Lighting	Prefers subdued lighting. Used LED RGB to enhance coloration.	Prefers subdued lighting. Recommend LED RGB to enhance coloration.	Cool–light fluorescent (500–800 Lux) or metal halides. Lamps (175 W 6500 K). Photoperiod 12 L:12D
Temperature (°C)	20–22	28 (27–29)/25 ± 1	26 (22–28)
Salinity	33.5 (24–35)	31–33/35 ± 1	34 (24–35)
Dissolved oxygen (mg L^{-1})	>95%	4–8	<95%
pH	8.0–8.2	8.2	8.25
Ammonia (mg L^{-1})	0	0	0
Nitrite (mg L^{-1})	<0.01	<0.5/≤0.3	<0.1
Nitrate (mg L^{-1})	<15	<50/≤12.5	<10

Table 17.5b Aquarium parameters for seahorses.

	H. guttulatus	*H. abdominalis*	*H. zosterae*
Tank size	Minimum 100 L Height of tank 60 cm	Minimum 150 L Height of tank 75 cm	Minimum 40 L
Circulation	Weak to moderate currents	Weak to moderate currents	Weak current
Aeration	Aeration recommended, larger bubbles	Aeration recommended, larger bubbles	Aeration recommended
Lighting	Medium light	Prefers subdued lighting	Prefers subdued lighting
Temperature (°C)	18–21	17–20	20–22
Salinity	30–35	30–35	27–30
Dissolved oxygen (mg L^{-1})	<90%	<85%	<90%
pH	8.2	8.2	8.3
Ammonia (mg L^{-1})	0	0	0
Nitrite (mg L^{-1})	<0.01	<0.5/≤0.0	
Nitrate (mg L^{-1})	<10	≤15	<20

Habitat: Shallow tropical and sub-tropical lagoons, algal and seagrass beds or reef areas. It may also be seen together with sponges and floating *Sargassum*, and has been reported as deep as 73 m.

Husbandry: a wide range of life support systems have been described for this species. Wild specimens may be more prone to disease at higher temperatures (>26 °C). It is easily fed live enriched adult *Artemia* and very keen on live marine amphipods. It can be trained to accept frozen mysis.

Breeding: in Mexico occurs during the colder months (November to March, 23–26 °C). This species can reach maturity at 5–7 months, 6.3 cm (Lin *et al.*, 2008). Southern population day 0 juveniles measure 10–12 mm; 6–8 mg and tend to take hold of any branch-like structure or mates as soon as they hatch, contrasting with other, more pelagic initial juvenile phases. Being large seahorses they are more comfortable in aquariums with considerable height.

IUCN Status: Vulnerable.

17.7.3 Slender or Longsnout Seahorse *Hippocampus reidi*

Size: Up to 18 cm.

Range: from North Carolina (USA) and Bermuda, throughout most of the Caribbean and as south as Brazil.

Habitat: subtropical marine and estuarine shallow waters although it has been spotted at 55 m deep. The Slender seahorses have an affinity for coral reefs, seagrass beds where they can be seen attached to gorgonians, sponges, seagrass, mangroves and *Sargassum*. They tend to be very site-specific with reduced mobility.

Breeding: Reproduction in captivity may start as early as 4 months (equivalent to 8 cm) and gestation period lasts around 14 days in captivity at temperatures between 24–28 °C (Gomez-Jurado, pers. obs. 2005). Brood sizes as large as 1200 have been reported. Eggs have an oval shape (2.5 mm) with an orange coloration. Gómez-Jurado (pers. com.) suggests recently hatched juveniles are pelagic as they tend not to use the tail for anchorage until day 25 after born.

IUCN Status: Data deficient.

17.7.4 European Long-snouted Seahorse *Hippocampus guttulatus*

Size: Up to 23 cm.

Range: Species of European waters, which is present along the Atlantic coast from the UK, Ireland and Netherlands to the Mediterranean and Black Sea, mostly inhabiting small home ranges in shallow coastal waters, lagoon systems and estuaries (0.5–15 m).

Habitat: Occurs mostly in areas with high vegetation cover where it is usually found holding on to plant cover. The fish is well adapted to estuarine habitat and can tolerate a wide range of environmental conditions, such as salinity.

Husbandry: It is a temperate species but does tolerate a wide variety of temperatures (from 14 to 26 °C). It is a large species requiring at least 100 L aquaria (minimum 80 cm height). Juveniles from 6 cm can be easily trained to feed on frozen prey (prefers mysids). Adult enriched *Artemia* is adequate for on-growing but not so for reproduction. Breeds successfully on a mixed diet consisting of *Artemia* and mysids.

Breeding: Breeding occurs from March to October. In captivity, breeding is optimal at 18–21 °C. Shifted breeding seasons have been successfully applied to this species.

Average brood size is 400 (maximum recorded: 836) and newborn measure about 13–15 mm. Generally monogamous but occasionally may change partners within a breeding season or in consecutive breeding seasons.
IUCN status: Data deficient.

17.7.5 Pot-belly Seahorse *Hippocampus abdominalis*

Size: Up to 35 cm.
Range: Occurs in the marine waters of south-eastern Australia and all around New Zealand; populations have also been recorded from estuaries in Australia and may also occupy similar sites in New Zealand.
Habitat: Typically occurs in waters of less than 50 m depth in harbors and protected coastal bays. Usually found among algae, seagrasses, rocky reefs, and jetty piles and other man-made objects. Specimens found in deeper water are usually attached to sponges and colonial hydroids.
Husbandry: This is a temperate species. Temperature should be between 17–20 °C. Other water quality parameters are standard. Although live feed is preferred pot bellies will readily accept frozen foods. Adult brine shrimp is a favorite food.
Breeding: Readily mate when aquarium parameters are optimum. Larger specimens require a deeper tank to allow mating dance. Health and size of fry are directly related to the adult's health and nutritional feeds. Gestation time is 8 weeks, depending on water temperature. Young are large at birth, about 12 mm.
IUCN Status: Data deficient.

17.7.6 Dwarf Seahorse *Hippocampus zosterae*

Size: Up to 5 cm.
Range: Found in the coastal Gulf of Mexico, Bahamas, Bermuda, Florida and Texas.
Habitat: They are restricted almost totally to seagrass microhabitats in shallow water, particularly in association with the seagrass *Zostera*. They are most common in bays during periods of high salinity.
Husbandry: Generally regarded as hardy in comparison to many other seahorse species although, in fact, their requirements are not dissimilar. They are tropical with a temperature of 20–22 °C. Although they may be kept using very simple aquarium arrangements, they will do better in a complete aquarium system of 75 to 150 L. They feed on amphipods, copepods, and enriched *Artemia* and prefer a holdfasts, eelgrass or facsimile to wrap its tail on.
Breeding: Will breed all year long in captivity, although they slow down during times of shortened day length. Adults produce two broods per month. The gestation period within the male's brood pouch is usually about 10 days with 5 to 25 juveniles.
IUCN Status: Vulnerable.

17.8 Rearing of Selected Pipefish

There are many genera and species of pipefish. The majority of these rarely make their way into the aquarium industry. The most common species in the aquarium trade are (see also Tables 17.6a and b): *Corythoichthys intestinalis* (dragonface pipefish),

Table 17.6a Aquarium parameters for pipefish genus.

	Corythoichthys	*Doryrhamphus*	*Dunckerocampus*
Tank size	Minimum 75 L	Minimum 75 L	Minimum 75 L
Circulation	Weak current recommended	Prefer a moderate current	Weak current recommended
Lighting	Use LED RGB to enhance coloration	Prefers subdued lighting Recommend LED RGB to enhance coloration	
Temperature (°C)	20–22	28 (27–29)/25 ± 1	
Salinity	33.5, 1.018–1.026	31–33/35 ± 1	
Dissolved oxygen ($mg\,L^{-1}$)	>95%	4–8	
pH	8.0–8.2	8.2	
Ammonia ($mg\,L^{-1}$)	0	0	
Nitrite ($mg\,L^{-1}$)	<0.01	<0.5/≤0.3	
Nitrate ($mg\,L^{-1}$)	<0.15	<50/≤12.5	

Table 17.6b Aquarium parameters for pipefish genus.

	Haliichthys	*Solenostomus*	*Syngnathus*
Tank size	Minimum 150 L with 60 cm water column height	Minimum 30 L with 60 cm water column height	Adults require a minimum of a 75 L tank, larger for multiple specimens
Circulation	Weak/fluctuating current promotes activity	Weak/fluctuating current promotes activity	Prefer a moderate current
Lighting	Prefers subdued lighting Use LED RGB to enhance coloration	Prefers subdued lighting Recommend LED RGB to enhance coloration	Prefers subdued lighting
Temperature (°C)	20–22	28 (27–29)/25 ± 1	26 (22–28)
Salinity	33.5 (24–35)	31–33/35 ± 1	34 (24–35)
Dissolved oxygen ($mg\,L^{-1}$)	>95%	<95%	<90%
pH	8.0–8.2	8.2	8.25
Ammonia ($mg\,L^{-1}$)	0	0	0
Nitrite ($mg\,L^{-1}$)	<0.01	<0.5/≤0.3	<0.1
Nitrate ($mg\,L^{-1}$)	<0.15	<50/≤12.5	<10

Corythoichthys sp. (red-tail dragonface pipefish). *Doryrhamphus dactyliophorus* (banded pipefish), *D. excisus* (bluestripe pipefish), *D. janssi* (Janss' pipefish), *D. multi-annulatus* (multi-banded pipefish), *D. pessuliferus* (yellow multi-banded pipefish), *Dunckerocampus baldwini* (red-stripe pipefish), *Festucalex* sp. (stubnose pipefish), *Solenostomus paegnius* (ghost pipefish), *S. paradoxus* (ghost weedy pipefish), *Syngnathoides* sp. (green pipefish), *Syngnathus californiensis* (bay pipefish), *S. leptorhynchus* (kelp pipefish), *S. scovelli* (American Gulf pipefish), *S. louisianae* (chain pipefish) and *Trachyrhamphus bicoarctatus* (bend stick pipefish).

The genera of pipefish commonly available in aquarium trade are the following:

17.8.1 Genus *Corythoichthys*

Primarily found on or in close proximity to coral reefs on rubble substrates, rarely seen swimming more than a few inches off the bottom. They are often found in pairs during day and congregate into groups at night. Color variations are usually related to habitat, feed, and breeding season. They go through an extensive courtship dance. Males have large brood pouches with up to 1000 eggs depending on size of the individual male. This genus is easily kept in captivity as long as their specialized and regular feeding requirements are met.

17.8.2 Genus *Doryrhamphus*

Found only in tropical waters, this genus is made up of free swimming benthic fishes, often found hanging in or around large corals and sponges. They have large, brightly colored tails that bare diagnostic color patterns. They are found in various reef habitats, usually staying close to cover. Many species, in this genus, are known to be active cleaners. The egg brood, usually 80–150 eggs, is semi-exposed on the abdomen. Some species can be very territorial in aquariums chasing any similarly colored pipefish until death (*D. jansii* and *D. excisus*).

17.8.3 Genus *Dunckerocampus*

Found only in tropical waters this genus is made up of free swimming benthic fishes. They are often found in front of caves or reef overhangs. Most species are distinctly barred and have moderately large caudal fins. Adults usually occur in pairs. Males incubate the eggs below the trunk and eggs are exposed to the outside, partly embedded in the skin. Brood sizes are usually 200–300.

17.8.4 Genus *Solenostomus* (Ghost Pipefish)

There are six species in this genus. They have a great degree of variability in color and dermal appendages, probably due to the different preferences with regard to benthic habitats. Pipefish in this genus are thought to be short lived, 2–3 years. Adults will pair but some species may be found in small groups. They feed on small shrimp-like crustaceans that use the same habitats, invertebrates such as gorgonians and crinoids. They are notoriously difficult to keep in captivity. This may be traced to the shyness of the genus, the difficulty in providing appropriate foods, and extended shipping times or specimens that must constantly eat.

17.8.5 Genus *Syngnathus* (Seaweed Pipefish)

This is a large genus with 32 species. They are found in temperate to tropical waters variously distributed in Atlantic and Pacific oceans. Some species may inhabit brackish and sometimes freshwater. They are commonly found in eelgrass, surf grass and various algae where they blend in perfectly. Breeding is seasonal, but the length of the breeding season is variable depending on the water temperature. Brood size is 200–400 eggs depending on the size of the male.

17.8.6 Genus *Haliichthys* (*Haliichthys taeniophorus*, Ribboned Pipehorse)

Size: Up to 30 cm.
Range: North-western Australia.
Habitat: Lives mainly in shallow water and in weedy zones bordering open substrates.
Husbandry: More tropical than the southern relatives makes it more suitable to many public aquariums.
Breeding: Bred successfully by Dallas World Aquarium (Carlson *et al*., 2008).
IUCN Status: Insufficient data.

17.9 Rearing of Seadragons

Even though they have not been treated until now in this chapter, seadragons deserve some consideration. Over the past 20 years husbandry of captive seadragons has improved to the point that most major aquariums display one or both species, the leafy seadragon *Phycodurus eques* and the weedy seadragon *Phyllopteryx taeniolatus*. There have been several successful propagations, but it is rarely duplicated. It takes great dedication to setting up the appropriate habitat, husbandry program, simulation of their natural environmental conditions, and a lot of luck to have obtained a male specimen that will breed in captivity. To date, successful propagation has been hit or miss. There is no set protocol that guarantees successful seadragon propagation. Wild caught and wild caught/raised in captivity specimens are still available, but with the "near threatened" IUCN status, this may change (L. Squire Jr., pers. comm.).

The key to keeping seadragons is the daily husbandry and maintenance of the exhibit. The seadragon tank should be large enough to allow the seadragons to comfortably swim, without being crowded. If adequate space is not provided they may injure their delicate snout while negotiating around the walls, tank mates, habitat, and currents of the tank. It is recommended that the system water be turned over 4–6 times per hour to maintain the appropriate water flows (Powell, 1998). Any more than this can cause undue stress to the specimens; any less may cause reduced activity. Water temperature should be maintained between 13 and 17 °C. Lighting of the seadragon tank is a key factor to success. Seadragons have been known to go into shock when startled with bright light. Most facilities inhibit flash photography on their seadragon display for this reason. Installing a light system that allows a gentle ramp up and down of lighting is highly recommended. Maintaining a weak night light, to allow the seadragons to settle at night and not be attracted to extraneous lights, is also highly recommended (Branshaw, 2005).

Upon arrival seadragons should receive an extended acclimation period. Changes in salinity, pH, and temperature may negatively affect them. These parameters must be matched exactly. During transfer, from various containers, the seadragon should never be removed from the water. Due to the risk of injury to their delicate snouts, a net should never be used. Quarantine of newly acquired specimens is highly recommended, especially if they will later be added to an existing population. Specimens should be examined closely for isopods. A prophylactic formalin treatment is a good idea. If protozoans are suspected a 0.2 ppm copper treatment is very effective. Seadragons require constant observation for snout injuries and resultant bacterial infections that may have developed during the handling or shipping.

Seadragons are known for being difficult to feed. They prefer live food, specifically live mysid shrimp. It is rare to get them to feed on frozen foods. It is important to have access to large amounts of mysid shrimp through collection, propagation, or purchase. It is a good idea to have a backup plan for an alternate source. Larger specimens may be fed once or twice a week, smaller specimens more often, depending on their size. Mysid shrimp should be fed nutritionally enriched *Artemia* beginning 24-hours before the shrimp are fed out.

It may take several days for all of the eggs, on the male's tail, to hatch. The newly hatched seadragons are approximately 2 cm in length. The yolk sac is still attached, and will be absorbed over the course of the next few days. At this time the seadragon fry are able to eat on their own. It is highly recommended that the seadragon fry be placed in an appropriately sized tank separate from the display or brood tank (Forsgren, 2005). Since the hatchings will eat all day and night, a 24-hour feeding bucket and night light should be set up (Forsgren, 2005). Seadragon fry can initially eat baby brine shrimp, followed by one-day old live mysids. Larger mysids can be added to the diet as they begin to grow. Young seadragons begin eating adult mysids at approximately 3–4 months of age (Forsgren, 2005).

The species of seadragons commonly available in aquarium trade are the following:

17.9.1 Leafy Seadragon, *Phycodurus eques*

Size: Up to 41 cm.
Range: Found in both South and Western Australia.
Habitat: Rocky shores with algae, has many camouflaged appendages that allow it hide in its algal habitat.
Breeding: Estimated to have 250–300 eggs covering most of the under and sides of the tail. Breeding may vary but usually occurs during November and December. Although successful egg transfer has taken place in captivity there is no record of successful hatching.
IUCN Status: Near threatened.

17.9.2 Weedy Seadragon, *Phyllopteryx taeniolatus*

Size: Averages 30 cm but can reach up to 45 cm.
Range: Found from South to Southwestern Australia.
Habitat: Occurs in kelp-covered rocky reefs at depths from about 3 m to 50 m. It is usually found in areas ranging from shallow estuaries to deep offshore reefs.

Breeding: The breeding season is early summer, with usually one brood per season. Male seadragons carry the eggs fixed to the underside of the tail until they eventually hatch. Estimated that 250 young are released from a single brood. Young may mature in one year, but usually will not breed until their second year when fully mature (Kuiter, 1999).
IUCN Status: Near threatened.

17.10 Conclusions

Important advances have been achieved in the rearing of some syngnathids in captivity, particularly in seahorses. Rearing techniques are already available for a few species of seahorses and, to a lesser extent, pipefish. However, available rearing procedures have not been optimized yet. On this regard, main efforts should be directed towards the increase in survival and growth rates. For that, aspects such as breeding success, breeder's management, food quality and nutritional requirements (breeders and juveniles) and diseases control need to be conveniently addressed. Furthermore, availability of inert food for weaning of juveniles and ongrowing continue to be an issue in this group of fish. Special attention should be also focused on the development of novel rearing techniques for species that still have not been cultured. This is particularly important for pipefish, as only a few species are currently cultivated with some success.

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18

Post-larval Capture and Culture of Ornamental Fishes

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Abstract

Post-larval capture and culture (PCC) is a sustainable technique whereby post-larval (PL) fish are collected from plankton in the open ocean without physically impacting the marine environment. Wild reef fish undergo extremely high natural mortality (>95%) during reef colonization and settlement as they transition from the planktonic to the juvenile phase of their life cycle. Post-larval capture collects a tiny proportion of post-larvae prior to this high natural mortality event, impacting minimally on overall plankton biomass. Post-larval culture rears and conditions captured post-larvae into healthy, superbly conditioned fish with a high survival rate. Thus PCC not only reduces environmental and human impacts of devastating wild fish collection methods, it also produces first-class specimens for aquarium hobbyists, a win-win situation for all concerned. Nevertheless, even after a decade of development, PCC is not yet widely implemented. The reasons are many but often unrelated to the technique itself.

Keywords *PCC; sustainability; marine aquarium trade; new species*

18.1 Introduction

Tropical marine animals (mainly fishes but also crustaceans and molluscs) are very highly prized by the Marine Aquarium Trade (MAT). Therefore marine resources are overexploited not only for food species, but also for popular reef species. Over 11 million reef fish and millions of other reef dwellers are taken each year to meet the huge demand of aquarium hobbyists worldwide.

Current market demand leads to extensive wild fish collection, with short-term economic factors usually taking precedence over conservation needs, resulting in major disadvantages for all stakeholders. Hobbyists and traders suffer from poor animal quality, while exporting countries suffer coral reef overfishing, biodiversity loss and habitat destruction; moreover communities form exporting countries suffer economic losses

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and face several risks to their health, and even death, from hazardous fishing methods (Wood, 2001).

Most fishing techniques target juveniles or adults, often breeders, diminishing not only current but also future stocks. The destructiveness of conventional open water fishing for food fish varies according to technique; less destructive methods (e.g. gillnets and long-lining), together with catch restrictions, can help to conserve both stocks and habitat. This is not the case for coral reef fisheries, where many fishermen use harmful chemicals, such as cyanide and dynamite, because these are fast and facilitate the catch of multiple fishes at once (Cartwright *et al*., 2012). Russ & Alcala (2004) found that 75% of Philippine coral reefs have been damaged by such practices. Not only is there overfishing, but habitats are also devastated by chemical poisoning or blast damage, leaving nature with no way to recover. It is an undoubted, and somehow inconvenient truth, that the MAT negatively impacts marine biodiversity and human heath, and therefore one of the main challenges facing the MAT today is obtaining specimens that are hardy and economically, as well as environmentally, sustainable.

Governments, conservation organizations, producer communities, dealers and hobbyists have all recognized the role that aquaculture could play in reducing harvesting pressure on wild stock and promoting species conservation (Tlusty, 2002; Koldewey & Martin-Smith, 2010). However, many popular ornamental species do not breed in captivity, and therefore PCC – which harvests wild-caught post-larvae – can significantly increase the number of sustainably-produced species. But does PCC represent a real hope? This chapter provides an overview of the technique, sums-up current PCC deployment and opportunities, and discusses hobbyists' roles and responsibilities.

18.2 PCC *Versus* Fish Life Cycle

Most coastal marine animals (coral reef fish, crustaceans, molluscs) have oceanic larval phases at the beginning of their life cycles (Sale, 1980; Leis, 1991; Leis & Carson-Ewart, 2000) (Figure 18.1). This phase allows them to colonize new habitats, thereby facilitating the species' broad distribution and, consequently, their persistence (Choat & Robertson, 1975; Lobel, 1978; Victor, 1986). Fairly passive during most of this phase, they finally become active in order to seek their new habitat (post-larval stage). The various stages of their life cycle are marked by high mortality, mainly due to natural predation, but also pollution or habitat destruction (Figure 18.2). It has been scientifically demonstrated that during the final colonization phase (when the post-larvae transform into juveniles on the reef habitat (c in Figure 18.2), post-larvae suffer catastrophic mortality rates during the settlement process: more than 90% disappear in the week following colonization (Doherty & McWilliams, 1988, Planes & Lecaillon, 2001; Planes *et al*., 2002; Doherty *et al*., 2004). The capture and culture of those post-larvae prior to settlement is called PCC (post-larval capture and culture), a new technique first introduced in 2000 and defined as "*aquarioculture*" when the post-larvae are cultured for sale to the MAT (Delbeek, 2006; Lecaillon & Lourié, 2007).

Eggs, larvae and post-larvae of marine fish and crustaceans are usually considered to be a non-exploitable marine resource, as opposed to juveniles and/or adults, which are actively harvested. Recent publications shed light on the rarely described life stages of those post-larvae (Maamaatuaiahutapu *et al*., 2006; Junker, 2007; Lecaillon *et al*., 2012).

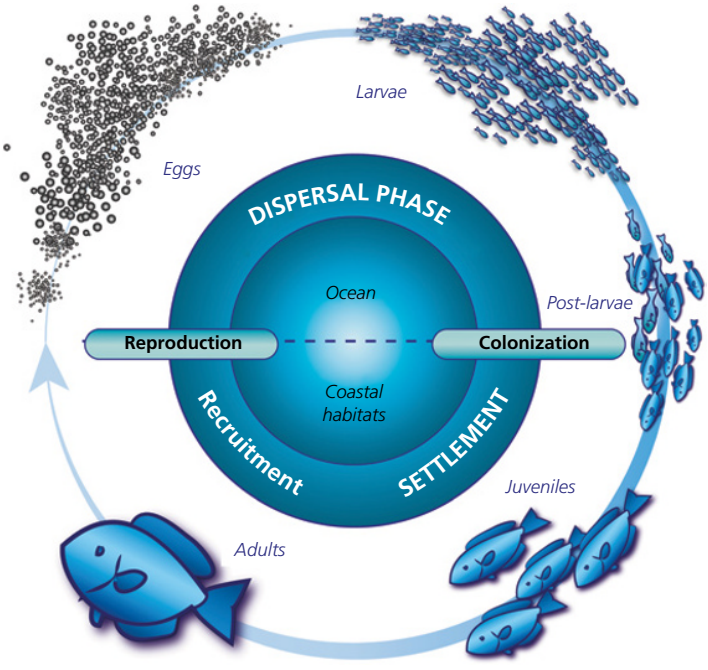


Figure 18.1 Reef fish life cycle (Lenfant *et al*, 2016).

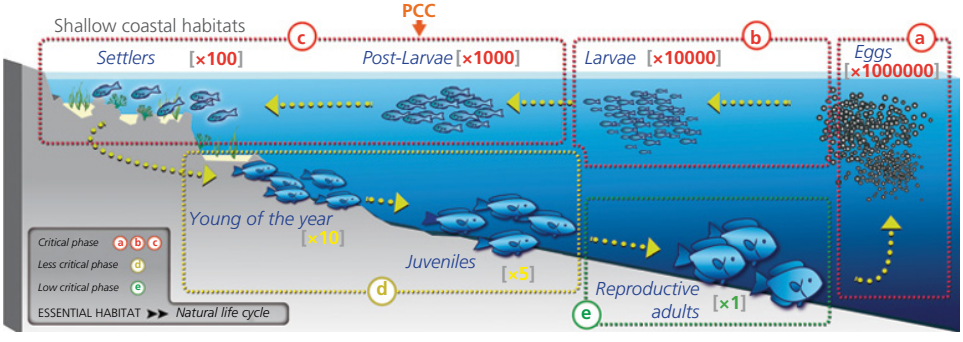


Figure 18.2 Level of mortality within fish life cycle and position of PCC intervention.

Today it is fully accepted that, given the very large number of post-larvae arriving from the ocean, collecting a small percentage of them has a negligible impact, which is also limited in time (Bell *et al*., 1999, 2009). Thus PCC can effectively exploit a resource that would otherwise be largely and naturally wiped out (Lecaillon, 2004; Lecaillon & Lourié 2007).

Thanks to a range of existing and new collection tools (Lecaillon & Lourié, 2007) and the know-how developed by certain private and public agencies, those post-larval fish can be kept alive, weaned, grown and shipped to supply the marine aquarium trade.

The various inexpensive PCC tools and the different steps required to reach saleable size (sorting, rearing, etc.) are described in various papers (Bell *et al*., 1999, 2009;

Hair & Doherty, 2003; Lecaillon, 2004; Lecaillon & Lourié, 2007) and are not detailed here. However, PCC technology involves the following major steps: (1) overnight catching of live, healthy and uninjured post-larval fish or crustaceans; (2) identifying and segregating/sorting species which could harm or consume one another; and (3) weaning and growing the fish to a saleable size (average of 3 months).

One of the most important advantages of PCC is that the rearing stage is easy to implement using intermediate technologies, thus reducing the need for technologically advanced and expensive laboratories and hatcheries. Sorted post-larval fish are initially fed with newly hatched *Artemia* (brine shrimp). Fish food granules can then rapidly replace the live food as the fish never develop an appetite for natural reef food. In short, PCC produces pre-conditioned, easy-to-feed marine aquarium animals.

18.3 Features Determining Species Suitability to the Marine Aquarium Trade

18.3.1 Catch Efficiency

Depending on the year, locality, season, wind direction, sea surface temperature, etc., about 30% of all post-larval fish caught is suitable for the ornamental market (e.g. butterflyfish, surgeonfish, colorful damselfish, etc.). Moreover, a further average of 40% can be used for local food fish aquaculture (e.g. rabbitfish, groupers, jacks or snappers). These data are based on experiences over more than one decade and can also differ from one specific site to another.

The catch rate of live, healthy post-larvae is a direct function of the fishing tools used (e.g., hoa net, crest net, light trap, etc.), with certain tools being more suited to specific sites and locations (Lecaillon & Lourié, 2007). However, assuming a site-appropriate choice of tool(s), for PCC to work profitably in a specific location, a combination of the following factors is required:

1) A large number of post-larvae caught alive per night per trap (catch per unit of effort, CPUE). This is directly related not only to the fishing tool used (as noted above) but also to the adult fish assemblage and the overall health of the local habitat. The coral reef fish catch can range from as high as 300 PL/night/trap in a French Caribbean island (Guadeloupe, on-going Zoé project, pers. comm.) down to 20 PL/Night/trap in poor Philippine areas (MAMTI project, pers. comm.).
2) Low internal cost of the fishing activity. This depends on ease of sea access, distance from rearing farm to fishing site, fuel prices and of course local salaries.
3) Easy market access. Direct flights to the main hobbyist markets (USA, Europe and Japan) are essential, as logistical delays can easily damage live animals, regardless of how well they are packed. It is also important to consider freight costs, which usually contribute 30% of the final price of fish arriving in the target markets. In some areas (e.g. French Polynesia), high freight costs were the main reason for the failure to profitably implement PCC.
4) Local political support, as political will and the necessary policies must be in place to help change behaviour and regulate destructive fishing techniques.
5) Availability of attractive and popular species. It is generally assumed that Southeast Asian species are much more colorful and desirable than Caribbean species.

18.3.2 High Quality of Fishes

Opportunities exist mainly in the developing countries of the Coral Triangle (specifically the Philippines, Indonesia and Papua New Guinea, but also East Timor and Solomon Islands), who currently export more than 80% of the wild-caught aquarium fish consumed by the world market (Hoeksema, 2007; Olazul, 2012 in Cartwright *et al.*, 2012). Those countries are not noted for the quality of their exported fish, with mortality rates at times peaking up to 90% between point of collection and final purchase by aquarium hobbyists (Schmidt & Kunzmann, 2005).

The know-how required for the various PCC steps is very well understood and several trials have been successfully carried out (e.g., in the Philippines, Madagascar, Mauritius, Reunion Island, French Polynesia and Fiji), with an astonishingly low mortality rate of less than 2% from point of shipment to one week after reception (Nausicaa Aquarium, pers. comm.). Because an average three months of grow-out are needed to obtain fish of a "small" marketable size (1 month for Pomacentridae but more than 4 months for Chaetodontidae), juveniles become accustomed to human handling and acclimated to elevated levels of fish by-products in their tanks. Their adjustment and acclimatization to the tank environment makes them far less prone to stress and therefore much easier to transport and keep in captivity. PCC aquarioculture also produces immunized and disease-resistant specimens, and thus their acclimatization, freedom from disease and general hardiness improves lifespan in marine aquaria. This in turn enhances demand for such eco-friendly fish while reducing pressure to restock aquaria due to fish mortality.

PCC aquariocultured fishes are considered to be as sustainable and resistant as aquacultured species such as clownfishes and dottybacks. In most cases, captive-bred species are very complementary to PCC species in terms of handling and feeding. Therefore breeders and PCC farmers have the opportunity to pool resources, share fixed expenses and offer a more extensive stock list to the market.

Nevertheless, and despite their many advantages, PCC aquariocultured species must be consistent and attractive.

18.3.3 Consistency

As explained above, PCC catches depend on natural fish recruitment, which is variable in abundance and diversity throughout the year. As shown in Figure 18.3, the over-year recruitment CPUE in Micronesia varies seasonally (Ellis, 2010). During June to August the post-larval catch is very low in some tropical areas, such as Micronesia and the Indian Ocean islands, likely due to low water temperature. These periods are therefore not profitable for PCC. However, from November to April, the CPUE is very high with three months (November, December and May) yielding CPUE of over 100 PL/night/trap.

To deliver a consistent supply of PCC fishes to the market over the entire year, the market needs to be supplied by various fishing sites from different biogeographical regions. The Caribbean region, for example, exhibits the best recruitment with the highest CPUE in July, when the southern Indian Ocean is at its lowest point; in July 2014, the Zoé project caught up to 224 fish per trap with a diversity of 40 species (Igrec Mer/Zoé project, pers. comm.).

While the marine aquarium trade may consider this seasonality to be a disadvantage for the PCC technique, it can be easily offset if PCC producers start exporting (and the

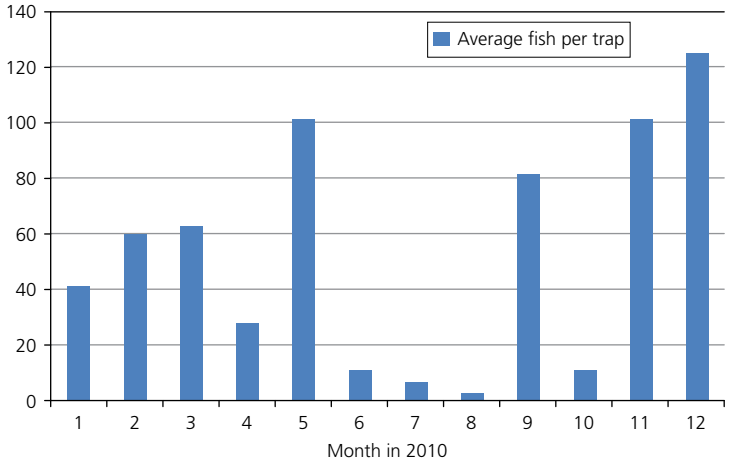


Figure 18.3 Micronesia CPUE over one year (2010). Fish traps used were CARE light traps and crest net.

MAT starts buying) from different sites at different times of the year. For example, *Chromis viridis* can be supplied from the French Polynesian islands or similar islands in the Pacific during the period from June to August and from Mauritius (or similar islands in the Indian Ocean) from October to December. However, if the MAT is to become sustainable it must accept a certain degree of seasonality. If we want PCC-caught yellow tangs we must fish in Hawaii during the natural peak of this species, which occurs from September to November each year with fish being available on the market only 2 to 3 months later.

Such seasonality is not necessarily a disadvantage for the MAT. For example, manufacturers and retailers involved in many other hobbies (such as fishing and hunting) leverage "closed seasons" when people are barred from pursuing their hobby (or aspects thereof) to encourage purchases of expensive items, such as new fishing rods and reels, new guns and bows, training equipment and so on. In the MAT's case, a hobbyist who can't buy new damselfishes until February should be hoping for a nice new aquarium for Christmas (or Hanukkah or Chinese New Year), in order to condition it in time for the arrival of new marine ornamentals.

18.3.4 Species Requested by the Market

Seasonal species composition can be as variable as CPUE. Some species recruit all year round, whereas others only peak once a year. Indeed, the PCC catch rate depends on annual conditions (El Nino *versus* La Niña), season (i.e. temperature of the water) and the inshore fish assemblage. In fact inshore fish species opportunities for PCC vary widely between countries: the French Polynesian archipelago has 633 species of reef fishes, the Marshall Islands up to 827 species and the Philippines a maximum of 2500 species (Myers, 1999).

It is widely but erroneously believed that PCC species do not appear in the top 10 or 20 lists of MAT species. This fallacy is evident from the work by Rhyne *et al.* (2012), where the top species and families of marine fishes entering the US MAT (Figure 18.4 and 18.5). As clearly shown in Figure 18.4 and Table 18.1, pomacentrids lead the list, with species

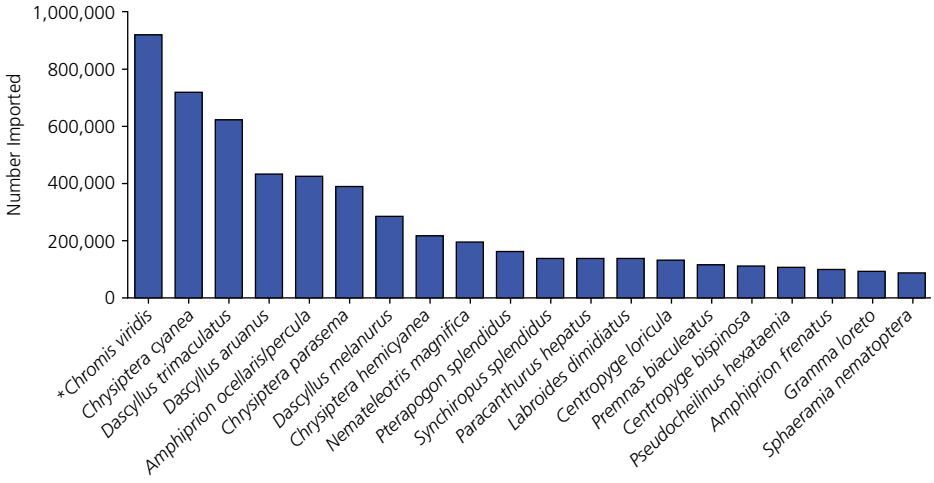


Figure 18.4 Top 20 marine aquarium fish species imported into the United State (from Rhyne *et al*., 2012). *Indicate species complexes, which could represent more than one species which are all traded under the same name.

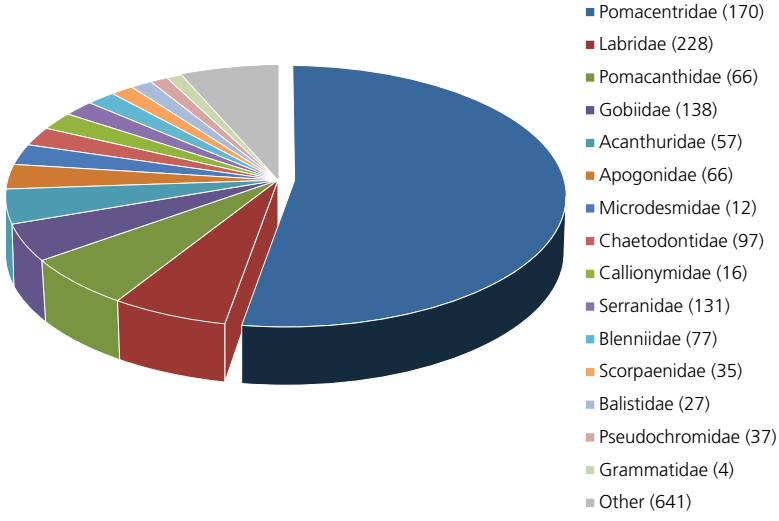


Figure 18.5 Top 10 marine families aquarium fish species imported into the United State (from Rhyne *et al*., 2012).

such as *Chromis viridis, Chrysiptera cyanea, C. parasema* and *C. hemicyanea, Dascyllus aruanus* or *D. melanurus* being among the top eight species. All of these species (or very similar ones) have already been collected at various PCC sites tested over the last decade. Moreover, if we look at the top ten families (Figure 18.5), the PCC catch is remarkably similar.

Nevertheless, PCC is not a miracle tool – it does not manufacture post-larvae! PCC can only harvest fish species where they are naturally present and at some relative

Table 18.1 Comparison of fish families collected from PCC from two sites versus wild-caught families listed in Rhyne *et al.*, 2012 and the Global Marine Aquarium Database. ■ cells show similarities, ■ cells the specificity of PCC and ■ cells refer to families poorly caught by PCC.

PCC				MAT			
Indian Ocean	**%**	**Caribbean**	**%**	**Worldwide**	**%**	**GMAD**	**%**
Briot (2012)		**Igrec Mer (2014)**		**Rhyne *et al.*, 2012**			
POMACENTRIDAE	43	POMACENTRIDAE	18	POMACENTRIDAE	52	POMACENTRIDAE	45
APOGONIDAE	17	HOLOCENTRIDAE	16	LABRIDAE	8	ACANTHURIDAE	7
GOBIIDAE	5	TETRAODONTIDAE	15	POMACANTHIDAE	7	LABRIDAE	6.5
ACANTHURIDAE	4.5	APOGONIDAE	9	GOBIIDAE	5	GOBIIDAE	6
BLENNIDAE	2	BALISTIDAE	8	ACANTHURIDAE	3	CHAETODONTIDAE	4
HOLOCENTRIDAE	1.8	ACANTHURIDAE	8	APOGONIDAE	2	BLENNIDAE	2.5
BALISTIDAE	1.6	BLENNIIDAE	5	MICRODESMIDAE	2	SERRANIDAE	2.5
SERRANIDAE	1.5	CHAETODONTIDAE	4	CHAETODONTIDAE	2	*OTHERS*	26
CHAETODONTIDAE	1	SYNGNATHIDAE	4	CALLIONIMIDAE	2		
SYNGNATIDAE	0.5	SERRANIDAE	2	SERRANIDAE	2		
LABRIDAE	0.3	DACTYLOPTERIDAE	1	BLENNIDAE	2		
OTHERS	22	*OTHERS*	10	SCORPAENIDAE	1		
				BALISTIDAE	1		
				OTHERS	11		

abundance, as the resident wild reef community. As described earlier in the chapter, PCC sites can also vary widely from one location to another. Therefore PCC needs to be targeted on those locations where the species assemblage and abundance either match existing market needs or can produce viable alternative species for the market.

Even marketable species, however, need to be potentially cost-effective. For example, a significant amount of wrasses (Labridae) can be collected with crestnet, but this family produces very small larvae which need a year of rearing before they can be sold in the market. Because of the comparatively high rearing costs, PCC efforts would be better targeted at less common wrasses that can demand a high market price, rather than common and abundant cleaner wrasses such as *Labroides dimidiatus*.

18.3.5 The Best Site on the Planet?

The closer to the Coral triangle and specifically the Raja Ampat Archipelago (RAA), the more suitable the inshore fish species list for sale to the MAT. Indeed, RAA is considered to have the planet's most biodiverse reefs (Jones & Shimlock, 2002). In November 2009, Ecocean was able to conduct a diagnostic for PCC development over a few days of collection with CARE traps (Lecaillon, 2010). The fishes collected (represented in Table 18.2) were amazingly interesting in terms of both diversity (64 species) and abundance (1739 PL collected in three nights with two CARE traps). The number of fish suitable for sale to the MAT represent 49% of the total catch, with 64% of these species already in demand by the market, including 19% of *Chromis viridis*, the MAT's most highly demanded species.

These results illustrate that if PCC is established in the right place with good reef conditions, and with the right fishing tools, it can provide economically viable opportunities for local communities to produce sustainably caught aquarium fish. The report by Cartwright *et al*. (2012) recommends implementing PCC in combination with a grow-out operation using Micropod™ ocean cages. We recommend this approach to be carried out in combination with the rearing of local food fish species.

In Figure 18.6, we can see the list of families caught from post-larval collection in the Indian Ocean from a 10 years data set from Ecocean (for additional information see Briot, 2012). Pomacentrids represent 43% of the catch and are the main family collected, as they are heavily requested by the MAT (Rhyne *et al*., 2012).

There is a difference in rank abundance of species in the 16 most abundant families between two islands in the Indian Ocean (Figure 18.7). Thus PCC has the potential to produce and deliver many different species for the MAT taking advantage of the Indian Ocean biodiversity.

18.4 Hobbyist Responsibilities

MAT wholesalers and retailers order and stock species according to hobbyist demand, which is relatively consistent throughout the year and across all major markets for most species. Unfortunately this is a powerful disincentive for the use of sustainable production methods. PCC aquarioculture is affected by seasonality as it relies on fishing for post-larvae at specific times of the year. Therefore, despite the availability of sustainable production methods, the MAT continues to demand wild-caught fish even though

Table 18.2 Fish species collected with CARE light trap during the Conservation International diagnostic.

Families	Scientific name	Qtv
Acanthuridae	*Acanthurus pyroferus*	1
Blenniidae	*Escenius* sp1	4
	Escenins sp2	2
Caesionidae	*Pterocaesio* sp	93
	Pterocaesio sp2	1
Carangidae	*Caranx sexfasciatus*	1
	Scombrides sp	2
Chaetodontidae	*Chaetodon* sp	1
Cirrithidae	*Paracirrithes* sp	2
Holocentridae	*Neoniphon sammara*	21
	Holocentrids sp	11
Labridae	*Stetojulis* sp	4
	Coris sp	2
Lutjanidae	*Macolor niqer*	2
Nemipteridae	Scolopsis lineatus	27
	Scolopsis sp	1
Mullidae	*Parapuneus bifasciatus*	4
Pomacanthidae	*Pomacanthus semicirculatus*	2
Pomacentridae	*Pomacentrus* spp	1
	Amblyglyphidodon tematensis	18
	Pomacentrus coelistis	75
	Dascyllus trimaculats	30
	Dascyllus reticulatus	35
	Pomacentrus chrysurus	35
	Pomacentrus dark spot, red line on	6
	Pomacentrus yellow tail	149
	Pomacentrus yellow tail+ black spot	28
	Chrysiptera talboti	3
	Chrysiptera rex	6
	Chromis viridis	109
	Chromis lepidolepis	55
	Plectroglyphidodon lacrymatus	50
	Neoglyphidodon sp1	1
	Neoglyphidodon sp2	1
	Chromis tematensis	2
	Pomacentrus yellow+ black spot	19
	Pomacentrus moccculensis	12

Table 18.2 (*Continued*)

Families	Scientific name	Qtv
Siganidae	*Siganus spinus?*	16
	Siganus sp2	6
Tetraodontidae	*Canthigaster benneti*	1
	Arothron hispidus	2
Special families	*Triacanthidae/monodactylus?*	1
	Caloplessiops altivelis	1
	Platycephalidae	1
	Pleuronectidae	1

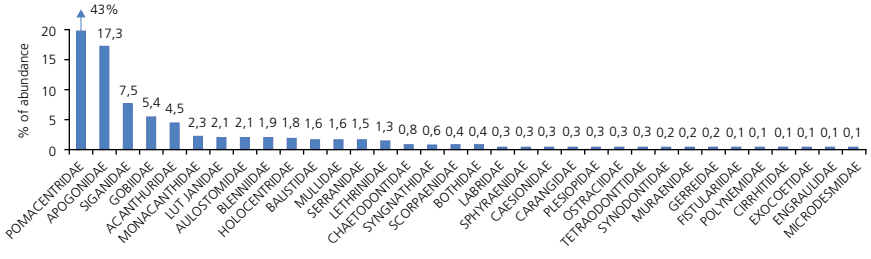


Figure 18.6 Rank-abundance diagram for the 35 families more abundant in the Indian Ocean (four islands grouped).

sustainably reared fish are available. This naturally has a catastrophic impact on reef population assemblages worldwide. For example, the yellow tang (*Zebrasoma flavescens*) is native to Hawaiian reefs and highly valued by aquarium hobbyists. Thousands of yellow tangs are taken by the trade annually. Unfortunately, only recently have yellow tangs been bred in captivity and the species only recruits in sufficient numbers for viable PCC collection from September to November. If every hobbyist wants this species anytime anywhere, the market will never become green, and ironically the high seasonality and predictability of breeding behaviour of such wild fish species actually contributes to the market's unsustainability.

In contrast to wild-fishing for adults, PCC actually leverages the seasonality and predictability of breeding behaviour to reduce its impact to negligible levels, and thus has a great advantage in terms of sustainability. However, PCC fishing is not strongly species selective, and yields quantities of post-larvae species in accordance with the respective species abundance at the fishing site. Thus PCC will invariably collect more damselfishes than angelfishes, because in the wild, damselfishes are much more common. This is not necessarily a disadvantage if hobbyists can be persuaded to adapt their behaviour accordingly. They can play a very positive role in reducing the destruction of the coral reef ecosystem by opening their minds (and wallets) to new original species produced by captive breeding or by PCC aquarioculture. A good example is that of *Chromis nigrura* from the Indian Ocean, which exhibits identical peaceful and unaggressive behaviour to the popular *Chromis viridis* and can be caught sustainably in large numbers at Reunion Island and Mauritius.

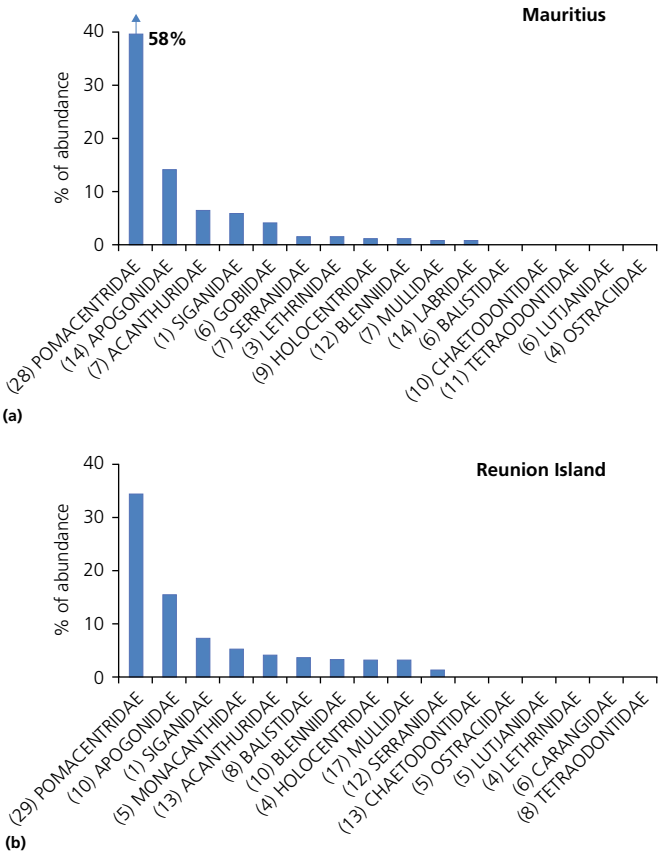


Figure 18.7 Rank-abundance diagram for the 16 first families more abundant in two different Indian Ocean islands. (a) Mauritius and (b) Reunion Island.

For the marine ornamental fish trade to become and remain sustainable, hobbyists must learn to accept a degree of seasonality in the availability of the most popular species, and acquire their new specimens during the appropriate period of the year.

Finally, hobbyists must be prepared to pay for sustainable PCC-caught and captive-bred fish versus unsustainable wild-caught adults. According to the recent Bren School of Environmental Science and Management study (Cartwright *et al.*, 2012), American hobbyists are willing to pay up to 25% more for sustainably caught fish compared to regular wild fish caught. If the MAT is prepared to address this apparent willingness, it will have a direct positive impact on the entire market chain: aquaculture operations and fisherman can increase their income; distributors and retailers can increase their turnover and profit margins; and hobbyists can enjoy higher quality fish with much-reduced mortality. However, the study referred above declared willingness and not an actual behaviour. Despite the best of intentions, when a hobbyist enters an aquarium store with his or her own hard-earned money, the resulting purchasing behaviour may well lean towards cheaper wild-caught fish, even though tank-raised fish are almost certain to stay alive much longer and far less likely to die within a couple of weeks!

It has been clearly demonstrated that both PCC aquarioculture and captive-breeding aquaculture can contribute to the sustainability of the MAT. However,

only a profound shift towards sustainability in hobbyist behaviour can actually green the market.

There are actually some precedents for such behaviour change in other areas. For example, sport hunters contributed to the extinction of the passenger pigeon and the near extinction of the American bison, while anglers (sport fishers) were principally responsibility for the near absence of the Atlantic bluefin tuna from the North Sea. However, at least in North America and Northern Europe, hunting and angling organizations are now amongst the most powerful and effective conservation lobbyists, and have themselves voluntarily introduced or proposed legislation for close seasons, catch reductions, size limits and restrictions on equipment used. It could be argued that this change of behaviour, which took place gradually over many decades, was entirely due to self-interest, as both hunters and anglers recognized the impact their behaviour was having on their choice of activity. Nevertheless their behaviour did become far more sustainable.

The major difference between hunters, angers and aquarium hobbyists is that the first two groups were able to experience their environmental impacts directly, in terms of scarcity of game and fish. The aquarium hobbyist, by comparison, is one step removed; he or she may be theoretically aware of the impacts of this hobby, but experiences these impacts only indirectly in terms of increased prices for certain species, and not directly by diving on a devastated reef. Thus more effective measures are required to raise awareness amongst marine aquarium hobbyists about the impacts of their activities and the mitigating strategies available to them.

18.5 PCC Experiences

A successful PCC project must collect a large number of post-larvae to be able to obtain sufficient numbers of the most desirable species for the MAT. In most cases, roughly 40% of the post-larvae collected are suitable for the MAT. However, unlike other fishing methods, the remaining 60% are hardly by-catch. Instead, they are also tank-reared and then aquacultured for two purposes. Firstly, they can supply the local (and occasionally regional) food fish market, thereby reducing commercial fishing pressure on reef species and also contributing to the profitability of the project as side income. Secondly, they can be reintroduced to the coral reef alive for restocking purposes (along with a proportion of the ornamental species where this would be beneficial) or be used for feeding the selected valuable species.

Over the last decade, attempts at building profitable businesses based on PCC have not entirely succeeded. Problems often originated from administrative or political interference or inertia (e.g., Mauritius and Reunion Island), because local stakeholders were not sufficiently involved and motivated (e.g., Madagascar) or because the distance from market, as well as local labor and material costs, were just too high (e.g., French Polynesia, New Caledonia). However, two Southeast Asian projects (Marine Aquarium Market Transformation Initiative – MAMTI and National Fish and Wildlife Foundation – NFWF) came very close to profitability, despite the local marine resource being too overfished in the targeted areas (Bohol Island), the species caught lacking in attractiveness to the MAT and the CPUE being too low (20 PL/night/trap).

In the Indian Ocean, Mauritius and Reunion islands produced excellent stock lists, such as the one obtained in November 2012 from the Eco Fish Mauritius PCC project (see Table 18.3). Up to 50 different species were available, with damselfishes being the

Table 18.3 Stock list example of available fishes and crustaceans from PCC from Mauritius islands in February 2012.

Scientific name	Common english name	Qty
Cardinalfishes		
Apogon apogonides/evermanni	Short-tooth cardinal	4
Apogon cyanosoma	Yellowstriped cardinalfish	80
Apogon kallopterus	Iridescent cardinalfish	4
Apogonichthys perdix	Perdix cardinalfish	170
Damselfishes		
Chromis dimidiata	Chocolatedip chromis	4
Chromis lepidolepis	Scaly chromis	113
Chromis nigrura	Blacktail chromis	615
Chromis viridis	Blue green damselfish	70
Chrysiptera brownriggii/leucopoma	Surge damselfish	168
Chrysiptera glauca	Grey demoiselle	55
Dascyllus aruanus	Whitetail dascyllus	70
Dascyllus carneus	Cloudy dascyllus	4
Dascyllus trimaculatus	Threespot dascyllus	158
Plectroglyphidodon dickii	Blackbar devil	14
Plectroglyphidodon imparipennis	Brighteye damselfish	145
Plectroglyphidodon johnstonianus	Johnston Island damsel	15
Pomacentrus caeruleus	Caerulean damsel	2290
Pomachromis richardsoni	Richardson's reef-damsel	670
Boxfishes		
Arothron nigropunctatus	Blackspotted puffer	2
Canthigaster coronata	Crowned puffer	1
Canthigaster solandri	Spotted sharpnose	1
Canthigaster valentini	Valentin's sharpnose puffer	6
Lactoria cornuta	Longhorn cowfish	2
Lactoria fornasini	Thornback cowfish	1
Ostracion cubicus	Yellow boxfish	1
Blennies & Gobies		
Cirripectes castaneus	Chestnut eyelash-blenny	6
Eviota albolineata	Spotted fringefin goby	26
Exallias brevis	Leopard blenny	3
Surgeonfishes		
Acanthurus nigrofuscus	Brown surgeonfish	1
Acanthurus triostegus	Convict surgeonfish	90

Table 18.3 (*Continued*)

Scientific name	Common english name	Qty
Triggerfishes & limefishes		
Cantherhines pardalis	Honeycomb filefish	3
Rhinecanthus aculeatus	White-banded triggerfish	10
Rhinecanthus rectangulus	Wedge-tail triggerfish	1
Butterflyfishes		
Chaetodon auriga	Threadfin butterflyfish	8
Chaetodon guttatissimus	Peppered butterflyfish	1
Chaetodon lunula	Rancoon butterflyfish	3
Chaetodon trifasciatus	Melon butterflyfish	1
Chaetodon xanthocephalus	Yellowhead butterflyfish	1
Divers		
Corythoichthys schultzi	Schultz's pipefish	10
Epinephelus fasciatus	Blacktip grouper	10
Epinephelus flavocaeruleus	Blue-and-yellow grouper	1
Grammistes sexlineatus	Goldenstriped soapfish	20
Histrio histrio	Sargassumfish	2
Lutjanus kasmira	Common bluestripe snapper	8
Myripristis murdjan	Pinecone soldierfish	3
Parupeneus bifasciatus	Doublebar goatfish	1
Parupeneus macronemus	Long-barbel goatfish	4
Priacanthus sp	Bigeye sp	1
Pterois volitans	Red lionfish	1
Solenostomus paradoxus	Harlequin ghost pipefish	1
Crustaceans		
Stenopus hispidus	Banded cleaner shrimp	10

most abundant. The color and tank behaviour of those species were really attractive but they were unknown to the market, and thus the MAT was not yet ready to accept them. Additionally, the local government failed to provide final authorization for commercial exploitation due to political disagreements. Export freight was also expensive, as local shipping companies and freight forwarders preferred to charge high one-off prices for shipments rather than help develop this sustainable market (and generate a repeat business for themselves) by offering regular freight prices. A PCC project is still operating in Madagascar but, at present, they have no authorization to export.

Some experiences have been more positive. In Hawaii an aquaculture company is collecting post-larvae fish for a local program named "adopt a baby fish" which is designed to raise awareness of marine conservation among local school students. (www.livingartmarinecenter.com). Sustainable Aquatics has proposed using PCC to harvest sustainable fish from the Solomon Islands using crest nets. Conservation International

is still looking of money to try developing PCC in the Raja Ampat archipelago, probably the most suitable location of all for this sustainable technology.

Indeed, PCC is an excellent tool with which to educate people, fisherman and even tourists about the state of our coral reefs and to research and better understand this little-known part of the life cycle of coastal marine species.

18.6 Conclusions

In summary, tank-raised fish from post-larval collection offer many benefits both for conservation and for the marine aquarium trade. They reduce the number of wild-caught fish, which in turn decreases the pressure from marine ornamental collectors on coral reef habitats. Even with such a fantastic principle, PCC has not yet become really successful worldwide. As described earlier in this chapter, for PCC to work a combination of local factors and market desire is needed, not all of which are yet in place at the right markets and locations. We also have to cope with unrealistic expectations; some countries have expected PCC techniques to work on reefs and fisheries that have already been overfished to the point of local extinction, when of course it is too late for this method to have any effect. Without genitors, there are no post-larvae, and with reef habitats in poor condition, there are insufficient genitors, so naturally PCC cannot be profitable under these circumstances. When implemented in healthier neighboring areas, however, it can be a valuable source of healthy live fishes for restocking. On the positive side – and as a result of the effort, expense and energy devoted to the development of this technology by a number of very dedicated people – PCC has already been labeled "good practice" by the International Coral Reef Initiative (www.icriforum.org). Organizations such as UNESCO, through its Man and Biosphere (MAB) Programme, have found PCC suitable for building quality economies through the conservation and sustainable use of coastal and marine biodiversity. The overwhelming majority of the technological challenges have already been overcome, and it is now fully feasible to transfer capacity building and technology of the various steps to local communities.

It is only fair to point out that funding for PCC has been somewhat limited – the CRISP project (500 K US$, AFD), the MAMTI project (300 K US$, World bank), a couple of other experiments funded by the NOAA and the French Polynesia Fisheries Department and about a dozen small private investments worldwide. In short, no more than US$ 3 million has been spent on developing PCC in over a decade. In contrast, many decades of research and development have been conducted and millions and even billions of dollars have been spent to overcome the technological barriers to global food fish aquaculture, with many millions more for aquacultured marine aquarium fishes (such as clownfish).

The stakes are enormous – the future survival of our tropical coral reefs! We must invest in this technique because it is fully sustainable and is proven to work when all the requisite factors are in place. We have the know-how and the technological barriers have been overcome. We only lack the necessary political will, a modest amount of funding and the MAT's sincere desire to green itself. If hobbyists can become more aware, if policy makers can make courageous decisions (Tissot *et al*., 2010) and if institutional donors and governments are willing to invest, PCC can be a powerful tool to help green the MAT and mitigate its impacts.

If not successfully mitigated, these impacts are far more likely to increase than to decline in the foreseeable future. Chinese aquarium hobbyists (and Koreans, Malaysians, Singaporeans, wealthier Indonesians and Filipinos) absolutely adore ornamental freshwater fish and are busy developing a similar passion for marine species. Asia's emerging economies are capable of bringing literally millions of new and acquisitive hobbyists to the global MAT, and thus greening the industry is a priority of the greatest possible urgency. PCC fish – along with other sustainably reared fish – have to become products that hobbyists demand from the MAT because they are not only eco-friendly but also better and more desirable! Consumer demand will green the market far more effectively than any legislation.

To conclude on a positive note, PCC serves to reduce the pressures on adult reef fish by offering access to a new and under-exploited or even unexploited marine resource in tropical seas and developing countries. However, PCC is also suitable for use by advanced nations in temperate seas as an additional economic, conservation and restocking resource (mitigation banking), especially where conventional conservation strategies have already failed. The heavily over-fished Mediterranean and North Sea fisheries are good examples of areas that could benefit from such an approach. Finally, PCC is also an excellent tool to study and understand this little-known part of the life cycle of coastal marine species; research projects using PCC, such as the SUBLIMO LIFE+ Project (www.life-sublimo.fr) are still on-going. Post-larval capture and culture is certainly not the only solution to the overexploitation of demersal marine aquarium trade species, but it is nevertheless a valuable one that directly complements other conservation strategies, such as the development of aquacultured marine aquarium species detailed in this book.

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19

Common Diseases in Marine Ornamental Fishes

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Abstract

Welfare and health of marine ornamental fish can be influenced by several environmental, nutritional and biological factors. Considering that the description "*marine ornamental fish*" includes over 1500 species of dozens of families with extremely different biological, nutritional and environmental requirements, it is obvious that extremely different health problems may be encountered by these animals during all the phases of their life, from capture or artificial reproduction to confinement into public or private aquaria. Keeping fish in a confined/artificial environment, whether in a farm tank or a private or public aquarium, generally results in their forced coexistence with stressful conditions that may adversely affect their wellbeing, making them prone to several diseases, primarily those due to parasitic and infectious agents. The factors which most commonly favor the development of a transmissible disease in a fish population held in a confined environment are numerous, such as water quality, nutrition deficiencies and/or imbalances, overcrowding, inadequate sanitization procedures and, of particular importance, the introduction of parasitized/infected fish into the tank without appropriate quarantine and health checks. This chapter focuses on the most common and important diseases caused by infectious and parasitic agents in marine ornamental fish kept in aquarium systems.

Keywords *Marine ornamental fishes; diseases; parasites; fungi; bacteria; viruses*

19.1 Introduction

Only a few of around 1500 marine ornamental fish species marketed worldwide (2–5%) are reproduced in captivity on a commercial scale. The remaining species are caught in the wild with stressful or, sometimes, destructive methods (e.g. cyanide) and subjected to a chain of stressful events (capture, handling, transport, etc.) culminating in their confinement in artificial environments that are often unsuitable or sub-optimal. This course of events deeply undermines the welfare and health of the fish, causing death either directly or predisposing fish to infections due to opportunistic pathogens which

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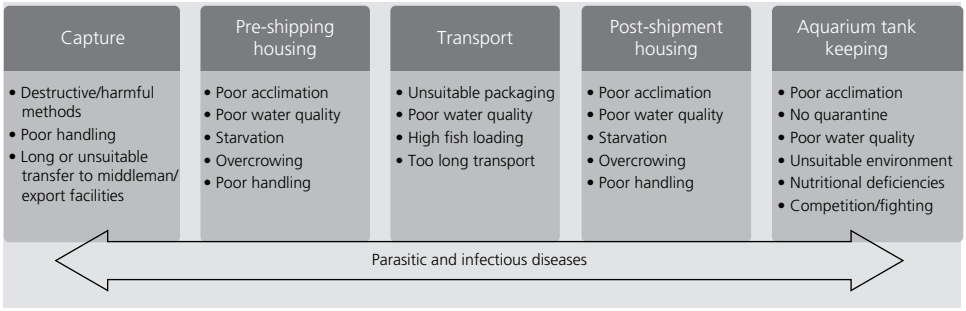


Figure 19.1 Main adverse conditions and risks encountered by marine ornamental fish along their chain of custody. Copyright owned by the authors, Maria Letizia Fioravanti & Daniela Florio.

are commonly found on/in the fish or in the aquatic environment. The effects of stress or damages related to capture and post-capture often emerge only later, after days or weeks, when the fish have already arrived at the retailers or even inside the aquarium, and are responsible for the "delayed mortality syndrome" (DMS). This syndrome includes all those episodes of mortality observed in marine fish in the period immediately after delivery, generally within 1 week, and which may be attributable to factors such as long-term toxic effects of cyanide, improper transport/packaging conditions, prolonged fasting, onset of opportunistic bacterial, fungal or parasitic infections, etc. In these phases, the application of best management practices that may be useful to avoid harmful forms of fishing (e.g., fishing net with optimal mesh size), unsuitable post-capture housing (e.g., low stock density, coexistence of compatible species, etc.), and prolonged and stressful transport (e.g., appropriate packaging systems) are needed. In addition, careful acclimation to all new environments (e.g., slow adaptation to water parameters changes, proper food supply, etc.) and quarantine for health monitoring purposes (e.g., isolation of newly introduced fish for a certain period in order to check their health status and avoid introduction of pathogens) can be crucial to minimize mortality rates, to ensure immunocompetence and to prevent the establishment of disorders, and parasitic and infectious diseases (Figure 19.1) (Lim *et al*., 2003; Bartelme, 2004; Rubec & Cruz, 2005; Francis-Floyd & Petty, 2010; Noga, 2010).

As ornamental fish spend most or even their entire life in confined artificial conditions, the tank of a public or private aquarium has to be regarded as a complex system. All the abiotic and biotic elements useful to the welfare of fish and other living organisms have to be introduced from the outside, kept at an optimal level and well balanced in order to prevent discomfort and health problems. In particular, in marine aquaria, the water parameters, which in tropical reef environments are remarkably constant, and the feed supply, that often replaces a complex trophic chain, are elements that may be difficult to ensure and maintain. This is especially important when considering the huge biological variability inherent in the "marine ornamental fish" category. In fact, besides the most commercially important species such as damselfish, anemonefish and angelfish, which together account for over 50% of the total volume of marine fish market, the remaining volume is made up of species from dozens of different fish families (Bruckner, 2005). Thus, marine ornamental fish may be affected by several disorders caused by environmental and nutritional factors which can directly result in disease or trigger the

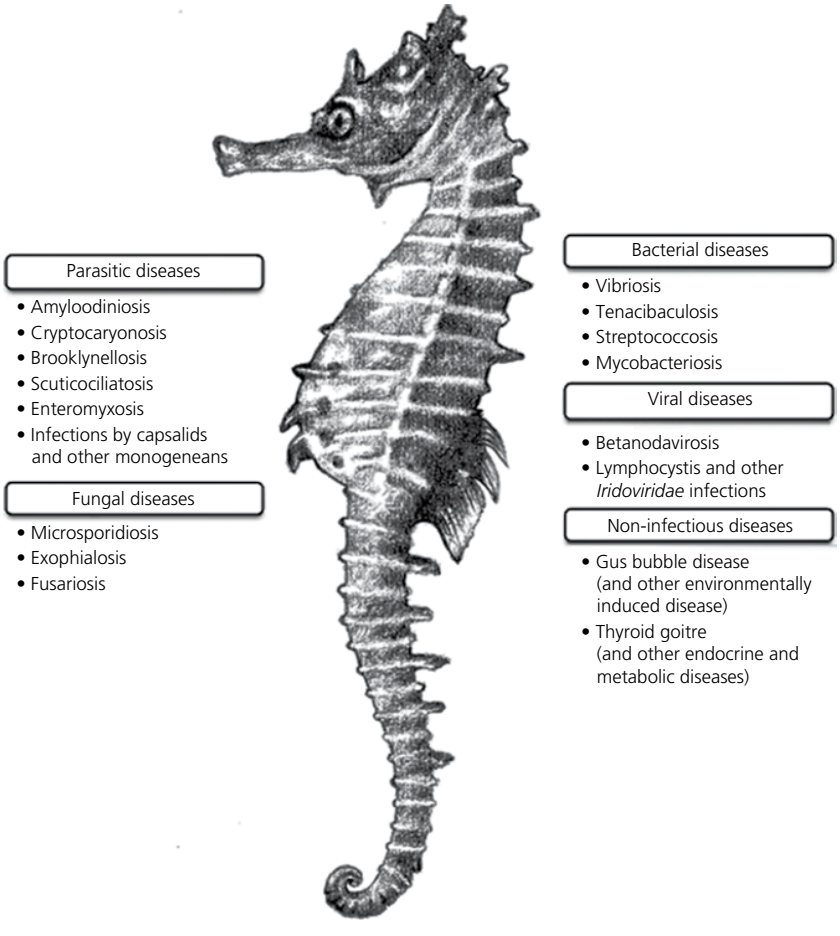


Figure 19.2 Synoptic scheme of main diseases of marine ornamental fish (seahorse as an example). Copyright owned by the authors, Maria Letizia Fioravanti & Daniela Florio.

outbreak of infectious and parasitic agents which are generally the outcome of complex relationships among the environment, hosts and pathogens (Hedrick, 1998) (Figure 19.2). Marine ornamental fish health issues should therefore be approached from a holistic perspective, which considers the aquarium as a "whole" and permits an integrated approach to prevent and control diseases of fish living there.

In the following sections the most common parasitic, fungal, bacterial and viral diseases of marine ornamental fish are presented.

19.2 Parasitic Diseases

Among the most important parasitic diseases in a confined environment, those caused by direct life cycle parasites (do not require intermediate hosts) are the most serious as they can easily spread in the tank population. In particular, the dinoflagellate

Amyloodinium ocellatum and the ciliate *Cryptocaryon irritans*, etiologic agents of "velvet disease" and "marine white spot disease", respectively, are of great importance as they find in the aquarium optimal conditions for their development and transmission. Among protozoans, other obligate parasites can cause severe disease in marine ornamental fish, i.e. *Brooklynella hostilis*. The potential pathogenic role of some facultative parasites (e.g. Scuticociliatida) which may become a true menace to ornamental fish health in the presence of environmental and host predisposing factors, should not be underestimated. In addition, several monoxenous helminth ectoparasites, such as Capsalid monogeneans, can rapidly spread in a captive fish population, especially when overcrowded, and cause severe diseases and mortality. However, the possibility should not be ruled out that even heteroxenous parasites (e.g., with an indirect cycle) once introduced into the tank through parasitized wild fish can cause disease in the host in captivity or may spread among fish thanks in the presence of invertebrates that may act as suitable intermediate hosts (e.g., some polychaetes for Aporocotylid trematodes). For these reasons all newly acquired fish should always be quarantined and subjected to health checks before their introduction into a new environment, with routine biosecurity measures always being applied.

19.2.1 *Amyloodinium ocellatum*

Amyloodinium ocellatum is a dinoflagellate of the Chromoalveolata supergroup, which is strongly adapted to a parasitic life style and is considered one of the most harmful parasites for marine fish kept in confined environments (aquaria and farms). *A. ocellatum* shows a wide host range and has been reported from hundreds of eurhyaline and marine fish species, elasmobranchs included (Álvarez-Pellitero, 2008; Noga, 2011). *A. ocellatum* has very rarely been reported in wild fish, but is well known since 1930 (Brown, 1931; Nigrelli, 1936) as a serious pathogen of aquarium marine fish. Since then, it has been reported in a wide range of food and ornamental fish kept in confined environments (Noga & Levy, 2006).

It has a polyphasic direct life cycle, with a parasitic trophic stage (trophont) attached to the gill and skin epithelial cells of a fish host and two environmental stages, a benthonic reproductive stage (tomont) and a free-swimming flagellated infective stage (dinospore). After adhesion of the dinospore to the gill or skin epithelium, the trophont attaches firmly to the cells through rhizoids and grows up to 350 μm. It then detaches, sinks to the bottom and quickly transforms into a tomont, the reproductive cyst, in which up to a maximum of 256 dinospores are produced (Paperna, 1980, 1984a; Lom & Dyková, 1992). Dinospores can remain infective for up to 6 days at 26 °C (Bower *et al*., 1987).

The life cycle is strongly regulated by temperature and salinity. Optimal temperature is 23–28 °C, although *A. ocellatum* can reproduce in a wider range. Concerning salinity, reproduction of tomonts occurs within a range of 12–50 ppt, although tolerance to salinity is dependent on temperature, being widest at 24–25 °C (Álvarez-Pellitero, 2008), and may vary in different geographical strains (Noga, 2011). Under optimal conditions, *A. ocellatum* can complete its life cycle in less than a week (Noga & Levy, 2006).

The deep attachment pattern of trophonts to gill and skin epithelial cells causes traumatic lesions and triggers frequent secondary infections. Inflammatory responses

include hyperplasia and hypersecretion of mucus, followed by epithelial sloughing off and tissue necrosis (Colorni, 1985; Noga & Levy, 2006; Noga, 2011). Respiratory and osmoregulatory disorders can lead to death of the host (Colorni & Padrós, 2011). Although gills represent the primary site of infection, skin, fins and eyes can also be infected. Massively infected fish show respiratory distress, irritation (flashing or skin rubbing), anorexia and lethargy. In cases of massive skin infection, the body surface appears velvety, and for this reason Amyloodiniasis is also known as "velvet disease" in marine ornamental fish.

Dinospores are susceptible to chemotherapy, but trophonts and tomonts show a greater resistance, making the control of Amyloodiniasis difficult (Colorni & Padrós, 2011; Noga, 2011). Several treatments have been studied for *A. ocellatum* including copper sulphate (Paperna, 1984b; Noga, 2010), caprylic acid (Hirazawa *et al*., 2001), hydrogen peroxide (Montgomery-Brock *et al*., 2001), benzalkonium chloride (Hirazawa *et al*., 2003), formalin (Fajer-Ávila *et al*., 2003), hyposalinity (Colorni, 1985) and changes in salinity (Pironet & Jones, 2000). Experimental trials have demonstrated the efficacy of chloroquine diphosphate at 5–10 ppm for 10 days against *A. ocellatum* infections in *Amphiprion ocellaris*, but this product is highly toxic for algae and some invertebrates (Noga & Levy, 2006). If feasible, 6–7 transfers of fish between tanks every second day, fallowing and disinfecting tanks after each change, may be an effective treatment (Colorni & Padrós, 2011). Biological control of *A. ocellatum* through introduction of brine shrimp *Artemia* spp. to the tank has been proposed, as they actively prey on dinospores (Oestmann *et al*., 1995). Fish surviving Amyloodiniosis acquire a certain degree of immunity (Cobb *et al*., 1998), indicating the potential for development of vaccines. A comprehensive review of treatments against *A. ocellatum* is reported in Noga (2010).

19.2.2 *Cryptocaryon irritans*

Cryptocaryon irritans is a pathogenic ciliate included in the order Prorodontida, Class Prostomatea, Family Cryptocaryonidae (Colorni, 2008). This parasite is responsible for "white spot disease" in marine fishes, the saltwater counterpart of freshwater "white spot disease" caused by *Ichthyophthirius multifiliis*. *C. irritans* shows low host specificity, infecting nearly all marine fishes, except for elasmobranchs, and is mainly reported in aquarium and cultured fish, since it finds the optimal conditions for its propagation there. In fact, it has a direct polyphasic life cycle with an obligate parasitic feeding stage (trophont), which lives encysted in host gills, eyes and skin epithelia, and some environmental stages: the reproductive cystic stage (tomont), the aciliate daughter cell (tomite), which develop inside the tomont and differentiate into the free-swimming ciliated infective stage (theront) (Dickerson, 2006) (Figure 19.3).

Once actively penetrated into host epithelia, the infective stage fast develops into a rounded or pear-shaped trophont that lives encysted in epithelial tissues, feeding on sloughed epithelial cells and body fluids and growing up to 450 µm in diameter (most often 100–150 µm). After 3–7 days the trophont spontaneously leaves the host, as a protomont, sinks to the bottom and develops into a cystic tomont within 1–2 days (Colorni, 1985), within which 200–300 tomites are produced by binary fission (Colorni & Burgess, 1997). When tomites complete their development into the ciliated infective stages (theronts) they exit the reproductive cyst and swim

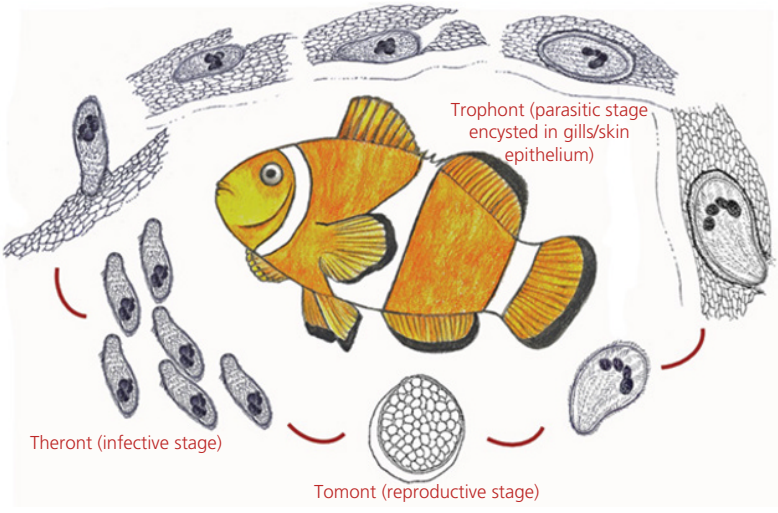


Figure 19.3 Life cycle of *Cryptocaryon irritans*. Copyright owned by the authors, Maria Letizia Fioravanti & Daniela Florio.

actively toward a fish host. Theronts (20–30 × 50–70 µm) are able to penetrate into fish epithelia in few minutes but, without a host to parasitize, survive less than 24 h (exceptionally up to 48 h) losing their infectivity after 6–8 h (Colorni, 1985). Both the exit of the trophont from the host and the excystment of theronts from the tomont are influenced by photoperiod, being favored by predawn darkness (Burgess & Matthews, 1994).

The length of life cycle depends on several factors such as the parasite strain, temperature, salinity and host species (Yanong, 2012); showing great differences (weeks or months) for a specific fish host or strain (Colorni & Burgess, 1997). Optimal temperatures for growth of most of the strains of *C. irritans* are 23–30 °C (Dickerson, 2006), but some strains are adapted to lower temperatures (Diggles & Lester, 1996; Jee *et al*., 2000). Experimental studies have demonstrated that trophonts and tomonts of *C. irritans* can survive dormant for 4–5 months at 12 °C, becoming infective again when the water temperature increases to 27 °C (Dan *et al*., 2009).

Completion of the life cycle can occur in 6 days, although most of the parasites take 11–15 days (Noga, 2011). Striking differences in virulence and development among strains have been observed (Diamant *et al*., 1991; Diggles & Lester, 1996).

C. irritans can cause severe disease and mortality in marine fish kept in captivity. Within the site of encystment, trophonts can continuously stimulate strong inflammatory reactions, with epithelial hyperplasia and mucus hyperproduction. Clinical signs of Cryptocaryonosis include the presence of small white spots/nodules on skin, fin, gills and eyes ("white spot disease") (Figure 19.4 a, b), cloudy eyes, dyspnea, abnormal swimming behaviour (flashing/scratching), lethargy and anorexia. Respiratory distress and osmoregulatory imbalance can be lethal to heavily infected fish (Colorni, 2008). White spots may not be visible in some fish species with lightly pigmented skin or in cases of exclusive gill involvement (Dickerson, 2006).

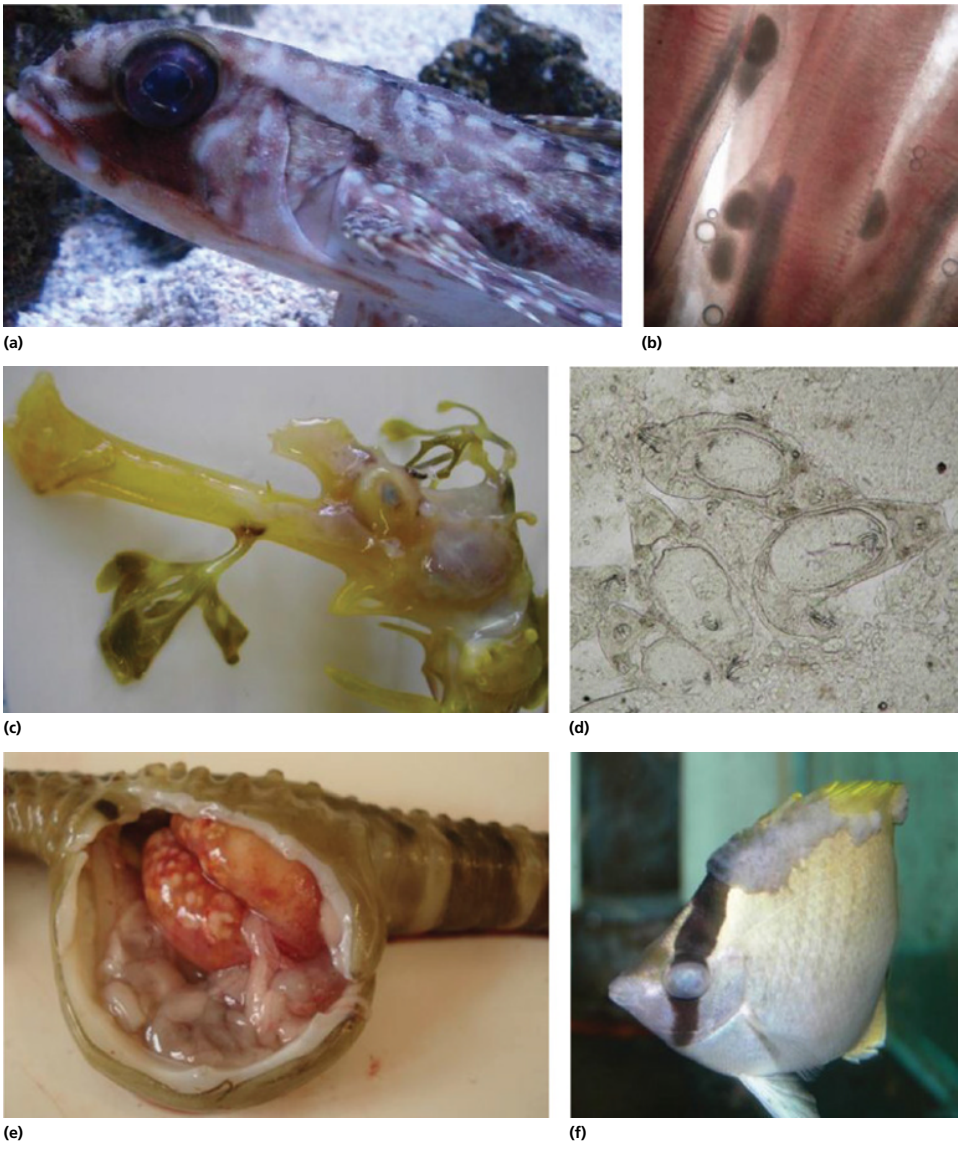


Figure 19.4 (a) Marine white spot disease by *Cryptocaryon irritans* in flying gurnard; (b) trophonts of *C. irritans* in gill wet mount; (c) lesions by Scuticociliatida on the head of a leefy seadragon; (d) Gyrodatylid monogeneans in skin wet mount from pipefish; (e) gross lesions referable to Mycobacteriosis in *Hippocampus abdominalis*; (f) Lymphocystis in *Chaetodon ocellatus*. Copyright owned by the authors, Maria Letizia Fioravanti & Daniela Florio. (*See insert for color representation of the figure.*)

Although few reports exist about *C. irritans* in wild fish populations, it has been isolated from several marine species traded internationally and chiefly from coral reef species (Burgess & Matthews, 1994). The coral reef environment is considered the natural habitat of *C. irritans* (Burgess, 1995).

Control of Cryptocaryonosis is based on breaking the life cycle of the parasite in order to avoid massive infections, since the parasite burden can increase about ten-fold every 6–8 days under optimal conditions (Burgess, 1995). Therapy is mainly aimed at killing the environmental stages, in particular theronts. Although traditionally copper and, to a less extent, formalin have been used for this purpose, over the last decades several studies have been carried out, with varying degrees of success, using other treatments such as caprylic acid (Hirazawa *et al*., 2001), benzalkonium chloride (Hirazawa *et al*., 2003) and peroxigenate compounds (Picón-Camacho *et al*., 2011).

Changes in salinity (Cheung *et al*., 1979; Colorni & Burgess, 1997; Pironet & Jones, 2000), consecutive transfers of infected fish to clean aquaria (Burgess, 1995), or regular removal of tomonts (Colorni & Burgess, 1997) may be also useful in the control of Cryptocaryonosis.

In general, all chemical treatments are more effective when applied late at night, since trophonts from the host and theronts from reproductive stages exit mainly in predawn darkness (Burgess & Matthews, 1994; Colorni, 2008). For a thorough presentation of currently available treatments against Cryptocaryonosis see Noga (2010).

19.2.3 Scuticociliatida

Scuticociliatida ciliates can cause severe diseases in marine ornamental fish, sometimes with onset of fatal systemic infections. Most scuticociliates are histophagous facultative fish parasites that can easily colonize superficial host tissues, mainly in the presence of superficial injuries in the host or environmental predisposing factors (e.g., sharp variations of the water temperature). In some cases they can enter underlying tissues and cause systemic infections.

Among scuticociliates, *Uronema marinum* and *Philasterides dicentrarchi* (syn. *Miamiensis avidus*) have been frequently reported from ornamental marine fish during disease outbreaks. *U. marinum* is considered the most common scuticociliatida in tropical marine fish (Noga, 2010); it acts as an opportunistic pathogen causing frequent infections in fish subjected to injuries and environmental stress (Colorni & Padrós, 2011). It can invade skin, muscle and internal organs causing severe pathology and mortality within a wide range of temperatures and salinities (Cheung *et al*., 1980). *P. dicentrarchi* has been reported as a causative agent of disease and mortality in several species of marine food and ornamental teleosts and, more rarely, in elasmobranchs kept in captivity (Stidworthy *et al*., 2014). It is regarded as a highly pathogenic species able to migrate via the bloodstream and connective tissues to various organs and tissues where it feeds actively on cells and tissue components (Iglesias *et al*., 2001).

Among marine ornamental fish, syngnathids (seahorses and seadragons) appear to be particularly susceptible to infections by different species of scuticociliatida (Cheung *et al*., 1980; Bombardini *et al*., 2006; Rossteuscher *et al*., 2008; Shin *et al*., 2011; Ofelio *et al*., 2014). Generally, the skin is the primary site of infection but underlying tissues, gills and other organs are successively involved (Bonar *et al*., 2013). Infected fish show superficial white patches (Figure 19.4 c) which can evolve into open wounds, abnormal swimming behaviour, anorexia and dyspnea. Respiratory distress and osmoregulatory imbalance are often the cause of death.

Several drugs and disinfectants (e.g., formalin) have been shown to be effective against scuticociliates (Iglesias *et al*., 2002), but only when administered at a very early stage of

infection. When the parasites are already deep in skin or muscle, or when they are disseminated in internal organs, no treatment is effective.

19.2.4 *Brooklynella hostilis*

Described first in 1970 from marine tropical fish (Lom & Nigrelli, 1970), the hartmannulid ciliate *Brooklynella hostilis* has been successively reported from wild, farmed and aquarium marine fish as an obligate parasite. Landsberg (1995) found it on wild fish in the Florida Keys without observing clinical signs referable to parasitosis except for fish showing immunosuppression and intercurrent diseases. Diamant (1998) reported a case of Brooklynellosis in gilthead sea bream (*Sparus aurata*) caged in Israel and linked the infection to pre-existent poor health conditions. Similarly, this parasite can cause severe pathology in stressed marine ornamental fish kept in aquaria where water parameters are not optimal (Lom, 1995; Noga, 2010). *B. hostilis* is 56–86 × 32–50 µm, kidney-bean shaped and displays a slow gliding movement (Lom & Nigrelli, 1970; Noga, 2010; Diamant, 1998). Although reported mainly as a gill pathogen, it has also been shown to cause skin lesions in ornamental marine fish (Noga, 2010). Infected fish show lethargy, anorexia, breathing difficulties and skin lesions. Outbreaks of Brooklynellosis are frequently reported in *Amphiprion* spp. kept in aquarium closed systems. Treatments with formalin are effective (Colorni & Padrós, 2011).

19.2.5 Other Potentially Pathogenic Ciliates

Trichodinids are mobile ciliates that occur in fish generally as ectocommensals (Basson & Van As, 2006). They use superficial tissues of fish as a substrate on which they can "graze" for obtaining food particles (bacteria, tissue detritus, etc.). Healthy fish generally are not subjected to massive infections, but trichodinids can colonize in great number the gills and skin surfaces of very young fish or fish kept in poor environmental conditions. At high intensity of infection, their rotating movement and "sucker-like" adhesive mechanism can seriously damage epithelial tissues. Clinical signs of disease are hyperproduction of skin mucus, frayed fins, lethargic swimming and dyspnea. Trichodinosis can be an important disease for fry of marine fish reproduced in captivity, such as *Amphiprion* spp., where it can cause heavy losses if not controlled.

19.2.6 Myxozoans

Most myxozoan parasites are highly host specific and almost always display an "alternate" complex life cycle. For these reasons they are generally not a significant health threat to marine ornamental fishes with the exception of *Enteromyxum* spp. It is unique among myxozoans in that it can be transmitted directly fish-to-fish (Diamant, 1997; Redondo *et al*., 2002) showing great potential for spreading in confined environments. Furthermore, *Enteromyxum leei* has low host-specificity and has been reported from several warm water marine fish, primarily sparids, but also non-sparid species (Diamant *et al*., 2006; Colorni & Padrós, 2011). Kent (1999) reported a myxozoan resembling *E.* (*Myxidium*) *leei* in the anemone fish *Amphiprion frenatus* and Padrós *et al*. (2001) described an outbreak of *E.* (*Myxidium*) *leei* in 25 different fish species kept in an exhibition aquarium in Spain, with Blenniidae and Labridae being very susceptible.

E. leei causes severe chronic or acute enteritis, depending on host species, often resulting in high mortalities among farmed and ornamental fish, especially when water temperatures are above 20 °C (Rigos *et al*., 1999; Padrós *et al*., 2001). It has also been described as one of the causative agents of the "emaciation disease" of tiger puffer (*Takifugu rubripes*). In this host, *E. leei* causes more serious enteric lesions at 25 °C than at 20 °C, with inhibition of its development/proliferation at temperatures <15 °C and resumption of the infection at 20 °C (Yanagida *et al*., 2006). Other *Enteromyxum* species (e.g., *E. scophthalmi* and *E. fugu*) are host specific for *Scophthalmus maximus* and *T. rubripes*, respectively.

Enteric infections due to *Enteromyxum* sp. in elasmobranchs needs to be investigated in the light of recent finding of *Enteromyxum* spores from the gut of a specimen of *Pteromylaeus bovinus* that died during the quarantine period (Florio, unpublished data).

In order to prevent and control Enteromyxosis, all introduced fish should be quarantined and early diagnosis, based on molecular methods (Yanagida *et al*., 2005), should be conducted mainly in the presence of enteric syndromes. Considering the direct orofecal transmission of *Enteromyxum* spp., prompt isolation of infected fish may reduce spread of the disease.

Effective treatments are not known, although some experimental trials showed some anti-coccidial substances such as salynomicin combined with amprolium to be effective (Golomazou *et al*., 2006).

Several other myxosporean parasites have been reported from marine ornamental fish, such as *Myxobolus* spp., *Ceratomyxa* spp., etc., but their complex life cycle (requiring an annelid as alternate host) makes their spread difficult in tank populations.

