

SUSTAINABLE
BIOFLOC SYSTEMS
— FOR —
MARINE
SHRIMP



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Preface

Reducing aquaculture's impact on the environment is now widely recognized by producers, retailers, researchers, and consumers alike as absolutely essential if the industry is to expand to meet the growing global demand for seafood.

Consumers have been prominent in driving this trend by demanding that their seafood purchases satisfy certain sustainability criteria. Their concerns relate to practices that not only ensure a healthy product, but also reduce aquaculture's environmental footprint. In no particular order, these concerns include:

- Discharge of untreated wastewater and pathogens into the environment
- Feed ingredients derived from stressed fishery stocks
- Antibiotics and artificial coloring agents used in production
- Inefficient use of diminishing freshwater resources
- Escape of cultured stock into wild populations
- Preference for locally raised, ultra-fresh products
- Farm-to-fork traceability

Fulfilling many of these criteria inevitably requires a shift from traditional flow-through systems to recirculating aquaculture system (RAS) technologies. Commercial adoption of RAS, however, is proceeding very slowly. Two reasons for this are as follows:

- It is more profitable to “externalize” the cost of water treatment by discharging waste directly into the environment.

- RAS management requires greater technical expertise.

Responsible environmental legislation and consumer preference for sustainably produced seafood both encourage growers to “internalize” water treatment, the former by regulatory enforcement and the latter acting through market forces.

The technical hurdle to expansion is lowered by providing the tools and training needed for modern RAS design and management. This is, in fact, the core motivation behind the present manual that describes the biofloc-dominated (BFD) system developed by Dr. Tzachi Samocha at the Texas A&M AgriLife Research Mariculture Laboratory (ARML) in Corpus Christi, Texas.

Dr. Samocha's system, the product of over 16 years of research, has reached a point at which it is ready for dissemination beyond the aquaculture research community. Parts of it have been reported in the scientific literature and some components have been implemented commercially (*Florida Organic Aquaculture, Fellsmere, FL, US; American Mariculture, St. James City, FL, US; Bowers Shrimp, Palacios, TX, US; several small-scale production operations throughout the US; LAQUA, Palotina, Parana, Brazil, and a number of shrimp farmers in South Korea*), but this manual is the first complete description made available for a wide audience of aquaculture stakeholders.

Among RAS technologies, Dr. Samocha's BFD system stands out by regularly yielding 7–9 kg/m³ of high-quality, marketable shrimp

after about two months of grow-out. This is roughly ten times the yield of traditional flow-through systems, with which well-run BFD systems are cost competitive. Further, this is achieved with effectively zero water exchange, an important feature that enhances this system's claim of environmental sustainability.

Texas A&M has a record of producing practical aquaculture manuals based on decades of research by its staff, students, and collaborators. These manuals (e.g., Treece and Yates, 1988, 2000; Treece and Fox, 1993) have had a recognized impact in advancing commercial aquaculture in Texas and beyond.

The present work aspires to continue that tradition but diverges in that it is not strictly a 'How-To' manual. While it does contain detailed instructions for carrying out procedures essential to BFD production of Pacific White Shrimp, it also provides a thorough account not only of what worked but—importantly—what did *not* work. This gives readers deeper insight into the process that resulted in the most recent BFD system and also alerts them to certain pitfalls to be avoided.

Much of the material in the manual thus does not fit the content and style required by typical scientific journals and so has not previously appeared in print. The text also is purposely written in a more narrative style intended to make it more accessible to a wider audience. The intent is to help aspiring entrepreneurs build and operate a scale version of Dr. Samocha's BFD system to get hands-on experience under the conditions of their site. Such experience will inform their decision of how—or whether—to incorporate BFD technology in

their business plans. The economic analyses of Chapter 13 will prove particularly useful in this regard.

Along with a set of helpful appendices, the manual also touches on more general aspects of closed systems, such as equipment and procedure options, that may be unfamiliar to those without experience with this type of aquaculture.

Finally, it is the hope of the author and his contributors that this manual will prove useful in stimulating adoption of this innovative shrimp production technology and, in some way, contribute to sustainable expansion of the US shrimp aquaculture sector.

Descriptions of procedures, equipment, and materials used in this work sometimes give the name of manufacturers. Mentioning supplier names does not, however, imply endorsement by the authors, Texas A&M AgriLife Research, or the Texas Sea Grant Program.

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All photos presented without credit were taken by former Texas A&M AgriLife Research staff members.

Introduction

Granvil D. Treece

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1.1 DEVELOPMENT OF BIOFLOC TECHNOLOGY FOR SHRIMP PRODUCTION

In the 1980s, most shrimp farms around the world were managed as extensive or semiintensive ponds with low postlarvae (PL) stocking densities (2–5 PL/m²), low yields (0.05–0.1 kg/m²), and high daily water exchange of up to 100% (but typically 10%–15%). Whenever a water quality problem arose—such as high levels of ammonia, low dissolved oxygen, dense algae blooms, or outbreaks of disease or parasitic organisms—it simply was flushed away by replacing a large fraction of poor-quality water with freshly pumped “clean” water. This practice exports water quality problems to the local environment, compromising the health of the surrounding aquatic ecosystem and the quality of intake water pumped by downstream aquaculture farms. This type of water quality management clearly is unsustainable.

Many of these flow-through systems gradually evolved toward smaller ponds (<10 ha) with greater stocking densities (5–20 PL/m²) and greater yields (up to 0.3 kg/m²). This initially worked very well, but in 1988 *Monodon baculovirus* (MBV) infected shrimp farms in Taiwan. Other viral and bacterial diseases soon

followed and this exacted a heavy toll on the worldwide shrimp aquaculture industry well into the 1990s. Some examples of noteworthy diseases include:

- Taura Syndrome Virus (TSV) infected shrimp in ponds in the Taura River area of Ecuador and rapidly spread to other parts of the country.
- White Spot Syndrome Virus (WSSV) started in Asia, arrived in the United States in 1995 and continues to cause problems in Mexico and many other countries.
- Early Mortality Syndrome (EMS), also called Acute Hepatopancreatic Necrosis Disease (AHPND), began in China in 2009 and subsequently spread to Thailand, Vietnam, and Mexico.

This naturally prompted much greater attention to biosecurity, which now became a central concern of shrimp producers. A common response to controlling disease outbreaks was to add a secure holding reservoir to isolate disease-free broodstock. In addition, many farms began treating incoming water. In a dramatic break with contemporary practices, some established farms even undertook a major reconfiguration from traditional flow-through to water-reuse systems.

Over this same period, efforts were made to develop a viable marine shrimp farming industry in the United States. The emerging US industry was faced with overcoming a number of obstacles, foremost of which is a limited growing season. Significantly higher labor costs, higher energy costs, lack of suitable coastal land, and more stringent environmental regulations than in many shrimp producing countries also contributed to the competitive challenge.

With limited potential for development of year-round pond culture, research focused on cost-effective recirculating aquaculture systems (RAS) that operate at much higher biomass ($>5\text{kg}/\text{m}^3$) and with minimal water exchange ($<10\%$ /day). Because these systems use considerably less land and water than traditional ponds, they promised enhanced sustainability, greater biosecurity, and a regular supply of ultra-fresh, high-quality shrimp to domestic markets.

Achieving this objective motivated advances in a number of related areas, especially development of genetically improved lines of commercial shrimp species that are more tolerant of elevated stocking densities, advanced aeration equipment and techniques, efficient ammonia management procedures, and manufactured dry feeds specially formulated for use in high-density closed systems. Regarding genetically improved shrimp, many generations of selective breeding resulted in the production of specific pathogen free (SPF) stocks of Pacific White Shrimp *Litopenaeus vannamei*. This species has since risen to become the primary species cultured in ponds and closed systems around the world. These genetic lines have been a key reason for achievement of the much higher yields in modern aquaculture systems.