19.2.7 Monogeneans

Monogeneans are ectoparasitic flat worms (Platyhelminthes) with a direct transmission pattern and generally high site and host specificity (Buchmann & Bresciani, 2006). Among them, the family Capsalidae (Monopisthocotylea) includes about 180 species of epithelium-feeding parasites that can infect different sites on marine teleosts and elasmobranchs (Whittington, 2011). The capsalid *Neobenedenia melleni* is one of the most important monogeneans affecting marine teleosts kept in captivity. *N. melleni* shows low host specificity, being reported from over 100 teleost species belonging to more than 30 families and 5 orders, and low site specificity, infecting primarily eyes and skin but also fins, gills and the nasal cavity (Buchmann & Bresciani, 2006; Whittington & Horton, 1996). It was first described in 1927 by MacCallum in several fish species (*Spheroides annulatus*, *Chaetodipterus faber*, *Pomacanthus* spp.) from the New York Aquarium (NYA), where it was probably introduced with one of the Caribbean chaetodontid fish that had been found to be naturally infected with this parasite (Nigrelli, 1947). Thereafter it spread to a large number of teleost families kept in captivity at NYA, causing severe problems for several decades (Thoney & Hargis, 1991). Successively, *N. melleni* has been reported from over one hundred marine teleost species in different geographical areas, mainly from aquaria and farms, and rarely from wild hosts (Bullard *et al*., 2000). Recently the exceptional broad host specificity and wide geographical distribution of *N. melleni* has been linked to the existence of a "*N. melleni* species complex" including species morphologically indistinguishable but genetically different (Whittington, 2004). Although *N. melleni* adults are large (2–7 mm long), they are not

easily detectable because they are transparent or lightly pigmented (Whittington, 2011). They are attached to the host surface by strong suction exerted by the discoidal attachment organ (haptor), probably assisted by penetration into the epithelia via the large anterior hooks (hamuli) (Whittington, 2011). The life cycle of *N. melleni* is direct; adults are oviparous and release filamented, operculated and tetrahedral eggs from which a ciliated larva, oncomiracidium, hatches and swims in the water in search of a host. Pathogenicity of *N. melleni* is linked to several factors, such as attachment and feeding activity, site preference, host susceptibility, and infection intensity. Eyes are the primary site of attachment; corneal opacity, lesions exacerbated by secondary infections, and blindness can occur. Scale loss and erosion of the epidermis and underlying tissues are often encountered during massive infestations (Buchmann & Bresciani, 2006; Whittington, 2011) which can cause mortalities under crowded conditions in aquaria. Marine angelfish are very susceptible to capsalid infections (Francis-Floyd & Petty, 2010). Quarantining fish is a basic preventive measure for avoiding the introduction of this adaptable monogenean into aquarium/exhibition tanks. The parasitosis may be controlled through short freshwater baths, useful for lowering infection intensity (Buchmann & Bresciani, 2006). Furthermore praziquantel has been proved to be effective against some capsalids (Hirazawa *et al*., 2004) and cleaner fish (gobies) and shrimp (i.e. *Periclimenes pedersoni*) may help reduce the number of parasites on the host (Cowell *et al*., 1993; McCammon *et al*., 2010).

Although *N. melleni* shows very wide host specificity among teleosts, it has never been reported from elasmobranchs. However a different capsalid species, *Neoentobdella diadema*, has been described in stingrays kept in captivity (Paglialonga *et al*., 2001; Marcer *et al*., 2004; Minardi *et al*., 2008) sometimes with associated lesions at the eyes and/or skin. This species may also be controlled through praziquantel or freshwater baths (Paglialonga *et al*., 2001). Attention should always be paid to application of prolonged freshwater baths both with marine teleosts and elasmobranchs, as they are stressful and could cause detachment of skin mucous which predisposes the treated fish to reinfections (Ohno *et al*., 2009).

Among capsalids, other species may represent a health threat to marine fish kept in confined environments and for this reason should be considered as potential pathogens, especially when showing low host-specificity and high fecundity. For example, *Benedenia epinepheli* has been described in more than 25 fish species belonging to different orders (Ogawa *et al*., 1995) and may cause disease and mortalities in confined marine fish (Hine & Diggles, 2005).

Furthermore, other monopisthocotylean monogeneans may be found in marine ornamental fish, sometimes associated with disease. For example, among the viviparous Gyrodactylids (Figure 19.4 d), some new species have been recently described in pipefish held in a public aquarium with high infection intensities and causing clinical disease (Paladini *et al*., 2010). The high reproductive potential of these viviparous monogeneans, together with their direct transmission route, make them an important health risk if introduced into the aquarium.

19.2.8 Other Potentially Pathogenic Helminthes

With main reference to marine ornamental fish captured in nature, there is a possibility that they are already parasitized by different species of digeneans, cestodes,

nematodes and acanthocephalans. Almost all of these helminthes are endoparasites characterized by high host-specificity and complex life cycles, requiring at least two hosts (one definitive and one or two intermediate hosts), so they generally cannot complete their life cycle in the aquarium and spread to other fish. Nevertheless, it cannot be ruled out that introduced fish which are already parasitized by some potentially pathogenic helminthes (e.g. Aporocotylid trematodes) can develop disease during their life in captivity.

19.3 Mycotic Diseases

Fungal infections are relatively rare among marine ornamental fish but in the last decades new diseases such as Exophialosis and Fusariosis have been reported. Most of these fungi are saprophytes in soil and water and sometimes they have been reported as opportunistic pathogens in fish. In fact, most fish fungal infections are thought to be secondary to injuries or to immunosuppression resulting from stressful conditions such as transport, confinement, etc. (Silphaduang *et al*., 2000). Poor water quality can also lead to an increase in fungal infections in an otherwise healthy fish population. Although generally fungal diseases affect external tissues of fish or their eggs, some systemic mycoses have been described in ornamental marine fish, especially in cases of immunosuppression and several detrimental conditions. Furthermore, since Microsporidia have been recently included in the kingdom of Fungi, Microsporidiosis will be dealt with in this section.

19.3.1 Microsporidiosis

Microsporidia are intracellular spore-forming organisms currently ascribed to the kingdom Fungi. Among them, several species have been described in fish, with different localizations and pathogenicity. Fish microsporidia have a direct life cycle and are commonly transmitted through ingestion of spores shed by lesions or by decaying carcasses of infected fish; for some species also vertical transmission has been indicated. Generally they show a high host-specificity, but some species can infect a wide range of hosts (e.g. *Glugea anomala*). Some fish microsporidia cause a peculiar hypertrophic reaction in the infected cell forming a so called "xenoma" (or xenoparasitic complex) which sometimes becomes macroscopically detectable. Concerning marine ornamental fish, the most important Microsporidiosis has been reported in seahorses. In fact infections due to the microsporidian *Glugea heraldi* have been described as a cause of severe disease in *Hippocampus erectus*, first by Blasiola (1979) then by other authors (Vincent & Clifton-Hadley, 1989; Bombardini *et al*., 2006). *G. heraldi* infects the subcutaneous connective tissues of seahorses, to which it seems to be specific, producing xenomas ranging from 100 to 800 μm in diameter that resemble boil-like lesions visible to the naked eye. In some cases very high mortality has been associated with infections of *G. heraldi* in seahorses (Vincent & Clifton-Hadley, 1989). No effective treatments are known, although fumagillin *per os* and toltrazuril bath have shown some effects against microsporidia in fish (Kent *et al*., 2014). Anyway it should be taken into consideration that toltrazuril effectiveness seems to be markedly lower in seawater (Iglesias *et al*., 2002).

19.3.2 Exophialosis

Exophiala spp. (Ascomycetes, Chaetothyriales) are ubiquitous melanized fungi known as "black yeasts" for the presence of melanin in the cell wall. The genus was first described in cutthroat trout (*Salmo clarkii*) in 1966 (Carmichael, 1966). In recent years *Exophiala* infections have been reported in a wide variety of freshwater and marine fish including elasmobranchs, sometimes with high mortality. Gaskins & Cheung (1986) found *Exophiala pisciphila* in brain and skin lesions from a smooth dogfish (*Mustelis canis*) housed in the New York Aquarium. The same species was also found in a juvenile zebra shark (*Stegostoma fasciatum*) with necrosis of liver, spleen and gills in association with the zygomycete fungus *Mucor circinelloides* (Marancik *et al*., 2011). Furthermore, Reuter *et al*. (2003) reported in King George whiting (*Sillaginodes puntuta*) an epidemic due to an unidentified *Exophiala* species. *E. angulospora* has been observed to cause mycosis in aquarium-maintained weedy sea dragon (*Phyllopteryx taeniolatus*) and leafy sea dragon (*Phycodurus eques*) (Nyaoke *et al*., 2009). Infected seadragons showed renal and vascular necrosis with fungal invasion of gill, kidney, and other internal organs with or without the presence of skin ulcers. More recently, a novel species of *Exophiala*, now described as *E. aquamarina*, has been isolated from the leafy seadragon (*P. eques*) showing systemic necrotizing lesions and invasion of blood vessels (Nyaoke *et al*., 2009; de Hoog *et al*., 2011). Clinical signs of infection with *Exophiala* spp. vary based on the species affected and on species of pathogen. Infected weedy and leafy sea dragons showed weakness, loss of appetite, lethargy, increased respiratory effort and rate, abnormal buoyancy, listing, piping at the surface of the water and death (Nyaoke *et al*., 2009). The clinical course lasted from 1 week to 6 months before death. The portal of entry of fungi was presumed to be *via* gill or skin by traumatic injuries with subsequent hematogenous dissemination to visceral organs or also by direct ingestion of infected food (Nyaoke *et al*., 2009). Diagnosis of *Exophiala* can be made by cytological examination of affected tissues or biopsy. At post mortem examination, the presence of olive to black brown fungal hyphae is indicative. Histopathology of lesions shows the presence of "annelated" zones with fungal hyphae. Isolation of *Exophiala* spp. into Sabouraud agar needs 1–3 weeks of incubation at 30–33 °C; the colonies will appear as velvety, olivaceous or black brown mold. Identification to species level of isolates should be based on molecular analysis. No treatment of *Exophiala* in fish is available, so prevention based on avoiding injuries and stress, and ensuring optimal hygienic conditions should always be applied.

The potential pathogenic role of some piscine *Exophiala* species to man should not be underestimated. In fact Yoon *et al*. (2012) recently isolated *E. salmonis* from a subcutaneous cystic mass in the ankle of an immunocompromised patient.

19.3.3 Fusariomycosis

Fungi belonging to *Fusarium* genus are described as saprophytes of soil and water, and in recent years have been reported to be opportunistic pathogens in humans and animals, including fish (Smith *et al*., 1989; Crow *et al*., 1995; Yanong, 2003). In particular one species, *Fusarium solani*, has been reported as the causative agent of diseases in freshwater and marine fish and in many animals maintained in aquaria (Ostland *et al*., 1987; Yanong, 2003). *F. solani* is considered an important pathogen of juvenile sharks and is thought to be an opportunistic organism severely infecting debilitated hosts

(Terrel, 2004). The disease has been reported in bonnethead (*Sphyrna tiburo*) and scalloped hammerhead sharks (*S. lewini*) maintained in public aquaria. The animals showed skin lesions, myositis, muscle necrosis and hyphal penetration into cartilages or granulomatous exudative mycotic dermatitis of the lateral line canal system (Muhvich *et al*., 1989; Smith *et al*., 1989; Crow *et al*., 1995). Recently a case of Fusariomycosis has been observed in a baby spotted dogfish (*Scyliorhinus canicula*) with severe cardiac mycosis (Florio, unpublished data). Yanong (2003) found these fungi in many species of ornamental fish such as angelfish (*Pomachanthus paru*, *P. arcuatus*, *Holocanthus bermudensis* and *H. ciliaris*) and Parrotfish (*Scarus coelestinus* and *S. guacamaia*).

Generally Fusariomycosis is associated with relatively low water temperatures; in fact increasing temperature (in the range of 15–35 °C) may decrease the growth rate and the pathogenic potential of these fungi (Palmero Llamas *et al*., 2008).

19.4 Bacterial Diseases

With regard to bacterial diseases, the main problems in the aquarium are represented by infections sustained by opportunistic pathogens.

Among these, several Gram-negative bacteria, ubiquitous in the aquatic environment (e.g. *Vibrio* spp., *Flavobacteriaceae*, etc.) are the cause of serious superficial and/or systemic diseases in the presence of predisposing conditions. Some Gram-positive bacteria (i.e. *Streptococcaceae*), have also been described as causal agents of disease and heavy losses in both wild and captive marine fish populations.

Furthermore fish mycobacterioses, caused by atypical mycobacteria such as *Mycobacterium marinum*, *M. fortuitum* and *M. chelonae*, are also frequently observed in fish kept in controlled environments where the optimal conditions for the replication of mycobacteria are found. This can lead to difficult to treat serious chronic systemic granulomatous diseases. As agents of minor zoonoses, their presence in the tank population should always be subject to prompt and appropriate control measures.

Moreover, bacterial infections can cause mortality in captive tropical marine fish eggs, whose mucosal surfaces provide a good substrate for the adhesion and colonization of bacteria. Nelson & Ghiorse (1999) found *Pseudoalteromonas piscicida* associated with high mortality in damselfish (*Amphiprion clarkii* and *Amblyglyphidodon curacao*) eggs.

19.4.1 Vibriosis

Species in the genus *Vibrio* (family *Vibrionaceae*) are responsible for serious disease and mortality of cultured marine finfish worldwide including ornamental fish (Thompson *et al*., 2004). These Gram-negative bacteria are normally found in the marine environment. Disease outbreaks occur when fish are exposed to these infectious agents in the presence of stress factors (Austin & Austin, 2007). Some of the common symptoms of diseases in fish caused by strains of pathogenic *Vibrio* include dark skin, exophthalmia, pale gills, hemorrhages on skin and at the base of fins, corneal opacity, skin ulcers, ascitic fluid in peritoneal cavity, splenomegaly and enteritis (Toranzo *et al*., 2005). Major predisposing risk factors for outbreak of most of Vibrioses are high temperature, crowding, organic pollution and other stressors. Among the *Vibrio* species that have

been isolated from marine ornamental fish during disease outbreaks *V. harveyi* (syn. *V. carchariae*) is considered one of the most important. It has been repeatedly described as a cause of meningitis in sandbar shark (*Carcharhinus plumbeus*), lemon sharks (*Negraprion brevirostris*), sand tiger (*Carcharias taurus*), spiny dogfish *Squalus acanthias* (Grimes *et al*., 1984; Stoskopf, 1993) and has often been isolated in association with chronic skin ulcers (Bertone *et al*., 1996). The monogenean *Dermophthirius nigrelli* has been indicated as a possible vector of *V. harveyi* infection from shark to shark (Grimes *et al*., 1984). Infected sharks show lethargy, anorexia, abnormal swimming and disorientation. As the infection progresses, convulsions, coma and death may occur. In some cases, the renal tissue is compromised. Among marine ornamental teleosts, Syngnathids are very susceptible to infections by *V. harveyi* (Alcaide *et al*., 2001; Tendencia, 2004; Raj *et al*., 2010). Alcaide *et al*. (2001) reported an outbreak of Vibriosis due to *V. harveyi* in *Hippocampus kuda* with mortalities >90%. The fish showed pale skin with hemorrhages, depressed abdomen, hemorrhagic liver, pale kidney and fluid in the intestine. Isolation of *V. harveyi* can be performed from cerebrospinal fluid, liver, spleen, kidney and skin lesions when present.

Other *Vibrio* such as *V. alginolyticus*, *V. anguillarum*, *V. fluvialis*, *V. pelagius* and *V. vulnificus* have been isolated from external lesions and deep ulcers during episodes of mortality reported in blue damselfish *Pomacentrus caeruleus*, often in association with other issues (Sonia & Lipton, 2012). *V. alginolyticus* and *V. splendidus* have been isolated from *Hippocampus guttulatus* and *H. hippocampus* with disease symptoms such as lethargy, lack of appetite, white spots on the skin and necrotic tail lesions (Balcázar *et al*., 2010). Martins *et al*. (2010) isolated *V. alginolyticus* from *H. reidi* during a disease outbreak and demonstrated its pathogenicity in experimentally infected seahorses that displayed lesions on the mouth, gills, liver and kidney and high mortality.

Other bacteria belonging to the family *Vibrionaceae* such as *Photobacterium* spp. were found in the course of fish disease. *Photobacterium damselae* subsp. *piscicida* (formerly *Pasteurella piscicida*) and *Ph. damselae* subsp. *damselae* (formerly *Vibrio damselae*) are frequently isolated from marine fish. The latter was initially isolated from damselfish, *Chromis punctipinnis* (Love *et al*., 1981) and is considered a pathogen of several ornamental marine species including sharks (*Carcharhinus plumbeus* and *Stegostoma fasciatum*) (Grimes *et al*., 1984; Han *et al*., 2009), and an emerging pathogen in marine aquaculture (Labella *et al*., 2011). Infected fish show hemorrhages on the body surface and ulcerative skin lesions. In damselfish, ulcers typically occur in the region of the pectoral fin and caudal peduncle (Love *et al*., 1981). Stress conditions, crowding and poor water quality may enhance the emergence of disease. Furthermore *Ph. damselae* subsp. *damselae* has been isolated in the course of necrotizing fasciitis and fatal infections in humans, so its zoonotic potential should not be underestimated (Rivas *et al*., 2013).

19.4.2 Tenacibaculosis

Tenacibaculosis is a serious bacterial disease affecting a great variety of cultured and wild marine fishes worldwide. The genus *Tenacibaculum* (Suzuki *et al*., 2001) belongs to the family *Flavobacteriaceae* (Reichenbach, 1992; Bernardet *et al*., 1996) and currently comprises 16 species of filamentous Gram-negative bacteria all isolated from marine environment. *T. maritimum* (previously *Flexibacter maritimus*) is a notorious

pathogen in the aquaculture industry and ornamental fish sector (van Gelderen *et al*., 2010). El-Galil & Hashem (2012) isolated *T. maritimum* from diseased black damsel (*Neoglyphidodon melas*), Picasso trigger (*Rhinecanthus assasi*) and broomtail wrasse (*Cheilinus lunulatus*). This microorganism is an opportunistic pathogen which can cause extensive skin and gill lesions and can rapidly colonise other tissues. The disease affects both adult and young fish, but is most severe in juveniles. The infection has been associated with characteristic gross lesions on the body surface of fish, such as ulcerative skin lesions, necrosis of skin, fin and gill, eroded mouth, frayed fins and tail rot (Avendaño-Herrera *et al*., 2006). The disease is influenced by a multiplicity of environmental (e.g., temperature above 15 °C and salinities between 30 to 35 ppt), management and host-related factors. Recently Declercq *et al*. (2014) reported *T. aestuarii* in *Hippocampus kuda* affected by *Uronema*-like parasites. The animals showed whitish patches on the distal body portion and a necrotic tail tip. The isolation of these bacteria is difficult and the most appropriate media is *Flexibacter maritimus* medium (FMM) (Pazos *et al*., 1996). The colonies grow after 48 to 72 h of incubation at 20 to 25 °C and appear flat, pale-yellow with uneven edges and strongly adherent to the medium.

19.4.3 Streptococcosis

Streptococcosis is a generic term used to indicate a complex of septicemic diseases caused by Gram-positive bacteria belonging to the family *Streptococcaceae* that affect freshwater and marine fish worldwide causing significant morbidity and mortality. Different genera are implicated as etiological agents of fish streptococcosis: *Streptococcus*, *Lactococcus* and *Vagococcus*. Some species belonging to the first two genera (e.g. *Streptococcus iniae*, *Lactococcus garvieae*) are able to cause infections in both cultured freshwater and marine fish at temperatures above 15 °C (warm water Streptococcosis) while other species (e.g. *Vagococcus salmoninarum* and *L. piscium*) are pathogenic exclusively to salmonid fish at temperatures below 15 °C (cold water Streptococcosis) (Eldar & Ghittino, 1999).

Streptococcus iniae was first isolated from the skin lesions of a captive Amazon River dolphin *Inia geoffrensis* (Pier & Madin, 1976), and since then has been reported in many species of fresh, estuarine and marine fish species, including ornamental fish, from many countries worldwide (Russo *et al*., 2006). *S. iniae* was found to be involved in two (1999 and 2008) massive fish mortalities of Caribbean reef fishes (Ferguson *et al*., 2000; Keirstead *et al*., 2014) and has been isolated from wild marine fish during a mortality outbreak in the Maldives in December 2007 (Fioravanti *et al*., 2008). It causes an acute bacteraemia and/or meningoencephalitis, panophthalmitis, pericarditis and can also infect other organs often resulting in high levels of morbidity and mortality (Agnew & Barnes, 2007; Keirstead *et al*., 2014). Transmission is horizontal (Evans *et al*., 2001). Clinical signs and mortality are often associated with an increase in water temperature and poor water quality, especially increased levels of ammonia, nitrate and nitrite, and low dissolved oxygen <4 mg/L (Agnew & Barnes, 2007). Ferguson *et al*. (2000) discussed several factors that could have influenced the occurrence and infection of reef fish by *S. iniae*. Among these, the fact that the coral reef provides means for concentrating and therefore leading to predisposing conditions for exposure of pathogens to fish, or a particularly virulent strain of *S. iniae*. The combination with other pathogens, like viruses or parasites, may also be important in the pathogenesis of

disease, especially in marine systems, but this is still poorly understood. Also the role of potential reservoir hosts in fish populations has to be clarified and needs further research. The zoonotic potential of *S. iniae* should not be underestimated, since it is able to cause disease in humans (Agnew & Barnes, 2007).

Among the bacterial species causing warm water streptococcosis, *L. garvieae* is an important pathogen of marine and freshwater fish worldwide. It can infect a wide range of fish species and has been also recorded from wild *Coris aygula* in the Red Sea (Colorni *et al*., 2003); the fish showed hemorrhaging in the kidney, spleen and liver but no other pathological lesions were visible.

19.4.4 Mycobacteriosis

Fish mycobacterioses are chronic progressive diseases characterized by systemic granulomatosis, leading to cachexia and unfavorable prognosis, which can be caused by several species of atypical mycobacteria such as *Mycobacterium marinum*, *M. fortuitum* and *M. chelonae* (Decostere *et al*., 2004). During the last decade or so, several surveys on diffusion and pathogenic effects of mycobacteria in ornamental fish have been carried out as a consequence of increasing interest in the aquarium fish industry, their zoonotic potential, and advancements in molecular diagnosis (Prearo *et al*., 2004; Lai *et al*., 2010).

Atypical mycobacteria are common in aquaria and the conditions of the aquarium itself, such as water recirculation, presence of organic material, overcrowding, etc., can favor their spread and trigger clinical diseases, mainly when the fish host is immunocompromised, a common condition in captivity. Giavenni *et al*. (1980) documented severe cases of *M. marinum* infection in 17 different genera of marine tropical aquarium fish, observing that Pomacentrids are especially susceptible to mycobacterial infections.

A survey carried out on batches of freshwater and marine ornamental fish imported into Italy (Zanoni *et al*., 2008) showed a significant prevalence of mycobacteria infections. *Mycobacterium* spp. were found in 29.9% of the 127 examined batches, although clinical signs and lesions related to mycobacteriosis were present in very few fish. In particular, 8 (25%) of the 32 batches of marine fish were found positive, but no fish showed symptoms due to mycobacteriosis. Surprisingly, several species of mycobacteria were isolated (e.g., *M. abscessus*, *M. chelonae*, *M. fortuitum* and *M. peregrinum*), but no *M. marinum*.

The disease is a particular problem for Syngnathidae housed in aquaria (Bombardini *et al*., 2006; Francis-Floyd & Petty, 2010), in which systemic granulomatosis can develop. Recently the new species *M. hippocampi* has been described in *Hippocampus guttulatus* and *H. hippocampus* showing lethargy, lack of appetite, white spots on the skin and necrosis of the tail (Balcázar *et al*., 2011, 2014). Also *Mycobacterium avium* has been isolated from granulomas in an epaulette shark, *Hemiscyllium ocellatum* (Janse & Kik, 2012) and *M. chelonae* was detected in splenic lesions of Atlantic guitarfish *Rhinobatos lentiginosus* (Anderson *et al*., 2012) and in a calvarial abscess in yellow stingray *Urobatis jamaicensis* (Clarke *et al*., 2013) kept in public aquariums.

The oral route of transmission, including ingestion of contaminated food (mysid shrimps) or dead fish, is very important in the epidemiology of mycobacteriosis (Jacobs *et al*., 2009) and freezing does not kill bacteria (Ross & Brancato, 1959). Harriff *et al*. (2007) demonstrated experimentally that the transmission of *M. marinum* and *M. peregrinum* occurred via the gut in zebrafish (*Danio rerio*). Furthermore water may act as

an important mycobacterial source for transmission to both fish and humans, in fact water and biofilms are natural habitats of atypical mycobacteria. Vertical transmission of mycobacteria has been suggested by Ashburner (1977) and transovarian transmission has been demonstrated only in the viviparous Mexican platyfish *Platypoecilus maculatus* (Conroy, 1966) and in *Poecilia reticulata* (Conroy & Conroy, 1999). Other possible routes of mycobacterial infection are through injured skin or through vector organisms such as protozoa and amoebae (Jacobs *et al*., 2009).

Clinical signs of mycobacteriosis are not specific to the disease and can vary in occurrence and severity. Affected fish may lose their appetite, appear debilitated and emaciated and become more susceptible to infections by other opportunistic bacteria. They may also be listless and lethargic or may separate from other fish to become solitary in the corner of the holding facility (Austin & Austin, 2007). Some fish show dermal lesions such as fin erosion and skin ulcerations, scale loss accompanied by hemorrhagic lesions penetrating the musculature in advanced cases, pigmentary changes, unilateral or bilateral exophthalmos and in some cases skeletal deformities (Colorni *et al*., 1998; Rhodes *et al*., 2001; Jacobs *et al*., 2009). Sometimes affected fish may delay sexual maturation and show problems with releasing eggs, but in many cases no external signs are detected (Jacobs *et al*., 2009).

Gross lesions in internal organs typically include grayish to white granulomatous nodules (1–4 mm) mainly in the spleen, liver and kidney (Figure 19.4 e). Sometimes atypical manifestations have been reported in acute and subacute mycobacteriosis of sygnathids and stingrays, with the presence of nodule-like abscesses within the skin and subcutaneous tissues (Bombardini *et al*., 2006; Clarke *et al*., 2013). Enlarged organs, peritonitis and edema may be present. Microscopically the granulomas are characterized by a central area of necrosis surrounded by macrophages, epithelioid cells and fibrous connective tissue (Diamant *et al*., 2000).

Presumptive diagnosis is usually based on symptoms and gross lesions; a careful microscopic observation of tissue smears stained with Ziehl–Neelsen method is recommended for detecting the presence of acid fast bacteria (Zanoni *et al*., 2008). Histology and immunohistochemistry can be used to support a diagnosis of mycobacteriosis (Sarli *et al*., 2005). Because culturing these organisms is quite difficult, needing special media and many days for isolation and characterization, several authors have described molecular techniques that allow rapid diagnosis and identification of organisms to species level (Jacobs *et al*., 2009). Atypical mycobacteria are potentially zoonotic and can cause in humans nodular cutaneous lesions characterized mainly by granulomas on the finger, hands and sometime nodular lymphangitis that can progress to tenosynovitis, arthritis and osteomyelitis especially in immunosuppressed individuals (Passantino *et al*., 2008).

19.4.5 Control of Bacterial Diseases

Control of bacterial diseases should not include only the treatment of infected fish with antimicrobials but also the resolution of all the factors which may have contributed to the outbreak of disease. In fact, an interaction between the pathogen, the host and the environment almost always concurs to trigger the clinical manifestation of a bacterial infection. Fish which are constantly subjected to stressful conditions, as typically happens in confined environments, have impaired defense mechanisms due to

immunosuppression and more easily develop bacterial diseases in presence of pathogenic and/or opportunistic microorganisms. For this reason the resolution of stressful factors through optimal husbandry practices has to be considered fundamental in controlling disease, as well as the administration of the correct antibiotic therapy. Furthermore, when a bacterial disease is suspected, etiological diagnosis should always be made and antimicrobial sensitivity test conducted in order to identify the antibiotics which are effective against that specific pathogen isolated in the course of the disease.

In the specific case of mycobacteria infections, treatment with antimicrobials may slow the progress of disease but will not cure infected animals and will not completely eliminate infection (Santacana *et al*., 1982; Lawhavinit *et al*., 1988; Conroy & Conroy, 1999; Noga, 2010). Moreover, vaccines are still in the experimental stage despite years of intensive research (Pasnik & Smith, 2005; Cui *et al*., 2010). For these reasons strategic measures aimed at eradicating the disease from the tank should always be adopted, with the understanding that several fish mycobacteria are potentially pathogenic to humans. Complete information on antimicrobial therapy in fish is available in Noga (2010).

19.5 Viral Diseases

Several viral diseases have been reported in marine ornamental fish. Most important etiological agents are included in *Nodaviridae* and *Iridoviridae* families. The related diseases will be presented hereafter, together with a brief description of the Damselfish neurofibromatosis due to an unclassified virus.

19.5.1 Viral Encephalopathy and Retinopathy (VER)

Viral encephalopathy and retinopathy (VER) or viral nervous necrosis (VNN) is a worldwide disease caused by *Betanodavirus*, family *Nodaviridae*, described in more than 50 fish species, mainly marine and eurhyaline.

Piscine nodaviruses have been classified into four major genogroups: striped jack nervous necrosis virus (SJNNV), tiger puffer nervous necrosis virus (TPNNV), barfin flounder nervous necrosis virus (BFNNV) and red-spotted grouper nervous necrosis virus (RGNNV) (Nishizawa *et al*., 1997; Dalla Valle *et al*., 2001). VER is most severe in larval or juvenile stages, but adult fish may also be affected in some species (Bovo & Florio, 2008). Furthermore the disease can be influenced by environmental factors such as water temperature (depending on genogroup, in fact RGNNV occurs at 25–30 °C, SJNNV at 20–25 °C, TPNNV at 20 °C and BFNNV at 15–20 °C), poor feeding, sub-optimal water quality, crowding and transport (Binesh *et al*., 2013). Transmission is horizontal although also vertical transmission has been demonstrated for some genogroups (Manin & Ransangan, 2011). VER can be spread from infected fish, invertebrates, water, live or frozen foods. Chi *et al*. (2000) have detected VER in live food organisms including crustacean nauplii such as *Artemia* sp., the copepod *Tigriopus japonicus* and the shrimp *Acetesinte medius*.

The affected fish show abnormal swimming behaviour, including vertical positioning and spinning motions in all directions, with body flexion and muscular tremors also being observed. Other signs include changes in skin pigmentation (darkening or paling), traumatic lesions caused by erratic swimming, exophthalmia, hyperinflation of the

swim bladder and moderate to high mortalities (Bovo & Florio, 2008). Moribund larvae of *Amphiprion sebae* showed darkening of the body, uncoordinated corkscrew-like swimming, hypersensitivity to stimuli, and assembly into large groups (Binesh *et al*., 2013).

VER has been detected in several marine ornamental fish including convict surgeonfish (*Acanthurus triostegus*), lined surgeonfish (*A. lineatus*), threespot dascyllus (*Dascyllus trimaculatus*), scopas tang (*Zebrasoma scopas*), blueband goby (*Valenciennea strigata*), tiger puffer (*Takifugu rubripes*), lemonpeel angelfish (*Centropyge flavissimus*), and several marine invertebrate (Yanong, 2010). In a Tahiti hatchery VER was found in cultured juveniles from wild-caught surgeon *Acanthurus triostegus*, the apogonid *Apogon exostgma* and the bat fish *Platax orbicularis* (Thieri *et al*., 2004; David *et al*., 2010). Furthermore, Gómez *et al*. (2006) found positivity (4.64%) at nested PCR in apparently healthy marine aquarium fish such as shrimp fish (*Aeoliscus strigatus*), milkfish (*Chanos chanos*), spot damsel (*Dascyllus trimaculatus*), Japanese anchovy (*Engraulis japonicus*), pinecone fish (*Monocentris japonica*), blue ribbon eel (*Rhinomuraena quaesita*), lookdown fish (*Selene vomer*) and the yellow tang (*Zebrasoma flavesenes*). Binesh *et al*. (2013) reported high mortality (80%) in 20–23 days old larvae of clownfish (*Amphiprion sebae*) affected by VER.

The genogroup RGNNV has been detected in larvae of *Amphiprion sebae* and in *Platax orbicularis* (David *et al*., 2010; Binesh *et al*., 2013).

Appropriate diagnostic tests include histopathology (most distinctive finding is the presence of vacuolation and necrosis in the brain, spinal cord and retina), virus isolation, immunological and molecular (RT-PCR) methods.

There is no treatment available for VER; low fish densities and reduction of stressful conditions can decrease the incidence of the disease. Disinfection of tanks between uses (by employing sodium hypochlorite, benzalkonium chloride or iodine) and appropriate hygiene and quarantine protocols are also recommended.

19.5.2 Infections Due to *Iridoviridae*

During the last two decades, viruses in the family *Iridoviridae* have been studied as causative agents of serious diseases in aquaculture and in the ornamental fish industry (Paperna *et al*., 2001; Jeong *et al*., 2008). Within the *Iridoviridae* family, the genera *Ranavirus*, *Lymphocystivirus* and *Megalocytivirus* include taxa infecting fish (Chinchar *et al*., 2005). Diseases due to the last two genera have been more frequently described in marine ornamental fish.

19.5.3 Lymphocystis

Lymphocystis is a viral disease described in over 140 species of freshwater, eurhyaline and marine fish (Pontejo *et al*., 2013). It is caused by *Lymphocystivirus*, family *Iridoviridae*, which infects fibroblasts causing strong hypertrophy with formation of greyish/pearl-like nodules on skin and fins of the affected fish (Figure 19.4f). Hypertrophied cells have also been detected in the oral cavity, gastrointestinal tract, ovaries, spleen and heart (Schäperclaus, 1992).

Clinical course is generally benign, although in some cases lesions on the mouth rims may interfere with feeding. Some marine species seem to show a higher susceptibility to *Lymphocystivirus* (e.g., Pomacanthidae and Pomacentridae) (Francis-Floyd & Petty,

2010; Pirarat *et al*., 2011). Red drum (*Sciaenops ocellatus*) juveniles may develop infections in internal organs, with more pathogenic effects (Colorni & Diamant, 1995).

The main transmission route of *Lymphocystivirus* is horizontal by direct contact with infected animals or through contaminated water and virus adsorption through gills and skin, especially in the presence of external trauma. *Artemia* sp. has been demonstrated as a possible vector of *Lymphocystivirus* (Cano *et al*., 2009). Stressful conditions such as high stocking density, water pollution, nutrition deficiencies, low dissolved oxygen, etc., can exacerbate the clinical manifestation of the disease (Cano *et al*., 2007). Incubation time of Lymphocystis depends on temperature: 6 weeks of incubation at 10–15 °C and 5–12 days at 20–25 °C (Plumb, 1999). *Lymphocystivirus* is the only virus in fish that can be diagnosed with microscopic examination of skin or fin scrapings and histopathology, although molecular methods have also been developed (Cano *et al*., 2007). So far, no effective therapy or vaccine is available, but stress reduction and good water quality may minimize the impact of the disease and facilitate the resolution of the lesions.

19.5.4 Megalocytivirus

Megalocytivirus is an Iridovirid genus that has emerged in the recent decades as a pathogen of freshwater and marine finfish. Members of this genus have been associated with serious systemic infections in a wide variety of fishes, and have had a negative impact on ornamental and food fish aquaculture worldwide (Weber *et al*., 2009; Yanong & Waltzek, 2010). Phylogenetic analyses support four separate species within the genus *Megalocytivirus* (Kurita & Nakajima, 2012; Waltzek *et al*., 2012), but most of the infections described in marine ornamental fish have been from; the infectious spleen and kidney necrosis virus (ISKNV). In particular the Banggai cardinalfish iridovirus (BCIV) and the orbiculate batfish iridovirus (OBIV) have been reported to cause disease respectively in *Pterapogon kauderni* and *Platax orbicularis* (Weber *et al*., 2009; Sriwanayos *et al*., 2013). *Megalocytiviruses* induce lethal systemic disease at water temperatures ranging from 20 to 32 °C and in presence of environmental stressors (Yanong & Waltzek, 2010, Waltzek *et al*., 2012). These viruses can spread horizontally from fish to fish directly or through the ingestion of infected food (Yanong & Waltzek, 2010). Fish infected with any *Megalocytivirus* often die without premonitory clinical signs or apparent gross lesions, or in some cases showing no specific symptoms such as lethargy, anorexia, darkening, exophthalmos, abnormal swimming, increased respiration, fin erosion, skin ulceration, haemorrhages in gills, coelomic distension, white faeces and heavy mortalities (up to 100%) (Weber *et al*., 2009; Waltzek *et al*., 2012). On post-mortem examination the spleen, kidney and liver show pathological alterations, and hemorrhagic fluid in body cavity is also present. Other organs and tissues, including muscles, gonads, heart, gills, and the gastrointestinal tract, may also be affected (Weber *et al*., 2009; Yanong & Waltzek, 2010; Waltzek *et al*., 2012). Histopathologically, *Megalocytivirus*-associated disease is characterized by the presence of cytomegalic cells, which contain large basophilic intracytoplasmic inclusions, in many organs (especially kidney, spleen, liver, gastrointestinal tract, gill and connective tissue) (Yanong & Waltzek, 2010; Waltzek *et al*., 2012). Presumptive diagnosis of *Megalocytivirus* infection can be based on clinical signs, post-mortem and histopathological findings. A definitive diagnosis can be reached through viral isolation, electronic microscopy or PCR testing (Chinchar *et al*., 2005). There is no known treatment for

Megalocytivirus infection. The disease should be prevented through good husbandry practices and biosecurity protocols.

19.5.5 Damselfish Neurofibromatosis

Damselfish neurofibromatosis (DNF) is a transmissible neoplastic progressive and fatal disease that has been documented in adult bicolor damselfish (*Stegastes partitus*) from coral reefs in South Florida with an occurrence of up to 23% on some reefs (Schmale, 1991). Affected fish develop neurofibromas, neurofibrosarcomas and chromatophoromas. Initially related to a retroviral etiology (Schmale *et al*., 1996), more recently DNF has been associated with an extrachromosomal DNA virus-like agent, named the *damselfish virus-like agent* (DVLA) (Schmale *et al*., 2002; Rahn *et al*., 2004; Coffee *et al*., 2013).

19.6 Conclusions

Welfare and health of marine ornamental fish can be constrained by several parasitic and infectious agents that find in the aquarium favorable conditions for disease outbreaks. Improving the knowledge of fish management, including diet and environmental and ecological requirements, at all stages of the marine ornamental trade is paramount for preventing diseases. Along with this, biosecurity measures such as health certification, quarantine and routine health management practices should always be applied to avoid the introduction, movement and spread of pathogens. To achieve this, collaboration among all the professionals involved in the marine ornamental fish trade, at both the private and public levels, should be strengthened and ensured. In particular, strong cooperation between farmers, wholesalers, retailers, aquarists and other professionals involved in the trade with veterinarians and diagnostic centers is crucial to better understand the etiopathogenesis and epidemiology of diseases and for better identification and development of appropriate control strategies. In this respect, future research aimed at finding appropriate treatments against pathogens of marine ornamental fish, generally kept in complex and fragile aquarium systems, should be focused on natural and innovative molecules that could replace chemicals that are harmful or destructive to many other living organisms usually hosted in the aquarium (invertebrates, biological filter, etc.).

Transfer of knowledge, together with education and training on fish health management and public health aspects of aquarium keeping, will have to be pursued at all the levels of the marine ornamental fish industry to ensure their welfare, health and safety.

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Part IV

Marine Ornamental Invertebrates Aquaculture

At present marine aquariums are no longer displays of colorful reef fishes dwelling among dead coral skeletons. Instead, with the advent of new technological solutions for water filtration and illumination, as well as a better understanding of coral reefs ecology, reef fishes are now stocked in miniaturized versions of this complex ecosystem – the modern coral reef aquarium (Nilsen & Fossa, 2002; Vargas, 2011). Natural reefscapes are created through the use of porous coral rocks (popularly termed as live rock), coral sand or gravel, macroalgae and seaweeds and a plethora of micro and macro-invertebrate organisms. Macro-invertebrates that are stocked deliberately in reef aquariums are commonly termed as marine ornamental invertebrates and include a remarkable range of distinct taxa (Wabnitz *et al*., 2003). The use of live rock is now a common practice in reef aquarium displays, as these limestones ensure a perfect recreation of reefscapes and harbor an abundance of microorganisms able to nitrify and denitrify nitrogenous compounds. Additionally, live rock is commonly colonized by sponges, small corals, polychaetes, mollusks, crustaceans and echinoderms, thus contributing to enhance the fauna of reef aquariums. A drawback of this feature is the fact that live rock may also act as a vector to the introduction of pest invertebrates (e.g., glass anemones *Aiptasia*) and macroalgae (e.g., bubble algae *Ventricaria*). Environmental awareness (at times unfounded) associated with the collection of live rock from the reef has prompted research on the production of artificial live rock (Chapter 20). The most popular and pricey of all marine ornamental invertebrates are certainly symbiotic photosynthetic corals, either stony reef building species (known as hard corals) or those without a calcareous (calcium carbonate) skeleton (known as soft corals). Both stony and soft symbiotic corals are now propagated worldwide, either *in situ* or *ex situ*, using sexual and asexual propagation (the latest being more commonly employed through the fragmentation of mother colonies) (Subchapter 21.1). The display of asymbiotic corals has also gained popularity in recent years among hobbyists and public aquariums, although their husbandry and propagation is considerably more challenging than that of symbiotic coral species. Anemones are also popular among hobbyists, being considered a "must have" when displaying clown fish. The initial steps in the propagation of sea anemones have already been taken, as the natural populations of some highly popular species seem to be unable to cope with current collection pressure (Subchapter 21.2). Jellyfish were once exclusively displayed in public aquariums. However, the improvement of husbandry techniques and the design of customized tanks now also make

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the culture and display of these remarkable organisms an option for hobbyists (Subchapter 21.3). Decapod crustaceans are also long time favorites of marine aquarium hobbyists, namely shrimp displaying fish cleaning behaviour and small crabs and hermit crabs performing "janitor" tasks in the reef display (e.g., eating unwanted algae and food leftovers). Several breakthroughs have been achieved in the last decade, but the supply of cultured specimens is still scarce compared to the volume of wild specimens that is currently traded (Subchapters 22.1 and 22.2). Mollusks are also commonly displayed in reef tanks, the most emblematic certainly being giant clams with their dazzling colored mantles hosting their photosynthetic endosymbionts. This group of mollusks is somehow unique in the marine aquarium landscape, as the majority of traded specimens is currently cultured (a consequence of the remarkable effort undertaken to save these unique mollusks from extinction due to their over-harvest for human consumption) (Subchapter 23.1). A multitude of snails and sacoglossan sea slugs is commonly used to keep the growth of unwanted algae under control, while beautifully colored nudibranchs are used to fight pest invertebrates (e.g., glass anemones of genus *Aiptasia*). Some of these species are already cultured in captivity, but the supply to the marine aquarium trade is still heavily dependent on the collection of wild specimens. The display of cephalopod mollusks is commonly reserved to public aquariums, with considerable breakthroughs currently allowing researchers to close the lifecycle of several species displayed (Subchapter 23.2). Colorful feather duster-like polychaetes are also commonly displayed in modern reef tanks and recent advances in their sexual and asexual propagation opens good prospects for the large scale production of these popular marine ornamentals in the near future (Chapter 24). Several species of echinoderms, with emphasis on algae grazing sea urchins are also traded. Macroalgae, seaweeds and even mangroves are also stocked in display or support tanks, either for their aesthetic value or for their ability to use excess nutrients present in the display system. The majority of macroalgae employed in the marine aquarium hobby can be easily propagated, with seaweeds and mangroves commonly being harvested from the wild (Chapter 25). Overall, significant steps have been undertaken towards an increase in the supply of cultured marine ornamental invertebrates (Olivotto *et al.*, 2011). Nonetheless, the remarkable diversity of marine ornamental species available in the hobby (Calfo & Fenner, 2003; Shimek, 2005), the lack of knowledge on the feeding behaviour of early life stages and a range of technical bottlenecks, require a new wave of research efforts similar to that recorded in the late 1990s and early 2000s. Only by engaging an effective collaboration between academics, commercial breeders, public aquariums and hobbyists will it be possible to increase the number of available species and specimens of cultured marine ornamental invertebrates in the aquarium industry.

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20

Live Rock

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Abstract

Live rock is widely used in marine aquariums and aquaculture systems all over the world for filtering and aesthetic purposes. The present chapter provides a general introduction and overview of the general types and classifications of live rock available in the market, as well as their prices and characteristics. A compilation and synthesis of the most widely used "recipes" to make your own artificial live rock, and review of the financial aspects related to the aquaculture of live artificial rock are provided. Scientific bibliographic information is reviewed and new data is added that confirm many long-assumed but seldom demonstrated roles of live rock in contained seawater systems. The pros and cons of the use of live rock are identified and briefly discussed. The chapter ends with a proposal of future directions and recommendations for the aquaculture of live rock.

Keywords *Aquascaping; base-rock; biofiltering*

20.1 Introduction

The term "live rock", generally speaking, applies to any piece of hard substrate that will assist the development of an architectural structure for aquariums and aquaculture tanks, and that has been underwater long enough to develop a community capable of processing and recycling water born waste chemicals such as nitrogen compounds, amongst others. The community consists of the attached bacterial biofilm together with a myriad of larger aquatic algae and invertebrate fauna. The term "live rock" is normally specific to the marine aquarium business. Live rock is acquired for tank water quality maintenance purposes, contributing a large surface area substrate that facilitates active biological filtration within the aquarium environment and stabilizes its water chemistry. The aesthetic part is also important, shaping the "layout" of the aquarium or "aquascaping", as well as providing a base to attach other corals, sponges, anemones and clams, and providing

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Figure 20.1 Holding tanks with moist live rock waiting to be shipped from a facility in Singapore (2009). (*See insert for color representation of the figure.*)

shelter for other inhabitants of the reef aquarium. These live rocks covered with pink or purple encrusting coralline algae tend to command a high retail price. Live rock is primarily collected from Indo-Pacific reefs, where its export is an important source of revenue for many small subsistence communities (Figure 20.1).

The Department of Fisheries of Western Australia (WA-DF, 2009) defines live rock as "a trade name for a substrate (generally a rock or dead coral) colonized by a range of benthic flora and fauna. Clearly the substrate itself is not alive but the veneer of organic matter living on or around it is". The U.S. government, on the other hand, defines live rock as "living marine organisms, or an assemblage thereof, attached to a hard substrate including dead coral or rock" (NMFS, 1995). Other definitions describe live rock as "a collection of calcium carbonate material that contains encrusting organisms such as coralline algae, other algae, and epibenthic invertebrates" (Delbeek & Sprung, 1994; Tullock, 1997; Falls *et al*., 2003). It is interesting that the first two, more official definitions, focus on "dead coral", as most of the traded live rock is for marine aquariums and tend to be colonized dead coral rock. Being dead hard coral skeletons, means that shipments must obey international trade regulations set up by CITES. A substantial amount of paperwork needs to be done before a shipment of live rock from traditional tropical countries can be sent, and then imported, and clear customs in the largest markets for these goods, the USA, Europe and Japan, where enough people have spare money to keep a seawater aquarium.



Figure 20.2 Example of an artificial hand-made base rock, ready to go into a maturation tank for a minimum of a 2-month period.

These natural and colonized rock from tropical coral reefs, are considered one of the most important items traded in the marine aquarium market. Live rock harvest continues to be an important business as the seawater aquarium market expands; the best known internationally is the live rock from Fiji. The cost of transporting such a heavy product, the lack of appropriate management that could lead to overharvesting of natural occurring rock and, more seriously, the potential to introduce disease, parasites and exotic species throughout the planet oceans, have raised the question: can aquacultured base rock product be a practical mitigation alternative? The aquaculture production of artificial base dry rock already takes place in Florida and Fiji, and could indeed provide an economically feasible solution to some of these problems (Figure 20.2). The goal is to create a low-cost, lightweight product with a porosity comparable to natural live rock and with its ammonia and nitrite–nitrate biofiltering capacity. This artificial product could be colonized with the beneficial bacteria and the region's native invertebrate biota, and should also be of sufficient aesthetic value to compete in the market with natural live rock. This chapter will deal with several of these issues in detail.

20.2 Trade of Live Rock: A Bit of History

Live rock is as old as marine aquariums themselves, both private and public. According to Green & Shirley (1999), at the end of the last century, more than 50,000 tons of live rock were thought to be maintained within U.S. aquariums. Before the closure of wild live rock harvest in U.S. waters in 1997 (Falls *et al*., 2003), the United States supplied much live rock to its own industry (Parks *et al*., 2003). The recreational fishery for coral and associated groups in Western Australia was banned in 2001 (WA-DF, 2009). The commercial extraction of wild live rock began in Florida during the mid-1980s reaching 300 tons per year during the mid-1990s (Falls *et al*., 2000). This was followed by wild extraction and retail in Hawaii. At present live rock is "cultured" in the US in Florida, Hawaii, Michigan and Idaho, using calcium carbonate rocks either collected from land or artificially constructed. These are "seeded" by placing them on leased areas of

the seabed or in land-based tanks that already contain live rock. According to the Fishery Management Plan for Regulating Offshore Marine Aquaculture in the Gulf of Mexico (NOAA, 2015), in 2013, there were 20 permit holders for live rock aquaculture in federal waters off the coast of Florida. However, since the prohibition of live rock and live coral extraction, the United States (the largest market in the world) has imported nearly all (95%) of the live rock traded within the marine aquarium industry (Moore & Best, 2001), representing millions of kilograms of live rock every year. Precise figures are difficult to estimate, as live rock data are generally not reported separately from other scleractinian imports (Bruckner, 2001) and this difference is not specified in the CITES database either. However, according to the results reported by Falls *et al.* (2001), the total value of U.S. live rock sales during the 1992–2000 period was estimated at US$14 million, representing a volume of 2.5 million kilograms at an average value of US$5.60 per live rock kilogram.

According to Bruckner (2001), Indonesia and Fiji have been the leading exporters of live rock and live coral. This pattern is confirmed by the CITES web-data-portal (www.CITES.org) until 2013, although CITES report coral trade as a whole, where the live rock trade is included. Hard dead-coral rock tend to be used for construction a little bit everywhere in the island nations and the methods of extraction can go from simple extracting harvest techniques to more drastic and impact techniques such as dynamite explosives. The use of the same material for the ornamental trade may represent another exploitation of the same resource, but seldom is its price increased to the local workers. CITES records show that the live rock trade increased annually by 15% to 30% in the 1990s. It is estimated that over 50,000 tons of rock is kept in aquariums in the United States (Parks *et al.*, 2003). Other consumer countries are Canada, Europe and Japan and, according to CITES, Brazil has recently increased their imports of corals. Difficulty with imports and the lack of natural legal rock, can create a small internal market for artificial live rock.

20.3 Types of Live Rock

Many different types of live rock are commercialized nowadays at very different prices (Table 20.1). It can be characterized according the country or region of origin (Fiji, Indonesia, Florida, etc.) or if it is wild-caught versus artificially made. For example, the enterprise Walt-Smith International (2015), one of the largest live rock producers, has both natural, wild collected live rock, together with artificially hand-made live rock that is left maturing for a year in Fiji's coral reef environments. It is difficult to tell them apart.

Igneous or volcanic rocks are very porous and may be excellent candidates although, depending on the exact geological origin, may over the long-term leach chemicals that create water quality problems. Some desert stones with nice convoluted forms have also been used as base natural rock left to mature in an aquaculture or sea-ranching facility. The problem with naturally occurring rocks is that they tend to be denser and have less void spaces which are key characteristics for good live rock.

Artificial, hand-made live rock can be sold as "base rock" or "starter rock", as opposed to fully matured live rock. Artificial live rock can also be further characterized according to the materials it's made of. These can range from natural rock broken into an appropriate size and left to mature in the sea, up to a myriad of different ways to

Table 20.1 Average live rock final retail prices in the USA (21), Europe (16), Brazil (14) and Mexico (9), from up to 98 different live rock varieties and brands traded in those four markets. Information was obtained during a November 2014 internet survey. Prices were converted into the US$/kg equivalent using the same-day Exchange rate of currencies other than US$ (euro – 1.24; Brazilian real – 0.382; and pesos – 0.0736).

	Price per kg (converted to US dollars)				
Variety	**Brazil**	**Europe**	**Mexico**	**USA**	**Average**
Reef live rock dried	11.1	9.1	12.1	7.2	9.9
Reef live rock wet	6.9 (5.4)	14.4	17.2 (6.2)	8.2	11.6
Artificial live rock dried	4.5	8.9	–	11.0	8.12
Artificial live rock wet	–	12.3	20.6	12.1	14.9
Desert live rock	6.3	–	–	–	6.3

produce and shape your own live rock, following the general procedure of binding several materials into a design shape (Table 20.2).

Several well-known examples of DIY live rock exist and perhaps one of the best known is the GARF aragocrete live rock (www.garf.org/class.html). Several examples of ingredients, bonders, shapes and curation procedures are summarized in Table 20.2. Another alternative is the plastic, dummy or fake rock. Many brands of aquarium decorations and ornaments include plastic rocks that try to imitate real natural live rock. The arguments tend to be that these products require less maintenance, are lighter, will not harden, crack or fracture, come at a fraction of the cost of the real live rock, and there is a huge assortment of different coral reef styles to capture a specific aquatic look. The filtration capacity, however, is reduced and we recommend them for "fish-only" aquariums with low biomass. Qualities to look for include 100% non-toxic and safe for all aquatic life, non-abrasive, UV and temperature resistant, detailed craftsmanship and durability.

Natural live rock originates from calcareous base matrix (aragonite crystals), deposited by hermatipic coral skeletons that can be posteriorly eroded by friction or excavated by a myriad of organisms such as bivalves, serpulid worms, sponges, and others. This rock tends to command a premium price due to the supposed excellence in variables such as beauty, lightness, porosity, filtration efficiency, durability and proper biota or startup bacterial community (Table 20.1). This may not be entirely true and if properly designed and engineered, artificial aquacultured live rock can reach these high standards as we demonstrate in another section of this chapter. Some traded natural dry rock tends to get trade names such as the popular "pukani" dry rock which really applies to any connected branching hard coral dead skeleton.

Some argue that dry base rock is better than curated wet live rock as with the former you have a greater level of control in avoiding introduction of nuisance species such as algae and pests which can cause complete tank shut downs. Most dry rocks are guaranteed to be free of *Aiptasia*, bubble algae, parasitic isopods, mantis shrimp, *Acropora* eating flat worms, little red bugs, fire worms, predatory nudibranchs, pyramidellid snails and other common aquarium pests. However, since they originated from

Table 20.2 A few of the readily available recipes to produce your own live rock (adapted from www.garf.org/). Supplies needed include 20-L plastic buckets, plastic mixing bowls, shovel, wheelbarrow or large plastic mixing tub, a small garden shovel and several small sticks of wood.

Recipes/ingredients	Proportion (Cement: Aggregate) material)	Mixing	Shaping	Curing
1 Part Portland cement 5 Parts oyster shells 2 Parts plastic shaving	1–7	The materials should be mixed homogeneously with the addition of a just little water (only enough to give grip to the mix). The materials should be mixed homogeneously with the addition of a just little water (only enough to give grip to the mix).	The mixture should be added (without compression) to the chosen mold. The most popular method uses a sandbox (digging up the sand to generate the desired shape). To ensure a more natural look, some people add solar salt in the mold before putting the mixture.	Can be done at sea or closed systems with regular seawater changes (which already promotes the colonization of the rock) or freshwater changes (until the moment when the rock does not change the water parameters). Recommended time is quite variable: 1–9 months. In closed systems, the addition of acid substances (e.g. vinegar) can accelerate the process.
1 Part Portland cement 1 Part aragonite 4 Parts crushed coral	1–5			
2 Parts Portland cement 4 Part oyster shell 1 Part aragonite 2 Parts solar salt	2–7			
1 Part Portland cement 5 Aragonite gravel	1–5			
1 Part Portland cement 1 Part aragonite sand 1 Part crushed coral 3 Parts crushed oyster	1–5			
1 Part Portland cement 5 Aragonite sand 1 Part plastic sawdust	1–6 (Aragocrete)			

wild-caught, dried natural coral rock, they may have dead material such as sponges, and other marine invertebrates that should be extracted before use. They also need curation, which increases the time window before the product is ready to use in an aquarium. Nevertheless, other than the guarantee of being pest free, dry rock has other advantages such as low initial cost on premium types of rock and better price for pound value as there is no water weight. This material can be shipped through standard ground which saves a fortune on shipping.

Dry rocks tend to be cheaper than curated and live rocks. On the other hand, artificial live rock is almost always more expensive than natural reef live rock (Table 20.1). Indonesia and Fiji live rock command the highest prices. Brazil and Mexico internet shops tend to provide less information on the origin, forms, and characteristics of their live rock products, when compared to those from Europe and the US, which perhaps is an indirect indication of more demanding customers from the latter. The US is the most diverse market with a greater variety of branded and variety products available. Brazil, however, appears to be the exclusive market for dry, natural "desert rock", although it might be more related to a problem of coding and naming the same category products with completely different common names. Most countries trade the rock product through specialized aquarium internet shops, except Mexico, where a considerable proportion of the available live rock is traded through a popular general auction website (MercadoLibre.com.mx).

20.4 Principal Recipes to Make Your Own Aquacultured Live Rock

The most commonly found recipes for artificial live rock use Portland cement as binder; it is alleged that some brands of cement have lower pH and therefore less potential to change water parameters in the aquarium. Many materials can be combined with the binder, in different proportions, according to the recipe in question (aragonite sand or gravel, coarse solar salt, coarse sugar, crushed oyster shell, crushed coral and plastic shavings, are some of these materials – Table 20.2).

Besides cement, there is still the possibility of using ceramic as binder. This material exhibits a lower potential to change the water pH. However, this technique has only been observed in industrial products, due to the need for special ovens to burn the ceramic that are not easily available to the average hobbyist. When using ceramic, it is important to attend to the composition, since it is extremely variable and the addition of certain chemicals can be undesirable in the aquarium.

Mixing – The materials should be mixed homogeneously with the addition of a just little water (only enough to give grip to the mix).

Shaping – The mixture should be added (without compression) to the chosen mold. The most popular method uses a sandbox (digging up the sand to generate the desired shape). To ensure a more natural look, some people add solar salt in the mold before putting the mixture.

Curating – can be done at sea or in closed systems with water changes of salt water (which already promotes the colonization of the rock) or freshwater closed systems with water changes (until the rock does not change the water parameters). The time

used in the process is quite variable, ranging from 1–9 months. In closed systems, the addition of acid substances (such as vinegar) can accelerate the process.

Alternatives – We developed a process in which small Styrofoam balls were added, to the Portland cement and aragonite sand mixture. After the drying process, the rocks were heated up at 300 °C, burning the Styrofoam. This increased the amount of void spaces and, consequently, the area available for bacterial fixation. Coarse sugar and large solar salt crystals can also be used in a similar way (instead of burning, they can be dissolved in the water), but these methods are still to be tested.

20.5 Decoration Versus Filtration: Myth or Reality?

Although most professional aquarium keepers agree that the acquisition of live rock is important for water quality control, they argue that the aesthetic factor and aquascaping potential tend to be more important when it comes to choosing whether the aquarium setup will have live rock and which type of live rock. Our point is that the industry does not have a standard regarding parameters to measure live rock efficiency in terms of biological filtering capacity, and that the market does not demand one, meaning that it is more a question of belief, rather than a matter of hard science. Variables such as average effective surface area, weight/volume relation, inferred density or porosity, oxygen permeability, nitrification capacity, biological starter capacity, and so on, are seldom measured and advertised in the marketing campaigns, always assuming that a particular product provides the best performance without confirming it. Customers are normally more interested in the coralline incrusting algae cover and the degree of "pink-color-cover", rather than the actual filtering and recycling performance. Therefore, once the basic structural efficiency of live rock problems are solved, time and research should be put into the protocols to further increase coralline algae colonization of artificial live rock, together with best conditions to promote growth of those algae.

Yuen *et al*. (2009) provided the first scientific evidence for the effectiveness of live rock in removal of nitrogen compounds from coral aquaria. Using comparative experiments they demonstrate that live rock is capable of removing nitrogen compounds and reduces the mortality of a reef-building coral (*Acropora digitifera*) in the presence of a nitrogen producing marine invertebrate (the sea cucumber *Holothuria atra*). The presence of live rock remarkably reduced the nitrogen concentrations and sustained high coral photosynthetic activity. Furthermore they detected the functional genes "amoA" and "nirS" within the live rock, suggesting the occurrence of both nitrifying and denitrifying bacteria. This study finally put an end to the discussion on the effectivity of live rock as a promoter of biological filtration in closed marine systems.

As previously said, the presence of encrusting algae covering its surface is a desirable characteristic for aesthetic reasons. Most of these encrusting algae belong to crustose coralline algae that play a fundamental role in the formation of coral reefs (Littler and Doty, 1975). An interesting new finding is that these crustose coralline algae seem to facilitate larval settlement and metamorphosis of a wide variety of marine invertebrates including reefbuilding corals (Morse & Morse, 1991). There are already some products that claim to enhance red coralline algae growth such as PurpleUp^{tm} (US patent No. 7,565,883) from the company Carib Sea. This is not a fertilizer and therefore does not promote the growth of nuisance algae. Instead it works on the ionic calcium

concentrations, together with other minerals (strontium, magnesium, and carbonate), as well as delivering iodine. This kind of product could be used to finish up some artificial aquacultured live rock to increase its market value.

The fact is that in most live rock the original bacteria communities will shift and adapt to the new conditions from the moment the rock is collected from the wild to the moment it reaches the final aquarium. The bulk of the marine biofilm and other invertebrates growing and prospering in the tropical reef dead coral rocks, die off during air-exposure, water pressure hose cleaning and scraping, and temperature, oxygen and salinity oscillations along the product distribution chain. Only the deep, internal interstitial bacteria communities may remain and those are the prime product, as they will serve as inoculum and kick start all over again, shaping the new rock community. This ecological succession process following major biomass loss tend to branch in different directions as each community will adapt to the specific new aquarium or tank conditions.

Effective surface area is perhaps the most important variable, as most bacteria require a surface on which to grow, whilst they feed on an organic substrate. Surface area is increased when the rock is more porous, less dense. This has great business advantages since the main cost of live rock comes from the air-shipment cost for transporting something heavy over long distances. A less dense more light-weight, fully porous product will have a competitive advantage even when distributed over short distances.

However, lightness is not the only factor. The surface-area-to-volume ratio is also crucial. Not only must the rock be light-weight in order to reduce shipment costs, but is also needs to be sufficiently porous to allow more bacteria to grow on the surfaces. The depth and diameter of the channels are also important as this controls oxygen permeability. The deeper communities tend to run anoxic as the oxygen provided by diffusion eventually depletes, giving space to different bacterial communities that could, for example, process excess organic nitrogen in the form of nitrates.

The filtration service may be accomplished with efficient ceramic biomedia such as MarinePure^{tm} spheres or plates, Bioballs^{tm} or any other biofilter media – although these lack the aesthetic plus and aquascape possibilities and are normally kept in the sumps, holding reservoirs or refugiums.

20.6 Problems in Use

Live rock commonly serves as the ecological foundation within living coral reef aquaria and is therefore heavily traded in the ornamentals market. Perhaps the most important issue with live rock trade for the ornamental marine aquarium industry is that of the spread of invasive species plus the potential for introduced diseases and parasites. The interstitial water and the inherent complex structure of live rock enable a myriad of different refuges for a variety of animals and therefore promotes the transport of unnoticed organisms. This is a central point of discussion and sooner than later, stricter regulations may be implemented in most trade countries.

The trade in high-value ornamental marine species for the aquarium industry is increasingly recognized as important source for biological invasions, which result from the inadvertent or deliberate release of marine organisms (Semmens *et al*., 2004). The live rock trade, specifically, poses a serious threat of bioinvasion because of the

potential for release of the organisms that reside on its surfaces and interiors, as this trade is not subject to quarantine restrictions in its principal markets. This threat was clearly demonstrated by the transportation of jellyfish polyps of a species of *Cassiopea* (upside-down jellyfish) on live rock imported to the USA from the Indo-Pacific (Bolton & Graham, 2006). The invasion of tropical jellyfish in the Gulf of Mexico has been reported to have several ecological and economic implications in the region (Graham *et al*., 2003) and several changes in regulations have been proposed (Doelle, 2003). Along with this transversal problem, comes another issue that is more internal: introduction of nuisance species into a balanced aquarium. Unless properly processed, some rocks will introduce nuisance species into the aquarium. This can go from species specific bacterial, virus and fungal infections, to larger menaces such as parasites and predators. These include zooanthids, sponges, tunicates, bryozoa, anemones, and soft and hard corals that all commonly encrust live rock, as can chitons, mollusks, serpulids, gastropods, echinoids, and even small crustaceans and fishes.

Another problem with the live rock trade is the cost of air-freight and its carbon footprint. Dry rock reduces the magnitude of this problem and local production of aquacultured live rock close to the demand market also helps to decrease the costs and reduce the carbon footprint.

Yet another problem is the bad public impression that the marine ornamental trade somehow supports destructive dead coral extraction. While the coral making up the bulk of live rock is indeed dead, it provides a substratum for recolonization by corals and other organisms, and thus for recovery of coral populations over time. However, current harvesting techniques for the ornamental trade are environmentally sound, and have benefited by the introduction and promotion of best practices. The difference of dead coral rock as material for building construction needs to be clearly separated from the collection for aquarium use.

Large live rock aquaculture ventures in the sea should carefully plan their deployments according to the regulations and in close cooperation with the authorities in order to avoid navigational hazards and achieve full legal compliance. The depths at which the rock have been maturing should be reported to the consumer. There is a tendency to prefer shallow sites where more light gets to the bottom and stronger wave action, although this increases the chances of catastrophic loss as a result of disasters (hurricane damage, disease, poaching) and navigational hazards.

20.7 Financial Issues?

Live rock aquaculture operations may seem to be an attractive economic activity to entrepreneurs or resource managers and decision makers seeking sustainability relief through aquaculture promotion. However, the results of an economic study on the feasibility of a joint live rock live-coral aquaculture venture in either the US or an Indo-Pacific island country (Parks *et al*., 2003) were not particularly encouraging. Under early 2000s market conditions, due to competitive pricing structure, the absence of a green premium, and deliberate regulatory intervention restricting or limiting wild-caught live rock and coral, it does not appear overall that strict live rock and/or live coral culture operations are economically feasible or risk free. Parks *et al*. (2003) argue that under such conditions, the strict-culture operations will alone be insufficient to

solve the marine ornamentals sustainability issue as an industry enterprise alternative; therefore wild stock collection needs to be sustainably managed and best practices certification should be implemented. In order to favor a strict-culture supply, the same authors suggest improving marketability and profitability of cultured products via green labeling and green premium capture through third-party certification. This together with funding to support research into the science and impacts of live rock and live coral culture, including: (a) the testing of ideal site conditions and locations for live rock seeding and grow-out of propagated live rock and corals; (b) ideal vertical (water column/depth) and horizontal (reef versus seafloor) placement of propagated pieces; and (c) the ecological impacts of such culture operations is needed.

However challenging strict-culture of live rock may be, several benefits arise from its operation, beyond the promotion of a more sustainable alternative for the international marine ornamentals trade than wild extraction that collectively may outweigh the financial costs. According to Parks *et al.* (2003), the first set of these benefits is related to improved livelihoods and livelihood opportunities in the producing countries, including: (1) salaries for commercial employment staff; (2) an important source of household income for the families of commercial staff or for rural village community residents, including income generation by women and children (Wheeler, 1996; Counterpart-International, 2000); (3) an occupational alternative in fisheries with strong reported job satisfaction; (4) group income toward community projects from coral farm ecotourism fees (Heeger *et al.*, 2000); and (5) improved fish habitat (coral reefs) resulting in replenished and/or larger food fish stocks (Herndon, 1998).

Following the argument of Parks *et al.* (2003), the second set of benefits is related to the biological and non-livelihood social outputs from culture operations. The most commonly cited biological benefit would be coral reef rehabilitation and restoration (Herndon, 1998; Heeger *et al.*, 2000), resulting in improved ecological functioning and biodiversity maintenance. Some live rock and coral culture advocates claim that after culture operations, the *in situ* coral reef habitat was healthier and in better condition than before culture operations (Sweeney, 1992; Spears, 1995). In this regard, Parks *et al.* (2003) argue, the costs of operational subsidy may be less than the cost of coral reef loss or degradation. Acknowledgment of such conservation incentives may provide culture operators with sources of investment capital otherwise not considered. Important social benefits not associated with livelihood are improved marine resource management, increased environmental awareness and education, and strengthened local ownership (tenure) and/or responsibility over the area being cultured.

20.8 Observations on the Differences of Live Rock

20.8.1 Experiments with Different Types of Rock and Maturation Times

A brief experiment consisted of measuring and comparing porosity and the oxygen consumption from different types of live rock. We used two sets of hand-made, artificial live rock submitted to two different conditions. One set was allowed to age and fully mature for more than 2 years in a seawater exposition aquarium, whilst the other only matured for 2 months in a running seawater live rock maturation aquaculture tank.

Table 20.3 External volume and void space of three different rock types, tested for their biofilter efficiency. Two artificial, hand-made rocks using one part cement, five parts beach sand, matured for either 2 or >24 months, and a third treatment with natural coral skeleton from a coral reef in southern Gulf of Mexico.

		Artificial rock 2 months matured	Artificial rock >24 months matured	Natural live rock
External rock volume (mL)	50 g	11.4 ± 2.33	14.34 ± 2.43	34.06 ± 5.23
	100 g	10.74 ± 2.66	31-04 ± 4.92	46.88 ± 7.1
Internal rock volume (void space)	50 g	6.03 ± 0.83	5.74 ± 0.6	14.61 ± 1.12
	100 g	9.19 ± 1.01	5.6 ± 1.2	24.07 ± 2.31
Rock dry weight (mg)	50 g	44.32 ± 0.21	44.58 ± 0.07	35.47 ± 0.44
	100 g	91.40 ± 1.06	94.47 ± 0.45	76.27 ± 0.52

The third set consisted of a Gulf of Mexico, dead-coral live rock collected directly from the Madagascar-reef crest (Yucatan, Mexico) as a control. The objective was to quantify physical differences in the origin of the rocks, detect changes in batches of artificial live rock, and assess the effect of the maturation time in the oxygen consumption efficiency of the artificial rocks. In order to see if there was any volume-dependent variable, two sizes of each rock were tested (50 and 100 g).

Five smaller replicate pieces of each of the three rock treatments were cut until they all were either 50 or 100 g approximately. Dimensions, exterior volume and internal volume (void space) were measured by water displacement, and differences in weight through evaporation. Results clearly show that for the same weight, natural live rock tends to have twice the volume, and is therefore half as dense as the artificial counter-parts tested in this short experiment (Table 20.3). Internal void space was two to three times larger in natural rock than in artificial rock. Differences in volume and void space between the artificial rock treatments may be due to small differences in the share of materials when the mix was prepared (Table 20.3). However, maturation time may also increase porosity of artificial rock, through biological erosion by organisms that excavate galleries, or dissolve the raw materials, especially if enough time is allowed.

Oxygen consumption of each rock fragment-replicate, from each rock treatment was measured with a simultaneous 10 channel oxymeter (PreSens, gmBh, Germany) in a 500 mL closed chamber system. Internal chamber volume was continually mixed with a small internal powerhead-pump and temperature was controlled with a water bath. Oxygen concentration was measured with 20 second frequency readings and each fragment-replicate was followed for 25 minutes. Results show low oxygen consumption rates and larger volumes of rock may be needed to increase the signal in the future. However, we demonstrate that there is indeed a live community living and breathing in the rocks (Figure 20.3), further validating the findings by Yuen *et al.* (2009). No differences were found amongst all three treatments, although this is surely a result of the low oxygen consumption rate that correlates to a low biomass. Other ways to measure biological activity may make use of ammonia and nitrite reduction capacity and will be closer to the purpose of using live rock.

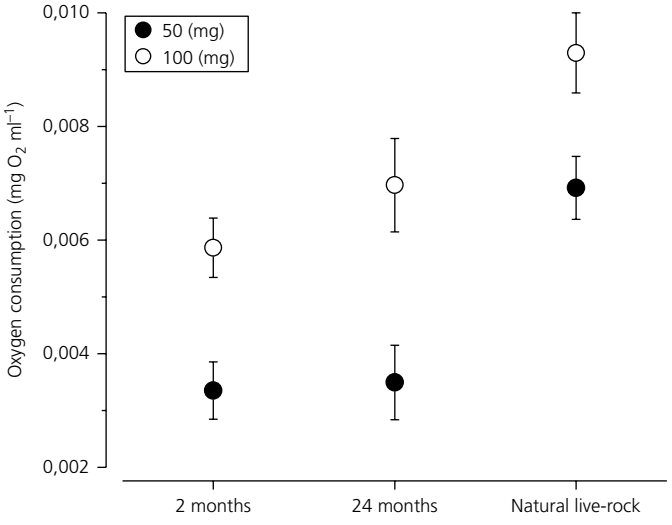


Figure 20.3 Graphs showing oxygen consumption rate (mg O_2 mL^{-1}) of three different sets of live rock.



Figure 20.4 Example of manipulation of the void space in artificial, hand-made rock using Styrofoam (see text for details). This can be attained with other dissolving materials such as large salt or sugar grains. Scale bar 10 cm.

20.8.2 Experiments with Different Level of Porosity

In another experimental trial, we tested the effect of increasing levels of porosity and void space in artificial live rock on the microflora oxygen consumption as an indirect measure of the colonization (unpub. data). Artificial live rocks were made using a mixture of white cement and sea sand (2:1). Styrofoam balls (5 mm diameter) were added in different percentages (0, 15, 30 and 45%) of the total standard volume of a model container (50 mL). The mixture of cement, sand and Styrofoam was left to dry at room temperature and then burned at 300 °C for 2 hours in order to vaporize the Styrofoam and form the intended internal and external porosity pattern (Figure 20.4).

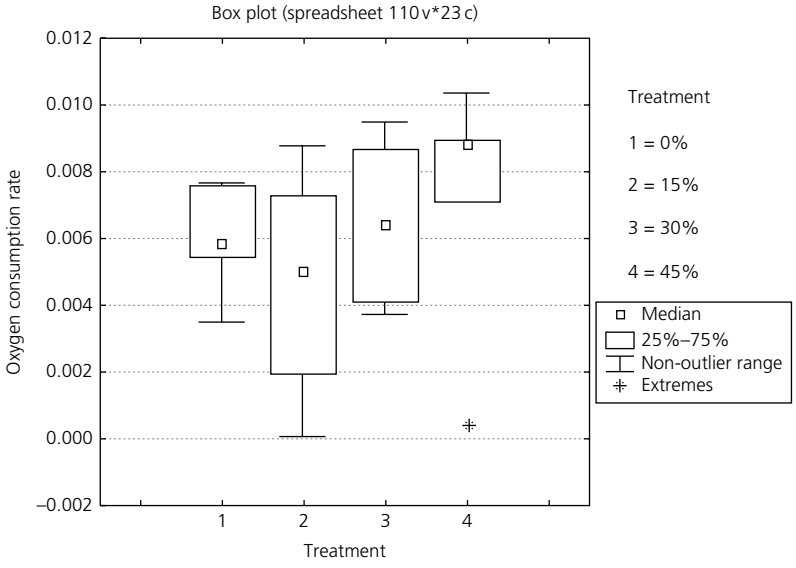


Figure 20.5 Oxygen consumption rate (mg O_2 mL^{-1}) for each Styrofoam % inclusion treatment (six rock replicates per treatment).

Rocks were then coded and left on an outdoors 5000 L open seawater flow rate system to be colonized over 30 days. The damp weight of each rock was measured, together with its volume, by water displacement to calculate density. The oxygen consumption of six rocks of all % Styrofoam treatments, were measured using a 10 simultaneous channels oxymeter (Presens, Germany) and a closed chamber experimental design. Oxygen concentration was recorded every 15 seconds for a minimum of 25 minutes, or until a drop to less than 80% of the saturation level. Each rock replicate was measured three consecutive times within a few hours.

Results show a clear direct proportionality trend of the internal rock volume (mL) or porosity, to the level of each Styrofoam % inclusion treatment (22.67 ± 0.76; 28.94 ± 1.14; 26.99 ± 2.17 and 37.96 ± 2.4, for 0, 15, 30 and 45%, respectively), thus confirming that internal void volume can indeed be manipulated using this technique, enabling lighter and more porous rocks. After one month of incubation in running seawater, we could measure oxygen uptake and hence bacterial colonization (Figure 20.5). The oxygen consumption rate, however, did not vary significantly between Styrofoam inclusion treatments (ANOVA, $p > 0.005$). This was due mainly to a large heterogeneity of variance and large intra-treatment variability between rocks related to the small rock mass, short curation period, inadequate cement mix pH, and other small details in the experimental readings such as light and lack of disinfection of the reading system tubing. Oxygen consumption can be used as an indirect proof of bacterial colonization and, with some modifications of the current protocol, could be also used to quantify and monitor microflora colonization. Future trials are needed with other inclusion materials such as rock salt, sugar crystals or other water-dissolving solids.

20.9 Conclusions

Better off with an artificial, custom and hand-made product, instead of natural dead coral-rock. Create a hard, durable, but light, porous, product.

Maximize surface-area relation and minimize volume as most aquariums out there are relatively small.

Use pH neutral products and materials during fabrication and know the pH of your rock well.

Do not rush to put the product in the market – allow for sufficient curation time and maturation of the bacterial communities and if possible calibrate your maturation phase to be sure not to affect the water pH of the tanks where the rock will be used. Be as close to your potential market as you can to reduce shipment distances and costs. Research the best method to inoculate coralline red incrusting algae into artificial hand-made live rock and to grow it as biologically fast as possible. A proper definition and standard of measurement of variables that allow objective comparison of live rock efficiency as contributors to biofiltration is needed. We propose the use of Rock density; Rock porosity and void space; Curation time; If possible, oxygen consumption rate and/or ammonia and nitrite removal rate per unit of weight. Research the use of different tints and colors to artificially produce a more appealing and aesthetic product. Research the metagenomics description of the microflora present in the interstitial spaces and gene-activity related to the seawater nutrients turnover to enable precise comparison between products.

Research methods to ensure elimination of undesirable pests and diseases without killing beneficial microflora, in the source region to mitigate spread of invasive and nuisance species. Several geographically separated sites should be used to cure rock in the sea to avoid catastrophic loss as a result of disasters (e.g., cyclone damage, disease, poaching). Be prepared and expect an increase in legislative restrictions regarding import of live rock as a mitigation to extraction form the wild and control of potentially invasive species.

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21

Cnidarians

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Phylum Cnidaria hosts a group of simple but remarkably diverse animals that display a range of common features: radial symmetry, planula and polyp life stages and organelle-like capsules that are unique in marine organisms – the cnidae. Nematocysts are the sole cnidae common to all cnidarians and to date no loss of this feature has ever been documented (Daly *et al*., 2007). Popularly known as stinging cells, the presence of cnidae is certainly the most easily recognizable diagnosing feature of cnidarians.

Corals, sea fans, sea anemones and jellyfish are some of the most popular marine ornamentals in the trade, but their importance in the industry is certainly different (Wabnitz *et al*., 2003). While corals, sea fans and small anemone-like polyps (e.g., zoanthids) harboring photosynthetic endosymbionts (commonly termed zooxanthallae) are the most heavily traded and cultured group of invertebrates at present, jellyfish have only recently become popular in the aquarium industry (although public aquariums have been breeding and displaying them for several years). The conservation issues associated with the collection of hard corals from the wild (e.g., their listing on CITES, the Convention on International Trade in Endangered Species of Wild Fauna and Flora), and their high demand and market value in the trade (e.g., Rhyne *et al*., 2012) have fostered culture efforts worldwide. From low tech *in situ* aquaculture initiatives in the coral reefs of exporting countries to high tech *ex situ* propagation in the main cities of importing countries, from small backyard businesses to large enterprises, coral aquaculture is now a global, well-established industry. The propagation of soft and hard corals, sea fans and zoanthids through fragmentation (an asexual form of reproduction) became a generalized practice among professional breeders and hobbyists, and while most propagation efforts have been successful there is still room for improving current culture protocols (e.g., using innovative illumination systems such as LEDs during grow-out) (Rocha *et al*., 2013). The breakthroughs achieved by academic and public aquarium research groups on the sexual reproduction of corals also increases the prospects for large scale production of some highly priced species. Moreover, the growing interest in the use of farmed corals for coral reef restoration (Rinkevich, 2014) and the bioprospecting of new marine natural compounds (Leal *et al*., 2013) opens a window of opportunity for entrepreneurs willing to venture into coral aquaculture.

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Sea anemones are also long-time favorites of marine aquarium hobbyists – if clownfish are displayed in a reef tank, someone will always ask where their anemone is. Anemones are likely to be one of the most negatively impacted marine ornamental invertebrates in the industry due to the rather unregulated collection of specimens from the wild (Shuman *et al*., 2005). Thus, it is urgent to establish large scale propagation protocols for the most heavily traded anemones in order to have a marine aquarium industry that promotes sustainable practices and contributes to the conservation of marine biodiversity. But not only are the "beautiful" sea anemones being targeted by aquaculture, the "ugly" anemones that everyone in the industry dreads are also being cultured – the glass anemones *Aiptasia*. These pest anemones are commonly used as a simplified version of corals to investigate a range of topics related with the association between the cnidarian host and its photosynthetic endosymbionts (Weis *et al*., 2008). To perform such studies a large number of monoclonal specimens are usually required for statistically robust experimental designs. Large number of *Aiptasia* are also needed to culture one of the most favored biological weapons for controlling outbreaks of glass anemones in private and public aquariums – the highly priced sea slugs popularly termed *Berghia* (Dionísio *et al*., 2013).

Jellyfish are likely one of the most "in fashion" marine ornamental invertebrates in the aquarium industry today. The mesmerizing and peaceful effect that jellyfish tanks have has granted these animals a niche market outside of public aquariums. As jellyfish are relatively short-lived, require customized stocking tanks and specific husbandry needs, the aquaculture of these remarkable cnidarians is likely to experience a significant increase in the next few years. The large-scale production of these organisms will certainly benefit from a range of technical improvements on the life support systems employed for their culture, as well as on the accumulated know-how of researchers associated with public aquariums. Additionally, the use of jellyfish in integrated multi-trophic aquaculture (IMTA) (Ren *et al*., 2014) targeting their production for human consumption may also have a positive impact on the supply of lower priced organisms to supply the aquarium trade.

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21.1

Corals

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Abstract

Coral reef degradation worldwide and the concomitant increasing demand for corals to supply the marine ornamental trade have been driving the growing interest for coral aquaculture. Captive breeding and propagation of corals among aquarium hobbyists and public aquariums is a well-known and established activity. In contrast, the scientific community has poorly explored coral production methods. Nevertheless, research on coral biology has dramatically increased in the past decades and resulted in numerous scientific studies that are highly relevant to further advance coral aquaculture. Here we provide an overview of coral aquaculture with particular emphasis on *ex situ* production methods. A comprehensive review of current asexual and sexual propagation techniques is provided, together with qualitative aspects of corals, such as coral shape and coloration, and quantitative production parameters, such as growth and volumetric productivity. Finally, we thoroughly discuss how the manipulation of abiotic and biotic conditions can maximize coral quality and volumetric productivity.

Keywords *Ex situ aquaculture; growth; reproduction; symbiosis*

21.1.1 Introduction

Coral reefs have a high economic value, mainly as a provider of food and natural products and as a tourist attraction (Moberg & Folke, 1999). Additionally, these extremely productive and biodiverse ecosystems provide hundreds of species for the marine ornamental trade (MOT), which has been increasing over the last decades to a point that is becoming a threat for reef organisms (Rhyne *et al*., 2012a). Corals are included in the list of principal exploited species for the MOT, mostly because of their attractive colors and forms (Delbeek, 2001).

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Over the last decades the demand for corals to supply the MOT has been increasing to a point that is becoming a threat for reef organisms (Rhyne *et al*., 2012b). Consequently, increased efforts have been made to advance *ex situ* (aquarium-based) and *in situ* (sea-based) coral aquaculture (Pomeroy *et al*., 2006; Leal *et al*., 2016; Rocha *et al*., 2015). This strategy is mostly aimed toward turning coral trade into a more sustainable practice and to decreasing harvesting pressure on coral reefs. Coral scientists and producers have focused on both culture methods as they display different features and have their own advantages and disadvantages. *In situ* aquaculture greatly benefits from nature to supply essential elements, such as light and nutrients. Manpower is only needed to create and maintain infrastructures, fragment mother colonies and harvest full-grown corals. However, the manipulation of culture conditions is relatively inflexible, as the principal flexibility in this culture process is the selection of the place where the structures are positioned. Nonetheless, this decision is extremely important, as different places may have different light levels, nutritional sources, wave exposure and current regimes. Contrastingly, *ex situ* aquaculture involves controlled environments in culture systems that allow for manipulation of abiotic and biotic features to maximize coral production and guarantee their careful husbandry. Furthermore, it is possible to control the origin of the corals in *ex situ* aquaculture and thus avoid mass culturing single genotypes, which may ultimately lead to genetic pollution of natural populations.

Although extensive literature has been published on coral husbandry practices (e.g., Fossa & Nilsen, 1996; Borneman, 2001; Sprung & Delbeek, 2005), scientific studies aimed at *ex situ* aquaculture are still comparatively scarce (Leal *et al*., 2016). Nevertheless, coral aquaculture is already observed in several countries and has been a practice mainly associated with partnerships between research institutes and public aquariums (Osinga, 2008). Also, aquarium hobbyists and several companies that are farming corals have been improving coral propagation and culture techniques. Their expertise is sometimes disseminated, but most information is unsystematically distributed and often available in grey literature, such as aquarium magazines, web, forums, etc.

This chapter aims to introduce and develop the key issues to understand how to grow and propagate corals *ex situ*. We first review coral propagation methods, both asexual and sexual, as this is the first step for culturing corals. Then we focus on the factors that influence the qualitative and quantitative aspects of coral culture and their maximization through the manipulation of chief abiotic and biotic variables, such as light, nutrition and flow, among others. Important factors for commercial aquaculture are defined, with particular focus on shape, color, growth and productivity. While this chapter tries to embrace all types of corals, some coral groups have been more studied than others, particularly symbiotic corals. This chapter thus provides special emphasis on symbiotic stony corals (see definition below), as these corals display greater aquaculture potential and are in high demand by the MOT.

As any other production process, coral aquaculture aims to produce great quantities of high-quality organisms, two points that are thoroughly discussed in the following sections. While quantity can be easily defined, quality may be more challenging. Coral quantity is associated with coral biomass and growth, whereas coral quality refers to its physiological condition, color, shape and lack of diseases and parasites. It may also be associated with trade value or price, as high-quality coral is expected to be more expensive than a low-quality ones. Although aquaculture usually deals with high-density of organisms that carry risks associated with pathogen virulence and communicability, the

cause of diseases, its prevention and treatment are not embraced in this chapter. Indeed, several good reviews already exist on this subject (Borneman, 2001; Raymundo *et al*., 2008; Bourne *et al*., 2009; Sweet *et al*., 2011; Sheridan *et al*., 2013).

Before introducing coral physiology and ecology to understand coral quality and quantity, one needs to define corals into groups and introduce key definitions that help better defining them. While a large set of popular definitions has been used to group corals, they are often discordant to taxonomical groups. Moreover, not all definitions currently used are universally approved. In summary, all corals belong to the class Anthozoa and most of them belong to the orders Scleractinia and Alcyonacea. Scleractinian corals (stony corals) build a calcareous skeleton and are usually known as hermatypic or reef-building corals. Alcyonacean corals are usually known as soft corals, and contain spiny skeletal sclerites rather than a hard skeleton. Other common definition concerns the presence of photosynthetic dinoflagellates within the genus *Symbiodinium* in the coral tissue (popularly known as zooxanthellae). These corals are known as symbiotic corals, while corals without zooxanthellae are named asymbiotic corals. Although these definitions are not unanimous, we here use symbiotic and asymbiotic, and stony and soft corals.

21.1.2 Coral Propagation

21.1.2.1 Asexual

The most popular way to asexually propagate corals is to cut adult colonies into smaller fragments that will grow and develop into functional colonies. Fragments, also known as nubbins, can be as small as single polyps. Detailed protocols for coral fragmentation are already available in (grey) literature (e.g. Borneman, 2001; Borneman & Lowrie, 2001; Calfo, 2001; Shafir *et al*., 2006; Leewis *et al*., 2009) and the selection of the most appropriate method is species-specific. A two-step procedure to optimize fragmentation methods for a particular species has been recently published (Osinga *et al*., 2012), where the optimal sample size for comparative experiments is first determined in order to allow calculating growth rates and kinetics. Afterwards, optimization is addressed through studies on fragment size, fragment orientation, fragmentation materials and fragmentation procedures. This procedure is detailed in the CORALZOO Book of Protocols (Leewis *et al*., 2009).

Three important aspects of asexual propagation should be taken into consideration. The first is the selection of optimal fragment size, which should be based on growth kinetics data. Small fragments are better to propagate massive corals, whereas fragment size is irrelevant for branched corals. However, small fragments are more vulnerable and often display higher mortality rates (Heyward & Collins, 1985; Soong & Chen, 2003; Okubo *et al*., 2005; Edwards & Gomez, 2007) due to a higher sensitivity to wave action (Clark & Edwards, 1995) and fouling (in particular by algae), predation (Edwards & Gomez, 2007) and smothering by sedimentation (Soong & Chen, 2003). Particularly for *in situ* aquaculture, it is beneficial to use larger fragments because predation, sedimentation and water movement cannot be controlled. For *ex situ* aquaculture, small fragments are more appropriate because it is possible to use husbandry techniques that control sedimentation and fouling. A popular approach is to propagate

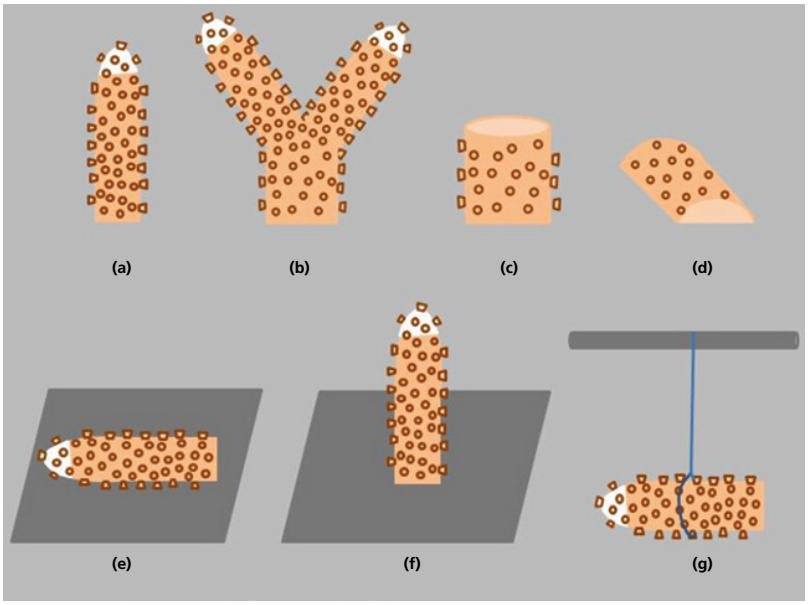


Figure 21.1.1 Type of fragments and orientation of fragments in branched corals. (a) Single branch tip. (b) Dichotomous branch. (c) Cylindrical middle section of branch. (d) Flat fragment. (e) Horizontal orientation. (f) Vertical (upright) orientation. (g) Suspended fragment.

small fragments *ex situ* and transfer them to sea-based nurseries to further grow-out (Epstein *et al*., 2001; Rinkevich, 2005). One advantage of using small fragments is that less material from donor colonies is required for initial propagation. This is relevant because cutting more than 10% of the mother colonies of *Stylophora pistillata* reduced the viability of the donor colonies (Epstein *et al*., 2001). Furthermore, removing long branches from mother colonies may render the original colonies infertile or reduces fecundity, as observed for *Acropora palmata* after fragmentation (Lirman, 2000).

Type and orientation of fragments are also relevant for the success of asexual reproduction. Fragment type refers to branched species, which can be fragmented in (Figure 21.1.1a–d): single branch tips, dichotomous branches, cylindrical sections or flat fragments (i.e. cylindrical cuttings that are laterally cut into two equal pieces). Fragment type affects growth and survival rates. Dichotomous branches of *Stylophora pistillata* grow faster than single branch tips (Rinkevich, 2000), whereas single branch tips display better results for *Montipora digitata* (Osinga *et al*., 2012). Orientation refers to the position of the fragment. Fragments may be positioned horizontally, vertically (upright or upside down) or suspended (Fig. 21.1.1e–g). Different morphogenetic patterns resulting from differences in orientation may be observed (Shaish & Rinkevich, 2009), whereas growth rates may be similar regardless of orientation (Soong & Chen, 2003). Fragment size and orientation may also interact and affect growth and survival rates, as observed for *Acropora formosa* (Okubo *et al*., 2005). Particularly, higher mortality rates were observed in small fragments when oriented horizontally, in opposite to large fragments. Furthermore, higher growth rates were observed for large fragments when vertically oriented as compared to fragments oriented horizontally.