RAS may be classified in several ways. One that is useful for present purposes distinguishes between those that raise the target species separately from the bio-treatment processes and those in which the target species is raised in the same water volume as the bio-treatment organisms.

The first includes typical “clearwater” and IMTA (Integrated Multi-Trophic Aquaculture) systems, both of which maintain separate compartments for grow-out and removal of dissolved inorganic nitrogen. Clearwater systems use a traditional biofilter (Timmons and Ebeling, 2013) and IMTA uses macroalgae and bivalves for essential water-treatment tasks (Samocha et al., 2015).

In the second category, the target species is raised together with organisms that remove ammonia and recycle waste products. These may be a mixture of phytoplankton in so-called greenwater systems, or floc aggregates with their microbial community in “brownwater” systems. The biofloc system that is the subject of this manual belongs to the latter type.

1.2 THE BEGINNINGS OF BIOFLOC

In general terms, flocculation is a physical process by which, under favorable conditions, small particles suspended in a fluid coalesce to form aggregates. It has long been employed in wastewater treatment and has an even longer history in food processing, especially in beer and cheese production.

One of the first references in the popular scientific literature to what now is referred to as “biofloc” by the aquaculture community might be traced to a short piece entitled “Food Bubbles” that appeared in the November 1964 issue of the *Scientific American* magazine. It introduced what previously was an unappreciated path in the marine food web: wave-generated bubbles that stimulated formation of organic-rich aggregates. The article stated:

...molecules from the vast supply of organic chemicals dissolved in seawater adhere in large numbers to the “air bubbles” two-dimensional boundary layers. They form clumps of organic material that are eaten by the smallest members of the marine animal population. It was pointed out that the quantity of organic matter in the oceans is at least 50 times greater than that contained in all living plankton.

Application of this natural process to biofloc aquaculture did not immediately follow, of course, as modern aquaculture itself was still in its infancy. One of the first applications of biofloc technology for aquaculture was in the early 1970s at the IFREMER-COP (French Research Institute for Exploitation of the Sea, Oceanic Center of the Pacific) research facility in Tahiti (Emerenciano et al., 2013).

This groundbreaking work focused on the suitability of Pacific White Shrimp, Black Tiger Prawns *Penaeus monodon*, Banana Shrimp *Fenneropenaeus merguensis*, and Western Blue Shrimp *L. stylirostris* for production under biofloc conditions (Aquacop, 1975; Sohier, 1986). Western Blue Shrimp and Pacific White Shrimp subsequently were reared successfully in Aquacop-style systems in Tahiti and Crystal River (USA), thereby demonstrating the feasibility of producing healthy shrimp in biofloc (Bob Rosenberry, personal communication).

These innovative systems encouraged development of suspended aggregates comprised of fragments of shrimp molts, uneaten feed, and feces, along with an attached community of heterotrophic and chemoautotrophic bacteria, microalgae, cyanobacteria, and even micro- and macro-invertebrates. The net effect is that, in a well-operated biofloc unit, these organisms recycle waste material and also provide a supplemental feed for the shrimp (Ray and Lotz, 2014). This both eliminates the need for a dedicated biofilter and reduces use of formulated feed.

At about the same time as the French work began, the Ralston Purina Company in the United States started development of a culture system for marine shrimp. Information from an interview with Harvey Persyn by Bob Rosenberry summarized their early work using the biofloc culture (Bob Rosenberry, personal communication). In a 60-day, temperature-controlled nutrition study using handmade diets, they documented better shrimp performance in tanks with the least water exchange. Follow-up tests using diets with tracer dyes

showed that the feed passed through the shrimp in less than 30 min. The improved performance suggested active shrimp consumption of the feces and floc made of undigested fragments of grain colonized by filamentous bacteria, fungi, and other small organisms. The work also showed that the floc acted as a mini biofilter, serving to detoxify nitrogenous wastes. Adding sugar into culture tanks stimulated the growth of bacteria that were consumed by the shrimp.

Other groups also worked to advance biofloc aquaculture. Steven Serfling and his business partner at the time, Dr. Dominick Mendola, scribbled down some aquaculture concepts that led to trials with “biofloc” shrimp farming. They then built a zero-exchange, intensive, biofloc shrimp farming system in the late 1970s that was capable of producing roughly 22.5 t/ha/yr. This was an unheard-of yield at a time when contemporary production methods produced about one-tenth of that amount. The reported production levels were so extraordinarily high that no one believed them. As a result, they were unsuccessful in attracting investors (Bob Rosenberry, personal communication). Serfling and Mendola were before their time but fortunately the shrimp farming industry finally embraced many of their ideas, especially those related to biofloc shrimp production (Bob Rosenberry, personal communication).

IFREMER initiated a research program in 1980 to advance their initial success by investigating the details of biofloc dynamics. Comprehensive studies explored, among other topics, the relationship between floc bacteria and water quality and the nutritional physiology of shrimp reared in biofloc.

Interest in biofloc continued to spread. Although it had been looked at earlier (see Persyn above) Leber and Pruder (1988) showed that juvenile shrimp reared in organically rich, hypereutrophic pond water and fed a commercial diet ad libitum grew 48%–89% faster than shrimp fed an identical diet but maintained in clear well water devoid of natural productivity. Hopkins et al. (1993) demonstrated that high

shrimp production rates could be achieved with low rates of water exchange and that production could be increased with intensive aeration.

Biofloc culture of tilapia *Oreochromis* sp. and Pacific White Shrimp began in the early 1990s at the Waddell Mariculture Center, Bluffton, South Carolina, US (Hopkins et al., 1993) with shrimp and in outdoor ponds for production of tilapia in Israel (Avnimelech et al., 1992, 1994).

One of the first commercial applications of super-intensive outdoor biofloc shrimp culture was in 1988 at the Sopomer facility in Tahiti, where 20–25 t/ha in two annual crops was produced in 1000-m² concrete tanks operated with limited water exchange (Garen and Aquacop, 1993; Bob Rosenberry, personal communication).

Biofloc technology in outdoor ponds and indoor raceways continues to advance as a result of the work of a number of research teams and commercial groups. This manual deals with indoor biofloc systems, but a brief description of outdoor biofloc ponds is informative and discussed next.

1.3 BIOFLOC POND CULTURE

Belize Aquaculture Ltd. (Fig. 1.1), owned by Barry Bowen and first managed by Robins McIntosh, began experimenting with biofloc shrimp production in 1997 with 660-m² lined ponds. They eventually scaled up to 1.6-ha commercial ponds operated as closed biofloc systems with no water exchange (Boyd and Clay, 2002; Burford et al., 2003). This was a dramatic break from traditional pond practices. Their yields of 11–26 t/ha/crop—much higher than those obtained with the traditional methods of the day—along with lower feed conversion ratios (FCRs) and a more stable culture environment generated a great deal of interest around the industry.

The Belize Aquaculture technology was applied in Indonesia at C.P. Indonesia (now P.T. Central Pertiwi Bahari, C.P. Indonesia).



FIG. 1.1 Belize aquaculture. (McIntosh, R., 2010. Sir Barry Bowen: the Belizean who changed shrimp farming. *Glob. Aquac. Adv.* 13 (3), 6–9, Used with permission.)

They achieved an average production more than 20 t/ha per year in 0.5-ha lined ponds. Research trials yielded more than twice as much: 50 t/ha per year. Combined with partial harvests during a crop, this technology yielded even better results in Medan, Indonesia (2008) and also was successful in Java and Bali (Fig. 1.2).

These techniques since have been refined and adapted to satisfy the requirements of several species raised in different local environments. Outdoor biofloc technology has been applied successfully to production of tilapia in Israel, Pacific White Shrimp in Belize and Indonesia, and Black Tiger Prawns in Australia (Taw et al., 2008).

The exact number of outdoor shrimp farms currently using biofloc technology is not known (Taw, 2010a), but the innovative work at Belize Aquaculture remains at the foundation of all such facilities now in operation.