Coral fragments for aquaculture purposes are often attached to a solid substratum, such as rock, concrete, plastic, ropes or lines, which is important to maximize survival rates (Heyward & Collins, 1985; Nagelkerken *et al*., 2000). Unattached fragments usually show comparatively higher mortality *in situ* because the time needed for attachment can vary from several months up to a year (Guest *et al*., 2011). The preferential substratum to attach coral fragments is species-specific and varies with the final purpose of the corals produced as well as the culture approach, i.e. *in situ* or *ex situ*. For instance, the suspension of coral fragments on ropes (e.g. Soong & Chen, 2003) is certainly a viable option for mariculture as it reduces sedimentation and provides "clean" coral colonies that can be directly positioned on reefs or in aquaria without requiring the removal of unattractive supports. Attaching corals to plastic plates or other flat supports is a preferential technique for scientific purposes, as it keeps young colonies in a fixed orientation and allows the researcher to rearrange the position of the fragments according to the requirements for metabolic measurements. The most popular methods to attach coral fragments to the substrate are gel-based cyanoacrylate glue and two-component epoxy (Leewis *et al*., 2009). Fragments attached with marine epoxy show lower detachment rates and high rate of concurrent natural attachment (Dizon *et al*., 2008). Alternatively, coral fragments can be bound using tie-raps or nylon lines, or nailed to their support (Soong & Chen, 2003; Okubo *et al*., 2005).

Seasonality should also be considered for asexual reproduction of corals (Okubo *et al*., 2005), as well as broodstock selection, i.e. genotypic variability, and preservation of genetic diversity (Rinkevich, 2000; Osinga *et al*., 2012). While cloning a single genotype may be appropriate to provide reproducible materials for the MOT, it may not be the most appropriate strategy for reef restoration purposes. Alternatively, sexually obtained recruits may be more suitable to maintain genetic diversity. Sexual reproduction of symbiotic corals will be further outlined in the next section of this subchapter.

21.1.2.2 Sexual

Coral sexual reproduction has only been recently addressed for aquaculture purposes as it is much more complex than asexual propagation. Different methods have been used to produce and culture larvae, control settlement and raise juveniles of a limited number of species. However, in order to understand the underlying methods for coral sexual, it is essential to first know the reproductive biology of the particular species.

There is a great diversity in sexual reproduction of corals as they can be either classified as gonochoric or hermaphroditic, with the latter often being simultaneous, i.e. a single polyp produces male and female gametes in the same breeding season. The two known reproduction modes are illustrated in Figure 21.1.2 and are described as: (1) internal fertilization and release of settlement competent planula larvae (also known as brooding); and (2) external fertilization with a 1–4 days planktonic embryogenetic stage depending on the species and water conditions (also known as broadcast spawning). External cues, such as seasonal temperature (spawning usually occurs when the water temperature reaches its annual maximum of 28–30 °C) and the lunar cycle, are often important triggers of the reproductive cycles. Larval release by coral brooders may occur seasonally for several months, or may be released year round in a few species. Broadcast spawners usually have annual cycles where they spawn over a few consecutive nights of one or more consecutive months. Baird *et al*. (2009) reviewed the diversity

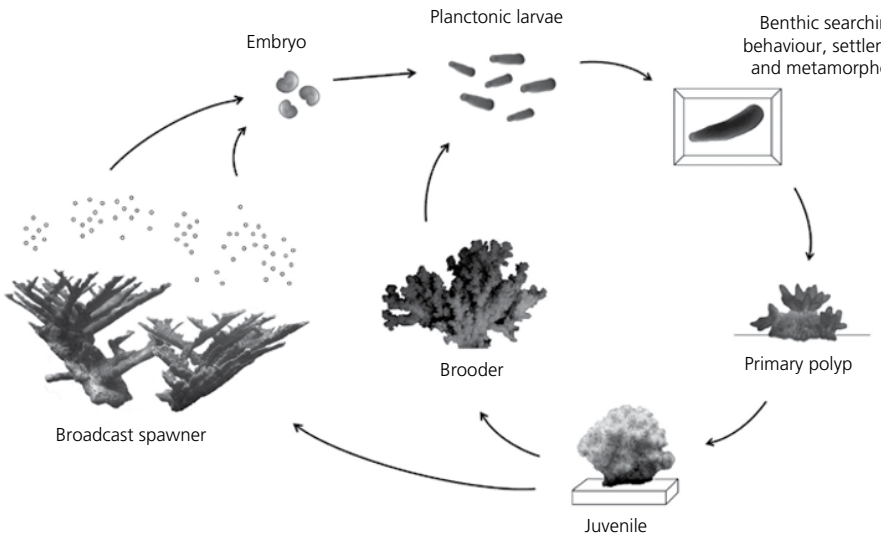


Figure 21.1.2 Reproduction cycle of broadcast spawners and brooders.

of sexual reproduction in corals and reported that approximately 400 scleractinian corals are brooders and 800 species are gonochoric. Some brooding species, such as *Pocillopora damicornis*, may exceptionally produce larvae asexually, whereas some broadcast spawners and brooders may display self-fertilization. Detailed information on coral reproduction is available in Harrison and Wallace (1990), Baird *et al.* (2009) and Barton *et al.* (2015).

Sexual reproduction in corals includes six fundamental steps that are required to produce coral recruits: (1) gametogenesis; (2) spawning; (3) fertilization; (4) planktonic larval stage; (5) settlement including metamorphosis; and (6) post-settlement development (Figure 21.1.3). As every stage can act as a bottleneck, the total production of coral recruits may be drastically reduced or even destroyed if a single step fails. All steps need to be optimized to maximize coral production, which requires adjusting methods to species-specific differences (i.e. brooder *versus* broadcast spawners; see Petersen *et al.* (2007a, b) and Petersen (2008)).

Internal factors such as age (maturity) and external factors such as environmental variations affect the energy available for reproduction and gamete production. Although species-specific, corals may reach maturity within one year, such as the opportunistic brooder *Favia fragum* (Petersen *et al.*, 2007a), or take several years after settlement (see Harrison and Wallace (1990) and Baria *et al.* (2012)). External factors such as competition (algae, corals, etc.), disease, predation, insufficient water quality and inappropriate water movement may lead to reduced energy resources available for gamete production. Nevertheless, *ex situ* coral culture may allow the use of particular conditions that trigger sexual reproduction. Furthermore, the brood stock may be artificially fed to enhance gamete production. A clear determination whether a coral is in the process of producing gametes can be done through histology, as described by Okubo *et al.* (2007). However, this process is time consuming and invasive to the coral. Therefore, a less precise but popular alternative method to predict spawning time is the use of spawning

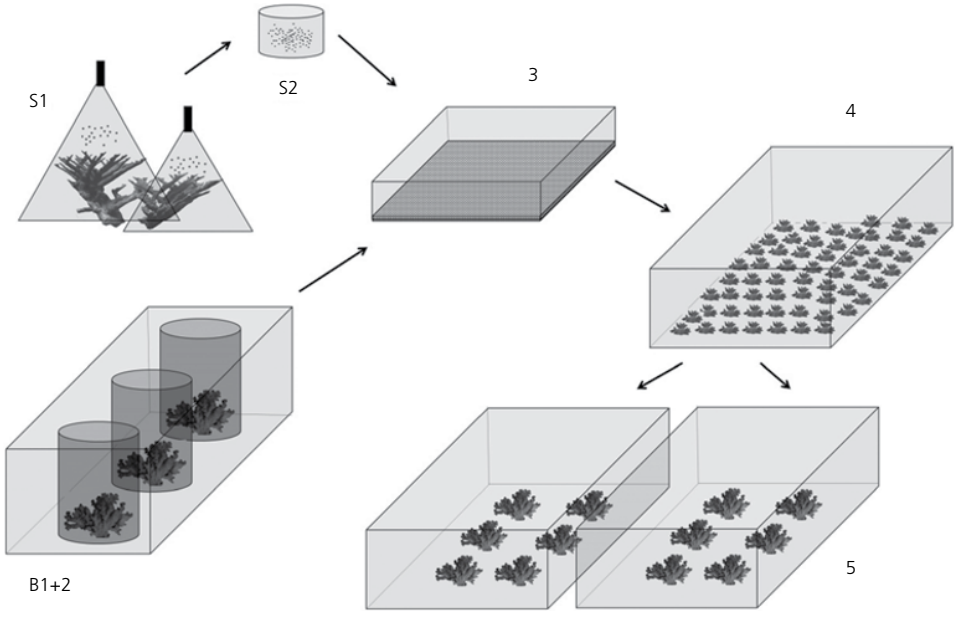


Figure 21.1.3 Typical design for producing sexual coral recruits in aquaculture: gametes of broadcast spawning species are collected in the field using plankton nets (S1). Gametes are fertilized *ex situ* and reared for several days (S2). Brooders usually release settlement competent larvae following internal fertilization and embryogenesis; larvae can be collected from the water surface with cone-shaped larvae traps (B1+2). Regardless of the reproductive group, larvae are similarly treated and added to an aquarium with settlement tiles without filtration system and with water movement provided by airlifts (3). After larval metamorphosis, primary polyps are transferred to recirculation systems for grow-out (4), and further transferred as juveniles increase and require more space (5).

records. Please note that it is not recommended to use a broodstock coral for both asexual and sexual propagation as fragmentation may decrease fertility (Okubo *et al*., 2007).

While sexual reproduction of a limited number of broadcast spawning species has been reported *ex situ*, as reviewed by Petersen *et al*. (2007b) and Petersen (2008), it is not yet possible to induce spawning *ex situ* without natural sun- and moonlight. In nature, spawning occurs at the physiological limits of corals. When this process is simulated under aquaculture conditions it may lead to negative effects, such as the outbreak of temperature induced diseases (Gil-Agudelo *et al*., 2004). While inducing spawning *ex situ* still involves notable challenges, gametes can be relatively easily collected during natural spawning events and grown in aquaculture tanks.

Most coral species are hermaphroditic and release egg-sperm bundles, which are usually positively buoyant and float to the water surface where fertilization occurs. They can be relatively easily collected in the reef using cone shaped tents with a sample bottle on top in which the bundles get trapped (Figure 21.1.3). These tents are placed above the coral a few minutes before spawning. Alternatively, gametes may be collected from broadcast spawners by transferring donor colonies to culture tanks a few days prior to spawning. During the spawning night, the coral colonies can be momentarily moved from the holding tanks to containers with standing water; released gametes can be

carefully skimmed off the surface using plastic pipettes or small cups. However, this method should only be applied if culture conditions are appropriate and the donor colonies can be safely placed back in the reef after spawning due to conservation and sustainability reasons. The egg-sperm bundles will break apart and become fertile within half an hour after spawning and sperm is then applied at an approximate concentration of $10^6\,mL^{-1}$. One or two hours after incubation the fertilized eggs should be transferred to fresh seawater in plastic pins with standing seawater. Frequent water changes are recommended to maintain water quality. Hagedorn *et al.* (2009) successfully culture hundreds of thousands of developing embryos in flow-through chambers during field work as part of SECORE project (see www.secore.org). Larvae are usually fully developed after 1–4 days and ready to be used for settlement at the field location or transported over long distances via airmail or air cargo to any aquaculture facility at concentrations of <4000 larvae L^{-1} (Petersen *et al.*, 2005a).

Harvesting larvae from brooders is much easier because competent larvae are released right away. Larvae can be collected from cultured parental colonies using different methods. For instance, donor colonies can be placed close to the water surface and with cylindrical shaped plankton-mesh cylinders around the colony so that the top end of the cylinder is a few centimeters above the water surface (Figure 21.1.3). Released larvae drift to the water surface where they can be easily collected using a plastic pipette. Larval release usually occurs soon after sunset or after the light has been switched off. Alternatively, plankton-mesh cups may be placed at the water overflow of the culture system to catch larvae that will stay submerged while the water passes towards the filtration system.

Competent larvae require environmental triggers to initiate settlement and metamorphosis. One of the most important and intensively studied cues is the biofilm of the substrate. Crustose coralline algae (CCA) and associated bacteria usually induce settlement, whereas cyanobacteria and turf algae prevent it (Harrison & Wallace, 1990). In *ex situ* aquaculture, settlement tiles should be incubated for one or two months prior to larvae settlement. Tile incubation should be performed in controlled settings that favor "clean surfaces" with CCAs and low cover of other algae or cyanobacteria. Any sediment should be avoided as it will likely inhibit larvae from settling or it might cover and kill early settlers. Water quality and water movement should be carefully monitored, and effective grazing by surgeon fish and small herbivorous hermit crabs is suggested (Petersen *et al.*, 2005b). The shape and surface relief of the substrate may be important to induce and spatially control settlement (Petersen *et al.*, 2005c).

Depending on the scale, several hundred to thousands of these tiles can be placed together using egg crates to create large surfaces for settlement (Petersen *et al.*, 2005c). After settlement, the coral-tile units can be placed further apart as the juveniles develop and need more space. High water quality is critical for the whole process of sexual reproduction, as well as water movement and light conditions, which may play an additional role during the settlement process. Moderate water movement provided by air lifters increases the chances for larvae-substrate to contact with the tiles and consequently enhance settlement. It is recommended that low light levels (<150 $\mu E\ m^{-2}\ s^{-1}$) are used for any settlement set up as settlement commonly occurs on more sheltered and less light exposed substrate; juveniles grow towards more light exposed areas with increasing size (Harrison & Wallace, 1990). However, it is important to note that the

role of water movement and light has been poorly studied *ex situ* and needs further investigation to maximize settlement rates.

Due to their limited size, juvenile corals are more fragile to suboptimum culture conditions compared to semi-adult or adult corals. Therefore it is essential to quickly grow coral recruits up to a larger and more stable size, which may be promoted by external nutrient sources. For example, Petersen *et al*. (2008) showed that growth of early settlers of *Acropora tenuis* and *Favia fragum* increased ten times when fed with live *Artemia* nauplii, and consequently reduced hatchery time significantly. Coral growth both in biomass and numbers is, therefore, reviewed in the following section.

21.1.3 Coral Productivity

Coral productivity is characterized by the production of both numbers (coral nubbins and colonies) and biomass (size/volume) of the target organism. Coral productivity is thus closely related to volumetric productivity, i.e. the amount of product produced per volume of culture system per time. It is, therefore, imperative to have information on growth rates and growth kinetics of the organism being cultured. Productivity is determined by growth rates while the optimal size of the organism for cost-efficient production will be affected by growth kinetics. In this view, it is the maximization of coral quantity production that will be largely based on information on their growth rates and kinetics.

Quantification of coral growth rates for aquaculture purposes have been reviewed by Dullo (2005). Overall, linear extension (expressed in mm $year^{-1}$ or similar) has been used to measure coral growth, which has been thoroughly investigated. Contrastingly, the study of coral growth kinetics is relatively scarce (Bak, 1976; Crabbe, 2007; Osinga *et al*., 2011) and only the study by Osinga *et al*. (2011) has addressed growth rates and kinetics and its association with productivity.

Growth rates vary over time depending on the underlying growth kinetics, as shown in Figure 21.1.4. Linear growth kinetics (not to be confused with linear extension!) is characterized by an increase in biomass over time independent of the size of the coral fragment (Figure 21.1.4a). In contrast, exponential growth suggests that the increase in biomass is positively correlated with increasing fragment size (Figure 21.1.4b). While a constant specific growth rate (SGR; usually expressed in % growth per unit of time, or simply as $time^{-1}$) is observed in corals that grow fully exponentially (first order kinetics, see Osinga *et al*. (2011)), corals displaying linear growth rates will have SGR decreasing over time. However, most coral species neither follow first order kinetics nor linear growth. For instance, tabular and boulder corals, such as *Galaxea fascicularis*, tend to display an exponential growth but a constant linear extension rate. Consequently, the SGR slowly decreases over time (Figure 21.1.4c) (Schutter *et al*., 2010).

Linear extension rates are a useful measure for growth as colony size is an important parameter to the MOT. It is possible to calculate the time needed for a coral fragment to grow to commercial size from randomly measured linear extension rates (Dullo, 2005) if the target coral species displays a constant linear extension. However, culture design should be based on growth kinetics because linear extension rates may vary over time (Figure 21.1.4d–e) and, consequently, random measurements may not be representative for the entire life span of the coral. Furthermore, information on growth kinetics allows analysing size-dependent growth. Higher SGR suggests a concomitant

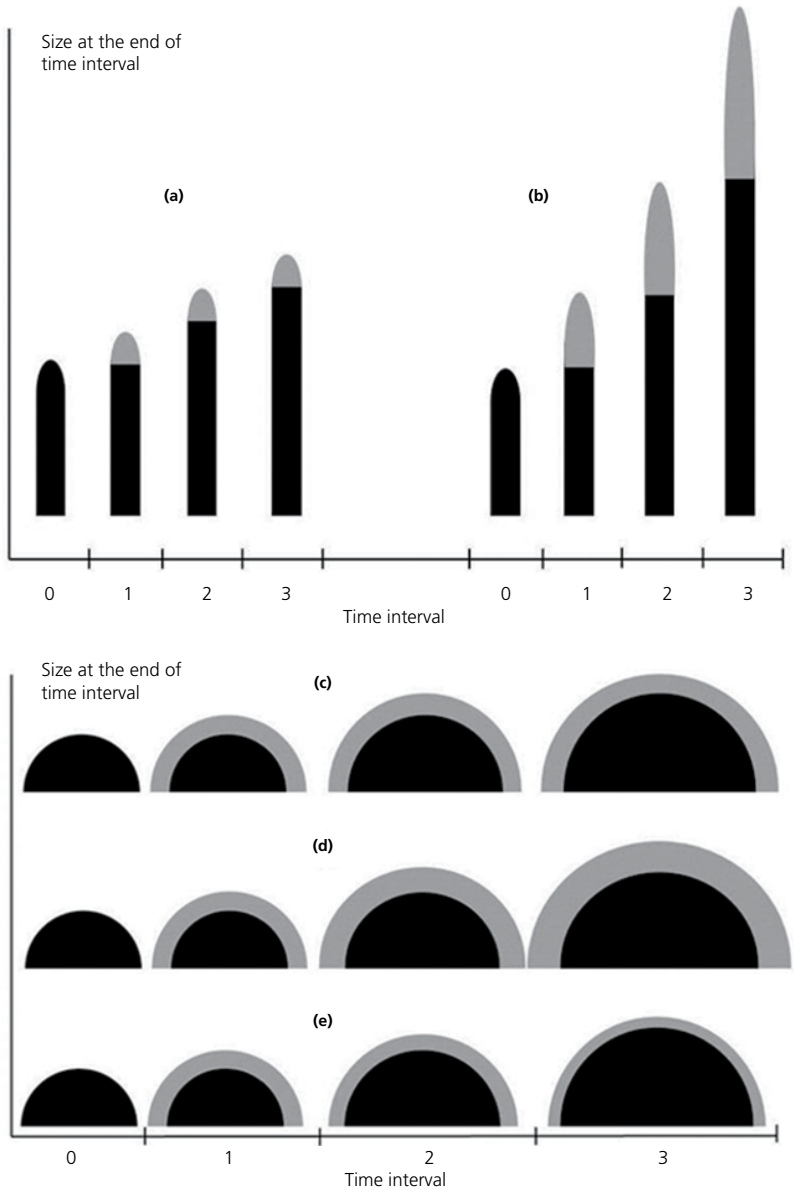


Figure 21.1.4 Conceptual overview of growth kinetics in relation to linear extension rates and coral morphology, representing the growth of hypothetical corals during three time intervals of equal length. (a) Single branch morphology, linear growth kinetics. The linear extension rate is constant over time, the growth increment (grey area) is constant over time, and specific growth rates (SGR) decrease over time. (b) Single branch morphology, exponential growth following first order kinetics. The linear extension rates increase over time, the growth increment (grey area) increases over time and SGR remains constant. (c) Boulder morphology, constant linear extension rate, the growth increment increases over time (exponential growth), but SGR slowly decreases over time. (d) Boulder morphology, exponential growth following first order kinetics. The linear extension rate increases over time, the growth increment increases over time and SGR remains constant. (e) Boulder morphology, linear growth kinetics. The linear extension rate decreases over time, the growth increment is constant over time and SGR strongly decreases over time.

increased growth per coral size and therefore, a higher productivity. For example, the coral *G. fascicularis* shows a SGR that decreases over time. Therefore, the best strategy is to start with very small fragments, or even single polyps if possible. In opposite, branched corals usually grow following first order kinetics during the first two years of maturation. Therefore, fragment size is not influencing culture efficiency, as their growth is size-independent.

Mortality and the need for broodstock materials to initiate the next culture cycle are additional aspects that should be taken into account for commercial coral aquaculture. The following procedures, similar to those outlined by Schippers *et al*. (2012) for designing sponge aquaculture, are therefore recommended. The equations proposed by Schippers *et al*. (2012) can be applied as follows to coral aquaculture:

$$NIF = Y/P_F \quad (21.1.1),$$

where NIF is the number of initial fragments, Y is the target harvest (i.e. the number of commercial sized colonies to obtain) and P_F is the average productivity per fragment. P_F can be calculated from Equation 2:

$$P_F = (1 - M)F_t - Y_2/Y \times F_0 \quad (21.1.2),$$

where M is the mortality factor that is calculated as (100 – percentage survival)/100, Y_2 is the target harvest for the following production cycle, F_0 is the initial fragment size and F_t is the fragment size at the end of the production cycle.

Using *G. fascicularis* colonies as an example, initial fragments may be seeded as single polyps ($F_0 = 1$) and commercialized as colonies with an average size of 100 polyps ($F_t = 100$). If a coral aquaculture endeavor is aiming at producing 100 colonies per culture cycle, the yield Y is the total number of polyps to be produced (i.e. 10,000 polyps). Mortality was calculated to be 10% ($M = 0.1$). Equation 2 will be calculated as follows: $P_F = (1–0.1)*100 – 10{,}000/10{,}000*1 = 89$. This value will consequently be used in Equation 1 to calculate the number of initial fragments needed for a repeated production of 100 commercially-sized colonies of *G. fascicularis* (10,000/89 = 112.36; i.e. 113). From these 113 initial fragments, 11 will be lost during grow-out, 100 can be commercialized and 1.36 will be used to prepare fragments for the subsequent culture cycle.

Prior to any endeavour involving the aquaculture of a particular coral species, we therefore recommend that producers gather information on coral growth and kinetics. If there is no such information, a growth experiment can be executed with the target species to assess the time needed for a small fragment to grow to the desired commercial size to provide the required data to calculate growth kinetics. Moreover, mortality rates and broodstock material for the following culture cycles are also important to consider. While all these factors should be taken into account to setup a coral aquaculture, the culture conditions of the aquaculture system are also of utmost importance, as they will provide the means to maximize coral quality and quantity production.

21.1.4 Coral Quality

Anyone who attempts to culture corals aims to increase productivity, but also has the goal of producing high-quality organisms. However, the definition of coral quality may be controversial. According to Delbeek (2009), the perfect coral in the MOT should be

easy to keep, colorful, display a good appearance and high growth rate. It should also be resilient to variations in water quality, tolerant to different light intensities, not too expensive and readily available. Price and availability rely on market demand, while other characteristics, such as resilience to water variations, vary with coral species. Some other characteristics may also be genetically determined, as a single species may have different genotypes displaying, for instance, different growth rates and different colors. Nonetheless, most of these characteristics are also shaped by the environmental conditions where the coral lives. For instance, growth rates can be maximized, colors can be enhanced and coral appearance can be adjusted. These latter characteristics play an important role in determining coral quality and, ultimately, its price (Delbeek, 2001). For instance, *Turbinaria* and Pocilloporidae species are good candidates for aquaculture as they display high growth rates and resilience to abiotic and biotic variations. Further, *Stylophora* species are also fast growers and usually display an array of attractive colors. In contrast, *Porites* species are massive and exhibit low growth rates.

Coral color is also an important feature. In nature, coloration patterns have a wide range of functions, whereas in the ornamental industry they are only important in decision making and in setting high-retail prices (Delbeek, 2001). Coral color derives primarily from pigments and proteins, through light absorption, reflection or diffraction. Although coral color has been primarily associated with the presence of symbionts, it is now known that coral has several substances that also contribute to its color, such as fluorescent proteins, astaxanthin, carotenoids, melanin and ommochromes. Some pigments may be dietary, for example carotenoids, related to vitamin A, may produce red, orange, and yellow-orange coloration (Borneman, 2001). Besides diet, it has been suggested that color variations may not be a true polymorphism, but rather a manifestation of phenotypic plasticity determined by a homologue to the green fluorescent protein (Kelmanson & Matz, 2003). Asymbiotic corals usually display very diverse colors, which may derive from food sources or pigmentation of their sclerites, which are probably genetically defined or environmentally shaped.

Coral quality can also be associated with shape, which can vary broadly, with some shapes being more attractive than others. Coral shape is difficult to categorize and most popular categories are often arbitrary, as the range of coral morphologies is very wide and the definition of categories is based on personal judgment. Nevertheless, one can follow the categories suggested by Borneman (2001) and Todd (2008). Accordingly, the shape of hard corals can be defined as: boulder, foliaceous, encrusting, branching, arborescent, columnar, tabular and turbinate (Figure 21.1.5). Coral shape may be genetically determined and/or changed by environmental conditions, which is usually known as phenotypic plasticity (Todd, 2008). This plasticity allows corals to acclimatize, for example, to changes in light and water movement, which are among the most important environmental drivers affecting coral shape and branching (Muko *et al.*, 2000; Kaandorp & Kubler, 2001).

One basic concern for coral quality is the use of water with the correct physio-chemical properties in order to meet the corals' preferences (Borneman, 2008). Parameters such as salinity, temperature, pH and oxygen are crucial to maintaining healthy corals, whereas other chemical elements, such as calcium and magnesium, are also important. The use of correct light quantity (usually measured as photosynthetically active radiation; PAR) and quality (with a spectral range that suits the photosymbionts) are other key issues, although only for symbiotic corals, which need light for the symbiont's photosynthetic activity. In order to sustain metabolism and support coral growth, it is

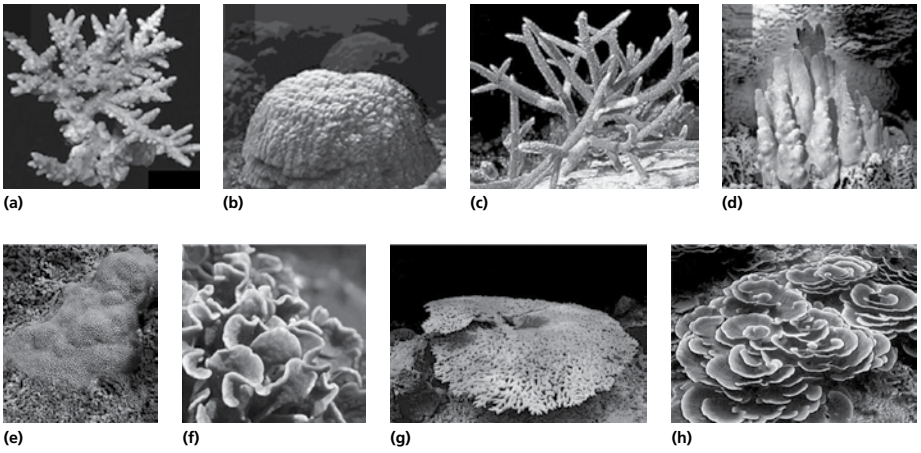


Figure 21.1.5 Different morphologies of scleractinian corals: arborescent (a); boulder (b); branching (c); columnar (d); encrusting (e); foliaceous (f); tabular (g); and turbinate (h).

also important to nourish them. While asymbiotic corals rely entirely on external food sources (i.e. heterotrophy), symbiotic corals can use nutrients provided by the endosymbiotic dinoflagellates and by capturing planktonic organisms. Water flow is also a key environmental parameter that has a considerable effect on coral quality (Kaandorp & Kubler, 2001). It is closely associated with coral nutrition because corals are sessile organisms, and water motion is responsible for carrying away waste products and sediments, as well as transporting dissolved and particulate nutrients to the corals. In nature, although currents and waves usually dominate water flow in shallow reef waters, the hydrodynamic processes also include many other factors, such as wind-driven currents, internal-waves, etc. Such complex water flow characteristics are impossible to replicate *ex situ*.

All these key factors for coral quality are reviewed in the following section, particularly the biological and physiological information based upon which coral quality and quality can be manipulated through changes in culture conditions. The focus is on light, nutrition and water flow, but some other aspects will be shortly discussed as well.

21.1.5 Manipulating Coral Quality and Growth

21.1.5.1 Light

Light is a central factor for symbiotic corals, as it supports the photosynthetic endosymbionts inhabiting the coral host and, therefore, contributes to keeping both symbiotic partners healthy. Although some corals can be facultatively symbionts (Piniak, 2002), most symbiotic corals need appropriate light to be in good condition and grow. However, the selection of appropriate light conditions is species-specific and it may be ambiguous, as a key feature of symbiotic corals is the acclimation to different light environments. Light affects coral quality through its physiological condition, shape and color. It also affects coral quantity because it promotes the production of

photosynthetates from the zooxanthellae and consequently its translocation to the coral host (Leal *et al*., 2015a). This process will, ultimately, contribute to coral calcification and growth.

Light manipulation in *ex situ* aquaculture includes quantitative (irradiance), qualitative (light spectrum), and technological aspects (types of light sources). Light quantity is usually measured through PAR intensity (photosynthetic active radiation), which is a measure that quantifies the light that is available for a symbiotic coral. Light quality is characterized by its spectrum, which can vary with depth and light source. The main light sources commonly used in aquarium corals are: fluorescent lamps, metal halide lamps and LEDs (light-emitting diodes). Please consider other reviews for a more complete analysis on light properties and light sources (Delbeek & Sprung, 1994; Fossa & Nilsen, 1996; Osinga *et al*., 2008).

Most coral ecologists addressing the effect of light on coral physiology have focused on quantity aspects, rather than its quality or source. In contrast, the use of different light qualities and technologies is more interesting for aquaculture and aquarium hobbyists, as they have been continuously searching for more energy-efficient light sources. Simultaneously, different light qualities or technologies are also interesting for aquarists trying to boost coral colors. While for many years the main goal of aquarists was to find the ideal light source that would reproduce natural sunlight, nowadays the main goal is to find the most economic light source that yields the best coral quality and quantity results.

Light Quantity

In the tropics, light irradiance has a bell shape over the day and may reach 2000 $\mu E\ m^{-2}\ s^{-1}$ in surface waters at midday. This quantity decreases in the morning and evening, and with depth. For instance, it can be reduced by 90% at 15 m depth (Trevor-Jones, 2002). Up to a certain limit, the increase in light intensity, and therefore in photosynthetic rates, induces an increase in calcification rates (Houlbrèque *et al*., 2004; Moya *et al*., 2006). Heterotrophic feeding also increases the photosynthetic capacity of corals, as corals obtaining additional food have higher amounts of chlorophyll, and therefore higher photosynthetic rates than starved corals (Houlbrèque *et al*., 2004). Moreover, maximal photosynthetic rates are achieved at a higher PAR intensity when corals are fed. These results imply that for efficient coral growth, a combination of high PAR intensity and regular feeding is needed. Besides heterotrophic feeding, water flow also acts synergistically to the two previous factors to increase coral growth (Schutter *et al*., 2011). However, relatively high light levels (e.g. >600 µmol quanta $m^{-2}\ s^{-1}$) may trigger coral bleaching and concomitantly decrease its growth or even induce mortality (Osinga *et al*., 2008).

Light changes usually induce short-term adaptations in photosynthetic rates, while medium and long-term adaptations are observed through other mechanisms, such as coral morphology, as corals change their growth pattern with changes in ambient light conditions (Titlyanov & Titlyanova, 2002). A coral exposed to low light levels will try to increase its horizontal surface to maximize incoming light. Consequently, the coral will become more flattened as compared to a coral of the same species exposed to high light irradiance. Light variations may also induce changes in color. Overall, the total amount of chlorophyll present in the symbionts living in coral polyps will increase when moved to low light, which will intensify the brownish color in the coral surface (Rocha *et al*.,

2013a,b). These changes in pigment content and/or symbiont cell density usually occur in medium-term scale, as it takes several weeks to observe notable differences (Titlyanov & Titlyanova, 2002). However, this will only be observed in corals exposed to low light levels, yet high enough to support photosynthesis. Further, if photon capture efficiency is already close to 100% there is no need to further intensify pigmentation with lower light and no changes are expected in coral color.

The opposite variation in light intensity, i.e. from low light to high light, will reduce the intensity of the brown coloration as pigment content will be reduced to prevent oxidative stress (Gorbunov *et al*., 2001). However, it is important to note that this photophysiological defence mechanism is only expected to occur when light levels increase beyond 200 µmol quanta m^{-2} s^{-1}. This will affect coral color, as well as changes in the content of photoprotective pigments and proteins that are usually observed in corals following an increase in light intensity (Leal *et al*. 2015b). Such proteins are usually known as fluorescent proteins, which are defined by their excitation and emission spectra and display a wide range of attractive colors. D'Angelo *et al*. (2008) showed that colonies exposed to low light intensities appeared brownish, whereas those exposed to moderate light levels displayed distinct colorations, such as blue, green and red. The increase in pigmentation of the latter corals was notable when the tissue was excited with blue or green light. While these proteins are essentially absent in corals grown under low light intensity, their concentration proportionally increases up to irradiance levels higher than 400 µE m^{-2} s^{-1}. It has therefore been suggested that fluorescent proteins have a photoprotective role, as well as other host pigments that are responsible for the intense bluish, green, or reddish hues (Salih *et al*., 2000; D'Angelo *et al*., 2008). This photoprotective role was supported by a more intense green fluorescence of *Acropora nobilis* in light-exposed parts of the branches, as well as an increase in pigmentation of a pink morph of *Pocillopora damicornis* upon exposure to high light intensity (Takabayashi & Hoegh-Guldberg, 1995; D'Angelo *et al*., 2008).

Light Quality and Source

Light quality is primarily defined by the light source, as different light sources can produce different light spectra.

Symbiotic corals have their zooxanthellae well adapted to light, as the pigments involved in photosynthesis absorb certain wavelengths. A large variety of pigments exist in microalgae, including zooxanthellae, and all have specific capacities with respect to light capture. Zooxanthellae living in coral tissues can adapt their pigment composition in order to maximize the use of the light that reaches them. Because different pigments have different absorption spectra and different organisms may contain different combinations of pigments, phototrophic organisms can inhabit areas with a wide range of light conditions. This explains why even the same coral species inhabiting different places on the same reef can be adapted to different light conditions.

The most well-known pigment in zooxanthellae is chlorophyll *a*, which is the most efficient with respect to energy transfer at wavelengths of 400–500 and 630–700 nm. Hence, light spectra with intensity peaks in these ranges are useful to grow organisms with high chlorophyll *a* content (Osinga *et al*., 2008). Almost all symbiotic corals can also use blue light (400–500 nm), which penetrates deeper in the water. In contrast, not all corals are suited to efficiently use red light (600–700 nm) (Wijgerde *et al*., 2014). Nevertheless, symbiotic corals generally adapt to their light availability by making modifications in

the pigment densities of their zooxanthellae (Leal *et al*., 2015b). Corals inhabiting shallow water environments tend to be lighter in color, and most have UV absorbing pigments that tint them with beautiful colors, such as purple, pink, blue and green (Delbeek & Sprung, 1994; Osinga *et al*., 2008). Corals are very sensitive to UV-A and UV-B light, which can destroy their genetic material. Therefore, the production of such colored pigments and proteins will protect them and their photosynthetic endosymbionts from damaging light. This adaptive response is not immediate, and placing corals that were adapted to low light, or temporarily light starved, into a location with high irradiances can induce light-shock, which may damage the coral beyond its capacity to repair itself. A study by D'Angelo *et al*. (2008) showed that the molecular response of *Acropora pulchra* and *A. millepora* after 8 hours of light stimulation was still lower than a control group of coral exposed to blue light for 4 weeks. This relatively slow increase in the genetic expression of corals suggests that the accumulation of pigments is a medium/long-term adaptive process.

Another study investigated the individual and combined effect of blue and red light on coral growth and photophysiology (Wijgerde *et al*., 2014). According to the results of this study, red light negatively affects the health, survival, symbiont density and chlorophyll *a* content of the symbiotic coral *Stylophora pistillata*. The response process to light stress that usually involves increasing the amount of photoprotective pigments and fluorescent proteins will simultaneously provide the coral with intense and stunning colors. For instance, some corals display a violet color, which can indicate they are reflecting or filtering excessive UV light and protecting themselves from harmful UV wavelengths (Delbeek & Sprung, 1994). Although in these situations coral quality will likely not be the best, because the coral is under stress, it will display more attractive colors, which will make the coral more attractive and more expensive. It is important to note that although these fluorescent proteins have been considered as mechanisms to protect the corals and their symbionts from harmful effects of stressing light, several studies have shown that such proteins may also have other roles in coral physiology. For instance, pocilloporin, a pink pigment present in some corals may function in the coral's immunological and chemical defence systems (Dove *et al*., 1995).

As previously mentioned, light quality can also be changed through the use of different light sources. Each type of illumination systems has its own advantages and disadvantages. They all vary in initial and maintenance cost, light spectrum, longevity, efficiency, and power. Metal halide lamps and fluorescent lighting are the two most commonly used types of aquarium lighting (Osinga *et al*., 2008). Metal halide lamps have been widely used because of their relatively close resemblance to the sunlight spectrum. However, for aquaculture purposes, they are not the most energy efficiently light source. Fluorescent lights, as well as LEDs, allow greater manipulation of the light quality provided to the corals. This feature may be a great advantage for those looking to maximize coral quality and quantity through light manipulation. Metal halide lamps are point sources of light, which provide a scattering that resembles sunlight. Fluorescent light sources provide a more diffuse, uniform light pattern. Relatively recent in aquarium technology is the use of new LED light technologies, which provides a uniform light pattern and an unlimited range of color spectra. These have the potential to be much more efficient than any other technology. Furthermore, they can be easily adjustable to different power levels and light spectra. Although LED popularity is rapidly increasing, to our knowledge only few studies have been published regarding the use of

different light sources and its effect on the photobiological condition of zooxanthellae living in coral tissues (Wijgerde *et al*., 2012; Rocha *et al*., 2013c; Wijgerde *et al*., 2014). Indeed, such crucial information for coral aquaculture is still missing. Most coral scientists have been focusing their research on ecological questions rather than aquaculture applications. Nevertheless, a recent study investigated the effect of using light emitting plasma (LEP) or LED light sources, as well as different irradiances, on the growth of the hard symbiotic coral *Galaxea fascicularis* (Wijgerde *et al*., 2012). According to this study, significantly higher growth rates were observed for corals reared using LEP for two of the tested higher irradiances (125–150 and 275–325 μE m^{-2} s^{-1}). In contrast, another study comparing LEP, LED and T5 illumination systems to grow the stony corals *Acropora formosa* and *Stylophora pistillata* suggests that LED increases growth rates of *A. formosa*, whereas growth of *S. pistillata* is maximized using either T5 or LED (Rocha *et al*., 2013c).

21.1.5.2 Nutrition

While asymbiotic corals only use heterotrophic resources (particulate and dissolved organic matter) to supply their energetic needs, symbiotic corals rely on heterotrophy and autotrophy (synthesis of organic molecules from inorganic ones). The animal host is heterotroph and ingests a wide range of particles, while the autotrophic capacity is provided by photosynthetic symbionts, i.e. zooxanthellae.

Symbiotic corals indeed benefit from the association with photosynthetic symbionts, which transfer most of their photosynthates to the host for its own nutrition (Muscatine *et al*., 1981). Theoretically, such corals may survive without heterotrophic nutritional sources. However, photosynthates are rich in carbon but often deficient in some organic and inorganic molecules, such as nitrogen and phosphorus, which are essential for growth (reviewed by Houlbreque and Ferrier-Pagès (2009)). Therefore, the utilization of external nutrient sources is extremely important to endorse coral growth, even in symbiotic corals. External nutrient sources of corals are very diverse (Figure 21.1.6) and can be separated in organic and inorganic compounds. Organic nutrients usually contain carbon and are associated with matter that has come from a once-living organism, while inorganic nutrients are compounds necessary to sustain life processes and are not chemically carbon-based.

Organic Nutrients

Since inorganic carbon (mainly carbon dioxide and bicarbonate) is not a limited source in seawater, and is transformed into organic molecules via photosynthesis performed by zooxanthellae, it has been suggested that corals are not carbon limited. This is often the case for surface living corals, which have high rates of photosynthesis. However, corals living in deeper, turbid or temperate environments might be light limited, and photosynthesis may not supply enough carbon for the coral's needs, particularly for calcification. The host has therefore to rely on external carbon molecules. Depending on its form, organic matter is usually categorized in dissolved and particulate matter. The latter can be divided into detrital or living particulate matter. Dissolved organic matter includes sugars, free amino acids and urea, among others (Grover *et al*., 2006, 2008). Detrital organic matter is mainly in the form of marine snow, or sediment particles, which are deposited and can be re-suspended near the corals (Anthony, 1999). Finally,

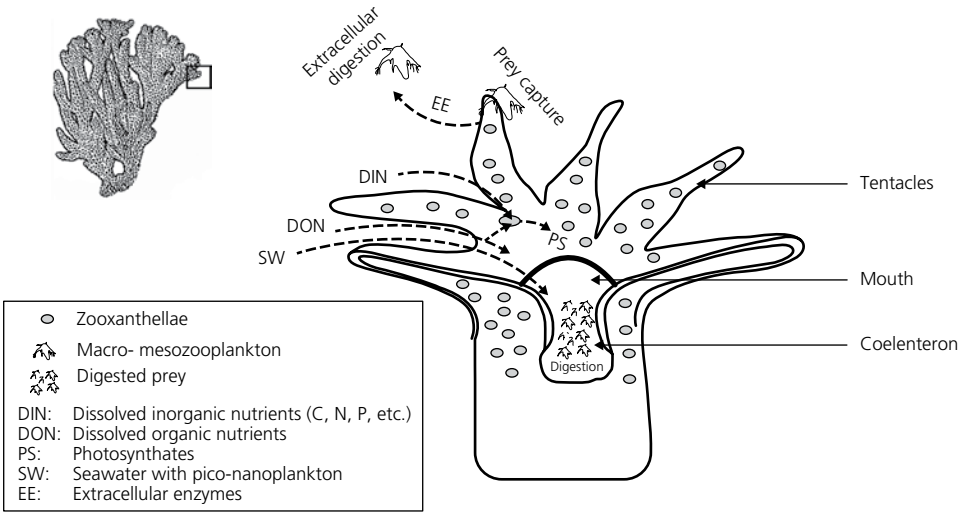


Figure 21.1.6 Nutritional sources of symbiotic corals. Strait lines indicate different parts of the coral polyp. Dashed lines indicates processes of nutrient acquisition, either through the zooxanthellae or the coral the tissue (inside or outside the coelenteron).

live particulate matter includes all sorts of planktonic organisms, from pico- to nano- and mesozooplankton. Corals are able to ingest all these sources of organic matter, and also particles from a large size range (Houlbrèque & Ferrier-Pagès, 2009). However, due to physical constraints or to their feeding selectivity, not all coral species can ingest all ranges of particles (Sorokin, 1991; Sebens *et al*., 1996). While some asymbiotic soft corals, such as *Dendronephthya* species, feed almost exclusively on phytoplankton, the capacity that symbiotic hard corals have to feed on such microalgae was only recently demonstrated (Leal *et al*., 2014a, b). It is important to note that there is no ideal prey to nourish corals, as feeding preferences, prey capture and digestion rates vary with coral and symbiont species (Leal *et al*., 2014c, 2015a).

It has been demonstrated that heterotrophic feeding increases photosynthetic rates of the symbionts, which could also mean that more photosynthetic products are available to the animal host (Houlbrèque & Ferrier-Pagès, 2009). Corals fed with live prey also have twofold greater organic matrix synthesis, calcification, protein and lipid content than starved corals (reviewed by Houlbrèque & Ferrier-Pagès, 2009). Moreover, skeletal growth can be 30% higher in fed corals, as verified for the symbiotic corals *Stylophora pistillata* and *Turbinaria reniformis* (Ferrier-Pagès *et al*., 2003; Treignier *et al*., 2009). Heterotrophic feeding also improves coral quality as it contributes to maximize resilience to stress and potential for recovery (Grottoli *et al*., 2006). This is important to increase the tolerance of corals to changes in water quality and light variation that often occur in the MOT.

Live prey is an important source of key nutrients (mainly nitrogen, carbon and phosphorus) that are available in an appropriate biological ratio. While providing live prey is not expected to disturb the nutrient balance inside the coral, feeding frequency may alter this balance if the nutrient load in the surrounding water decreases water quality and interferes with the natural inorganic nutrient concentrations. For example,

Forsman *et al*. (2011) observed a significantly slower growth of *M. capitata* and *P. damicornis* when feeding doses increased too much and deteriorated water quality. Another important result was the observation of interspecific differences on growth rates. This indicates that each species has unique requirements and that coral colony health or genotype prior to fragmentation may affect subsequent growth and survivorship.

Besides live diets, there are also artificial diets commercially available often made of one, or a combination of several, of the following components: planktonic eggs or larvae, such as oyster larvae, meso- and macrozooplankton, such as rotifers and copepods, microalgae and cyanobacteria. Such diets are usually rich in amino and fatty acids, proteins and carotenoids. Unfortunately, as the supply of artificial diets is mostly important for aquaculture and aquarium hobbyists, the investigation of the effect of such artificial diets on coral quality and quantity has been relatively scarce. Nevertheless, artificial diets may also contribute to coral quality through coral color. Such properties are regularly justified by the presence of trace elements and amino acids that boost coral growth and color. However, it is still unclear how such ingredients enhance coral color. For instance, it is known that the presence of carotenoids affects the color of the asymbiotic soft corals (Cvejic *et al*., 2007; Maia *et al*., 2011). Although their ecological role and physiological function is still highly debated, it is known that different carotenoids concentrations produce different coral colors. Carotenoids are some of the most widespread pigments found in nature and cannot be synthetize *de novo* by animals. Therefore, carotenoids are either dietary or are present in zooxanthellae of symbiotic corals. A key question, however, is whether the dietary pigments are translocated to the coral tissue or skeleton. In the case of the asymbiotic corals of genus *Corallium*, such as the red coral *C. rubrum*, their coloration is associated with the organic matrix. Since corals are slow growers, coral nutrition should not have an immediate effect on coral color, because the pigments must be assimilated and incorporated in the coral matrix as it grows. Therefore, in the case of the red coral, its color results from the assimilation of pigments throughout its life history (Cvejic *et al*., 2007). In contrast, stony corals usually have a white skeleton, made of calcium carbonate in the form of aragonite. Therefore, the color of these corals derives from the tissue layers existing above the skeleton, where coral and symbiont pigments and fluorescent proteins are present. Although, to our knowledge, the manipulation of coral color though nutrition has never been studied, Borneman (2001) states that some pigments, such as carotenoids related to vitamin A, may be dietary, and produce red, orange, and some yellow-orange coloration. Besides carotenoids, there is evidence that nutrient enrichment affects coral colors. For instance, potassium additions resulted in intensified colors together with better growth of all photosynthetic organisms (Pawlowsky, 2008). Also, the application of trace elements may lead to better colors, as well as increased growth rates (Balling *et al*., 2008). Accordingly, the addition of trace elements specifically enhances green fluorescent colors in corals.

Inorganic Nutrients

Inorganic nutrients, particularly phosphorous and nitrogen are important building blocks for coral biomass production. However, this is a process mediated by the symbionts and, therefore, only symbiotic corals can take up inorganic nutrients. Inorganic nutrients can also be artificially supplied (nutrient enrichment) to improve coral quality and quantity.

Nitrogen is usually a limiting nutrient in oligotrophic coral reef environments (concentrations <0.5 µM, (Atkinson *et al*., 1995)). It is mainly found as nitrate, ammonia and urea, and is needed for growth. Both internal and external nitrogen sources are available to corals. Indeed, urea and ammonia, two forms of nitrogen that are by-products of coral host metabolism, are not excreted and released by the symbiotic association, but are re-used by the symbionts and transformed into photosynthates (Muscatine & D'Elia, 1978). The uptake of seawater inorganic nitrogen is also mediated by the symbionts, although the coral host has adapted to transport these molecules through its membranes (Grover *et al*., 2002; Godinot *et al*., 2009). The availability of nitrate (NO_3^-) and ammonia (NH_4^+), which are the two main forms of seawater inorganic nitrogen, is critical for coral growth and particularly important during heat-stress (Godinot *et al*., 2011a). The latter study further showed that the stony coral *Stylophora pistillata* decreases its capacity to take up nitrogen under such conditions. However, this same coral species is able to maintain its photosynthetic capacity if nitrogen is supplied (Béraud *et al*., 2013). Thus, nutrient enrichment can maximize coral quality and quantity (Godinot *et al*., 2011b). In contrast to the supply of live prey, where the acquired nutrients are first available to the coral host, the artificial supply of inorganic nutrients primarily affects the symbionts hosted in the coral tissue. This occurs through an increase of protein, lipid and chlorophyll concentration in symbiont cells and by stimulating proliferation of the symbionts (reviewed by Houlbrèque & Ferrier-Pagès, 2009). Nevertheless, nitrogen assimilated by the symbionts is rapidly translocated to the coral host (Piniak & Lipschultz, 2004). Nitrogen enrichment only works up to a certain level, above which eutrophication problems (chaotic development of bacteria, phytoplankton and algae) may affect the culture system. In addition, although most corals readily use inorganic nitrogen, both ammonia and nitrate are highly toxic to most marine life and can damage sensitive animal tissues at high concentrations (Muller-Parker *et al*., 1994; Grover *et al*., 2003).

Phosphates are also taken up by the symbionts and are usually found in minute quantities in coral reef waters, thus becoming limiting for reef biological processes. Phosphorus needs vary from one species to another, and some symbiotic corals, particularly *Stylophora pistillata*, may have greater limitation in phosphate than in nitrogen under culture conditions (Godinot *et al*., 2011c). However, phosphate tends to accumulate in aquaria and can even become problematic, especially because it may affect coral calcification (Borneman, 2008). High phosphate level is usually associated with overfeeding of marine organisms, particularly fishes, and is usually accompanied by increasing proliferation of algae.

Micronutrients also have an important role in coral physiology. Trace elements, such as metals, can be incorporated into corals by a variety of mechanisms, such as trapping of particulate matter within skeletal cavities, uptake of organic matter from coral tissue and coral feeding (Hanna & Muir, 1990). While micronutrients may be limiting when present in low concentrations (Ferrier-Pagès *et al*., 2005), elevated trace metals concentrations may be harmful to corals (Ali *et al*., 2011).

Before considering nutrient enrichment to improve coral quality and productivity, it is important to consider the water properties in the culture system. One key issue is the source of seawater, as natural seawater is different from artificial seawater, prepared with commercially available sea salts. This issue is particular important for micronutrients and trace elements that are available in natural seawater but may be scarce in

artificial seawater. Regardless of the water source, nutrient enrichment may be considered. It is also important to note that bacteria are likely to benefit more from nutrient enrichment than cultured corals. Contrastingly, the supply of live prey does not usually induce the anarchic development of bacteria unless bacteria use food that is degrading in the tank.

21.1.5.3 Water Flow

Water flow can be turbulent or laminar. Turbulent flow is the most common type of flow and is characterized by uneven fluctuations and irregular particle direction and speed. In contrast, laminar flow is characterized by its unidirectionality where fluid particles move in smooth straight paths or layers. Wave-making systems create a bi-directional flow in order to simulate the wave flow commonly observed in coastal regions. Although it is difficult to create laminar flows, the idea of the wave-making systems is to create a laminar flow that goes back and forth. Most *ex situ* coral studies attempt to create a laminar flow system, as it maximizes food capture rates and recreates natural flow conditions. In this view, the use of laminar flow is recommended, as it allows corals to improve their feeding rates and, therefore, improve coral quality and productivity. Although no study has ever investigated wave-making systems on coral nutrition and shape, it should produce similar results to laminar flow systems. Besides different flow systems, water flow can also be manipulated through speed.

Without sufficient water flow, corals, as other benthic organisms, become isolated from the surrounding environment. If water flow is not suitable, a motionless water layer may be created around the coral. This layer will affect the coral metabolism, as water flow aids the exchange of respiratory gases, dissolved food, and waste across coral surface (Delbeek & Sprung, 1994). In nature, corals are adapted to their environment, and it is common to observe different species in different reef areas. Therefore, different coral species have different water flow preferences, and it is not possible to define the ideal water flow to use in coral aquaculture systems. Nevertheless, the relation between corals and water flow has been thoroughly investigated, and the available information can help to predict how corals respond to water flow changes (Sebens, 1984; Sebens *et al*., 1997; Finelli *et al*., 2006). For instance, branching and cylindrical coral species increase food capture with increasing flow. In contrast, flat coral species show no effect of flow speed on particle capture due to the tendency of the coral's tentacles to be flatten under high flow speed (Kaandorp & Kubler, 2001). The increase of flow speed will maximize the particle delivery rates to the coral surface. However, particle capture efficiency often decreases with increasing flow. This is due to the tendency for the coral's tentacles to flatten under high flows, which diminishes the capture of particles moving across their surfaces. Therefore, particle capture rates are often highest at intermediate flow speeds, where particle delivery rate is high, but coral polyps are still capable of retaining food particles. Some species are able to partially circumvent this limitation through the formation of aggregations in which spacing between branches varies as a function of ambient flow. It has been suggested that plasticity in branch spacing represents a means of acclimatizing to the characteristics of the local flow environment (Sebens *et al*., 1997; Kaandorp & Kubler, 2001; Todd, 2008). Accordingly, laboratory studies with laminar flows confirmed that particle capture increased with branch spacing in low flows, but decreased with branch spacing in higher flows. Space between

coral branches affects colony compactness. Therefore, compact growth forms are generally found in environmental conditions with large exposure to water movement, and the growth forms usually gradually change into a branching shape when the amount of water movement decreases (Kaandorp *et al*., 1996). Moreover, thickness of coral branches is higher in high-flow conditions, as observed for the hard symbiotic coral *Pocillopora damicornis* (Lesser *et al*., 1994). Besides spacing between branches, the presence of neighbouring coral colonies also affects particle capture. For the soft coral *Alcyonium*, lower particle captures were observed under low flow conditions and when surrounded by other colonies (McFadden, 1986). In contrast, higher flow speeds increased particle capture rates.

Overall, the effect of water flow on coral quantity is reflected by the sum of the flow effects on particle capture, nutrient uptake, photosynthesis and respiration (Sebens *et al*., 2003). Water flow enhances photosynthetic rates of zooxanthellae and increases respiration rates of coral tissue, as well as calcification, growth rate, uptake of dissolved nutrients and waste disposal from coral surfaces. For instance, water flow will interact with light, because it determines both the rate of supply of nutrients needed for photosynthesis and the efflux rate of oxygen and oxygen radicals that may inhibit photosynthesis (Osinga *et al*., 2011). For example, in the symbiotic coral *Galaxea fascicularis*, a combination of high light and flow have a stronger positive effect on growth than the individual factors (Schutter *et al*., 2011).

Water flow characteristics, particularly type and speed, are also important for the production of asymbiotic corals in quality and quantity (Janes, 2008). Adequate water flow is necessary to encourage polyp expansion and allow high prey capture rates. According to Janes (2008), several studies showed that asymbiotic soft corals are able to capture prey in a wide range of velocities, and many species showed a near optimal velocity range of 8 to 12 $cm\,s^{-1}$.

In conclusion, the use of correct water flow characteristics is critical to keep corals in good condition. The manipulation of water flow can also produce different coral shapes, as morphological variations, particularly in hard corals, are induced by plastic responses to the environmental conditions or by genetic constraints (Todd, 2008). Generally, high-density branching can be obtained with high flow conditions, whereas low-density branching can be achieved with low water flow. In naturally branching species, low flow tends to create thin branches, while high flow tends to induce more robust branches. Flow speed is also important to improve coral growth rates, mostly because of its interaction with light and nutrition. While indirectly, water flow may also promote coral productivity by removing sediment and by preventing settlement of fouling organisms such as algae (Fabricius, 2005).

21.1.5.4 Others

The use of substrate in the culture tank may affect coral mortality, particularly of coral nubbins immediately after fragmentation. Lirman (2000) observed higher mortality when sand, rubble and hard substrates were used, whereas no mortality of *Acropora palmata* nubbins was observed when fragments were placed on the top of live colonies of the same species. While sediment may be negative when rearing recent-fragmented corals, its presence may be beneficial as a nutrient sink (Fossa & Nilsen, 1996). Sediments

help keeping nutrients in relatively low concentrations and allow corals to thrive without being overgrown by algae and bacteria. Moreover, sediments rich in detrital organic matter and bacteria may provide an additional nutrient source for corals (Leal *et al*., 2014a). In this regard, it is beneficial to use sediment together with benthic organisms, such as gobies, sea cucumbers and/or shrimps that actively rotate the substrate and contribute to maintaining a natural equilibrium in the sediment (Fossa & Nilsen, 1996). A thick layer of sand will also create anoxic zones below aerobic ones present in the sediment, which will support the occurrence of biological processes as in live rock. The bottom sand layer will act as a denitrifying filter due to the presence of specific groups of bacteria. The use of herbivorous fish and invertebrates, such as tangs and sea urchins, may also offer another nutrient source for corals. Their grazing of undesired algae will release large quantities of detrital-sized algal fragments, which may be taken up by corals. A portion of the nutrients fixed by fishes is released back into the water in the form of feces, which become available as nutrients for corals.

The interaction of different coral species within the same culture system may also affect coral quality and productivity. Corals are known to produce bioactive compounds (Rocha *et al*., 2011; Leal *et al*., 2013) that are often used to compete for space and resistance against fouling organisms. However, this particular issue is still poorly investigated under controlled conditions. Nevertheless, chemical extracts from the stony coral *Tubastraea faulkneri* are known to kill larvae of other coral species (Koh & Sweatman, 2000). Although this study focused on a symbiotic stony coral, a larger and increasing number of new molecules have been discovered in soft corals (Leal *et al*., 2012). In this regard, combining different coral species, either stony or soft corals, in the same culture system should be assessed before hand to avoid a "chemical war" between the cultured species that will likely induce coral mortality and deteriorate water quality.

One last factor to consider for manipulating coral quality and productivity is broodstock selection. Different specimens/genotypes may have different growth rates, color- and morphotypes, and acclimation potential. Genetic manipulation and selection of genotypes exhibiting a particular coral shape may also be possible. However, this practice is nor as fast nor as flexible as phenotypic plasticity (Kaandorp & Kubler, 2001). Other factors that also affect coral quality and productivity are the size of the coral fragments and origin of the fragment, i.e., location in the mother colony, as previously discussed in the coral propagation section.

21.1.6 Conclusions

The aim of any aquaculture is to work efficiently at low costs and low labor. While coral aquaculture is a growing practice, it is in its infancy as compared to other marine ornamental organisms (Olivotto *et al*., 2011). Nevertheless, the current knowledge on coral physiology and ecology can be used to understand the mechanisms shaping coral quantity and quality, and to develop culture methods accordingly. It is also important to note that most knowledge of coral husbandry and culture practices is present in grey literature and mostly disseminated among aquarium hobbyist forums. The interaction between coral aquaculture scientists and marine aquarium hobbyist and stores should be expanded to contribute to substantiate and improve current knowledge on coral aquaculture *ex situ* and its efficient application to the MOT.

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21.2

Sea Anemones

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Abstract

Sea Anemones that form an iconic association with anemonefishes are highly sought after by marine aquarists. Currently, no commercial culturing protocols have been established for these host anemones, and individuals are sourced from the wild, causing localized depletions in some areas of the Indo-Pacific. This chapter details the reproductive biology of sea anemones, and then outlines potential approaches to culturing host anemones, and future research and industry development needs. Characteristics that appear favorable for captive breeding through sexual reproduction include having predictable annual spawning seasons, releasing large numbers of broadcast spawned gametes that readily fertilize, and larvae with a relatively short planktonic period that settle onto a variety of substrata and require little feeding to optimize growth. Alternatively, propagation using fission, by natural or artificial means, could produce individuals throughout the year. Although, much work remains to be done, aquaculture is a possible solution for addressing concerns about overexploitation, and individuals could be used to supply the trade or restock depleted reefs.

Keywords *Reef restoration; aquarium trade; captive breeding; sexual reproduction; asexual reproduction; spawning; longitudinal fission; clownfish*

21.2.1 Introduction

Sea anemones belong to the order Actiniaria in the class Anthozoa, one of five classes that comprise the phylum Cnidaria (Fautin, 1992; Fautin, 2007). There are over 1000 species, which are exclusively solitary, and have a cylindrical body with an oral disc that has a central mouth surrounded by tentacles, and a pedal disc that attaches to the substrate (Fautin & Mariscal, 1991; Fautin, 1999; Daly *et al*., 2007). They are found throughout the world's oceans in tropical, temperate and polar regions; they occur at all depths, from the shallow intertidal to the deep-sea; and occupy various habitats such as rock pools, muddy or sandy substrates, seagrass beds, and coral reefs (Thomas & Shepherd, 1982; Dunn, 1983; England, 1987; Fautin & Allen, 1997; White *et al*., 1999).

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Only ten species are known to provide essential habitats for 28 species of obligate symbiotic anemonefish (and hereafter referred to as host anemones) (Table 21.2.1) (Dunn, 1981; Fautin & Allen, 1997; Ollerton *et al*., 2007; Allen *et al*., 2008; 2010). This symbiosis typically occurs on, or near coral reefs, in the suitable shallow and mesophotic waters of the Indo-Pacific (Mariscal, 1970; Dunn, 1981; Fautin & Allen, 1997; Bridge *et al*., 2012). Owing to the unique and fascinating interactions between the anemonefish and their hosts, these anemones are highly prized for the aquarium trade (Fautin & Allen, 1997; Wabnitz *et al*., 2003) and will be the primary focus of this chapter. Most other sea anemones are of little commercial value, although some are traded as marine ornamentals (e.g. *Condylactis gigantea* and *Bartholomea annulata*, which form associations with crustaceans and several fishes other than anemonefish), and some are sold for human consumption (Hanlon & Kaufman, 1976; Fautin, 2000; Gasparini *et al*., 2005; Rhyne *et al*., 2009).

Lack of regulation within the marine ornamental fishery makes it difficult to obtain quantitative data about the number of species and organisms collected, as well as the locations of these collections (Dufour, 1997; Wood, 2001; Wabnitz *et al*., 2003; Cohen *et al*., 2013). However, it is estimated that 9 to 10 million marine invertebrates (excluding corals) are traded annually, with host anemones among the most popular species (Wabnitz *et al*., 2003). Developing island nations such as Indonesia and the Philippines tend to be the main exporters, with most animals going to developed nations such as the United States, the European Community, and Japan (Wood, 2001). Host anemones represent high-value species for collectors. In the Philippines, the price paid to fishers for anemones can be up to 13 times the amount for anemonefish (Shuman *et al*., 2005), while in Indonesia it can be more than 50 times the amount (Madduppa *et al*., 2014). This means that they are often preferentially harvested, and thus incur substantial fishing effort (Edwards & Shepherd, 1992; Shuman *et al*., 2005; Madduppa *et al*., 2014).

Host anemones are typically rare on reefs (Dunn, 1981; Chadwick & Arvedlund, 2005; Hobbs *et al*., 2013; Scott & Baird, 2014) and are currently sourced entirely from the wild. High levels of harvesting from limited extraction areas have caused localized depletions (Edwards & Shepherd, 1992; Shuman *et al*., 2005; Madduppa *et al*., 2014). For example, excessive harvesting has adversely affected both the size and density of anemone populations near Cebu in the Philippines (Shuman *et al*., 2005), and depletions and a localized extinction have been reported for *C. gigantea* (Gasparini *et al*., 2005; Rhyne *et al*., 2009).

Host anemones have a number of life history characteristics that make them particularly vulnerable to overexploitation. They are potentially very long-lived and slow-growing (Fautin & Allen, 1997). Most species rely solely on sexual reproduction as a way of maintaining and renewing their populations, having limited annual breeding seasons, with density-dependant fertilization that can be adversely affected by overexploitation, and subsequent larval recruitment that is thought to be rare (Dunn, 1981; Babcock *et al*., 1992; Fautin & Allen, 1997; Scott & Harrison, 2005; Scott & Francisco, 2006; Scott & Harrison, 2007a,b; 2008; 2009). Thus for aquarium collection rates to be sustainable, exploitation must not exceed the ability of the anemones to repopulate impacted regions (Shuman *et al*., 2005).

The susceptibility of host anemones to overexploitation is exacerbated by ease of collection (Edwards & Shepherd, 1992). Host anemones are sedentary, typically occur in relatively shallow water, and their bright coloration and association with anemonefish

(which hover above them), make them easy to locate (Dunn, 1981; Fautin & Allen, 1997). Poor survival in captivity leads to added pressure on populations as more anemones are collected to compensate for stock loss.

There are a number of other factors that can adversely impact host anemone populations. Specifically, the collection of anemonefish can indirectly impact anemone abundance (Shuman *et al*., 2005), because this may leave them vulnerable to predation by reef fishes such as chaetodontids (Fautin, 1986; Godwin & Fautin, 1992; Porat & Chadwick-Furman, 2004; Holbrook & Schmitt, 2005). Host anemones are also sensitive to environmental perturbations (such as increased water temperatures), as they form a lesser-known symbiosis with microalgae from the genus *Symbiodinium* (Dunn, 1981; Hill & Scott, 2012; Hobbs *et al*., 2013). These algae are found within the gastrodermal layer of the anemones, and translocate photosynthetically fixed carbon to their host, which is then available for growth and respiration (Muscatine, 1971; Davy *et al*., 1997; Muller-Parker & Davy, 2001). When stressed, this association can break down, and the host may bleach and die (Hattori, 2002; Hill & Scott, 2012; Hobbs *et al*., 2013). Furthermore, threats such as large storm swells and land reclamation have reduced anemone abundance in some areas (Richardson *et al*., 1997; Hattori & Kobayashi, 2009).

The continued popularity of host anemones in the aquarium trade, and the range of other pressures experienced by these iconic species, highlights their vulnerability and the need for appropriate conservation and management. Aquaculture could make a valuable contribution and has the potential to integrate both conservation and development goals (Tlusty, 2002). If reliable, cost-effective techniques can be developed, captive-bred anemones could be used to supply the aquarium trade, thus reducing pressures on wild populations (Scott & Harrison, 2005; Scott, 2012; Scott *et al*., 2014). Indirect environmental benefits would confer to non-target species such as anemonefishes, which cannot survive in the wild without their hosts, and other commensals such as shrimps and crabs (Fautin & Allen, 1997; Porat & Chadwick-Furman, 2004; Shuman *et al*., 2005). If aquaculture occurred within the countries currently exporting anemones, it could provide an alternative livelihood for fishers (Wood, 2001; Tlusty, 2002; Wabnitz *et al*., 2003) and may have flow-on benefits such as augmenting the technical capacity of communities and fostering awareness for better resource stewardship (Purcell, 2012).

Accordingly, this chapter highlights progress made towards developing captive breeding methods for host anemones, as well as identifying constraints and knowledge gaps that need to be addressed. It provides a general overview of the reproductive biology of sea anemones, before detailing possible approaches for culturing host anemones using sexual and asexual methods.

21.2.2 Reproductive Modes of Sea Anemones

Sea anemones use both sexual and asexual strategies and, within each, numerous modes are used (Shick *et al*., 1979; Fautin, 1999; Fautin, 2002). In many cases, both strategies may be utilized by a single species, and may occur concurrently (Ford, 1964; Shick *et al*., 1979; Chia *et al*., 1989; Hand & Uhlinger, 1991; Lin *et al*., 1992; Hand & Uhlinger, 1995; Billingham & Ayre, 1996). Furthermore, a single species, or even an individual, may use more than one mode of asexual reproduction to proliferate its genotype (Atoda, 1976; Johnson & Shick, 1977).

21.2.2.1 Sexual Reproductive Strategies

While most sea anemones are gonochoric, having separate sexes and producing either eggs or sperm, hermaphroditic species that produce sperm and eggs simultaneously, or sequentially, may occur (Spaulding, 1974; Dunn, 1982a; Fautin, 1999). Sequential hermaphrodites can be protandrous, maturing first as male and later as female, or protogynous, maturing first as female and later as male (Rossi, 1975; Bronsdon *et al*., 1993; Bronsdon *et al*., 1997). Occasionally, hermaphroditic individuals may be found in a species that is predominantly gonochoric (Jennison, 1979). Gynodioecy (a breeding system that consists of hermaphrodites and females but no males) occurs in at least two species of anemone (Dunn, 1975a; Shaw, 1989; Fautin, 1999).

Gonochoric or hermaphroditic species may broadcast their sperm and eggs into the water for external fertilization and development, or brood their young to a later developmental stage (Fautin, 1992; 1999). Brooding can occur internally within the gastrovascular cavity (Spaulding, 1971; Siebert & Spaulding, 1976; Dunn, 1982b), or externally with the developing anemones being attached to or enfolded by the exterior of the parent (Uchida & Iwata, 1954; Dunn, 1975b; 1983; Edmands, 1995; Ishimura & Nishihira, 2003). For internal brooders, fertilization generally occurs in the gastrovascular cavity by sperm from other individuals, carried into the cavity with water. For external brooders, the eggs may be fertilized before or after being released by the anemone (Fautin, 1999). Brooded larvae or juveniles often settle quickly, which is thought to reduce the high mortality associated with planktonic development and increase the chance of finding appropriate substrata at time of settlement (Spaulding, 1971; Siebert & Spaulding, 1976; Ottaway, 1979).

21.2.2.2 Asexual Reproductive Strategies

Longitudinal fission is the most widespread form of asexual reproduction amongst sea anemones, and occurs by the anemone stretching in opposite directions, which thins and tears the tissue along the oral–aboral axis and generally creates two roughly equal-sized fragments (Stephenson, 1929; Atoda, 1976; Shick, 1991; Mire, 1998). In contrast, transverse fission is considered rare, and begins on one side of the column and progresses to the opposite side resulting in the formation of an oral and aboral portion (Stephenson, 1929; Schmidt, 1970; Shick, 1991; Shostak, 1993; Hand & Uhlinger, 1995; Fautin, 1999).

New individuals can also be produced by pedal laceration or tentacle regeneration. Pedal laceration involves the tearing, or constriction and pinching-off of small fragments of the lower column and pedal disc that subsequently regenerate (Atoda, 1973; Shick, 1991). Similarly, tentacles can be shed externally and regenerate to form new individuals, or they may be shed and then brooded within the parent until such time that they are sufficiently mature (Okada & Komori, 1932; Cutress, 1979; Pearse, 2002). In some cases, regeneration can occur before the tentacle is shed, and is considered a form of budding (Fautin, 1999). Budding is a rare phenomenon in sea anemones, where the hollow outgrowth of part of an existing organism differentiates into a polyp (Hand & Uhlinger, 1995; Fautin, 1999).

Brooded young can be produced asexually; however the underlying mechanisms remain unclear (Fautin *et al*., 1989; Fautin, 1999; Yanagi *et al*., 1999). Some authors have suggested parthenogenesis (non-recombinational form of reproduction, whereby

individuals are produced from sex cells without the intervention of fertilization); while others have proposed some form of internal budding as the main type of asexual reproduction (Shick, 1991; Shostak, 1993; Monteiro *et al*., 1998).

21.2.3 Culturing Host Sea Anemones

To date no commercial culturing protocols have been established for host anemones. The lack of published information about the sexual reproductive biology of these species has previously precluded captive breeding using this approach (Fautin & Allen, 1997; Wabnitz, *et al*., 2003). However, more recently, fundamental biological research on the sexual reproductive biology of two species of host anemone, *Heteractis crispa* and *Entacmaea quadricolor*, has been completed (Table 21.2.1) (Scott and Harrison, 2007a,b; 2008; 2009), and could form the basis of protocol development. Examination of museum specimens (Dunn, 1981), and incidental observations of spawning in *Heteractis magnifica*, *Stichodactyla gigantea*, and *S. haddoni* (Table 21.2.1) (Babcock *et al*., 1992; Scott & Francisco, 2006) suggests that all other host anemone species are likely to be gonochoristic broadcast spawners.

Table 21.2.1 Scientific name, common name, and reproductive biology of the ten species of sea anemone that provide habitat for anemonefish.

		Reproductive biology	
Scientific name[1]	**Common name**[1,2]	**Asexual**	**Sexual**
Cryptodendrum adhaesivum	Adhesive anemone	No	Gonochoric[3#]
Entacmaea quadricolor	Bulb-tipped/tentacle or bubble tip anemone	Longitudinal fission[3]	Gonochoric, broadcast spawner, planula[3#,6–10]
Heteractis aurora	Beaded anemone	No	Gonochoric[3#]
Heteractis crispa	Leathery anemone	No	Gonochoric, broadcast spawner, planula [3#, 6–9]
Heteractis magnifica	Magnificent or ritteri anemone	Longitudinal fission[3,4,5]	Gonochoric[3#], broadcast spawner[11]
Heteractis malu	Delicate anemone	No	Gonochoric[3#]
Macrodactyla doreensis	Corkscrew tentacle anemone	No	Gonochoric[3#]
Stichodactyla gigantea	Gigantic or giant carpet anemone	No	Gonochoric[3#], broadcast spawner[11]
Stichodactyla haddoni	Haddon's, carpet, or saddle anemone	No	Gonochoric[3#], broadcast spawner[12]
Stichodactyla mertensii	Mertens', carpet, or saddle anemone	No	Gonochoric[3#]

[1] Fautin & Allen, 1997; [2] Fautin, 2013; [3] Dunn, 1981; [4] Brolund *et al*., 2004; [5] Holbrook & Schmitt, 2005; [6] Scott & Harrison, 2005; [7] Scott & Harrison, 2007a; [8] Scott & Harrison, 2007b; [9] Scott & Harrison, 2008; [10] Scott & Harrison, 2009; [11] Babcock *et al*., 1992; [12] Scott & Francisco, 2006; [#] Museum specimens only.

Alternatively, asexual propagation may be possible. Asexual reproduction is known to occur in two of the ten species of host anemone, with both *E. quadricolor* and *H. magnifica* producing clusters of individuals in the field via longitudinal fission (Table 21.2.1) (Dunn, 1981; Brolund *et al*., 2004; Holbrook & Schmitt, 2005). In captivity, natural longitudinal fission has produced individuals for research purposes (Chadwick & Delbeek, personal communication), and cutting has been used as a culture method by scientists and aquarium hobbyists (Porat & Chadwick-Furman, 2004; Scott *et al*., 2014).

21.2.3.1 Captive Breeding using Sexual Reproduction

21.2.3.1.1 Spawning

Sea anemones are often referred to as tissue grade organisms as they lack organs, and therefore do not truly possess gonads. The structures termed gonads are actually aggregates of germ cells in various stages of gametogenesis that occur in the mesenteries (Campbell, 1974; Conn, 1991; Fautin & Mariscal, 1991; Fautin, 1999). To examine gametogenesis and infer field spawning times of *E. quadricolor*, biopsy samples of the gonads can be taken through the column, and histologically sectioned and analysed (Scott & Harrison, 2009). In the Solitary Islands, Australia, inferred spawning occurs in the austral summer and autumn, between January and April (Scott & Harrison, 2009). Females displayed asynchronous oocyte development, both within and among individuals, and unlike scleractinian corals, where the oocytes become highly pigmented when mature (Babcock *et al*., 1986; Harrison & Wallace, 1990), *E. quadicolor* oocytes were green throughout development. Males provided the most easily interpreted indicator of spawning times, because they showed a single cycle of spermary growth, development and spawning (Scott & Harrison, 2009).

As spawning times are likely to vary among locations and species; species and location specific information is needed on the timing of reproduction to be able to source and rear gametes (Guest *et al*., 2010). The biopsy sampling technique developed by Scott & Harrison (2009) could be used to determine this information. However, trials of this technique would be needed to determine survival of other species (especially those that do not reproduce using asexual reproduction and may not have the ability to recover from sampling) before undertaking such research.

Alternatively, observations of spawning and the subsequent collection of gametes could be achieved by maintaining anemones in captivity. Scott & Harrison (2007a, b; 2008) kept *E. quadricolor* and *H. crispa* (30–40 individuals of both species) in separate 3000 L outdoor tanks with natural photoperiod and lighting, and flow-through seawater ($20\,L\,min^{-1}$). Broodstock numbers would need to be carefully considered by culture facilities as this will affect the total number of gametes obtained. Relying on a small number of individuals would be risky, as some anemones may not spawn, or the gametes produced may not be genetically compatible (Scott & Harrison, 2007b; Guest *et al*., 2010). Furthermore, as the sex of an anemone cannot be determined from external appearance, facilities will need to rely on large numbers of individuals to ensure adequate numbers of males and females, or they will need to undertake biopsy sampling (if the species can survive this) to determine sex ratios.

Scott & Harrison (2007b) fed their broodstock every second week on a varied diet of fish, prawns and scallops. However, as fecundity has been shown to be influenced by nutrition (Fritzenwanker & Technau, 2002; Moorhead & Zeng, 2010), research is

needed to determine the most appropriate diet and feeding regime for host anemones. Most anemones were collected at least one week before expected spawning times to enable them to acclimatize to tank conditions, with individuals being replaced at regular intervals to ensure some had recently experienced field conditions (Scott & Harrison, 2007b). Keeping broodstock in captivity for extended periods may alter spawning times (Guest *et al.*, 2010), decrease reproductive success, or even cause the condition of the animals to decrease to a point where spawning will not occur. If this is the case, then repeated collections would be needed and this may actually contribute towards the demise of wild populations (Tlusty, 2002).

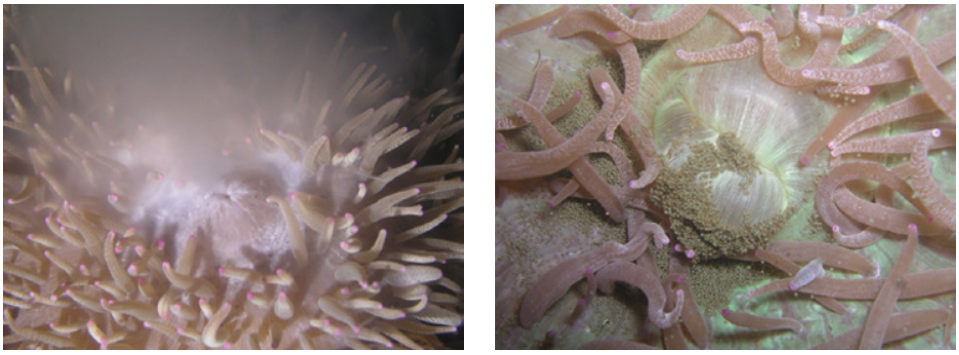
To determine spawning times, Scott & Harrison (2007b) monitored host anemones from the night of the full moon until 12 nights afterwards. During this time, the water flow was reduced or turned off, and observations were made every half hour from dusk until 11 p.m. each night or until spawning activities ceased, using a flashlight covered with a red filter. Egg catchers (consisting of a 200 L plastic tub with 250 µm mesh panels immersed in a holding tank) were fitted to the outflow of each tank to ensure that eggs would be retained if spawning occurred outside of the predicted times (Scott & Harrison, 2007b).

Observed spawnings coincided with inferred spawning periods, with male and female anemones broadcast spawning their gametes for external fertilisation and development on a few nights of each year in late austral summer and autumn (Scott & Harrison, 2007b, 2009). *Entacmaea quadricolor* usually spawned during a single month at the beginning of the year between 1 and 7 nights after the full moon (NAFM); whereas in *H. crispa* spawning was often split between lunar months, and occurred between 0 and 9 NAFM (Scott & Harrison, 2005, 2007b). In both species, spawning occurred in the evening, between dusk and six hours after sunset, and lasted for up to 3 hours (Scott & Harrison, 2007b).

Reproductive seasonality and synchrony of gamete release suggests that spawning in both species is coordinated by similar responses of individuals to a range of environmental factors such as seawater temperature, photoperiod, lunar phase, and food availability (Giese & Kanatani, 1987; Harrison & Wallace, 1990; Fautin, 1992; Olive, 1995; Scott & Harrison, 2007b). For this reason, keeping broodstock indoors for long periods of time might not be conducive to achieving spawning.

Male *E. quadricolor* and *H. crispa* generally released gametes before the females, which has also been found for a number of other anemone species (Uchida & Yamada, 1968; Clark & Dewel, 1974; Spaulding, 1974; Marshall *et al.*, 2004). This suggests that sperm or some associated product may provide the final trigger for females to spawn (Scott & Harrison, 2007b). Methods of inducing spawning should be investigated to allow for easier access to gametes for aquaculture. Knowledge of possible chemical and environmental cues used by host anemones could help elucidate reliable ways to stimulate spawning. Other sea anemones have been induced to spawn by altering factors such as light, temperature, and feeding regimes (Gemmill, 1920; Hand & Uhlinger, 1992; Fritzenwanker & Technau, 2002; Weis *et al.*, 2002). Desiccation, the addition of macerated testes, and water exchanges have also been found to stimulate spawning (Clark & Dewel, 1974; Siebert, 1974; Carter & Miles, 1989; Hand & Uhlinger, 1992). Increased fecundity may also be achieved by manipulating environmental variables (Fritzenwanker & Technau, 2002).

Changes in anemone posture occur before spawning, and are a good indicator that spawning is likely to occur (Scott & Harrison, 2007b). The column becomes fully



(a) (b)

Figure 21.2.1 Broadcast spawning *Heteractis crispa*: (a) male; and (b) female. (*See insert for color representation of the figure.*)

extended, raising the oral disc above its usual position near the substratum; and in some individuals the mouth becomes cone shaped above the oral disc. Increased hydrostatic pressure may facilitate gamete release, and is aided by rhythmical contractions that start near the base and travel up towards the oral disc (Scott & Harrison, 2007b).

Entacmaea quadricolor and *H. crispa* commonly released sperm in milky white streams or clouds, whereas their eggs were green to brown, and released singly or in large masses (Figure 21.2.1) (Scott & Harrison, 2007b). It is thought that several thousand eggs may be released by each female; however, the exact number has not been quantified. Spawned *E. quadricolor* and *H. crispa* eggs were roughly spherical and averaged 794 and 589 µm in diameter, respectively. They contained *Symbiodinium*, which indicates vertical transmission of symbionts (Scott & Harrison, 2007a). Because these anemones are highly fecund, sexual reproduction has the potential to produce very large numbers of juveniles.

21.2.3.1.2 Embryogenesis and Larval Rearing

After fertilization, embryos can be collected using 250 µm plankton mesh sieves or with a beaker from the water surface where they accumulate. At this stage, it is important to clean the fertilized eggs to remove excess sperm (Scott & Harrison, 2007a). Excess sperm can lead to polyspermy (where many sperm attempt to fertilize one egg), and can also reduce water quality when it breaks down, leading to embryo mortality (Guest *et al.*, 2010). Scott & Harrison (2007a) reared *E. quadricolor* and *H. crispa* embryos in large plastic tubs (60 L) containing seawater and aerated with a slow stream of bubbles from Pasteur pipettes located at the base of each corner of the tub. Tubs were placed indoors with a 12 h light/dark cycle.

For the first week, 10–20% water changes may be required at least once or twice a day to avoid any cloudiness in the water, this can be done using a plankton mesh sieve and siphon hose. After which, exchanges can be reduced to every 2 to 3 days (Scott & Harrison, 2007a). Conditions that maximize larval survival during culture need to be investigated, for example the use of specific antibiotics or different larval rearing set ups may assist (Scott, 2008), and there may be optimal temperatures for development and survival of early life history stages (Koldewey & Martin-Smith, 2010).

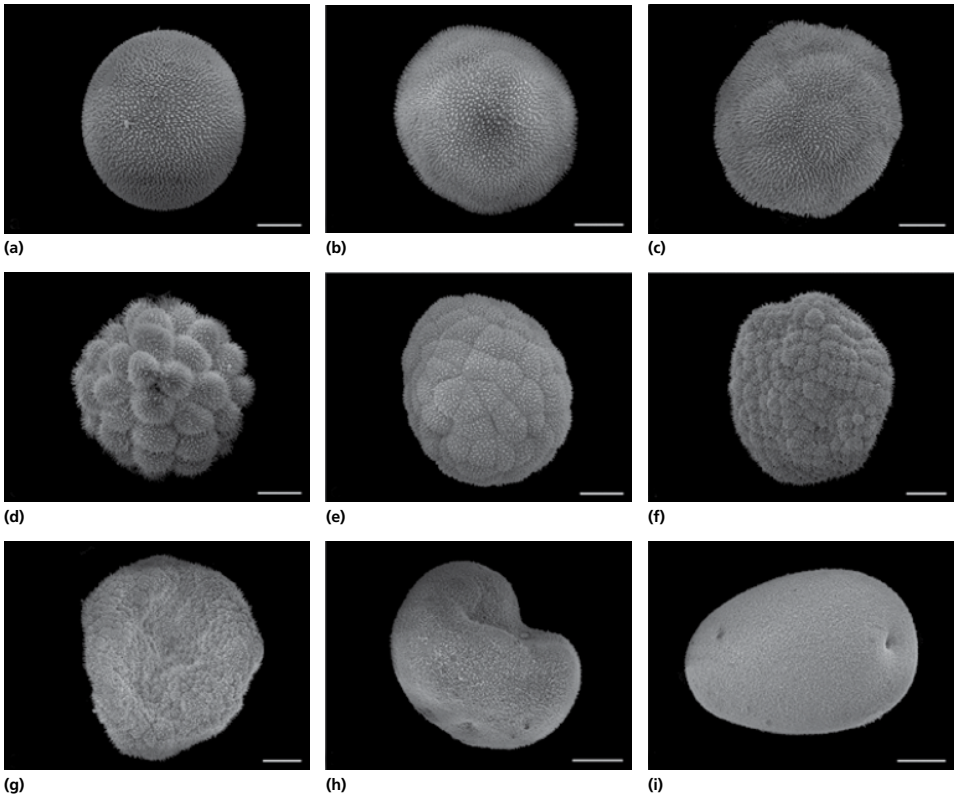


Figure 21.2.2 Embryonic development of *Heteractis crispa*: (a) newly spawned egg; (b) embryo undergoing superficial cleavage; (c) individual cells becoming more visible; (d) early blastula with clearly defined cells; (e) ~64 cell stage; (f) flattening of blastula before gastrulation; (g–h) basin-shaped gastrula; (i) ciliated larva with oral pore visible. Scale bars: 150 μm.

The embryos of *E. quadricolor* and *H. crispa* undergo superficial cleavage, with no evident cell separation on the surface before the 32–64 cell stage (Figure 21.2.2a–c) (Scott & Harrison, 2007a). As development continue, individual blastomeres become readily distinguishable (4–5 hours after spawning) and round to ovoid blastulas were formed (6–7 hours), which flatten with further divisions (Figure 21.2.2d–f). The edges of the blastula thicken creating a concave-convex dish-shaped gastrula (7–8 hours), before the outer margins roll inward (10–12 hours) leading to the formation of an oral pore and a ciliated planula larva (14–22 hours) (Figure 21.2.2 g–i) (Scott & Harrison, 2007a). Before the larval stage, embryos are not robust and great care needs to be taken during cleaning and with aeration. Turbulence and/or high aeration can break the embryos into irregular groups or individual blastomeres, which has also been documented for coral (Heyward & Negri, 2012). *E. quadricolor* and *H. crispa* larvae are motile, at first they spin due to uncoordinated ciliary beats; however, as development progresses ciliary beats become coordinated resulting in directional movement (Scott & Harrison, 2007a).

21.2.3.1.3 Settlement and Metamorphosis

Entacmaea quadricolor and *H. crispa* larvae readily settle on a variety of surfaces, and settlement is generally preceded by intensive larval searching and testing of the substratum (Scott & Harrison, 2008), suggesting microhabitat selection (Fadlallah, 1983; Harrison & Wallace, 1990). Once settled, the larvae undergo metamorphosis, which by definition, comprises the loss or transformation of larval structures and the elaboration of adult structures (Müller & Leitz, 2002).

Peak settlement of *E. quadricolor* and *H. crispa* larvae occurs 10 d after spawning, with between 33 and 50.3% of larvae settling onto biologically conditioned settlement tiles (Scott & Harrison, 2008). Settled individuals received supplementary feeding with liquid invertebrate food, were able to feed on small invertebrates that may have been present, and would have also received photosynthate from their endosymbiotic algae (Scott & Harrison, 2008).

Optimizing larval settlement and reducing mortality post-settlement will be important for ensuring successful culture using sexual reproduction (Petersen *et al*., 2005). Because coral settlement has been shown to have specific biological and environmental requirements (Morse *et al*., 1988; Leitz, 1997; Mundy & Babcock, 1998; Heyward & Negri, 1999; Petersen *et al*., 2005; Nozawa & Harrison, 2007; Schlacher *et al*., 2007), the role of factors such as biofilms, light, and temperature in anemone settlement deserve research attention. Likewise, information on the stocking densities that maximize settlement and survival of juveniles, and feeding regimes that optimize growth rates of newly settled anemones are required (Scott, 2008). Petersen *et al*. (2008) found that providing *Artemia* nauplii to new settled coral polyps can potentially enhance both growth and survival during early development.

Optimal feeding practices can lead to increased growth and decreased food wastage during culture (Moorhead & Zeng, 2010). Sea anemones are carnivorous, actively capturing prey with their tentacles, and in the field their diet consists of a variety of organisms due to opportunistic feeding behaviour (Purcell, 1977; Peterson & Black, 1986; Acuña-Maurizio & Zamponi, 1996; Kruger & Griffiths, 1996). Scott (2012) did a 12-week feeding experiment to determine how often feeding was required to maximize growth in 34 months old captive-bred *H. crispa*. Anemones that were fed once per week with small pieces of prawn meat achieved growth rates equivalent to those fed three times a week. Unfed anemones survived, and even showed significant pedal and oral disc growth over time, albeit at a lower rate than their fed counterparts (Scott, 2012). Given that low levels of feeding were required to maximize growth, this bodes well for potential aquaculture ventures, as food costs and associated labor would be relatively low (Moorhead & Zeng, 2010).

While Scott (2012) addressed the effects of feeding frequency on growth of *H. crispa*, this study did not consider diet. Identifying diets that optimize growth would help potential aquaculture operations maximize profitability by producing saleable sized individuals within the minimum amount of time (Daggett *et al*., 2005). Both natural and commercial feed types should be considered. The most appropriate feeding rates and diets could be species specific and are most likely to vary throughout development.

21.2.3.2 Captive Breeding using Asexual Reproduction

Fission has been used to produce a range of sea anemone species, such as *Aiptasia pallida* and *Nematostella vectensis*, which are often used as biological models for scientific research (Leal *et al*., 2012; Bossert *et al*., 2013; Stefanik *et al*., 2013); and

E. quadricolor and *C. gigantea*, which are important in the aquarium trade (Porat & Chadwick-Furman, 2004; Centurión Fernandéz *et al*., 2009; Scott *et al*., 2014). This has been done by either allowing fission to occur naturally in the laboratory or by fragmenting the anemones artificially.

Holbrook & Schmitt (2005) documented fission in 48 *H. magnifica* over 3 years in the field in Moorea, French Polynesia. During this time they recorded 26 divisions, with most incidences occurring in winter and when two anemonefish were present (Holbrook & Schmitt, 2005). Low natural fission rates suggest that culturing host anemones using this approach would be insufficient to meet the demands of the aquarium trade. However, as fission activity is known to be influenced by factors such as temperature (Miyawaki, 1952; Johnson & Shick, 1977; Minasian, 1982) and feeding regime (Minasian, 1976; Tsuchida & Potts, 1994), it may be possible to increase division in captivity by manipulating these variables.

Alternatively, host anemones may be able to be cultured by cutting them into fragments (Porat & Chadwick-Furman, 2004; Scott *et al*., 2014). Porat & Chadwick-Furman (2004) cut six *E. quadricolor* in half to generate individuals for a laboratory experiment with anemonefish, and found that the majority survived (67%). Whereas, Scott *et al*. (2014) completed experiments aimed at establishing whether *E. quadricolor* could be cultured by cutting them in halves or quarters. Anemones were removed from aquaria and cut using a steel knife on a plastic cutting board, whilst wearing latex gloves. Tentacles were moved toward the outer edge of the oral disc, and cuts were made through the anemones mouth (along a random plane to the directional mouth). The resulting fragments were immediately placed back into aquaria, with the pedal disc directed downwards. Anemones that were cut in half were larger in size, and healed and grew quicker than those cut in quarters. However, even though survival was lower when the individuals were cut in quarters (80% vs. 89–94%), this treatment did produce the greatest number of anemones (Scott *et al*., 2014).

Scott *et al*. (2014) also examined whether feeding was needed to maximize survival and growth of the resulting fragments over 105 days. Feeding resulted in increased oral disc diameter growth and reduced wet weight loss, but did not significantly influence pedal disc diameter. Because the anemones took up to 56 days to form an off-center mouth it is likely that feeding may have produced greater effect if the experiment was run for longer (Scott *et al*., 2014). Given that temperature has also been shown to affect sea anemone growth (Chomsky *et al*., 2004), it would also be interesting to examine the effects of varying temperatures on the regeneration and growth of fragments.

Artificially fragmenting anemones provides a low technology solution that would allow for the year round production of individuals (Scott *et al*., 2014). Furthermore, it would allow desirable color morphs, which attract higher sale prices, to be cloned, thereby increasingly potential profitability. Given the simplicity of the technique, it could easily be used without personnel needing a large amount of training, and thus may allow livelihoods to be maintained in a more sustainable manner in developing islands nations. It may be possible to apply this technique in the field (either in cages or on the reef flat), which would negate the need for captive breeding facilities that are expensive to set up and maintain. However, it is important to note that the findings of this study might not apply to all sea anemones, as species that do not naturally asexually reproduce may not be able to recover from cutting (Scott *et al*., 2014). Trials of this technique across a greater range of species are needed to determine the broader suitability of this culture method.

21.2.4 Conclusions

Aquaculture provides a potential solution for reducing aquarium collecting pressures that are impacting host sea anemone abundance in some areas of their distribution, and may also allow for the restoration of areas already impacted by human or natural disturbances (Edwards & Shepherd, 1992; Shuman *et al*., 2005; Scott *et al*., 2014). Anemones could be cultured using sexual reproduction, which has been studied in two (*Entacmaea quadricolor* and *Heteractis crispa*) of the ten species of sea anemones that host anemonefishes (Scott & Harrison 2007a,b; 2008; 2009). Alternatively, it may be possible to propagate anemones using asexual reproduction, as *Entacmaea quadricolor* and *Heteractis magnifica* can reproduce asexually using longitudinal fission. While natural fission rates would not be sufficient, fragmentation by cutting has been shown to be a low technology solution that could produce large numbers of individuals (Scott *et al*., 2014).

Due to high product value and the benefits to biodiversity conservation, the potential for host anemone aquaculture seems promising. However, economic feasibility needs to be evaluated as large- or small-scale commercial viability has not been demonstrated. While some consumers may be willing to pay a premium for cultured marine ornamentals, aquaculture will only be viable if individuals can be competitively with price to wild-caught animals (Koldewey & Martin-Smith, 2010). If aquaculture does prove viable, it would be preferable if ventures were located in the developing nations that are currently exporting anemones to allow livelihoods to be maintained in a more sustainable manner (Wood, 2001; Tlusty, 2002; Wabnitz *et al*., 2003).

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21.3

Jellyfish

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Abstract

Medusae belonging to the Phylum Cnidaria, hereafter referred to as jellyfish, are interesting due to the fact that they lack simple organs such as a brain, heart, skeleton and gills that most people would consider essential for animals to function. Nonetheless, jellyfish have been very successful as a group, surviving for more than 540 million years. Methods have been developed over the last 100 years for culturing jellyfish, especially the common moon jelly, *Aurelia* spp., for research and public displays. This chapter presents considerations for the aquaculture of moon jellies, which includes acquisition, culture systems, tank designs and maintenance, nutrition, disease recognition and treatment and transportation techniques.

Keywords *Aurelia aurita; life cycle; maintenance; nutrition; moon jelly; tank designs*

21.3.1 Introduction

Jellyfish have bell-shaped, gelatinous bodies and possess tentacles attached to the rim of the bell that are covered with tiny nematocysts or stinging cells. Stinging cells can shoot microscopic barbs into prey and many deliver toxins to help immobilize prey. The mouth, centered under the bell, has frilly oral arms that extend to the rim of the bell. Scyphozoans, or "true jellyfish", have a life cycle that features an alternation of generations consisting of a planktonic, sexual medusa stage that is bell-shaped and a sessile, asexual polyp stage that reproduces by cloning. There are about 200 recognized species of true jellyfish, they are all marine and are found in all oceans of the world. Moon jellies are perhaps the most common and best known of the scyphozoan jellyfish.

People have been fascinated with jellyfish for many reasons, including: their ability to sting swimmers, fishermen's nets are sometimes ruined by them, people have been eating them in Asia for more than 1000 years, researchers study them to understand their

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biology, and people being mesmerized by simply watching their gentle pulsations as they move through the water.

Moon jellies (*Aurelia* spp.) have been kept in cultures for many years. Dorothy Spangenberg, a cellular biologist, studied moon jellies to understand their development and metamorphosis of the various life history stages (Spangenberg, 1964, 1967, 1971, 1974). Dr. Spangenberg and her colleagues even sent moon jellies into space on Space Shuttle missions in the 1990s to understand their accommodation to microgravity in the hopes of applying that knowledge to human accommodation to long-term space travel (Spangenberg *et al*., 1994). Among her many publications, she published a paper on cultivation of the different life stages of *Aurelia aurita* (Spangenberg, 1965).

The first aquariums to show moon jellyfish were in Japan. Yoshitaka Abe at the Ueno Zoo worked out a culture system and display tanks for moon jellies (Abe & Hisada, 1969). In 1975 he shared his knowledge with colleague Paul Van Den Sande from the Antwerp Zoo in Belgium who helped spread the techniques to Europe (Lange & Kaiser, 1995). In 1984, Mr. Abe also shared his knowledge with staff at the Monterey Bay Aquarium who helped spread the techniques to aquariums and zoos in North America (Sommer, 1992a,b).

Since that time, many zoos and aquariums have successfully started husbandry programs in order to fill their expanding jellyfish exhibits. The Association of Zoos and Aquariums published the Jellyfish Animal Care Manual in 2013 detailing the husbandry of various species of jellyfish in public aquaria (AZA, 2013), however, moon jellies remain the most cultured and displayed to date.

Hobbyists have been exploring the idea of jelly keeping, but sourcing stock has been problematic as there have been only a handful of commercial breeders worldwide and wild collection is not only seasonal, but sporadic and for most, geographically impossible. A book about how to keep jellyfish in aquariums (Widmer, 2008) has served to expand the notion of successful jelly keeping at hobbyist level and at the same time, familiarize the marine ornamental community with a new invertebrate to consider for trade. Commercial jellyfish breeding is currently on the rise to meet the growing demand for this delicate and graceful animal.

Although this chapter is focused on moon jellies, a large majority of the details outlined are similar to the husbandry techniques of other jellyfish species. Good general references to jellyfish biology can be found in Arai (1988) and Brusca & Brusca (1990).

21.3.2 Anatomy of Moon Jellyfish Medusae

The body of an adult moon jellyfish medusa resembles a flattened bell or umbrella (Figure 21.3.1). The exumbrellar surface is smooth. The central mouth, called a manubrium, is subumbrellar and normally has four frilly oral arms extending to about the rim of the bell. The gelatinous part of the bell is called the mesoglea. The bell of moon jellies can be clear as juveniles but becomes more opaque with age. Hundreds of tiny, thin, retractable tentacles are attached to the rim of the bell. Although there is variation in moon jellies, normally there are eight rhopalia that contain sense organs (light and gravity sensing) attached to indentations at the rim of the bell. Normally four semicircular

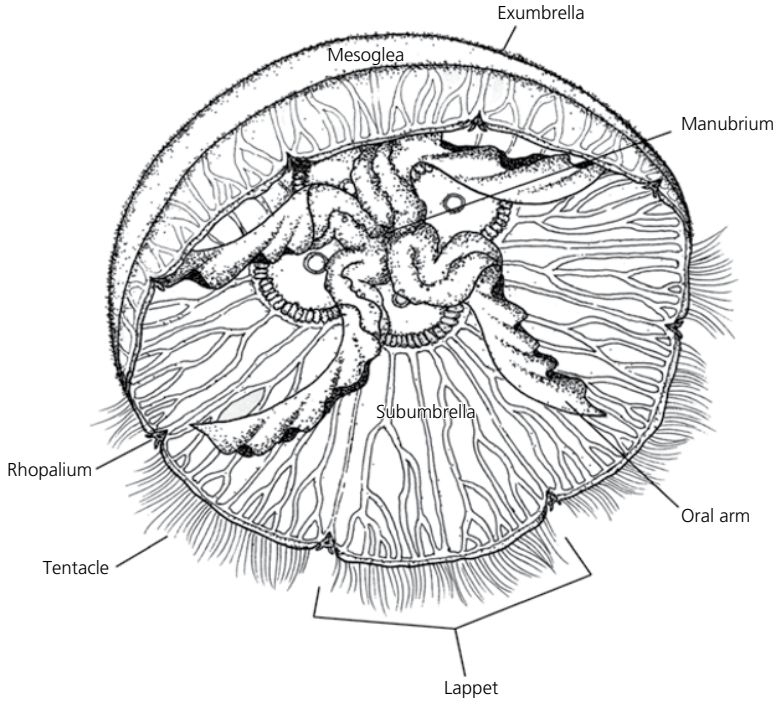


Figure 21.3.1 Adult moon jelly medusa.

reproductive organs attached to the subumbrellar surface are easily visible through the mesoglea. Subumbrellar to each reproductive organ is a stomach pouch that is visible only when it contains food.

21.3.3 Lifecycle of Moon Jellyfish

The lifecycle of moon jellies (Figure 21.3.2) consists of a planktonic, sexual medusoid form and a sessile, asexual polypoid form. Mature medusae reach about 40 cm and are separate sexes. Mature male medusae release sperm strings from channels that extend to the tip of the oral arms. Mature female medusae catch sperm strings while passively feeding. As the sperm strings get close to the female's mouth, individual sperm break free of their strings and fertilize mature eggs in the female's ovaries. Fertilized eggs develop into motile planulae larvae and move to folds in the female's oral arms. Planulae form aggregations in the folds that give a white lace appearance to the bases of the oral arms. Planulae regularly release from the female to swim in the water column until finding a suitable place to settle (a rock, the underside of a boat dock or the sides of tanks). A settled planula grows into a polyp (or scyphistoma) which resembles a small sea anemone (Figure 21.3.3). Polyps reach a mature size of about 2 mm in diameter and 3 mm tall. Polyps bud new polyps from their bases and stolons. At certain times of the year, usually late winter or spring, polyps undergo strobilation whereby ephyrae or larval medusae are produced. A polyp undergoing the process of strobilation is referred

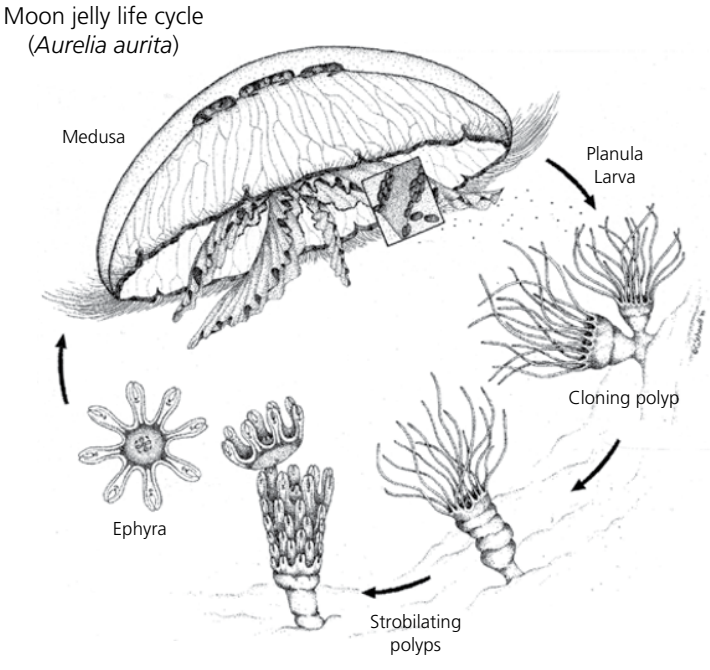


Figure 21.3.2 Moon jelly (*Aurelia* sp.) life cycle.

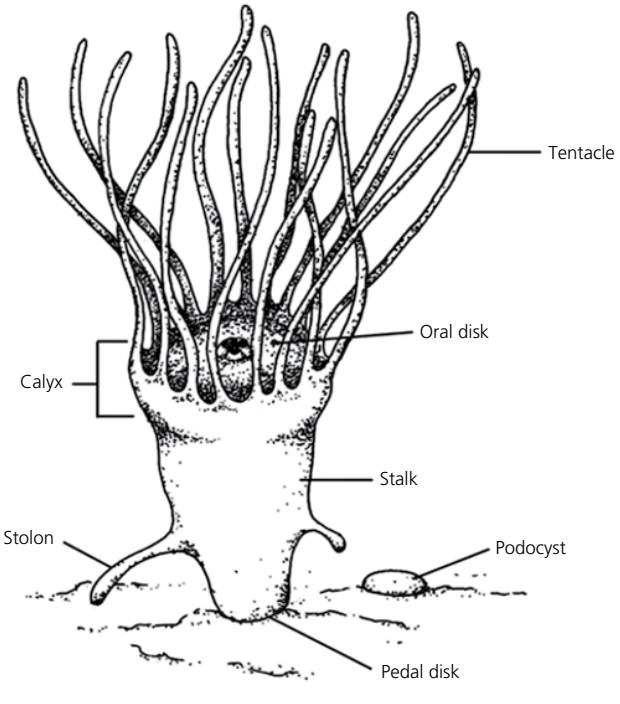


Figure 21.3.3 Moon jelly polyp.

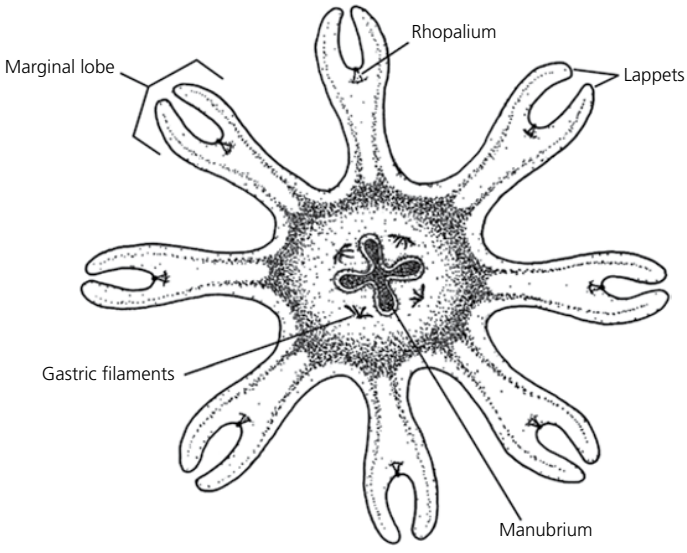


Figure 21.3.4 Moon jelly ephyra.

to as a strobila. Strobilation is a type of asexual reproduction where the stalk of the polyp becomes constricted into segments. Each segment metamorphoses into a tiny larval medusa called an ephyra. Strobilation ends with each ephyra releasing from the stack to become free-swimming. In optimal conditions in the wild, an ephyra can grow from about 2 mm in diameter (Figure 21.3.4) to maximum adult medusa size of 40 cm in about 2–3 months. In the wild there is evidence that medusae live one to two years (Albert, 2005), but it is interesting to note that moon jellies in many public aquariums and zoos can live many years (up to nine years at Cabrillo Marine Aquarium, MS unpubl. observ.).

This chapter will present methods for the display of healthy moon jellies including: environmental requirements, jellyfish acquisition, general tank designs, maintenance, nutrition, disease recognition and treatment, and transportation techniques.

21.3.4 Environmental Requirements

Choosing the proper environmental requirements for keeping jellyfish ultimately depends upon the goals of the aquarist. Most people are usually interested in the environmental parameters required for keeping medusae healthy and displayable, but those parameters depend upon the species of jellyfish being kept, and where they occur naturally in the world. Consider for example the common moon jellyfish. Members of the genus *Aurelia* may be found in global waters ranging from the tropics to the poles. In the past, all moon jellies were known as *Aurelia aurita*. Many jellyfish keepers cling to this outdated idea when making jellyfish husbandry decisions today. Those using molecular tools have shown that up to 12 distinct species of moon jellyfish exist (Dawson, 2003), and more are likely to be revealed. Therefore when deciding upon optimal water quality parameters for medusae, it is essential to keep the ecology of the

target species in mind. For example the moon jellyfish *Aurelia labiata* from the northeast Pacific Ocean experience temperatures ranging from 7–15 °C in an annual cycle whereas moon jellyfish, *A. aurita*, from Taiwanese waters experience temperatures ranging from 19–32 °C (Liu *et al.*, 2009). Populations of *A. aurita* occurring in Gullmar fjord, Sweden experience temperatures as low as 1 °C during winter (Hernroth & Grondahl, 1985), and during a normal annual pattern jellyfish inhabiting British waters typically encounter temperatures ranging from about 4–19 °C (Russell, 1970). Therefore it may not be wise to make water quality generalizations regarding husbandry of closely related jellyfish species based upon ones that inhabit different latitudes because moon jellies originating from one location may require warmer or cooler temperatures than those from another locale.

People who are interested in growing their own jellyfish are usually keenly interested in requirements for inducing strobilation in polyps in order to obtain juvenile medusae. In order to derive that information thinking like an ecologist will serve nicely. In naturally occurring colonies, jellyfish polyps are adapted to generate and release their ephyrae at a time that is advantageous for juvenile growth and development. Juvenile jellyfish eat zooplankton (Arai, 1988), and for many species increasing temperatures in the presence of abundant food enhances ephyral growth (Bamstedt *et al.*, 1999; Widmer, 2005). In the sea the period during which these two things are co-occurring is spring, and field studies often find juvenile medusae in the spring. Spring is also the period when they are observed in the Puget Sound, Washington, USA (CW, unpubl. observ.). Therefore, strobilation must take place before then, perhaps during winter when sea temperatures are at their minima. Temperature has been shown to play a key role in initiating the molecular machinery required for strobilation induction in jellyfish polyps (Fuchs *et al.*, 2014). Several species have been experimentally shown to strobilate only after experiencing critical minimum low temperatures for sufficient durations, usually lasting periods of weeks (CW, unpubl. results). The minimum temperature thresholds and durations are species specific. Most jellyfish aquarists will need to conduct a bit of research about the annual sea temperature patterns that occur in the regions from which their jellies originate.

21.3.5 Nutritional Requirements and Feeding

Good nutrition is absolutely essential for successful jelly keeping, and poor diet should be an issue to investigate if things appear to be slowly going amiss in your jelly collection. Natural populations of jellyfish eat zooplankton which is comprised of a small host of diverse organisms including larval invertebrates, juvenile fish and their eggs, and a wide variety of small shrimp-like animals. Natural populations of jellyfish swim in the same waters as the nutritious plankton soup, and when food is abundant jellyfish can grow very large very quickly. Jellyfish also have the ability to fast for extended periods, gradually shrinking in size as they metabolize their own body tissues. When jellies encounter food again, they can begin the process of growing again (Hamner & Jenssen, 1974). In an aquarium setting it is best to avoid the shrinking and growing cycle and attempt to maintain gradual and progressive growth. Since proper nutrition is an essential requirement for jelly keeping (Bamstedt *et al.*, 1997) one option for achieving a proper diet is to collect wild plankton from the sea daily. However, while it is an easy

thing to write, in practice the daily harvesting of wild plankton is far more challenging to accomplish with proximity to the sea being a major factor. Therefore, jelly keepers tend to rely on more easily obtainable foods.

Newly hatched brine shrimp, *Artemia* sp., nauplii larvae are one of the staple foods used in modern jellyfish keeping. Nauplii are roughly similar in size (450–500 microns) to the zooplankton foods jellyfish naturally encounter and eat in the sea. They are relatively easy to acquire, and with a bit of practice easy to hatch and feed out to collections as well. For a good overview of the use of *Artemia* in aquaculture systems see Plankton Culture Manual (Hoff & Snell, 2004; see also Chapter 8). Jelly keepers have several options for hatching *Artemia* depending on their preference and time: naturally hatching the eggs by rehydrating *Artemia* cysts; decapsulating the *Artemia* nauplii by burning away the outer shell of the cysts with bleach and then stopping the chemical reaction by neutralizing the bleach; or purchasing pre-decapsulated *Artemia* cysts that then are simply rehydrated. There are pros and cons to each method, all of which should be considered when choosing a method to use. One of the golden rules of jellyfish husbandry is that good nutrition leads to good looking jellyfish.

Regardless of the hatching method chosen, nauplii that are not fed to jellies within 24 hours of hatching should be enriched. When nauplii hatch from their cysts they survive for the first hours of life using the energy stored in a yolk sac that was provided by their mother. Feeding jellies nauplii that still have their yolk sacs attached would be optimal for nutrition, but the timing may not be practical for all involved. Therefore enriching nauplii that are a bit older than 24 hours is suggested. One way is to feed phytoplankton to the nauplii such that they have a bit of food in their guts making them more nutritious. Another way to enrich nauplii is using an aquaculture product such as Super Selco or Algamac 3050, which are made up of carefully crafted blends of nutritious foods for nauplii and therefore, the jellyfish. Feeding nauplii phytoplankton, as well as enriching them in Super Selco or Algamac 3050 produces very good results.

Small krill are another appropriate food for jellyfish in aquariums. In the sea, small krill are eaten by nearly everything that can catch them because they are very nutritious. Whenever possible, krill should be included two to three times per week in the moon jellyfish diet. Small krill can be gently squirted directly onto the tentacles of larger jellyfish with a turkey baster. When feeding smaller jellyfish, jelly keepers may want to consider blending the krill into a paste, and using the turkey baster to squirt small amounts of "krill shake" onto the tentacles of the jellies. Loose fragments from krill shakes can cause water flow problems by clogging screens, so screens covering the outflow ports of the exhibits should be cleaned gently with a brush about an hour after feeding to avoid clogging issues. Uneaten krill and other detritus left to accumulate on the bottoms of the tanks could lead to problems with fouling organisms, and damage to jelly bells due to mechanical abrasion, so leftover krill should be siphoned away.

In the sea zooplankton occur in large groups of individuals that resemble large, midwater, drifting clouds. One can imagine that when a jellyfish passes through a zooplankton cloud food is very abundant for jellies, but when the cloud moves away food is scarce. This idea is referred to by scientists as patchy distribution, and jellyfish are well adapted for taking advantage of it. Some jelly keepers attempt to enhance jellyfish growth by utilizing the ability of the jellyfish to eat and digest a lot of food quickly by first removing them from larger enclosures and then isolating them in smaller ones. Next, the jelly keeper super saturates the smaller enclosure, which is often a bowl or a

dish, with food which allows the jellyfish to collect it very quickly due to the increased predator-prey encounter rates that occur inside the smaller enclosure. This "power feeding" method is followed by returning the food-saturated jellyfish to the larger enclosure. If jellies are allowed to remain in the small super saturated enclosure for too long, water fouling issues may result.

21.3.6 The Chain of Production

A chain of production needs to be established and maintained such that as older medusae reach desired adult size and move out of the chain of production, younger smaller ones are being culled and graduated. The moon jelly culture system consists of three main sections; the polyp tank, the ephyrae grow out tanks and the juvenile/adult holding/shipping tanks. A number of different tank types are utilized ranging from polyp culture tanks, juvenile medusa harvesting tanks and so on, all of which are designed to grow developing medusae from one size class to the next until they ultimately reach desired medusae sizes of 2.5 cm and larger. A review of the various tank designs and their construction is given in Widmer (2008).

Polyps can be maintained in any variety of small vessels and are remarkably forgiving and hardy. However, polyps cannot thrive unless provided a proper maintenance routine which includes regular feeding and cleaning of the culture tanks. For optimal polyp health arrange polyps such that their tentacles dangle from their plastic petri dishes or acrylic plates where they were settled. That will allow for optimal feeding and waste removal, and therefore better health for the polyps and your jellyfish medusae in the end. Small rectangular plastic aquariums with small holes drilled at the top of one side make for excellent flow-through polyp tanks. Water leaving the polyp tank can exit the small hole through a tube and be deposited in a small tank designed for collecting juvenile medusae called an ephyrae catch tank.

Ephyrae catch tanks (Figure 21.3.5) are small plastic tanks that are usually placed after a polyp tank in a flow-through sea water system. They provide aquarists the benefit of not having to chase ephyrae around the polyp tank with a pipette in order to collect them. Instead ephyrae are swept out of the polyp tank into the ephyrae catch tank. The tank has a large parabolic arc shaped mesh screen glued with silicone to one side of the vessel. There is a hole in the ephyrae catch tank following the mesh screen allowing water to leave the catch tank whilst leaving juvenile medusae remaining. Juvenile moon jellies may be kept in successively larger ephyrae catch tanks as they grow.

For those with abundant time and patience, and for times when every ephyra is sacred, they should be harvested daily from their ephyrae catch tanks and placed inside dishes filled with standing sea water and maintained at a constant temperature, then fed enriched *Artemia* nauplii. Each day the ephyrae should be pipetted into a different dish filled with new sea water and fed. Raising newly released moon jellies in this manner is more time consuming than just leaving them in their ephyrae catch tanks to grow. However, this method usually produces a higher success rate in the beginning stages of juvenile medusa growth. If one chooses to use this method it is imperative that daily water changes are conducted, because water left standing in a dish for longer periods may foul having deleterious effects on ephyral growth.



Figure 21.3.5 Polyp and Ephyrae catch tanks. (Grey) Polyp tank: 330 mm × 254 mm × 102 mm (13″L × 10″W × 4″H). (White) Ephyrae catch tank: 457 mm × 305 mm × 76mm (18″L × 12″W × 3″H).

Moon jellies may be grown all the way to small adults in progressively larger ephyrae catch style tanks. When the tanks reach larger sizes (>3 L) they are referred as modified boxes, but in reality they are all variations on an ephyrae catch theme. There is also a delicate balance that exists between properly balancing currents in a tank to keep moon jellies up and off tank bottoms, and also using that balance to allow water to enter and leave a tank, whilst leaving the jellies behind and in good condition. Pseudokreisels, based on the shipboard planktonkreisel design published by Hamner (1990) are again variations on the same theme with the added benefit of having elongated viewing ports. The main idea when balancing the current in any jellyfish tank is to use the least amount of current required to keep the jellyfish suspended whilst minimizing contact with any surface.

21.3.7 Jellyfish Acquisition

Starting a moon jellyfish culture begins with acquiring adult moon jellies, polyps or planula larvae. Ultimately, a polyp colony should be established and this can be accomplished by way of collecting adult moon jellies, purchasing adult moons, polyps or planula larvae. If local collection is an option, specimens should be collected by hand and while submerged. Do not use a net to lift the animal out of the water, but keep the jelly underwater, bring it to the surface and hold it in place while positioning a fish collection bag near it and gently move the animal into the bag. Initially filling the bag with approximately 20% of seawater helps anchor the bag and allow for a smooth transition of the jelly into it. Typically, collection bags for adult moons range in size from 20 cm × 38 cm to 25 cm × 45 cm. Fill the remainder of the bag with seawater and cinch it

down with a rubber band leaving no air in the bag. The animal should have enough room to open its bell completely and pulse freely. Collected jellies should be acclimated into their holding tanks within 24 hours. Further collecting methods and practices are discussed in Raskoff *et al*. (2003).

If planulae are settled onto acrylic plates for polyp colony propagation the plates should be sized according to what size tank they will inhabit. Do not make them too large as to diminish the flow of water around them and do not crowd them for the same reason. Smaller plates allow for efficient water flow and are easier to manage over time. Polyp plates should hang as opposed to lying flat and positioned perpendicular to the flow. This allows for the tentacles to hang down into the cross current. This configuration makes for easy weekly cleaning of the polyp colonies and efficient feeding and strobilation, as well. It is recommended to have an ephyrae catch tank plumbed into the outflow of the polyp tank so as to collect any newly released ephyrae.

There are a few companies commercially breeding moon jellies for the marine ornamental trade, with more to come, and obtaining polyp cultures and adults by way of purchase is possible. Know the species, regional origin and water quality parameters such as temperature and salinity before purchasing. Collecting or purchasing adult specimens will yield the opportunity to harvest planula larvae, settle polyp plates and begin a culture. If local collection is not an option, there are a handful of commercial marine ornamental collectors knowledgeable in the jellyfish collecting arena. It is recommended to review their jelly collecting practices ahead of time to ensure viable adults on the receiving end. If the wild caught or cultured jellies obtained are adults, they will most likely be fertile and ready to spawn. The males and females should be kept together in a large modified box or planktonkreisel for natural spawning (Raskoff *et al*., 2003). The males will typically spawn in the morning hours. Continue to feed them as per normal on a daily basis. Within a few days after a spawning event, a concentration of planulae may be observed on the folds of the upper oral arms of the female, noticeable by the white or brownish-purple lattice forming. Using a small 2 mL pipette the planulae can be gently removed from the oral arms and transferred to a Petri dish for settling. It is wise to have one or two culture plates line the Petri dish so the planulae will directly attach to them as opposed to the Petri dish. Since *Aurelia* planulae are light sensitive and have a tendency to settle on the undersides of surfaces (Custance, 1964; Brewer, 1978, 1984; Raskoff, unpublished data), elevate the plates slightly in the Petri dish. Once the planulae attach, in approximately 4–7 days (Sommer, 1986), hang the plates in the flow-through polyp tank and encourage them to bud or clone with daily feedings (Raskoff *et.al*., 2003; Sommer, 1986).

Maintaining a stable environment and routine feeding protocol will produce healthy polyps ready to strobilate given the right parameters. When required there are a few methods to induce strobilation. Adjusting the temperature and salinity, either up or down, depending on the species, can induce strobilation. Allow one to two weeks for the polyps to respond to the adjustment to temperature and/or salinity changes before attempting again. In most cases, given a stable and healthy polyp culture, modifying just one water chemistry parameter can instigate a strobilation event. On the other hand, the combination technique of feeding restriction coupled with increased light intensity can also induce strobilation. If the natural approach does not yield any strobila, iodine levels can be increased. Lugol's solution or potassium iodide may need to be added to your system to instigate strobilation (Spangenberg, 1967, 1971; Olmon & Webb, 1974).

21.3.8 Transportation of Moon Jellies

Moon jellies can be transported readily across the globe. As long as they are packed properly and arrive within 24–36 hours, they are great world travelers, arriving at their destination alive, intact, healthy and ready to be introduced into their new environment.

There are a few good ways to pack moon jellies for transportation. First, consider how long they will be in transit, how many and what size will be going, and what climate they will be traveling to, from and through. The use of cold or heat packs should be considered to accommodate either the warm or coldwater strain of moon jellies traveling.

Water quality in the culture system is extremely important when the time comes to pack up the jellies for transportation. Shipping jellies in poor quality water results in deformities of the bell and sloughing of the mesoglea. This is also the reason jellies are not to be fed the day they are scheduled for shipping. If they have been fed, a minimum time of 4 hours should be allowed before packing them. This is dependent on the volume of food that was fed. No food should be on the tentacles, in the oral arms or stomachs prior to being packed and shipped.

The pH, salinity and temperature of the culture system from which the jellies are being packed should all be noted and relayed to the receiving party to expedite the acclimation process of the jellies on arrival.

The moon jellies can be individually packed or packed two or more to a bag depending on the size of the medusae. When packing individually, consider the diameter of the jelly and allow for a complete extension of the bell in the bag and allow at least 2.5 cm of water surrounding the bell. The animal should be able to bell freely inside the bag. Once inside the bag, exhaust all the air, twist the bag and rubber band or clip the end. It is critical that there be no air inside the bag. Air left in the bag can result in tiny bubbles that can get caught under the bell or ingested. Also, jellies' oxygen requirements can easily be met over the time they spend in the shipping bag. The shipping bag should not be rigid, but with a slight give. Plastic bags should be at least 3 mm in thickness, and even so double bagging is recommended.

Individual bags can be arranged side by side inside a Styrofoam box lined with a plastic bag that serves to maintain the integrity of the individual bags and protect the outer boxes from accidental leakage. Packing material such as newspaper should be used to fill any cavities inside the Styrofoam box to eliminate shifting or tumbling of the shipment. If shipping moon jellyfish originating from warm locales use a heat pack or two to maintain the temperature during transit. Likewise, if shipping cold water moon jellies cold packs should be utilized. Styrofoam boxes should be placed inside a larger cardboard box and labeled "Perishable."

Multi-animal bagging can be useful when transit times are shorter than 24 hours or the medusae are under 7.5 cm diameter. In general, the rule of thumb that 7.5 cm of "mesoglea" per liter of saltwater should not be exceeded when placing several animals in a single shipping bag. Double or even triple bagging is recommended.

Keep in mind the weight of the box as the shipment is being prepared. Do not exceed 18 kg per box when transporting via UPS or FedEX, or 27 kg per box via air cargo. Shipping live moon jellies domestically is best accomplished via air cargo. Airlines generally offer rapid turnaround times and insurance to cover any loss or damage due to delays that result in animal loss. Although it may not be convenient to go to the airport

to drop off the shipment or to pick it up, the overall trip for the jellies is very easy and without the turbulence, tossing, sorting and dropping presented when shipping via carriers such as UPS and FedEX. Consider the fact that UPS and FedEX recommend that all shipments be packed to withstand a 1.8 m flat drop onto concrete. When using standard carriers, the shipment must arrive within 24 hours and usually will if shipped "Overnight Delivery". This is a premium service with UPS and FedEX. Moon jellies will not survive the immobility of "2nd Day Delivery" or later. They must ship "Overnight Delivery".

When booking with an airline, always choose the direct flights when possible. If you must book the shipment with layovers or a change of planes, allow for enough time for the shipment to make its connecting flight. Airlines offer different levels of service for their live animal shipments. Make sure that the level of service you choose delivers your jellies to their destination in under 24 hours. Thirty-six hours is an outside maximum time limit. Jellies are essentially immobile in the shipping bags and long stays in shipping bags can cause deformities in the animal due to prolonged inactivity.

Although domestic shipping of live moon jellies does not require any permits, airlines do require a cargo account and "Known Shipper" status. International shipping is more involved. Beyond having a cargo account and "Known Shipper" status, Import/Export permits are required, and in most countries a "Certified Health Certificate" is required for the importation of shipments consisting of animals: live or dead, animal products or by-products, for human consumption or not, for education or exhibit. Because these requirements vary from country to country and are changing sometimes from month to month, it is the responsibility of the shipper/exporter and the consignee/importer to contact their respective ministry such as the Department of the Interior or Department of Agriculture for up to date information and requirements. Inspections of the shipment will most likely take place at some point along the way. Pack jellies in such a way that if the box needs to be opened and inspected, it can easily be repacked and secured without difficulty or disruption to the jellies.

If the transportation of moon jellies requires more than 3 days including one or more stops to reach their final destination, they should be unpacked, acclimated, circulated and fed for a minimum of 48 hours in a proper plankton system before repacking and shipping to the next destination.

21.3.9 Maintenance and Disease of Moon Jellies

On average, the water quality parameters should be monitored daily. A stable environment is the best scenario for healthy moons. For both the warm water (22–28 °C) and the cold water moons (15–20 °C) maintain pH between 8.0–8.2, salinity 32–33, through water changes and RO/DI top off. Protein skimming is not recommended as the removal of proteins and beneficial organic and inorganic trace elements rob the jellyfish of nutrition (Moe, 1992; Widmer, 2008).

The polyp tank can be incorporated into your grow out system or it can be a separate system. Keeping the polyp tank separate allows for independent manipulation of the water chemistry in order to induce strobilation when needed without compromising the water quality parameters of the grow-out system.

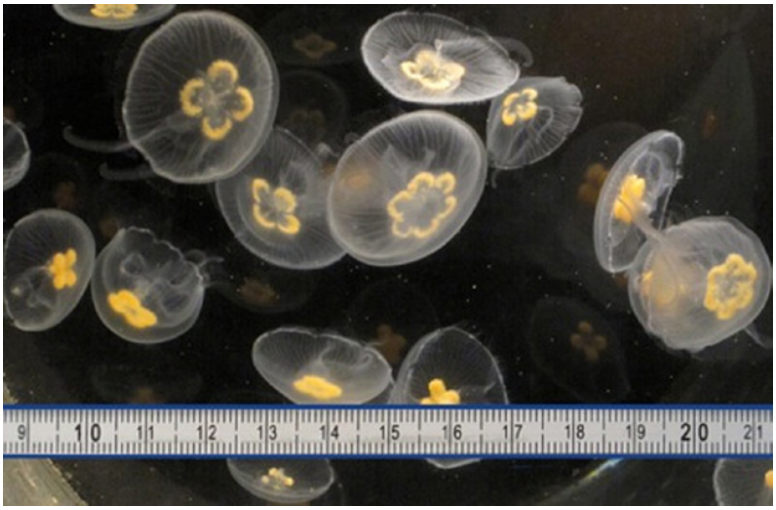


Figure 21.3.6 *Aurelia aurita* gut loaded juveniles: 3–4 cm diameter (7 weeks old).

The necessary multiple daily feedings of enriched nauplii will create the need to groom, brush, clean and siphon each section of the system on a regular basis. The polyp tank will develop hair algae, hydroids like *Bougainvillea* or *Cladonema*, and diatoms, which all need to be removed manually in order for the polyps to develop well. Depending on how the polyps are being cultured, whether on plates or screens, hanging vertically or horizontally, will determine which tools are best to remove the unwanted growth. Tweezers, pipettes, small paintbrushes and the like are the most effective at keeping the cultures groomed.

Once strobilation has occurred and the ephyrae have been effectively collected via the ephyrae catch tank, ephyrae should be removed to the grow-out tanks for development. Ephyrae tanks have nylon mesh screen that needs attention daily. With the amount of enriched nauplii being fed each day, these screens can become clogged and restrict flow. Daily, gentle brushing of the screens maintains the proper flow rate through these micro-flow tanks. Also, on a daily basis, any uneaten nauplii should be removed from the ephyrae tanks with a pipette. With the proper flow established, stable water quality and filtration in place, the growth rate depends on what and how much is being fed. The strongest ephyrae will double in size in 2 weeks.

Once the ephyrae reach 1.25 cm they are graduated to small 11 liter grow out pseudokreisel tanks where they remain until they reach 2.5 cm and are ready for market (Figure 21.3.6). They can also be graduated to intermediate jellyfish grow out tanks of 38–75 L and grown to larger diameters. Typically, they will grow approximately 2.5 cm per month after reaching the 2.5 cm mark.

Each week the juveniles should be sorted, culled and graduated according to size and development. With each sorting, a 10–20% water change on the system takes place.

Although there are no known diseases for the moon jelly, there are other problems that can occur during the aquaculture process that can inhibit growth rates and overall quality of the moons being produced. If it is common practice to bring in natural seawater or wild caught specimens to the facility, hyperiid amphipods are a possible pest

(Laval, 1980). Natural seawater should be mechanically and chemically filtered before use, as well as, UV sterilized. Any wild caught specimens should be inspected for the amphipod and removed manually with tweezers. The hyperiid amphipod does not pose a threat to the cultures, ephyrae or juveniles, but left unchecked they can infest and become a nuisance to production.

The majority of problems moon jellies encounter during development have to do with unstable water quality, non-nutritive food and/or inadequate flow rates at one or more stages of development. Some of the results of poor water quality, food quality and flow are bell deformities such as pitting, curling and everting of the bell. The oral arms can become elongated and stretch past the bell perimeter or simply just hang. If more than 50% of your population is reflecting any one or more of these characteristics, a systematic check of your set up and procedures should be reviewed. Overcrowding may be another reason for the production of deformed jellies. Proper flow rates cannot be achieved if the grow out tanks are so overcrowded they do not allow for freedom of movement of the jellies. As the jellies grow past 2 cm bell diameters they should have enough room around them to freely bell in any direction they choose without directly interacting with another individual.

Polyp cultures should always have adequate flow and be fed daily with enriched nauplii to ensure healthy colonies and individuals with long feeding tentacles. Small, thin, withering polyps with no apparent budding activity can indicate a water quality issue and/or filtration problem. If that is not the problem, the quality of the food being fed may be the issue. Bacteria can foul a batch of *Artemia* nauplii and subsequently cause issue with your polyp cultures and your juveniles. Adequate aeration in the *Artemia* hatchers is crucial. Bleaching and dechlorinating the hatchers monthly is recommended.

Water quality parameters must remain stable for the proper development of the moon jelly from the ephyra stage to the healthy and uniform juvenile stage. High salinity levels can overburden the jellies, which in turn, impedes movement and slows feeding patterns and growth rates. Maintain stable salinity levels at all times with an RO/DI automatic top-off system.

21.3.10 Conclusions

Some of the challenges to the future of jellyfish husbandry, as we see them, are establishing responsible goals when developing new exhibitions, enhancing sustainability of collections, and offering appropriate educational messages. There is a potentially disturbing trend occurring in some large public aquariums that display jellyfish. It is a race to be the institution that can display the greatest number of jellyfish species without regard for the "whys" of the exhibition. The trade off with the increased number of species is at times a decrease in the quality of the exhibitions. Often displays contain only one or two specimens, or are badly lit and poorly maintained. If the purpose of "the race" is to exemplify the staggering diversity that exists amongst gelatinous zooplankton then bravo! We would challenge those with large collections to do a good job of it. Visitors do not want to see acrylic glare, kreisel screens, spray bars or hydroids. Think about the end-product, what will the visitors pictures look like? We are in the business of creating life time memories.

For the commercial ornamental hobbyist market, the introduction of new technology in the way of home jellyfish aquariums has sparked a marketing wave of misinformation about jellyfish. Toy-like tanks that lack filtration are being sold under the guise that jellyfish are cheap and easy to maintain and they come very close to using the word "disposable" when speaking of jellyfish. Cnidarians are delicate invertebrates that require proper filtration, specific foods, and for the case of jellyfish, specialized tanks, in which to survive. The emerging jellyfish hobbyist market has created that same rush to the top as seen in the zoo and aquarium industry with regard to exhibitions. Taking a step back, slowing down and setting some responsible goals about species management and education is the challenge at hand.

Jellyfish are often portrayed by the media as being problematic when large blooms of them appear unexpectedly. Blooms of jellyfish can clog sea water intakes of nuclear power plants, fishing nets, sting swimmers at beaches and kill farmed fish. Since most jellyfish species complete their entire life cycles in one year medusa abundance can vary greatly each year depending upon environmental conditions. Human actions also have the potential to greatly affect jellyfish medusa abundance though overfishing, pollution and urbanization on the coastal margin. We would challenge those developing new jellyfish exhibitions to consider weaving these themes into their educational content. Therefore, the challenge we bring to the industry is not more jellyfish exhibitions, but better ones.

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22

Decapod Crustaceans

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Over the last decades, decapod crustaceans have become one of the most popular groups of invertebrates currently traded in the marine aquarium industry. Their "ornamental status" is certainly not due exclusively to their dazzling coloration and delicate looks. These organisms commonly are quite hardy in captive conditions, are "reef safe" (not harming other organisms also being displayed in the same aquarium set up), display associative behaviour with fish and other invertebrates, and/or control nuisance organisms (e.g., glass anemones, hair algae) in the reef display (Calado, 2008).

Caridean shrimp are by far the most popular group of decapods in the marine aquarium trade. This popularity is largely due to the species of fish cleaning shrimp within the genus *Lysmata*. Other shrimp species, such pistol shrimp of genus *Alpheus* that associated with watchman gobies, and the sea star eaters harlequin shrimp of genus *Hymenocera*, also make marine ornamental shrimp a "must have species" in coral reef displays. Along with stenopodid shrimp of genus *Stenopus* and dwarf reef lobsters of genus *Enoplometopus*, caridean shrimp command the highest prices in the trade (Calado *et al*., 2003a). Their market value, rather than the impacts associated with their collection from the wild, is likely to be the main rationale that has prompted intense research on their aquaculture (Calado *et al*., 2003b, 2007, 2008). Curiously, these species are not necessarily the ones being collected in highest numbers from the reef. Small majoid brachyuran crabs and hermit crabs are likely experiencing the most intensive fishing effort among all decapod crustaceans supplied to the marine aquarium trade (see Rhyne *et al*., 2009). Reaching lower unitary values than ornamental shrimp, the large number of specimens traded could eventually make them appealing for aquaculture. Nonetheless, their culture presents some bottlenecks (Calado, 2008). Unless ovigerous (or sperm carrying) females of majoid crabs of the genus *Mithraculus* are collected from the wild, a regular supply of high quality larvae is unlikely to occur. For hermit crabs the main constraint is the need to supply growing juveniles with suitable shells. Thus, as long as the harvest of wild specimens continues to be largely unrestricted, aquaculture efforts will likely continue to be biased towards the most highly priced species – ornamental shrimp within the genera *Lysmata* and *Stenopus*.

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22.1

Shrimp

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Abstract

Ornamental shrimp are among the most popular invertebrates stocked by reef aquarium keepers, with a handful of species within genus *Lysmata* and *Stenopus* representing the majority of shrimp traded in the marine aquarium industry. The majority of traded specimens is still collected from the wild, with the cleaner shrimp *L. amboinensis* and *L. debelius*, a number of *Lysmata* species popularly termed as peppermint shrimp and the banded boxing shrimp *Stenopus hispidus*, being the most iconic species. Despite nearly two decades of research on the larviculture of these highly priced organisms, commercial scale protocols are still unavailable for the ones most heavily traded. The main bottlenecks impairing large numbers of cultured shrimp to enter the marine aquarium industry are related to their long larval development period and low survival at metamorphosis. The present chapter presents an overview of the current state of the art of *Lysmata* and *Stenopus* aquaculture, while also critically discussing the potential that post-larval capture and culture (PCC) may have to supply the industry and decrease the fishing effort targeting adult specimens.

Keywords *Cleaner shrimp; growout; larviculture; Lysmata; maturation; Stenopus*

22.1.1 Introduction

Marine ornamental shrimp are among the most heavily collected groups of ornamental invertebrates in the marine aquarium industry (Wabnitz *et al*., 2003; Chapter 4). While ornamental decapod crustaceans have always been popular among marine aquarium hobbyists (Calado *et al*., 2003a), no other group matches the status of marine ornamental shrimp. What features make these organisms so appealing for reef aquariums? Most marine shrimp deserving the "ornamental status" display a beautiful coloration and/or unusual external morphology and are considered to be "reef-safe" (they do not damage

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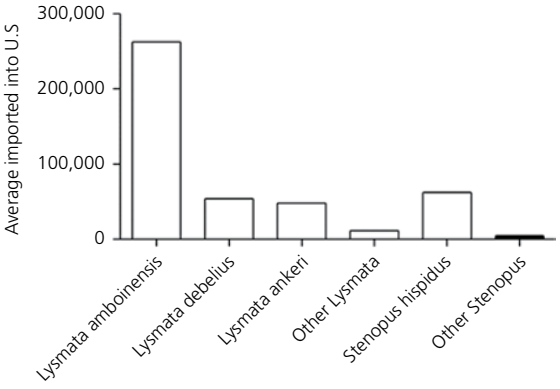


Figure 22.1.1 The average number of *Lysmata* and *Stenopus* imported into to the United States annually between 2008 and 2011. *Source:* www.aquariumtradedata.org

other inhabitants commonly stocked in coral-reef displays). It is also common for these shrimp to exhibit symbiotic behaviour (e.g., associating with anemones and fishes) or display fish cleaning behaviour. Some shrimp traded as ornamentals are also employed for their ability to control nuisance organisms in marine aquariums (e.g., glass anemones of genus *Aiptasia*). However, above all these features, coloration is probably the most important one, with brightly colored red, orange or yellow specimens being favored by the industry (Calado, 2008). Shrimp displaying unusual feeding habits that may not be easily fulfilled through commercially available diets are less appealing to the marine aquarium trade. As always, there are exceptions to this rule; harlequin shrimp *Hymenocera* spp. are readily available in the list of any supplier trading marine ornamentals, even though they solely feed on sea stars (Wickler, 1973). Nonetheless, with the growing level of information on the husbandry requirements of marine ornamentals, along with an unprecedented awareness of marine aquarium hobbyists towards environmental and ethical issues, fewer and fewer species that are destined to slowly starve in captivity are currently traded.

As for several other groups of marine ornamentals, the culture of the most heavily traded species of marine ornamental shrimp has been advocated as an alternative to the collection of wild specimens (Calado, 2008). Over a million ornamental shrimp in genus *Lysmata* Risso, 1816 and *Stenopus* are harvested every year from coral reefs and other marine habitats (Figure 22.1.1) (Wabnitz *et al*., 2003; Rhyne *et al*., 2009; Chapter 4). Given the high fishing pressure targeting the populations of these two genera in the wild, along with their high commercial value, it comes as no surprise that most culture trials to date have focused on the production of *Lysmata* and *Stenopus* (Calado, 2008).

Species in genus *Lysmata* are commonly reported as being members of family Hippolytidae Bate, 1888, with most available literature on their culture following this classification (see Calado, 2008). However, a recent study by De Grave *et al*. (2014) assigned the genus to family Lysmatidae Dana, 1852. This genus likely hosts the highest number of marine ornamental shrimp species being traded for marine aquariums. While some of these species are popularly termed as peppermint shrimp, others are known as cleaner shrimp. Peppermint shrimp refer to a large number of *Lysmata* species, some of them closely related and not easily recognizable as different species by non-specialists (Rhyne & Lin, 2006). These are unquestionably the most heavily traded group of marine ornamental shrimp, with approximately one million specimens being traded per year (2010–2013) in the USA alone (Rhyne *et al*., 2015). Peppermint shrimp

commonly display species specific variations in their coloration patterns, exhibiting semi-translucent bodies with red longitudinal, transverse, and/or oblique red bands. Some species display a more or less intense blue tinge on the posterior region of their telson and uropods. While most species are generally traded under the scientific name of the western Atlantic species *Lysmata wurdemanni* (Gibbes, 1850), most specimens are likely to belong to other species present in the same region, *L. boggessi* Rhyne and Lin, 2006 or *Lysmata ankeri* Rhyne and Lin, 2006 or sometimes to species occurring in the Pacific (e.g., *L. kuekenthali* (De Man, 1902) and *L. vittata* (Stimpson, 1860)). All of these species are well known *Aiptasia* eaters, the pest glass anemone that all reef keepers aim to eradicate from their display tanks (Rhyne *et al*., 2004). The Monaco shrimp *Lysmata seticaudata* (Risso, 1816) inhabits the warm temperate waters of the Mediterranean and the North-eastern Atlantic, presents similar color patterns to other peppermint shrimp and only in the last 10 years made its debut in the trade as suitable species to control *Aiptasia* (Calado & Narciso, 2005). Cleaner shrimp refer to a clade within *Lysmata* (Baeza, 2009; Feidler *et al*., 2010) commonly observed "cleaning" fish species both in the wild and in reef aquariums. The trade mark of ornamental shrimp in general, and cleaner shrimp in particular, is *L. amboinensis* De Man, 1888, also known as skunk cleaner shrimp. A closely related species *L. grabhami* (Gordon, 1935), popularly known as lady cleaner shrimp, may sometimes be confused with *L. amboinensis*. The first occurs in the Indo-Pacific, the Red Sea and Hawaii, while the second is present in both sides of the tropical and subtropical Atlantic. The two species display conspicuously long white antennae, a white third pair of maxillipeds and bright yellow legs and bodies. They both display a longitudinal white stripe from head to tail in their dorsal region, bordered on each side by a red longitudinal stripe. Nearly identical at first sight, they can be distinguished *in vivo* by the patterns on their telson and external branch of the uropods: the longitudinal white stripe exhibited by *L. amboinensis* is discontinuous on the telson and forms an inverted white triangle in its posterior region, while each external branch of the uropods displays two conspicuous white patches on their outer margins; for *L. grabhami*, a longitudinal white stripe runs continuously from the anterior tip of the rostrum to the posterior end of the telson, and each external branch of the uropods has a continuous white line bordering its outer margin (Debelius, 2001). *Lysmata amboinensis* is the most heavily traded cleaner shrimp in the marine aquarium industry with more than 300,000 specimens imported into the United States annually (Rhyne *et al*., 2015), while *L. grabhami* is not commonly available in the trade (which allows traded specimens to reach higher values). Fire shrimp *Lysmata debelius* Bruce, 1983, are also known as cleaners and originate from the Indian Ocean, primarily exported from Sri Lanka, but also occurring in the Central Pacific. Their body displays a brilliant red coloration, which contrasts with their white antennae and terminal segments of their third to fifth walking legs. Fire shrimp also have large white dots on their cephalothorax and pleopods. A closely related species, rarely available in the trade that fetches higher prices, is *Lysmata splendida* Burukovski, 2000. This species displays white dots on the dorsal surface of the abdomen and is so far only known from its type locality, the Ary Atoll in the Maldives (Burukovski, 2000).

It is ironic that some of the most heavily collected and traded shrimp are not actually true shrimp. Species within genus *Stenopus* Latreille, 1819, popularly known as boxer shrimp, are emblematic of the marine aquarium hobby and differ from true shrimp in a number of traits. True shrimp, such as those in genus *Lysmata*, are also

referred to as caridean shrimp given their current classification in infraorder Caridea Dana, 1852 (for a detailed description of the life history of these animals see Bauer, 2004). The genus *Stenopus* is currently placed in infraorder Stenopodidea Claus, 1872, differing from "true shrimp" (among other features) by the presence of chelae (claws) on the third pair of pereiopods (walking legs). All stenopodidean species traded are members of family Stenopodidae, being free living and occurring either alone or as mated pairs in relatively shallow waters. The most popular stenopodidean species is certainly *Stenopus hispidus* (Oliver, 1811), also known as the barber pole or coral banded boxing shrimp. This circumtropical species is mainly collected in the Caribbean, Eastern Africa and Southeast Asia. It displays a white body with conspicuous orange transversal bands on the abdomen and enlarged chelipeds (claws); the basal area of their pereiopods displays a bluish hue. Commonly observed "cleaning" moray eels, it is referred to as a "fish cleaner" (Debelius, 2001). One of the smallest sized species in the genus, *S. tenuirostris* De Man, 1888, is popular in the hobby. Distributed through the Indo-Pacific, this species can be easily recognized by the orange bands it displays on its abdomen and chelipeds and by the bluish tone of its carapace. Females display metallic blue ovaries, which can be easily observed when fully mature in the dorsal region of the cephalothorax. Newly extruded embryos of *S. tenuirostris* are also as bright blue as the ovaries, slowly fading to a greyish-blue during the incubation period in the ventral portion of the female's abdomen. The golden boxer shrimp *S. scutellatus* Rankin, 1898 is collected throughout the Caribbean and, as its common name indicates, can be identified by its golden yellow body coloration.

The researchers attempting to raise marine ornamental shrimp were optimistic at first (e.g., Fletcher *et al*., 1995) given the know-how already available at the time for the aquaculture of penaeid shrimp. Indeed, it was foreseen that the production of these species would be possible in the short-term. However, it rapidly became evident that the lack of knowledge on the reproductive and larval biology of most traded species caused a number of bottlenecks that impaired commercial scale culture using protocols for penaeid shrimp aquaculture (Calado, 2008). To date, none of the most heavily collected species (e.g., *L. amboinensis*, *L. debelius* and *S. hispidus*) are produced in aquaculture at commercial levels.

The present chapter addresses broodstock maintenance and maturation, larviculture and growout, including the culture of post-larvae collected from the wild, of the most emblematic *Lysmata* and *Stenopus* species in the marine aquarium trade. Readers seeking additional information on the species discussed above, or any other marine ornamental shrimp, should refer to Calado (2008) and references cited therein.

22.1.2 Broodstock Maintenance and Maturation

The majority of decapod species have separate sexes. Nonetheless, certain caridean shrimp species may change sex during their lifetime and several different patterns have already been described (e.g. Bauer, 2000).

Stenopodid shrimp display separate sexes and commonly occur in heterosexual pairs. *Stenopus hispidus* detect each other either by visual, olfactory and tactile sense; while olfactory cues strengthen the increase in courtship and agonistic motivation, visual cues inhibit such motivational rise, and tactile contact decreases agonistic motivation

due to sex recognition (Johnson, 1966, 1969). Individual *S. hispidus* behave differently when paired with conspecifics with which they have been previously paired or with individuals with which they had no previous interaction, thus supporting the existence of individual recognition in this species (Johnson, 1977). Large *Stenopus* females are commonly aggressive towards smaller males (Johnson, 1969), but this does not impair successful pair formation (Yaldwyn, 1966). A detailed description of *S. hispidus* mating behaviour has been provided by Zhang *et al*. (1998a) and can be briefly described as follows: (1) antennule contact – both specimens establish antennules contact and slowly wave their large third pair of chelipeds at each other (this step lasts from 10 minutes to 6 hours); (2) erection of the female body – the female turns around with the abdomen raised; (3) grasping – the male rapidly approaches the female, also with its abdomen raised, and holds the female abdomen to abdomen; (4) mating – the male turns rapidly (150 to 180°) to face the female abdomen to abdomen and head to tail, with copulation occurring next (it lasts for approximately 10 seconds); and (5) spawning – after mating, the male leaves the female, with spawning taking place in the next 15 to 25 minutes (it may last 10 minutes). The male deposits a simple spermatophoric mass in the female's posteroventral surface, with the fertilization of mature oocytes occurring externally as these are spawned (Gregati *et al*., 2014).

Marine ornamental caridean shrimp in genus *Lysmata* display an unusual sexual system: simultaneous hermaphroditism (SH) (shrimp initially reproduce as males and later in their life become simultaneous hermaphrodites, but are not able to self-fertilize, nor reverse back to male) (Bauer, 2000). To date, all *Lysmata* species investigated are known to display this puzzling sexual system (including the heavily collected and highly priced species *L. amboinensis* and *L. debelius*) (Baeza, 2013). This sexual system is certainly interesting from a production point of view, as mating pairs may easily be assembled since "any two will do" (Lin & Zhang, 2001a). Moreover, breeders culturing *Lysmata* and stocking the same number of breeding pairs that they do for species with separate sexes will produce twice the number of larvae (as long as they solely use simultaneous hermaphrodites). Once considered to be restricted to genus *Lysmata*, SH has already been recorded in other closely related genera (e.g., *Exhippolysmata* and *Lysmatella*) (Fiedler, 2010). In *Lysmata* mating can be described as follows (see Bauer, 2004 for further details on caridean shrimp in general): (1) Contact – the shrimp at male stage or the hermaphrodite shrimp playing the male role (from now simply called male for clarity) detects a receptive hermaphrodite playing the female role (those with mature ovaries, which have recently molted, from now simply called female for clarity) through contact chemoreception after touching with their antennal flagellum; it must be highlighted that in *Lysmata* a distance pheromone that is detected by receptors on the outer flagella of the antennules may also be involved in this detection (Zhang & Lin, 2006); (2) Climb – after contact, the male crawls to the dorsal midsection of the female; (3) Straddle – while clutching the female with the pereiopods, the male is astride the female's dorsal midline with the anterior ends of the shrimps facing the same side; (4) Mount – the male positions its abdomen along the female's anterior abdominal region; (5) Dip – the male swings under the female and positions its thoracoabdominal junction beneath and perpendicularly to the female's first abdominal sternite; (6) Pleopod beat – the male briefly beats the pleopods in the dip position, with the spermatophore being deposited on the underside of the female's first abdominal segment; (7) Disengagement - male and female separate just after the pleopod beat.

Females or hermaphrodites in genus *Stenopus* and *Lysmata*, respectively, may still spawn in the absence of males if they exhibit mature ovaries, although these unfertilized eggs may remain attached to their pleopods only for the next 48 hours (or less) before being aborted (Calado, 2008). Both stenopodidean and caridean shrimp addressed in this chapter, under stable abiotic conditions, behave as continuous breeders (see Bauer, 1989): as females are incubating developing embryos in their abdomen their ovaries continue to mature (when embryos are about to hatch as free swimming larvae large vitellogenic oocytes can be perfectly identified in the dorsal portion of the female's (or hermaphrodite's) carapace, where the ovaries are located); after larval hatching the female will molt, be receptive to males, mate, spawn a new clutch of oocytes that are fertilized externally and the cycle is repeated.

While eyestalk ablation may be successfully used to trigger the maturation of oocytes in ornamental shrimp (Zhang *et al*., 1997a), there is no need to employ this intrusive technique that may easily result in the loss of highly priced broodstock. Ovarian maturation in *Lysmata* and *Stenopus* may be triggered simply by assuring good water quality, stable water temperatures (26–28 °C), a photo period of 12 hours of light and providing breeding pairs with a variety of available fresh, frozen and/or dry foods. This includes enriched and regular *Artemia* nauplii and adult biomass, clam, mussel, krill, shrimp, squid, polychaetes and dry diets (e.g. Marine Cuisine® produced by San Francisco Bay Brand® and commercial Kuruma prawn diet produced by Ebi Star®, Higashimaru, Japan) (Simões *et al*., 1998; Lin & Zhang, 2001b; Lin & Shi, 2002; Calado *et al*., 2007a, 2010; Tziouveli *et al*., 2011, 2012). Frozen and fresh food items commonly contribute to the rapid decay of water quality and may be a vector for the introduction of pathogens. As already highlighted by Lin *et al*. (2002) and despite all research trials targeting the commercial culture of marine ornamental shrimp, no formulated diets designed for these organisms are currently available.

It is important to note that the maturation of *Lysmata* and *Stenopus* may still be triggered when water quality is poor. Nonetheless, the following issues may be recorded: (1) a reduction in the number of embryos produced; (2) molting by female/hermaphrodites while numerous embryos are still present in the abdomen and remain attached to the shed exoskeleton; and (3) larvae that do not "inflate" after hatching, remaining curled and commonly dying in a few hours (Calado, 2008). An initial warning signal of unsuitable water quality, which can be easily detected, is the presence of "wrinkled" antennae in newly molted shrimp.

An important aspect when selecting broodstock of *Lysmata* or *Stenopus* is to buy mated pairs whenever they are available. While mated pairs are commonly more expensive than specimens bought isolated, assembling breeding pairs of stenopodid shrimp and a few *Lysmata* species, such as *L. debelius* (Rufino & Jones, 2001), may be a time consuming process. It is also possible that some specimens may be lost due to unpredictable agonistic behaviours between members of newly mated pairs. Another important issue when selecting broodstock is to avoid specimens that have damaged appendages or abnormal swells in the lateral regions of the carapace. These swells are commonly caused by the presence of bopyrid isopods that are known to negatively affect the reproductive performance of these marine ornamental shrimp (Calado *et al*., 2006, 2008a). Different species of bopyrid isopods may also be present at least in the abdomen of *Lysmata* and they are also known to decrease the reproductive performance of their host (Calado *et al*., 2005a). Therefore, all broodstock should be carefully inspected for the presence of

these parasitic isopods. It is important to highlight that if a specimen carrying a bopyrid isopod is introduced into the system it is very unlikely that other conspecifics become infected, as these isopods require an intermediate host (commonly a copepod) before being able to infect their final decapod host (O'Brien & Van Wyk, 1985).

Breeding pairs are occasionally stocked in captivity in isolated aquariums which are not connected to a recirculated life support system. When using such a display it is necessary to perform regular surveys on the embryonic stage of developing embryos in the abdomen of females/hermaphrodites, in order to determine when larval hatching is about to occur. This task is not always easy and some stress may be induced to the breeding pair when trying to examine ovigerous specimens. Shrimp carrying embryos in the last stage of development (when yolk is nearly invisible in the embryo, see Calado *et al*., 2005b) must be captured and are commonly placed in larval rearing tanks inside a mesh basket until hatching (Palmtag & Holt, 2001). After larval release, these shrimp are returned to their home aquarium to pair once again. Needless to say that apart from being time consuming, this approach may also induce stress to ovigerous specimens when they are collected and placed in the new container where larvae are to hatch (significant losses of embryos may occur). In *Lysmata* and *Stenopus* species the bond developed between the pair may be broken if prolonged separation occurs (this may trigger agonistic behaviours once the pair is reunited). Additionally, females and late stage embryos may be exposed to unnecessary osmotic and thermal stress when they are placed in a different container than their home aquarium, which may negatively impact larval hatching mechanisms. Females may also cannibalize their newly hatched larvae if no physical barrier separates them. Moreover, females may molt while still separated from their breeding partner and the time window that allows breeding pairs to copulate be lost until the next molt. In order to overcome these constraints, Calado *et al*. (2007a) developed a recirculated maturation system for ornamental decapods (see Chapter 6, Figure 6.3). The main features of this system are the shorter periods of time needed to perform routine tasks (e.g., siphoning, inspecting broodstock, and monitoring water quality), better water quality for broodstock ensured by a proper filtration system, and the fact that it is not necessary to displace ovigerous females before larval release (and therefore risk disrupting reproductive pairs and breeding cycles). Briefly, this system employs a movable plastic mesh inside each breeding tank that separates the breeding pair from the region where newly hatched larvae will be concentrated immediately after release. This separation is achieved by using a unidirectional flow (from the area housing the breeding pair to the region where larvae will be collected), as well as by placing a source of light in the area where newly hatched larvae are protected from cannibalism by the breeding pair. Newly hatched larvae will actively swim towards the light source (commonly a dim actinic light that is turned on after the system lights are switched off, so that breeding pairs are not stressed), with this movement also being favored by the unidirectional flow present in the tank. The outflowing pipe of the tank housing the breeding pairs, located in the region where newly hatched larvae concentrate, is protected by a mesh screen that not only keeps the larvae from being drained from the tank, it may also allow live preys to be provided to larvae immediately after hatching. By providing food to the larvae immediately after hatching it is possible to eliminate the negative impacts of short term larval starvation known to occur in ornamental shrimp (see Calado *et al*., 2005c, 2007b). For further details on this recirculated maturation system please see Calado *et al*. (2007a) and references cited therein.

22.1.3 Larviculture

Species of *Stenopus* and *Lysmata* display a large number of larval stages (termed zoeae), making larval staging a challenging task for those unfamiliar with the morphological features of stenopodidean and caridean larvae. These larvae in particular can delay their larval development through mark-time molting (among other mechanisms; see Gore, 1985). Mark-time molting occurs when a larval stage performs a sequence of molts in which very little change in morphology is perceptible, although small increments in size may occur. Larvae may "mark time" for long periods of time, eventually dying or completing larval development. Once considered a laboratory artifact resulting from suboptimal feeding and/or poor culture conditions, evidence from material collected from the plankton indicate that this larval ability to delay metamorphosis may commonly occur in the wild (see Gurney & Lebour, 1941 for an example in *Stenopus* larvae). Ultimately, mark-time molting can be labeled as a maintenance mode: energy is used to keep the larva alive, and once the energetic requirements are fulfilled, growth first and morphogenesis latter may resume their normal mode (Anger, 2001).

It is paramount that those addressing the larviculture of *Lysmata* or *Stenopus* are familiar with larval morphology. The works by Calado *et al*. (2004) and Bartilotti *et al*. (2012) on *Lysmata* larvae and those from Gurney (1936), Williamson (1976) and Fernandes *et al*. (2010) on *Stenopus* larvae are good starting points for those seeking information on the larval morphology of these two genera. The most remarkable larval features displayed by *Lysmata* larvae are the long stalked eyes and the huge paddle-shape fifth pereiopod of latter zoeal stages (see Bartilotti *et al*. 2012) (Figure 22.1.2). Concerning *Stenopus* larvae, these are known to exhibit a remarkably long rostrum, along with a number of spines and hooks in their abdominal segments (see Fernandes *et al*., 2010) (Figure 22.1.2).



Figure 22.1.2 Late larval stage of *Lysmata* (left) and newly hatched larvae of *Lysmata* (upper right) and *Stenopus* (lower right). Scale bar: 1 mm.

Marine ornamental shrimp can be cultured using either static or circulated systems. Static culture may only be suitable when targeting the culture of a reduced number of larvae (<100) in small water volumes (<20 L). This approach is rarely used for commercial purposes, being mostly employed for research studies evaluating larval stage duration, morphological shifts during larval development, resistance to starvation and feeding behaviour (Zhang *et al*., 1997b, 1998b, c; Simões *et al*., 2002; Calado *et al*., 2007b, 2008b). Flow-through culture systems may decrease the risks of poor water quality that occur in static culture tanks when suitable partial water changes are not performed. Unfortunately, flow-through systems may only be operated in facilities nearshore where seawater with reliable quality is available throughout the year. Recirculated culture systems are the most common choice for raising marine ornamental shrimp, as they can be easily set up in small areas, either nearshore or inland, and operated with natural or synthetic seawater.

Shape and water circulation patterns are likely the most relevant features to successfully select suitable tanks to culture larval decapods. Marine ornamental shrimp are certainly no exception to this rule. The most successfully employed culture system to raise *Lysmata* and *Stenopus* larvae to date was inspired in the "planktonkreisel" described by Greve (1968). This system was described by Calado *et al*. (2003b) and employed cylindrico-conical tanks (10 and 200 L in volume), as well as feeding and cleaning mesh screens inspired by those described by Illingworth *et al*. (1997) that allowed the daily flush of uneaten prey and the supply of new ones exhibiting a higher nutritional profile without disturbing the shrimp larvae being cultured.

A few years later, Calado *et al*. (2008c) described some technical improvements to the system described above, namely the use of cylindrico-spherical instead of cylindrico-conical tanks (Figure 22.1.3). This change in tank shape significantly reduced the number



Figure 22.1.3 Cylindrico-spherical 20-L larviculture tanks supplied with inflowing water at the bottom of the tank through a PVC pipe with a valve to regulate the intensity of the upwelling circulation; fine mesh screens submerged and large mesh screens (employed to drain uneaten prey) outside the water (Photo by R. Calado).

of late *Lysmata* larvae with damaged large paddle-shaped fifth pereiopods or *Stenopus* larvae with their rostrum damaged. Additionally, the time consuming procedure associated with the changing of mesh screens described by Calado *et al*. (2003b) was significantly improved by eliminating the need to lower the tank water level below the outlet; by attaching the cleaning and feeding mesh screens in opposing extremes of a PVC "T", and fitting the free and threaded portion of the "T" to the outlet, it is possible to easily replaced either of the screens simply by rotating the "T" and the screens. It is important to assure that the mesh screens are simply fitted, and not glued, to the "T" so they can easily be detached and cleaned to avoid clogging.

Recently, it has been suggested that larval decapods with frail appendages prone to mechanical damage may be successfully cultured using kreisel tanks commonly employed for the production of gelatinous zooplankton (e.g., jellyfish; see Subchapter 21.3) (Goldstein & Nelson, 2011). Indeed, this type of culture vessel has already been successfully used to culture small numbers of *L. amboinensis* to metamorphosis (Sérgio Leandro, pers. comm.). Most large sized public aquariums around the world have their own "in house" production of jellyfish to stock their displays. In this way, it may be relatively easy for researchers to team-up with aquarium curators and perform trials to further evaluate the suitability of these tanks to culture the fragile larval stages of *Lysmata* and *Stenopus*.

An interesting feature described by Calado (2008) is the use of several artificial grass strips suspended in the water column of culture tanks, to increase tank settlement area and provide shelter to newly settled shrimp. These strips can also be used to easily remove young juvenile shrimps from the culture tank, as they firmly cling to these structures. This procedure no longer requires culture tanks to be drained, which avoids damaging the fragile larval appendages of late zoeal stages.

The initial selection of newly hatched larvae to start a culture trial is certainly one of the most important issues for successful larviculture. Larvae showing a pale coloration or an odd shape (e.g., bended abdomens, "uninflated" carapace spines) usually exhibit poor performances during culture trials and experience mortalities from 90 to 100% in only 48 hours (Calado, 2008). From the various tests available to evaluate larval quality at hatching, light response and swimming ability are the ones most commonly used for marine ornamental shrimp (e.g. Simões *et al*., 2002; Calado *et al*., 2003b, 2005c). While starting culture trials with high quality larvae is important for success, it does not assure *per se* optimal survival at metamorphosis.

Water quality issues and unsuitable feeding protocols may negatively affect the larviculture of *Lysmata* and *Stenopus*. Newly hatched larvae of some (not all) marine ornamental shrimp may endure variable periods of starvation when deprived of live prey, some even being able to molt from zoea I to zoea II (Calado *et al*., 2007b). In some studies (e.g., Fletcher *et al*., 1995; Zhang *et al*., 1998b), this ability has been used as rationale to advocate that there is no need to feed newly hatched larvae of marine ornamental shrimp until they have reached the second zoeal stage. However, Calado *et al*. (2005c) have clearly shown that not feeding the first larval stage of *Lysmata* may extend larval duration, negatively affect survival at metamorphosis and result in a more asynchronous settlement.

The larvae of *Lysmata* and *Stenopus* have been successfully cultured using a multitude of dietary items. Microalgae, rotifers, newly hatched *Artemia* nauplii and enriched metanauplii, copepods, minced/pureed shrimp and squid, as well as commercial flakes, have all been used in different combinations to culture the larvae of these marine

ornamental shrimp (Calado, 2008). While newly hatched *Artemia* nauplii may be employed from hatching to metamorphosis to successfully culture the Monaco shrimp *L. seticaudata* (Calado *et al*., 2005c), this prey may be too big for the first larval stages of other *Lysmata* and too small for late stage larvae near metamorphosis (the same rationale applies to *Stenopus*). Calado *et al*. (2008b) showed how the first larval stages of different *Lysmata* species display distinct abilities to capture and ingest *Artemia* nauplii and metanauplii. These findings reveal that species-specific feeding schemes may have to be implemented for culturing marine ornamental shrimp, even if they are closely related. Simões *et al*. (2002) also suggested that using "green water" to supplement a diet of rotifers/and *Artemia* may improve larval survival of *Lysmata*. The first zoeal stages of *L. amboinensis* appear to benefit when a diet of newly hatched *Artemia* nauplii is supplemented with nauplii and copepodites of the calanoid copepod *Acartia tonsa* (Sérgio Leandro, pers. comm.). Concerning late zoeal stages, it is already known that they are able to feed upon large live preys (e.g., adult *Artemia*) (Calado, 2008), as well as pieces of peeled shrimp (Palmtag & Holt, 2007). These last authors report the successful use of feeding rings inside culture tanks to allow late zoeal stages of *L. debelius* to feed upon shrimp pieces, also allowing easy removal of uneaten portions, preventing the decay of water quality. These records reinforce the claims by Rhyne and Lin. (2004) of the potential to use inert diets to culture late zoeal stages of *Lysmata*, given their positive response to inert diet particles when these are co-fed with live prey (e.g., *Artemia* nauplii or enriched metanauplii). While few studies are currently available on the culture of *Stenopus* larvae, it is likely that most of observations made above for *Lysmata* are also valid for the larviculture of boxer shrimp.

With the advent of molecular tools, it is now possible to gain a new insight on the natural diets of larval decapods in the plankton (O'Rorke *et al*., 2012, 2014). In this way it will be interesting to follow a similar rationale to that already employed for spiny lobsters used for human consumption (Wang & Jeffs, 2014). This approach allows researchers to identify the nutritional requirements of target marine ornamental shrimp species through the biochemical analysis of natural prey identified from larvae collected from the wild.

22.1.4 Growout

To date, the sole marine ornamental shrimp species being regularly produced at a commercial scale is the Monaco shrimp *L. seticaudata*. While the culture of a few thousand *L. debelius* and hundreds of *L. amboinensis*, as well as a few thousands of *Lysmata* spp. from the peppermint species complex described by Rhyne & Lin (2006), have already been reported (see Calado, 2008), they are certainly far from the tens of thousands of *L. seticaudata* produced in captivity. There is presently no record on the successful culture of *Stenopus* at a commercial scale. The unpredictable survival and time to metamorphosis of *Stenopus* and the most highly priced *Lysmata* pose serious bottlenecks to the commercial production of boxer shrimp.

Juvenile ornamental shrimp do not commonly swim in the water column, thus growout tanks should present a high surface area/volume ratio. Shallow growout tanks reduce the water volume of recirculated systems, allow better monitoring of cultured specimens and make routine cleaning tasks significantly easier. Nonetheless, higher

evaporation may also occur and a higher energetic expense may be required to control water temperature. The surface area of growout tanks may be increased by placing stacked PVC pipes inside the tank, mesh bags or artificial grass mats (Calado, 2008). It is recommended that these structures be placed in a way that does not interfere with water flow, nor promote the existence of dead-spots where debris may accumulate. These structures play a key role in the minimisation of cannibalism, as larger specimens readily attack and kill smaller conspecifics during the molt period. Recently, Patel *et al*. (2015) described a growout system for fire shrimp, where shrimp were maintained in enclosures either in pairs or singletons from settlement until market size. Fire shrimp proved to be highly territorial during the male phase of their life cycle and mortality rates in pairs was nearly 60%. Furthermore, growth rates were slow, averaging 0.12 mm/day. Patel *et al*. (2015) recommended growing shrimp out in mass until they are ready for market and then holding singleton shrimp for a few molt cycles in enclosures to ensure damage from territorial fighting healed prior to sell. As already highlight for fire shrimp, not all marine ornamental shrimp species are suitable for communal rearing. *Stenopus* may have to be raised individually soon after metamorphosis given their agonistic behaviours towards conspecifics. The same is also valid for *L. amboinensis*. The growout systems already described for clawed lobsters of genus *Homarus* may be a potential solution for these territorial ornamental shrimp (e.g. Mickelsen *et al*., 1978; Kristiansen *et al*., 2004).

The culture of ornamental shrimp suitable for communal rearing (e.g. those known to occur in groups of several individuals in the wild) can also present some interesting challenges, namely if high stocking densities are employed during growout. *Lysmata seticaudata* is a gregarious species in the wild but prone to cannibalism during growout (Calado & Dinis, 2007). By adding live *Artemia* nauplii, metanauplii or adults to growout tanks it is possible to minimize cannibalism or even completely avoid it (Calado, 2008). When employing live *Artemia* nauplii or metanauplii as live preys in growout tanks it is necessary to regularly clean the screens protecting outflows so they do not clog and cause the tank to overflow.

During the growout period it is very important to minimize heterogeneous individual growth, as larger specimens will become even larger in the short term and smaller conspecifics will be more prone to be cannibalized. In this way, always stock shrimps in growout that do not have an age difference of more than five days. If a reduced number of shrimp is produced, individual culture should be preferred, as this more labor intensive approach is compensated by higher growth rates and survival (Calado & Dinis, 2007). Sort cultured shrimp at least 4 weeks after they were initially stocked and every two weeks after until cultured specimens reach commercial size. Sorted shrimp should be re-distributed through growout tanks according to their size, in order to minimize heterogeneous individual growth, and adjust stocking density with increasing shrimp size.

Juvenile marine ornamental shrimp are known to accept an incredible variety of foods: decapsulated cysts, live or frozen newly hatched nauplii, enriched metanauplii or adult *Artemia*, fresh or frozen chopped squid, shrimp, clams, mussel or fish roe, mixed commercial frozen foods (e.g. Marine Cuisine™ and Emerald Entree™), flakes or freeze-dried Cyclop-eeze™, shrimp pellets, as well as fish flakes and pellets (Calado *et al*., 2005d; Calado & Dinis, 2007). Since the work by Calado *et al*. (2005d) on *L. seticaudata* no study addressed any issue related with growout diets for marine ornamental shrimp.

As referred by Calado (2008), it is legitimate to question if formulated diets will ever be needed for growing juvenile marine ornamental shrimp to commercial size given the satisfactory results achieved with a multitude of fresh, frozen and dry foods. Nonetheless, if an enterprise is willing to undergo commercial scale culture and out-compete less expensive specimens collected from the wild, it must have at its disposal suitable formulated diets. As soon as commercial scale larviculture protocols are established for the most heavily traded species (e.g., *L. amboinensis*, *L. debelius* and *S. hispidus*), formulated feeds that optimize juvenile growth will certainly start to be investigated. The methodologies already available for the formulation of diets for crustaceans cultured for human consumption (Wickins & Lee, 2002) will certainly be a valuable help when the formulation of growout diets for marine ornamental shrimp starts to be addressed.

22.1.5 Collection of Post-Larvae from the Wild

An alternative to circumvent the bottlenecks impairing the larviculture of marine ornamental shrimp is the collection of post-larvae from the wild. Crest nets (nets put on the reef crests) (Lecchini *et al*., 2006), light traps (Doherty, 1987; Dufour & Galzin, 1993), or specially designed collection devices such as the one described by Lecaillon (2004) have already been successfully employed to capture large numbers of wild post-larval marine ornamental fishes (for further detail on this topic please refer to Chapter 18). From 0.5 to 1% of the post-larvae captured are crustacean and in some areas such as Guadeloupe (Caribbean) or Madagascar, *Stenopus* post-larvae can be collected alive (Lecaillon, pers. observ.). Only nine specimens were collected in Guadeloupe with three CARE traps in 6 months (2014) and only six specimens with three CARE traps in Madagascar in 6 months (2011).

Those collected specimens grown in captivity, and weaned from a very early stage to pelleted diets, are less sensitive to shipping and more easily adapted to life in captivity. All these features represent added value for their trade in the marine aquarium industry. For *S. hispidus*, the capture size is about 10–15 mm and can reach a saleable size, up to 5 cm in 2–3 months of growout (Figure 22.1.4) For *Stenopus tenuirostris*, the EcoFish Mauritius Company was able to offer the shrimp after 1 month of growth at 3 cm. Other species such as anemone shrimp can also be collected but *Lysmata* has never been captured with light traps (Lecaillon, pers. comm.).

Hair *et al*. (2004) described how crest nets could be easily employed to harvest newly settled *Stenopus*. By performing a small modification to their nets, employing a rigid water retaining cod end, these authors collected more shrimp with minimum mortality. Collected *Stenopus* were then cultured in submerged cages with coral rubble that were employed on trestles to keep them off the sea floor. Shrimp were fed twice a day with a diet of minced fish, crustaceans and molluscs. Individual culture trials using 200 mL black plastic jars that were previously drilled to facilitate water circulation, clearly outperformed communal rearing (mortality peaked to nearly 90% in these trials) (Hair *et al*., 2004). After only four weeks of growout, *S. hispidus* were ready for export. Crest nets appear to be more efficient for *Stenopus* compare to light-traps in the Fiji area.

If the capture of post-larval ornamental shrimp starts to occur at a significant scale, it may be necessary to employ local/regional measures to reach "biological neutrality" (e.g., decreasing the collection of adult specimens) (see Gardner *et al*., 2006). Briefly, if



Figure 22.1.4 *Stenopus hispidus* 30 days after post-larval capture (Photo by I. Mer). (*See insert for color representation of the figure.*)

increased "fishing mortality" is induced by the collection of post-larvae it may be necessary to either decrease the number of adult specimens being harvested or return a certain percentage of cultured juveniles to the areas where they were initially collected after the growout period. If the approach advocating the release of juveniles is employed it may easily over-compensate the previous harvest of newly settled specimens (as mortality during growout has been drastically reduced). As specimens to be released where harvested from the wild, the risk for promoting genetic bottlenecks through this approach is commonly lower than that resulting from stocking specimens raised from a few broods in captivity.

22.1.6 Conclusions

The commercial scale culture of the most heavily traded species of marine ornamental shrimp is still far from being a reality. The surge in research addressing this topic in the late 1990s and the first decade of the 21st century appears to have faded in recent years. An important boost to once again fuel research on *Lysmata* and *Stenopus* aquaculture may be future limitations to the harvest of these organisms from the wild. If the collection and trade of wild specimens of these ornamental shrimp is somehow restricted, this would definitely push the industry towards the culture of these highly priced species. The production of *Lysmata* and *Stenopus* species/color morphs only occasionally available in the aquarium trade, may also be an interesting approach to add value to the organisms being produced and facilitate the traceability of cultured organisms (Cohen *et al*., 2013). It is also important to highlight that the marine aquarium trade should not be flooded with cultured specimens that may end the "rarity" issue driving the higher prices and demand for those rarer species/color morphs (see Rhyne *et al*., 2014)

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22.2

Other Marine Ornamental Decapods

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Abstract

Herbivorous marine decapods have become a staple in the marine aquarium industry. The control of algae in reef aquaria makes herbivores highly prized members of "cleaning crews". The most heavily traded species in the marine aquarium trade are two genera of western Atlantic hermit crabs (*Clibanarius* and *Paguristes*), with several million *C. tricolor* being traded each year. These small hermit crabs are prized for their ability to eat "hair algae" in reef aquaria. The low per unit price combined with difficult life history features make hermit crabs challenging candidates for commercial aquaculture. Herbivorous brachyuran crabs are equally important members of cleaning crews. *Mithraculus sculptus* and *M. forceps*, traded as green emerald crabs or ruby red crabs, respectively, are ideal candidates for commercial aquaculture. This genus has a short larval period and grows to market size within a few months. While herbivory is the most important factor predicting the popularity of decapods, porcelain crabs and reef lobsters (*Enoplometopus* spp.), among other highly ornamental species, do not fit this role. This chapter reviews the current efforts in the culture of non-shrimp decapod crustaceans, with emphasis on those with shorter larval periods and higher demand due to their role in the maintenance of algae in reef aquaria.

Keywords *Cleaning crews; hermit crabs; Mithraculus; reef lobsters*

22.2.1 Introduction

Caridean and stenopodidean shrimp have always been among the most popular ornamental decapods in the marine aquarium industry (Calado *et al*., 2003a; Chapter 4 and Subchapter 22.1). Nonetheless, several other decapod groups have also been increasingly traded. This trend has led researchers to widen their study scope of potential target ornamental decapods for aquaculture purposes. The present chapter will address the

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culture of porcelain crabs, hermit crabs, true crabs (also known as brachyuran crabs) and lobsters (namely, dwarf reef lobsters). The main issues addressed in this chapter include broodstock management, larviculture and growout of these decapod groups.

22.2.2 Marine Ornamental Porcelain Crabs

Porcelain crabs receive this popular name because of their porcelain-white coloration and their ability to rapidly autotomize their chelipeds when stressed (in other words, break easily) (Debelius, 2001). Apparently identical to true crabs, these organisms are in fact in infraorder Anomura MacLeay, 1838, and are closer relatives to hermit crabs. All traded species are within family Porcellanidae Haworth, 1825 and can be distinguished from true crabs by their reduced fifth pair of pereiopods (it is common that hobbyists are only able to count 8 of the typical 10 legs displayed by decapods).

Several porcelain crabs in genus *Petrolisthes* Haig, 1962 and *Porcellana* Lamarck, 1801 display beautiful colorations that would likely make them suitable candidates for display in reef tanks. However, the cryptic habits displayed by most of these species largely impair their market potential in the marine aquarium trade. The most popular species of porcelain crabs are unquestionably *Neopetrolisthes maculatus* (H. Milne Edwards, 1837) with less than 15,000 specimens reported to be imported into the U.S. per annum. It is likely that several species of *Neopetrolisthes* are traded with *N. ohshimai* being reported on several internet sources. *Neopetrolisthes* are commonly associated with sea anemones (e.g. *Entacmaea* Ehrenberg, 1834 and *Stichodactyla* Brandt, 1835), commonly being present as mated pairs (females are commonly larger than males). While *N. ohshimai* exhibits large red dots scattered through the carapace and chelipeds, *N. maculatus* displays numerous small pink or violet dots. Neither species is presently cultured, although they both display a reduced number of larval stages (two zoeas and one megalopa) (Fujita & Osawa, 2003) that would make them suitable candidates for culture. The two zoeal stages can be identified through the number of setae they display in the exopods of their first and second maxillipeds (the first stage solely displays four setae) (Lebour, 1943). Both zoeal stages can be fed with newly hatched *Artemia* nauplii, although provisioning newly hatched zoeas with rotifers, at least until they have molted to the second zoeal stage, may improve survival. The megalopa will accept enriched *Artemia* metanauplii, as well as minced squid or mussel gonad, with these dietary items likely increasing survival at metamorphosis to the first crab instar if compared with a diet exclusively relying in newly hatched *Artemia* nauplii. It is important to highlight that due to their associative behaviour with sea anemones the metamorphosis from megalopa to the first crab instar may be influenced by the presence of settlement cues released by host anemone species. As the zoeal stages of porcelain crabs display an unusually long rostrum and two long spines in the posterior region of the carapace (e.g. Lebour, 1943; Fujita & Osawa, 2003), they require specially designed larval culture systems to avoid damaging these structures. The larviculture systems described by Calado *et al*. (2008) may be successfully employed to raise these larval stages without damaging their frail appendages.

The husbandry of *Neopetrolisthes* broodstock is not too challenging, as these organism readily ingest a multitude of food items, including the mucus produced by their host anemone (Sprung, 2001). As *Neopetrolisthes* specimens tend to survive poorly

when separated from their cnidarian host (pers. observ.), it may be necessary to stock breeding pairs with their symbiotic sea anemone inside the maturation tank. To decrease the chances of porcelain crab larvae being captured by the host sea anemone it is important to employ efficient larval collectors that rapidly remove the newly hatched zoea form the vicinity of the anemone tentacles (e.g. Calado *et al*., 2007).

The latter stages of *Neopetrolisthes* growout may be marked by intraspecific aggression, a feature already known from other anemone dwelling porcelain crabs (e.g. Baeza *et al*., 2002). This feature may require the use of customized growout tanks that impair intra-specific interactions.

22.2.3 Marine Ornamental Hermit Crabs

Hermit crabs are members of infraorder Anomura and are currently the most popular decapods in the marine aquarium trade as members of "cleaning crews" (see Chapter 4). These decapods are employed by hobbyists as reef aquarium janitors, controlling nuisance algae and eating food leftovers (Sprung, 2002). The most popular species in the trade are those within family Diogenidae Ortmann, 1892, namely the Atlantic scarlet red legged hermit crab (*Paguristes cadenati*) and the Atlantic blue legged hermit crab (*Clibanarius tricolor)*. These two species represent the bulk of the trade with several million *C. tricolor* traded annually (Rhyne *et al*., 2009, Chapter 4) and nearly a million *P. cadenati* imported from Haiti in 2008 (Chapter 4). These are followed up by the electric blue and/or pumpkin hermit *Calcinus elegans* (H. Milne Edwards, 1836), blue eyed hermit *C. laevimanus* (J.W. Randall, 1840), the Pacific red legged hermit *C. tibicen* (J.F.W. Herbst, 1791), the green legs hermit *Clibanarius erythropus* (Latreille, 1818). Hermit crabs popularity is mostly due to their efficiency in controlling the proliferation of unwanted algae and for being reef-safe (not damaging other species displayed in the reef tank). Species deemed the most reef safe are the most traded and in the most demand. These organisms commonly occur in large aggregations over reef flats, as well as lagunar and rocky intertidal areas (e.g., Gherardi & Benvenuto, 2001). This grouping behaviour makes it possible for marine ornamental collectors to capture hundreds to thousands of hermit crabs in a couple of hours (e.g. Calado & Dinis, 2008). It is now acknowledged that such fishing pressure driven by the need to fulfil the increase in demand for these ornamental decapods could result in population impacts (Rhyne *et al*., 2009). Fishers acknowledge that some shallow water populations of the Atlantic red legged hermit crabs in Florida have likely been overexploited, "the low hanging fruit has been picked" (Rhyne, personal observation and anonymous fisherman personal communication). There is limited fishery and life history data with regards to the impacts of this harvest levels on hermit crab populations.

Ovigerous female hermit crabs can be easily collected from the wild and stocked in maturation tanks until larval hatching (see Calado *et al*., 2007). Whenever possible, larger females should be selected over small ones, as body size can influence embryo production in these species (Mantelatto & Garcia, 2000; Contreras-Garduño & Córdoba-Aguilar, 2006), as well as shell architecture (Fotheringham, 1980; Bertness, 1981). Avoid selecting ovigerous females using damaged shells as shelter as they may negatively affect stereotypical hatching behaviours (Ziegler & Forward, 2006).

Species within genus *Clibanarius* display four (sometimes five) zoeal stages plus one megalopa (Bartilotti *et al*., 2008), while those in genus *Calcinus* display five (sometimes six) zoeal stages plus one megalopa (Provenzano, 1962). The megalopa of hermit crabs is sometimes termed in the literature as the glaucothoe. The larval stages of both genera can be cultured in tanks similar to the ones employed for marine ornamental shrimps (Calado *et al*., 2008). All larval stages will readily accept newly hatched and/or enriched *Artemia* metanauplii as a live diet. Under suboptimal culture conditions extra zoeal stages may occur and larval development can be extended (Provenzano, 1962). Near metamorphosis, the megalopa will dramatically change their swimming behaviour and actively start seeking gastropod shells near the bottom of the culture tank (Reese, 1968; Harms, 1992). This is unquestionably one of the main constraints for the culture of marine ornamental hermit crabs. Failing to provide suitable gastropod shells to the megalopa may significantly delay metamorphosis (Harms, 1992). Supplying suitable shells to hermit crabs megalopa, in order to prevent the delay of metamorphosis is time-consuming and it will likely impair commercial scale production of these organisms, as this constraint also occurs during growout. Unsuitable shell size may negatively affect growth of juvenile hermit crabs (Sato & Seno, 2006). An important area of research would be the use of artificial shells for settlement habitat, as well as alternative shelters, as hermit crabs may at times be recorded using small pieces of coral rubble instead of shells.