Plastic liners play an important role in outdoor biofloc farms. They are necessary to eliminate the high scouring rates that would occur with the high aeration rates that are necessary to keep biofloc in suspension. They stabilize

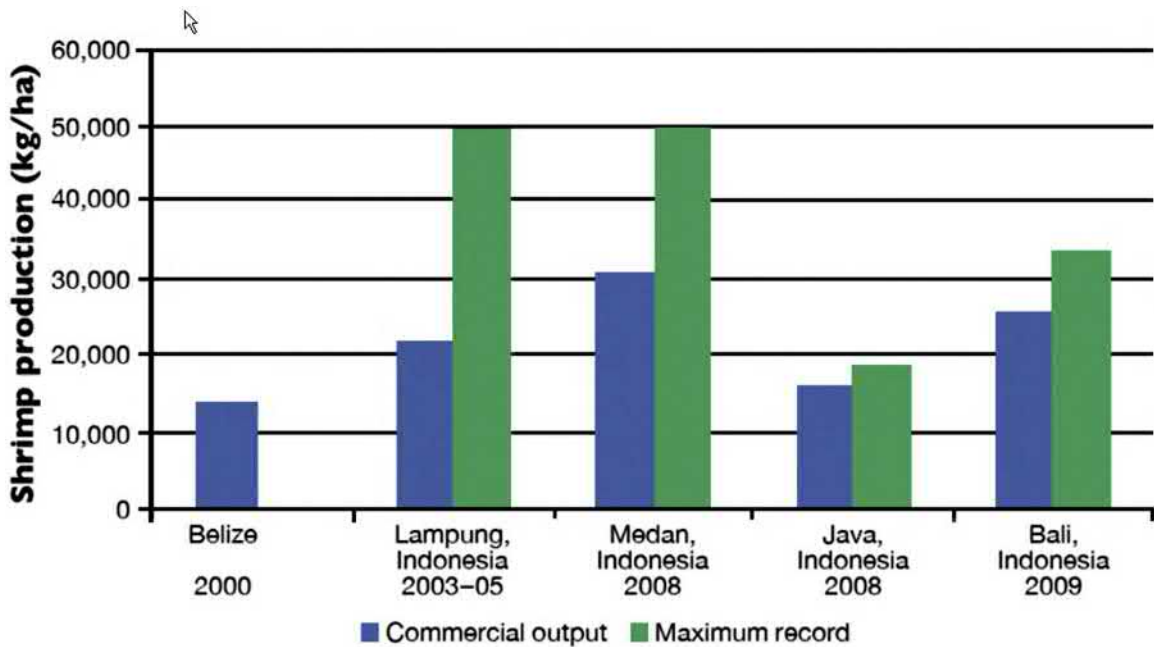


FIG. 1.2 Production at outdoor shrimp biofloc farms. (Taw, N., 2010a. *Biofloc technology expanding at white shrimp farms*. *Glob. Aquac. Adv.* 13 (3), 20–22, Used with permission.)

pond dikes and supply canals and, more importantly, contribute to reducing the disease problems that have plagued traditional operations by enhancing biosecurity (Taw, 2010b; Bob Rosenberry, personal communication). Under no water exchange, lined ponds typically have production and carrying capacities 5%–10% greater than earthen ponds. Shrimp also grow larger and FCRs are desirably lower (1.0–1.3), reducing production costs by as much as 15%–20% (Taw, 2010a).