While some marine ornamental hermit crab species are the most traded invertebrates (Rhyne *et al*., 2009, Chapter 4), the low unitary value of wild specimens may impair the profitability of commercial scale ventures to culture these organisms.

22.2.4 Marine Ornamental Crabs

True crabs belong to infraorder Brachyura Latreille, 1802 and as several other "non-shrimp" decapods have lately gained popularity in the marine aquarium hobby. Species within genus *Mithraculus* White, 1847 are the ones most heavily traded, as they are commonly part of "cleaning crews" employed to control nuisance algae (Sprung, 2002). The present chapter solely addresses the culture of these species, with information on the culture of other genera (e.g., *Percnon*, *Stenorhynchus*, and *Lybia*) being available elsewhere (e.g., Calado *et al*., 2003a, 2008).

Genus *Mithraculus* is currently placed under family Mithracidae Balss, 1929 and is closely related to genus *Mithrax* Desmarest, 1823. Older literature refers to *Mithraculus* as a subgenus (see Wagner, 1990). The green emerald crab *M. sculptus* (Lamarck, 1818) originating from the Caribbean is the most popular brachyuran crab in the aquarium trade with nearly 400,000 specimens landed in Florida in 2013 (Rhyne *et al*., 2009, Chapter 4). Traded with *M. sculptus* are the sister species *M. forceps* and *M. coryphe*. These importantly congener species range from a muted to bright red coloration and are often termed ruby red crabs by the trade. The red congeners offer the most promising commercial aquaculture potential as detailed below. The larval development of these two species (two zoeas plus one megalopa) is already known from laboratory work (Wilson *et al*., 1979; Rhyne *et al*., 2006), with zoea II being easily differentiated from newly hatched zoea by its mobile eyes (zoea I has sessile eyes) and six long plumose

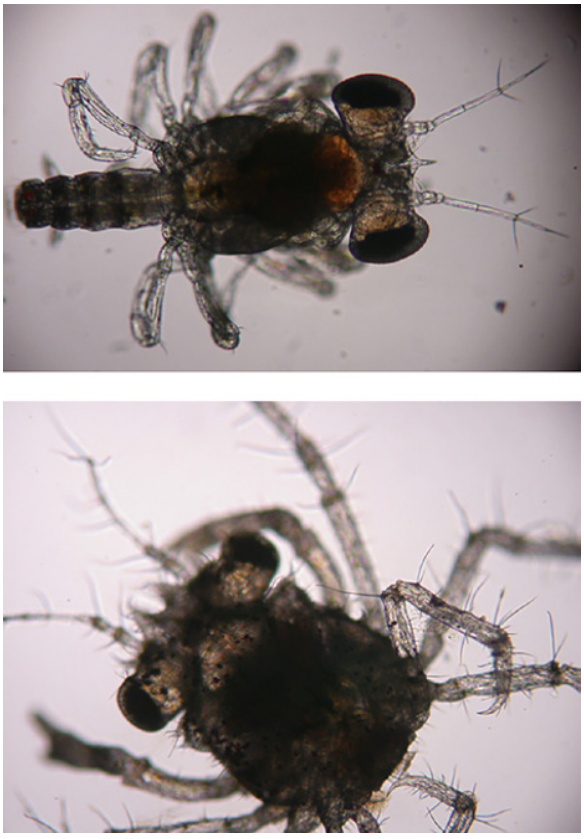


Figure 22.2.1 The megalopa (top) and first crab stage (bottom) of *Mithraculus sculptus*.

natatory setae in the terminal region of the exopod of the first and second maxillipeds (zoea I only has four plumose natatory setae). Rhyne *et al.* (2005) reported how species could be successfully raised to the first crab instar on a diet of newly hatched *Artemia* nauplii. In the same study, *M. forceps* and *M. sculptus* were reported to reach the stage of megalopa in only 5 days with a survival >90%. Nonetheless, while nearly 80% of *M. forceps* megalopa (Figure 22.2.1) were able to molt to first crab (Figure 22.2.1), less than 26% of *M. sculptus* megalopa were able to successfully metamorphose (Rhyne *et al.*, 2005). Such levels of mortality at metamorphosis have also been reported for other crab species (Lárez *et al.*, 2000; Hamasaki *et al.*, 2006), being commonly associated with deficiencies in the fatty acid composition of larval prey (Suprayudi *et al.*, 2004). Hence, providing *Artemia* metanauplii previously enriched on essential fatty acids (e.g. DHA, docosahexaenoic acid), at least to developing megalopa, may contribute to improve survival at metamorphosis. The absence of suitable settlement substrates may also negatively affect metamorphosis in brachyuran crabs (Forward *et al.*, 2001). *M. sculptus* associates with the coralline algae *Neogoniolithon strictum* (Foslie) and the branching coral *Porites* (Pallas, 1766), with this crab species playing an important role in the control of algal epibionts that may affect these organisms (Coen, 1988; Stachowicz & Hay, 1996). Rhyne *et al.* (2005) suggested that the less satisfactory metamorphosis results recorded for *M. sculptus* could be due to the lack of specific settlement cues. Developing

brachyuran megalopae are unable to delay metamorphosis indefinitely, either dying or metamorphosing into smaller sized juveniles (Gebauer *et al*., 2003). Penha-Lopes *et al*. (2005) optimized the larval culture of *M. forceps*, employing the culture system described by Calado *et al*. (2003b) to the stage of megalopa at density of 40 larvae L^{-1}, water temperature of 28 °C and the supply of seven newly hatched *Artemia* nauplii mL^{-1}. Following this period, the megalopa (Figure 22.2.1) should be stocked in a different culture system designed by Tunberg & Creswell (1988) for culturing *Mithrax spinosissimus*. Briefly, this system can be described as follows: a large and shallow recirculated water table supplied with approximately 20 L per minute of 5 µm filtered seawater housing several 300 mm diameter polyvinyl chloride (PVC) rings kept above the bottom of the water table; each PVC ring is fed by gravity from a head tank through a 2 mm inlet positioned in the central area of the ring, at a constant flow of 0.5 L min^{-1}, and with a 200 µm mesh screen attached to the bottom of the PVC ring (Penha-Lopes *et al*., 2005).

The growout phase of juvenile crabs may be differently affected by temperature, namely the intermolt increment of the carapace, if these display (determinate species) or do not display (indeterminate species) a terminal molt (Hartnoll & Bryant, 2001). As all majoid crabs (Hartnoll, 1982), *M. forceps* is a determinate species. Penha-Lopes *et al*. (2006a) reported that while an increase in water temperature did not promote significantly different molt increments, the shorter intermolt period promoted a higher growth of juvenile *M. forceps*.

By employing the modified version of the culture system designed by Tunberg & Creswell (1988), Penha-Lopes *et al*. (2005) were able to raise *M. forceps* communally and did not record any cannibalistic interactions (dead specimens were intact). Penha-Lopes *et al*. (2006a) reported that high stocking densities during growout may enhance the number of interactions between conspecifics, which may result in physiological stress and contribute to a lower growth rate and survival. Cultured juvenile crabs readily ingest inert diets, such as frozen newly hatched *Artemia* nauplii and penaeid shrimp growout pellets (Penha-Lopes *et al*., 2006a).

Ovigerous females of *M. forceps* may occur in the wild throughout the year (Mantelatto *et al*., 2003), thus making it possible to have a regular supply of larvae for captive culture. Nonetheless, Penha-Lopes *et al*. (2006b) demonstrated that females kept in captivity could produce viable embryos fertilized from stored sperm, thus reducing the number of wild females that need to be collected from the wild. Despite the ease of rearing, high market demand and ready supply of broodstock there has been no reported commercial aquaculture success of these species.

22.2.5 Marine Ornamental Lobsters

In the marine aquarium industry there are two main groups of taxonomically distinct decapods being traded under the common name of lobsters: spiny lobsters, within infraorder Achelata Scholtz & Richter, 1995 and dwarf reef lobsters, infraorder Astacidea Latreille, 1802.

The most commonly traded spiny lobster in the marine aquarium industry is the juvenile morph of the blue spiny lobster *Panulirus versicolor* (Latreille, 1804) (as even young adults will rapidly grow too big for regular sized reef tanks), along with small adults of the long handed spiny lobster *Justitia longimanus* (H. Milne Edwards, 1837).

The bottlenecks impairing the commercial culture of spiny lobsters for human consumption are well documented (Phillips, 2006). However, commercial aquaculture of tropical spiny lobsters may provide a supply of juveniles for reef aquariums.

The most commonly imported species of dwarf reef lobsters are Daum's reef lobster *Enoplometopus daumi* Holthuis, 1983, Debeliu's reef lobster *E. debelius* Holthuis, 1983 and the red reef lobster *E. occidentalis* J.W. Randall, 1840. Invoice data is difficult to access for *E. daumi* and *E. debelius*, as these species are often misidentified at the exporter and importer level. Nevertheless, U.S. import volumes of reef lobsters remains low, with less than 15,000 individuals imported annually. These organisms are incredibly aggressive towards same sex conspecifics and cheliped-to-cheliped contact appears to be obligate for *Enoplometopus* specimens to recognize conspecifics from a different sex (Cushing & Reese, 1988). These features may be a challenge for their husbandry and anyone attempting to form mated pairs must be well familiarized with the sexual determination and mating systems in clawed lobsters to avoid unnecessary losses (see Waddy *et al.*, 1995). When pairing any of the three species discussed above it is important to always place the female in a tank that is already stocked with a male, as not doing so will always elicit aggressive behaviours from the female (Penha-Lopes *et al.*, 2004). Mating appears to be tightly coupled with molt cycle and females can store sperm for more than one spawning event. Females carry eggs for 20–30 days post-spawn (Figure 22.2.2).

The morphology of *Enoplometopus* larvae is still poorly understood (see Abrunhosa *et al.*, 2007 for larval description), as well as their average larval development period. Three species of reef lobsters (*E. occidentalis, E. daumi* and *E. debelius*) have been raised to metamorphosis (Penha-Lopes *et al.*, 2004) by the leading author. The larvae of reef lobsters can consume both newly hatched *Artemia* nauplii and enriched



Figure 22.2.2 An ovigerous Daum's reef lobster, *Enoplometopus daumi*.

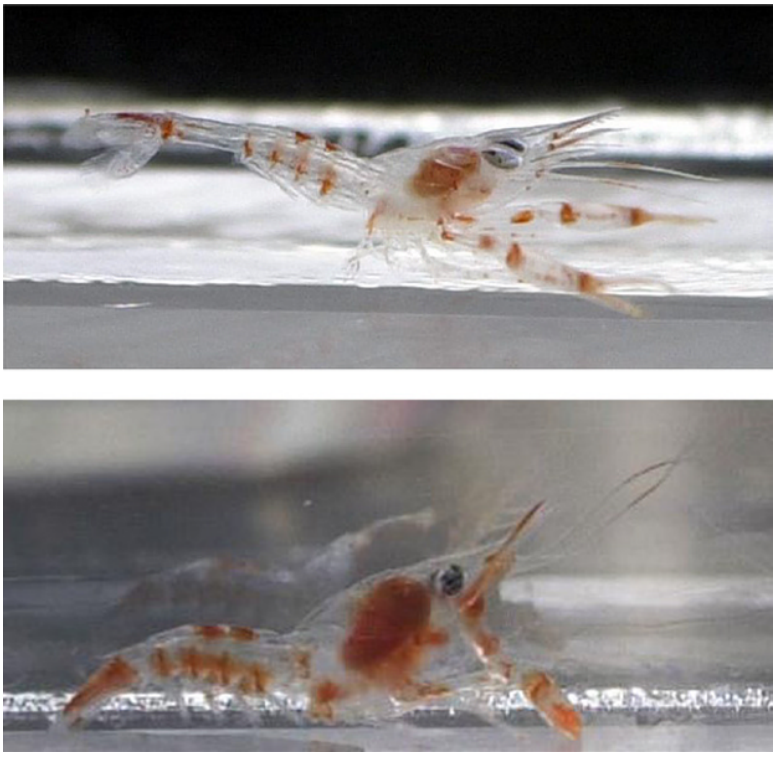


Figure 22.2.3 Late zoea (top) and post-larvae (bottom) of Daum's reef lobster, *Enoplometopus daumi*. (*See insert for color representation of the figure.*)

metanauplii. Overall survival was low (less than 10%) but was greatly increased by enriching prey and providing late zoeal stages (Figure 22.2.3) with larger prey (e.g., *Lysmata* larvae, A.L. Rhyne, pers. observ.). The larviculture of these species must be performed using low stocking densities in order to avoid cannibalism in late zoeal stages. Newly settled *E. debelius* are approximately 18 mm (total length) (Figure 22.2.3) and communal rearing of *Enoplometopus* may only be possible during early post-settlement stages if a large number of suitably sized shelters are available. At larger body sizes, *Enoplometopus* must be grown individually using similar approaches to those commonly employed for other clawed lobsters such as *Homarus* (e.g. Mickelsen *et al*., 1978; Kristiansen *et al*., 2004). Long larval stages and high cannibalism are large barriers for entry in commercial aquaculture of reef lobsters.

22.2.6 Conclusions

While significant numbers of several "non-shrimp" decapods are already traded for marine aquariums it is unlikely that cultured specimens will be available any time soon to alleviate the current fishing pressure on natural populations. The sole exceptions to this trend are likely to be brachyuran crabs in genus *Mithraculus*, given the relatively simple requirements for their larviculture and juvenile growout. Wild stocks of some of

the decapod species referred in this chapter may already be under heavy collection pressure (Rhyne *et al*., 2009). Nonetheless, even for species with easy to rear larvae and gregarious life history patterns the relatively low unitary price of wild specimens is a serious constraint to their culture.

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23

Molluscs

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Molluscs traded in the marine aquarium industry range from dazzling colored giant clams and sea slugs, to shimmering cephalopods and drab colored snails. While several mollusc species are traded for their appealing coloration, others are sold as marine aquarium janitors who tirelessly consume food leftovers and graze upon unwanted algae that cover aquarium displays and may threaten sessile invertebrates.

The most popular marine ornamental bivalve molluscs are unquestionably the giant clams of genus *Tridacna*. Once heavily collected for human consumption and threatened by extinction, tridacnid clams are now listed under CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) since the 1980s. Given the strict regulation and monitoring associated with the international trade of these organisms, along with the poor condition of most wild stocks, it was not surprising that the aquaculture of these highly priced bivalves would play a key role in solving the supply issue and the recovery of wild stocks. Rapidly, those culturing tridacnid clams realized that even small sized specimens could fetch high market values in the aquarium industry (Foyle *et al*., 1997). Valued for their remarkable coloration, tridacnid clams are also esteemed for their ability to thrive much better in captivity than other bivalves. This is due to the trophic plasticity provided by their symbiotic association with photosynthetic microalgae that these molluscs host in their mantle. This group of marine ornamental organisms is also unique in the trade due to the fact that nearly all traded specimens originate from aquaculture.

Sea slugs are traded for marine aquariums either because of their beautiful colors or their ability to control unwanted pest organisms (e.g., algae, glass anemones, flatworms) (Dionísio *et al*., 2013). Unfortunately, the majority of these colorful sea slugs are stenophagous feeders and slowly starve to death in reef tanks. The exceptions to this rule are those employed to control pest organisms, at least as long as those pests are not eradicated. At present, the culture of these pest controlling sea slugs is restricted to those popularly termed as *Berghia* (employed to control the outbreaks of glass anemones *Aiptasia*). A key issue for this scenario is that unlike *Berghia*, which display an abbreviated or direct larval development, most traded sea slugs have planctotrophic larvae. This is true for the majority of species in the genus *Elysia*, a remarkable group of

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sacoglossan sea slugs that control unwanted green hair-like algae. The popularity of these sacoglossan sea slugs in the aquarium trade is also partly due to their puzzling ability to steal the choloroplasts from the algae they feed upon and incorporate them fully functional in their own body. This feature to incorporate stolen plastids that are able to photosynthesize (from a few days to several months) when trapped in the slugs gastric diverticula has granted them the nickname of crawling leafs or solar powered animals (Cruz *et al*., 2013).

Cephalopods are most commonly displayed in public aquariums theme tanks, as their predatory behaviour makes them unsuitable for reef tanks. Due to their relatively short life cycles, several public aquariums breed some of the cephalopods they display (e.g., cuttlefish and nautilus).

In terms of number of specimens, gastropod snails are likely one of the most heavily collected and traded groups of molluscs in the marine aquarium industry. These specimens are commonly sold as part of "aquarium cleaning crews" – groups of marine invertebrates traded for their ability to consume food leftovers and feces of other aquarium organisms (e.g., fish), as well as their tireless grazing behaviour upon unwanted algae. The alarming rate at which some of these grazers are being collected from the wild to supply the aquarium trade may contribute to a decrease of coral reefs resilience and eventually push these ecosystems towards an eventual collapse (Rhyne *et al*., 2009). A good example illustrating the important role that these gastropods may have for coral growth was described by Toh and co-authors (2013); coral juveniles cultured *ex situ* in the presence of grazing snails grew up to eleven-times faster than conspecifics cultured without any grazers. While some of the snails in the marine aquarium trade have already been culture (e.g., *Trochus* and *Astraea*) (Salas-Garza *et al*., 2009; Dolorosa *et al*., 2013), they are yet to make a regular appearance in the species list of marine ornamental wholesalers. The most logical explanation for this generalised lack of interest in the trade of cultured gastropod snails, in opposition to other molluscs such as tridacnid clams, is likely due to the relatively low unit price of wild specimens in the marine aquarium industry. Unless the collection of these snails from the wild is strongly regulated and drastically reduced, it is unlikely that in years to come cultured specimens will be available for sale.

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23.1

Giant Clams

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Abstract

Giant clams are the largest bivalves on the planet and conspicuous inhabitants of Indo-Pacific coral reefs. Harvested for the food and aquarium trade markets, giant clams are symbiotic with dinoflagellates and possess large and colorful mantles. The aquaculture of giant clams can be divided into five sections: system, broodstock, induced spawning, larval rearing and growout. The larval rearing is the most critical step for success considering high natural mortality. Larvae are reared by means of special techniques, including feeding and offering symbionts until metamorphosis is attained. The most significant challenges in giant clam aquaculture involve the acquisition of adequate broodstock, successful restocking programs and stimulation of research and production of statistics.

Keywords *Coral reef; larvae; symbiosis; Tridacna; tridacnid clams*

23.1.1 Introduction

Giant clams are sedentary and benthic bivalve mollusks, belonging to the order Veneroida, which includes scallops and cockles. The largest extant bivalves, these organisms are distributed across the shallow reefs of the Indo-Pacific (Rosewater, 1965). The shells of a specimen of the giant clam *Tridacna gigas* were once measured at 137 cm (Ruscoe, 1962) and weighing 200 kg (Lucas, 1988). What distinguishes giant clams from other bivalves is the presence of a colored and greatly expanded mantle. Traditional bivalves such as oysters and scallops possess small and thin mantles, while in giant clams the mantle is the most visible organ, extending beyond the shells. Symbiotic dinoflagellates are harbored within the mantle tissue, translocating photosynthetically fixed carbon to the giant clam host, similar to the symbiotic relationship found in corals. The presence of such dinoflagellates coupled with accessory pigments is what gives the mantle its intense coloration. Giant clams and heart cockles of the genus *Corculum* are the only bivalves able to establish a symbiotic relationship with dinoflagellates (Farmer *et al*., 2001). Mixotrophic filter-feeders, giant clams find their sources of organic carbon

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in the metabolites supplied by symbionts and also by passively filtering live and dead organic compounds from the water column. Giant clams are hermaphrodites and develop indirectly, going through three larval stages before completing metamorphosis and becoming a juvenile. Once adults, individuals can live for over 50 years (McKoy, 1979).

23.1.2 Collected and Traded Species

There are eleven species among the Tridacnidae, from the smallest *Tridacna crocea* to the largest *T. gigas*. The following paragraphs briefly describe the main characteristics of all tridacnid species. The only exception is *T. rosewateri*, a species that has been described based only on the finding of dead shells and there are no other reports on this organism. Therefore this species will not be addressed in this chapter. A simplified key for the identification of the main species of giant clams is given in Table 23.1.1.

***Tridacna crocea* (Boring or Crocus Clam – Lamarck, 1819)**

The Crocus Clam is the smallest giant clam species, attaining no more than 16 cm in shell length. The mantle lobes are generally bulbous when comparing to other giant clams and its coloration is generally towards green or blue. Specimens are normally found burrowed in intertidal reef flats near shore at depths between 0 and 5 m. Its distribution ranges from the northeastern Indian Ocean to Fiji. The Crocus Clam is the most abundant giant clam in the world and its conservation status is listed as "least concern" by the International Union for Conservation of Nature (IUCN, 2012).

When aquaculturing this species, one must consider that *Tridacna crocea* is the giant clam that becomes fully mature at the smallest size, around 9 cm of shell length (Mies *et al*., 2013). However its juvenile mean growth rate is also the slowest, *ca.* 1.4 mm month^{-1} (Hart *et al*., 1998).

Table 23.1.1 Simplified identification key to the main species of tridacnid clams comparing shell length and morphology, location and size of scutes, size of byssal orifice and mantle characteristics. Maximum shell size is approximate.

Species	Maximum shell length (cm)	Shells	Scutes	Byssal orifice	Mantle
Tridacna crocea	15	Slightly tall	Small, near the rim	Very large	Bulbous, extended
T. maxima	35	Very elongated	Several	Very large	Extended
T. squamosa	40	Slightly elongated	Large, distanced	Small	Irregular lobes, extended
T. derasa	60	Slightly elongated	Absent	Very small, narrow	Extended
T. gigas	135	Elongated	Very small, near the rim	Small	Roughened, slightly extended
Hippopus hippopus	45	Triangular, abrasive	Absent	Vestigial	Not extended

***Tridacna maxima* (Rugose or Small Giant Clam – Röding, 1798)**

The Rugose Giant Clam reaches a maximum size of 35 cm and it is the species with the largest variation in mantle coloration. The shell is very elongated and contains several scutes. It is the most horizontally widespread giant clam, being found all the way from the Red Sea to Polynesian waters. This wide distribution has permitted the observation of four genetically distinct populations of *Tridacna maxima* across the Indo-Pacific (Nuryanto & Kochzius, 2009). These populations seem to have generated several hybrid specimens (Figure 23.1.1). While high densities of *T. maxima* are found in shallow reefs, this species is listed as "conservation dependent" (IUCN, 2012).

Tridacna maxima reaches maturity at 11 cm (Jameson, 1976) and its juvenile seed growth rate is twice that of *T. crocea*, at 2.9 mm month^{-1} (Hart *et al*., 1998).

***Tridacna squamosa* (Fluted Clam – Lamarck, 1819)**

The Fluted Clam reaches about 40 cm and it is the species that presents the relatively largest scutes of all tridacnid species. *Tridacna squamosa* is found in both near shore and offshore coral reefs, with a similar horizontal distribution to *T. maxima*, from the Red Sea to Polynesia. This species is also listed as "conservation dependent" (IUCN, 2012). The Fluted Clam becomes mature presumably with a shell length of approximately 17 cm. The growth rate for this species has been reported to be around 3.0 cm year^{-1} (Beckvar, 1981), or *ca*. 2.5 mm month^{-1}.

***Tridacna derasa* (Smooth Giant Clam – Röding, 1798)**

The Smooth Giant Clam is the second largest of all tridacnids with a maximum shell length of 60 cm. The shell does not possess any scutes and the byssal orifice is very



Figure 23.1.1 A hybrid specimen (*Tridacna maxima* × *T. squamosa*) with rare mantle pattern and coloration surrounded by acroporid corals at Fitzroy Island, Cairns, Australia. Photo by Luiz Fernando Furlan. (*See insert for color representation of the figure.*)

small. This species is found in the eastern Indian Ocean and from the central Pacific Ocean to Fiji at depths between 0 and 25 m. *Tridacna derasa* is one of the most harvested species, listed as "vulnerable" (IUCN, 2012). This species becomes fully mature presumably at around 23 cm and has a rapid seed growth rate of 6.0 mm month^{-1} (Hart *et al*., 1998), which is about twice as fast as *Tridacna maxima* and four times faster than *T. crocea*.

***Tridacna gigas* (The True Giant Clam – Linnaeus, 1758)**

The True Giant Clam is the largest living bivalve and one of the largest of all invertebrates. The shells grow more than 1.0 m in length and the scutes are very small and near the rim. The mantle is very thick and roughened. Specimens are found on both intertidal and offshore coral reefs, from the eastern Indian Ocean to Fiji. The True Giant Clam is the most harvested of all species and it is also listed as "vulnerable" (IUCN, 2012).

This species seems to be fully mature between 50 and 60 cm (Heslinga *et al*., 1984; Crawford *et al*., 1988). The growth rate of *Tridacna gigas* is the highest among giant clams varying from 8.0 to 10.0 mm month^{-1} (Beckvar, 1981).

***Tridacna squamosina* (Red Sea Giant Clam – Sturany, 1899)**

This species, synonymous to *Tridacna costata*, has shells with large scutes similar to those of *T. squamosa* and the brownish-green mantle extends very little. There are no reports on maximum size, but a single individual was measured at 32 cm (Richter *et al*., 2008). This rare species seems to be restricted to intertidal and offshore coral reefs of the Red Sea. The stocks of this organism have reportedly crashed more than 100,000 years ago (Richter *et al*., 2008) and there is still no conservation status determined by the IUCN. There have been no aquaculture attempts for this species and there are no reports on its maturity and growth.

***Tridacna mbalavuana* (Devil or Tevoroa Clam – Ladd, 1934)**

The Devil Clam, synonymous with *T. tevoroa*, reaches a maximum size of 50 cm and is easy to identify because of its pitch-black mantle. This species is restricted to deeper coral reefs (20 to 30 m) in Fiji and Tonga. The Devil Clam is a rare specimen, listed as "vulnerable" (IUCN, 2012).

There have been few culture attempts and there are no reports on size at maturity or growth rate.

***Tridacna noae* (no Popular Name – Röding, 1798)**

This species had been regarded as synonymous with *T. maxima*. However, a recent molecular study based on COI and 16S rRNA supports that this is in fact a separate species (Su *et al*., 2014). Morphological examinations do not show significant differences between *T. noae* and *T. maxima*, making it very difficult to distinguish them.

This species is distributed mainly in shallow reefs in Taiwan and in a few Japanese islands (Su *et al*., 2014). There has been no aquaculture studies published on *T. noae*, making this species still very much unknown.

***Hippopus hippopus* (Horse's Hoof Clam – Linnaeus, 1758)**

Specimens of the Horse's Hoof Clam reach a maximum size of 45 cm. Its shells are different from those of genus *Tridacna*, being slightly triangular, abrasive and

without byssal orifice or scutes. The mantle is commonly yellow or dark grey and does not extend beyond the shells. This species inhabits shallow coral reefs, often intertidal associated with seagrass beds. The distribution of this species includes Southeast Asia, northern Australia and Polynesia. It is listed as "conservation dependent" (IUCN, 2012). *H. hippopus* is fully mature at 15 cm (Alcazar *et al*., 1993). A growth rate of 4.0 cm year^{-1} is reported by Beckvar (1981).

Hippopus porcellanus ***(China or Porcelain Clam – Rosewater, 1982)***
This species can reach 40 cm in shell length and it is distinguishable from *H. hippopus* by having a thinner and less triangular shell. The mantle also does not extend and its coloration is generally yellow or green. Specimens can be found in shallow coral reefs in Palau and Southeast Asia, mainly Indonesia and the Philippines. This species is also listed as "conservation dependent" (IUCN, 2012).

While there are no specific reports on size at maturity or growth rate, individuals larger than 20 cm seem to spawn efficiently (Alcazar *et al*., 1987).

23.1.3 Habitat

Coral reefs are coastal environments located within the euphotic zone of tropical and subtropical continental shelves. While there is a certain availability of particulate and dissolved organic matter, the nutrient concentrations are low. The water column is well mixed with little stratification and stable physical–chemical parameters. Average temperature values range from 24 to 30 °C and salinity is around 34 (specific gravity of 1025 kg m^{-3}), with little or virtually no run-off influence.

A giant clam is only one of many organisms that inhabit coral reefs. The Great Barrier Reef is a perfect example of the remarkable diversity found in such habitats, sheltering specimens representative of almost all animal phyla. It is estimated that 25% of all marine species can be found in coral reefs. Along with that diversity comes an intense competition for space.

Tridacna spp. and *Hippopus* spp. inhabit a wide variety of coral reefs throughout the oceans at different depth ranges. They can be found in intertidal and shallow reefs, as well as in slightly deeper and offshore reefs. Different natural substrata are accepted by these species, including sand, rock and coral rubble.

23.1.4 Importance of Trade

Countries in Micronesia have started selling giant clams in the 1980s after perceiving the international interest in its meat, as well as on live specimens for the aquarium trade (Leung *et al*., 1994). Giant clams have long been harvested as a source of protein for human consumption and the adductor muscle is considered a delicacy, usually sold dried or steamed to flavor soups. Currently, restaurants in Japan serve giant clam mantles as sushi. However, restaurants and wholesalers require large organisms that are often five or more years of age (Hambrey & Gervis, 1993). The aquarium trade market has been growing over the past few decades and giant clams are one of the most desired invertebrates (Olivotto *et al*., 2011). The most desired ornamental species are *Tridacna*

crocea, *T. maxima* and *T. derasa*. Some countries (e.g. the Solomon Islands) established aquaculture farms in the 1990s specifically to supply giant clams for the aquarium trade (Bell *et al*., 1997; Hean & Cacho, 2002). In 2001 alone, more than 100,000 live giant clams specimens were internationally traded (Wabnitz *et al*., 2003) and that number doubled in 2007 (Friedman & Teitelbaum, 2008). To a lesser extent compared to the aforementioned, giant clams are also harvested and commercialized for decoration purposes. Their shells are bleached white and used as dishes, plates and even sculptures.

With the worldwide interest in both live and dead giant clams, the increase in fishing pressure on the natural stocks is evident. *Tridacna gigas* and *T. derasa* are by far the more harvested species for human consumption given their large sizes. In the mid 1970's, more than 98% of the population of *T. gigas* and 87% of *T. derasa* had already been harvested at Helen Reef in Papua New Guinea (Pearson, 1977). *T. gigas* has also been reported extinct in Fiji and some Micronesian Islands (Heslinga *et al*., 1984), while local extinctions have been reported in some islands in the Philippines (Gómez *et al*., 1994). Even more recently, natural giant clam sanctuaries in Tahiti had a yearly harvest of 70 tons of clam meat and the Tatakoto protected area had a population decrease of 90% (Andréfouët *et al*., 2013). Successful aquaculture and restocking research programs have been activated since, but still not enough to restore natural stocks. The larval cycle of giant clams was first described in laboratory conditions in Fiji (LaBarbera, 1975), Guam and Palau (Jameson, 1976). In the following years the first mass culture of tridacnid clams was established in the Micronesian Mariculture Demonstration Center (MMDC) in Palau (Heslinga *et al*., 1984) and the first guidelines for restocking were also published (Munro, 1993). Past restocking programs have been reasonably successful in several Micronesian countries (Heslinga *et al*., 1988) and less successful in the Philippines (Gómez & Mingoa-Licuanan, 2006) and other countries.

23.1.5 Anatomy

The physiology of a tridacnid clam is similar to most veneroid bivalves. The most evident differences are the presence of a hypertrophied mantle, a ventral hinge and a considerable distance between incurrent and excurrent siphons. This section describes the basic anatomy and functions of the main organs found in giant clams (Figure 23.1.2). A thorough anatomical and histological description can be found in Norton & Jones (1992).

The external skeleton is comprised of two valves, or shells, made of $CaCO_3$. On the outer face of each valve are rows of four to six scutes aligned vertically. The two valves are held together by a brown-colored ligament, which goes all the way down to the umbo, a bump located near the hinge. The clam orientation is perpendicular to the seabed and the hinge in which the ligaments are bound is located ventrally. Immediately next to the umbo, there is an aperture, called byssal orifice, from which the byssal threads and foot are sometimes exposed. The shells comprise most of the weight of the clam, thus being important in sustaining the organism.

While most veneroid clams possess two adductor muscles, tridacnid clams have only one. The adductor muscle in the Tridacnidae is located at the posterior end and is made of both smooth and striated tissue, being firmly attached to each valve. This organ controls the opening and closing of the valves, as well as the speed and intensity when doing so.

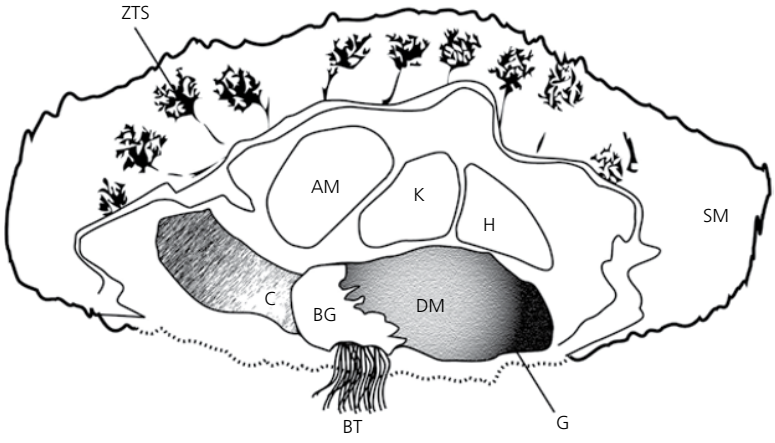


Figure 23.1.2 A longitudinal section of a tridacnid clam and the basic anatomical features. AM: adductor muscle, BG: byssal gland, BT: byssal threads, C: ctenidia, DM: digestive mass, G: gonads, H: heart, K: kidney, SM: siphonal mantle and ZTS: zooxanthellar tube system. Illustration by Daniel Motta.

As mentioned previously, the mantle of giant clams is much larger than the ones found in other bivalves. There are many functions performed by this organ, including protection of soft organs, water transport and maintenance of the symbiotic relationship with dinoflagellates. On the external surface of the mantle, there are two siphons for water transport. The incurrent siphon is located on the posterior end of the mantle and is responsible for water intake. The edges of the incurrent siphon have small guard tentacles (absent in *T. gigas* and *H. hippopus*). After flooding the internal chambers and bathing the organs, water exits through the cylindrical-shaped excurrent siphon. Several retractor muscles located between the mantle and the valves control retraction and extension of the mantle. Small protuberances called iridophores can also be observed on the surface of the mantle. These structures reflect light and have also been suggested to diffract light to symbionts (Griffiths *et al*., 1992). The symbionts are harbored extracellularly (Yellowlees *et al*., 1993), in a network of diverticulae called the zooxanthellar tube system located within the mantle (Norton *et al*., 1992). This system is bathed by hemolymph and emanates from the stomach, where giant clams originally acquire the symbionts. Having a large mantle is an important ecological adaptation. More mantle surface area allows a larger symbiotic population that will perform photosynthesis and secrete larger amounts of organic compounds to the host giant clam.

The ctenidia, or gills, are comb-like (*ktenidion*, diminutive of comb in Greek) structures that perform dissolved gas exchange and food particle sorting for the organism. This organ is easily spotted because it is located immediately after the incurrent siphon opening and beneath the mantle. The ctenidia contain two pairs of arches filled with lamellae and are immediately attached to the digestive mass.

The gonads are found in a thin connective tissue between the digestive mass and a muscular wall. As clams become mature, the gonads increase in size and eventually surround the digestive mass almost in its entirety. Therefore, a mature clam would have a thin gonadal tissue layer enclosing the solid digestive mass. Giant clams are

hermaphrodites; the female and male follicles lay next to one another throughout the gonadal tissue (Norton *et al.*, 1992).

The byssal gland is an organ that produces thread-like filaments, called byssus, that are used for attachment to a consolidated substrate. The byssus is exposed through the byssal orifice, located underneath the clam. The filaments are coated with an adhesive protein in order to facilitate anchoring. Larger clams such as *T. gigas* and *T. derasa* have a relatively small byssus compared to their size. Considering their larger and heavier shells, they rely more on gravity; older specimens may even discard the byssus altogether.

Aside from the aforementioned, giant clams have many other organs with multiple functions, but similar to those of any other bivalve or invertebrate. They include heart, digestive mass, nervous ganglia, enlarged kidneys and the foot. The latter is used for substrate sensing and occasional crawling; it is extended through the byssal orifice.

23.1.6 Reproduction

The reproductive strategy of giant clams is quite peculiar when compared to other invertebrates. They are protandrous simultaneous hermaphrodites (Nash *et al.*, 1988). This means that an individual first matures the male gametes and can only act as a male during a spawning event; eventually, the female gametes are also developed and an individual can act both as a male and a female in a single spawning event. A similar mode of reproduction has been described for species of caridean shrimps (Bauer, 2002).

Giant clams are broadcast spawners, gametes are released into the water column and externally fertilized. While giant clams are simultaneous hermaphrodites, an individual does not release both gametes at the same time. Sperm release precedes egg release and never the other way around. Self-fertilization can happen, but larvae do not survive beyond the first stages (Crawford *et al.*, 1986; Alcázar *et al.*, 1987). Along with the gametes, not yet described pheromones are also released. These substances provoke other clams to also begin releasing gametes resulting in an epidemic spawning, an event that can last for several hours. While most spontaneous spawns take place in the late afternoon (Beckvar, 1981; Heslinga *et al.*, 1984), there is a lot of variance in the frequency in which giant clams spawn. It has been reported that *T. maxima* can spawn every six months (Gwyther & Munro, 1981), while other studies report that giant clams spawn only once a year (Shelley & Southgate, 1988). Some authors report that giant clams can spawn for consecutive months and sometimes in consecutive days (Fitt & Trench, 1981; Heslinga *et al.*, 1984).

Spawning is marked by contraction of the valves and expulsion of gametes through a jet impulse by the excurrent siphon. This behaviour occurs many times during a spawning event. Spawning adults first release sperm and after about half an hour reverse gamete expulsion to eggs. Spermatocytes develop inside the gonads and give origin to the sperm cells, which are released in the water column measuring 5 µm (M.S. Scozzafave, unpublished data). Once released, sperm cells are active for one hour (Ellis, 1998) and have a milky, dense appearance. Oocytes require more time to mature and undergo vitellogenesis before the release of ova (eggs). The ripe egg has a granular appearance and averages 100 µm in size (Lucas, 1988), remaining viable for about thirty minutes after release (Ellis, 1998). The amount of eggs spawned by a single individual has been reported for

Table 23.1.2 Histological description and classification of the reproductive stages of male and female gonadal tissue of giant clams according to Nash *et al*. (1988).

Stage	Male tissue	Female tissue
0: Immature	No gonad tissue; predominance of connective tissue	No gonad tissue; predominance of connective tissue
I: Early gametogenesis	Follicles empty; lined with spermatogonia and spermatocytes	Follicles empty; lined with developing oocytes
II: Mid-gametogenesis	Predominance of spermatocytes, small amount of spermatozoa	Small and elongate ova; developing oocytes attached to follicle wall
III: Late gametogenesis	Mostly ripe spermatozoa, spermatocytes lining the follicle	Densely packed ova, most round; follicle walls thick and smooth
IV: Partly spent	Sparseness of spermatozoa; space filled with amoebocytes	Spaces in ovarian follicles, thin wall; some ova in cytolysis
V: Spent/regressing	No sign of gametes or few residual strands of spermatozoa	Follicles mostly empty or collapsed; residual ova and developing oocytes

some species. *H. porcellanus* released five million eggs in a single event (Alcazar *et al*., 1987), while *H. hippopus* spawned 25 million eggs and *T. gigas* an impressive amount of 500 million eggs (Crawford *et al*., 1986). However, giant clams do not spawn until they become totally spent in most spawning events (Heslinga *et al*., 1990). Sometimes, ripe eggs are stored in the gonads even after a spawning event has ceased. The development of the gonads of giant clams has been classified in six stages (Nash *et al*., 1988, Table 23.1.2), ranging from immature to spent.

The evaluation of whether a clam is mature or not (without taking gonad samples) is many times determined upon size. However, there are studies using age and not size as an indicator of full gonadal maturity. It has been reported that *T. derasa* is mature for male gametes at three years of age and at five for female gametes (Heslinga *et al*., 1984). There are also reports of specimens of *T. maxima* being fully mature at five years of age (Jameson, 1976; Mckoy, 1979).

23.1.7 Diseases

The majority of giant clams casualties are related to inadequate environmental conditions. The greatest symptom (but many times called a disease itself) is the bleaching phenomenon. Adverse physical–chemical conditions such as increased temperature, decreased pH and photoinhibition are the main causes. The underlying cause of this disease is the loss of symbiotic dinoflagellates, causing the mantle to look pale white. Many times the bleaching event is local and the organism can overturn the illness, but sometimes the condition is generalized. Massive losses of symbionts commonly lead to atrophy of the zooxanthellar tube system (Norton *et al*., 1995) and are fatal. Another environmental condition is the gasbubble disease. If there is a supersaturation of dissolved gases in the surrounding water, giant clams may develop bubbles in the mantle.

Severe cases may lead to death. The third main environmental condition is caused by hyposalinity, causing ctenidia inflammation and damaging the feeding apparatus. This symptom is commonly shown by clams submitted to prolonged freshwater dips and can also be fatal.

The infectious diseases in giant clams are mainly of bacterial or protozoan origin, considering that there are no reports of viral or fungal diseases among the Tridacnidae (Braley, 1992). The most common bacterial infection in giant clams is caused by *Rickettsia* sp., which heavily infects the ctenidia and the digestive epithelium. The observation of yellow cysts in the ctenidia tissue when viewed under a stereomicroscope allows for a positive diagnosis. Protozoan infections are commonly related to the presence of *Marteilia* sp. or *Perkinsus* spp. *Marteilia* infections are externally asymptomatic and slowly cause superficial lesions on the kidney. *Perkinsus* spp. are protozoans that cause the most concerning of diseases in giant clams. Perkinsosis, or pinched mantle syndrome, is almost always fatal and there is no treatment for this condition. This disease is widespread in all oceans (Dungan & Reece, 2006) and has been reported to destroy entire populations of other bivalves (Villalba *et al*., 2004). Once infected, a giant clam is asymptomatic until any stressful event causes immunosuppression, when the protozoan population increases and slowly causes the clam to become ischemic. After becoming symptomatic, the clams show curved mantle edges and slow response to stimuli. Once the clams die, the 10 µm long trophozoites of *Perkinsus* spp. become waterborne and infect surrounding clam populations. Trophozoites are extremely resistant to both salinity and temperature, tolerating salinity levels between 15 and 40 and temperature ranges between 5 and 37 °C (Ordás & Figueras, 1998), thus being hard to kill. Diagnosis can be made using the fluid thioglycollate medium (Burreson *et al*., 2005). International shipments of giant clams have been infected with *Perkinsus* sp. have been reported (Sheppard & Phillips, 2008). The most affected species by far is *T. crocea*, while there are no reports of this condition in *T. derasa*. Freshwater dips and antibiotics are of no use against this disease. Most unexplained deaths in giant clams held in captivity are a result of Perkinsosis. The extremely high mortality rate and lack of treatment are an indication of the necessity for research on the matter.

The last and less life-threatening group of diseases is comprised of external parasites. The most intrusive parasites are pyramidellid gastropods. These snails extend their proboscises into the clam tissue and feed on the hemolymph inside the siphonal mantle of the clam (Braley, 1992). While juvenile clams can eventually perish, it is hard for an adult clam to be killed by pyramidellid snails. The other external parasites are more of a nuisance. Sponges of the genus *Cliona* can bore holes into the valves, and algae such as *Gracilaria* sp. can overcrowd the shell and prevent the mantle from extending, but none cause mortality.

The traditional symptoms of a sick giant clam are quite visible. A retracted and not extended mantle is the first sign of something wrong. When a condition is in advanced stage, the incurrent siphon may open wide, a symptom called "gaping". In smaller clams, shortly before death, the individual may discard part of the byssal gland. As already discussed, bleaching is a symptom of inadequate physical–chemical water parameters. When a giant clam is sick, the first troubleshooting measure is checking if the water parameters and conditions are fine. This is also a preventive measure. Some infectious diseases may respond to antibiotics, but concentrations and dosages for giant clams have not been reported. Regarding external parasites, manual removal or freshwater dips commonly solve the problem.

23.1.8 Ecological Aspects

23.1.8.1 Ontogeny

Giant clams are quite fecund bivalves, producing millions of eggs for a single spawning event. After fertilization, a succession of larval stages occurs before metamorphosis. The three stages are trochophore, veliger and pediveliger (Figure 23.1.3, Table 23.1.3). The larval development is similar for all tridacnid species, with the timeline varying slightly according to temperature, culture techniques and broodstock gametogenic condition.

The fertilized egg contains approximately 20% of lipids as yolk reserve (Southgate, 1988), but this amount may vary according to broodstock ripeness. After fertilization, a protective membrane is formed in order to partially prevent other sperm cells entering the egg and causing polyspermy. Eggs are planktonic and have no locomotion abilities, being completely dependent on the ocean hydrodynamics for dispersal. The size of ripe eggs is extremely variable even for individuals of the same species, especially due to broodstock gametogenic conditions. In general, eggs are 100 µm in diameter, with the smallest reported size of 75 µm for *T. squamosina* (Richter *et al.*, 2008) and the largest size of 130 µm for *H. hippopus* (Jameson, 1976). At two hours

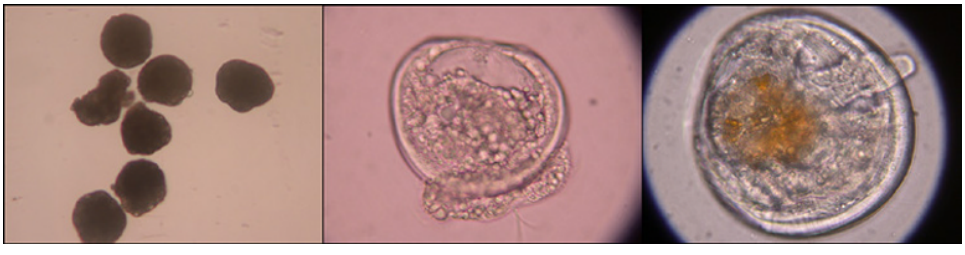


Figure 23.1.3 The three larval stages in giant clams, shown for *Tridacna crocea*. Left: swimming early trochophore larvae at 15 h post-fertilization (pf), Center: veliger larva at 43 h pf showing exposed velum and calcareous shells and Right: pediveliger larva at 7 days pf with exposed foot. Photos by Felipe Braga and Miguel Mies.

Table 23.1.3 Detailing of the main characteristics of the embryo and each one of the larval stages of giant clams, including average size, approximate time of development, life style and specific ecological and physiological developments.

Stage	Size (µm)	Time post-fertilization	Life style	Ecological developments	Specific organs	Phototaxis
Embryo	100	0 h	Planktonic	–	–	–
Trochophore	110	12 h	Planktonic	Limited locomotion	Apical tuft and cilia	–
Veliger	150	24 h	Planktonic	Locomotion and feeding	Shells, velum and digestive tract	Positive
Pediveliger	190	7 days	Benthic	Settlement and metamorphosis	Foot	Negative

post-fertilization (pf) the first embryonic cleaving takes place (Jameson, 1976; Mies *et al*., 2012). Within three to six hours an eight-cell blastula is developed and a gastrula at nine hours pf (Fitt & Trench, 1981).

The free-swimming trochophore stage is developed around 12 hours pf (Lucas, 1988; Mies *et al*., 2012). Locomotion in trochophore larvae is aided by developed apical tuft and cilia. The movements are limited, mainly swirling in circles. While planktonic, this larval stage is not light-oriented. Trochophores are unable to feed since their digestive tract is not yet fully formed. The size of a trochophore larva of *T. crocea* is *ca*. 110 µm (Mies *et al*., 2012).

At 24 hours pf the trochophores morph into veliger larvae (Fitt *et al*., 1984; Mies *et al*., 2012), marked by the genesis of the calcareous shells. Veligers are positively phototactic, swimming towards light using organs that are extended beyond the shells, the velum and cilia. Size may vary, but at four days pf a veliger larva of *H. hippopus* has been reported measuring 187 µm (Fitt *et al*., 1984). Veliger larvae have fully formed digestive tract and start feeding almost immediately (Heslinga *et al*., 1984; Crawford *et al*., 1986). Picoplankton and nanoplankton are filtered by the velum and transported into the larval stomach. Besides food, veligers also acquire symbionts through filtering in the water column. The veliger stage is the longest lasting larval stage in tridacnid clams, ranging from a few days to a few weeks.

The third and last larval stage is the pediveliger stage, evidenced by the presence of the foot. Pediveligers are the transition from a planktonic to a benthic life style. Larvae are negatively phototactic and alternate between swimming and crawling in search of an appropriate substrate for settlement. This stage is attained by day 7–12 pf (Fitt *et al*., 1984; Crawford *et al*., 1986), but has been reported as early as day 4 pf (Alcazar *et al*., 1987).

Metamorphosis is attained between days 9–18 (Fitt *et al*., 1984) and is marked by the presence of the statocyst, an organ responsible for orientation and balance. The size of a metamorphosed juvenile is about 200 µm (Heslinga *et al*., 1984). Once a juvenile, a giant clam has a physical appearance similar to an adult, including the presence of a siphonal mantle. Heavy mortality is experienced during metamorphosis, especially because of the drastic and sudden rotation and re-positioning of the organs. Furthermore, it has been reported that larvae cannot feed during metamorphosis (Southgate, 1988; Fitt, 1993), increasing the mortality rate. The high lipid content is a requirement for larvae to successfully become juveniles. The lipid and protein contents increase during larval development as larvae feed, but during metamorphosis lipid levels decrease and protein levels increase given that muscular tissue must be developed (Southgate, 1988; Lee, 1993). After metamorphosis, growth rates increase significantly until the clam attains full gonadal maturity. Afterwards, growth rates decrease as energy starts being allocated for gametogenesis and reproduction rather than growth (Heslinga, 1989).

The major issue in culturing giant clams is overcoming the low larval survival. Considering their *r*-strategist profile, most larvae are subject to natural mortality and maybe simply unfit for successful development. Generally less than 1% of fertilized eggs survives larval development and become juveniles (Beckvar, 1981; Fitt *et al*., 1984). The best result obtained so far has been 1% survival from egg to 3-month old juvenile (Heslinga *et al*., 1984). Survival has been examined at different life stages for *H. porcellanus*, with survival of 25% for trochophore stage, 10% for veliger stage and 0.76% for juveniles (Alcázar *et al*., 1987).

23.1.8.2 Feeding and Nutrition

There are few studies on the nutrition of giant clams, either larvae or adults. The adult individuals are mixotrophic filter-feeders; live organic carbon is obtained by filter-feeding on phytoplankton and bacterioplankton. Dead organic compounds are also ingested and have a significant contribution to the metabolic requirements of juvenile and adult clams (Klumpp *et al*., 1992; Klumpp & Griffiths, 1994). The second source of nutrition for giant clams comes from the secretion of photosynthates produced by the symbionts. It has been estimated that the symbiotic relationship with dinoflagellates may contribute more than 50% of the carbon requirements of an adult giant clam (Klumpp & Lucas, 1994). Studies on the composition of the hemolymph of giant clams have determined that the main compounds translocated from symbiont to giant clam host are glycerol and glucose (Muscatine, 1967; Griffiths & Streamer, 1988; Ishikura *et al*., 1999). Given the autotrophic character of the dinoflagellates, strong light is an indispensable requirement for the optimum growth of tridacnids.

T. gigas and *T. derasa* have been mass-produced without any additions of food (Heslinga *et al*., 1990), demonstrating a lecithotrophic character. However, many reports show that giant clams hardly make it through metamorphosis and present poor growth rates if unfed (Fitt *et al*., 1984; Fitt, 1993). The discrepancy in these results may be a reflection of the variations in the gametogenic conditions of the broodstock. Therefore, giant clam larvae may have a more planktotrophic character and the acquisition of food may be necessary to survive larval development without constraints. Veliger and pediveliger larvae readily accept a wide variety of microalgae, especially haptophytes such as *Isochrysis galbana* and the green alga *Nannochloropsis oculata* (Fitt *et al*., 1984; Mies & Sumida, 2012). These microalgae are suitable given their content of *n*-3 fatty acids such as DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid), essential elements for the growth of invertebrate larvae (Nevejan *et al*., 2003). Veliger larvae begin feeding early in the development, at 72 hpf (Heslinga *et al*., 1984). Despite the absence of any quantifications, it has also been determined that the symbionts are able to supply veliger larvae with products of photosynthesis (Mies *et al*., 2016), thus enhancing larval growth.

23.1.8.3 Symbiosis

A juvenile or adult giant clam without symbionts in the mantle tissue has never been documented, thus making the symbiotic relationship obligatory for the host. Yonge (1936) published the first report on the symbiosis between giant clams and dinoflagellates almost a century ago. These dinoflagellates are photosynthetic autotrophs and belong to the genus *Symbiodinium*. Commonly referred to as zooxanthellae, these organisms are alveolates (Wisecaver & Hackett, 2011), a group comprised mostly of protozoans. Zooxanthellae can be found in two different stages, either a planktonic zoospore or a vegetative cyst. The latter stage is the one found in endosymbiotic association with multiple phyla, including Cnidaria, Mollusca and Porifera (Stat *et al*., 2006).

The symbiotic relationship between giant clams and zooxanthellae is mutualistic and has many advantages for both sides. Apart from protection, zooxanthellae harbored within the mantle of a giant clam have access to CO_2, nitrogen and phosphorus (Smith *et al*., 1969). In exchange, host clam are supplied essential metabolites (Leggat *et al*.,

2003) through metabolic processes associated with carbonic anhydrase (Venn *et al*., 2008) and can function normally in the absence of particulate or dissolved organic matter if appropriate lighting is available.

Zooxanthellae are not transmitted vertically in the Tridacnidae (Fitt & Trench, 1981; Fitt *et al*., 1986; Mies *et al*., 2012), that is, they do not pass from one generation to another. Gametes spawned by giant clams are aposymbiotic and larvae must acquire the symbionts from the surrounding environment. Zooxanthellae cells are approximately 10 μm in diameter and are first acquired by veliger larvae. Zooxanthellae are ingested by veligers, but resist digestion (Fitt & Trench, 1981; Fitt *et al*., 1986). Giant clam larvae accept zooxanthellae of different origins, such as strains extracted from corals, anemones, jellyfish and other giant clams (Fitt *et al*., 1986). However, it has also been determined that the more motile the zooxanthellae strain, the higher is the acquisition rate by veligers (Fitt *et al*., 1986). There are nine different clades of zooxanthellae (A–I), five of which can be found associated with giant clams (Stat *et al*., 2006), especially clades A and C (Rowan *et al*., 1996; Carlos *et al*., 2000). There are no reports of giant clams symbiotic with any dinoflagellates other than *Symbiodinium* sp..

The symbiosis between zooxanthellae and giant clams was first described as active only after larval metamorphosis, when healthy zooxanthellae migrated from the stomach to the zooxanthellar tube system (Fitt *et al*., 1986; Hirose *et al*., 2006). However, recent studies have shown that zooxanthellae express a symbiosis-specific gene 24 hours after their acquisition by the veliger larva (Mies *et al*., 2016). This information indicates that the symbiosis is already active during larval development and highlights that acquiring symbionts early in the development may have an important effect on survival and growth. It has also been demonstrated that veliger larvae containing symbionts in the digestive tract grow much faster than aposymbiotic veliger larvae (Mies *et al*., 2012). Furthermore, larval growth is enhanced if the source of zooxanthellae offered is a conspecific (Fitt & Trench, 1981).

23.1.9 Aquaculture

23.1.9.1 Systems

The first requirement for successful aquaculture of any marine organism is maintaining seawater quality. Since giant clams are coral reef inhabitants, the physical–chemical parameters of the water must simulate tropical reef waters (Table 23.1.4). Both natural and artificial seawater can be used. Considering the small size of the larvae and that particulate contaminants and live microorganisms are abundant in dense aquaculture systems, all seawater used in giant clam aquaculture must be filtered to 1 μm. Both mechanical and ultraviolet filters should be installed at the pipeline located immediately before the water input in the larval culture tanks.

The most successful systems in giant clam aquaculture have been intensive and composed of five different sets of tanks: broodstock tank, spawning tank, hatching tank, raceway and growout tank. This system can be large-scale, outdoors and connected to the ocean such as the one in the MMDC (Heslinga *et al*., 1990; Braley, 1992) or small-scale, indoors and recirculating (Mies & Sumida, 2012; Mies *et al*., 2012). A sump tank with a protein skimmer, heaters and/or chillers should be installed if a small-scale culture will be performed. Artificial lighting with a photoperiod of 14 L: 10D is suitable.

Table 23.1.4 Recommended values and concentrations of the physical–chemical parameters for the seawater used in giant clam aquaculture systems.

Parameter	Range	Unit
Temperature	26.0–29.0	°C
Specific gravity	1022–1025	$kg\,m^{-3}$
pH	8.1–8.4	–
Carbon hardness	8.0–9.0	dKH
Calcium (Ca^{2+})	370–430	$mg\,L^{-1}$
Magnesium (Mg^{2+})	1100–1200	$mg\,L^{-1}$
Ammonia (NH_3)	<0.1	$mg\,L^{-1}$
Nitrate (NO_3^-)	0–10.0	$mg\,L^{-1}$
Nitrite (NO_2^-)	0	$mg\,L^{-1}$
Phosphate (PO_4^{3-})	<0.05	$mg\,L^{-1}$

Broodstock Tank

The broodstock tanks keep the adult and mature clams. Tanks can be made of concrete and should have a shallow water column of 30 to 50 cm so that intense light can penetrate the water and stimulate zooxanthellae growth. Tanks can be over 2 m in length and 1 m wide. Small-scale tanks can be made of glass, with length and width depending on the amount of broodstock.

Spawning Tank

Only the spawning adults are placed in this tank that serves the purpose of gamete collection. It is appropriate to have one set of tanks for sperm and another one for eggs. Many times this tank is isolated from the ocean or a recirculating system; therefore temperature must be taken into consideration and heating or cooling provided if necessary. Spawning tanks can be made of fiberglass or plastic.

Hatching Tank

The first set of hatching tanks stock the fertilized eggs and trochophore larvae once they hatch. A round, flat-bottomed tank made of plastic or fiberglass is adequate given that its hydrodynamics can aid in keeping the embryos in motion. Volume depends on the culture scale, but each tank should hold at least 500 L of water containing fertilized eggs if necessary. Gentle aeration can be activated after the first cleaving and a central standpipe covered with a 40-µm mesh should be provided for central draining. Once all larvae have reached veliger stage, they can be drained in a 40-µm mesh and transferred to a second set of tanks or immediately transferred to raceways.

Raceway

The raceways stock veliger or pediveliger larvae and should be rectangular and shallow, with no more than 30 cm of water height. Large-scale raceways can be made of concrete and small-scale raceways made of glass. Prior to stocking the larvae, appropriate

substrate for settlement should be provided. Anything that is hard, irregular and easily handled can be used as substrate. Plates of ceramic, cement and coral rubble are all adequate. Another possibility is carborundum (silicon carbide) plates, reported to have high settlement rates (Lee, 1993). The installation of a substrate is fundamental, considering that giant clam larvae are extremely chemosensitive and actively search for an appropriate substrate (Dumas *et al*., 2014). Moreover, it has been shown that bivalve larvae may delay metamorphosis and eventually die if a settlement cue such as the presence of an appropriate substrate is not available (Yu *et al*., 2008). If possible, substrates are cut into small squares in order to be removable and analyzed under magnifying lenses for settled juveniles.

Growout

The growout tanks can be made of any material and size as long as they are shallow, with no more than 30 cm of water height. Strong lighting and moderate to strong water flow should be provided.

23.1.9.2 Broodstock

Adult clams held as broodstock should always be tagged for recording of spawning information. Epoxy resin is suitable for this purpose. When choosing clams for broodstock, size should always be highly regarded. Larger clams are mature enough to produce quality eggs, while medium-sized broodstock are not trustworthy and can often spawn unripe gametes. A slightly invasive but often precise technique to avail broodstock ripeness is the performing of a gonadal biopsy. A syringe with a thin needle is inserted in the excurrent siphon all the way to the gonads and a small amount of tissue is sampled (Braley, 1984). Mature eggs should be spherical while developing eggs should appear irregularly shaped and with increased perivitelline space (Crawford *et al*., 1986).

The recommended amount of broodstock is as much as possible. Some farmers recommend a minimum amount of hundreds of broodstock clams (Heslinga *et al*., 1990). However, many times this is not possible and a rotation of broodstock must be employed. In order to produce constantly throughout the year, adult broodstock should be divided in groups in a manner that one individual is not induced to spawn in intervals shorter than six months (Heslinga *et al*., 1990).

Before spawning, mature clams must be submitted to a conditioning regimen. This procedure has the objective of optimizing gametogenesis and is employed in many bivalve hatcheries around the world (Sühnel *et al*., 2012). A few months prior to induced spawning, broodstock clams are kept in exceptionally stable physical–chemical conditions and supplied with additional food sources. Live cultured microalgae or lyophilized marine algae are supplied twice or three times a week to enhance lipid contents and stimulate gametogenesis. If broodstock is kept under artificial lighting, the light cycle of the photoperiod can be slightly increased.

23.1.9.3 Induced Spawning

The shells of broodstock clams must always be rinsed clean before an induced spawning event. This procedure prevents associated fauna such as polychaetes and crustaceans to enter the spawning tank. One of the first methods employed in induced spawning of giant clams was the heat stress method. Giant clams often spawn under stressful

conditions and this method is based on laying individuals on their sides in the sun for a few hours (Heslinga *et al.*, 1990). It may take a few hours more for spawning to take place after clams are reintroduced to the spawning tank. While mortality records are low, so is the reported success rate. Fitt & Trench (1981) report a success rate of only 20% using this method.

Another induced spawning method is squirting 20 mL of a 3% hydrogen peroxide solution into the incurrent siphon (Beckvar, 1981). This method may result in spawns but normally clams are not responsive (Fitt & Trench, 1981; Gwyther & Munro, 1981). Additionally, this is a procedure that may cause stress signs, such as closing of the valves and excessive mucus production (Fitt & Trench, 1981).

The macerated gonads method is based on the sacrifice and removal of the gonads of a mature clam. Approximately 20 g of gonadal tissue are macerated, diluted in 500 mL of seawater and filtered. Up to 10 mL of the resulting solution are introduced to the incurrent siphon of each broodstock clam (Heslinga *et al.*, 1990). Clams may take a few minutes to half an hour for spawning and this method has good success rate (Fitt & Trench, 1981; Gwyther & Munro, 1981). This technique is sometimes combined with the heat stress method (Fitt *et al.*, 1984; Hirose *et al.*, 2006).

Another successful induced spawning method is the intragonadal injection of serotonin, a neurotransmitter naturally found in giant clams (Braley, 1992). A syringe containing 1 mL of a 1.0 g L^{-1} serotonin (5-hydroxytryptamine) solution is inserted through the mantle cavity and the contents injected in the gonadal tissue (Braley, 1985; Mingoa-Licuanan, 2000). Reported time for spawn averages three minutes and the success rate is 75% (Braley, 1985). However, there is a risk of broodstock mortality due to misplaced injections that pierce vital organs (Ellis, 1998). Besides the injection *via* mantle cavity, it is possible to reach the gonads through the byssal orifice. Injections of serotonin through the byssal orifice yield a spawning response of less than a minute and there are no reports of broodstock mortality (Mies *et al.*, 2013). The absence of mortality is related to the fact that there are no major organs in between the byssal orifice and the gonads.

23.1.9.4 Larval Rearing

Adult clams are immediately transferred to spawning tanks after induction. Once spawning initiates, individuals release sperm for variable time intervals averaging 20 minutes. After sperm release is ceased, the gamete reversion process takes place and eggs are released for a few minutes up to several hours. The first egg spawns commonly contain deformed eggs and ruptured gonadal tissue (Gwyther & Munro, 1981) and should not be collected. After a further few contractions the tank becomes laden with eggs ready for fertilization. Considering the short period of viability of eggs, these should be quickly collected and placed in hatching tanks at densities close to 25 eggs mL^{-1} (Heslinga *et al.*, 1990; Braley, 1992; Mies & Sumida, 2012) and subsequently fertilized with concentrated sperm. The egg: sperm volumetric ratio of 1:200 (Heslinga *et al.*, 1990; Mies *et al.*, 2012) should be considered when fertilizing in order to avoid polyspermy. Fertilized eggs sink to the bottom and after one hour, eggs sampled under a stereomicroscope show germinal vesicle breakdown and first cleavage (Murakoshi, 1986; Mies *et al.*, 2012). A successful batch should yield a minimum of 80% fertilized eggs (Ellis, 1998).

Gentle aeration should be turned on at two hours pf. Dead and decaying eggs will lay at the bottom and a bacterial population will flourish, producing H_2S and reducing both

pH and oxygen. Therefore antibiotics should be added at a concentration of 5 ppm (Fitt *et al*., 1992). Neomycin, streptomycin and cephalosporin are a few appropriate examples. Antibiotics should be added daily or once every two days during the first week of culture. Tanks should be left undisturbed during embryonic development and also after the hatching of trochophore larvae. At 30 hours pf all larvae should be at veliger stage and must be transferred to the raceway. Draining should be slow and gentle, preferably using a hose and a 70-µm mesh submerged in a bucket of seawater.

The stocking density of veliger larvae should be corrected to 10 larvae mL^{-1} (Fitt *et al*., 1984; Mies & Sumida, 2012). Day three veliger larvae begin feeding and the green-water technique can be employed. A variety of microalgae smaller than 10 µm is offered, including *Isochrysis galbana* and *Nannochloropsis oculata*. Species such as *Platymonas subcordiformis, Dunaliella tertiolecta* and the diatom *Phaeodactylum tricornutum* can also be added (Fitt *et al*., 1984; Murakoshi, 1986), but in smaller quantities than *I. galbana* and *N. oculata*. Offered microalgae can be either cultured or purchased as concentrated products and the final concentration of algae in the raceways should be in the order of 10^4 cells mL^{-1} (Fitt *et al*., 1984). Feeding may be tasked every two days until metamorphosis, on days three, five, seven and so on (Mies *et al*., 2012).

At day four pf symbionts must be seeded in the veliger larvae. Optimum survival and growth rates have been found with larval tank concentrations of 10^3 zooxanthellae cells mL^{-1} (Fitt *et al*., 1986). Offered zooxanthellae can be freshly isolated from another organism or cultured. Better results have been produced with freshly isolated zooxanthellae (Fitt *et al*., 1986), presumably because larvae are able to establish symbiosis easier with the vegetative cyst than with the zoospore stage. Freshly isolated zooxanthellae can be obtained from anemones, corals and other giant clams (Fitt *et al*., 1984; Mies *et al*., 2012). The tissue containing symbionts is excised with a pair of tweezers, blended in seawater and filtered through a 40-µm mesh (Murakoshi, 1986; Knop, 1996; Fatherree, 2006). The brownish resulting solution is then ready to be added to larval tanks. The best larval growth rates have been achieved with freshly isolated zooxanthellae from a conspecific (Fitt & Trench, 1981). However, that requires cutting a piece of the mantle of an adult clam and may require the removal of the whole mantle if a large volume is being cultured. Symbiont addition should happen every two days, alternated with microalgae addition (Table 23.1.5). Therefore, zooxanthellae must be added on days four and six (Mies *et al*., 2012) and a few additional doses after day eight are recommended (Crawford *et al*., 1986). If the culture is performed indoors, artificial lighting must be turned on after offering zooxanthellae. After larvae have reached pediveliger stage, feeding and zooxanthellae addition regimens must continue in order to obtain optimal harvest of juveniles. It is wise to perform daily checks and counts to evaluate the progress of the culture.

23.1.9.5 Metamorphosis and Growout

Once pediveligers have completed metamorphosis, the shells will be positioned upwards and a thin mantle will be present. This is the moment when substrates containing juveniles can be removed and transferred to the growout tank. At this stage zooxanthellae populations must be well established. About 1000 zooxanthellae cells supply the carbon requirements of a 400-µm juvenile clam (Fitt *et al*., 1986). At this point, dissolved nitrogen compounds should be added to stimulate zooxanthellae growth and photosynthetic

Table 23.1.5 Standard protocol for the first week of larval rearing of giant clams. Duration of stages is approximate and based on a culture temperature of 27 °C.

Day	Procedure	Tank	Stage	Maximum stocking density	Add microalgae	Add zooxanthellae	Add antibiotics
1	Induce spawning	Spawning tank	–	–	–	–	–
	Fertilization	Hatching tank	Embryo	30 ml^{-1}	No	No	Yes
2	Transfer	Hatching tank – raceway	Veliger	10 ml^{-1}	No	No	Yes
3	Routine check	Raceway	Veliger	–	Yes	No	Yes
4	Routine check	Raceway	Veliger	–	No	Yes	Yes
5	Routine check	Raceway	Veliger	–	Yes	No	Yes
6	Routine check	Raceway	Veliger	–	No	Yes	Yes
7	Routine check	Raceway	Pediveliger	10 cm^{-2}	Yes	No	No

rates (Hastie *et al.*, 1992). Nutrient enrichment increases the density of zooxanthellae cells and also the pigment content within the host (Falkowski *et al.*, 1993). An elevated nutrient concentration is also known to enhance soft tissue production in juvenile clams (Belda-Baillie *et al.*, 1998). Therefore periodical addition of a solution of NH_4NO_3 at a final concentration of 4.0×10^{-4} g L^{-1} is recommended (Fitt *et al.*, 1993; Grice & Bell, 1999).

A good batch of juveniles produced is about 0.1% of the amount of fertilized eggs; individuals at 1.0 cm in shell length can be harvested and removed from the substrate. Juveniles at that size can be stocked at 1000 individuals m^{-2} (Heslinga *et al.*, 1984). Feeding is no longer necessary but good if done occasionally. Herbivore cerithid and trochid gastropods can be added to prevent algal overgrowth from smothering juveniles (Heslinga *et al.*, 1990; Grice & Bell, 1999).

Considering that juveniles are known to grow faster *in situ* than in artificial conditions (Munro *et al.*, 1993), an open ocean growout can be employed. If cultures are held near the coast, 2 cm individuals can be placed in large cages and grown in clear and shallow areas. Cages with structures made of plastic or PVC can measure $1 \times 0.5 \times 0.3$ m and are covered with a 25-mm mesh (Calumpong, 1992). The cages covered with fine mesh prevent carnivorous fishes and benthic predators from harming the juveniles. At 20 cm, the giant clams can survive normally without the protective cage.

23.1.10 Conclusions

The main obstacle today in giant clam aquaculture is acquiring reliable broodstock. An appropriate broodstock population is characterized by having several large and pathogen-free individuals. The few existing (only 10 fully operational giant clam hatcheries exist today – M. Mies, unpublished data) and older hatcheries hardly sell larger

clams, only producing and selling small individuals and seldom growing spat to full maturity. Added to the fact that acquiring wild-caught clams is forbidden in most countries, new and smaller hatcheries have a difficult time acquiring broodstock. Some Pacific hatcheries currently run operations with less than 30 broodstock clams (P. Dor and S.R. Lindsay, personal communication). The situation is more difficult for hatcheries outside the Pacific, that rely solely on imported clams, that often are small, immature and infected with diseases such as Perkinsosis. As a result, hatchery production of giant clams for the international market is low and does not supply the demand (Friedman & Teitelbaum, 2008).

A second issue in giant clam aquaculture is successful restocking. The most successful restocking program was performed in the 1980s by the MMDC. However, other restocking initiatives have not had much success. Especially because clams were introduced at small sizes, were subject to mortality in transport and handling, had heavy fish predation and effects of natural climate events (Calumpong & Solis-Duran, 1993; Bell *et al*., 2005). Added to the naturally slow recruitment rates (Braley, 1988), enhancing natural populations has been a challenge.

Research is also lacking in the field of giant clam aquaculture. A wealth of material was produced during the 1980s by the MMDC-associated researchers, but studies have become rare ever since most of the lead scientists left. Restocking programs could improve if studies on genetics and behaviour were performed. Research could also help battle the major threat of Perkinsosis, a common and lethal disease still without described treatment. Gonadal studies regarding time for the completion of a gametogenic cycle could also help the giant clam aquaculture industry. Therefore, investing in quality research groups throughout the world is essential. The ultimate goal in giant clam aquaculture is to make it become accessible to small breeders. Since collecting is not a solution, hatcheries must help each other and broodstock needs to become more available. Research must be stimulated along with the collection of data and statistics on giant clam production, export and sales.

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23.2

Snails, Slugs and Cephalopods

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Abstract

This chapter reviews the major aspects about the culture of sea snails, slugs and cephalopods of interest to the ornamental industry. Knowledge on rearing and breeding of these marine molluscs has steadily increased in the last decades, but while the well-established sea snails culture protocols already allow commercial scale culture, sea slugs and cephalopods rearing is still in its infancy, i.e. conducted at a small scale mostly for academic purposes. Thus, there is a need for developing feasible culture technology for these two mollusc groups and suitable larval diets and settlement cues that can induce competent larvae. Here we provide relevant biological data to assist the development of culture techniques, information on suitable husbandry, larviculture, nursing of young and growout, presented from an academic and commercial perspective. Emphasis is given to some of the most relevant groups: the cleaning crews (*Trochus* spp., *Turbo* spp., *Haliotis* spp.), nudibranchs and the "solar powered" sea slugs (*Berghia stephanieae* and *Elysia* spp.), and keystone cephalopods (*Sepia* spp. and *Octopus* spp.).

Keywords *Cleaning crews; nudibranchs; sea slugs; octopus; abalone; breeding; feeding; embryogenesis; metamorphosis; culture systems*

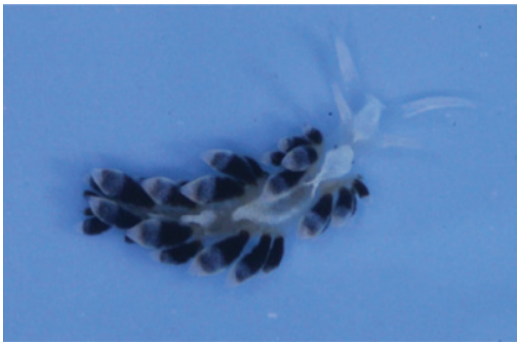
23.2.1 Introduction

Sea snails, slugs and cephalopods are a very diversified and successful group of marine molluscs that have worldwide distribution, and occupy several and distinct ecological niches. Gastropod sea snails have been commercially exploited over the last decades for making jewellery, mostly in the sub-tropics and tropics (Heslinga *et al*., 1984). Yet, nowadays, they are also commonly traded for marine aquariums as part of cleaning crews to reduce epiphytic algae (Olivotto *et al*., 2011). Some of the most traded species are those from genus *Trochus*, *Turbo* and *Haliotis*, with well-established large-scale culture protocols (Hahn, 1989; Heslinga *et al*., 1981; Murakoshi *et al*., 1993).

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Substantial culture efforts have been made for restocking declining populations (FAO, 2014), although their potential use for the marine aquarium trade has long been recognized (Bell *et al*., 1999).

Sea slugs have been reported as experimental tools and models in many research areas, particularly in neurosciences and physiology (Schacher *et al.*, 1983; Pierce *et al.*, 2012). Their potential has increased interest in developing suitable culture protocols for: mass production (Dionísio *et al*., 2013); bioprospecting for new biocompounds (Leal *et al*., 2012a); and for the study of chemical ecology and photosymbiotic associations (Pawlik, 1993; Rumpho *et al.*, 2000; Cimino *et al.*, 2009; Cruz *et al.*, 2013; Dionísio *et al.*, 2015, 2016). Besides the academic and medical interest, some species are also very



(a)



(b)



(c)

Figure 23.2.1 Ornamental sea slugs. (a) *Berghia verrucicornis* adult cultured at Guia Marine Laboratory, Cascais, Portugal; (b) *Elysia clarki* adults; (c) *Elysia clarki* juveniles cultured at Guia Marine Laboratory, Cascais, Portugal. Photo credits: a and b: Gisela Dionísio; c: Cláudio Brandão. (*See insert for color representation of the figure.*)



(a) (b)

Figure 23.2.2 Ornamental cephalopods. (a) *Sepia officinalis* adult; (b) *Octopus vulgaris*. Photo credits: Armindo Pereira. (*See insert for color representation of the figure.*)

popular in the marine aquarium trade. Already a number of organisms are being produced in captivity and employed as biological weapons to control organisms that are considered pests by marine aquarium hobbyists. The nudibranch *Berghia stephanieae* is one of the best examples (Figure 23.2.1a), as it preys exclusively upon glass anemones of genus *Aiptasia* (Carroll *et al*., 1990). Other commonly traded specimens such as *Elysia clarki* eliminate the unwanted green hair-like algae *Bryopsis* (Figure 23.2.1b, c). These specialized sea slugs feed by slicing or puncturing algal cells and sucking their contents. Chloroplasts are retained as functional plastids (kleptoplasts) within the digestive cell of the host (Rumpho *et al*., 2000). Most sea slugs, traded as ornamental species, are vividly colored beautiful organisms (e.g. *Chromodoris* spp., *Hypselodoris* spp.) with stenophagous feeding (most food items are difficult to collect, e.g. sponges, corals, cnidarians, bryozoans), and have low chances of surviving in captivity (Calfo *et al*., 2003; Sprung, 2001).

Cephalopods are masters of camouflage and are thought to be the most intelligent among all invertebrates. Their culture began around five decades ago, sparked mainly by the use of these neurologically complex and highly intelligent animals as model organisms in neuroscience research. More recently, the rapid growth of the ornamental industry has made cephalopods an increasingly desired aquarium pet (Vidal *et al*., 2014). In the ornamental market, the production of cuttlefish (*Sepia officinalis* and *S. pharaonis*) and octopuses (*Octopus maya* and *O. vulgaris*) is noteworthy (Figure 23.2.2). They have many appealing features for aquaculture, including their fast growth and high feed conversion rates, short life cycles, and production of developmentally advanced hatchlings that are miniatures of the adults (Vidal *et al*., 2014). Small-scale cephalopod culture is already possible for some species (Vidal *et al*., 2014), and others are on the verge of moving to industrial scale production in the coming years (Sykes *et al*., 2014a).

This chapter reviews the major questions concerning the culture of these three mollusc groups as marine ornamental species. Relevant information is provided to

Table 23.2.1 Stocking conditions employed for the successful husbandry and grow out of marine sea snails, slugs and cephalopods with emphasis on temperature, diet and density.

	Broodstock husbandry			Growout		
Species	**Temperature (°C)**	**Diet**	**Density**	**Temperature (°C)**	**Density**	**References**
Sea snails						
Haliotis spp.	9–16	Giant kelp[a]; *Gracilaria* spp. *Gelidum* spp.	1 kg per 50 L^{-1}	9–15	88–227 ind. m^{-2}	1, 2
Trochus niloticus	27–30	*Navicula* spp.	–	30–31	>30 ind. m^{-2}	3, 4, 5
Turbo spp.	21–26	*Gracilaria* spp; *Euchema* spp.	20–30 ind. m^{-2}	–	–	6, 7
Sea slugs						
Berghia verrucicornis	24–26	*Aiptasia pallida*	–	–	–	8
Chelidonura varians	26	Planarian	–	–	–	9
Elysia clarki	26	*Penicillus* spp.; *Halimeda* spp. *Bryopsis* spp.	–	–	–	10
Elysia crispata	26	*Bryopsis plumosa*; *Derbesia tenuissima*	–	–	–	11
Cephalopods						
Sepia pharaonis	25–28	Crustacean based diet[b]	–	–	200 ind. m^{-2}	12
Sepia officinalis	15–27	Crustacean based diet[c]	2 to 76 ind. m^{-2} ind. m^{-2}	19–25	120 ind. m^{-2}	13, 14
Octopus vulgaris	14–25	Mix of crustaceans and fish[d]	5 kg/m^3	13–28*	8–14 kg m^{-2}	15, 16, 17
Octopus maya	24–26	Mix of crustaceans and fish[e]	–	24–29**	10–25 ind. m^{-2}	18, 19

1) Lloyd *et al.* (2008); 2) Capinpin Jr. *et al.* (1999); 3) Heslinga *et al.* (1981); 4) Clarke *et al.* (2003); 5) Amos *et al.* (2003); 6) Dwiono *et al.* (2001); 7) FAO (2014); 8) Carroll *et al.* (1990); 9) Gomes (pers. comm.); 10) Curtis *et al.* (2006); 11) Curtis *et al.* (2007); 12) Minton *et al.* (2001); 13) Sykes *et al.* (2003); 14) Forsythe *et al.* (1994); 15) Vidal *et al.* (2014); 16) Delgado *et al.* (2010); 17) Aguado Giménez *et al.* (2002); 18) Vidal *et al.* (2014); 19) Noyola *et al.* (2013);

[a] mainly *Macrocystis pyrifera* and *Nereocystis luetkeana*;

[b] several shrimps and crabs species;

[c] mainly the grass shrimp *Palaemonetes varians* and occasionally the crab *Carcinus maenas*;

[d] several crab species and low commercial value fish such as *Boops boops;*

[e] mainly the blue crab *Callinectes sapidus* and fish as *Lutjanus* spp.;

* 16–21°C is the optimum temperature range;

** 24.3°C is the optimum temperature for growth.

assist in the development of culture techniques, suitable husbandry, larviculture, rearing of young and growout, from both an academic and commercial perspective.

23.2.2 Sea Snails

23.2.2.1 Broodstock

There are three options for obtaining sea snail mature broodstock: (i) collect mature snails during the spawning season; (ii) collect adults for conditioning before the spawning season; (iii) use farm-raised individuals (Castell, 2012). Sea snails are easily detected and captured by SCUBA diving in coastal reefs areas. After collection, specimens should not be out of water for more than eight hours during extended transport over long distances (Lee *et al*. 1996a), and should be placed in well-aerated containers (Wabnitz *et al*. 2003).

Culture Systems

Breeders are generally held in cement tanks (Heslinga *et al*., 1981) with flow-through or recirculating systems (McBride *et al*., 1996; Teitelbaum *et al*., 2008). The incorporation of a seawater reuse system within a controlled temperature broodstock conditioning system offers considerable energy savings over simple flow-through alternatives (Heasman *et al*., 2004). The broodstock is reared in plastic cages with mesh made to fit inside the concrete raceway tanks, with running seawater and strong aeration. The cage bottom is elevated from the raceway tank bottom, keeping the snails away from the waste and silt (FAO, 2015).

Culture Conditions

In order to attain consistent spawning success together with high fecundity levels, high quality larvae and reliable re-spawning, the broodstock should be maintained at optimum temperature, stocking density and water quality (Hahn, 1989; Dwiono *et al*., 1997). Sea snails require seawater at salinity of 33–36, pH between 8.0 and 8.3, with water temperature being dependent on the sea snail origin (Heasman *et al*., 2004; Heslinga *et al*., 1981). Table 23.2.1 summarizes the most relevant parameters for successful broodstock culture of some of the most important sea snails species for the marine ornamental trade.

As the scale of snail culture has increased, problems of deterioration of water quality, contamination and diseases have also intensified (Castell, 2012). Regular inspection of broodstock is recommended to remove death or moribund animals. Also, cleaning and flushing of uneaten foods and faeces will assure healthy breeding pairs (Heasman *et al*., 2004).

Spawning events are variable between species. For instance, *Trochus* spawning in captivity occurs spontaneously on a predictable monthly schedule. Gravid broodstock is available year-round and females are sufficiently fecund to ensure easy manipulation of larval culture densities (Heslinga *et al*., 1981). On the other hand, one of the major bottlenecks in abalone husbandry is controlling spawning events. Several artificial exogenous factors [e.g. UV-irradiated seawater, temperature shock, hydrogen peroxide (Hahn, 1989)] can stimulate females to release unfertilized eggs (Heslinga *et al*., 1981; McBride *et al*., 1996). Thus, there is an enormous variation in the length of the spawning season and the number of spawning events per season (Hahn, 1989).

Feeding

Although there is some research on the effects of diet on the reproduction of sea snails, breeding of captive animals is difficult to control (Heasman *et al*., 2004). Generally, sea snails broodstock are fed *ad libitum* with fresh algae (e.g., *Macrocystis* spp., *Bryopsis* spp., *Gracilaria* spp., *Euchema* spp., *Gellidum* spp.) (Capinpin Jr. *et al*., 1999; Lloyd *et al*., 2008; Ng *et al*., 2013). For *Haliotis asinina*, either artificial diet or a combination of natural and artificial diet showed a higher reproductive performance regarding spawning rate, fecundity and larval hatchability than feeding only a natural diet (Castell, 2012).

23.2.2.2 Embryonic and Larval Development

Fertilized eggs hatch into free-swimming trocophore larvae in a few hours (Murakoshi *et al*., 1993). This planktonic larval stage is brief and differs among and within species and with water temperature. Lecitotrophic larvae (e.g. in *Trochus niloticus* and *Haliotis rufescens*) do not feed during this period (Jaeckle *et al*., 1989). Pediveliger larvae require suitable substrate for settlement, such as solid substrate covered with sessile diatoms (Dwiono *et al*., 2001). Larval competence is normally determined by the presence of three or four ramifications of the cephalic tentacles (Hahn, 1989).

Culture Systems

Embryos can be held in cylindro-conical fiberglass tanks from 20 to 360 L, or plastic baskets with 60–70 μm mesh net inside (lined to retain the embryos) until hatching (Murakoshi *et al.*, 1993; McBride *et al.*, 1996; Heasman *et al.*, 2004). After hatching, *Trochus* veligers can be transferred to outdoor fiberglass raceways with flat polycarbonate plates distributed on the floor of the tanks (Clarke *et al*., 2003). Abalone larvae can be held in flow-through water systems equipped with screens to prevent their escape (McBride *et al*., 1996).

Culture Conditions

The use of filtered and UV-treated seawater is recommended to prevent bacterial infections. Temperature is species-specific, and it is known to affect the rate of development (McBride *et al*., 1996). Various natural and chemical substances have been shown to induce settlement and metamorphosis of larvae. From encrusting coralline algae, benthic diatoms, bacterial films or pre-grazed settlement plates are all commonly used (Heslinga *et al.*, 1981; Ebert *et al.*, 1984; Salas-Garza *et al*., 2009; Castell, 2012). Also, the amino-acid neurotransmitter γ-aminobutyric acid (GABA) has been found to induce settlement and metamorphosis in several species of abalone.

Feeding

Benthic diatoms are the principal food for post-larval sea snails in hatcheries (Heslinga *et al.* 1981; Clark *et al.*, 2003; Gordon *et al.*, 2006). In spite of the increasing number of studies on the nutrition of newly settled snail larvae, their growth and survival rates during the early postlarval stage are usually low (Searcy-Bernal *et al*., 1992). Poor performance is attributed to food variability (different diatoms and their composition) and growout conditions (Kawamura *et al*. 1998). Benthic diatoms (e.g., *Navicula* sp., *Nitzschia* sp., *Amphora luciae*) (Table 23.2.2) are commonly used since they offer high levels of lipids and polyunsaturated fatty acids – PUFAs (Brown *et al*., 1997); both *n*-3 and *n*-6 families are essential for growth (Mai *et al*., 1996). Free amino acids (FAA) are

Table 23.2.2 Rearing conditions employed to raise sea snails, slugs and cephalopods early stages with emphasis to temperature, salinity, diet and density. E – Embryonic phase; H – Hatchling phase; densities are expressed in pdv: pediveligers; ind: individuals.

Species	Temperature (°C)	Salinity	Diet	Density	References
Sea snails					
Haliotis spp.[c]	15	30–35	Diatoms[h]	–	1, 2
Trochus niloticus	28–31	30–35	Diatoms[i]	25,000–100,000* pdv. m^{-3} 100,000	3, 4
Turbo marmoratus	19–25	30–35	Diatoms[j]	–	5
Sea slugs					
Bhergia verrucicornis [a]	24–26	35	No exogenous food required	–	6, 7
Elysia clarki [b]	25–26	35	No exogenous food required	–	8, 9
Cephalopods					
Sepia pharaonis [d]	25–28 (E)	24–36*	Mysids, shrimps and small fish[k]	200–500 ind. m^{-2}	10, 11
Sepia officinalis [e]	18–25 (E); 13–27 (H)	34–37	Grass shrimp or mysids[l]	<500* ind. m^{-2}	12, 13
Octopus vulgaris [f]	18 (E); 18–25 (H)	32–35	Crabs, gammarids, crustacean zoeae, Artemia[m]	10–48 ind. L^{-1}	14, 15
Octopus maya [g]	24–25 (E); 24 (H)	32	Crabs, gammarids[n]	150 ind. m^{-2}	16, 17

[a] Cultured in Petri dishes and in recirculated systems (described by Banger, 2011) using Millipore-filtered aged natural seawater and artificial seawater, respectively;
[b] cultured in culture dishes containing old natural sea water with rifampicin (5 µg mL^{-1}) in an incubator 12 h light:12 h dark cycle;
[c] cultured in flow-through system equipped with screens to prevent their escape;
[d] cultured indoor in plastic baskets with 5 mm mesh size, at lower temperatures, paralarvae may be less tolerant to low salinities;
[e] raised in tanks from 10 to 1000 L^{-1} or more;
[f] cylindro-conical tanks are recommended with stagnant water in the first week;
[g] paralarvae are cultured in 3000 L^{-1} tanks with 7.5 m^2 of bottom area;
[h] *Amphora luciae* and *Navicula* cf. *lenzii;*
[i] *Navicula* spp. and *Nitzshia* spp.;
[j] *Navicula* spp.;
[k] mysids (e.g. *Mesopodopsis orientalis*, *Eurobowmanilla simulans* and *Mysidopsis* sp.), shrimps (e.g. *Penaeus indicus*, *P. merguiensis*, *Metapenaeus dobsoni* and *Palaemonetes pugio*) and small fish (e.g. *Lates calcarifer* and *Poecilia reticulate*);
[l] crabs (e.g. *Palaemonetes varians)*, mysids (*Paramysis nouvelli*);
[m] crabs (*Callinectes sapidus*), mysids (*Hyale azteca*), crustacean zoeae (*Maja brachydactyla*, *Palaemon serrifer*, *Liocarcinus depuratus*, *Pagurus prideaux and Grapsus adcensionis*) and enriched *Artemia*;
[n] crabs (*Callinectes sapidus*) and crustacean zoeae (*Hyale azteca* and *H. media*);
1) McBride *et al.* (1996); 2) Gordon *et al.* (2006) 3) Dwiono *et al.* (1997); 4) Gimin *et al.* (1996); 5) Murakoshi *et al.* (1993); 6) Carroll *et al.* (1990); 7) Banger (2011); 8) Dionísio, unpublished data; 9) Curtis *et al.* (2007); 10) Nabhitabhata *et al.* (2001a); 11) Anil *et al.* (2005); 12) Sykes *et al.* (2006); 13) Forsythe *et al.* (1994); 14) Iglesias *et al.* (2014a); 15) Iglesias *et al.* (2006); 16) Vidal *et al.* (2014); 17) Rosas *et al.* (2014).
* Higher hatching rate at 32.

easily absorbed by postlarvae (Manahan *et al*., 1992), a fact that is especially important at early stages, before the complete development of the gut enzymes involved in protein digestion (Takami *et al*., 1998).

23.2.2.3 Juvenile Growout

Culture Systems

There has been intensive research into the design of growout tanks to maximize survival and growth rates of snails, while minimizing the need of labor (Castell, 2012). The growout of sea snails can be carried out in cages, land-based flow-through tanks (McBride *et al*., 1996). Cages consist of PVC frames covered with heavy gage plastic mesh. Land-based flow-through production usually consists of reinforced concrete or fiberglass tanks of 3800 to 7000 L capacity. In both cages and flow-through tanks, it takes 3 to 4 years for abalone to grow to a commercial market size (7.6 to 8.9 cm) (McBride *et al*., 1996). For abalone culture, the rectangular cages have proven to be more economic, efficient, easier to handle, and produce better survival rates (FAO, 2015). For *T. niloticus* juveniles, reef-based cages are more problematic (e.g. juvenile escape) than floating cages, but can yield faster shell growth (Amos *et al*., 2003).

Improved methods such as the addition of coral rubble inoculated with benthic diatoms enhance food production in the growout tanks (Lee *et al*., 1996b). Co-culture has been successfully applied to *Trochus* spp. and *Turbo* spp. For example, transferring *T. niloticus* juveniles to concrete giant clam tanks halved the time needed to rear *Trochus* to 30 mm (Clarke *et al*., 2003).