Blue Archipelago's Arca Biru shrimp farm in Malaysia eliminated viral disease outbreaks by redesigning the farm to operate with limited-exchange biofloc technology in lined ponds (Taw et al., 2011, 2013). This initiative substantially increased growth and production (Table 1.1). An added benefit was reducing the typical 110- to 120-day crop cycle to 90 to 100 days. This improved capital efficiency and

TABLE 1.1 Production Performance of Arca Biru Farm in 2010

Production Parameter	0.4-ha Pond Lined, Biofloc	0.8-ha Pond Lined, Semi-Biofloc	0.8-ha Pond Lined Dikes
Number of ponds	2	19	119
Aerator energy (hp)	14	24	20
Stocking density (shrimp/m ²)	130	110	83
Cycle (days)	90	101	111
Survival (%)	89	81	83
Mean body weight (g)	18.8	18.3	17.8

Continued

TABLE 1.1 Production Performance of Arca Biru Farm in 2010—cont'd

Production Parameter	0.4-ha Pond Lined, Biofloc	0.8-ha Pond Lined, Semi-Biofloc	0.8-ha Pond Lined Dikes
Feed conversion ratio	1.39	1.58	1.77
Average daily growth (g)	0.21	0.18	0.16
Average harvest (t)	9.0	12.9	9.6
Production (kg/ha)	22.5	16.2	12.0
Production/power input (kg/hp)	643	540	481

(Taw, N., 2011. Malaysia shrimp farm redesign successfully combines biosecurity, biofloc technology. *Glob. Aquac. Adv. March/April*, 74–75, Used with permission.)

production by increasing the number of annual crops from 2 to 2.5.

Outdoor biofloc pond technology continues to expand. Avnimelech's (2015) practical manual on biofloc pond culture should be consulted for details on pond biofloc practices.

1.4 INDOOR BIOFLOC

Whether operated with traditional methods or biofloc practices, outdoor pond management differs from indoor tank management. This is partly a matter of scale: the more compact size of indoor culture allows greater control over the culture environment and more attentive husbandry, both of which combine to allow much higher stocking densities. This point is illustrated clearly by comparing the grow-out area needed by a traditional shrimp farm (50shrimp/m², 1crop/yr) to match the production of a super-intensive recirculating system (600shrimp/m², 3.5crops/yr), with the much



FIG. 1.3 Traditional farm compared to the area required for comparable super-intensive production [red area—(light gray square in print version)]. (Photo by Craig Browdy, Waddell Mariculture Center, Bluffton, South Carolina, USA. Used with permission.)

smaller area of the latter indicated by the red (light gray square in print version) area in Fig. 1.3.

The decision to invest in outdoor ponds or indoor tanks rests primarily on regional climate and availability of land. The general unsuitability of both these factors in the United States has motivated research into development of indoor systems rather than outdoor ponds.

Extensive work in high-density, biofloc-dominated, no water exchange in greenhouse-enclosed raceways was initiated by the Waddell Mariculture Center (WMC), Bluffton, South Carolina, USA in early 2001 (Fig. 1.4). The studies conducted at the center over a decade and a half focused on system design and management practices refinements in order to make these systems more economically viable.

There are advantages and disadvantages of using indoor biofloc systems. High stocking densities are an advantage that promises greater yields and more efficient use of space, but limited water exchange produces water quality problems that do not always arise in traditional systems. These problems can arise very quickly in densely stocked systems and, if not quickly and correctly addressed, can decimate a crop in a matter of hours. Noteworthy advantages



FIG. 1.4 Biofloc technology in practice at Waddell Mariculture Center in Bluffton, South Carolina, USA. (Craig Browdy, Waddell Mariculture Center, Bluffton, South Carolina, USA. Used with permission.)

and disadvantages of indoor biofloc systems compared to traditional ponds—some of which also apply to nonbiofloc indoor systems and outdoor biofloc systems—are itemized as follows.

1.4.1 Advantages of Indoor Biofloc Systems

1. Water conservation: Water use is greatly reduced, recycled, and available for multiple crops (Table 1.2; Tacon et al., 2002).