It was not surprising that growth is density-dependent, as this has been demonstrated for *Trochus* (Clarke *et al*., 2003) and abalone (Capinpin Jr. *et al*., 1999; Lloyd and Bates, 2008) in response to intraspecific competition for food and/or space (Parsons *et al*., 1992; Capinpin Jr. *et al*., 1999; Lloyd *et al*., 2008). Recent studies point out that even when snails are fed *ad libitum*, food may be limited if the tank design requires the animal to crawl to the food from dense aggregations (Lloyd *et al*., 2008). Table 23.2.1 summarizes the most relevant parameters for the successful growout of some of the most important species.

Feeding

Sea snails growout strongly depends on the availability of specific food to maintain the animals under correct nutritional conditions. When juvenile abalone reaches a size of approximately 10 mm, the type of nourishment changes from benthic diatoms to seaweed (*Macrocystis pyrifera*, *Nereocystis luetkeana*, *Egregia menziesii*, *Gracilaria* sp.) (Simental *et al*., 2004; Castell, 2012). The biochemical composition of seaweeds varies among species and generally is poor comparing to benthic diatoms (Simental-Trinidad *et al*., 2001, Simental *et al*., 2004). This variability in nutritional value produces heterogeneous growth rates (Fleming *et al*., 1996) and high mortalities in organisms under culture conditions. Yet, the biochemical composition can be enhanced by colonizing the seaweed blade with benthic diatoms (Simental *et al*., 2004). The great interest from academic research and industry (i.e. to reduce manufacturing costs and improve diet performance) has prompted the development of artificial diets that are already successfully used in juvenile growout (Table 23.2.1). The combination of natural seaweed and artificial diets can result in faster growth than a single-seaweed diet (Chen & Lee, 1999).

23.2.3 Sea Slugs

23.2.3.1 Broodstock Husbandry

Sea slugs thrive in intertidal and coastal areas and are generally easily found during low tides. Organisms can either be captured manually or with some underwater suction device. Moreover, collection of the most frequent prey of the target species is a good option to harvest small sized or mimetic animals, which may be easily found after collection. Sea slugs can be transported in aerated containers for short distances, or stocked in plastic bags with round bottoms with one third of their volume filled with seawater and two thirds with oxygen for long distances (as described by Wabnitz *et al*., 2003).

Culture Systems

The optimal type system to stock and breed sea slugs is species-specific and depends on the purpose of its production. Recirculating systems using artificial seawater are already allowing the culture of sea slugs in limited spaces (e.g. *Berghia stephanieae*), with biological, chemical and mechanical filtration guaranteeing optimal water quality and little system maintenance (Banger, 2011). Sea slugs may be housed in glass, fiberglass or plastic tanks (Capo *et al*., 2002; Schlesinger *et al*., 2009). The most successful system employed so far for ornamental sea slugs, at small scale, is the recirculating "breeding chamber" culture system described by Banger (2011) for *B. stephanieae*. More recently, Dionísio (unpublished data) developed an adaptation of this system to successfully culture sea slugs from genus *Elysia* (Figure 23.2.3).

Culture Conditions

Sea slugs require seawater (either synthetic or natural) at a salinity of 30–35 and pH between 8.0 and 8.2, with water temperature being dependent on the sea slug origin (Dionísio *et al*., 2013). Since temperature is a key factor in gonadal maturation, it is imperative to adjust this parameter. Also, whenever sea slugs with photosynthetic endosymbionts are stocked, such as the commonly used with *Elysia* spp. and *B. stephanieae*, temperature and light are important factors to consider. Additionally, stocking density is an important issue for the successful culture of sea slugs (Capo *et al*., 2002). The release of animal pheromones and toxic compounds, high prey biomass and concomitantly high ammonia levels will deplete water quality and are indicated as potential growth inhibitors (Capo *et al*., 2009; Handy *et al*., 1993).

Feeding

Sea slugs display different feeding preferences. Some species are known to be stenophagous (preying on a single species), others are able to feed on a range of prey, and even shift their dietary preferences throughout development (Trowbridge *et al*., 2001). In order to assure suitable feeding for stocked organisms, prey must be easily collected from the wild or cultured in captivity. The diet of most traded ornamental sea slugs, namely *Elysia clarki*, *B. stephanieae*, *Chelidonura varians*, is composed of algae, anemones and flatworms, respectively. Different macroalgae culture protocols are available (Winberg *et al*., 2011) and species such as *Codium* and *Bryopsis* can be easily purchased. Anemones and acoel flatworms have also been successfully propagated in captivity to

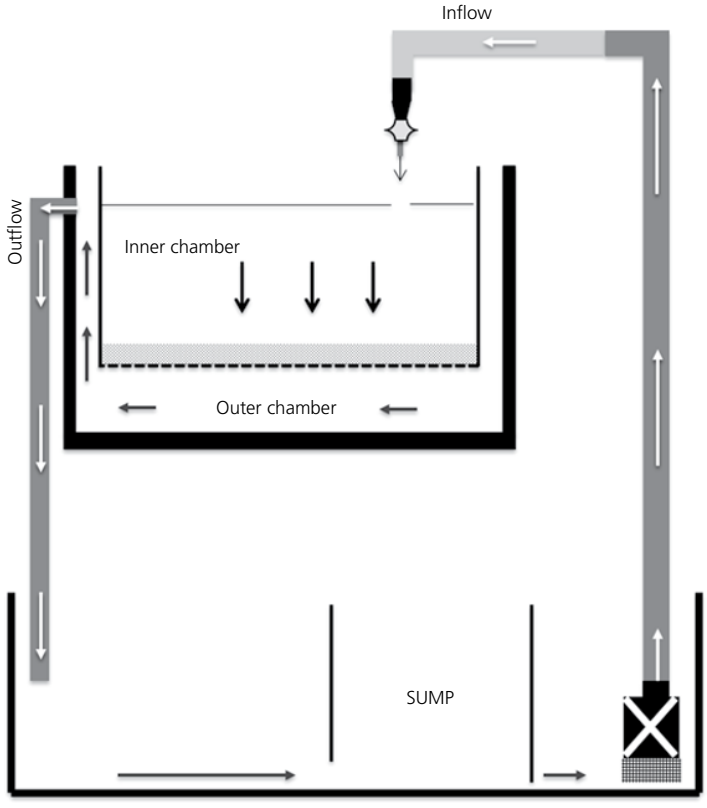


Figure 23.2.3 Illustration of a recirculating "breeding chamber" culture system for *Berghia verrucicornis* (adapted from Banger, 2011). Filtered seawater enters the inner chamber from the sump, passes through the substrate, and exits the breeding chamber via a drain located on the side of the outer chamber and returns to the sump for filtration; broodstock, larvae and juveniles remain inside the inner chamber.

maintain nudibranch cultures (O'Keefe 1990; Schlesinger *et al*., 2009; Leal *et al.*, 2012b). As with most marine invertebrates, sea slugs cannot synthesize certain FA *de novo*, and they must be derived from their prey or provided through symbiotic relationships with microalgae or algal chloroplasts (Zhukova, 2007, 2014).

Reproduction

Sea slugs possess complex reproductive systems and strategies (Ghiselin, 1966). They are hermaphrodites with internal cross fertilization (Beeman, 1970). Standard insemination mode (where sea slugs donate and receive sperm reciprocally in the head-to-tail cross position) or hypodermic insemination (in which sperm is introduced through the partners' body surface) are common among sea slugs (Angeloni, 2003; Schmitt *et al*., 2007). Other species alternate sex roles (Anthes *et al*., 2006), form mating chains (Angeloni, 2003), or transfer sperm with externally attached spermatophores (Karlsson *et al*., 2002). Different strategies or gender preferences can be assumed by sea slugs depending on the conditions related to each mating encounter (Anthes *et al*., 2006).

Stocking groups of breeders of the same size contributes positively to the mating behaviour and number of embryos produced (Angeloni *et al*., 1999; Capo *et al*., 2002).

23.2.3.2 Embryonic and Larval Development

The initial phase of development of sea slugs occurs in protected egg masses, often followed by a free-swimming larva – the veliger. Sea slugs may undergo metamorphosis inside the capsule and crawl out as adults (direct development) or metamorphose outside the capsules (indirect development) (Carroll *et al*., 1990). Although the nutritional modes of marine invertebrate larvae are classified between planktotrophy and lecitotrophy (Strathmann, 1985), sea slugs are known to display poecilogony (variable larval developmental modes within the same species) (Vendetti *et al*., 2012) (Figure 23.2.4). In this way, the type of larvae produced can be modulated in captivity with mild adjustments of some abiotic factors (e.g. temperature, salinity) (see Krug *et al*., 2012).

Although there are several studies on nudibranch (e.g. Hadfield *et al*., 1987; Todd *et al*., 2001) and sacoglossan species (Clark, 1975; Trowbridge, 1998, 2000) with planktotrophic life stages, the more interesting species from the aquaculture point of view will be those displaying lecitotrophy or direct strategies.

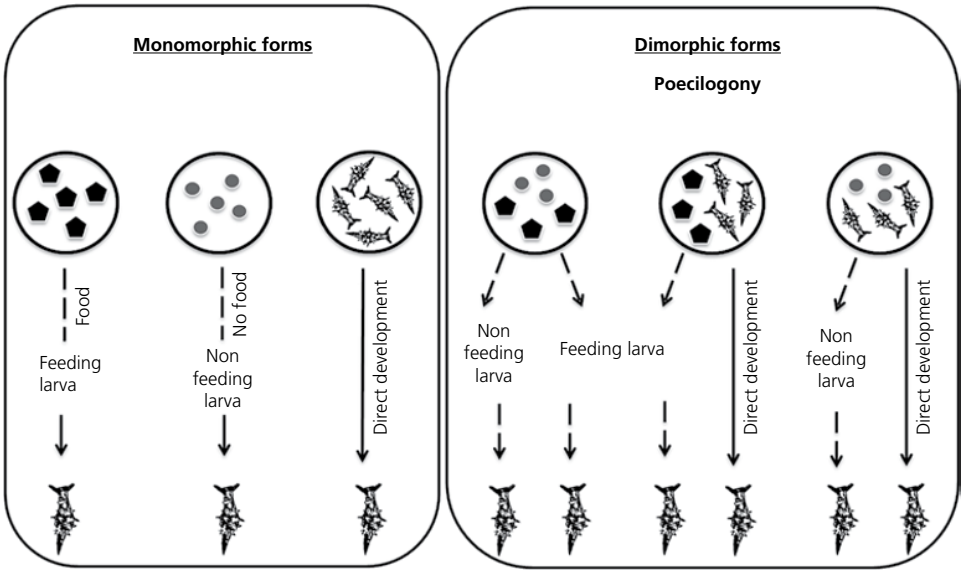


Figure 23.2.4 Overview of monomorphic and dimorphic forms of development in sea slugs (adapted from Dionísio *et al*., 2013). **Monomorphic forms:** Feeding larvae (planktotrophic larvae that require a suitable supply of phytoplankton after hatching to reach metamorphosis). Non-feeding larvae (lecitotrophic larvae, no exogenous food is required after hatching to reach metamorphosis), and direct development (metamorphosis occurs inside the egg capsule and an *imago* of the adult crawls from egg mass). **Dimorphic forms** (different modes of development can occur within the same egg mass – poecilogony): feeding and non-feeding larvae can hatch from the same egg mass; the release of feeding larvae and *imagos* of the adult – direct development – can be recorded from the same egg mass, and the release of non-feeding larvae and *imagos* of the adult – direct development – can be recorded from the same egg mass.

Culture Conditions

Egg masses are commonly detached artificially from the substrate and transferred to sterile beakers with filtered seawater and gentle aeration (Carroll *et al*., 1990). The water is changed generally three times a week using mesh screens to retain cultured larvae and then poured into a new culture beaker (with new filtered seawater and algal food) using a pipette (Carroll *et al*., 1990; Trowbridge, 2000). Recent advances such as the "Banger breeding chamber" (Banger, 2011) are very interesting choices for embryos incubation. This device consists of an inner aquarium holding breeding sea slugs and a flow-through sand bed that avoids newly hatched larvae or juveniles being drained through the outflow to the filtration system. One of the most frequent problems described is that sea slug larvae are positively phototactic and may be trapped at the air–water interface by the surface tension. This phenomenon can be avoided by adding specific compounds to the water surface, such as acetyl alcohol or iodine-based surfactant or by keeping the culture beakers in the dark (e.g., Capo *et al*., 2009; Franz, 1975). Another major bottleneck is the susceptibility of cultured larvae to infections by bacteria, fungi and virus. Some authors reported the use of antibiotics, such as penicillin and rifampicin (Franz, 1975; Curtis *et al.*, 2007), anti-septic solutions (Capo *et al*., 2009), chloramphenicol and EDTA (Sisson, 2005) to suppress microorganism's growth.

Some studies have already addressed the effect of initial larval density on growth, metamorphic competency and survival of sea slugs (Avila *et al*., 1997; Capo *et al*., 2009; Schlesinger *et al*., 2009). The general trend of lower survival rates at increasing larval densities is attributed to collisions among larvae, which may promote feeding inhibition, and physical injuries, among others (Avila *et al*., 1997; Capo *et al*., 2009)

Feeding

Sea slugs larvae can hatch as feeding or non-feeding forms. While "non-feeding" veligers rely on yolk reserves provided by parental organisms to fuel their energetic demands until metamorphosis, "feeding" larvae commonly depend on the ingestion of phytoplankton for a variable period of time. The period of this larval phase is species-specific and mostly controlled by abiotic factors, such as temperature and water quality. Species that display direct development (e.g. *B. stephanieae*) usually produce larger sized juveniles than those resulted from lecithotrophic or planktotrophic veligers. There are several feeding protocols, currently available that can be used for sea slugs larvae based on small sized phytoplankton species (e.g., *Isochrysis*, *Tetraselmis*, *Rhodomonas*). These microalgal diets, as well as the algal concentration and size should be adapted to the target species (for details see Dionísio *et al*., 2013). Some sea slug cultures proved to be unsuccessful in static rearing conditions, as water movement is needed to keep food in suspension. Equipment items such as the "roller bottle", that keep phytoplankton in constant suspension and promote a homogenous non-turbulent environment are important advances for successful larval growth.

Settlement and Metamorphosis

The habitat shift from plankton to benthos (called "settlement") is often followed by changes in feeding modes, community composition, organismal physiology and consequent morphological changes, the first critical phase of metamorphosis (Chia, 1978).

Still, many studies confound the terms "settlement" and "metamorphosis" (see Chia, 1978 for clarification of those concepts). In captivity, sea slugs larvae may settle and metamorphose in reaction to a variety of natural and artificial cues (e.g. aqueous extracts from preys). The majority of sea slugs are feeding specialists, with adult prey commonly acting as the metamorphic trigger for developing larvae.

Some species will also metamorphose in the presence of neuroactive agents, such as coline, serotonin and nitric oxide (Todd *et al.*, 1991; Leise *et al.*, 2001) or organic compounds (e.g., methanol, ethanol) (Avila, 1998). Water agitation and a slight temperature increase may also be effective to increase metamorphosis when natural inducers are present (Pechenik *et al.*, 1995; Avila, 1998). The introduction of algal or non-algal species, known to trigger metamorphosis, must be carefully timed, as only competent larvae will be receptive to any potential cues (Avila *et al.*, 1997). Also, culture differences in the timing of larval competence are inevitable and inherent to larval variability (Kempf, 1981). Such variability has been attributed to inadequate dietary requirements and water quality deficiencies, antibiotics usage and genetic differences (Avila, 1998).

23.2.3.3 Juvenile Growout

Successfully controlling water temperature, light, stocking density and food quantity and quality are key issues for culturing juvenile sea slugs to a commercial scale (Capo *et al.*, 2002). Growout systems are usually the same as used for broodstock maintenance. The most successful system employed so far for growing sea slugs are the ones described by Banger (2011) for the mass growout of the ornamental nudibranch *B. stephanieae* (Figure 23.2.3).

Feeding

Most sea slugs are specialized feeders that may switch their food preference during growout (Trowbridge, 2004). Some studies also point out that feeding preferences for certain species are related to secondary metabolites they acquire by feeding upon certain prey (Hay *et al.*, 1988). Artificial diets have only been used to successfully raise sea hare juveniles with agar and algal extracts as basic raw materials (Carefoot, 1980).

Studies specifically addressing the nutritional requirements of sea slugs during growout seem to be nonexistent in the literature. The information from ecological research is also limited to Zhukova and co-workers findings (2007, 2014) about lipid classes and fatty acid (FA) composition. Phospholipids were shown to be dominant, followed by sterols and monoalkyldiacylglycerols. The FA composition of nudibranchs differs greatly from other marine gastropods, which is determined by food supply, inherent biosynthetic activities and intracellular symbiotic microorganisms (Zhukova, 2014).

23.2.4 Cephalopods

Cephalopods require excellent water quality. In open systems, it is important to have a high-quality seawater source. Closed or semi-closed systems require strong mechanical, chemical and biological filtration, and consistent water changes to reduce the build-up of organic compounds (Sykes *et al.*, 2014b). Ammonia and nitrite levels should be kept below $0.1\,mg\,L^{-1}$, nitrates below $20.0\,mg\,L^{-1}$ (Boletzky *et al.*, 1983)

and UV sterilization is also important to remove potentially harmful organisms (Vidal *et al*., 2014). Constant aeration is essential to keep the oxygen levels above 5 mg L^{-1} (Rosas *et al*., 2014). Water oxygenation can be achieved by using air stones and airlifts (Vidal *et al*., 2014). Nevertheless, care should be taken with air bubbles (especially extra-fine bubbles), since cephalopods are quite susceptible to air entrapment, especially in the mantle cavity (Rosas *et al*., 2014).

Cephalopods are fast-growing animals, active swimmers and top predators that require substantial amounts of food to satisfy their voracious appetites, relying on an exclusively carnivorous diet and protein-based metabolism (Lee, 1995; Navarro *et al*., 2014). They have high protein requirements (over 60%), but also need high-quality lipids (around 4%) rich in essential fatty acids, phospholipids and cholesterol (Vidal *et al*., 2014). The amino acid profile of eggs and early embryos shows the importance of having the non-essential amino acids glutamate, aspartate and taurine and, especially, the essential amino acids leucine, arginine and lysine in the diet (Villanueva *et al*., 2004). Lipids are required in considerably lesser amounts, but phospholipids and PUFAs such as the essential fatty acids DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) are key nutrients in cephalopod nutrition (Navarro *et al*., 2000; Madeira *et al*., unpublished data). Nevertheless, most enrichment products and artificial diets currently available were not developed for cephalopods and have proven to be unsuitable to fulfil their nutritional requirements (Vidal *et al*., 2014).

23.2.4.1 Broodstock Husbandry

Cephalopods can be collected using creels, pots and traps, or lines with crabs as bait but without hooks (Iglesias *et al*., 2014a; Nabhitabhata, 2014; Vidal *et al*., 2014). The transport of cephalopods is challenged by their high metabolism, great sensitivity to water quality degradation, and the common release of ink. Nonetheless, they can tolerate quite extreme transport conditions (such as densities up to 165 kg m^{-3}), as long as the water temperature is kept low and the level of dissolved oxygen high (Fuentes *et al*., 2005; Sánchez *et al*., 2014). The presence of individual shelters (such as mesh bags, PVC tubes or other containers) is also important to avoid aggressive behaviours, stress and subsequent mortality (Iglesias *et al*., 2014a).

Culture Systems

Tank design has progressed from rectangular-cubic to circular-cylindrical, to allow proper water circulation and avoid the establishment of anoxic areas (Sykes *et al*., 2014b; Vidal *et al*., 2014). Since most cuttlefish and octopuses are benthic or semi-benthic, tanks should have large bottom areas (Domingues *et al*., 2010). The volume of the tanks used varies greatly within and among species. In cuttlefish culture, it has gradually increased from 250 up to 9000 L (Sykes *et al*., 2014b). Given the high mobility and curious nature of some cephalopods, in particular octopuses, it may be beneficial to make the tanks escape-proof.

Culture Conditions

Habitat enrichment is essential to reduce stress (Beigel *et al*., 2006). Sandy substrates well represent the natural environment but are not recommended (Forsythe *et al*., 1994). PVC tubes, fiberglass boxes, pots and cages have been used as shelters for

octopuses (Rosas *et al*., 2014), also sunken pieces of fishing net for cuttlefish (Nabhitabhata, 2014). Light conditions should be kept low (200 lx or less at the water surface). In outdoor systems, excess lighting may be avoided by using shading nets (Sykes *et al*., 2014b).

Cephalopods are stenohaline. Salinities outside the range of 27–37 can be fatal, salinity above 30 is recommended (Vidal *et al*., 2014). pH should be kept between 7.8–8.2 (Boletzky *et al*., 1983). Temperature and stocking densities vary greatly within and among species. Table 23.2.1 summarizes the broodstock conditions employed for the most traded cephalopods.

With cuttlefish, a sex ratio of 1 male to 2–3 females is suggested to avoid aggressive behaviours (Nabhitabhata, 2014; Sykes *et al*., 2014b) but high fecundities can be also achieved at a 3 males:1 female ratio (Sykes *et al*., 2014b). Male and female octopuses are kept at a sex ratio of 1:1 in *O. maya* (Vidal *et al*., 2014) and 1:3 in *O. vulgaris* (Iglesias *et al*., 2007).

Feeding

The broodstock diet is very important since it can influence the biochemical composition and size of the hatchlings (Quintana *et al.*, 2007; Márquez *et al.*, 2013). Adults may need a daily ration of 20–50% WW (Hanlon, 1987), which should be reduced during the spawning period since females stop eating (Iglesias *et al*., 2014a; Rosas *et al*., 2014).

Cuttlefish are generally fed a crustacean-based diet (mainly the grass shrimp *Palaemonetes varians*) (Sykes *et al*., 2014b). Higher fecundity and fertility were achieved with frozen rather than live grass shrimp (Domingues *et al*., 2003). An appropriate feed for octopuses is a mix of crustaceans and fish of low commercial value, with crustaceans comprising at least 30% of the diet (Vidal *et al*., 2014) (Table 23.2.1). Diets with crustacean and bivalve protein, complemented with arginine or leucine, are also proposed as improved feeds for cephalopods (Cerezo Valverde *et al*., 2012). Pelletized diets are not recommended since they have led to poor growth (Vidal *et al*., 2014).

Reproduction

Reproduction in captivity seems to be easily achieved when males and females are kept together under suitable environmental conditions (Iglesias *et al*., 2014a; Vidal *et al*., 2014). Mating involves a colorful courtship followed by the transfer of a spermatophore to the female. Nevertheless, *in vitro* fertilization may be an alternative strategy (Nabhitabhata *et al*., 2001b).

Cephalopods, in general, die naturally after reproduction. This implies new individuals must replace that broodstock every new season. In order to avoid the negative effects of inbreeding, it is suggested that the genetic bank in captivity needs to be refreshed every year with adults from wild populations (Boletzky *et al*., 2014).

23.2.4.2 Embryonic Development

Females lay their eggs in clusters, generally attached to substrates. Fecundities are around several thousands in cuttlefish or hundreds of thousands in octopods (Boletzky *et al*., 2014). After reproduction, octopus females and their eggs are generally separated from males, and transfered to individual tanks to avoid being disturbed by conspecifics

(Iglesias *et al*., 2014a; Rosas *et al*., 2014), but in cuttlefish it is not advisable to separate them (Vidal *et al*., 2014).

Most cephalopods do not have parental care (Boletzky *et al*., 2014). Female octopods are, however, an exception. They generally take care of their eggs by cleaning and ventilating them (Iglesias *et al*., 2014a). Nevertheless, artificial incubation can be as effective (Rosas *et al*., 2010). The embryonic phase may last 11–16 days for *S. pharaonis* at 25–28 °C (Minton *et al*., 2001), 45–50 days for *O. maya* at 24–25 °C (Rosas *et al*., 2014) or 38 days for *O. vulgaris* at 18 °C (Iglesias *et al*., 2014a).

Culture Systems

Cuttlefish eggs can be maintained in plastic baskets with 5-mm mesh (Nabhitabhata, 1978) or within suspended net bags of 10-mm mesh size (Anil *et al*., 2005), placed inside 4000 L concrete tanks with a diameter of 1.8 m and an initial water depth of 60 cm. After hatching, hatchlings can pass through the basket mesh into the tank (Nabhitabhata, 2014). On the other hand, octopus females and their clusters can be individually conditioned in 80 up to 500 L tanks (Rosas *et al*., 2014).

Culture Conditions

Lighting should be kept low (11–30 lx; Vidal *et al*., 2014) to avoid the growth of algae and fungi on the surface of eggs (Nabhitabhata, 2014). The photoperiod is also important, especially in species without maternal care, since it has a key influence on the timing of hatching (Vidal *et al*., 2014). Egg masses without maternal care should be suspended in an upwelling water flow or exposed to high flow rates to keep the surface of the eggs clean and well aerated (Vidal *et al*., 2014). Air bubbles should be avoided since it may be harmful to embryos (Van Heukelem, 1977). Eggs should be visually checked throughout embryogenesis so that the ones with dead or abnormally developed embryos can be discarded to prevent infection of the remaining (Iglesias *et al*., 2014a; Nabhitabhata, 2014). Table 23.2.2 summarizes the embryonic conditions employed with the most traded cephalopods.

The pH range recommended for egg incubation is 7.8–8.4 (Vidal *et al*., 2014). Abrupt changes in culture conditions should be avoided to prevent premature hatching (Iglesias *et al*., 2014a).

23.2.4.3 Hatchling Rearing

Regardless of how small they are, all cephalopods hatch as complete organisms that look like miniature adults (Boletzky *et al*., 2014). Cephalopods lack a truly larval stage, but in some benthic species, such as *O. vulgaris*, hatchlings are pelagic before settling later on and are called paralarvae (Young *et al*., 1988). This planktonic period lasts around 47–54 days at 21.2 °C (Villanueva, 1995). In contrast, *O. maya* and cuttlefish are already benthic or semi-benthic as hatchings (Vidal *et al*., 2014).

Culture Systems

The volume of the tanks varies greatly from experimental (as small as 10 L) to large (up to 6000 L) tanks (Iglesias *et al*., 2014b). Nevertheless, higher survival and growth rates can be obtained when using larger tanks (Sánchez *et al*., 2013) or with larger bottom areas (Domingues *et al*., 2010). Low water depth in the tanks of benthic hatchlings

favors prey encounters and reduces the energy allocated to catch prey, but should be gradually increased with increasing biomass (Forsythe *et al*., 1994). The color of the tanks is also important. Dark tanks are recommended (Iglesias *et al*., 2014a; Vidal *et al*., 2014), even though hatchlings can be successfully reared in completely white tanks (Carrasco *et al*., 2005). The use of black tanks with white bottoms is a good alternative since it improves paralarvae observation of prey (De Wolf *et al*., 2011).

Culture Conditions

Benthic hatchlings may need some kind of substrate as soon as they are born (Nabhitabhata, 2014), but it seems that *S. officinalis* can be successfully reared without substrate (Forsythe *et al*., 1994). In octopuses, refuges such as gastropod shells and PVC tubes are important to avoid cannibalism and reduce stress (Rosas *et al*., 2014; Vidal *et al*., 2014). Cephalopods require low light intensities, but optimal light conditions seem to depend on the type of tanks that are used. In general, higher light intensities and longer photoperiods are used in black tanks (e.g., 500–700 lx for a 24 h photoperiod) than in light-colored tanks or white-bottom tanks (e.g., 60–250 lx for a 14 L:10 D photoperiod (Iglesias *et al*., 2014a).

The water flow in the tanks is important to ensure good water quality. In open seawater systems, flow rates may be set at 2 L min^{-1} in 60 L tanks and 20 L min^{-1} in 500 and 1000 L tanks (Anil *et al*., 2005). Nevertheless, in tanks holding *O. vulgaris* paralarvae, the water flow should be very gentle to reduce the risk of injuries and subsequent mortality (Vidal *et al*., 2002). Stagnant water is commonly used with a green-water system for the first week (Iglesias *et al*., 2014a).

Abrupt changes in water parameters should be avoided to reduce mortality (Vidal *et al*., 2014). Cephalopods show low tolerance to low salinities. *S. officinalis* has been cultured at salinities of 34–37 (Sykes *et al*., 2014b), and salinities of 32–35 are recommended for octopuses (Iglesias *et al*., 2014a). The optimal temperature varies greatly with the species, but also with the natural location of a given population (Forsythe *et al*., 1994). Within the optimal temperature range of a species, the temperature should be higher to maximize growth over a shorter time period (Vidal *et al*., 2014). Temperature and initial larval density are variable and species-specific (Table 23.2.2).

As an example, the initial density in *S. pharaonis* tanks is 500 cuttlefish m^{-2}, reduced to 375–400, 280–300 and 200 individuals m^{-2} after 10, 20 and 30 days, respectively (Nabhitabhata, 2014). For octopus, the voracity and predatory behaviour of juveniles will increase considerably as they grow and must be taken into account (Vidal *et al*., 2014).

Feeding

Hatchlings still have some yolk reserves that enable them to survive without food for some days (Boletzky *et al*., 2014), but they may begin ingesting food and should be fed from the first day of life (Iglesias *et al*., 2006; Rosas *et al*., 2014). During this period, only a half ration of feed should be given (Vidal *et al*., 2014). Hatchlings may require a daily feeding ration of 30% of their body weight (Nabhitabhata *et al*., 1996; Quintana *et al*., 2011), but in massive culture conditions they should be fed 90–100 % WW (divided into 2–3 portions) to avoid cannibalism (Rosas *et al*., 2014; Vidal *et al*., 2014).

Live prey is recommended for hatchlings (Iglesias *et al*., 2014a). Cuttlefish are commonly fed mysids, shrimps and small fish (Domingues *et al*., 2003; Nabhitabhata 2014). In octopuses, higher growth and survival rates can be obtained with crustaceans, mainly

crabs or gammarids for *O. maya* (Vidal *et al*., 2014), and diverse crustacean zoea for *O. vulgaris* paralarvae (Iglesias *et al*., 2014a). Nevertheless, given the limited availability of live crustacean zoea, *Artemia* might be an alternative prey for *O. vulgaris* paralarvae under massive culture conditions (Iglesias *et al*., 2014a) (Table 23.2.2). Enrichment with a combination of DHA-rich *Isochrysis galbana* and EPA-rich *Nannochloropsis* sp. is, however, important to cover the PUFA requirements of the paralarvae (Fuentes *et al*., 2011). A green-water system based on *Nannochloropsis* sp. is recommended (Iglesias *et al*., 2014a).

Pelletized diets have produced poorer results (Vidal *et al*., 2014), but other inert diets can be successfully used. *S. officinalis* with 20–30 days-old can be fed frozen grass shrimp (Sykes *et al*., 2014b). *O. maya* can be fed frozen crabs and shrimp, as well as squid or crab pastes bound with gelatine (Rosas *et al*., 2014).

23.2.4.4 Juvenile Growout

The growout of cephalopods can be carried out in a great variety of systems, but if sufficient food is available and other important conditions are met, they can grow faster outdoors than indoors (Vidal *et al*., 2014).

S. pharaonis can be transferred to growout tanks (from 500 to 65,000 L) at 30 days after hatching (Anil *et al*., 2005), or can be kept in the nursing tanks to avoid injuries (Nabhitabhata, 2014). *S. officinalis* can be cultured in semi-intensive conditions, either in concrete tanks or net cages in ponds (Vidal *et al*., 2014). They can also be cultured in intensive systems, ideally in 9000 L cylindrical fiberglass tanks (Sykes *et al*., 2013), but smaller tanks can also be used (Sykes *et al*., 2014b). As for octopuses, 60-day-old *O. maya* juveniles can be transferred to recirculation systems composed of 5 m diameter tanks (Domingues *et al*., 2012). *O. vulgaris* juveniles are generally cultured in tanks (0.65–3.50 m^3) or floating cages (2.5–27.0 m^3) (Sánchez *et al*., 2014).

Culture Conditions

Habitat enrichment is important but some cuttlefish may grow and survive successfully in the absence of substrates (Nabhitabhata *et al*., 1999). Nevertheless, refuges such as PVC pipes and conch shells are important to reduce interaction and cannibalism among juveniles (Rosas *et al*., 2014; Vidal *et al*., 2014). Tanks should be kept under low light intensities and if placed outdoors, tanks should be protected with masking nets to reduce light intensity (Vidal *et al*., 2014).Optimal flow rates vary greatly with tank volume (Minton *et al*., 2001) and water temperature (Sánchez *et al*., 2014).

Cephalopods are generally cultured at salinities of 35–37 (Sykes *et al*., 2014b). Substantial mortality has been recorded when salinity falls below 29 (Chapela *et al*., 2006). Temperature and stocking densities vary greatly within and among species. Table 23.2.1 summarizes the growout conditions used for the most traded cephalopods.

Growth rates of cephalopods are so high that the initial biomass can triple in a few weeks, generating problems with oxygen consumption, detritus production and cannibalism (Sánchez *et al*., 2014).

Feeding

Several food items have been used, but currently, the growout of *S. officinalis* juveniles uses a crustacean-based diet, mainly *Palaemonetes varians* (Sykes *et al*., 2014b) and

preferentially frozen (Domingues *et al*., 2003). In regards to octopuses, *O. maya* is commonly fed with squid and crustacean pastes (Rosas *et al*., 2014; Vidal *et al*., 2014), while *O. vulgaris* is generally fed crustaceans and fish, and occasionally mussels (Chapela *et al*., 2006). The best combination of food seems to be 50% crab and 50% fish (García *et al*., 2006). The bogue (*Boops boops*) and squids have proved to be an excellent alternative to crustaceans (Sánchez *et al*., 2014). Formulated diets have had poor results when compared to natural diets (Sánchez *et al*., 2014). Nevertheless, some formulated feeds based on fish, crustacean or mollusc pastes mixed with binders (preferentially gelatines) have been accepted by octopuses and have promoted good growth (Sánchez *et al*., 2014). However, a major challenge will be to develop a sustainable artificial diet independent from fisheries products that sustain good survival and growth (Xavier *et al*., 2014).

23.2.5 Conclusions

Knowledge on rearing and breeding of sea snails, slugs and cephalopods have steadily increased in the last decades. While well-established snail culture protocols allow commercial scale culture, the rearing of slugs and cephalopods is still restricted to small scale. Concerning sea slugs and cephalopods, recent works have revealed fundamental principles of life cycle, physiology and behaviour that can provide the basis for a commercial scale culture of some species (e.g. *Berghia stephanieae*, *Elysia clarki*, *Octopus maya* and *Sepia pharaonis*). The application of new technologies to improve production, such as genomics, has been recognized for aquaculture in general but is still lacking for molluscs aquaculture in particular. The use of selective breeding as a tool to improve biological efficiency is yet to be thoroughly addressed. Furthermore, innovative technological approaches that combine the principles of mollusc traditional aquaculture with better management practices and a more efficient use of water and land need to be implemented, benefiting local communities and minimizing environmental impacts.

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24

Polychaetes

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Abstract

Sabellids (commonly known as feather-duster worms) are the most popular polychaetes on the ornamental market and are routinely collected from coral reefs to supply the trade. Consequently, there are growing concerns about harvest methods, habitat destruction and long-term sustainability and management of their collection. Efforts to culture sabellids are well under way utilizing their ability to reproduce sexually, as well as their capacity to regenerate. Reproductive studies on *Sabellastarte spectabilis* reveal a broadcast spawner with lecithotrophic larvae that develop for a period of 7–8 days followed by settlement and metamorphosis. Regeneration experiments on *Sabella* and *Sabellastarte* exhibited more than 75% survivorship in many cases. Both methodologies show great potential, but are currently not commercial operations. With a growing body of literature on both the reproductive biology and regenerative capacity of a variety of sabellid species, opportunities for applying culture techniques to a greater number of traded species shows much promise.

Keywords *Regeneration; reproduction; Sabellastarte; Sabellidae*

24.1 Introduction

Despite their abundance and ecological importance in all marine systems (Scoffin, 1972; Bailey-Brock, 1976; Rouse & Pleijel, 2001; Pernet, 2002) polychaete worms are often not well known outside of the relatively small group of marine biologists who study them and the limited number of marine aquarium enthusiasts who purchase them. Therefore, this diverse marine invertebrate group usually benefits from an introduction, even among some biologists and hobbyists.

Polychaete worms are a class of soft-bodied animals with four major body sections: a head region (prostomium), a mouth region (periostomium), a trunk region (metastomium) and a hind region (pygidium) (Beesley *et al*., 2000). The majority of the body is usually found in the trunk which is composed of a series of repeating segments and a

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Figure 24.1 Examples of sabellids or feather duster worms in the wild (photographs by J.M. Murray). (*See insert for color representation of the figure.*)

wide array of interesting morphological structures including: spine-like chaetae, sensory palps, tentacles and cirri, fleshy paradodia, ornate, chitinous jaws and colorful, feathery gills (Brusca & Brusca, 1990; Rouse & Pleijel, 2001). This pattern of repeating segments along with the suite of diverse morphological structures is combined into an almost endless variety of form and function which enables adaptation of polychaetes to a wide range of ecological niches as tube-dwellers, burrowers, borers, errant, planktonic, interstitial, commensal and parasitic organisms (Flint & Rabalais, 1980; Brusca & Brusca, 1990; Rouse & Pleijel, 2001). Trophic groups include carnivores, herbivores, omnivores, deposit feeders and filter feeders (Fauchald & Jumars, 1979).

Polychaetes are found in marine habitats from the upper reaches of the intertidal to the depths of hydrothermal vents (Rouse & Pleijel, 2001). Some live in brackish water (Cognetti & Maltagliati, 2000) and a few have even ventured into freshwater habitats (Glasby & Timm, 2008). They are one of the most abundant and diverse groups in coral reef communities (Fauchald & Jumars, 1979) and it should come as no surprise that those species with aesthetic appeal, have found a niche in the marine ornamental trade. While a variety of segmented worms can be found in the reef aquarium, the most popular polychaetes on the market by far are the sabellids (commonly known as feather-duster worms) which are often delicate and brightly colored (Figure 24.1). Other less charismatic species, such as those associated with live rock and live sand, are also being traded for their important ecological role in aquarium mini ecosystems including: scavenging (detritivores); substrate agitating; reef binding and reef recycling (consuming organic and inorganic matter) (Calfo & Fenner, 2003). As the shift from "fish only" aquaria to reef "mesocosms" continues

(Olivier, 2003), the presence of polychaetes in the marine ornamental trade is likely to increase over time.

As with many other marine ornamental species there are growing concerns about harvest methods, habitat destruction and long-term sustainability and management of collecting ornamental polychaetes from the wild (Wabnitz *et al*., 2003). In particular, sabellid worms build their own mucoid-sediment tubes and tend to attach to hard substrates on the surrounding reef framework making removal a delicate procedure that can damage both the worm and surrounding reef habitat (Bybee *et al*., 2006a). In addition, with many more individuals being traded than previously estimated and a lack of accepted taxonomy for many of these groups, it is difficult to arrive at precise trade figures and manage the wild stocks to collection for the aquarium trade (Murray *et al*., 2012). As the pressure on wild stocks increases so does the urgency for aquaculture alternatives to provide a sustainable future for the rapidly growing industry. This chapter summarizes the current status of the trade for marine polychaetes, knowledge about reproduction (sexual and asexual), regeneration capacity of target species, and concludes with recommendations for future research.

24.2 Worms Sold in the Trade

The vast majority of ornamental polychaetes sold in the trade are warm-water species although there is a growing market for cold-water species. Some European studies have begun to explore the potential for trading local species as an alternative to importing their tropical counterparts (Calado, 2006; Watson *et al*., 2012).

Of the feather duster worms, the genus *Sabellastarte* is particularly well known in reef aquaria (Fossa & Nilsen, 2002). Species from the genus regularly cited in aquarium literature include; *Sabellastarte japonica*, *S. magnifica*, *S. sanctijosephi* and *S. spectabilis* (Tullock, 2001; Fossa & Nilsen, 2002; Calfo & Fenner, 2003; Shimek, 2004). High intra-specific variation of morphological characters renders their accurate identification problematic (Knight-Jones & Mackie, 2003; Capa *et al*., 2010). A recent attempt to identify globally traded *Sabellastarte* suggests that there may be a number of un-described or cryptic species with a diverse mixture of characters from a number of known species present within an individual (Murray *et al*., 2012). Improving scientific understanding of ornamental polychaete taxonomy is a priority goal for the future. There is an urgent need to know what species are being collected and from what locations in order to facilitate the effective management of wild stocks and highlight which species may be at risk from over exploitation.

24.3 An Introduction on Ornamental Polychaete Culture

There are a limited number of references within the aquarium literature for the conscientious hobbyist exploring the possibility of culturing fan worms in their home aquaria. Small, colonial "feather-dusters" such as *Bispira brunnea* have been found to breed successfully in the hobbyist's tank with larvae settling on rockwork, glass and substrate (Fenner, 2001; Tullock, 2001; Fossa & Nilsen, 2002). Conversely, Calfo &

Fenner, (2003) recognized that it is currently unlikely for fan worms to successfully fertilize and rear larvae to the settlement stage in the filtered confines of a closed aquarium system.

Although almost no commercially cultured ornamental polychaetes are currently available within the trade, two research groups have, over the last decade, begun to implement pilot-scale production of feather-dusters by means of sexual reproduction (Bybee *et al*., 2006a, b; 2007; Tamaru *et al*., 2008, 2011) and regeneration (Murray, 2010; Murray *et al*., 2013).

24.4 Sexual Reproduction as a Method of Culture

Polychaetes are among the most reproductively diverse metazoan groups (Rouse & Fitzhugh, 1994; Giangrande, 1997) with 18 different sexual reproductive modes identified to date (Wilson, 1991). Some species have even shown variability in reproductive patterns and larval developmental modes within a single population (Gibson, 1997). This multiplicity of reproductive modes exhibited by polychaetes can be a formidable challenge for breeders in the commercial aquaculture sector.

24.4.1 Sexual Reproduction: Culture Status and Success

Some success has been reported in the commercial culture of less popular ornamental polychaetes. Indo-Pacific Sea Farms, located at the Natural Energy Lab of Hawaii Authority (NELHA) market "100% captive bred" worms sold as two different "forms" of polychaetes: Mama Mia Worms™ (also known as spaghetti worms from the family Terebellidae) and multiple varieties of Baby BristleWorms™ which could include families such as Nereididae, Amphinomidae, Eunicidae Polynoidae, Maldanidae and Dorvilleidae.

Years of work investigating sexual reproduction in *Sabellastarte* (*S. spectabilis*) has been crucial in developing culture methods for this popular feather-duster. Bybee *et al*., (2006a; 2007) have shown that this species reproduces via broadcast spawning correlated with seasonal changes in water temperature. Individuals appear to be protandric hermaphrodites (Bybee *et al*., 2006b) becoming sexually mature once the tube diameter exceeds 6 mm. Eggs are fertilized in the water column (or on the bottom of the tank) and embryonic development ensues for approximately 7 hours (Bybee *et al*., 2007). Hatching occurs at that point and lecithotrophic (nonfeeding) trochophore larvae emerge (Figure 24.2). Larvae drift and swim in the water column for 3 to 4 days using bands of cilia. Metatrochophore larvae with visible body segments are present for another 3–4 days until the ciliary bands resorb and settlement and metamorphosis take place 7–8 days post-spawning (Bybee *et al*., 2006a).

Spawning induction experiments were most successful when ablation was used whereby the bottom centimeter of the worm is removed using scissors. Ablated worms are returned to their aquaria for a period of four days after which they spawn (Bybee *et al*., 2006a). Developing embryos and hatched larvae are subsequently removed from the spawning aquaria and placed in 200 L conical fiberglass tanks for grow out (Tamaru *et al*., 2011). This has been done successfully in both sock filtered and UV filtered water. *Sabellastarte spectabilis* larvae will settle on a wide variety of substrates such as red and

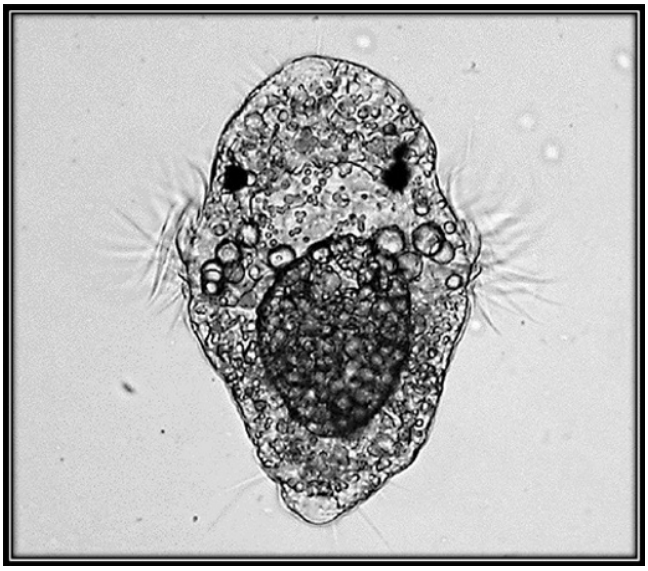


Figure 24.2 A newly hatched trochophore larva of *Sabellastarte spectabilis*.

black cinders, fiberglass, plastic mesh, rock, cement, macro algae (Tamaru *et al*., 2006; 2008) but controlled experiments indicate a significant preference for silicate air stones and coral chips (Tamaru *et al*., 2008).

There is a limited body of information on feeding in sabellids. Although there are a few species that touch their feathery crown to the substrate for "selective deposit feeding" (Fauchald & Jumars 1979), filter feeding is by far the most important feeding mode within the Sabellidae. Some sabellids possess a mechanism which allows them to sort filtered particles by size (Fitzsimons, 1965; Merz, 1984; Nicol, 1930). In *S. magnifica*, particles are sorted into three size classes. Small particles (3–8 μm) are ingested, medium size particles (9–18 μm) are often used for tube construction and larger particles are rejected (Fitzsimons, 1965). Ingested particles are composed mostly of diatoms, dinoflagellates, unicellular algae, small invertebrates and pelagic larvae (Fauchald & Jumars, 1979).

Similar observations for *S. spectabilis* showed that ingested particles (microalgae) were of 2–7 microns (Tamaru *et al*., 2011). It is not understood how selective (if at all) *Sabellastarte* are in sorting the plankton for specific types however preliminary observations in wild caught worms indicate that phytoplankton makes the greatest contribution to the contents of the gut with some limited evidence of zooplankton ingestion (DRB personal observation).

In the context of aquaculture, a feeding experiment of 132 days revealed significantly higher survival (>80%), among *S. spectabilis* juveniles fed *Isochrysis* sp. (Tahitian strain), compared to *Nannochloropsis* sp. at a density of approximately 1 million cells/mL (Tamaru *et al*., 2008, 2011). This was true for both live and commercially available preserved treatments and was due to the higher energy or fatty acid content of *Isochrysis* sp. (Tamaru *et al*., 2011). Worms fed live *Isochrysis* sp. were significantly larger than worms from all other treatments. The preserved *Isochrysis* sp. treatment resulted in less growth but was still comparable to those fed live *Nannochloropsis* sp. and worms exposed to only raw seawater (Tamaru *et al*., 2011). Growth and survival

trials with *Chaetocerus* sp. as a food source also showed promising results (Tamaru *et al*., 2006) but further research on feeding and nutritional requirements of sabellids is crucial for the development of their culture.

24.4.2 Sexual Reproduction: Culture Obstacles

The biggest obstacle facing the commercial culturing of ornamental polychaetes is that, for most species, reproductive information is lacking in the literature. An existing obstacle for *S. spectabilis* is that although a single female can produce tens of thousands of eggs, most of which are successfully fertilized, a significant number of these did not survive to settlement and metamorphosis in the studies cited above (Bybee, unpublished data). Initially, this was due to water quality issues associated with small volumes (18 L, 85 L) of unfiltered water. Larvae were observed under a microscope being attacked by ciliates and other microbes. The use of sock filtered and UV filtered water improved larval survival in later trials. Increasing filtered water volume to 200 L further improved larval survival. When water volume was increased to 2000 L however, larval survival did not increase. This was unexpected but could have been due to contaminants or environmental differences experienced by the larvae in the large 2000 L outdoor tanks. Further work should focus on identifying optimal water quality parameters as well as developing a method to grow larvae out in the ocean. Other obstacles to address include; dependence on wild brood stock, the cost of materials, supplies and facilities, and temporal limitations due to seasonal maturation cycles.

24.4.3 Sexual Reproduction: The Future

There is a continually growing body of literature on polychaete sexual reproduction including the sabellids, though much of this is for non-ornamental species (Giangrande, 1997; Murray *et al*., 2011; Pires *et al*., 2012; Smart *et al*., 2012; Kolbasova, 2013). If sexual reproduction as a culture method is to become a significant contributor to supplying the marine polychaete ornamental trade in the future, the obvious place to focus efforts is on species with highest demand and most comprehensive reproductive information. Murray *et al*. (2012) showed that over 70% of polychaetes imported into the UK over a two-year period were from the genus *Sabellastarte*. Although not all of those were *S. spectabilis*, the information now available on this species (Bybee *et al*., 2006a,b, 2007; Tamaru *et al*., 2011) could serve as a springboard for understanding sexual reproduction and dietary requirements in other traded members of the genus such as *S. japonica*, *S. magnifica*, *S. sanctijosephi*, and *S. samoensis*. It could also lead to production of these species in areas of the world where they are naturally abundant such as in the Philippines, Japan, the Caribbean, Kenya, Indonesia, Samoa and Singapore.

Another species on which efforts are being focused is the colonial feather-duster *Bispira brunnea*. Researchers in Mexico are currently planning a study to investigate the reproduction and life history of this species with a view to its commercial-scale culture in the future (Marianna Tovar, pers. comm.). Very little reproductive information is available in the literature on tropical *Bispira* species although there are several that appear in the trade. Published information on reproduction in related temperate species such as *Bispira volutacornis* (Nash & Keegan, 2003) could be helpful in determining reproductive characteristics of warm-water members of the genus and even in temperate species if the market for cold-water aquariums was to grow.

Other species of potential interest to the trade include feather-dusters of the genera *Branchiomma*, and *Sabella* (McEuen *et al*., 1983; Wilson, 1991; Licciano *et al*., 2002; Tovar *et al*., 2009; Tovar *et al*., 2011) and serpulid tubeworms (Smith, 1984; Marsden, 1992; Rouse 1996; 2005; Qian, 1999; Selim *et al*., 2005).

24.5 Asexual Reproduction and Regeneration as Methods of Culture

Asexual reproduction and regeneration both refer to the subdivision of an existing body into two or more multicellular parts. The only obvious difference between asexual reproduction and regeneration appears to be associated with how they are stimulated (e.g. asexual reproduction may occur in the presence of sub-optimal environmental conditions while regeneration is initiated by injury). Many animals can regenerate but cannot reproduce asexually, indicating that the two phenomena are distinct processes (Bely, 1999; Sánchez Alvarado, 2000).

In addition to reproducing sexually, some polychaetes can also reproduce asexually whereby offspring are produced without the recombination of genetic material but by the subdivision of an existing body into two or more multicellular parts (fission and budding) (Barnes *et al*., 2001). Some sabellid species reproduce throughout the year using this reproductive mode. For example, 11% of a population of *Branchiomma bairdi* studied was reproducing asexually providing a constant source of offspring (Tovar *et al*., 2011).

Sabellids can also regenerate both caudally (produce a new tail) and cephalically (produce a new head) from any segment (Berrill and Mees, 1936a; Licciano *et al*., 2012), with some species being capable of regenerating an entire individual from a single segment taken from the mid-body within a matter of weeks (Bely, 2006). The majority of studies concerning regeneration in sabellids were carried out in the early 20th century and were limited to the morphological aspects of regeneration in the temperate sabellid *Sabella pavonina* (Berrill, 1931, 1952, 1978; Gross & Huxley, 1935; Berrill & Mees, 1936a,b) and nervous control mechanisms in *Sabella melonstigma* (Fitzharris & Lesh, 1969; Fitzharris, 1976).

Interest in sabellid regeneration has been renewed recently with studies on *Sabella pavonina* (Murray, 2010; Murray *et al*., 2013), *S. spallanzanii* (Licciano *et al*., 2012), *Branchiomma luctuosum* (Licciano *et al*., 2012) and species of *Sabellastarte* (Murray, 2010; Murray *et al*., 2013). These studies have not only reported on the morphological details of regenerative development in these species but also considered the level of mortality induced following the sectioning procedure. Understanding the robustness of a species to the sectioning procedure is critical when assessing the potential for using regeneration as a method to culture those species.

Members of genus *Sabella* have been found to exhibit high levels of survivorship with more than 75% of individuals of both *S. spallanzanii* and *S. pavonina* cut into as many as eight fragments surviving the sectioning process. Species of *Sabellastarte* also exhibited high levels of survivorship (≥75%) but only when individuals were sectioned once. Higher mortality was recorded with increasing number of cuts and only 20% of fragments from individuals cut into eighths survived the four-week regeneration period assessed (Murray *et al*., 2013). The lowest survivorship rates recorded have been for

B. luctuosum with 100% mortality in individuals sectioned during October and low levels of survivorship during January (Licciano *et al*., 2012).

Surviving individuals of all species studied were capable of regenerating cephalically and caudally within a four week period. Caudal regeneration involves the healing of the cut surface, reconstruction of the pygidium and subsequent segment addition, while cephalic regeneration is a more complex process of wound healing, reconstruction of a new mouth and the development of the branchial crown structure. There are however apparent variations in the specifics of regenerative development between species. In *Sabella*, regeneration involves the production of a smooth, rounded surface upon which blastema formation takes place and seemingly proceeds at a faster rate than development in species of *Sabellastarte* and *B. luctuosum* where early regenerative development of the blastema is concealed within an invagination at the amputation plane. In addition, although morphallactic reorganization from abdominal segments to segments of thoracic nature (previously described by Gross & Huxley, 1935) was observed in *S. pavonina* and *S. spallanzanii*, no evidence of reorganization was observed in *Sabellastarte* and *B. luctuosum* with the most anterior segments remaining abdominal in character.

24.5.1 Regeneration: Culture Methodology

Over the last decade, there have been efforts to explore the potential for utilizing the regenerative capacity of sabellids as an alternative method of culture which is free from the complexities and bottlenecks of sexual reproduction. The process for regenerating sabellids was first developed using a temperate model species, *S. pavonina*, before being transferred to members of the popular ornamental genus *Sabellastarte* from a number of exporting regions: Batam Islands, the Philippines and the Indian Ocean. The method involves the removal of an individual from its tube by applying gentle pressure at the most posterior end of the tube and moving towards its anterior. The tube-less worm is then bisected anterio-posteriorly using dissecting scissors to produce up to eight fragments including; an anterior, a posterior and mid-body fragments (Figure 24.3).

In initial lab-based trials (Murray *et al*., 2013), individuals of *Sabellastarte* were sectioned into two worms; an anterior (regenerating caudally and composed of both abdominal and thoracic segments) and a posterior (regenerating cephalically and composed of abdominal segments only). Sectioning of worms into quarters and eighths to produce an anterior, a posterior and mid sections has been investigated with the aim of maximizing the culture methodology of each sectioning cycle. Mid sections were cut roughly the same length as anterior and posterior sections and like the posteriors, are composed of abdominal segments only. While anterior and posteriors are required to wound heal and regenerate only one cut surface, mid sections must heal and regenerate both the anterior and posterior cut surface.

Each fragment was maintained individually in biologically filtered seawater tanks (under-gravel filter grids, airlifts attached to twin outlet pumps and coral gravel) of temperate, filtered seawater at 25 °C with constant light conditions (12L:12D; intensity 40 μmoles m^{-2} s^{-1}) for a four-week period. Additional food was not provided and no anesthetics or antibiotics were used during these initial trials to ensure the procedure was "low-tech" and easily transferable to communities that depend on wild collection for the trade (Murray *et al*., 2013).

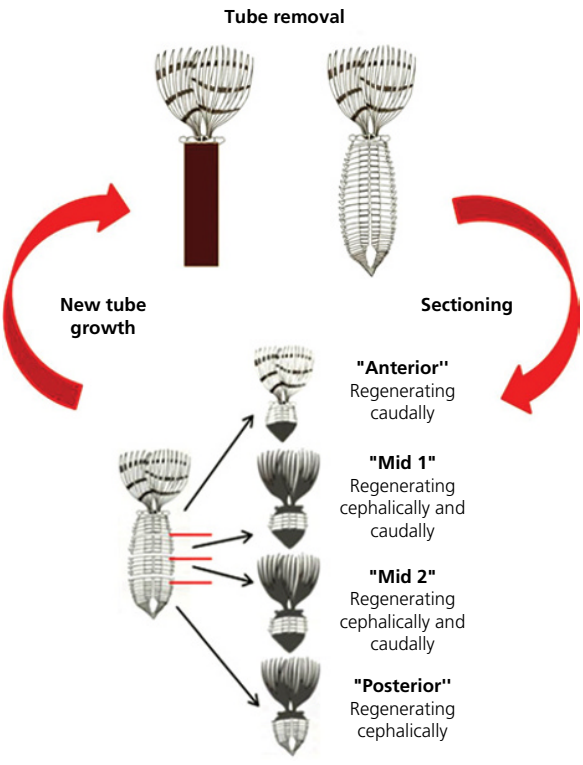


Figure 24.3 Schematic drawing of the process of sectioning. Specimens removed from their tube were cut using dissecting scissors into: (a) halves (b) quarters (shown) and (c) eighths, from the first thoracic segment to last abdominal segment. Fragments containing the head (prostomium) are referred to as anterior fragments; those containing the tail (pygidium) are referred to as posterior fragments; all other fragments are referred to as mid-body fragments. Anterior fragments are regenerated caudally, posterior fragments are regenerated cephalically, and mid-body fragments, containing only abdominal segments, are regenerated both cephalically and caudally. Tissue replaced by regeneration is shaded grey.

24.5.2 Regeneration: Culture Status and Success

Species of *Sabellastarte* from the three tested geographic regions (Batam Islands, the Philippines and the Indian Ocean) exhibited more than 75% survivorship when sectioned into just two fragments, although anterior fragments regenerating a new tail demonstrated higher mortality than posterior fragments regenerating a new head. All individuals that survived the sectioning procedure went on to regenerate missing appendages. By day 7, more than 67% of individuals regenerating a new head had completed the wound healing process and the emergence of rudimentary radioles was observed in over 10% of fragments. Between individual variability in development was greatly reduced by day 21 and by the 28th day of the grow-out phase, 100% of individuals had produced a branchial crown structure with at least one pigmentation spot present on the radioles.

Development of a new tail was a variable process following initial wound healing and blastema formation at day 7. On day 14, formation of the pygidium around the anus was the most commonly observed stage of development although wound healing to chaetae formation were observed concurrently. Similar to cephalic regeneration, day 21 was the most variable for regenerative stages with some individuals showing only the initial development of the pygidium, while others exhibit defined segments with emerging chaetae, which had begun to merge with a process of normal growth.

Fragments of *Sabellastarte* sp. sectioned into multiple fragments experienced high levels of mortality. Worms cut in half had the highest survival rate (75%) while those in

quarters and eighths had significantly lower survival than in other treatments. Survival appears to decrease with the increasing number of fragmentation and only 35% of mid-body quarter fragments survived and 19% of mid-body eighth fragments survived in these initial trials. The posterior fragments always exhibited a higher survivorship relative to the anterior fragments. Mid-body fragments displayed different rates of survivorship and regeneration depending on where in the 'parent' body they originated. For specimens cut into eighths, mortality was 100% in fragments Mid 1, Mid 2, Mid 3 and Mid 4, but from Mid 5 onwards, survivorship increased. The rearmost segment of all eight (the one with the pygidium) exhibited the highest level of survivorship (70%).

Sabellids which have been removed from their original tubes and have replaced missing appendages through regeneration must then be able to re-construct a new tube that is both hardy enough and aesthetically appealing for sale in the marine aquarium trade. Species of *Sabellastarte* quickly form an anchorage point to the substrate and regenerated individuals can construct a new tube within ten days when exposed to suspended sediment (Murray, 2010).

24.5.3 Regeneration: Culture Obstacles

The use of regeneration as a novel method to culture ornamental sabellids shows great promise in these pilot studies however, there are still a number of issues which must be addressed before the methodology could be considered at a commercial scale. Firstly, pilot-scale results have revealed some survival advantage in the most posterior fragments. Higher mortalities have been found in anterior fragments despite an initial assumption that the energetic demands of cephalic regeneration (by posterior fragments) involving new tissue growth and rearrangement would have been greater. These higher mortalities were often associated with the deterioration of the test tank water conditions and included the highest recorded levels of complete ammonia and the lowest concentrations of dissolved oxygen. This is a process of negative feedback where crown degradation or shedding in anterior fragments results from the stress of sectioning. This, in turn, increases the concentration of ammonia in holding tanks, reducing the availability of oxygen which ultimately promotes deterioration of condition. Murray *et al*. (2013) predict that by improving water quality parameters and providing additional nutrition, survivorship would be enhanced to maximize productivity. The next step in the development of this culture methodology is field-based trials (in flow-through holding tanks (*ex situ*) or within protective frames which are put back into the sea (*in situ*). This is likely to avert water quality issues.

24.5.4 Regeneration: The Future

While regeneration has the scope to provide a simple method for culturing ornamental sabellids, further optimization of the technique is required to improve the levels of survivorship, and the efficiency and extent to which regeneration occurs. Manipulation of temperature has the potential to influence the speed of regeneration. Berrill & Mees (1931a, b) found that at higher temperatures the time between amputation and the appearance of the regeneration blastema in *S. pavonina* was reduced. Investigating incremental temperature changes in culture conditions could elucidate the optimal temperature range for the fasted rates of regeneration and increase the efficiency of the methodology.

Feeding regimes in regenerating or post-culture worms need to be optimized to produce large and hardy specimen fit for sale. Studies on regenerating brittle stars found that they can utilize dissolved organic matter present in natural seawater to offset the costs of regeneration on the non-regenerating tissues and reduce overall weight lost in individuals (Dobson *et al*., 1991). If this were true for regenerating sabellids, a feeding regime could be introduced to the culture process, even to crownless fragments to aid the energetic demands of tissue addition.

24.6 Conclusions

Efforts to culture sabellids, the most popular of the polychaetes sold in the marine aquarium trade, are well under way and are utilizing their ability to reproduce sexually as well as their capacity to regenerate. Both methodologies show great potential but are currently not commercial operations. With a growing body of literature on both the reproductive biology and regenerative capacity of a variety of sabellid species, the potential to apply culture techniques to a greater number of traded species shows much promise.

A key issue which spans across both methodologies is the need for improved scientific understanding of their taxonomy. A greater resolution of taxonomy would aid the implementation of effective stock management (you need to know what species are being collected to understand any risk of potential exploitation) and would identify groups of species which share similar life history characteristics to those for which we already have a knowledge base.

Improved water quality has been highlighted as an area which requires further research and development in order to achieve higher larval survival post-settlement and for the survival of regenerating fragments. Murray *et al*. (2013) suggest water quality issues could be overcome when the methodology is transferred to field-trials. Additional research into the feeding and nutritional requirements of sabellids is another factor which has been raised as a development area by both groups working on their culture. Some work has already been done by Tamaru *et al*. (2011) and preliminary results have found that faster growth and larger worms can be produced when they are fed a diet of *Isochrysis* sp. or *Chaetocerus* sp.. Murray *et al*. (2013) suggest that there may be the potential for crown-less regenerating fragments to gain nutritional value from dissolved nutrients in natural seawater.

Regular collection of *Sabellastarte spectabilis* in Hawaii has been the catalyst for research efforts into its reproductive biology. Progress made to date has shown great potential to manipulate their reproduction as a method to culture them and relieve collection pressures on Hawaii's wild stocks. Regeneration methods were developed based on simple techniques which could be used in developing countries where communities rely on wild collection of ornamentals but where access to equipment and infrastructure for culture initiatives is limited. Murray *et al*. (2012) recommend that continued efforts to commercialize regeneration as a method should be made within the context of a community-based management (CBM) in small-scale community-led projects. Coastal communities could propagate fan worms as an alternative to wild collection and alongside other activities, such as coral or live rock farming.

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25

Other Invertebrates and Macroalgae

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Abstract

Reef aquarium displays currently try to mimic as close as possible tropical coral reefs. In this effort a growing diversity of reef organisms are currently traded to supply the marine aquarium trade. While fishes and corals form the bulk of the reef species commonly traded in this industry, invertebrates such as sponges, tunicates and echinoderms are also stocked for aesthetic and functional purposes. Macroalgae, namely those from genus *Caulerpa* and *Chaetomorpha*, are also employed to avoid the build-up of nutrients in reef set-ups. The present chapter presents an overview on the state of the art and aquaculture potential for these groups of invertebrates, as well as for macroalgae, for ornamental purposes. Special emphasis is given on the culture of *Diadema* sea urchins given their popularity in the marine aquarium trade and endangered status of some populations.

Keywords *Caulerpa; Chaetomorpha; Diadema; echinoderms; macroalgae; sponges; tunicates*

25.1 Introduction

Modern reef aquariums currently host a remarkable diversity of organisms, as they try to mimic the physical, chemical and trophic processes of coral reefs. While fishes and corals still occupy the spotlight of any display, a growing number of other marine invertebrates are now also selected by hobbyists to be displayed in their tanks. These invertebrates are stocked not only for their beautiful colors or odd shaped bodies, but also by the trophic role they play on reef tanks. Invertebrates that graze on unwanted macro or microalgae, stir the top layer of sand beds used in reef aquariums, prey on unwanted pests, eat food leftovers and manage to incorporate dissolved organic matter (DOM) are now sold by the thousands in the marine aquarium trade. The use of these cleaning crews of marine invertebrates has put significant fishing pressure on their populations in the wild, with the ecological impacts resulting from this rush in their collection still being largely unknown. A number of marine invertebrates are also involuntarily

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introduced into reef tanks as hitchhikers on live rock employed for functional (e.g., to improve biological filtration) and aesthetic reasons (Bolton & Graham, 2006).

Along with the use of invertebrate reef janitors, aquarium keepers also employ marine macroalgae (also known as seaweeds) to control the build-up of nutrients inside their marine aquariums. A smaller sized tank or "refugium", is often stocked with these macroalgae and receives part of the nutrient rich water that flows from the main display tank. Often, macroalgae growing on these tanks become a highly complex matrix that favors the proliferation of small sized crustaceans and polychaetes that reduce the load of solid organic particles in the system.

While the culture of some of the invertebrate species employed in reef cleaning crews was already covered in previous sections, the aquaculture of several others (e.g., sponges, tunicates, echinoderms) will be presented in this chapter. Lastly, the culture of macroalgae commonly employed in the marine aquarium trade will also be addressed.

25.2 Sponges

Sponges (phylum Porifera) are commonly present on the species lists of most wholesalers supplying livestock to the marine aquarium hobby. They are occasionally purchased by hobbyists, with species displaying an intense coloration (e.g., blue, red, yellow and orange) and/or unusual shapes (e.g., tubular and barrel-like) being the most popular ones (Sprung, 2001). However, the vast majority of sponges recorded in marine tanks are introduced unintentionally with live rock or any other hard substrate employed to attach other marine ornamental invertebrates (e.g., ceramic and cement plugs employed for growing corals in mariculture). Over the years, aquarium hobbyists have wondered how some of those sponges were able to thrive and reach remarkable sizes in less illuminated areas of their reef tanks. Indeed, in those microhabitats available amongst live rock, where water flow is slower and less turbulent and organic detritus tend to accumulate, a perfect environment is created for sponges to grow. Anecdotal reports on how sponge refugiums promoted a dramatic drop on the build-up of organic matter in reef displays sparked new interest in the use of sponges in the aquarium hobby.

To date, there is no production of sponges specifically to supply the marine aquarium trade. Nonetheless, recent studies (e.g., de Goeij *et al*., 2013) reporting the trophic role that sponges play on coral reefs may create the demand necessary to drive sponge aquaculture for marine aquariums. It has always puzzled researchers how the flux of DOM produced in coral reefs is transferred to higher trophic levels allowing these luxurious ecosystems to exist in what is commonly characterized as the marine equivalent of a terrestrial desert (Hatcher, 1990). The study by de Goeij *et al*. (2013) revealed that there are sponges that make DOM available to reef fauna in the form of detritus. This detritus originates from the extensive loss of sponge cells (choanocytes) to the water, as the rapid production of these specialized cells is counterbalanced by the shedding of extraordinary numbers of old choanocytes in the form of particulate organic matter (or detritus) (de Goeij *et al*., 2009; Alexander *et al*., 2014).

The nutrient recycling mechanisms present in coral reefs, in which microbial processing plays a key role, are a remarkable source of inspiration for researchers that may try to mimic such mechanisms in reef aquariums. With these new findings on "the sponge loop", it is possible that hobbyists may once again turn to a "more biology and

less technology" approach that has allowed for the generalized use of live rock and sand in reef displays. Hobbyists may start to use sponges to keep reef displays with a myriad of invertebrates (including hard corals) and large numbers of fishes, while simultaneously having oligotrophic waters that do not favor the growth of nuisance algae.

Sponge aquaculture has been reviewed by Duckworth (2009) and Schippers *et al.* (2012). The culture of whole sponges, the culture type of relevance for the marine aquarium trade, has been most successful in the sea (production *in situ*). This culture approach requires the use of wild donor sponges, which are fragmented by cutting to produce a number of small explants (Schippers *et al.*, 2012). These explants are secured to an artificial substrate, which may range from longlines with lanterns for bivalve culture to vertical ropes and underwater frames (Duckworth, 2009). As for coral mariculture, sponge production *in situ* is vulnerable to predation, fouling, disease and storms. Additionally, optimal conditions for growth are species specific (Schippers *et al.*, 2012), which may be a constraint for producers aiming to develop a multi-species sponge production. A detailed description of the success and pitfalls of sponge mariculture over a time series of 7 years and three different generations is provided by Page *et al.* (2011). In this study the authors highlight the dramatic impact that cryptic predators (e.g., sponge eating sea slugs) may have on sponge mariculture, being able to decrease sponge biomass by nearly 80%!

25.3 Tunicates

Tunicates (phylum Chordata, subphylum Tunicata), also known as ascidians or seasquirts, are occasionally available in the marine aquarium trade, with colorful specimens being the ones most commonly available. As for sponges, there is no record to date of any culture activity targeting tunicates for ornamental purposes. Nonetheless, these filter feeding organisms may also contribute to the significant decrease of DOM in heavily stocked reef displays. It is common practice for reef tank hobbyists to record the presence in mature reef tanks of species entering as "hitchhikers" (see above for sponges). These organisms may occur as solitary or colonial individuals and can be maricultured in longlines (*in situ*) (the sea pineapple *Halocynthia roretzi* is an ascidian maricultured in Korea for human consumption) (Lee *et al.*, 2012). The *ex situ* culture of tunicates is also possible employing flow-through culture systems or recirculated systems supplied with exogenous food sources (e.g., commercial diets for filter feeders, microalgae) (Rinkevich and Shapira, 1998; Joly *et al.*, 2007). If the use of tunicates in reef aquariums begins to be "in fashion", it is likely that available protocols for *in situ* and *ex situ* culture can be replicated to fulfil any increase in the demand for these organisms in the marine aquarium trade. Species able to reproduce asexually (Manni *et al.*, 2007) should be favored when selecting target species for production.

25.4 Echinoderms

Several echinoderms are currently traded in the marine aquarium hobby, either for their aesthetic features or for their role in cleaning crews employed for keeping healthy reef displays (Sprung, 2001). Sea stars, sea urchins, sea cucumbers and serpent stars are

the most commonly traded groups of echinoderms (Wabnitz *et al*., 2003; Martins *et al*., 2012). To date, none of these groups of echinoderms is cultured to supply the marine aquarium trade, with all specimens available being collected from the wild. Nonetheless, the aquaculture of sea urchins and sea cucumbers for human consumption has experienced significant breakthroughs in the last decades.

Sea stars aquaculture for ornamental purposes has never been addressed, at least at a commercial scale. However, the culture of sea stars may be important for the commercial production of some of the most beautiful marine ornamental shrimp available in the hobby, the harlequin shrimp of genus *Hymenocera*. The aquaculture of these organisms has already been established with the only constraint impairing their large-scale production being the growout period. Soon after metamorphosis these shrimp become stenophagous, with juveniles solely feeding on sea stars. While frozen and freeze dried sea stars can be used to feed these shrimp, the large numbers of sea stars required for a commercial scale production could represent a significant impact on natural populations if wild specimens were used as prey (Calado, 2008).

Serpent stars are also popularly known as brittle stars, with specimens mostly from genus *Ophioderma* and *Ophiarachna* being collected in large numbers from the wild (Fossa & Nilsen, 2002; Martins *et al*., 2012) to be used in cleaning crews. While there are no known attempts to commercially produce serpent stars to supply the marine aquarium trade, *Ophiarachna incrassata* may hold great potential for aquaculture since reproduction and growout has already been recorded in reef tanks (Fossa and Nilsen, 2002). Overall, serpent stars known to reproduce by fissiparity (an asexual form of reproduction by division through the splitting of the disc followed by the complete regeneration of both halves; common in six-armed species), or by brooding their embryos, larvae and small juveniles inside the gonadal chambers (bursae) of their disc (and thus not exhibiting free living larval stages) (Stohr *et al*., 2012) may be suitable candidates for aquaculture ventures aiming to supply the marine aquarium trade.

Sea cucumber farming has grown rapidly in recent years, mostly targeting pricey temperate species, such as *Apostichopus japonicus*. Nonetheless, some tropical species, with emphasis on *Holothuria scabra*, have also been successfully cultured (Purcell *et al*., 2012). Even so, the information on nutritional requirements, feeding mechanics and food assimilation in their larvae and juveniles is still scarce. Spawning can be induced through a variety of stressors, such as drying and thermal shock, with variable success (Agudo, 2006). Fertilized embryos should be stocked in 1-μm filtered and UV-irradiated seawater at a density less than one embryo per liter, with a mix of microalgae (e.g., *Chaetoceros*, *Isochrysis*, *Rhodomonas* and *Tetraselmis*) at a density of 20,000 (to 40,000 in late stage larvae) cells/mL being a suitable diet for feeding the free swimming auricularia larvae. It is worth highlighting that commercial algae paste mix has already been successfully used to culture sea cucumber larvae, a feature that may significantly decrease production costs. Auricularia larvae will advance to a non-feeding stage (doliolaria larvae) which will search for a suitable substrate to settle as a pentactula larvae (Agudo, 2006). Substrates employed (e.g., plastic, fiberglass) need to be suitably conditioned to induce settlement (e.g., by immersing them in a culture of diatoms).

After settlement, petactula larvae need to be supplied with fresh or dried algae (e.g., fresh cultures of *Nitzschia* and *Navicula*, as well as dried *Spirulina*). This diet will also be suitable for young juveniles. Around day 30, the young juveniles (<5 mm length) are ready to be stocked in nursery tanks and continue to be provided with fresh and dried

algae (as described above) in bare tanks, and later moved to tanks with a 5 mm layer of sand (Agudo, 2006). At about 25 mm the juveniles are commonly stocked for growout. However, for species used in marine aquariums this size may already be suitable for the trade. As tropical sea cucumbers commonly take a long time to reach commercial sizes (Purcell *et al*., 2012), selling small sized juveniles to supply the marine aquarium trade could be an alternative source of income for the early phases of sea cucumbers production. It is important to highlight that appealing color morphs (e.g., purple, white) occur in some species and progeny that inherit such features are easily bred (Bai *et al*., 2015) opening good prospects for the use of cultured sea cucumbers in the marine aquarium trade.

Sea urchins are an integral part of the ecology of marine ecosystems. Their ecological role is primarily herbivory, and by being efficient herbivores they have considerable importance in shaping the ecology of their environments. Sea urchins are also the world wide target of large, heavily overfished commercial fisheries and important marine aquaculture ventures (Lawrence, 2013). Despite the ecological importance of sea urchins, their role in the marine ornamental aquarium hobby/industry is somewhat confused. Several species are routinely offered by wholesalers and retailers and many marine aquarists have included them in their aquariums. However, there are many species of sea urchins, about 700, from many quite different areas of the world with different biological and ecological characteristics and environmental requirements. Consequently it is not surprising that aquarists often report different experiences in their success in keeping sea urchins. In many instances they make an interesting and beneficial addition to a marine/reef aquarium and in some other instances their presence may cause problems for the aquarist. While in some situations they thrive and grow, in other situations they persist and grow very little, or even decline and die. They require abundant algal or other vegetative food and this is often limited in a marine reef and/or fish aquarium. Differences in species and size of sea urchins, as well as on the size and ecology of the captive environment, will greatly affect the survival and growth of these organisms in captivity.

Sea urchins are an interesting and functional group of marine animals for marine aquarists and are a valuable part of the marine aquarium experience. Concerning the culture of sea urchins, many different species of microalgae have been employed to raise their larvae in captivity. Larvae of sea urchins in genus *Diadema*, one of the most popular in the marine aquarium trade, have been successfully cultured using a combination of *Rhodomonas lens*, *Isochrysis galbana*, and *Chaetoceros gracilis*. Nonetheless, species within this genus are very difficult to culture, as *Diadema* display small larvae with greatly extended post-oral spines; these become long, delicate arms that apparently function to provide the larva with some directional capacity for extended oceanic travels.

Developing larvae are also very susceptible to water quality issues, which combined with a long larval development period make their culture a challenging task. In sea urchin culture, both small scale experimental culture and large scale commercial culture, it is essential to develop a method that maintains the larvae in the water column without damage or growth restriction from micro turbulence. Additionally, it is also necessary to allow for water exchange over the duration of the larval period, which can extend to 50 days or more depending on the species being cultured. These culture requirements can be fulfilled by employing small 1 to 5-L containers with slow aeration or more effectively, an arrangement of paddles attached to a low RPM electric motor

that slowly stirs the water in the containers and maintains the larvae being cultured in the water column (Strathmann, 1971; Walcott, 2002). Large conical tanks with aeration uplift and central water exchange have also been used by many large scale sea urchin hatcheries (Dworjanyn, 2007). Sea urchins occasionally spawn in marine aquariums but there have been few, if any, efforts to culture sea urchins for marine ornamental purposes. It may be worthwhile for projects that culture tropical sea urchins for restoration and/or commercial purposes to include the marine ornamental market in their planning. Recent advances in the small scale technology for sea urchin culture will certainly make it easier for small scale facilities to produce large numbers of tropical sea urchins (Moe, 2014).

The successful captive culture of *Diadema antillarum* required several years of experimentation to create a small 50-L culture vessel system that could provide a wide range of laminar water current conditions (smooth, horizontal flow) with the capability of vessel cleaning if necessary, as well as unlimited water exchanges, including constant flow. In the larval stage *Diadema* urchins develop two long (up to 1.5 mm) arms that provide lift for riding oceanic currents but severely limit their adaptability to artificial culture environments. This culture vessel design has functioned to produce thousands of both *D. antillarum* and *Tripneustes ventricosus* juveniles. The culture vessel is relatively simple in construction and can be modified in many ways without changing the basic structure and operation. It can be scaled up or down in size to provide for different types of larvae and different production and operation requirements.

The prototype (Figure 25.1) was constructed from two 6 mm acrylic plates on the sides and one 3 mm acrylic piece formed the ends and bottom; one end and the bottom is curved, while one end is vertical. In operation an air wand extends horizontally along the bottom of the flattened vertical end and creates an air current (large bubbles are much better than micro bubbles) that flows up this end and past the 105 µm screened

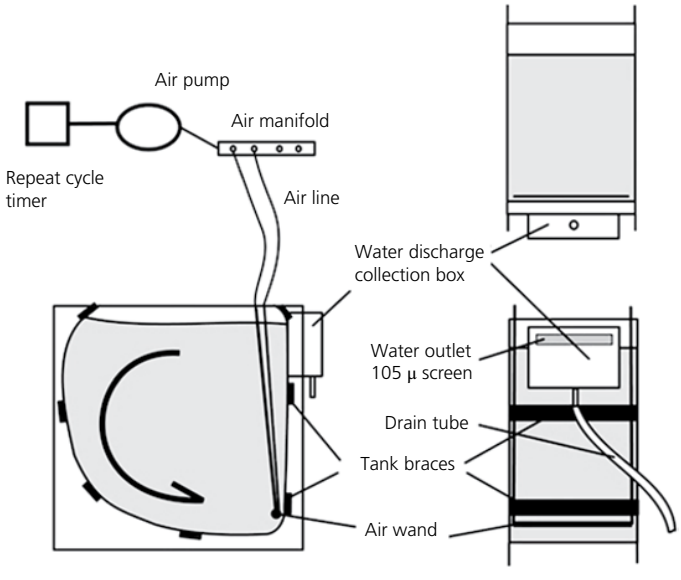


Figure 25.1 Structure of the timer controlled, rotating current, 50 L larvae culture vessel.

horizontal window that discharges a slow flow for water exchange. The air is introduced into both sides of the air wand to create an air curtain that rapidly forms completely across the vertical end of the culture vessel. This air uplift creates a circular current that revolves around the enlarged, circular "belly" of the culture vessel. It is important to highlight that if this air flow was continuous, the circular current in the culture vessel would become too strong and turbulent for most larvae, especially *Diadema*, to navigate.

The strength and turbulence of the current is controlled by a repeat cycle timer that controls either the air pump or a solenoid valve that releases the air into the air wand. The on and off time of the air release can be controlled to the second. I (Moe) found that for sea urchin larvae, primarily *Diadema*, an on time of 4 s followed by an off time of 35–40 s creates the right balance between current and turbulence that keeps larvae in the water column. An increase of on time to 6–8 s and/or a decrease in the off time near the end of the run when the larvae are larger and heavier with a mature rudiment keeps them in suspension.

The 50-L reservoirs located above the culture vessels provide for a one volume water exchange over a time period controlled by a small valve, the slower the flow the longer the time of the exchange. Typically the valve is adjusted to allow a one volume exchange over a period of 6 hours. This allows the larvae to continue feeding throughout the water exchange period and then micro algae concentrations are adjusted after the water exchange. If the supply of microalgae is sufficient, water exchange can be continuous. Water discharge from the culture vessel through the 105 μm mesh window is collected to a small box under the window and discharged by a drain tube. The flow of air and water over the mesh screen prevents the capture of larvae. For a schematic representation of this culture system please see Figure 25.2.

The characteristics of the water currents within the culture vessel can be controlled by the location of the air wand on the vertical side of the vessel, the bubble size released by the air wand, the volume of air released by the air wand, the duration of the on/off time of air release, along with the size and shape of the larviculture vessel. The characteristics of water exchange can be controlled by the size of the water exchange reservoir, size and setting of the water release valve, size of the outflow window mesh. A constant water flow can also be employed for continuous water exchange. Increasing the size of the culture vessel would increase the production from a maximum of about 80 to 100 thousand larvae at the 50-L tank to up to one million or more at a 2000-L tank.

The curve of the distal end side and bottom is very important since it controls the characteristics of the induced water current, namely on how it relates to the bottom of the tank. A flatter bottom with a slight decline toward the air wand worked better than a steeper decline with a sort of V-shaped bottom. Increasing the size of the culture vessel may change the pattern of water flow-through and around the tank and some experimentation may be necessary to fine-tune larviculture. The juvenile sea urchin begins to form within the larvae in the left side near the gut at some point in development. This is somewhat variable but usually occurs around culture day 19 to 22 for *Diadema* larvae. After culture day 28 to 35 the rudiment in *Diadema* is large and tube feet extend out from the side (Figure 25.3). The rudiment remains internal as for some other species such as *Tripneustes*, with tube feet not emerging from the larvae until after settlement.

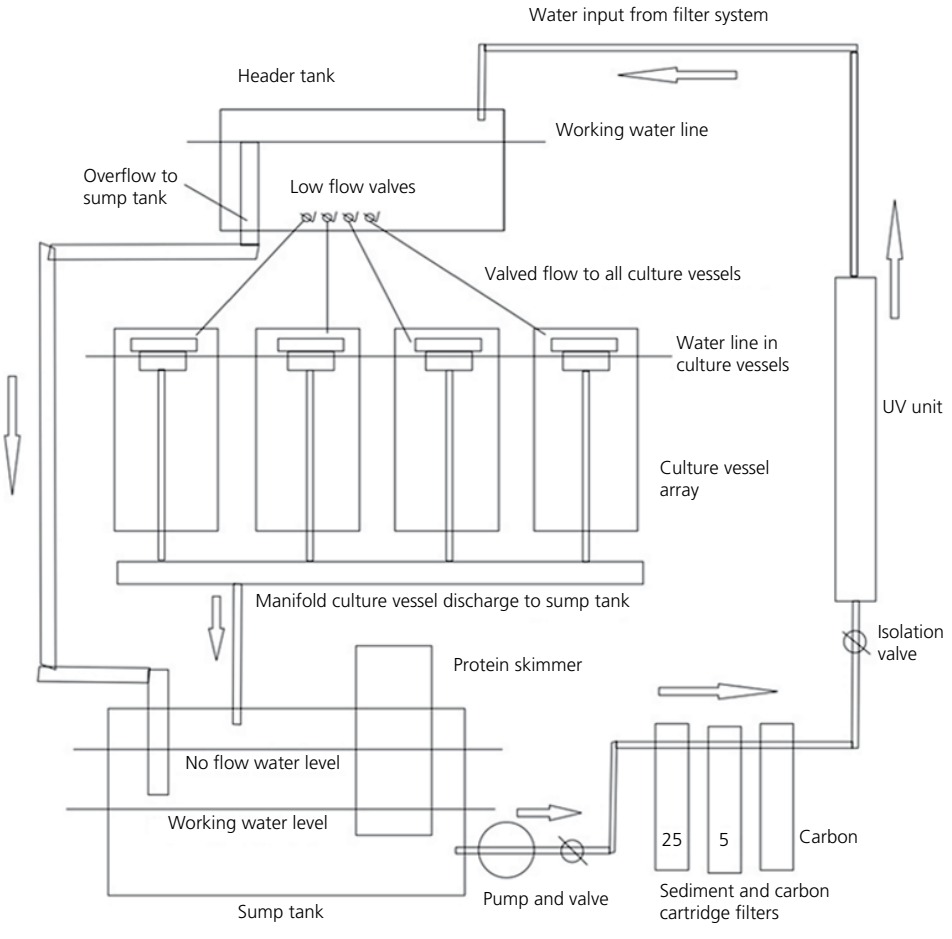


Figure 25.2 Structural and operational elements of a potential sea urchin larvae rearing system for larvae culture vessels varying in size and number.

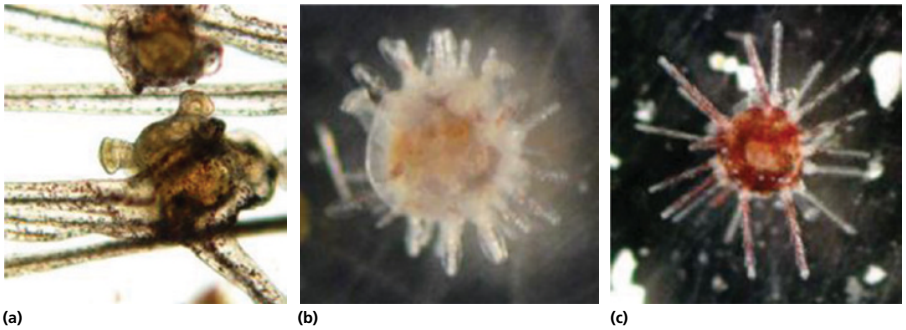


Figure 25.3 Late stages of *Diadema* larvae competent for settlement and metamorphosis: (a) mid metamorphic stage between larvae and early juvenile; (b) early juvenile just after completion of metamorphosis but before completion of test and spine development and development of internal organs; and (c) a functional, first feeding stage of the early juvenile. (*See insert for color representation of the figure.*)

At the point when larvae are competent and ready to settle and undergo metamorphosis, a biologically conditioned substrate with plated diatoms and/or algae can be introduced into the culture vessel where competent larvae will attach. The settlement plate can be later removed and placed in a growout environment. Another option is to introduce competent larvae into an aquarium without filtration but with good aeration that contains live rock and other substrates where larvae can settle and metamorphose. Metamorphosis can occur as rapidly as within 1 h or not being completed even after 24 h. Apparently, the better the larval history the more quickly metamorphosis occurs and is completed. During metamorphosis the larval tissues "melt" down into the developing body of the juvenile and in *Diadema*, the now naked internal supports of the long arms fall off near the body of the new juvenile. The new juvenile has extensive internal development that has to be performed rapidly, if the juvenile is to be able to feed and protect itself. Internal organs must be developed, external spines and test must be calcified, and the mouth parts and gut have to be formed before feeding can occur.

All this must be accomplished with the energy that the new juvenile has acquired during its larval life. Thus the quality and quantify of nourishment from the larval stage that flows into the juvenile is critical for survival at metamorphosis and early benthic life. If all is well and juvenile urchins survive to and through first feeding in 4 to 8 days, there is a good chance that they will survive through growout. Growth of juvenile sea urchins depends on the physical, chemical, and nutritional environment. The most rapid growing juveniles of both *Diadema* and *Tripneustes* placed under the most favorable environments can grow to about 4 to 5 cm in one year, being capable of spawning at that point. Although sea urchins are not major players in the hobby/industry of developing and maintaining captive marine environments, they will certainly be an important part of the expansion of the species of marine invertebrates that are cultured for the marine aquarium industry.

25.5 Macroalgae

Macroalgae are utilized by marine aquarium hobbyists to export nutrients from reef tanks rather than for their aesthetic value as ornamentals (Sprung, 2002). Indeed, macroalgae are rarely stocked in the display tank. Macroalgae are commonly placed in smaller tanks (refugiums) connected in series or in parallel to the main tank (Calfo & Fenner, 2003). The most commonly employed macroalgae for marine aquariums are those in the genus *Caulerpa* (Sprung, 2002). However, several of those species are well known to have successfully invaded a range of habitats outside their native distribution and negatively impacted marine ecosystems (Schaffelke *et al*., 2006; Williams & Smith, 2007). The impacts that invasive *Caulerpa* have already caused in the Mediterranean Sea (Streftaris & Zenetos, 2006) and in California (Anderson, 2005) have raised an awareness of how the marine aquarium trade can play a key role in such marine invasions (Jousson *et al*., 1998; Stam *et al*., 2006). The state of California, USA even approved a ban on the possession and sale of potentially invasive *Caulerpa* species (e.g., *C. taxifolia*, *C. racemosa*), although a large number of aquarium stores continued to make them available to hobbyists (Díaz *et al*., 2012). Under this scenario, conscientious hobbyists began favoring other macroalgae, with the green

filamentous *Chaetomorpha* becoming one of the most popular in the marine aquarium hobby (Walters *et al*., 2011). However, given the hardy nature and bloom-forming ability of *Chaetomorpha*, which has a propensity for vegetative fragmentation and is able to grow from a fragment as small as 0.5 mm, it is possible that marine aquarium keepers may be replacing well known invasive species (*Caulerpa* spp.) with other potentially invasive species (*Chaetomorpha* spp.) (Odom & Walters, 2013).

The culture of these popular green macroalgae to supply the aquarium trade is unlikely to be a profitable option. Not only do a large number of hobbyists not stock macroalgae in their systems, *Caulerpa* and *Chaetomorpha* are so prolific that they are very easily cultured by hobbyists. In fact, hobbyists need to harvest those macroalgae periodically to truly export from their system the phosphates and nitrogenous compounds that have been sequestered and turned into algal biomass. Therefore hobbyists frequently give away those macroalgae to other fellow hobbyists, or simply discard them (Odom *et al*., 2014).

A potential scenario that may make the culture of these (or other) macroalgae financially appealing is where they are employed to support the growout of ornamental sea slugs currently traded (ex. *Elysia crispata*). The production of these sea slugs is commonly hampered by the lack of suitable biomass of their algal diet to satisfy their stenophagous feeding regimes (Dionísio *et al*., 2013). This type of production can be interesting if an integrated multitrophic approach is implemented. Nutrients in the outflowing water of tanks holding ornamental fish during growout may be employed to grow such macroalgae, simultaneously decreasing the load of nitrogenous and phosphorous compounds in the water and producing food for stenophagous algae eating sea slugs.

Given the risk of accidentally introducing alien species that may rapidly become invasive in adjacent marine ecosystems, it is advisable to always target the culture of autochtonous macroalgae. Exotic species should only be targeted if these are illegal, or already known to have become invasive elsewhere. Only when cultured macroalgae are destined solely for use as food for other organisms being produced, and strict protocols are employed when those macroalgae are discarded (see Odom *et al*., 2014), should the production of exotic species be considered.

25.6 Conclusions

Overall, it is not likely that any of the groups of marine ornamental species discussed above will be cultured in the near future specifically to supply the aquarium trade and alleviate fishing pressure on wild populations. Nonetheless, with growing environmental awareness and potential restrictions on the harvest of some of these organisms from their natural habitats (namely echinoderms), it is possible that available culture protocols for species used for human consumption may start to be adapted for marine ornamental species. It is also possible that, as for tridacnid clams in the past, aquaculture ventures targeting tropical species of the groups discussed above can make cultured juveniles available for the marine aquarium trade. By trading juvenile specimens that have not yet attained commercial sizes for human consumption, enterprises may diversify their activity and generate an additional source of income that trades specimens on a unitary basis rather than on a weight basis.

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Part V

Other Issues in Marine Ornamental Species Aquaculture

Previous chapters in this book are devoted to general marine ornamental species aquaculture, to facilities, culture systems and other specific requirements, as well as to specific culture features of some marine ornamental fishes and invertebrates. However, there are other important issues directly or indirectly related to ornamentals aquaculture that deserve special consideration. The increasing demand of marine ornamentals entails huge activities of packaging and worldwide transportation of millions of organisms. Exporting large amounts of ornamentals is a task that requires previous knowledge on practical procedures, as well as on the biological characteristics of the specimens being traded. The main bottleneck in the shipping of marine species to buyers away from their culture location is the potential for mortality due to high stress levels promoted by physiological disorders. Transport success is not only dependent on the transport itself, but also on the packaging process and its characteristics. The duration of the trip is a key issue to be considered for shipment and all variables and administrative issues must be properly assessed. Chapter 26 describes in detail some techniques to minimize mortality risks during road, air or sea transportation. In addition to private aquarium owners and hobbyists, public and private aquariums are important target institutions for the reception and exhibition of ornamental specimens, contributing to education and playing a conservation role (Subchapters 27.1 and 27.2). Furthermore, many husbandry and breeding programs for vertebrate and invertebrate species are carried out in public and private aquariums. The information available from these programs is extremely important for the development of new rearing techniques for many species and the production of numerous specimens, contributing to reduce the pressure on wild populations. Collaborations among research organizations, hobbyists, traders and public and private aquariums would certainly provide solutions to the sustainability of the marine aquarium trade, to ecosystem preservation and the promotion of benefits to local communities. Large public aquariums have exhibits that allow the representation of ecosystems and the maintenance of large specimens that cannot be kept in small home aquariums. Most aquarium hobbyists want to simulate coral reefs and other ecosystems in their displays. Recent advances in husbandry and aquarium design and equipment (Olivotto *et al*., 2011) have given rise to home nano and mini tanks which require minimal knowledge of the owner on aquarium maintenance. Chapter 28 explores how the trend toward keeping nano tanks can contribute to the sustainability of the marine aquarium trade and to coral reefs preservation. Compared

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to larger aquariums which require a higher number of specimens to simulate a busy reef, nano tanks can be fully stocked solely with small sized captive-bred or cultured organisms. This approach lowers the demand in terms of diversity and quantity for reef organisms that need to be collected from the wild. Some solutions to the overharvesting of the world's coral reefs for the marine aquarium trade are also discussed (Chapter 28).

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26

Packing and Shipping

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Abstract

The aquarium industry as we know it today is greatly influenced by the possibility of transporting aquatic species worldwide. For most of the common marine species in this industry, their natural habitat is often located half a world away from their final destination. The transportation process, however, used to be a synonym for high mortality rates. High standards for collection and premium post-harvest husbandry practices are of paramount importance to achieve success in marine species transportation. Still, and despite the adoption of modern high standard practices, this may not be sufficient to ensure low mortality rates. Transport itself can be highly stressful and physiologically challenging to the organisms and may lead to mortalities. In order to reduce these mortality numbers, numerous techniques have been tested and applied globally and, in some cases, with great success. Some of these techniques are here described in detail.

Keywords *IATA Live Animal Regulations; polystyrene boxes; transport methods; tanks*

26.1 Introduction

Trade in live aquatic ornamental animals for the aquarium industry is a global activity that allows a hobbyist in North America to keep a clownfish from Polynesia in his living room's nano-reef tank, or a public aquarium in Asia to exhibit Barber gobies from South America. Ornamental marine species (corals, other invertebrates and fish) are collected and transported mainly from Southeast Asia, but also increasingly from several island nations in the Indian and Pacific Oceans, to consumers in main destination markets: United States of America (USA), European Union (EU) and, to a lesser extent, Japan (Wabnitz *et al*., 2003).

Although some marine aquarium fish and invertebrates are aquacultured by the industry and hobbyists, most marine ornamentals – over 90% – are from wild-caught fisheries (Chapman *et al*., 1997; Cato & Brown, 2003). Considering that most of these fisheries occur in coral reefs (commonly regarded as amongst the most biologically rich and productive ecosystems on Earth), it is extremely important that marine ornamental

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fisheries are managed in such a way that they are biologically sustainable, do not conflict with other resource uses and keep post-harvest mortalities to a minimum (Wabnitz *et al*., 2003).

Marine organisms often experience high post-harvesting and transport mortalities. These mortalities typically range from a few to more than 80%, as a result of a combination of the use of cyanide to stun fish, poor capture and handling techniques, inadequate husbandry practices, facilities and transportation, and the inclusion of unsuitable species in the trade (Johannes & Riepen, 1995; Wood, 2001; Sadovy & Vincent, 2002).

Even assuming that good practices of collecting and handling have been adopted, it is important to ensure that all organisms are perfectly healthy and stabilized before packing for transport. The collection process, as well as the organism's introduction to captivity, may cause mechanical abrasions, stress and discomfort, amongst multiple other problems. Therefore a "stabilization period" is critical for the animal to recover from these symptoms and, consequently, maximize its chances of survival during and after transport. This period may differ considerably according to numerous factors, including species resilience and adaptability and seriousness of the lesions on the organism. The adoption of good husbandry practices is crucial and may decrease this stabilization period. Only once the animal is healthy and fully recovered from the harvesting/introduction to captivity/stabilization period factors, should one consider packing it for shipping.

While preparing for shipping, a myriad of details has to be carefully analyzed and various options should be considered in order to transport animals safely for a reasonable cost. One of the first questions to be considered is the container where the animal will travel. For example, transporting a 1 m long Blacktip reef shark entails completely different logistics (and container) than transporting a 4 cm Pacific cleaner shrimp. The shark most likely requires a round polyethylene vat with at least 1500 L of seawater, constant water flow, oxygen supply, and preferably a filtration system, so that the animal may survive 24 h en route safely. The cleaner shrimp, on the other hand, may travel well inside a sealed plastic bag with 1 L of seawater and 0.5 L of oxygen above the seawater. This "closed system" would be enough for the animal to survive a 72 h journey safely. In general, animal transport can be divided into two different categories according to packing option: bag (sealed) transport and tank (open) transport.

26.2 Packing

Transporting live animals is a delicate operation that involves some risk. Poorly planned and executed transport may cause mortalities during transit or during the early hours that follow acclimation at the destination. The stress (both physiological and behavioural) felt by an animal that undergoes transport must always be taken into consideration and all measures that minimize this stress should be adopted in order to reduce (or, preferably, eliminate) mortalities. As such, some useful techniques are commonly used worldwide to reduce animal stress.

26.2.1 Container Size and Shape

The use of an adequate container is absolutely critical since this will be the animal's home during the following hours/days. The container must be of an adequate size for the animal to be transported. The size of the container depends not only on the

animal's size and weight, but also on the specific animal behavioural and physiological characteristics. For example, it is possible to transport a certain bioload (kilograms of body weight per liter of water) of Madeira rockfish, *Scorpaena maderensis*, in a sealed plastic bag with 5 L of water and 5 L of oxygen for 48 h. However, it is not possible to transport in the same conditions bioload of Chub mackerel, *Scomber colias*. The behavioural and physiological characteristics of rockfish are completely different from those of the mackerel. The first has a sedentary lifestyle and a relatively low oxygen consumption rate, while the latter is a typical pelagic free-swimming fish that requires lots of space to swim and exhibits high rates of oxygen consumption. In this case, the plastic bag packing would be a good option for the rockfish, but not for the mackerel. For the mackerel, a tank with permanent oxygen supply should be used, as described by Correia *et al*. (2011).

The shape of the container is also very important in terms of animal welfare and comfort. For example, when an animal is transported inside a plastic bag, it is important to ensure that it does not get stuck if the bag folds during the transport movement. In this sense, the plastic bags should be fully inflated and corners must be eliminated. This can be done by using round bottom bags, by taping the corners of the bag or simply by inverting the bag into another bag.

As for a tank transport of, for example, a Dolphinfish *Coryphaena hippurus* – a typical open ocean species – it would be necessary to use a round tank, with no corners, as described in detail by Correia (2001), Young *et al*. (2002), and Correia *et al*. (2008, 2011), to prevent the animal from colliding against the walls while swimming, and with no obstacles, so that the fish can swim freely inside the tank, as suggested by Rodrigues *et al*. (2012).

26.2.2 Water Parameters

When packing an animal, top quality water should be used and its parameters should meet the specific animal requirements. Under a normal situation, water parameters will change during transport, and possibly affect animal welfare. Dissolved oxygen, for example, will decrease during transport; temperature may increase or decrease according to external factors; pH will also decrease over time; ammonia, on the other hand, will increase during animal transport. These are the most important water parameters to monitor during transport as they are both highly likely to fluctuate. These fluctuations can, however, be minimized or even eliminated, which will be described afterwards.

26.2.2.1 Dissolved Oxygen

Oxygen depletion in transport water is caused by animal metabolism and it is a common cause of mortalities in transit. By using an oxygen cylinder and an airline tube while packing, bags can be inflated with oxygen, supersaturating the water (i.e. driving the oxygen saturation rate, in the water, far above 100%), thus eliminating this problem. The amount of oxygen volume added during packing will depend on the species' consumption rate, but usually 1/3 water and 2/3 oxygen is an acceptable ratio for most organisms. As transport progresses and the animals consume the oxygen dissolved in the water, the atmosphere of pure oxygen above the water, sealed inside the bag, will dissolve into the water, which typically drives saturation rates to the high 100s or even 200% over transport.

As for tank transports, one can use the same system as with bags, i.e. fill the tank to 1/3 with water and then seal the tank. Afterwards, a small hole is made in the tank lid, just large enough to fit an airline through which oxygen is pumped until it eliminates the 2/3 of air left in the tank above the water, replacing it with pure oxygen. The inner side of the tank lid may be fitted with an air pump coupled to an air stone, which will diffuse the oxygen into the water progressively. Once the tank is supersaturated, this hole must be sealed with silicone or another isolating material. One other option is to travel with an oxygen cylinder along with the tank, thus providing oxygen continuously through an airline and an air stone.

26.2.2.2 Temperature

Temperature shifts in transport water are common during transit and this happens mostly because of changes in ambient surrounding temperature. It is important, therefore, to keep temperature as stable as possible in order not to jeopardize the animals' wellbeing. This is true for both tropical and cold water species. During bag shipments, putting the bags into polystyrene boxes is a very common technique, as this material offers excellent thermal insulation properties, while also providing adequate mechanical protection. Adding newspapers or plastic bags inflated with air to the polystyrene box, between the bags with animals, is also common and efficient both for thermal and mechanical protection. Newspapers will also absorb any residual water that may be on the outside of the bags. One other common technique that is used to maintain temperature of transport bags is the use of ice (or heat) packs inside the polystyrene transport box, depending on the temperatures expected to be experienced during transit and animal requirements. If temperatures during transit are expected to be much lower than water temperature in the transport bag, heat packs can be used. On the other hand, if temperatures during transit are expected to be much higher than water temperature in the transport bag, the use of ice packs is advisable.

Controlling water temperature in tank transports is not practical with any of the aforementioned techniques, since none of them is effective for a large volume of water, typically above 1000 L. On the other hand, in large water volumes, temperature oscillations are much less likely to occur, unless tanks are exposed to extreme outside air temperatures for a long time. As long as this is avoided, temperature oscillations should not constitute a significant problem in tank transport.

26.2.2.3 pH and Ammonia

During transport, water pH will gradually decrease, whereas ammonia will increase, as a result of carbon dioxide buildup and the release of nitrogenous waste and miscellaneous stress-related metabolites. Both of these tendencies need to be counteracted through the use of filtration (only possible in tank transports using filters) and/or chemical supplements. Ammonia buildup may also be minimized by fasting the animals before transport.

26.2.3 Filtration

Water filtration is possible, and advisable, during tank transports. As described by Correia (2001), Young *et al.* (2002), Correia *et al.* (2008, 2011), filtration units can be used to help maintain water quality during transit. These units can be relatively simple,

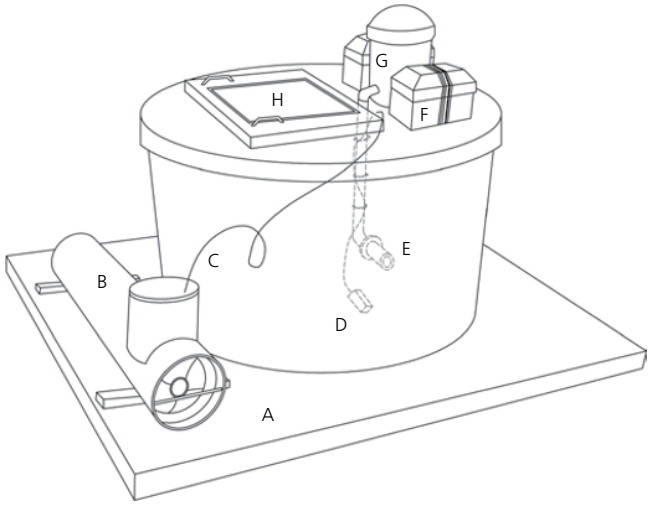


Figure 26.1 Transport tank for live fish. (A) Wooden, or plastic, pallet; (B) Stainless steel cylinder to hold oxygen cylinder (inside); (C) Airline tubing, connected to pressure regulator, in turn connected to oxygen cylinder; (D) Air stone (inside the tank); (E) 12 Volt bilge pump; (F) 12 Volt dry-cell battery; (G) Filter unit (usually laminated cartridge paper and one mesh bag with activated carbon inside); (H) Inspection and access window.

consisting of containers with laminated cartridge paper and one mesh bag with activated carbon. But more complex units have been used, such as foam fractionators (Rodrigues *et al*., 2012), which provide additional and continuous removal of organic matter from the water, thus eliminating the source of pH drops and ammonia buildup. All these systems are, as described by the authors, powered by 12 Volt dry-cell batteries (i.e. appropriate for air-travel), wired to bilge pumps (Figure 26.1).

26.2.4 Chemical Agents

The control of pH can be achieved by the use of buffering agents, such as the tribuffer described by McFarland & Norris (1958), common baking soda (i.e. sodium bicarbonate – $NaHCO_3$) and/or soda ash (i.e. sodium carbonate – Na_2CO_3). Ammonia (NH_3 and NH_4^+) may be removed with the assistance of quenching agents such as AmQuel® ($HOCH_2SO_3$) (Novalek, Inc., Hayward, CA, USA) which binds to ammonia and transforms it into nontoxic aminomethanesulfonate ($H_2NCH_2SO_3^-$) and water. The addition of these three agents (AmQuel®, sodium bicarbonate and sodium carbonate), according to the formula 20/50/50 g per 1000 L (Correia *et al*., 2011; Rodrigues *et al*., 2012) to the water while packing, and before adding the animals, will help prevent pH drop and ammonia buildup. Other types of chemical agents such as sedatives and other anesthetic agents can be used in transport; however, their influence will be on the organisms instead of treating the water. The stress in the animals (especially fish) caused by the transport process, many times in crowded conditions, can be reduced with chemical anesthetics, by producing a light sedation.

Various products have been widely tested for fish sedation (e.g. Quinaldine, Benzocaine, Tricaine methanesulfonate, 2-phenoxyethanol, clove oil) (Munday &

Wilson, 1997; Neiffer & Stamper, 2009) but the most commonly used in transports is Tricaine methanesulfonate, commonly known as MS222. This chemical is effective for most species of fish, but concentrations to produce light sedation vary for each species and depend on size and weight of the animals (Neiffer & Stamper, 2009).

26.2.5 Fasting

One other commonly used technique to prevent ammonia buildup is animal fasting prior to shipping. Not feeding the animals that are about to be packed for shipping is an effective way to reduce excretions and, therefore, reduce ammonia production. This fasting period depends on the species to be shipped but, generally, 48 h is considered adequate for most species. As a general rule, the smaller the animal, the shortest the fasting period needs to be. Also, if the animal is a constant swimmer (e.g. sardine) or a relatively sessile animal (e.g. scorpionfish), different fasting periods should be considered. The sardine should undergo a shorter period of fasting than the scorpionfish since the first will spend its energetic reserves faster than the latter. Usually, tropical species also fast for shorter periods than closely related cold-water species, since the first usually have higher metabolic rates and spend energy faster (e.g. tropical and cold-water *Anthias* spp. can have remarkably different habits and metabolic rates). It is important to remember that this fasting period will continue through transit and may be extended for some time after arrival to the destination. On arrival, the animals may not feed for various reasons such as stress, unfamiliar environment, and new food items, amongst others. Therefore, all measures should be taken so that the animal does not go without food for too long, thus compromising its health.

26.3 Shipping

26.3.1 Road, Air, Sea Transport

Shipping live marine animals may be done by air, road or sea. All three methods entail common concerns, highlighted in the previous pages of this chapter, but they also host specific aspects that will be addressed individually next.

26.3.1.1 Road Transport

Moving live marine animals by road usually involves long trips, typically over 12 hours. During this period of time, water quality deteriorates, as described above, but road transports allow for water changes (i.e. blowdown) to be performed in transit. It is therefore advisable, if possible, to pack extra clean water and pumping equipment (i.e. pump, adequate length hose and power source, such as batteries). One should note that saltwater is a highly corrosive substance, so care should be taken during water changes, specifically while saltwater is being drained from a transport tank. This wastewater should be channeled, with a hose, to a sewage intake whenever possible. Dumping saltwater onto a non-regulated destination could, not only cause harm or malfunction to the equipment that comes into contact with such saltwater, but may also land the person a heavy fine, with unwanted and unnecessary delays.

Moving tanks by road also offers unusual high level of vibration, which needs to be addressed by ensuring that all equipment is securely fastened. Should temperature

constitute a concern, trucks equipped with a heater (or chiller) unit should be used. Other additional concerns come in the form of simple logistics that may prove to be extremely important. For example, transport tanks are typically loaded on top of pallets, which are then loaded onto a truck's flat bed. When loading, it is important to learn whether the truck will be offloaded from the side or the back, so that the pallets are oriented in the correct way. Note that both tanks and pallets are easily moveable by hand when empty, but become virtually impossible to be moved by hand when filled with water. The proper orientation is therefore critical, the penalty for neglecting this aspect being the offloading of the fish from the tanks by hand while they are still loaded on the truck, and carrying the heavy bags, or nets, with fish.

Road transport offers multiple advantages, the most significant being their relatively lower cost compared to air transport. Additionally, road transport allows for filtration units to be wired directly to the trucks' power supply, which means no batteries are required to run the filtration pumps wired to the transport tanks. Alternatively, batteries may still be coupled to all filtration units and be charged on the road during transport, ensuring they are fully charged upon offloading the truck. This may prove extremely convenient if, for example, the tanks have to be left unattended for some time. However, great care must be taken to ensure both voltage and amperage are compatible. For example, most bilge pumps used in transport run on 12 Volts DC current, while some trucks have 24 Volts DC batteries. Wiring one to the other would result in burned pumps. The same concerns need to be observed for both amperage (measured in Amps) and frequency (measured in Hertz or cycles/second) to avoid equipment damage and/or, loss. In addition, voltage inversers may be coupled to the filtration system, converting the 12 Volt DC power of the vehicle to 110/220 Volt AV, which allows for the use of standard 110/220 Volt pumps and therefore more powerful and stable filtration, since the use of 12 Volt batteries is unfortunately coupled with a decrease in charge over time, which causes filtration to lose momentum and requires constant fine tuning of the equipment, particularly protein skimmers. Care should be taken to use "pure wave" inversers which, albeit more expensive, provide for a more stable and constant flow of voltage, and therefore a more smooth operation by all pumps and less need to constantly fine tune them.

26.3.1.2 Air transport

Air transport offers speed to reach the destination, but comes with two often deterring disadvantages: cost and regulations. Air-travel is indeed substantially more expensive than road travel and also entails a rather heavier administrative burden. For example, the use of compressed oxygen cylinders on board aircrafts is highly controlled, as it is considered a "Dangerous Good". Oxygen may still be used but a "Dangerous Goods" declaration needs to be filled out and the airline will have to request multiple permits from official aviation agencies in all countries over which the aircraft will fly.

Another major concern while flying transport tanks is that absolutely no leaks are allowed inside an aircraft, particularly of saltwater, which is not only extremely corrosive but highly conducting as well. There is a "famous" (or "infamous") story in the industry where a leaky tank, during take-off, allowed for some saltwater to seep into the avionics compartment of a cargo Boeing 747, triggering all sorts of alarms and malfunctions, forcing the crew to do an emergency landing immediately after take-off. This means that the shipper using transport tanks and/or polystyrene boxes, needs to ensure that not one single drop of saltwater will leak during transit. Leak-proofing transport

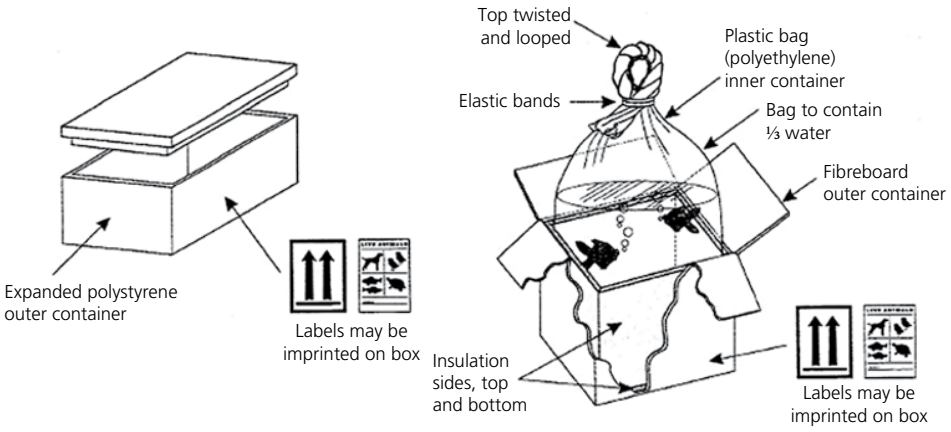


Figure 26.2 IATA (International Air Transportation Association) Live Animal Regulation number 51, for transporting live fish inside plastic bags, which travel inside polystyrene boxes that, in turn, must travel inside a cardboard box.

tanks basically involves a generous dose of sealant material (such as silicone) and a large number of nuts and bolts (typically 20 cm apart along the edge) securing the lid to the tank itself. When moving polystyrene boxes the bags inside need to be doubled, sometimes even tripled, often with newspaper, or another insulating material, in between bags. The polystyrene box, with the bags inside, is then placed inside a cardboard box, for added thermal and mechanical insulation, as well as safety. These requirements are described in great detail under "Live Animal Regulations" section 51 of the International Association of Air Transport guide for moving live animals (Figure 26.2).

Temperature on board commercial aircrafts' cargo holds is usually kept stable at 15 degrees Celsius, which is adequate for the vast majority of specimens, and both heat, or ice, packs may be included in the boxes, as described before. When flying cargo aircrafts, the crew often has the ability to control the cargo hold's temperature, enabling attendants who fly with the animals to request their optimal temperature.

26.3.1.3 Sea Transport

Moving live marine animals by sea offers two major advantages: cost and water. Cost of maritime freight is substantially lower than air, or even road. Traveling on a vessel allows for continuous, or frequent, water changes provided that the water being navigated offers enough quality and parameters for it to be used with the animals being moved. Sea freight is typically only used for transport tanks, as moving styrofoam boxes is easily done for a reasonable cost by air or road. Major concerns, while moving transport tanks by sea, involve safety of both the animals and persons attending to the animals' needs in transit. All parts and equipment need to be secured properly to avoid movement during rough seas. People should also take great care while moving around on a large ship, which even during flat seas, offers a multitude of possibilities for injury.

Temperature control is often not available, unless temperature controlled containers are used during the trip. Another potential disadvantage in sea-freight is "time", as oceanic trips typically last longer than 48 h with extremely limited opportunity for procuring supplies or materials. Great care therefore needs to be taken upon boarding

the vessel, as every single item that may possibly be required must be shipped with the transport tanks and their animals. Sea freight should therefore be limited to species that may handle 48+ h in transit, if not much more.

26.3.2 Administrative Issues

Shipping live animals to international destinations involves a myriad of support documentation, described next.

26.3.2.1 CITES

CITES (Convention on International Trade in Endangered Species – www.cites.org) acts along the borders and is controlled by customs agencies worldwide. As such, CITES licenses and declarations are not required for domestic or intra-European transports, but are required when borders are crossed to, or between, third countries. CITES lists animal and plant species in appendices I, II and III, depending on the level of protection from over-exploitation each species is attributed. CITES listed species require a license issued by CITES agencies in both the origin and destination countries. Species not listed in CITES appendices do not require a license but still require both an export and import declaration issued by the origin and destination country's agencies, respectively.

26.3.2.2 Veterinarian Certificate

Most countries require a veterinary certificate upon importation but be aware that veterinarian certificates have multiple formats in different countries. When applying for a veterinarian certificate, the exporting country's governmental veterinarian agency will request advice from their counterpart in the importing country, then issue the certificate according to the importing country's agency requirements. Be aware that often governmental veterinarian agencies take an extremely long time to communicate amongst each other, and direct communication between the exporter and importer might be advised regarding this issue. Most veterinarian agencies will require a veterinary declaration, issued by a certified veterinarian doctor, before the governmental official document is issued.

Intra-European shipments require an "International Transport Permit", which is issued by an online system known as "TRACES", which stands for "Trade Control and Expert System". This permit may be issued by the exporter directly from the Europa.eu website (http://ec.europa.eu/food/animal/diseases/traces/index_en.htm), although an official seal is often required by that country's governmental veterinarian agency. Once again, the seal is not issued until a veterinarian declaration, signed by a certified veterinarian doctor who has physically inspected the animals, is provided.

26.3.2.3 Certificate of Origin

Most non-European Union countries require a Certificate of Origin from the exporting party, which should be checked with the importing party. These Certificates are typically issued by the exporting country's Chamber of Commerce.

26.3.2.4 Customs Documents

Customs documents are required for all non-European Union shipments and come in multiple formats. We therefore strongly advise all those exporting, or importing, live

animals, to/from a non-European Union country to procure the assistance of a customs agent who is certified to handle all customs related documents.

26.4 Conclusions

Shipping live marine animals is no easy task, but it is also perfectly achievable for most animals with a reasonable degree of effectiveness, provided all logistical and administrative aspects are addressed adequately. Communication between both exporting and importing parties is crucial to ensure all required documents are taken care of in a timely fashion. Logistic concerns then follow, with choosing the packing method that ensures the highest standard in animal care, taking into consideration the behaviour, and special needs, of all specimens that will travel. Length of the trip is a key aspect to be factored in during the decision making process and adequate weighing of all variables will no doubt ensure a positive outcome and no mortalities in transit, or after.

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27

The Role of Public and Private Aquaria in the Culture and Conservation of Marine Ornamentals

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Public and private aquaria are the drivers of the marine aquarium industry, as they both rely on the trade of wild and cultured marine ornamental organisms to set up their displays and mimic the beauty of coral reefs. While the conservation role played by public aquariums on the protection of the marine environment is commonly well perceived by society, private aquariums are more often considered as "part of the problem" and rarely associated with coral reef conservation. Private and public aquariums are known to develop a number of breeding programs targeting marine ornamentals species (Petersen *et al*., 2006; Osinga *et al*., 2012) and may partner with players in the marine aquarium trade to help develop codes of best practice promoting a more sustainable industry (e.g., using non-destructive fishing techniques and improving husbandry along the supply chain to decrease the number of specimens that need to be collected from coral reefs to fulfil demand) (Tlusty *et al*., 2013). Public aquariums may also make available to research institutions, professional breeders and hobbyists the eggs of marine ornamental species that will rarely spawn in small-sized private aquaria, but readily reproduce in large-sized exhibition tanks. Overall, these actions may directly or indirectly shape the perception of private aquarium owners. Indeed, it may contribute to their education as conscientious buyers and start shifting their preference from inexpensive organisms collected using destructive practices and poorly shipped from reef to retail, to sustainably harvested or cultured specimens that may provide social benefits to exporting nations. Global economics and technological breakthroughs may also contribute to the shaping of the marine aquarium hobby by shifting hobbyists' preference towards smaller and less expensive displays (e.g., nano-tanks) which can be operated using innovative solutions for the husbandry of marine ornamentals (e.g., LED technology to light symbiotic corals) (Rhyne & Tlusty, 2012). The use of smaller sized private aquaria may promote the selection of cultured specimens by hobbyists and contribute to a more sustainable trade of marine ornamental species.

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27.1

Public Aquaria

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Abstract

Public aquaria have an intimate relationship with the commercial trade in ornamental aquatic species. They rely in part on the global aquatic animal trade to acquire their exhibit specimens, both directly sourcing from the trade itself or by collecting animals in the same locales as commercial collectors. Being centers of research, education and conservation, public aquaria should practice model behaviour when sourcing marine fishes, working with fisheries to ensure that stocks are well managed, ecosystems are preserved, and local communities benefit from collection activities. Model behaviour also implies that indirect participation in wild fisheries (e.g., when using paid or commercial collectors) must also adhere to these sustainability goals. Public aquaria have large multi-taxa exhibits, in which animals frequently exhibit reproductive behaviour. This behaviour allows for guided and opportunistic breeding programs, which can reduce the number of animals sourced from the wild. Maximum benefit to the ornamental aquatic animal trade will come from a balanced approach to *in situ* conservation and *ex situ* aquatic culture.

Keywords *Aquaculture; fisheries; ornamental fish; sourcing; sustainability; wildlife trade*

27.1.1 Introduction

The basic premise of a public aquarium is that visitors will spend their disposable income to be entertained and to learn more about the "blue" planet. A 2008 global survey showed that 700 million people visited zoos and aquariums, and US$350 million was spent on nature conservation, annually. These impressive figures demonstrate the high potential for zoos and aquariums to play an essential role in education and conservation (Gusset & Dick, 2011). To ensure there is a strategic approach to research and

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conservation, the regional associations of aquariums and zoological gardens (e.g., the World Association of Zoos and Aquaria (WAZA), the European Association of Zoos and Aquaria (EAZA) and the Association of Zoos and Aquariums (AZA)) have been working toward enhancing the education, conservation and research efforts of their member institutions. These Associations define standards, policies and procedures, which help zoos and aquariums improve their work practices, including, but not limited to, sustainable collections management, best practice animal husbandry and welfare, immersive informal education and robust conservation research.

Focusing on public aquaria WAZA recently published *Turning the Tide – A Global Aquarium Strategy for Conservation and Sustainability* (Penning *et al*., 2009), which outlines a proposed implementation of the World Zoo and Aquarium Conservation Strategy by WAZA members. *Turning the Tide* describes the role public aquaria can play in the conservation of aquatic species and highlights the point that livestock acquisition operates within the context of a larger international hobby trade, not only for fishes but for reptiles and invertebrates as well (Penning *et al*., 2009). In 2005 the AZA noted that 1375 species were held by public aquaria (AZA, 2010), of which 54.3% were declared on import documentation as a "…marine aquarium tropical fish…" (Rhyne *et al*., 2012). Public aquaria undeniably overlap with the aquarium hobby trade, leading Penning *et al*. (2009) to suggest that aquariums should look to the "trade" to "…draw on their expertise and engage in partnership initiatives…". Tlusty *et al*. (2013) examined these partnership opportunities and identified four key mechanisms for public aquaria to increase the sustainability of the ornamental fishes trade. These mechanisms include: (1) providing technical knowledge (Tlusty *et al*., 2013); (2) participating in research activities (Brittsan & Jones, 2008; Tlusty *et al*., 2013); (3) collaborating with national regulatory authorities, such as CITES (Jones, 2008); and (4) educating the general public about the need for the sustainability of the ornamental fish and coral trade (Gamain, 2008; Jones, 2008; Tlusty *et al*., 2013).

Many suppliers used by public aquaria provide animals for the ornamental fish trade too (Thoney *et al*., 2003; Tlusty *et al*., 2013). We posit that because of their conservation, research and education commitments, public aquaria are well placed to model best practices for the trade. Given that the main criticism of the trade surrounds issues related to the fishery itself, public aquaria should be leaders in developing and supporting fishery best practices that equally value intact habitats, sustainable wild collections, and animal welfare.

27.1.2 Increasing the Sustainability of Animal Collections – the Overlap with the Ornamental Trade

The AZA has developed an *Aquatic Sustainable Collection Plan* and Thoney (2011) enumerated nine "needs" for the *Plan* to become reality (Table 27.1.1). These needs can be summarized as follows: assessing the variety of production methods (i.e., wild caught vs. propagated), for each species, to ensure continued availability of animals in a manner that optimally benefits the natural environments from whence they originate. It is not a simple decision of whether all species should be cultured or not, but rather how culture can impact the overall conservation of the species in question.

Table 27.1.1 The needs of the Association of Zoos and Aquariums (AZA) Aquatic Sustainable Collection Plan (after Thoney, 2011).

1	Need to ensure that wild collection of fishes and invertebrates will remain an option into the future where sustainability is proven.
2	Need to develop a certified list of aquaculture operations, sustainable fish collectors and distributors, and encourage all AZA members to purchase through these sustainable suppliers when appropriate.
3	Need to support *in situ* sustainability efforts (i.e., Project Piaba, Project Seahorse) to support local sustainable fisheries and avoid unsustainable collecting methods/operations.
4	Collaboratively operated extractive reserves should be considered.
5	Need to purchase captive reared fishes and invertebrates when available; however, culture operations should be evaluated to ensure that they are operating sustainably.
6	Need to continue, and expand our ability to breed and rear fishes and invertebrates at our institutions. Staff training in aquaculture techniques and budgetary commitments are important components of institutional support. However, raising fishes will not be the only solution considering the large diversity of animals required for our exhibits.
7	Need to pursue further research into the breeding and rearing of difficult marine tropical fishes with small larvae. More effort needs to be put into forming partnerships with academia that will result in funding and technical assistance that zoos and aquaria cannot provide on their own. There has been inadequate attention devoted to fostering this sort of collaboration, and aquaria might benefit from AZA assistance in facilitating such relationships.
8	Need to develop techniques (i.e., artificial fertilization) that will increase our ability to breed large sharks and sawfishes (an area that also may benefit from AZA support).
9	Need to build large elasmobranch exhibits with the intent of breeding. This initiative will mean a serious re-thinking of how exhibits are themed to achieve success beyond display and public viewing outcomes.

27.1.3 Conservation – *In Situ* Fisheries Improvements and Ecosystem Preservation

A majority of AZA-accredited zoos and aquaria refer to "conservation" and "education" in their mission statements (Patrick *et al.*, 2007). The same is true for accredited institutions in other regions (e.g., EAZA members, etc.). Many public aquaria directly participate in, finance, and support, *in situ* conservation projects.

The overall working hypothesis for many public aquaria is: that numerous aquatic species have specific life history characteristics that make them well-suited to be harvested from the wild; that wild populations will not be threatened as long as there is adequate fisheries management (or an adequate balance between harvest levels and stock sizes); and that capture methods ensure animal welfare and ecosystem health (Tlusty, 2002; Thoney *et al.*, 2003). Ecosystems where these species originate can even benefit from the collection of fishes, since local communities will better care for the environment in order to maintain associated economic activities through time (Tlusty *et al.*, 2013). If these fisheries are closed and this income stream lost, the result will likely be an increase in the biomass removed from the ecosystem, given that the fishers may switch to higher biomass, lower value, food species (Rhyne *et al.*, 2014). In addition to

the increased biomass extraction, the local communities may also switch to more environmentally-destructive practices (e.g., reef removal for building materials, etc.).

The interplay between collection of exhibit animals and the trade in marine ornamental fishes cannot be down-played nor ignored. Field-collection activities by public aquaria typically taps into existing local export resources, available only through the trade (e.g., shipping supplies, paperwork skills, etc.; B. Shepherd, California Academy of Sciences, pers. comm.). Many species on exhibit are purchased through vendors that also supply the home hobby sector of the trade.

Within this context there are some key mechanisms available for public aquaria to advance the sustainability of the ornamental aquatic trade (Tlusty *et al*., 2013). Public aquaria can influence the sustainability of the trade by choosing collectors that meet specific criteria. The AZA proposes an "A-list" of collectors that "…have sustainable conservation plans, follow all governmental and international organization regulations, and can guarantee known sustainable chain of custody of aquatic animals from capture to final destination…" (Thoney, 2010). However, to best and positively influence the continued effort to increase sustainability, we suggest that the AZA and WAZA create an aspirational "preferred collector" category. This category would stipulate mandates that reduce environmental impacts, including, but not limited to, participating in population surveys and fishery management schemes, certified assurance of environmentally benign animal capture techniques, and responsible quarantine chemical use and disposal. Creating aspirational goals will ensure the aquatic trade does not stagnate, but rather, continues to advance animal collection sustainability. In this spirit, the European Union of Aquarium Curators (EUAC) recently formulated *Guidelines for Acquisition* to assist public aquarium personnel when making informed and responsible choices about sourcing display specimens.

Another way to advance the sustainability of the ornamental trade is the creation of extractive reserves (Firchau *et al*., 2013), whereby large tracts of marine and freshwater habitats are managed and effectively protected for the purpose of regulated specimen collection by stakeholders. These reserves can build on the positive social and environmental aspects of the trade in ornamental fishes, and represent a major step toward ecosystem sustainability because of the combined and balanced benefits to the environment, and to local economies and communities. Extractive reserves should result in a well-managed and balanced socio-ecological system that accentuates the benefits of an intact biodiverse ecosystem.

Collaborative work with commercial collectors *in situ* can also have an important impact on sustainable wild extraction by helping organize fishermen and ensuring jobs for local communities. Although a freshwater example, and not a sole extractive reserve, Project Piaba had been working since 1991 on the ornamental fishery in the Rio Negro, Amazonas, Brazil (Chao *et al*., 2000). The main fishes extracted from this region are the cardinal tetra (*Paracheirodon axelrodi*) and the discus (*Symphysodon discus*). Despite the enormous amount of fishes harvested annually (i.e., >20 million individuals) there are no demonstrated negative impacts on wild populations. The harvest methods are benign, with selective harvest, and no by-catch mortality. The communities of this region depend on these fisheries for their economic livelihood and understand that an intact ecosystem will provide for a resilient fishery. Project Piaba works with collectors to improve their animal capture, handling and transport methods, and also works with commercial wholesalers and pet suppliers encouraging use of animals that have been sustainably extracted. Project Piaba

highlights an example fishery where maintenance of wild capture is more beneficial to the environment than wholesale replacement by *ex situ* aquaculture.

Marine ornamental fisheries do not yet have a true Piaba-like model, yet it is proposed that this model can be just as relevant as it is for their freshwater analogs. One promising initiative, described elsewhere in this volume (see Chapter 18), is a collaborative effort initiated by Olazul (www.olazul.org) and the New England Aquarium. In this case, post-settlement reef fishes are collected in Bali and grown out in modified aquaculture cages. This project has the benefit of engaging local fishermen, as well as harvesting fishes prior to the high mortality threshold associated with larval settlement. In addition, the fishes are preconditioned to handling and to dry foods so, once grown, they can enter the commercial supply chain, experience lower stress and ultimately lower mortality.

To advance sustainability of collection species, public aquaria must work to ensure adequate resource management, ecosystem preservation and community benefit. Activities can include the hiring and training of local collectors, conducting fisheries surveys in support of local management plans, and the education of local peoples. Where field-collections are not conducted directly, public aquaria must ensure commercial collectors adhere to rigorous resource sustainability protocols and animal handling practices. Public aquaria can also inform their visitors of the benefits of sustainable extraction activities and promote sustainable choices by consumers.

27.1.4 Culture – *Ex Situ* Conservation and Improvements to Husbandry and Breeding

Overall, collection of wild fishes should be supported for their larger societal and ecosystem benefit. However, for those species whose habitats are threatened, *in situ* conservation efforts should be complemented with *ex situ* projects like breeding programs in zoos and aquaria (IUCN, 2002).

Because the majority of marine species have never been assessed, to determine their conservation status in the wild, and taking into account the general trend in marine habitat degradation, it is anticipated that many marine species will be classified as threatened in the near future (Thoney *et al*., 2003). The implication for public aquaria is that much of global aquatic biodiversity is threatened (Helfman, 2007) and that the collection of wild specimens will not be able to continue as currently practiced (Conway, 2010), therefore highlighting the importance of breeding programs, even for species that are not currently assessed as threatened (Conde *et al*., 2011).

One benefit of the natural exhibits within zoos and aquaria is that many species do reproduce while on display, providing these institutions with an opportunity to lead breeding programs and participate in head-start partnerships. WAZA has mandated the development of sustainable collections of aquatic animals and plants through in-house breeding programs (Penning *et al*., 2009). The AZA is similarly interested in the captive rearing of aquatic species and in 2010 awarded the prestigious Edward Bean Award to two institutions (i.e., the Cabrillo Marine Aquarium and the New England Aquarium) for the captive propagation of marine fishes. In 2010 the AZA, through the Conservation Endowment Fund, supported the "Rising Tide" initiative, to help construct a central rearing facility and establish a supply chain of eggs and larvae from public aquaria. The intended goal of this initiative was to improve the commercial

production of select marine fish species for the home hobbyist. While the rearing of fishes in one central location can certainly assist in the sustainability of collections for public aquaria, a significant effort needs to be made to materially impact the enormous diversity of species currently maintained on exhibit. In 2010 approximately 1375 marine fish species were held in North American public zoos and aquaria (AZA, 2010), yet only about 2% of these species have ever been reared in captivity. Marine fishes hatch in an undeveloped state, have notoriously small larvae, and require small and specific live foods for first feeding. While is it unlikely there will ever be a point in time in which *all* species on exhibit can be raised in captivity – in part as a result of the substantial space and resources required for captive breeding populations – husbandry staff at aquatic institutions need to develop the knowledge, skills and abilities to rear any species breeding at their respective institutions. The MoLaRS (Modular Larval Rearing System) project, funded by the Institute of Museum and Library Services (IMLS), and coordinated by the New England Aquarium and Roger Williams University, was developed to address this need. MoLaRS created common protocols and physical systems for rearing marine fish larvae that are readily scalable and shared with a large number of institutions. To date, aquarists from 20 different facilities have been trained to rear larval fishes, eggs have been collected from nearly 60 species, and approximately 3500 individual fishes have been returned to exhibit, the details of which have been summarized in Table 27.1.2. This outcome is a significant step toward the goal of sustainable collections.

Some target species in zoos and aquaria are managed through studbooks, as well as breeding programs like the European Endangered Species Programme (EEP) and the North American Species Survival Plan (SSP). The goal of these management plans is to maintain sustainable, captive populations over time, which "…must be demographically robust, genetically representative of wild counterparts and able to sustain these characteristics for the foreseeable future…" (www.waza.org). Under the auspices of EAZA, the first two European studbooks for fish species were created in 2007, the Zebra Shark European Studbook and the Blue-Spotted Stingray European Studbook. By 2013, the number of studbooks managed by the EAZA's Fish and Invertebrate Taxon Advisory Group (FAITAG) had increased to 10. These studbooks have resulted in positive outcomes for many species. Before the creation of the Blue Spotted Stingray Studbook breeding events were rare, but now several institutions breed this species, the number of captive births has increased significantly, and captive bred animals are being sent to several aquaria throughout Europe. Increasing the number of studbook-managed species will promote the dissemination of successful husbandry and breeding information; reducing specimen mortality rates, increasing captive breeding and reducing pressure on wild stocks.

A number of invertebrate species have also been reared. In recent years important advances in coral husbandry and propagation has allowed many public aquaria to maintain large coral reef exhibits. Species that were difficult to maintain in the past, are now flourishing and are even propagated by fragmentation (Thoney *et al*., 2003). As the exchange of coral fragments between institutions has increased, many public aquaria have started exhibiting corals without the need to collect from wild stocks. In 2004, 24 species of corals were identified as displaying sexual reproduction in aquaria and 16 of those had established an F1 generation (Petersen *et al*., 2007). A survey conducted three years later identified 45 scleractinian species reproducing sexually in aquaria, with successful recruitment for 29 of those species. (Petersen, 2008). Despite this demonstrable

Table 27.1.2 Species collected from on exhibit spawning, reared, and returned to exhibit as part of the MoLaRS (Modular Larval Rearing System) project (as of March, 2015, see http://www.larvalcultureproject.org).

Scientific name	Common name	*Scientific name*	Common name
Acanthochromis polyacanthus	Spiny chromis	*Haemulon* spp.	Grunts (unknown)
Amblyglyphidodon ternatensis	Ternate chromis	*Heterostichus rostratus*	Giant kelpfish
Amphiprion clarkii	Clark's anemonefish	*Hippocampus bargibanti*	Pygmy seahorse
Amphiprion frenatus	Tomato clownfish	*Hippocampus erectus*	Lined seahorse
Amphiprion ocellaris	Ocellaris clownfish	*Hippocampus reidi*	Long snout seahorse
Amphiprion percula	Orange clownfish	*Kuhlia mugil*	Barred flagtail
Amphiprion sebae	Sebae clownfish	*Lysmata amboinensis*	Pacific cleaner shrimp
Apogon dovii	Tailspot cardinalfish	*Lythrypnus dalli*	Lythrypnus dalli
Astraea tecta	Astraea snail	*Melanotaenia boesemani*	Boeseman rainbow
Atherinopsis californiensis	Jacksmelt	*Neoglyphidodon melas*	Bowtie damsel
Diodon holocanthus	Balloon fish	*Pomacanthus* sp.	Marine angelfish
Doryrhamphus dactyliophorus	Banded pipefish	*Pygocentrus nattereri*	Red belly piranha
Dunckerocampus pessuliferus	Yellow multibanded pipefish	*Stephanolepis hispidus*	Planehead filefish
Elacatinus oceanops	Neon goby	*Syngnathus floridae*	Dusky pipefish
Eumicrotremus orbis	Pacific spiny lumpsucker	*Syngnathus leptorhynchus*	Bay pipefish
Haemulon chrysargyreum	Smallmouth grunt	*Syngnathus scovelli*	Gulf pipefish
Haemulon flavolineatum	French grunt	*Trachinops caudimaculatus*	Southern hula fish
Haemulon sciurus	Blue-striped grunt	*Trachinotus falcatus*	Permit

success, more work is yet to be done to develop breeding techniques for the many other species of corals that are not yet propagated in *ex situ* conditions (Petersen, 2008).

An interesting collaborative project in the realm of coral propagation is CORALZOO, a SME-friendly (micro, small and medium-sized enterprises-friendly) European Breeding Program for hard corals. The Sixth Framework Project Program for Science and Technology of the European Union financed this project between 2005 and 2009. Three Universities, one research institution, 10 zoos and aquaria, a coral culture company, and the European Association of Zoos and Aquaria (EAZA) participated in this project, consisting of several work packages, including: (1) bioassay development; (2) fragmentation; (3) sexual reproduction; (4) abiotic factors; (5) nutrition; (6) health control; (7) transport and acclimation; (8) morphogenetic modeling; (9) protocol composition and dissemination; and (10) training (Osinga, 2008). One of the most important deliverables from this

project was the *Book of Protocols for the Breeding and Husbandry of Scleractinian Corals*, which includes 70 protocols on various topics related to hard coral husbandry and breeding (Osinga *et al*., 2012). The CORALZOO *Book of Protocols* is available on-line through a dedicated website (www.coralzoo.org) and demonstrates that zoos and aquaria can add great value to scientific research efforts (Osinga *et al*., 2012).

Another initiative, SECORE (SExual COral REproduction), was started in 2001 coordinated by the Rotterdam Zoo and the University of Duisburg-Essen, with the participation of several public aquaria and research institutions. The primary goal of SECORE is the sustainable management of captive coral populations through sexual reproduction and the sharing propagated corals between institutions (Petersen *et al*., 2006). In 2005 the first SECORE workshop was held in Rotterdam, where participants were taught techniques for coral larvae collection and settlement. This workshop represented the first step for a number of aquaria in Europe to start collecting and settling the larvae of several different species of coral. The Oceanopolis, in Brest, collected planulae from nine different species of corals on display in their aquaria: *Favia fragum, Pocillopora damicornis, P. verrucosa, Stylophora pistillata, Seriatopora hystrix, Porites* sp., *Euphyllia paradivisa, Stylocoeniella guentheri* and *Pavona cactus* (Barthelemy, 2008).

A follow-up SECORE workshop was held in Puerto-Rico, where participants from European and North American institutions had the opportunity to collect gametes and larvae during mass spawning events, and to improve techniques for coral fertilization and settlement. Primary coral polyps resulting from the workshop were distributed to several public aquaria to aid in the development of elkhorn coral husbandry techniques. In more recent years, SECORE workshops have been held in Guam and the Philippines in support of *ex situ* culturing programs that ultimately have (or in the future will have) transitioned to *in situ* restoration projects. In this way, SECORE has grown to be a globally important coral conservation initiative involving both conservation scientists and public aquarium professionals. The combined knowledge and experience of dozens aquarium professionals was essential for the success of this important initiative (www.secore.org).

In addition to breeding animals, public aquaria have a wealth of technical expertise related to the husbandry and care of aquatic life. Species that were difficult or even impossible to maintain in aquaria years ago, can now be maintained with relative ease through advances in animal husbandry. Research in the field of animal husbandry and captive breeding, as well as other associated investigations, is frequently conducted within public aquaria, the results of which are disseminated at professional meetings and via scientific journals and the grey literature (Hutchins & Thompson, 2008). In recent years, dedicated professional symposiums have focused on a particular aspect of aquarium science. Key meetings have included: the *1st International Elasmobranch Husbandry Symposium* held in Orlando in 2001, *Aquality: Water Quality and Water Treatment in Zoos and Aquaria* held in Lisbon in 2004, the *1st International Symposium of Coral Husbandry in Public Aquaria* held in Arnhem in 2007, and the *Husbandry, Management and Conservation of Syngnathids* held in Chicago in 2011. With support from public aquaria, commercial industry peers and other philanthropic organizations, proceedings from some of these meetings have already been published as dedicated husbandry manuals – the *Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays, and Their Relatives* (Smith *et al*., 2004) and *Advances in Coral Husbandry in Public Aquaria* (Leewis & Janse, 2008). These industry handbooks are available to buy

in hardcopy form or to download for free as PDF documents (refer to www.elasmobranchhusbandry.org and www.burgerszoo.com/about-burgers-zoo/coralhusbandryorg/). Follow-up elasmobranch husbandry and water quality meetings were held in 2013 and 2014, respectively, and proceedings from these meetings are currently in review. These manuals not only act as a bridge between public aquarium professionals and dedicated research scientists, they represent an invaluable source of information, compiling the latest advances in husbandry, care and breeding of several marine species.

27.1.5 Creating a Comprehensive Collections Plan

The culture of marine ornamental fishes and invertebrates by public aquaria is of growing importance as ecosystems become increasingly impacted by human activities. As we have reiterated throughout this chapter, however, the role of aquaculture and captive culture needs to be balanced against the benefits of, and the risks to, wild fisheries (Tlusty, 2002). Any comprehensive collection plan needs to draw on animals from many sources – i.e., originating from well-managed wild fisheries, from responsible collectors and aquaculturists, from the growout of species breeding on exhibit, and through trading of surplus animals with sister institutions (Figure 27.1.1). Balanced sourcing, however, is only one of

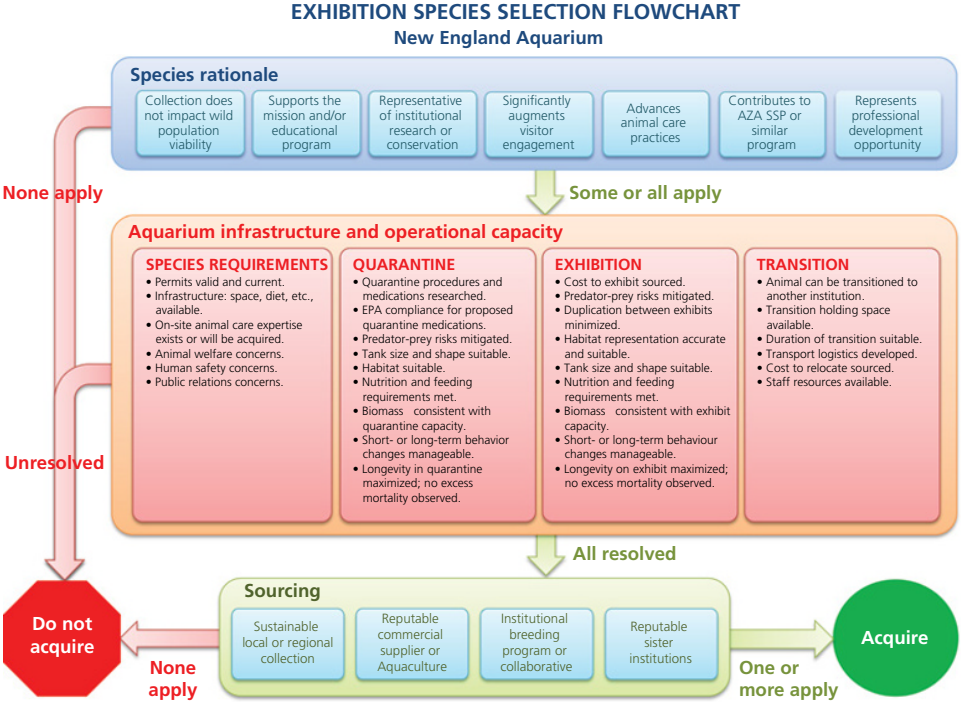


Figure 27.1.1 An exhibition species selection flowchart as developed by the New England Aquarium. The purpose of this flowchart is to provide a broad overview of the collections plan, and to ensure exhibit animals are selected to meet the institutional mission, can be cared for appropriately, and are sourced in a sustainable manner, including the use of responsible wild fisheries and/or *ex situ* cultivation of species. (*See insert for color representation of the figure.*)

three core considerations within a comprehensive animal collection plan, the others being the rationale for species choice, and the available infrastructure and operational capacity. The first consideration for a collection plan is to determine whether a species should be on exhibit at all. Criteria for this choice have been summarized in Figure 27.1.1. If some or all of the species rationale apply, an assessment then needs to be made of the infrastructure and operational capacity of the aquarium in support of the chosen species (Figure 27.1.1). This assessment includes animal husbandry and welfare considerations, as well as long-term deaccession plans. All three of these core choice categories are important components of a broader collections plan. If any is found to be deficient, then the responsible choice may well be to not acquire the species for exhibition.

27.1.6 Conclusions

Modern public aquaria have large exhibits that allow for the representation of ecosystems, and the maintenance of species, that typically cannot be managed in a smaller home aquarium. Many of these species are not easy to study in their natural habitat, so public aquaria present a great opportunity for scientists to study aspects of their biology, physiology and behaviour. Many researchers contact public aquaria in order to complement their field research with information drawn from animals in human care. Information gathered from public aquaria can provide important support information for *in-situ* conservation work on threatened species. The increasing number of species maintained by public aquaria provides an increased number of opportunities for fruitful research collaborations and contributions to a wide array of disciplines including, but not limited to, conservation biology, wildlife medicine, animal physiology, genetics, ethology and taxonomy.

In order to engage the public and instill a sense of care about marine and freshwater ecosystems, public aquaria need to display a wide variety of aquatic species. The ongoing viability of wild collection is critical and public aquaria must model behaviour by developing sustainable criteria for sourcing animals. Natural aquatic theming and high-quality water frequently induce species to produce eggs or young on exhibit. For many species this opportunity can result in institutional or multi-institution breeding programs. For other species, reproduction can represent a unique opportunity to learn about the life history of an organism for the first time. It is clear, however, that the large number of species exhibited in public aquaria cannot *all* be bred in captivity. It is for this reason that public aquaria need to collect animals from well-managed wild fisheries and establish best practices to ensure that the needs of the animals, the ecosystems, and local communities are satisfied. It is only through this balance of *ex situ* culture and *in situ* conservation that public aquaria will be able to reach their sustainability goals and realize their conservation missions. In so doing, public aquaria will positively impact the ornamental fish trade.

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27.2

Interaction Between Public and Private Aquaria

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Abstract

The challenges of marine species sustainability faced by the hobby and public aquarium communities can only be overcome by an approach that includes them both. The aquaculture program of Rising Tide Conservation is a model of such an integrated approach. Rising Tide is an integration of key stakeholders from academic institutions and both the hobby and aquarium sectors. The program addresses the needs of research, technology transfer, and market forces to advance marine fish aquaculture. There are four main areas of focus: egg collection from public aquaria, broodstock management and larval rearing research, technology transfer and consumer/visitor education. Given the success of cultured coral species as an alternative to collecting and for reef re-establishment, it is the goal of Rising Tide Conservation to enable a similar cultural shift for marine teleost fish.

Keywords *Pelagic spawning; aquaculture; conservation; sustainability; public aquaria; egg collecting; broodstock management; live feeds*

27.2.1 Introduction

Between 1.5 and 2 million people worldwide are believed to keep marine aquaria. The trade which supplies this hobby with live marine animals is a global multi-million dollar industry, worth an estimated US$ 200–330 million annually, and operating throughout the tropics (Wabnitz *et al*., 2003). The pet industry estimates the value of supplies, feed, and livestock in this industry around US$ 1 billion (Cato & Brown, 2003). Currently, as many as 30 million coral reef fish belonging to 1000 species are collected annually to supply private and public aquaria around the world (Wood, 2001). The proportion of marine organisms in the global hobby industry is increasing. In 1975, marine fish amounted to just 1% of total fish traded. By 1999, saltwater fish were at 10% of the 350 million fish traded (Baquero, 1999).The import value has increased from US$ 24–40 million annually in the 1980s to US$ 200–330 million in 2001 (Chapman *et al*., 1997;

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Baquero, 1999; Larkin & Degner, 2001) with imports to the USA representing 80% of the trade (Larkin & Degner, 2001). Clearly, the hobby industry is of significant size.

Beyond the home aquarium, public aquarium facilities extend throughout North America to provide a glimpse into the ocean world on a larger scale. These facilities vary from zoos to marine research facilities to theme parks and even dining and shopping venues with tanks holding hundreds of thousands if not millions of gallons of water. Be they public or private in financial terms, all face the same challenge as the home aquarist – how can they maintain a sustainable collection of animals while minimally impacting or even conserving the reef environments they present? Reef collecting has a variety of potential and real concerns including overharvesting, shifting reef ecology due to collecting pressures, reef degradation from physical trauma or toxins such as cyanide, with loss of biodiversity resulting from any of the above actions (Wood, 2001).

Approaches towards sustainability of marine ornamental fish include regulated collecting programs and aquaculture. These approaches are a reasonable solution to some of the problems of collecting wild fish and provide some good benefits (Rhyne *et al*., 2014; Fujita *et al*., 2013). Controlled collecting with trace back capabilities like the Marine Stewardship Council (http://www.msc.org/) designed to certify sustainable seafood have been effective. But this model presents challenges in oversight when applied to ornamentals. A similar, but no longer active program for marine ornamentals was the Marine Aquarium Council (MAC) certification program. This program created standards and certifications for those engaged in the collection and care of ornamental marine life from reef to aquarium (Shuman *et al*., 2004). A few commercial suppliers of marine ornamental fish currently track the origin of their stock, but these companies represent a small minority within the trade.

The second option, aquaculture has worked well to reduce collecting pressures for a variety of demersal species and can be developed to expand into pelagic species as well. By overcoming the hurdles of marine aquaculture, we can effectively shift from obtaining fish from reefs to one of obtaining fish from farms. Should efforts for repopulation become necessary, the skill and ability will have been developed to address such challenges. However, concerns about the negative impact of aquaculture on reef-based industries remain (Tlusty, 2002).

27.2.2 Marine Fish in the Hobby Industry

Advances in tank design, lighting, filtration and foam fractionators have expanded the capabilities of aquarists to address the challenges of simulating complex marine systems. Recent advances in coral and live-rock management and propagation, and the rise of nano- and mini- reef tanks have reinvigorated the hobby. At the same time, movies featuring marine fish have fueled the desire for marine species in homes, offices, and restaurants. Changing interests in animal welfare and environmental conservation have shifted the focus of the marine hobby. Where once the goal was to possess the largest tank displaying the most exotic and rare specimens, newer goals focus on establishing reef communities of coral, live rock, invertebrates and reef fishes. The role of the committed hobbyist in ornamental aquaculture has shifted to a more involved one. Social media has changed the hobby to a more communal event even for the fish keepers working in their garages. The active hobbyist now has options like choosing between

wild-caught or captive-reared fish and opportunities to learn and communicate on this topic through a variety of media. The hobbyists themselves are driving interest in captive propagation of reef species.

A study conducted in 2004 revealed that marine hobbyists gave considerable importance to conservation of coral reefs and wild stocks, and showed a particularly high level of involvement in, and knowledge about, their hobby. About 80% reported keeping marine ornamental fish as their primary hobby, 59% were members of an aquarium society, and 88% had researched the specimens they keep (Alencastro *et al.*, 2005).

Commercial production of marine demersal species for the hobby industry has a long history and strong foundation. Efforts began in the 1950s and progressed slowly but in a committed fashion. In 1981, SeaWorld, AquaLife Research, and Instant Ocean Hatcheries paved the way for discussing large scale production of clownfish (*Amphiprion* spp.) at a joint conference held in Dade City, Florida (Hoff, 1996). At that time, all three were developing large-scale production of these fish. The key issues they identified as limiting commercial aquaculture of clownfish were poor juvenile survival, high investment costs, difficulty in realizing a return for investment on aquaculture fish, inadequate water quality, and nutritional concerns. Restricted, rather than open sharing of information further impeded development of new techniques (Hoff, 1996). Interestingly, these challenges have been overcome in clownfish production; now 35 years later, cultured clownfish are abundant. Yet frustratingly, the same hurdles remain as a general rule for overall aquaculture of marine fish.

Since the early 1980s marine hobbyists and producers like Oceans, Reefs & Aquariums (ORA) and Sustainable Aquatics have made production of numerous clownfish species common. Large scale wholesalers are able to import these fish from international aquaculture facilities. These captive-bred specimens have a variety of advantages. Species variety and year-round availability are good. At least seven species are regularly available and another three to five are sporadically offered. Selective breeding has produced a number of popular color morphs that are either uncommon or absent in collected specimens, and fish that are very accustomed to people, aquariums, and commercial feeds. Cultured fish demonstrate reduced aggression in home aquaria. While cost was previously not comparable, increasing costs for collecting and shipping, along with advances in aquaculture techniques, have significantly reduced the difference between collected and reared animals.

While clownfish may serve as the icon of marine ornamental fish aquaculture, they are a small part of a growing trend. Commercial production for the hobby wholesale market now includes clownfish, dotty backs, as well as a few gobies and dragonets. Texts like *The Complete Illustrated Breeders Guide to Marine Aquarium Fishes* by Wittenrich (2007) describe propagation for members of many demersal marine fish families. These include clownfishes of the subfamily Amphiprioninae, damsels (family Pomacentridae), dotty backs (family Pseudochromidae), fairy basslets (family Grammatidae), gobies (family Gobidae), assessors and comets (family Plesiopidae), jawfish (family Opistognathidae), cardinal fishes (family Apogonidae), sea horses (family Sygnathidae) and blennies (family Blennidae). The techniques described focus on small scale production but serve as the basis for commercial advances. Beyond the techniques for demersal species, the text foreshadows the advances coming in pelagic spawning species with discussions of small successes with dragonets and angel fish. A study in the Philippines has demonstrated that a hatchery for clownfish production can be a sustainable

business (Pomeroy & Balboa, 2004). Growth in production facilities in Indonesia and the Philippines can shift the focus without detracting from economies.

Communication between hobbyists and hobby professionals has grown as well, taking advantage of expanded opportunities to share interests and achievements. Internet forums discuss breeding efforts and achievements and track first rearing successes. These include the Marine Ornamental Fish & Invertebrate Breeders, MOFIB (http://www.marinebreeder.org/forums/) and the Marine Breeding Initiative, MBI (http://www.mbisite.org/). The Rising Tide program has a regular blog to update individuals on developments real time (http://risingtideconservation.org/). These sites present a forum for sharing information on breeding, spawning, and larval rearing nuances. Points are awarded to participants for successes in breeding relative to the difficulty of various species. In addition to online discussions, the MBI hosts an annual meeting to bring together those with a passion for marine aquaculture with researchers and producers. A larger, related conference is the Marine Aquarium Conference of North America (MACNA). This national event is hosted each year by one of the many local marine aquarium societies and features advances on a variety of reef topics in addition to the regular focus on the propagation of marine fish, coral and other invertebrates.

27.2.3 Marine Fish in Public Aquaria

Public aquariums can take an interest in the ocean and produce wonder and awe for visitors of all ages. There are over 50 aquaria within the United States currently recognized by the Association of Zoos and Aquariums (AZA). These facilities in aggregate with zoos experience more than 143 million visitors annually (Falk *et al.*, 2007). There are approximately 50 additional large venues in the United States with public display aquariums that are not members of this professional association. These facilities range from research institutes to private enterprises where profit from dining and shopping is a primary goal. Regardless of the foundation, all of these establishments face the same challenges of sustainability woven into the marine fish hobby.

The maintenance of wild animal populations for education and wildlife conservation purposes is a unique responsibility of AZA member zoos and aquariums. To accomplish these goals, it may be necessary to acquire wild-caught specimens. When acquiring animals from the wild, careful consideration must be taken to evaluate the long-term impacts on wild populations and ecosystems. Any capture of free-ranging animals should be done in accordance with all local, state, federal, and international wildlife laws and regulations and not be detrimental to the long-term viability of the species. Current acquisition best practices for zoo and aquarium marine fish include aquaculture, highly controlled and regulated collecting trips, purchase and trade from other facilities, and purchase from the wholesale market. This is the same wholesale market that provides fish for the hobbyist industry. This wholesale market is the common link between public aquaria and the home hobbyist. Because of this link, this is the area where efforts can impact both the hobbyist and the display communities.

While there are links, there are also walls. The AZA provides professional support and direction for member facilities. Specific limitations restrict transfer of fishes from AZA facilities into the pet trade. Fishes that grow too large or require extraordinary life support (such as chillers), those that are deemed invasive, those capable of inflicting a

serious bite or envenomation, and those with wildlife conservation concerns are all restricted from exchange. These guidelines leave ample room for exchange of many species but create a series of barriers limiting exchange of specimens. But these restrictions focus on the aquarium facilities themselves. By focusing on propagation in research institutes with technology transfer to producers and ultimately wholesalers, the challenges of animal movements placed on display aquaria no longer apply.

Zoos and aquariums have developed limited internal aquaculture programs. For example, Birch Aquarium at Scripps in San Diego, California has had a seahorse propagation program since 1994. The program has raised 12 species of seahorses and shipped more than 3000 specimens. Similarly, the Shedd Aquarium in Chicago is a major player in the program Project Seahorse. Many display aquaria have set the standards for care and propagation of this family of fish (Bull & Mitchell, 2002). On the grand scale, these highly successful programs are very small when compared with the size of global demand. The majority of seahorse aquaculture involves small-scale operations in developed countries, employing relatively few personnel and selling live animals for the home aquarium market (Koldewey & Martin-Smith, 2010).

Additional challenges facing public aquariums include limited funding, space (for holding broodstock, larval rearing tanks and feeding systems) and specialized aquarists with a focus on production. In order to have a successful and sustainable program with a large-scale impact, zoos and aquariums need an umbrella program with a focus outside of their facilities. Beyond in-house efforts, AZA facilities support aquaculture production of ornamental species when species are difficult to obtain from the wild, when breeding supports a conservation program, when there is environmental benefit or reduced environmental damage, or to enhance the further production of domesticated species (Tlusty, 2002). Syngnathid production has shown that committed biologists at zoos and aquariums have an important role in advancing ornamental aquaculture. By channeling their efforts into larger research facilities, we can use their husbandry and management base while overcoming limitations.

27.2.4 Opportunities Through Collaboration

A non-traditional pathway was devised to address the challenges of space, funding, and trained personnel for developing ornamental aquaculture on a commercial scale. That pathway is Rising Tide Conservation, a constructive affiliation of stakeholders committed to developing techniques in aquaculture, transferring those techniques into commercial production, and fostering a market for the end product. The stakeholders in Rising Tide Conservation consist of engaged marine hobbyists, ornamental fish producers, wholesalers, retailers, public aquariums throughout North America and reef-focused non-governmental organizations. These stakeholders drive the program which benefits all of them. Rising Tide Conservation can be divided into three interconnected elements: production research, industry implementation and consumer and visitor education (Figure 27.2.1).

The collaborative nature of the program is critical to its success. The retail, wholesale, and zoo consumers of the fish determine where efforts for research will focus. As advances are made, scientists communicate these to the stakeholders. Producers work with the scientists to develop sustainable and profitable new species for aquaculture.

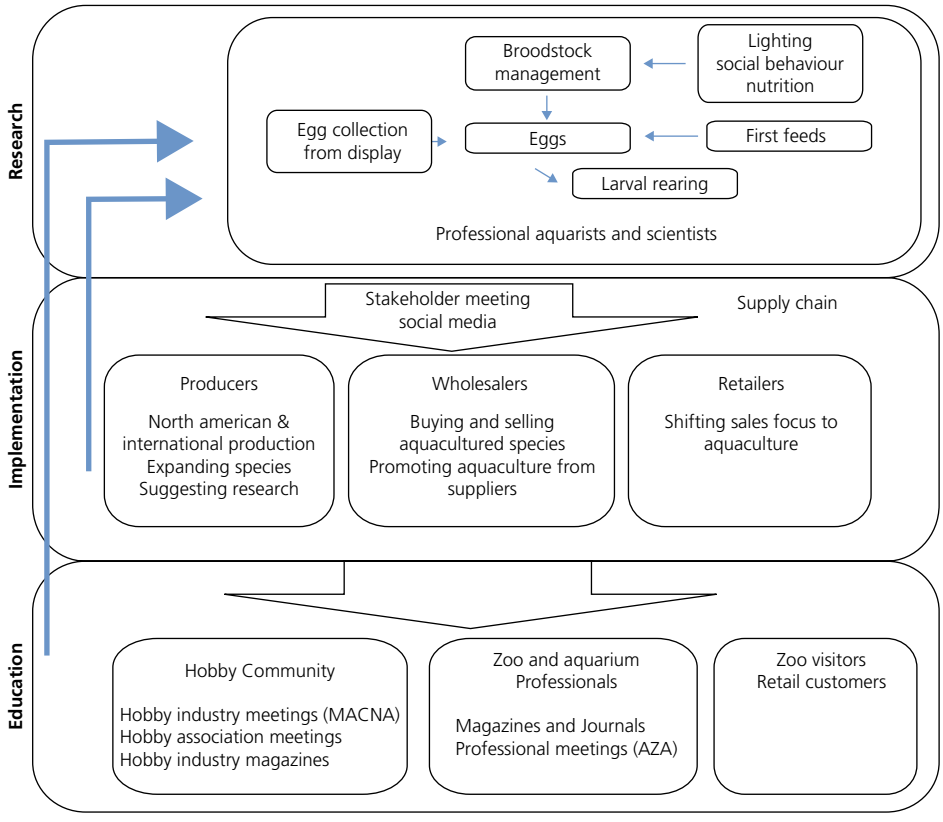


Figure 27.2.1 Schematic representation of the main interconnected elements of Rising Tide Conservation. The three major elements: research, implementation and education are all integrated to drive useful aquaculture technology.

At the same time, wholesalers and retailers communicate the market and consumer demands and close the loop by marketing the newly produced fish. In 2015, fish raised for the zoo and aquarium market were pork fish (*Anisotremus virginicus)*, striped grunts (*Haemulon striatum*), and golden trevally (*Gnathanodon speciosus*). Those raised for the consumer market included Banggai cardinalfish (*Pterapogon kauderni*), and purple masked angelfish (*Paracentropyge venusta*). Semicircle angelfish (*Pomacanthus semicirculatus*) were produced for both the hobby and public aquarium markets. Throughout the process, efforts are made through publications in hobby and zoo magazine and presentations to educate both communities about the program.

Funding for the program is diverse but generally starts with contributions from the stakeholders. Rising Tide Conservation is a program of the 501c3 non-profit, the SeaWorld-Busch Gardens Conservation Fund. As such, tax deductible donations can be done to the program. Funders thus far have varied from zoos and aquariums, to major pet retailers, fish wholesalers, hobby industry professionals, and both individual and hobbyist associations. When grant opportunities are available to support the scientists in the program, they have come from areas such as tourism and Sea Grant programs. As the program expands, opportunities for development in areas like the Coral Triangle

may open up funding from non-government organizations (NGOs), governments, and international development programs.

The integrated and collaborative nature of the Rising Tide Conservation program is based on the models initiated in efforts like agricultural extension (Leeuwis & Van den Ban, 2004) and adopted by groups like the World Wildlife Fund (Hughes & Flintan, 2001). In these programs, users direct research and researchers openly communicate results to afford large-scale adoption. Adoption is based on economic viability and sustainability. When this process is applied to development, a characteristic of the conservation and developments projects is that they seek to address biodiversity conservation objectives through the use of socio-economic investment tools. They were first introduced in the mid-1980s by the World Wide Fund for Nature (WWF) in an attempt to address some of the shortcomings of, and problems associated with, "fines and fences" approaches to conservation in protected areas (Hughes & Flintan, 2001).

27.2.5 Production Research

Production research starts with eggs. These come either from collection in public aquaria or production by broodstock. Broodstock are maintained at the research facilities where scientific investigations evaluate optimal nutrition, lighting, tank size and shape, water parameters (such as temperature) and fish variables (such as social factors). The goals of the research are to maximize egg quantity and quality. Species held specifically to develop best practices in broodstock management are listed in Table 27.2.1. These species represent primarily pelagic spawners, but a few demersal species are included to investigate efforts aiming to significantly enhance production.

Eggs are collected from exhibits at public aquariums across the United States. Facilities collecting eggs include SeaWorld of Orlando, SeaWorld of San Antonio, Discovery Cove, The Florida Aquarium, the Columbus Zoo and Aquarium, Omaha's

Table 27.2.1 Species maintained as broodstock for Rising Tide Conservation aquaculture investigations as of 2014.

Common name	Scientific name
Yellow tang	*Zebrasoma flavescens*
Pacific blue tang	*Paracanthurus hepatus*
Golden trevally	*Gnathanodon speciosus*
Bartlett's anthias	*Pseudanthias bartelettorum*
Bannerfish	*Heniochus acuminate* and *H. diphreutes*
Milletseed butterflyfish	*Chaetodon miliaris*
Bandit angelfish	*Apolemichthys arcuatus*
Purple masked angelfish	*Paracentropyge venusta*
Emperor angelfish	*Pomacanthus imperator*
Semicircle angelfish	*Pomacanthus semicirculatus*
Green chromis	*Chromis viridis*
Blue chromis	*Chromis cyanea*

Henry Doorly Zoo, the Steinhart Aquarium, Virginia Aquarium & Marine Science Center, the Birch Aquarium, the J.G. Shedd Aquarium, and the Georgia Aquarium. To collect the eggs, water flows through a collector and the small mesh net of the basket traps the eggs. Since most marine fish spawn at dusk, collectors are deployed in the evening and gathered in the morning. Eggs and debris are cleaned via a series of filtrations and settlings, placed into the bags without an air space to reduce agitation during transit and then shipped overnight to the rearing research center either in Ruskin or Vero Beach, Florida. Alternatively, collections have been performed in very large exhibits using hand nets and scuba gear. This method has been useful in attempts to collect eggs from a single species in a large, multi-species exhibit. Collected eggs are then processed and shipped as described above.

As of this writing, the research team has reared over 10,000 fish of 15 different species from egg to juveniles. These include: yellow tang (*Zebrasoma flavescens*), melanurus wrasse (*Halichoeres melanurus*), sea bream (*Archosargus rhomboidalis*), French grunt (*Haemulon flavolineatum*), smallmouth grunt (*Haemulon chrysargyreum*), Porkfish (*Anisotremus virginicus*), bluestripe grunt (*Haemulon sciurus*), Moonfish (*Monodactylus argentus*), schooling bannerfish (*Heniochus diphreutes*), semicircle angelfish (*Pomacanthus semicirculatus*), purple masked angelfish (*Paracentropyge venusta*), orbiculate batfish (*Platax orbicularis*), spadefish (*Chaetodipterus faber*), green chromis (*Chromis viridis*) and ternate damselfish (*Amblyglyphidodon ternatensis*). The program continues to grow and eggs are now collected at 20 display aquariums.

Determining what to feed the larvae is the next challenge, which is amplified by the size diversity of marine fish larvae. Because eggs from public aquariums typically come from multispecies reef tanks, shipments commonly contain eggs from many species. Upon receipt at the laboratory, eggs are sieved at 750–800 μm to separate smaller eggs of species such as tangs and anthias from larger eggs typical of grunts and angelfish. Wild zooplankton (including copepods, ciliates, tunicate larvae, polychaetes, tintinnids, and dinoflagelletes) is utilized as the first feed for these mixed species egg trials. Providing this zooplankton "soup" allows the individual larvae to select feeds that are appropriately sized for them but limits the researchers' ability to identify which species is eating what.

Another challenge within this "shotgun" approach to larval rearing is the identification of eggs and larvae. Fish identifications are based on larval characteristics, fish morphology after metamorphosis, and ultimately, molecular techniques (Cassiano *et al.*, 2014). Molecular techniques now allow positive identifications to be achieved from a single egg. A marine fish egg and larvae catalogue is currently under development and will be extremely helpful in the examination of new species. Once initial propagation techniques are developed, efforts are made to separate eggs or to establish broodstock in single species holding to facilitate growout of individual species.

First feed selection is more targeted for eggs and larvae from broodstocks maintained in single species holding tanks. Trials with diverse collected zooplankton are generally the first step, followed by selective feeding to better define food source components and optimal sizes. Determination of first-feed items can be guided by results from the larger aquaculture sector of marine food fish production (Ostrowski & Laidley, 2001). An example of this iterative process has been the rearing of the purple masked angelfish (*Paracentropyge venusta*). Initial efforts found success feeding larvae zooplankton from Hawaiian coastal areas. Subsequently, the species has been reared using 100% cultured feeds (K. Brittain, pers. comm).

27.2.6 Technology Transfer and Communication

Successes in production are communicated through regular blog posts, scientific publications, magazine articles, and presentations at industry meetings. A regular stakeholder meeting facilitates information exchange from research to producers, wholesalers and retailers and from them back to researchers. These events communicate results and direct new efforts. Topics addressed at these meetings include options for technology transfer and addressing specific questions generated from aquaculture partners. The stakeholder positions have created an atmosphere of interest and a desire for technology adoption. Researchers work closely with producers to create a seamless transfer of information and assistance. Not only are producers interested in exploring how to raise fish, but wholesalers are going back to their suppliers and promoting opportunities for aquaculture. Through this mechanism, aquaculture in areas like Indonesia and the Philippines can be promoted to both create jobs and provide alternative to reef collecting. An unexpected but critical result of these meetings has been a commitment by a major industry retailer to promote, purchase and sell aquacultured marine fish. This stance is changing attitudes for producers about future marketing possibilities. Now, the concern of cost of production over costs of collecting is becoming less significant. This shift is promoting producer investment.

Zoos and aquariums are educating visitors not only to this program but to the issues of reef conservation and diverse solutions. Through signage, tours, and popular websites, these facilities are showing that an integrated approach is the best way to face complex issues like reef protection and marine fish sustainability. Retail stores for many years have been quite about the origin of fish for sale. Now, as more cultured species are becoming available, customers are not only being given choices, they are being empowered through information.

Beyond communication, the program is also developing new specialists in ornamental aquaculture through graduate student programs. Students are encouraged to participate in internships and graduate programs that focus on propagation issues of single species or enhance knowledge relative to broodstock management or first feeds production.

27.2.7 Opportunities and Challenges for Conservation

Beyond reducing pressure on reef habitats, the Rising Tide approach offers opportunities to restock and repopulate reef habitats. The success of coral propagation to supply hobbyists and public aquariums, as well as replanting sea beds, is encouraging. Efforts in coral production involve a similar collaboration of the hobby industry, public aquariums and governmental agencies internationally. This interplay has resulted in development of large-scale coral farms as well as diverse techniques for both coral production and reef seeding (Garrison & Ward, 2008, 2012; Marharver *et al*., 2013). Commercial production is developing in countries hosting the species as native organisms. This diverse commitment and shared knowledge has made coral culture a success.

Thus far, no programs of ornamental marine fish propagation have focused on release for repopulation of wild stocks. Perhaps this is due to the resiliency of fin fish stocks. Perhaps it is due to the fact that propagation is still in the developing stages. Regardless,

these populations face the same common threats of coral reef communities. In situations where species abundance or distribution is limited, the need for propagation for release (stock enhancement and restocking) programs must be considered as an eventual likelihood. Establishing these programs in a responsible and effective manner can be developed for efforts for marine stock enhancement, restocking and sea ranching technologies. These efforts are currently growing in significance in the realm of food fish.

27.2.8 Conclusions

The slow pace of shifting to marine ornamental aquaculture reflects the challenges of this endeavor. However, new thinking as is seen in programs like Rising Tide Conservation shows great promise. There is increasing interest in sustainable populations for both home and public aquariums. These efforts in sustainability will likely include better managed specimen collection, as well as expanding aquaculture production. Efforts in aquaculture are seeing benefits of fundamental advances like understandings in larval feeding and broodstock nutrition. Facilities producing demersal species now have the ability to address global demands for many species. Changing abilities, as well as changing attitudes, are coming together to make the change needed a reality. In the future, political policies may restrict collection and movement of marine ornamentals. We must have aquaculture alternatives in place prior to such restrictions or the sustainability of keeping these species will erode in the face of new regulations.

Future efforts will continue to expand species diversity for marine ornamental aquaculture. Facility promotion in the regions of the Coral Triangle will integrate the native areas for these species into aquaculture thus maintaining their connections to the reefs. By promoting alternatives to collecting from reefs, these fish can be appreciated for their economic value, as well as their natural beauty.

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28

How Nano Tanks can Foster the Demand for Bred and Cultured Marine Ornamentals

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Abstract

Breeding and culture of marine ornamentals is often cited by hobbyists as the solution to sustainability issues related to marine aquarium fisheries, trade and hobby. However, when comparing capacity and availability of bred and cultured specimens in the trade with the variety and volume offered from wild resources, there is still a huge gap. Moreover traders report price competition between the sources, which makes bred and cultured specimens often only a second choice to hobbyists. This chapter explores how the trend toward keeping nano tanks can support the marketability of and demand for bred and cultured marine organisms and as such contribute to the sustainability of the marine aquarium trade.

Keywords *Conservation; fishery management; global trade; regulation; mini reef; source country; sustainability; breeding; culture*

28.1 Introduction

Coral reefs, the major source for the marine aquarium trade and hobby, face a plethora of human caused threats, among them rising ocean temperatures and acidity, pollution and sedimentation from coastal development, and overfishing (Burke *et al*., 2012). Exploitation of reefs through aquarium fisheries is often still perceived by the majority of society as a luxury hobby, not as meaningful and substantial as exploitation for food. The industry and hobby face campaigns over the unsustainable and unethical practices in use, and in some cases proposals for banning the aquarium fishery and trade are on the table, like in Hawaii. As Schmidt (2013a) summarizes, aquarium collection has been a controversial issue in Hawaii since 1973, followed in 1998 by a petition for a total ban on aquarium fishing in West Hawaii. The West Hawaii Fisheries Council was set up that year to facilitate negotiations between user groups and develop solutions. As a result of this work with involvement of fishery managers, the Hawaii legislature passed an act calling for the development of the West Hawaii Regional Fishery Management Area Plan. The plan designated 30% of the coastline as Fish Replenishment Areas where

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aquarium collection is prohibited. In December 2013 the West Hawaii Fishing Rules were signed into law. These rules now make the West Hawaii aquarium fishery one of the best-managed aquarium fisheries in the world; decisions are made based on scientific assessments and by involving different resource user groups. What started as a campaign questioning the sustainability of the aquarium fishery, turned into a turf war over the years and there are bills filed every year to request the ban of the fishery. However, the process resulted in a role model of aquarium fishery management.

But the aquarium trade is not only under threat caused by fights around the natural resource. Starting in November 2013 in Germany, the political Party Sozialdemokratische Partei Deutschlands proposed a general ban of all wildlife imports. While this was not advanced, it was offered as a move towards improved species conservation and animal welfare. The trade of wildlife, especially from resources that are under threat, is seen as unethical and the related hobby out of control (Schmidt, 2014a). While there is an ongoing debate on resource user rights and on the ethics of keep marine wildlife at all, it is generally accepted that coral reefs worldwide need to be preserved, if for the sake of conservation or to secure future supply for one of the most exciting hobbies.

Many traders have already recognized that an economically sustainable aquarium industry requires ecological sustainability as well, in order to safeguard their long-term livelihood. Many hobbyists agree that sustainability is also within their responsibility as the "consumer". The question ESAIA e.V. as an organization working to sustain the marine aquarium hobby and trade is often asked is how they can contribute to coral reef health and a sustainable use of wild resources while being far away and disconnected from sources. A justification for the marine aquarium hobby and trade brought up immediately when being questioned is the educational benefits aquariums provide and how this hobby raises awareness for coral reefs as a fascinating ecosystem. Closely related to these benefits is also the most cited solution to sustainability issues offered by the trade and hobby: breeding and culture of marine ornamentals to become independent from wild resources. While this is truly an alternative drawn from knowledge that aquarium keeping can provide, it is not the only solution required. Independence from wild resources will remain a dream for a long time, and when it comes to broodstock for breeding and culture it is even further in the future. As explored in more detail in the problem section below, this solution does not come without its own set of challenges. Therefore it is worth noting that breeding and culture should not be seen in isolation or as the only answer to sustain the marine aquarium trade and hobby. With a solid interest in coral reefs and their inhabitants, industry and hobbyists are tasked to contribute by all possible means to their well-being and sustainable use. Sufficient management and regulation of artisanal fisheries like the aquarium fishery is often problematic, especially in developing nations (pers. observation). Indonesia and the Philippines, however contribute significantly to the variety and volume (around 86%) of marine ornamental fish available to the hobby (Rhyne *et al.*, 2012). In both countries capacity and financial resources to regulate aquarium fisheries and, more importantly, to enforce such regulations are limited. This is not surprising as these are the world's largest archipelagos with extensive coastlines and a fishery that is highly fragmented. As recommended by Rhyne *et al.* (2014) it will require cross-political boundaries endeavours by both export and import countries to effectively manage the marine aquarium trade and fisheries. For management to be effective it will also need the support and collaboration of industry and hobbyists, demanding meaningful regulation.

This approach would certainly become a long-term solution for sustainable use of wild resources. However, breeding and culture represent another alternative on hand that can bring immediate benefits to reef health and reef species populations and includes the market side in a direct way. The following will thus focus on where we are with breeding and culture as an alternative to the supply of specimens collected from the wild, what challenges this solution poses and how the trend to smaller tanks can help foster the demand for bred and cultured marine ornamentals.

28.2 The Need

Most marine aquarium hobbyists want to mimic coral reefs in their displays. Thus a great variety of species need to make their way from tropical reefs into our homes. Most of them (fish) by volume, 86.6% (Rhyne *et al*., 2012) originate from the Philippines and Indonesia – hotspots of biodiversity in the coral triangle. Not only is there a great variety of fish and invertebrate species available from exporters there, but also the very competitive prices make those countries absolute export champions in the marine aquarium trade. Other countries like Sri Lanka (cleaner shrimps, *Lysmata* spp.) or the USA (namely the state of Hawaii) (Yellow tangs, *Zebrasoma flavescens*) are focusing on a smaller range of species, sometimes endemic, making them favourite sources of these specialties.

A baseline study by Wabnitz *et al*. (2003) suggests that 1471 species of fish are traded. A more recent analysis of invoices of imports into the USA only, based on data for a period of one year in 2004–2005 by Rhyne *et al*. (2012), found an even higher variety in fish species imported for marine aquariums: 1802 species from 125 families, not including those exported from the USA (e.g. Hawaii, Florida). Most of these fish are small fish and in the family Pomacentridae with Damsel and Clownfish species alone making the top 10 of most traded species (confirmed by Wabnitz *et al*., 2003 and Rhyne *et al*., 2012). The outstanding all-time favourite is *Chromis viridis*, in the trade a so-called bread and butter fish. The top 20 families include Gobiidae, Labridae, Blenniidae, Apogonidae, Callionymidae, Pseudochromidae, Microdesmidae, but also relatively bigger sized species of Pomacanthidae, Acanthuridae, Serranidae (mainly *Anthias*), Chaetodontidae, Balistidae, Syngnathidae and Scorpaenidae. Murray & Watson (2014) confirmed this trend when analysing the biodiversity in UK hobbyist tanks, although one has to assume the potential for market specific preferences. Among hobbyists the US market is always assumed to have, on average, larger tanks compared with European hobbyists, with available space dictating suitable sized species and species combinations for stocking. Nonetheless, as in nature, small sized fish species certainly dominate a coral reef tank, with fewer specimens of larger species being visible.

Furthermore 140 species of stony corals and 61 species of soft corals (these numbers should be treated with caution due to the complexity of coral taxonomy) are commonly traded. Wabnitz *et al*. (2003) identified the following genera of stony corals as most relevant: *Acropora, Catalaphyllia, Euphyllia, Galaxea, Goniopora, Heliofungia, Plerogyra, Trachyphyllia, Turbinaria*. In soft corals *Sarcophyton, Sinularia, Xenia, Cladiella, Clavularia, Anthelia, Lobophytum, Nephthea, Dendronephthya* and *Cespitularia* are the most traded genera.

However, all these organisms are only the more obvious components of a reef tank. To really build a reef requires hundreds of species of non-coral invertebrates like

shrimp, hermit crabs, star fish and sea urchins, snails, sea anemones and clams, as well as substrate for settlement and bio-filtration, so called live rock. According to Wabnitz *et al.* (2003): "516 species of invertebrates are being traded for the aquarium trade", but the same authors advise caution when interpreting this information due to the lack of a standard taxonomy for marine invertebrates when imports and export are listed by traders.

But this hobby has not only an appetite for diversity, but the volumes involved are also considerable. Wabnitz *et al.* (2003) estimated the annual global trade as between 20 million and 24 million specimens of marine ornamental fish, 11–12 million pieces of corals and 9–10 million specimens or other marine ornamental invertebrates. Although the study of US imports over 1 year by Rhyne *et al.* (2012) assumes that these previous assessments are likely overestimated, they found around 11 million fish specimens were imported within 1 year to the US alone. While the US is by far the largest market, we can nevertheless assume that all other markets together will show a similar need in volume of fish imports (see also Wabnitz *et al.*, 2003). As such, the estimate of 20 million fish being imported worldwide each year seems to be correct. Murray *et al.* (2012) suggests that in non-coral invertebrates the volume might rather be 10–20 fold underestimated. This confirms the author's insight into imports of one German wholesaler of thousands of hermit crabs alone on a monthly base (pers. observation). All reports identify an increasing appetite of the aquarium trade, although economic crises are reflected in the stagnation of trade volumes in certain markets. Traders and hobbyist associations alike report that hobbyist numbers are fairly constant, estimated to be around two million worldwide by Wabnitz *et al.* (2003), and the hobby lacks newcomers that continue for the long term. However, there are also trends of rising popularity, resulting in increased volumes for certain species, e.g. in the year following the release of "Finding Nemo" in 2003, wholesale of *Amphiprion ocellaris* (Nemo) jumped by 25% as a representative of Oceans, Reefs and Aquariums, a commercial hatchery in Florida, US, confirmed (Prosek, 2010). Over the past few years the trade has seen an even higher demand for small sized species, suitable for so called nano tanks – a natural response to rising electricity costs and economic crisis in the main markets in the US and Europe.

In summary the marine aquarium hobby depends on a plethora of reef associated fish and invertebrate species.