TABLE 1.2 Amount of Water to Produce 1-kg Shrimp

Shrimp Species	Water Exchange (%/day)	Stocking Density (#/m ²)	Water Use (L/kg shrimp)	References
<i>L. setiferus</i>	25.0	40	64,000	Hopkins et al. (1993)
<i>L. setiferus</i>	2.5	40	9000	Hopkins et al. (1993)
<i>L. setiferus</i>	0.0	20	6000	Hopkins et al. (1993)
<i>L. vannamei</i>	<0.5	100	483	Otoshi et al. (2002)
<i>L. vannamei</i>	<0.5	200	370	Otoshi et al. (2002)

Continued

TABLE 1.2 Amount of Water to Produce 1-kg Shrimp—cont'd

Shrimp Species	Water Exchange (%/day)	Stocking Density (#/m ²)	Water Use (L/kg shrimp)	References
<i>L. vannamei</i>	<0.5	300	352	Otoshi et al. (2002)
<i>L. vannamei</i>	0.4	301	195	Otoshi et al. (2009)
<i>L. vannamei</i>	0.1	408	163	Otoshi et al. (2009)
<i>L. vannamei</i>	0.2	450	98	Samocha (unpub. data)
<i>L. vannamei</i>	2.0	700	219	Moss et al. (2005)
<i>L. vannamei</i>	<0.5	828	402	Otoshi et al. (2007)

(USDA USMSFP presentation at Panel Review in Ocean Springs, Mississippi, USA.)

2. Stable water quality: Lower diel fluctuations in certain water quality properties, especially dissolved oxygen and pH.
3. Reduced fertilizer use: Many nutrients are recycled within the culture tank, greatly reducing the need for inputs of chemical fertilizers.

4. Small footprint: Occupies much less area than ponds per unit shrimp produced.
5. Year-round production: Can operate throughout the year, despite local climate.
6. Faster growth: Supports faster shrimp growth rates (Moss et al., 1999; Otoshi et al., 2001) because of greater control over feeding and temperature.
7. Lower susceptibility to disease: Shrimp are less susceptible to pathogens common in traditional systems (Taw, 2015) because of improved biosecurity.
8. More efficient use of protein in feed: Efficiency is 45%, compared to 25% in conventional ponds (Avnimelech et al., 1994; Boyd and Tucker, 1998; McIntosh, 2001) because waste nutrients are recycled into bacterial protein in floc that is consumed by shrimp.
9. Lower feed requirements: FCRs of 1.0–1.3 reduce production expenses by 15%–20% (Avnimelech, 2009).
10. Higher yields: Production is 5%–10% greater than that from traditional ponds (Avnimelech, 2009).
11. Sustainability: Less impact on the environment than open pond culture.

1.4.2 Disadvantages of Indoor Biofloc Systems

1. High capital investment per unit area: Compared to ponds, capital investment is greater, but much less land is needed for commercial levels of production.
2. Liner expense: Membrane liners are expensive and need constant maintenance.
3. High energy input: Higher energy expenses for aeration and pumping are incurred to operate biofloc facilities (Avnimelech, 2009).
4. Power failure is critical: More than an hour without power can result in crop loss.
5. Operating complexity: Management is more complicated than in traditional aquaculture, thus requiring a more technically trained staff and higher labor costs.
6. Toxins: Without adequate remediation, undesirable substances—nitrate, phosphate, and heavy metals—accumulate in reused culture water.
7. Disease risk: Disease, mainly *Vibrio*, has afflicted some closed systems. Despite the ever-present threat of disease common to all aquaculture systems, Horowitz and Horowitz (2002) found that limited-exchange systems reduce the threat and spread of pathogens.

In addition to the material contained in this manual, important aspects of indoor culture are described by Cohen et al. (2005), Hargreaves (2006), and Mishra et al. (2008). More technical perspectives are available in Ebeling et al. (2006) and Samocha et al. (2007).

1.4.3 Commercial Indoor Operations

Indoor biofloc technology recently has been applied successfully in insulated buildings and greenhouses in the United States, South Korea, Brazil, Italy, Germany, Australia, and China. The three main indoor facilities in the United States produced about 113.4t of shrimp in 2014. This did not include Marvesta Shrimp (Maryland), RDM Aquaculture (Indiana), and a few other small, indoor producers in Michigan, Massachusetts, Iowa, and Hawaii. Small-scale, super-intensive greenhouse shrimp farms such as Marvesta are capable of producing 45t/yr of fresh shrimp in a combined volume of $\sim 570\text{m}^3$ (Bob Rosenberry, personal communication).