28.3 Where We are Now

While we know now what species are important in the trade and hobby, we need to compare that with what our selected solution of breeding and culture has to offer as an alternative to wild sources.

There are varying reports on the number of species that are available from breeding. While they all agree that the number of available species from culture and breeding is increasing, there is no source for the number of species that are available on a commercial scale (volume and price). As Olivotto *et al.* (2011) report, advances in research of culture technologies have been made for many families and species. However, success in the lab unfortunately does not always translate into production at commercial scale. Barriers to accessing scientific literature and difficulties with understanding the scientific language used in breeding protocols by those who have the entrepreneurial

spirit to commercially culture marine ornamentals, are not to be underestimated. As such the efforts of hobby-based databases are good for spreading knowledge among those that have the potential to bring advances to a commercial scale. A successful breeding record e.g. in the Marine Breeding Initiative (MBI) database as Murray & Watson (2014) state: "*…can mean anything from 'inducing spawning' to 'keeping the larvae alive for 60 days post-larval settlement'*." With this in mind the 269 species with breeding success on the MBI list have to be taken with "a grain of salt". As a hobbyist, but also as a wholesaler or retailer, it is not easy to keep track of what is steadily available from breeding and culture. While Wabnitz *et al*. (2003) listed 38 fish species as being commercially available, Baensch at Reef Culture Technologies (2011a) already offers 112 fish species as accessible to the trade. A report by Krause (2012) in a German hobbyist magazine indicates only 12 bred fish species were regularly available in Germany and lists an additional 35 fish species as bred at commercial scale (most in US hatcheries). The CORAL Magazine's Captive Bred Marine Fish Species List for 2015 (Sweet, 2015) indicates a total of 272 species being bred, with 110 reaching retail level (grouped into commonly available, moderate to low availability and scarce). Still only 26 species from this list are commonly available to hobbyists. Of these 26 commonly available bred species, nearly 40% are clownfish. All reports align in that the commercially available species are mainly clownfish (Pomacentridae), dotty backs (Pseudochromidae), gobies (Gobiidae), blennies (Blenniidae), and sea horses (Syngnathidae), with angelfish (Pomacanthidae), Dragonets (Callionymidae) and Cardinals (Apogonidae) also advancing. Murray & Watson (2014) assume that the breeding successes in those families mean there is high potential for aquaculture in other species within these families; and identify the main breeding gaps remaining to be tangs (Acanthuridae) and wrasses (Labridae).

With invertebrates, especially stony and soft corals, exhibiting a much wider range of reproductive methods such as asexual reproduction, advances in their culture are greater. Many small polyp stony (SPS) corals are readily available from fragmentation, either from coral farms in traditional source countries or in the markets, or even from hobbyist tanks. However, successes are not yet translating in the same way for large polyp stony (LPS) corals, and especially not for non-coral invertebrates like sea anemones, hermit crabs, star fish and many gastropods used as the "cleaner crew" in reef tanks. Baensch (2011a) lists four shrimp species as commercially available, however, as Wabnitz *et al*. (2003) also implies, the vast majority of commercially traded non-coral invertebrates are still collected from the wild. A success story however is the breeding and culture of giant clams in farms located in the South Pacific (see Chapter 23.1).

28.4 Challenges

If fish or corals are available from breeding and culture, why are they also still sourced from the wild? Sometimes the sheer quantity of wild caught clownfish and wild collected stony corals at exporter facilities raises questions about the impact of the breeding and culture efforts undertaken.

So why do we see clownfish and *Acropora* spp. collected from the wild and moved to markets when bred and cultured specimens of the same species are commercially available? This issue is even more relevant if one considers that bred fish come with the

benefit of being raised in captivity, already conditioned to aquarium keeping, to readily accept processed feed, and thus are generally less stressed and healthier than wild conspecifics.

Anecdotal reports reveal that there is still resistance among hobbyists to pay the possibly higher price for bred specimens. But also concerns about the genetic fitness of bred specimens are still widespread, with references made to better coloration and shape in wild collected organisms. For many species, particularly fish, it is true that the price difference between wild and bred specimens can still be significant (Table 28.1). Species that are relatively easy to produce in a reasonable time and in significant volume, such as *A. ocellaris*, are sold to hobbyists at a similar price. However, at the import level the price difference is quite significant and explains why considerable volumes of wild clownfish are still being imported. Nevertheless, Nemo (*A. ocellaris*) remains the shiny success story of marine ornamental breeding (see Chapter 12). By breeding varieties with coloration and patterns distinct from wild specimens, the "artificial" family of designer clownfish has started a new trend in the marine aquarium hobby and has achieved higher prices. After some years of success, where demand was exceeding supply, the appetite seems to be decreasing, which might be a mixed effect of economic crisis on purchasing behaviour (as described by Rhyne & Tlusty, 2012), but could also be caused by a "back to roots" spirit with the want to be as close to nature as possible. Often prices of bred specimens can hardly compete with those provided by low paid fishermen in developing nations. This is especially true where prolonged life cycles and larval stages and difficult to meet dietary requirements make breeding and culture a time and capital intensive effort (e.g. *Synchiropus splendidus*). Given the lack of traceability along the trade chain (Cohen *et al*., 2013) there is even potential incentive for fraud in selling wild caught specimens of such species as bred to profit from the higher sales price.

Any aquaculture effort with its hefty initial investments and quite significant maintenance costs involved along the way (e.g. electricity, water, equipment, feed) will need to rely on those species that have established breeding protocols, which can be implemented in relative short time and produce significant volume to meet the trade's demand at comparable costs. As such not only is competitiveness in price a challenge, but also in volume. Baensch (2011b) summarizes: "*Many species have not been cultured because of their complex reproductive biology; others can only be raised*

Table 28.1 Comparison of average prices of wild vs. bred/cultured (NB *Euphyllia* corals are not sexually bred, but supplied from fragmentation and culture) specimens at import and retail level, (sources include pricelists of exporters from the Philippines, Indonesia, commercial hatcheries in the US, and retail prices of online traders in Germany).

	Purchase price at import in Euro		Sales price at retail in Euro	
Species	**Bred/cultured**	**Wild**	**Bred/cultured**	**Wild**
Euphyllia ancora	10.00	3.50	23.00	52.00
Synchiropus splendidus	12.40	3.50	44.00	22.00
Chrysiptera parasema	Not known	0.45	16.00	6.00
Amphiprion ocellaris	4.40	0.66	16.00	13.00

on an experimental scale, and for those that can be raised in large numbers, production is often not cost-effective."

For families like clownfish and damsels, gobies, blennies, dragonets and angelfish where breeding is quite advanced, volume and price are still not on competitive scales with wild sourcing. Commercial hatcheries will have a long way to go before they can find a market willing to pay them for their efforts to breed these fish, e.g. *Chrysiptera parasema*.

When investigating purchasing prices at the import level for damsels, importers in Germany did not know that they were available from culture and breeding (pers. observation). Thus, some species already listed as being commercially available might only be accessible to hobbyist in the USA from commercial hatcheries located in that country.

The only species in Table 28.1 where the higher price of bred specimens is not passed on to the retail level is an LPS coral, *Euphyllia ancora*. Just the opposite, here the wild specimen is more expensive to the hobbyist than the cultured coral. However, the sold specimens are not comparable in size in this example: whereas the wild coral is a colony of several branches, the cultured coral is only a fragment taken from such coral colony. Traceability might help to overcome these price differences and ensure that coral farming involves establishment of broodstock vs. fragmenting bigger colonies of wild collected corals to sell them as cultured corals, with a price premium for their sustainability efforts. Altogether it seems to be one thing to theoretically refer to breeding and culture as a solution to the sustainability dilemma of the marine aquarium trade, but is another thing to implement it.

Another challenge that breeding and culture is facing is that most of the trade volume producing commercial hatcheries are based in traditional market countries rather than the original producer countries. Whether this can be defined as bio-piracy might be discussed, but certainly it can give the purchase of bred specimens negative overtones for taking income from poor fishermen in developing countries that depend upon wild collection for their livelihood. With the marine aquarium trade being discussed as having potential to contribute significantly to reef conservation by incentivizing local coastal communities to safeguard reef health, culture activities in market countries put the required mechanisms into question as well. Naturally the incentive for local collectors to support aquaculture is low, when this only results in taking away badly needed income from themselves when aquaculture is conducted elsewhere (Tlusty, 2002).

28.5 Solutions

Depending upon the nature of the challenge different solutions are recommended.

As briefly introduced the marine aquarium trade is a global affair with many players involved. The trade chain is long, fragmented and quite complex (Cohen *et al*., 2013). This especially applies to situations where infrastructure and social development are not at an advanced level (developing nations) as represented in Figure 28.1.

Considering how much workforce and logistics are involved in collecting and moving marine ornamentals from coral reefs to home aquaria, a higher product price for these truly exotic species is to be encouraged. A higher price for wild caught organism would not only increase the value given to these animals and ensure fair compensation for those involved, when margins are shared with collectors, but would also make prices for bred and cultured organisms more competitive.

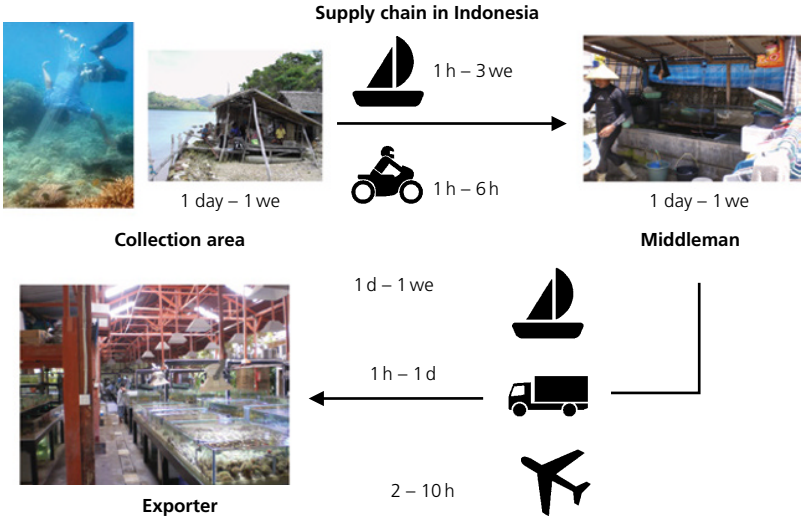


Figure 28.1 Local supply chain in Indonesia up to export level. (*See insert for color representation of the figure.*)

Another way to potentially lower the costs for bred and cultured organisms is to establish hatcheries in traditional source countries thus benefiting from the same reasonable priced work force and maintenance costs as wild collection and export.

Doing so will require the development and transfer of simple and cheap culture technologies to local communities (Murray *et al.*, 2013). Strengthened aquaculture production of marine ornamentals in traditional source countries will not only lower costs and price, but is also addressing the deprivation of income from marine aquarium collectors when their supply is replaced through market based hatcheries products. Collectors can be trained to become farmers to access an alternative livelihood to wild harvesting. However, it is mandatory to translate research advances into easy to understand culture techniques, which can be adapted to local circumstances. A close collaboration of sciences and trade will be needed to achieve successful commercial production.

Marketability of cultured products should also be supported by adequate regulation. Stony corals proven to originate from culture for instance should have easier and less costly access to CITES documentation. When full traceability is established, it is questionable if CITES documentation should still be required at all or if a CITES aligned quota to collect broodstock would not be sufficient. For CITES listed fish, like seahorses, breeding can become a nightmare for the required documentation alone. In Germany additional regulation by the federal agency for wildlife conservation requires breeders to record details of any successful reproduction up until sale. Any hobbyist buying a seahorse then needs to register with their local conservation authority. Clearly marketability of bred seahorses is suffering from this regulation in Germany. Regulation for rare and/or endemic species that are naturally more vulnerable to extinction could even include trade restrictions (e.g. only available as broodstock or for research) for wild collected specimens when these are readily available from breeding. Potentially this would also increase incentive to buy bred and cultured species. ESAIA e.V. supports this approach by educating hobbyists on sustainable choices of species that are

readily available from breeders and where purchases of wild organisms should be avoided (Schmidt, 2013b).

While regulation and the build-up of aquaculture in traditional source countries are again longer term measures, a market already exists that is destined to support aquaculture by its demand. In past years technological advances in lighting helped to efficiently light aquaria and in natural spectra without creating a significant heat load, previously a problem for small volume tanks especially (Rhyne & Tlusty, 2012). Technically it is now possible to set up a mini reef in so called nano tanks, with water volumes between 30 to 120 liters. More recently, nano reef keeping has become a natural response to rising electricity costs and economic crises, and thus popular in hobby and trade. Nano tanks help aquarists to focus on smaller critters taking centre stage, where they might be lost or overlooked in larger displays. The limitation in space restricts the selection of marine life suitable to live in a nano aquarium, and it also lowers the quantity of organisms needed to create the impression of a busy reef. Given these pre-conditions of relatively small organism size and low quantities required, the aquaculture sector is well prepared to meet the demand. A nano aquarium can realistically be fully stocked with captive-bred or cultured organisms only, making it a real Green Choice, in terms of maintenance (water and electricity use) as well as in stocking. Larger displays, on the contrary, often lead to increased demand in the variety and quantity of ornamental organisms, including specimens that grow large and are active swimmers (e.g., Acanthuridae and Labridae). These families were categorized by Murray & Watson (2014) as groups with high demand and a low potential for aquaculture to meet this demand.

Most organisms, especially fish, which are suitable for nano tanks (Schmidt, 2014b), come from families where culture technology is developed and production could satisfy demand (e.g. Pomacentridae, Pseudochromidae, Gobiidae, Blenniidae, Syngnathidae, Callionymidae and Apogonidae). Given that the quantity of purchases is restricted there might also be a greater willingness among nano reef keepers to spend more money on the organisms and pay the higher price for cultured specimens. Thus nano reef keeping can help foster the demand for specimens readily available from culture and breeding. ESAIA e.V. is supporting the marketing of aquaculture products for nano tanks with their new on line tool AquarioScenario (Figure 28.2).

Therefore it is recommended that future culture technology development focus not only on high priced species from families like Pomacanthidae, but also on bringing production of those families to the scale where some species can be successfully bred and cultured. As indicated above, even in families with high potential for commercial aquaculture, currently only a limited variety of species is successfully bred (e.g. in family Gobiidae of the 1703 registered species in Fishbase, only 28 have been bred in captivity and only five are commonly available from culture to the hobbyist (Sweet, 2015)). In other families, like Pomacentridae, production focuses on some, relatively easy to reproduce genera, with most other genera not being produced in a commercially relevant quantity. A wide selection of small sized and reasonably priced organisms readily available from breeding and culture will help aquarists to make the right choice when purchasing specimens for their nano reef tanks. Thus keeping nano tanks can support the marketability of and demand for bred and cultured marine organisms and as such contribute to the sustainability of the marine aquarium trade.

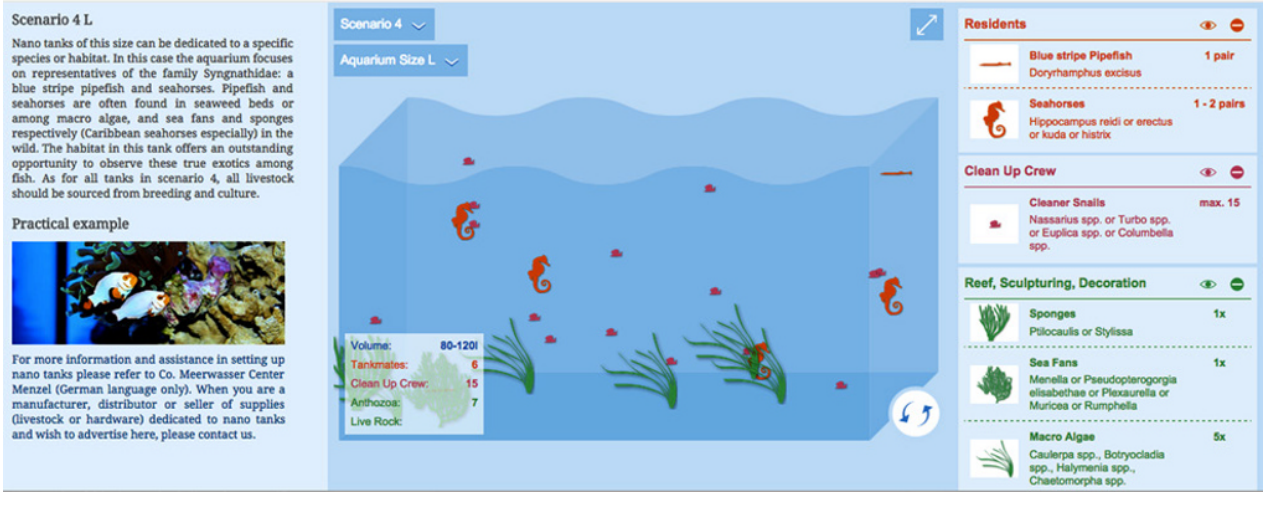


Figure 28.2 AquarioScenario by ESAIA e.V – an online tool to stock nano tanks. (*See insert for color representation of the figure.*)

28.6 Conclusions

In summary some central solutions to overharvesting of the world's coral reefs for the marine aquarium trade are (1) to develop higher prices for wild caught organism to not only increase the value given to these animals but to ensure fair compensation to the collectors involved as well as make prices of bred conspecifics competitive; (2) establish hatcheries in traditional source countries to benefit from the same reasonable priced work force and maintenance costs as wild collection and export, as well as not depriving them of livelihoods; (3) develop regulations and traceability for cultured products; and (4) support nano reef keeping because it would reduce the number and size of specimens required for the mini reef and help foster the demand for specimens readily available from culture and breeding.

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Part VI

Future Challenges and Concluding Remarks

Some remarkable achievements in breeding marine ornamental species have been documented in this book, as well as reminders of constraints still faced by marine ornamental aquaculture. Most marine ornamental species are still collected from the wild with issues of overharvesting and damage to the environment. At present, there is a lack of information on the global trade and collection of marine ornamentals, especially for invertebrates; thus accurate data are needed for the more than 2500 species that comprise the trade in order to truly understand this impact. Increasing the sustainability of the marine ornamental species trade should be considered a primary initiative for the whole trade chain, from collectors and producers to wholesalers, retailers and hobbyists. The continued viability of the marine ornamental species trade will undoubtedly depend on the ability to operate in a sustainable manner.

Marine ornamental aquaculture has an opportunity to play an instrumental role in achieving a balance between economic feasibility and environmentally sound practice. Less than 5% of marine ornamentals are currently cultured and even fewer are available in mass production to supply the ever growing demand of this trade. The development of aquaculture, especially in areas near collecting sites, would provide an alternative to collecting from the reefs and provide a new source of income. Coastal communities could propagate many species of fish as an alternative to wild collection alongside other activities, such as coral or live rock farming.

A balance of culture and conservation will be needed to make the marine ornamental trade sustainable. The challenges of marine species sustainability are faced by both the hobbyist and by private and public aquariums; thus the solutions could come from collaborations among these groups. An in-progress example is the Rising Tide Conservation model that includes key stakeholders from academic institutions and both the hobby and aquarium sectors. It has been successful in advancing knowledge of larval rearing and broodstock nutrition for several marine ornamental fishes.

Major issues with aquaculture for the majority of marine ornamentals remain. Research is specifically needed in: physical parameters of the culture environment, nutritional requirements of broodstock, larval rearing system design and larval feeds and nutrition, weaning diets and settlement cues and metamorphosis. For mass production there is a need for optimization of rearing techniques, and increased understanding of many aspects including breeding, nutritional requirements and diseases in high density production. Fish management, including diet and environmental and

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ecological requirements, at all stages of the marine ornamental trade is the key to preventing diseases.

New Developments

Some exciting new aquaculture developments have been reported recently and include new and more efficient equipment and the use of small or large system volumes (microcosm and mesocosm, respectively), and availability of live prey for larvae.

1) Nano-tanks or mini reefs offer solutions to overharvesting of coral reefs for the marine aquarium trade by reducing the number and size of specimens required and help foster the demand for specimens readily available from culture and breeding.
2) Recent breakthroughs include the inorganic fertilization method for producing high densities of small ciliates and flagellates for first feeding fish larvae with small mouth gape size.
3) Readily available commercially produced live copepods may lead to significant advancement in the culture of marine ornamentals that were previously limited by improperly sized first food items.
4) Several aquaculture businesses produce novel phenotypes through artificial selection opening new opportunities for marine ornamental aquaculture.
5) From an industrial and economical point of view, the development of recirculating aquaculture systems (RAS), whose technology and proficiency has been substantially improved in recent years both for small and large volume units is of special interest.

Future Challenges

- There is a major need to identify and culture new plankton species of the appropriate size (e.g., larvaceans) that can be cultured at high densities, and with the appropriate nutritional profile to sustain larval and juvenile development of many species, especially invertebrates. Research efforts are needed for finding appropriate treatments against pathogens of marine ornamental species. These efforts should be focused on natural and innovative molecules that could, at least partly, replace chemicals that are harmful or destructive to many other living organisms usually stocked in reef aquariums (e.g., invertebrates and beneficial bacteria providing biological filtration).
- The interaction between coral aquaculture scientists and marine aquarium hobbyist and stores should be expanded to substantiate and improve current knowledge about coral aquaculture *ex situ* and its efficient application to the marine ornamental trade. Future ornamental aquaculture efforts should also focus on species exhibiting high market potential and life history characteristics that lend well to aquaculture.
- It must be highlighted that after years of research one of the most emblematic fish in the marine aquarium trade, the yellow tang *Zebrasoma flavescens* has been successfully bred and cultured in captivity. This remarkable achievement is an important milestone in marine ornamental species aquaculture. This accomplishment allows us to anticipate that despite the many constraints to the culture of some of the most

heavily collected marine ornamental species, we will continue to witness significant breakthroughs in the aquaculture of marine ornamental species in the years to come.

- As a final caveat, we sincerely hope that with the release of the animated movie "Finding Dory" a new window of opportunity has opened to foster a sustainable and conscientious marine aquarium trade. The industry must learn from past mistakes, such as the ones prompted by the block buster "Finding Nemo", which led to unprecedented collection and irresponsible husbandry of marine ornamentals in general and clownfish in particular. The Dory cannot presently be bred in commercial numbers; thus the majority of fish that will enter the aquarium trade will be wild collected. Our hope is "Finding Dory" will promote the conservation, responsible collection, husbandry and aquaculture of the Dory *Paracanthurus hepatus*.

> "Remember that each Dori is unique and special and was likely collected from a beautiful coral reef. Provide it with a nice big tank, plenty of good food and a lot of love".

The Editors:
Ricardo Calado, Ike Olivotto, Miquel Planas Oliver and G. Joan Holt

Glossary

AA amino acids.

alkalinity Measures the resistance of a solution to pH variation when an acid solution is added.

amphipods Mostly marine crustaceans, members of class Malacostraca, commonly displaying a small body size (<1 cm) and used live or frozen as food for marine organisms.

Artemia Also known as brine shrimp, it is a relatively primitive form of crustacean of the class Brachiopoda. It has the ability to produce cysts (embryos whose development is temporarily "paused" and are encased in a complex "shell"), which are commonly traded in vacuum-packed cans. These cysts can be easily incubated to produce newly hatched *Artemia* nauplii, a very popular feed for marine larvae. Live, frozen, freeze-dried adult or newly hatched *Artemia* is commonly used as food for marine organisms.

buffer A solution that is resistant to pH changes despite the addition of small amounts of acid or basic solutions.

captive cultured A specimen collected from the wild at larval, post-larval or young juvenile stage and grown to commercial size in captivity.

captive bred A specimen grown from larvae to commercial size in captivity resulting from the offspring of a breeding pair also stocked in captivity.

CITES The Convention on International Trade in Endangered Species of Wild Fauna and Flora (also referred to as the Washington Convention) is a multilateral treaty to protect endangered plants and animals.

copepods Small crustaceans, members of class Maxillopoda, that may display a benthic or pelagic lifestyle and are often used as live prey (namely their first larval forms – the nauplii) for larval feeding a number of marine ornamental species. They are the feeding items preferred by most fish larvae, with the culture of calanoid copepods (order Calanoida) being considered as key-issue to solve current larviculture bottlenecks of marine larvae.

CPUE Catch per unit effort.

denitrification The process of conversion of nitrate to nitrogen gas through the action of anaerobic bacteria.

demersal species Species that live in the demersal zone, part of the sea comprising the water column that is near to the seabed and the benthos (organisms that live on, in, or near the seabed). The demersal zone is just above the benthic zone.

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diapause Delay in development in response to regularly and recurring periods of adverse environmental conditions.

dimorphism A phenotypic (generally applies to morphology) difference between males and females of the same species.

endogenous feeding In opposition to exogenous feeding (feeding on external nutrient supplies), endogenous feeding applies to the developmental phase (commonly early larval developmental stages) in which feeding is based exclusively on yolk sac reserves.

embryogenesis The process by which the embryo forms and develops.

epibenthos Community of organisms that live on top of the seabed, also known as the benthic zone.

ex situ In opposition to *in situ*, it means to examine a given process outside the place where it occurs (e.g. in the laboratory).

FA Fatty acids.

FAA Free amino acids.

fish only tank An aquarium solely stocked with fish.

gonochoric A species displaying separate sexes.

hermaphroditic A species displaying both sexes during its life cycle.

HUFA Highly unsaturated fatty acids. HUFA of both n-3 and n-6 series (DHA, EPA, ARA) are essential requirements for many marine species.

in situ In opposition to *ex situ*, it means to examine a given process exactly in the place where it occurs (e.g. in the ocean).

IUCN The International Union for Conservation of Nature and Natural Resources is an international organization working in the field of nature conservation and sustainable use of natural resources. Species are classified by the IUCN Red List into nine groups, set through criteria such as rate of decline, population size, area of geographic distribution, and degree of population and distribution fragmentation.

larva A distinct juvenile form many animals with indirect development undergo before metamorphosis into adults. Its diet and appearance is generally very different from the adult form.

larval settlement Transition to benthic life of pelagic free-swimming larvae.

lecithotrophy In opposition to planktotrophy, larvae displaying this mode of development do not require food to survive, relying on the degradation of internal reserves to fulfil energetic requirements. It is commonly considered an adaptation to low or unpredictable food levels in the environment where larval development takes place.

live rock Is the term used to designate the rock made from the calcium carbonate skeletons of long dead corals, or other calcareous organisms, forming the majority of coral reefs in the ocean. These rocks are commonly encrusted with coralline algae and inhabited by a multitude of marine organisms. The various forms of micro and macroscopic life living on and in the rock give it the name "live rock".

MAT Marine aquarium trade.

mesocosm Outdoor experimental system to evaluate the natural environment under controlled conditions. Generally used to estimate how organisms or communities might react to environmental changes.

metamorphosis In a strict sense, it describes a particular life history transition, from a larval to a juvenile (or adult) stage, accompanied by dramatic modifications on its morphology, physiology and ecology. It is usually regulated by thyroid hormones.

metanauplius This larval stage is very similar to the nauplius, also displaying a median eye and cephalic natatory appendages. However, it displays more than three somites and may also present thoracic appendages.

microalgae They comprise a vast group of photosynthetic, heterotrophic organisms that may be directly used in the rearing of larvae or to improve the nutritional profile of other planktonic preys (e.g. *Artemia* and rotifers).

monogamy A form of relationship in which an individual has only one partner during his or her lifetime or at any one time.

nano tanks An aquarium with a water volume ranging between 30 to 120 liters recreating a miniaturized version of a coral reef.

nauplius Is considered the most ancestral type of larvae in the Crustacea. It displays a median eye, no thoracic somites and its locomotion is achieved through the use of cephalic appendages (namely the antennules, antenna and mandibles).

nitrification A two-step process on which ammonia is converted to nitrite an latter to nitrate by nitrifying bacteria.

pelagic species Species inhabiting the pelagic zone (from the surface of the sea almost to the bottom). The pelagic zone is just above the demersal zone.

pH Is a measure of the acidity or alkalinity of a solution. Solutions with a pH less than 7.0 are considered acidic, while those with a pH greater than 7.0 are considered basic (or alkaline). When a pH level is 7.0, it is defined as "neutral".

prey enrichment Procedure to enhance the nutritional quality of live prey (rotifers, *Artemia*). It is commonly used in larviculture.

offspring Young born of living organisms.

PCC Post-larval capture and culture.

plankton A diverse group of organisms that inhabit the water column of large water bodies and that cannot swim against a current. Zooplankton and phytoplankton are the animal and autotrophic fraction of plankton, respectively.

planktotrophy In opposition to lecithotrophy, larvae displaying this mode of development require planktonic food to survive and fulfil their energetic requirements.

photoperiod The interval in a 24-hour period during which a plant or animal is exposed to light.

phototactism Concerns the behaviour of an organism in the presence of light. It can be positive if it moves towards the light source or negative if it moves away from it.

polyandry A form of relationship in which a female mates two or more males.

polygyny A relationship in which a male mates more than one female.

primary lecithotrophy Larvae displaying this mode of development use energy derived from endogenous egg yolk reserves to develop through some or all postembryonic larval stages. This mode of development commonly reflects the degree of female investment in reproduction.

protandrous A species that first matures as a male and with increasing size specimens may lose the external male features, stop producing sperm and become functional females.

protogynous A species that first matures as a female and with increasing size specimens may lose the external female features, stop producing sperm and become functional males.

reef safe A term used in the marine aquarium hobby to label a species that will not damage other organisms being displayed in the same reef aquarium.

reef tank An aquarium stocked with fish and invertebrates.

rotifers These are microscopic aquatic animals of the phylum Rotifera. The rotifer *Brachionus plicatilis* has been widely used as food for larval marine organisms due to its tolerance to the marine environment. It is commonly used to feed larval stages which have no or limited ability to capture and ingest *Artemia* nauplii.

secondary lecithotrophy A development mode displayed by a late non-feeding larval stage, which will moult to a juvenile that resumes feeding activity. During this period, the energetic requirements are fulfilled through the catabolism of plankton derived reserves accumulated by earlier larval stages

simultaneous hermaphrodites A species that is capable of producing male and female gametes simultaneously.

symbiosis Close and often long-term interaction between two different biological species (e.g. clownfish and sea anemone).

swim bladder An internal gas-filled organ that contributes to the ability of many bony fish to control their buoyancy.

SWOT analysis An acronym for Strengths, Weaknesses, Opportunities, and Threats (at times also referred as SWOT matrix) is a planning approach that evaluates these elements within the framework of a project or business venture.

UNEP-WCMC United Nations Environment Program -World Conservation Monitoring Centre, a collaboration between the United Nations Environment Programme, the world's foremost intergovernmental organization, and WCMC 2000, a UK-based charity (www.unep-wcmc.org).

viviparity A mode of reproduction that refers to development of the embryo inside the body of the parent, eventually leading to live birth.

yolk Material contained in a membranous sac (yolk sac) attached to an embryo that supplies nutrients for the development of the animal embryo (lecithotrophic embryo).

Appendix

List of public and private institutions (including non-academic) working on marine ornamental species aquaculture and researchers (with contacts and their fields of expertise)

Alma Mater Studiorum Università di Bologna, School of Agronomy and Veterinary Medicine
Dipartimento di Scienze Mediche Veterinarie, Via Tolara di Sopra 50, 40064, Ozzano Emilia (BO), Italy
Laboratory of Fish Pathology
Website: http://www.agricultureveterinarymedicine.unibo.it/en/
Contact person: Marialetizia Fioravanti; email: marialeti.fioravanti@unibo.it; Tel: +39-0512097045
Research interests: Research and diagnostic activities on fish diseases.

Azul Fish Farm
Marine Ornamental Aquaculture
Rua General Vitorino Monteiro 227 – Vila Romana, São Paulo – SP Brazil- 05053-060
Website: www.azulfishfarm.com.br
Contact person: Cássio Ramos; email: cassiorramos@hotmail.com
Tel: + 55 11 95255-1559
Interests: Marine ornamental fish aquaculture, coral propagation.

Brigham Young University-Hawaii
Department of Biology, 55-220 Kulanui Street, Laie, Hawaii, USA 96762
Website: http://byuh.edu/
Contact person: David Bybee; email: david.bybee@byuh.edu; Tel: (808) 675-4300
Research interests: Marine invertebrate reproduction and propagation, phylogenetics, coral reef ecology and conservation.

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Cabrillo Marine Aquarium
Aquatic Nursery
3720 Stephen M. White Dr., San Pedro, CA 90731, USA
Contact person: Mike Schaad; Tel: 310-548-7562
Research interests: Propagation and husbandry of sea jellies native to Southern California Bight; facilitating communication amongst institutions with public exhibitions of live sea jellies.

Center for Aquatic Sciences
Center for Aquatic Sciences 1 Riverside Drive, Camden, NJ 08103-1060, USA
Contact person: Alejandro A. Vagelli; email: avagelli@aquaticsciences.org; Tel: +01-856-361-1026
Research interests: Reproductive biology, ecology, and evolution of cardinalfishes (Apogonidae); aquaculture of marine ornamentals.

Centre for Environment, Fisheries and Aquaculture Science (Cefas)
Pakefield Road, Lowestoft, Suffolk, NR33 0HT, UK
Website: www.cefas.defra.gov.uk
Contact person: Joanna Murray; Joanna.murray@cefas.co.uk; Tel: +44 1502 524584
Research interests: Ornamental fisheries science, ornamental aquaculture, molecular biology applied to marine ornamentals, and socio-economics of the marine ornamental industry.

Centre Scientifique de Monaco
8 Quai Antoine 1er, MC-98000 Monaco
Website: http://www.centrescientifique.mc
Contact person: Denis Allemand; email: allemand@centrescientifique.mc; Tel: 00377 97774450
Research interests: Ornamental corals, physiology and ecophysiology, *Corallium rubrum*.

ECOCEAN
33 rue Chaptal, 34000 Montpellier, France
Website: www.ecocean.fr
Contact person: Gilles Lecaillon; email: gilles.lecaillon@ecocean.fr; Tel: +334 67 67 02 84 & +33 6 17 98 58 78
Main activities: Ecological engineering in marine environment; manufacturer of fish traps for post-larvae capture; manufacturer of dedicated wetlab for post-larvae culture; engineering in capture and culture of marine post-larvae for marine ornamental trade; restocking of damaged marines areas.

ESAIA e.V.
Marquardsholz D10, 91161 Hilpoltstein, Germany
Website: www.saia-online.eu
Contact person: Christiane Schmidt; chairman, email: fishfood@gmx.net; Tel: +49-01639655434
Research interests: Ornamental fish production and trading (breeding, culture, wild fishery) and sustainable use of the resource coral reef, promoting reef conservation by providing socio-economic incentives.

Florida Gulf Coast University
Department of Biological Sciences
Website: www.fgcu.edu
Contact person: Matt Palmtag; email mpalmtag@fgcu.edu; Tel: 1 239 590 1263
Research interests: Ornamental fish and crustacean breeding and rearing and zooplankton culture.

Flying Sharks, Ltd.
Rua Farrobim do Sul 116, 9900-361 Horta, Portugal
Website: www.flyingsharks.eu
Contact person: Nuno Rodrigues; email: nuno@flyingsharks.eu; Tel: +351 91 947 5411
Research interests: Promoting a sustainable use of the oceans, quality marine, education and research on the marine environment.

H2Ocean Aquaculture
Marine Ornamental Fish Aquaculture
Rua Piracicaba,40 Valparaiso – Santo André –SP – Brazil
Cep: 09060-180
Website: www.h2oceanaquicultura.com.br
Contact person: Alexandre da Cunha; email: contato@h2oceanaquicultura.com.br
Interests: Indoor marine ornamental fish aquaculture, larviculture.

Instituto de Investigaciones Marinas (CSIC)
Departamento de Ecología y Recursos Marinos, Eduardo Cabello 6, 36208 Vigo, Spain
Group of Biology and Physiology of Fish Larvae
Website: www.iim.csic.es
Contact person: Miquel Planas Oliver; email: mplanas@iim.csic.es; Tel: +34-986214457
Research interests: Marine species conservation, larval rearing and physiology of marine fish, seahorse breeding and rearing, molecular biology and microbiology applied to larval rearing, use of probiotics in aquaculture.

Instituto Oceanográfico – Universidade de São Paulo
Praça do Oceanográfico, 191 – São Paulo-SP, 05508-120 Brazil
Benthic Dynamics Laboratory, Room 139
Website: www.usp.br
Contact person: Miguel Mies; email: miguel.mies@usp.br; Tel: +55 11 3091 6543
Research interests: Marine aquaculture, larval development of coral reef organisms, zooxanthellae and symbiosis.

Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta
S.S. Laboratorio Specialistico di Ittiopatologia (Fish Diseases Laboratory), Italy
Website: www.izsto.it
Contact person: Marino Prearo; E-mail: marino.prearo@izsto.it; Tel: + 39-011-2686251
Research interests: Ornamental fish diseases; fish diseases; aquaculture, fish vaccination.

Istituto Zooprofilattico Sperimentale delle Venezie
Viale dell'Università, 10 – 35020 – Legnaro (Pd) – Italy
National Reference Center for Fish, Mollusc and Crustacean Diseases

Website: www.izsve.it
Contact person: Amedeo Manfrin; amanfrin@izsvenezie.it; Tel: +39 (0)498084211
Research interests: Diagnostics applied to marine ornamentals (bacteriology, virology, parasitology, histopathology).

La Casetta In Canada' Sas Di Amato Giuseppe & C.
Via Vittime delle fobie, 10036 Settimo Torinese, (TO), Italy
Contact person: Vito De Vita; email: info@lacasettaincanada.it; Tel: 0030- 011/ 8975088
Website: www.facebook.com/LaCasettaInCanada
Interests: Marine ornamentals suppliers.

National Museum of Marine Biology and Aquarium
Department of Biology, 2 Houwan Road, Checheng, Pingtung, 944 Taiwan
Fish Reproduction and Larviculture Laboratory
Website: http://www.nmmba.gov.tw/english/ResearchMemberDetail.aspx?CondID=38edcd1c-a455-449e-a4e7-24e1cdbb365a
Contact person: Ming-Yih Leu; email: myl@nmmba.gov.tw; Tel: +886-8-8825385
Research interests: Ornamental fish breeding and rearing, fish reproduction and larviculture, aquarium science, ichthyoplanktonology.

Oceanic Institute of Hawaii Pacific University
41-202 Kalaniana'ole Hwy, Waimanalo, HI 96795, USA
Website: Finfish Program; http://www.oceanicinstitute.org
Contact person: Chatham Callan; email callan@hpu.edu; Tel: +1-808-259-3149
Research interests: Marine ornamental aquaculture, larval fish feeding and ecology, fish reproduction and development.

Peixepalhaco.com
Marine Ornamentals Aquaculture
Rua Papa Paulo VI 27A Peruíbe-SP Brazil cep 11750-000
Website: www.peixepalhaco.com.br
Contact person: Igor Albergaria; e-mail: peixepalhaco@peixepalhaco.com.br; Tel: +55 13 997646872
Interests: Marine ornamental fish aquaculture, coral propagation.

Pelagosphera Soc. Coop.
Cooperativa di servizi in ambiente acquatico
Sede Legale: via M. Bigotti 1, 57037 – Portoferraio (LI), Italy
Sede Operativa: via Accademia Albertina 13, 10124 – Torino,Italy
Website: www.pelagosphera.com
Contact person: Nicola Nurra; email: info@pelagosphera.com; Tel: +39 011 6704578
Research interests: Physical and biological oceanography, biodiversity conservation, environmental analysis, zooplankton analysis, benthos ecology and taxonomy.

Piscicultura Tanganyika
Rua Angelica s/n, Parque das Flores – Aquiraz – Ceará – Brazil
Website: www.pisciculturatanganyika.com.br

Contact person: Ivan Oliveira; email: piscicuturatanganyika@hotmail.com; Tel: +55 8533612399
Research interests: Aquaculture of exotic and native marine ornamental fish and corals; freshwater ornamental fish aquaculture.

Point Defiance Zoo and Aquarium
Department of Husbandry, 5400 North Pearl Street, Tacoma, WA 98407, USA
Contact person: Chad L. Widmer, PhD; Tel: 253.404.3676
Research interests: Influences of climate variability on marine organisms; invertebrate zoology and ecology; husbandry of deep sea organisms; enhancing sustainability of global jellyfish collections.

Recife de Peixes
Av. Visconde de Albuquerque, 775 Recife-Pernambuco – Brazil 50610-090
Website: www.recifedepeixes.com.br
Contact person: Franciso Andrade; email: diretoria@recifedepeixes.com; Tel: +55 8199049297
Research interests: Aquaculture of exotic and native marine and freshwater ornamental fish.

Reef Culture Technologies
Website: www.rcthawaii.com
Contact person: Frank Baensch; email: rct@hawaii.rr.com
Research interests: Breeding marine fish; larval fish culture and development.

Rising Tide Conservation
SeaWorld Parks and Entertainment
500 SeaWorld Dr. San Diego, CA 92109, USA
Contact person: Judy St. Leger; Judy.st.leger@seaworld.com; Tel: 619-225-4259
Research interests: Ornamental fish rearing and broodstock management, technology transfer, *in situ* aquaculture of ornamentals.

SEA LIFE – Merlin Entertainments
Head of Merlin Animal Welfare and Development Europe, Weymouth, Dorset, DT4 7SX, UK
Website: www.merlinentertainments.biz; www.visitsealife.com; www.sealifetrust.org
Contact person: Chris Brown; email: chris.brown@merlinentertainments.biz; Tel: 0044 (0) 1305 761465
Research interests: Fish and elasmobranch breeding programs, fish and elasmobranch transportation, aquarium husbandry and elasmobranch conservation/research programs.

SeaWorld Orlando
SeaWorld Orlando, 7007 SeaWorld Dr., Orlando, Florida, 32821, USA
Website: www.seaworldparks.com/Orlando
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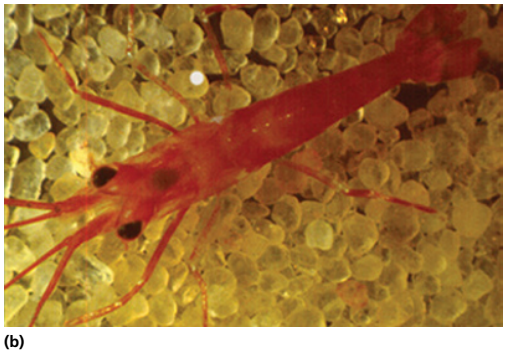
(a) (b)

Figure 1.1 Candy basslet (*Liopropoma carmabi*). (a) Adult pair of candy basslet; and (b) 58-day-old larvae raised by Todd Gardner at Atlantis Marine World in Long Island, New York in 2010.

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(a)



(b)



(c)

Figure 1.2 Fire shrimp (*Lysmata debelius*) raised by Matthew R. Palmtag and Dr. Joan Holt in the late 1990s at the University of Texas Marine Science Institute in Port Aransas, Texas, USA. (a) 68-day-old post-larvae; (b) 83-day-old newly metamorphose juvenile; and (c) adult shrimp.



(a) (b)

Figure 3.1 Protogynous female-to-male sex change (a) *Pseudochromis fridmani*; (b) *Pomacanthus semicirculatus*.

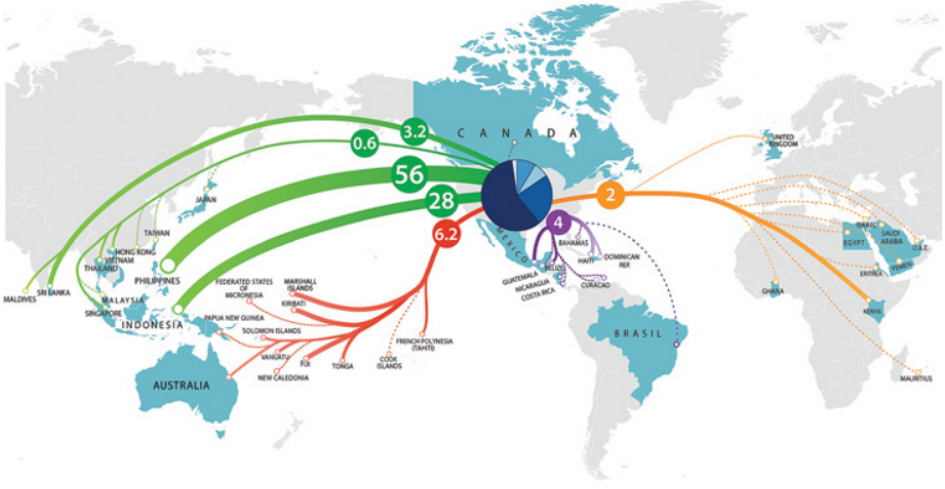


Figure 4.1 Trade flow of marine aquarium fishes from source nations to the United States. Numbers on lines represent percent total import. Pie chart within the United States represents ports of entry (with the Midwest starting at 0 degrees, and clockwise, NE, SE, SW and NW).

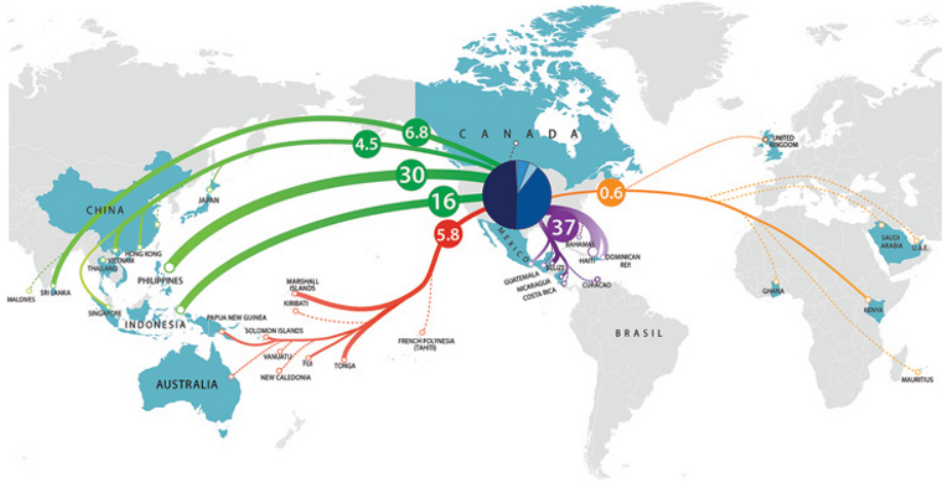
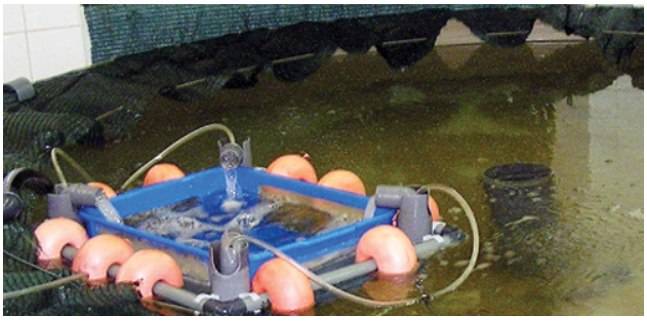


Figure 4.2 Trade flow of marine aquarium invertebrates from source nations to the United States. Numbers on lines represent percent total import. Pie chart within the United States represents ports of entry (with the Midwest starting at 0 degrees, and clockwise, NE, SE, SW and NW).



(a)

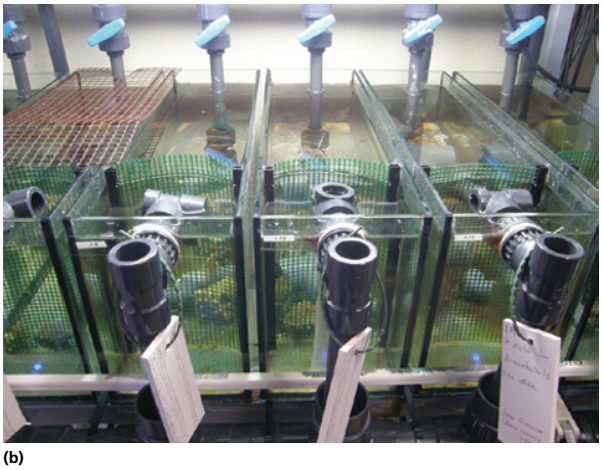


(b)

Figure 6.2 Egg collectors: (a) airlift collector placed inside the broodstock tank; (b) overflow collector placed outside the broodstock tank, receiving water with eggs through the overflow pipe. Photos by Aquaculture Research Group – CCMAR (www.ccmar.ualg.pt/aquagroup).



(a)



(b)

Figure 6.3 Modular maturation system for ornamental crustaceans broodstock: (a) general view of the 3-level modular maturation system; (b) rectangular-base glass tanks divided with a net into two compartments to avoid the passage of broodstock, illuminated with a blue LED spot in the small compartment.



Figure 7.1 Fiberglass larval rearing tanks at the Fisheries and Mariculture Laboratories, Port Aransas, TX, USA.



Figure 7.2 Small 50 L mesh baskets (rearing chambers) placed in large tanks (400–1000 L) have been used with success both for demersal and pelagic spawners.



Figure 7.4 Aquaria for seahorse breeding at Instituto de Investigaciones Marinas (CSIC) in Vigo (Spain).

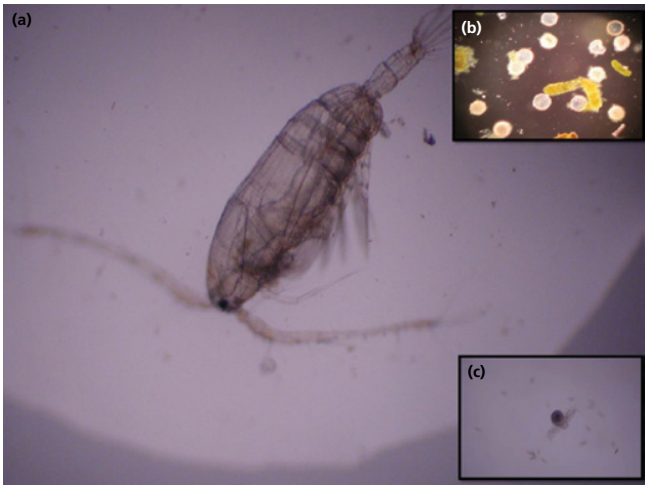


Figure 8.4 *Acartia tonsa* (a) adult; (b) embryos and (c) nauplii.



Figure 12.1 Symbiosis between *Amphiprion clarkii* and the sea anemone *Entacmaea quadricolor*.

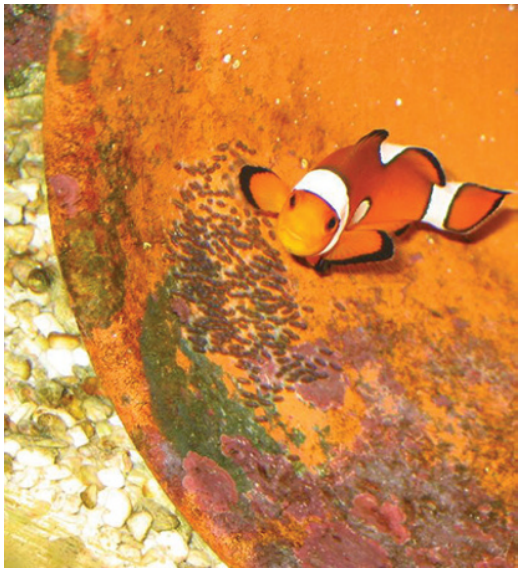


Figure 12.2 Sea-anemones can be easily substituted by clay flowerpots. Picture shows a male *Amphiprion ocellaris* guarding the egg clutch laid in the flowerpot.



Figure 12.3 Nest care performed by male *Amphiprion clarkii*.



Figure 12.4 Group of captive bred *Amphiprion ocellaris*.



Figure 13.2 Adult Banggai cardinal and its offspring sheltered in a sea anemone.

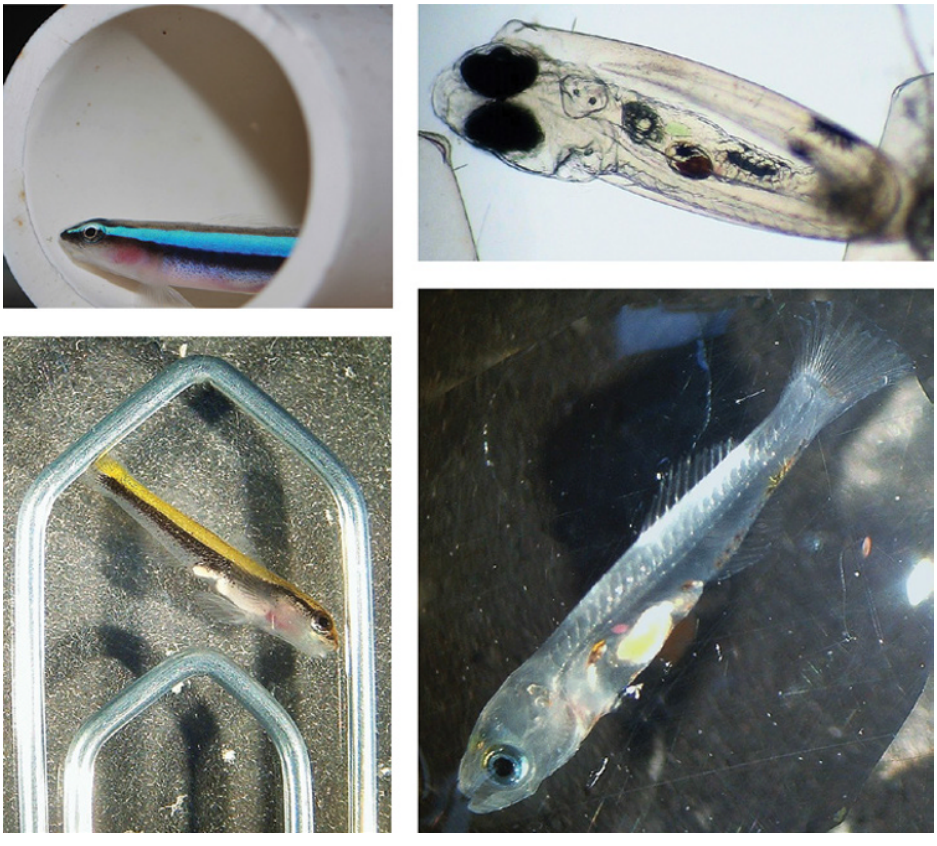


Figure 14.1 Clockwise from the top left: neon goby *Elacatinus oceanops* broodstock; pre-hatch embryo of *E. figaro* at 168 h post-fertilization; 25 days post-hatch larva of *E. evelynae*; post-metamorphosis individual displaying juvenile coloration. Photos by Marcelo Shei (top left and right) and Ike Olivotto (bottom left and right).

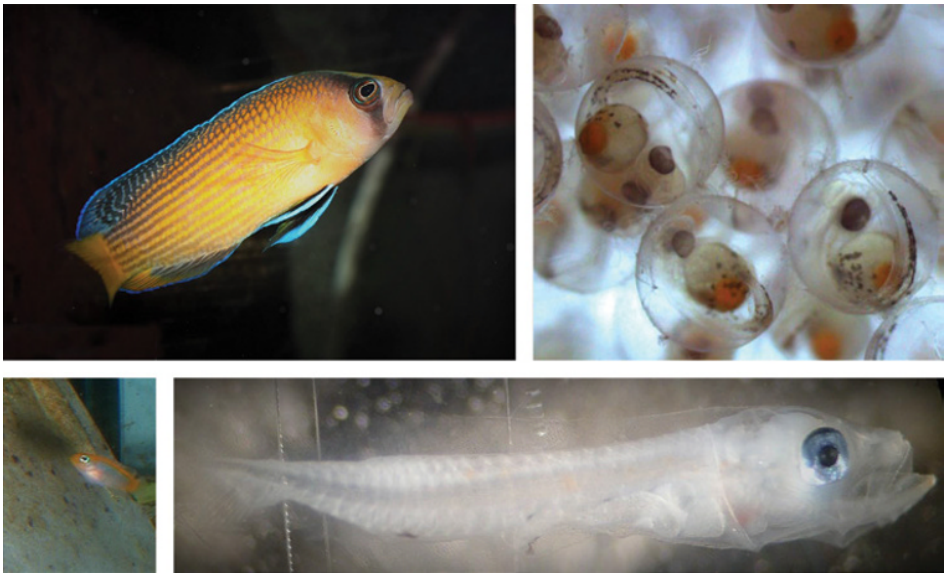


Figure 14.2 Clockwise from the top left: Splendid Dottyback *Manonichthys splendens* male broodstock; Close-up of an egg mass spawned by *Pseudochromis aldabraensis* at 96 h post-fertilization showing well-developed embryos; *P. fridmani* larvae at 7 days post-hatch and juvenile Sunrise Dottyback *P. flavivertex* two days post-settlement, showing yellow pigmentation. Photos by Marcelo Shei (top left); Arthur Z. Güth and Miguel Mies (top right) and Ike Olivotto (bottom left and right).

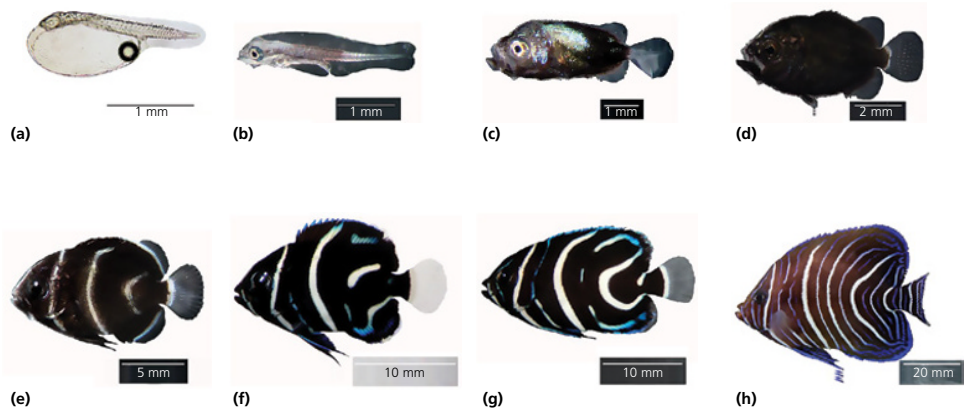


Figure 15.5 Photomicrographs of semicircle angelfish, *Pomacanthus semicirculatus*, larvae and juveniles reared at National Museum of Marine Biology and Aquarium. a = 0 day post-hatch (dph), b = 2 dph, c = 10 dph, d = 15 dph, e = 25 dph, f = 36 dph, g = 60 dph and h = 213 dph. Scale bar of a–c = 1 mm, scale bar of d = 2 mm, scale bar of e = 5 mm, scale bar of f–g = 10 mm and scale bar of h = 20 mm. Scale bars = 1 mm.

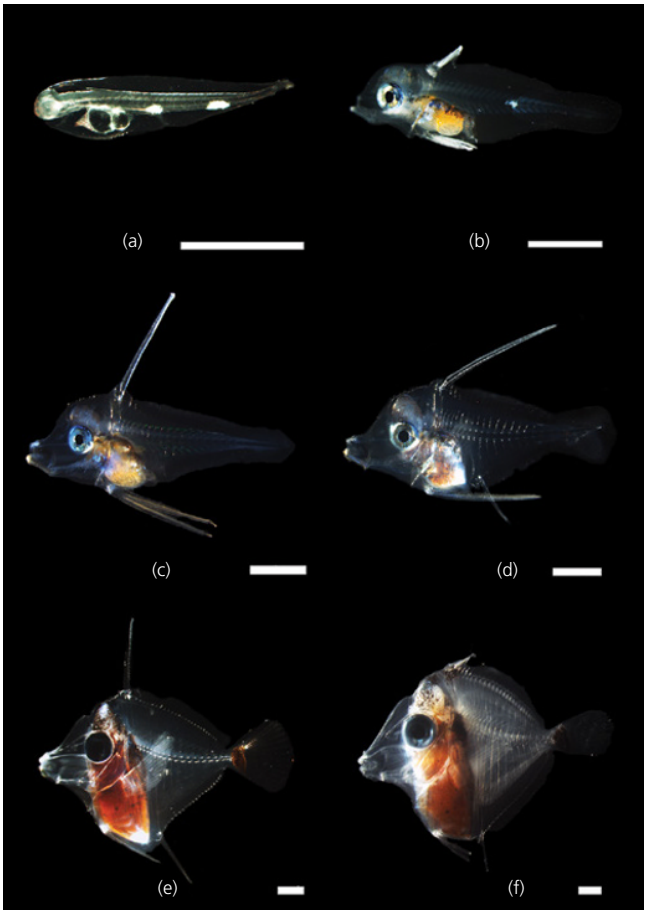


Figure 15.6 Photomicrographs of Yellow Tang larvae reared at Oceanic Institute. a = 1 day post-hatch (dph) b = 12 dph, c = 18 dph, d = 24 dph, e = 45 dph, f = 83 dph. Scale bars = 1 mm. Image credit David Hoy.

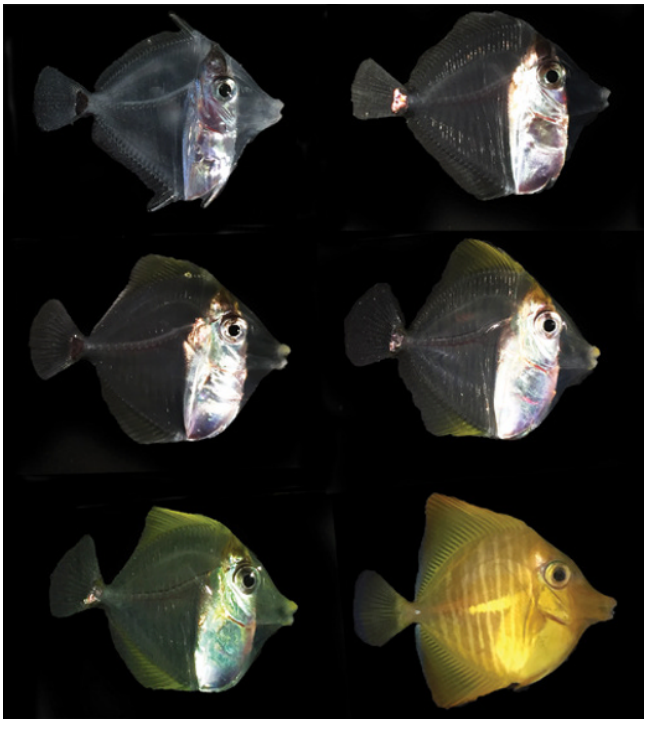


Figure 15.7 Photographs of Yellow Tang larvae from day post-hatch 40 to 65 showing the transition from a pelagic larva to a settled juvenile. Image credit Emma Forbes.

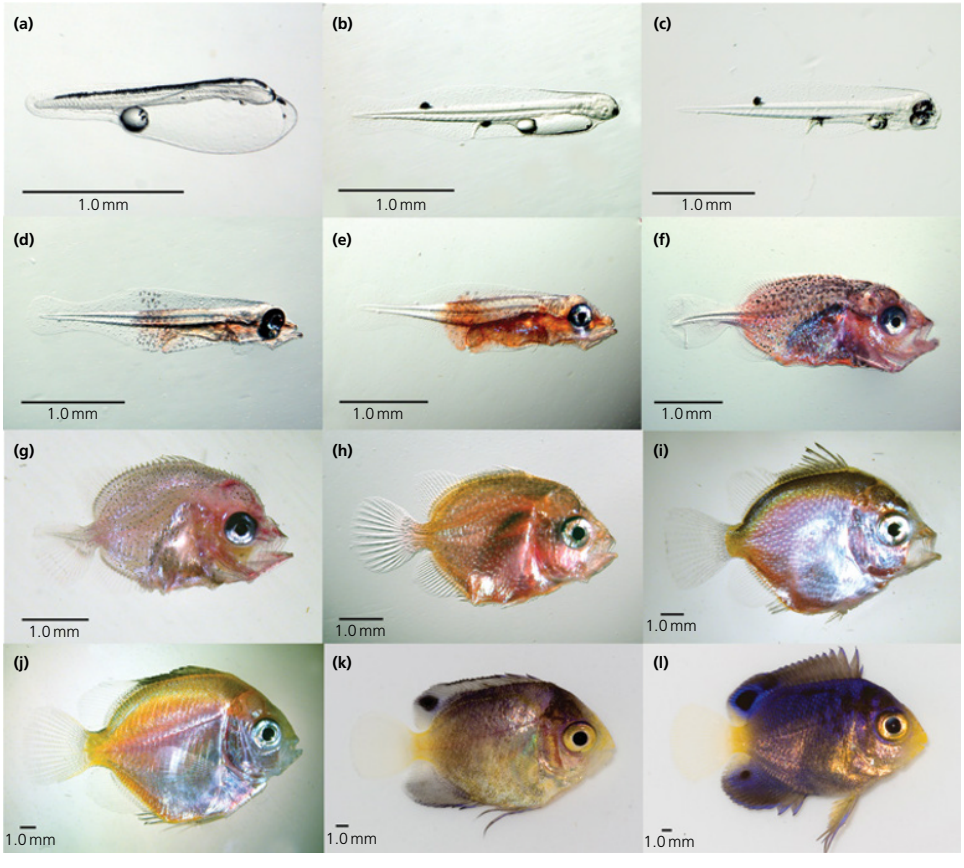


Figure 16.3 Photomicrographs and photographs of the various stages of development of a typical dwarf angelfish (e.g. *Centropyge debelius*).

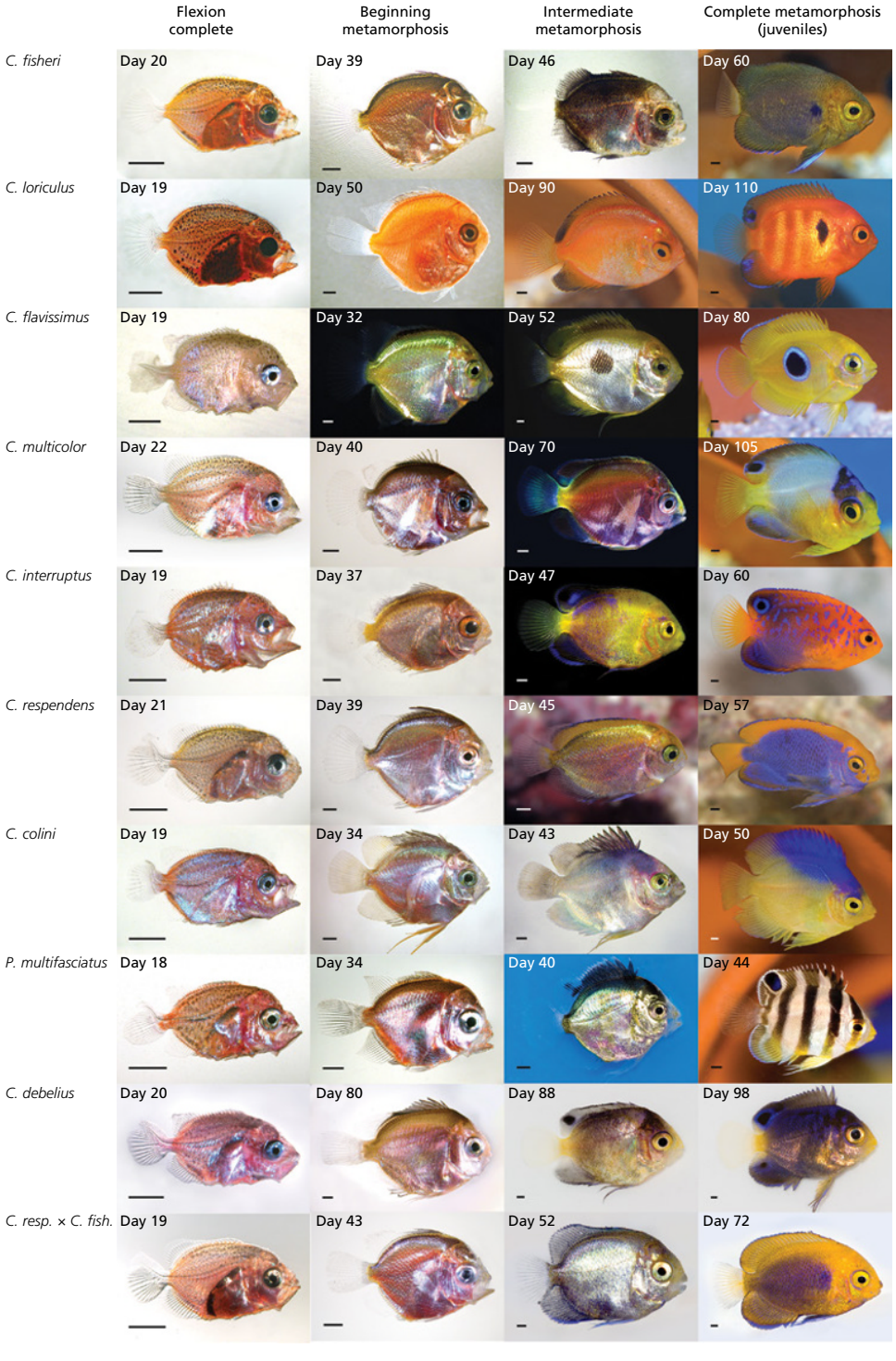


Figure 16.4 Late larval and juvenile development of nine dwarf angelfish species and one hybrid.



Figure 16.7 Four-centimeter dwarf angelfish juveniles (*Centropyge colini*) ready for sale.

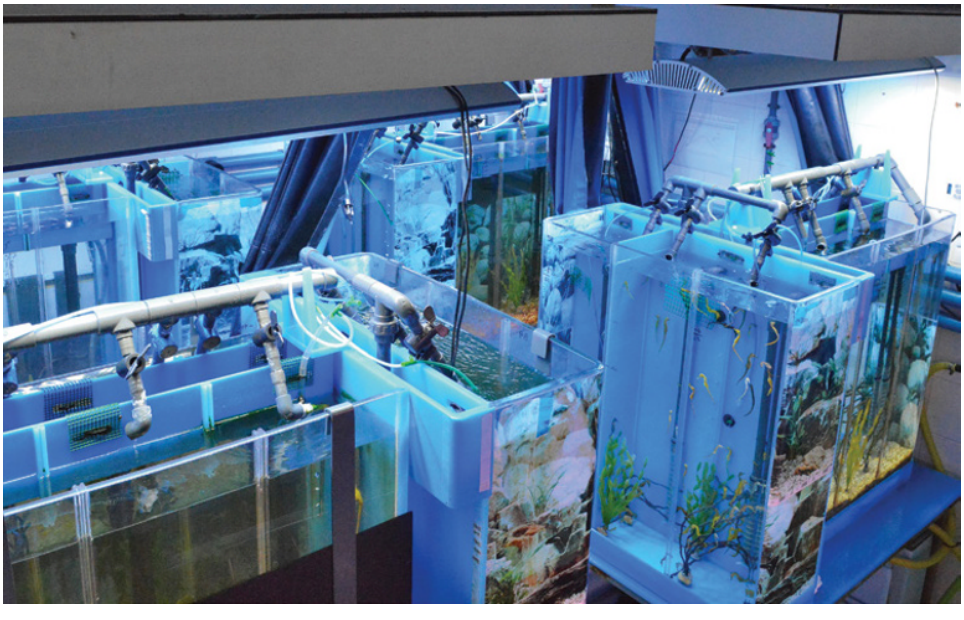


Figure 17.3 Seahorse breeding aquaria at IIM (CSIC) (description in Planas *et al*., 2008).

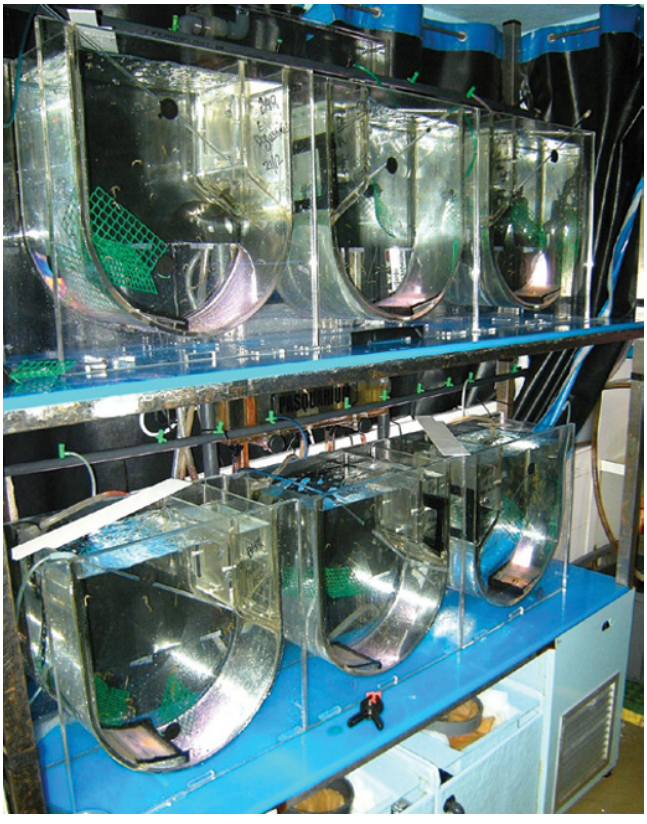


Figure 17.5 Self-working unit with six pseudo-kreisel aquaria at the facilities of the Instituto de Investigacones Marinas (IIM, CSIC) in Vigo (Spain) (description in Blanco *et al*., 2014).

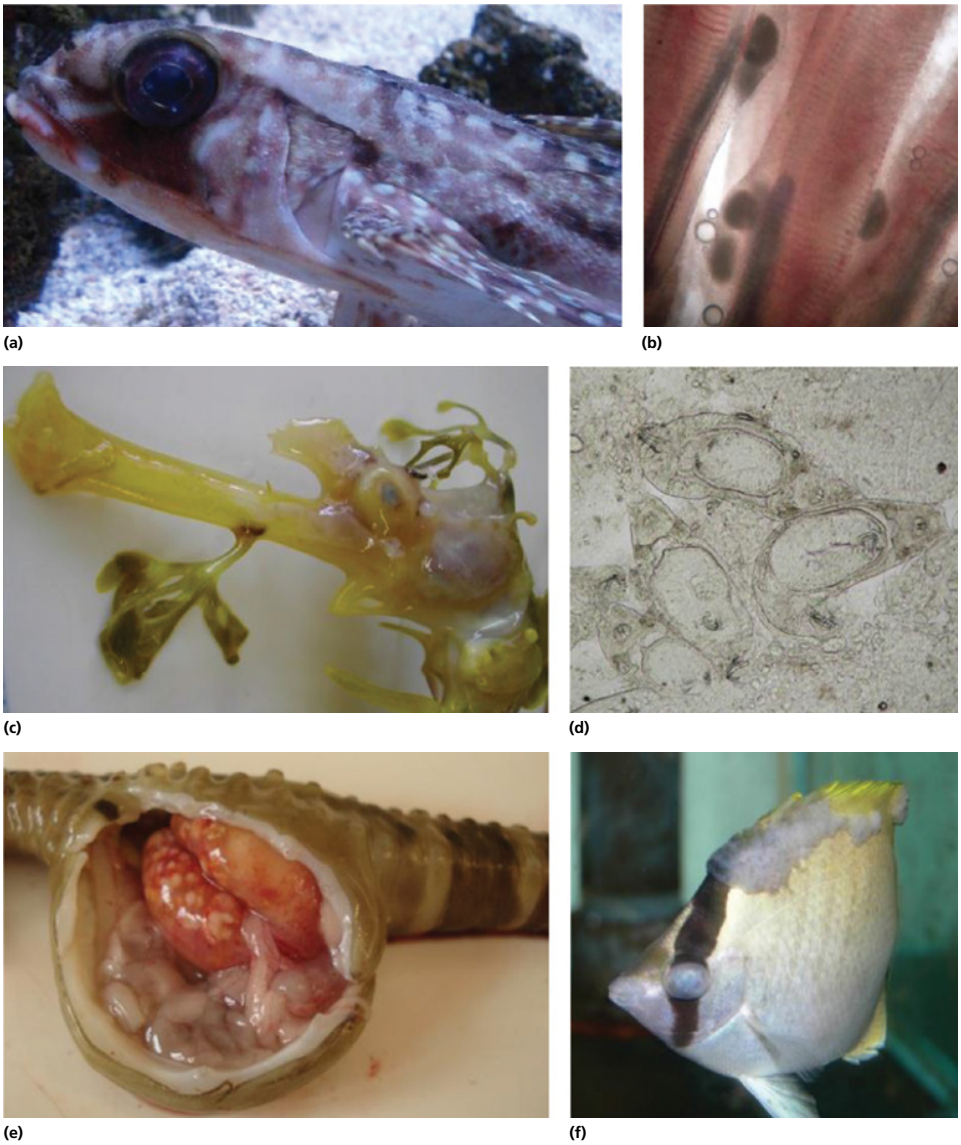
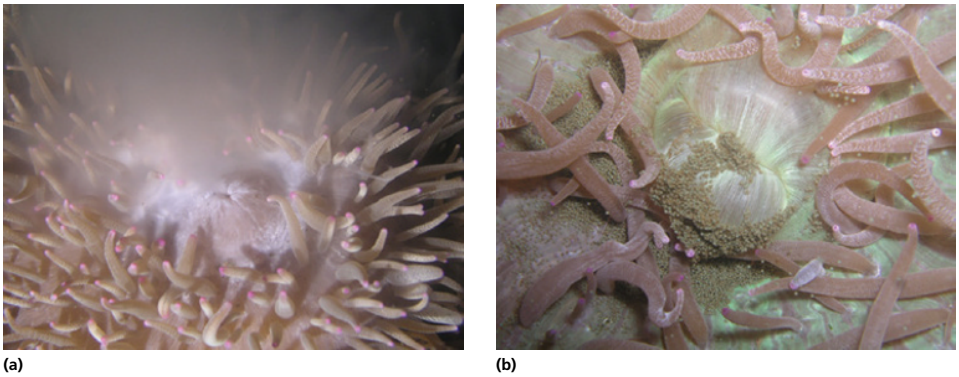


Figure 19.4 (a) Marine white spot disease by *Cryptocaryon irritans* in flying gurnard; (b) trophonts of *C. irritans* in gill wet mount; (c) lesions by Scuticociliatida on the head of a leefy seadragon; (d) Gyrodatylid monogeneans in skin wet mount from pipefish; (e) gross lesions referable to Mycobacteriosis in *Hippocampus abdominalis*; (f) Lymphocystis in *Chaetodon ocellatus*. Copyright owned by the authors, Maria Letizia Fioravanti & Daniela Florio.



Figure 20.1 Holding tanks with moist live rock waiting to be shipped from a facility in Singapore (2009).



(a) **(b)**

Figure 21.2.1 Broadcast spawning *Heteractis crispa*: (a) male; and (b) female.



Figure 22.1.4 *Stenopus hispidus* 30 days after post-larval capture (Photo by I. Mer).

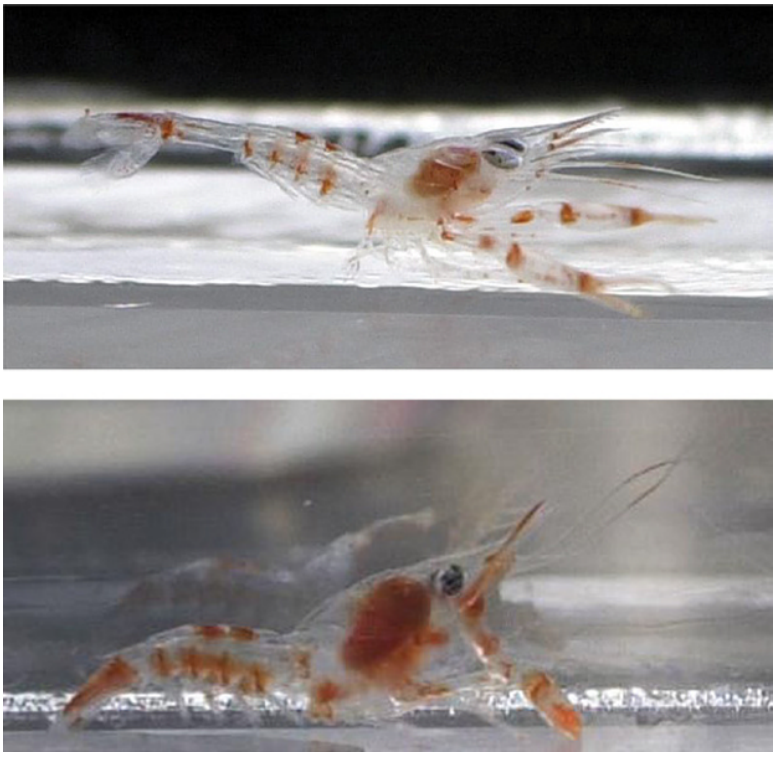


Figure 22.2.3 Late zoea (top) and post-larvae (bottom) of Daum's reef lobster, *Enoplometopus daumi*.

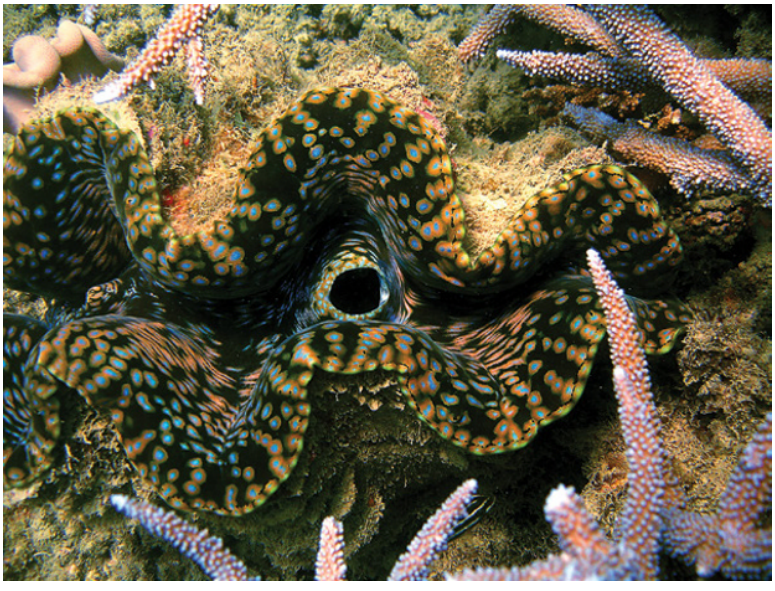
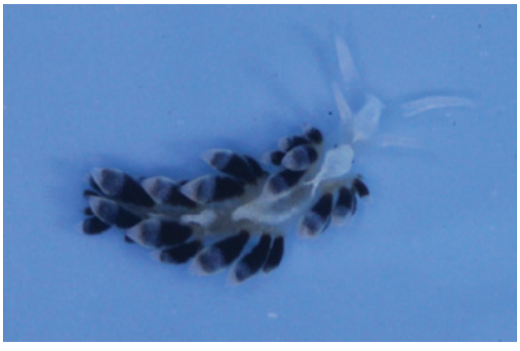


Figure 23.1.1 A hybrid specimen (*Tridacna maxima* × *T. squamosa*) with rare mantle pattern and coloration surrounded by acroporid corals at Fitzroy Island, Cairns, Australia. Photo by Luiz Fernando Furlan.



(a)



(b)



(c)

Figure 23.2.1 Ornamental sea slugs. (a) *Berghia verrucicornis* adult cultured at Guia Marine Laboratory, Cascais, Portugal; (b) *Elysia clarki* adults; (c) *Elysia clarki* juveniles cultured at Guia Marine Laboratory, Cascais, Portugal. Photo credits: a and b: Gisela Dionísio; c: Cláudio Brandão.

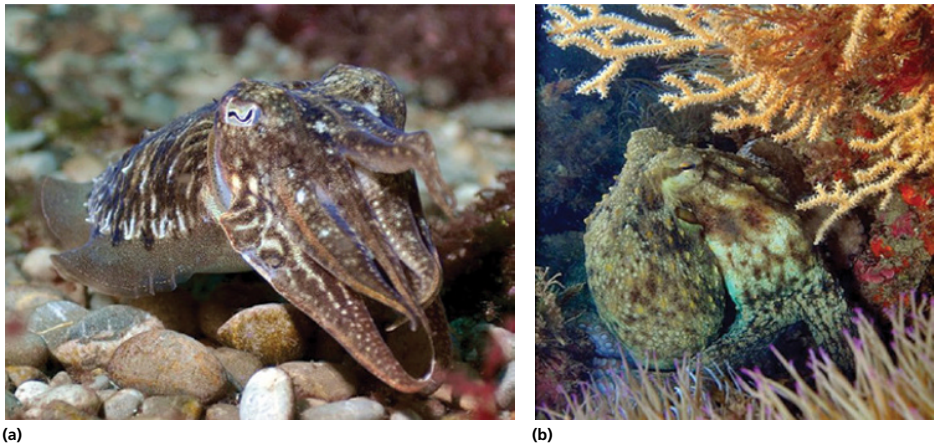


Figure 23.2.2 Ornamental cephalopods. (a) *Sepia officinalis* adult; (b) *Octopus vulgaris*. Photo credits: Armindo Pereira.



Figure 24.1 Examples of sabellids or feather duster worms in the wild (photographs by J.M. Murray).

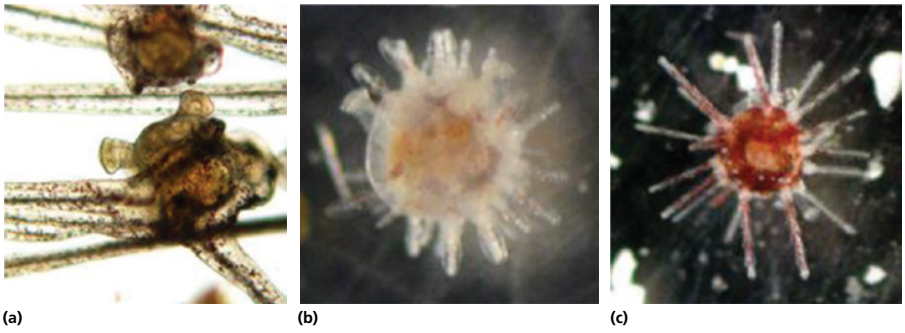


Figure 25.3 Late stages of *Diadema* larvae competent for settlement and metamorphosis: (a) mid metamorphic stage between larvae and early juvenile; (b) early juvenile just after completion of metamorphosis but before completion of test and spine development and development of internal organs; and (c) a functional, first feeding stage of the early juvenile.

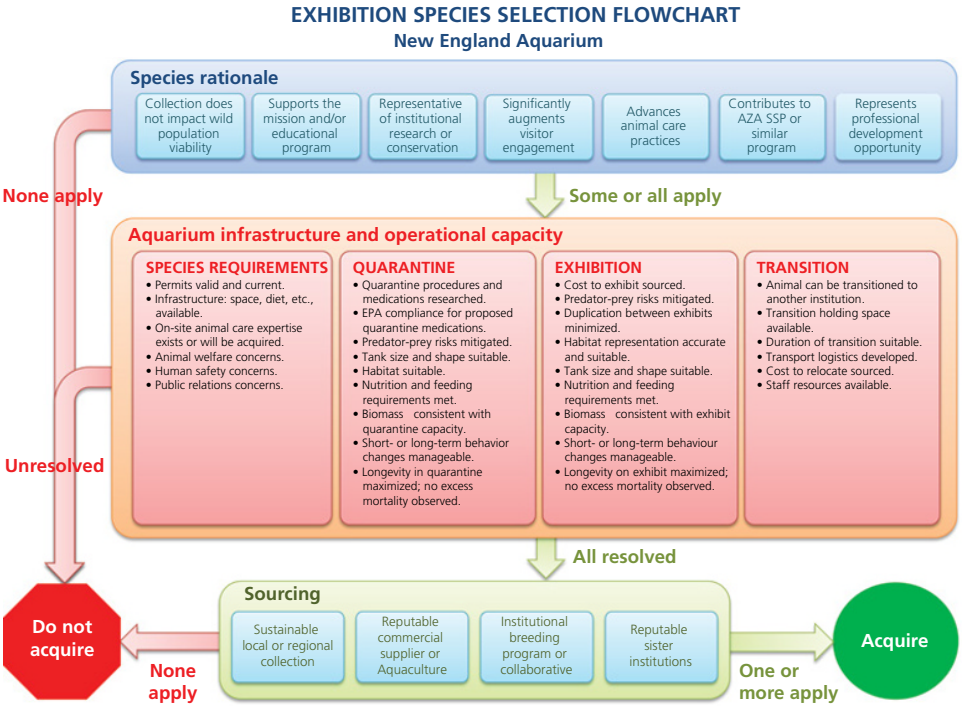


Figure 27.1.1 An exhibition species selection flowchart as developed by the New England Aquarium. The purpose of this flowchart is to provide a broad overview of the collections plan, and to ensure exhibit animals are selected to meet the institutional mission, can be cared for appropriately, and are sourced in a sustainable manner, including the use of responsible wild fisheries and/or *ex situ* cultivation of species.

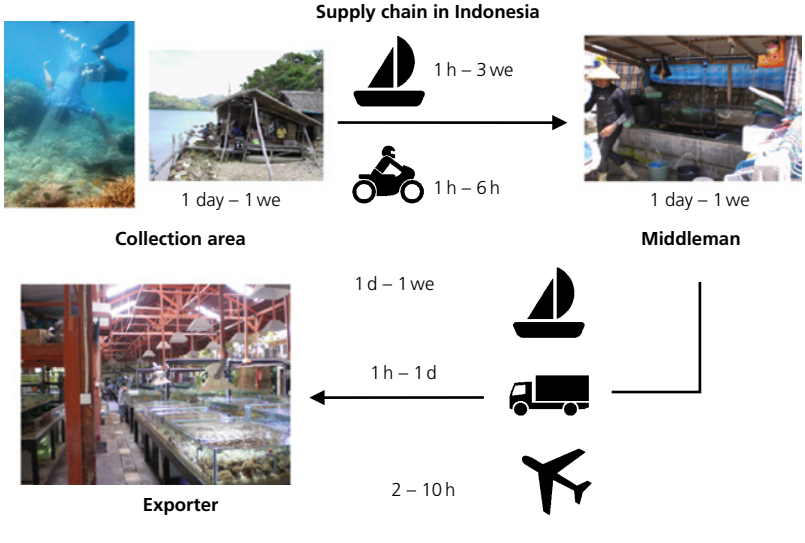


Figure 28.1 Local supply chain in Indonesia up to export level.

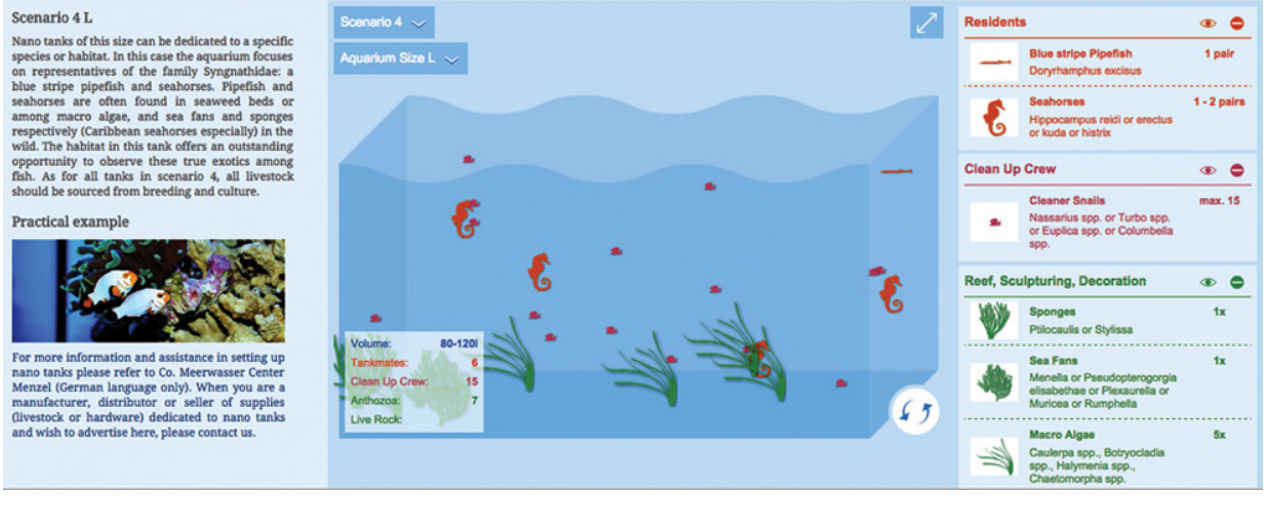


Figure 28.2 AquarioScenario by ESAIA e.V – an online tool to stock nano tanks.

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