As of 2016, facilities operating in the United States include Marvesta, RDM Aquaculture, Blue Ridge Aquaculture (Virginia), Global Blue Technologies (Texas), Ithuba Shrimp, Florida Organic Aquaculture (FOA), and American Mariculture (Florida).



FIG. 1.5 American Mariculture, Inc. on Pine Island, Florida, USA. (Robin Pearl, American Mariculture. Used with permission.)

American Mariculture (Fig. 1.5) runs a super-intensive biofloc farm for Pacific White Shrimp. Shrimp, marketed under the Sun Shrimp brand, reportedly are raised without chemicals, antibiotics, or preservatives in a biosecure facility consisting of 3.4ha of rectangular tanks in greenhouses (Bob Rosenberry, personal communication).

FOA was a large-scale indoor shrimp biofloc company (Fig. 1.6) that cultured Pacific White Shrimp in water drawn from a brackish water well with 32ppt salinity. FOA sold live and “fresh, never frozen” shrimp, and frozen tails in their farmer’s market retail store in Fellsmere. This farm closed its doors late 2017.

FOA’s maturation, hatchery, and nursery buildings are now being operated by Benchmark Genetics (<https://www.benchmarkplc.com/what-we-do/genetics/> Accessed 25 May 2019). They have set up a subsidiary company to run their site in Fellsmere. That company is called Akvagenetics, while the two grow-out raceway buildings with total area of 3.8ha is being operated by a new group named Pristine Water Aquaculture.

Several small-scale, family-run, indoor biofloc shrimp systems have been set up across the United States since 2010, mostly in inland locales. Many of these have been constructed in converted dairy, turkey, or hog production facilities. In 2013 Marvesta Shrimp Farms (Maryland, USA) partnered with Indiana-based RDM Aquaculture LLC to establish a franchise system for small-scale, zero-exchange indoor shrimp production. RDM reports that 18 such facilities have been established since 2010. Information can be found at the RDM and Marvesta websites, respectively: <http://www.rdmshrimp.com/> and <http://marvesta.Com/marvesta-partnership-with-rdm-llc/#shash.vo8AnydM.dpuf3> (Accessed 17 October 2018).

Commercial adoption of limited discharge in Texas began in the late 1980s with nursery ponds on farms in Olivia and the Rio Grande Valley. This practice initially was implemented to avoid seasonally low water temperatures experienced



FIG. 1.6 Florida Organic Aquaculture’s indoor biofloc shrimp culture raceways. (Granvil Treece. Used with permission.)



FIG. 1.7 Global Blue Technologies hatchery and grow-out cells near Rockport, Texas, USA. (Photo by Eduardo Figueras. Global Blue Technologies. Used with permission.)

in greenhouse nursery ponds when water from these ponds was mixed with water drawn from outdoor ponds. Global Blue Technologies built a pilot indoor facility in Port Isabel, Texas in 2012 and has since expanded to commercial scale under large inflatable greenhouses near Rockport, Texas (Fig. 1.7). Global Blue also has a hatchery capable of producing 20million postlarvae/yr. Another Texas company, Natural Shrimp in San Antonio, projected 2.7t/wk of fresh shrimp but the never reached this goal.

Bowers Shrimp Farm (Collegeport, Texas, USA) modified the Texas A&M AgriLife Research Mariculture Lab (ARML) biofloc-dominated nursery system described in this manual. They subsequently experienced significant production and farm efficiency gains (Morris, 2014, 2015). Their previous pond configuration limited their stocking flexibility because each grow-out only could be stocked from an adjacent nursery pond, and the nursery ponds could not be stocked until outdoor temperatures were sufficiently high to ensure good growth and survival. The farm added a 1250-m² indoor biofloc nursery in 2014 (Fig. 1.8) that eliminated this problem by:

- head-starting the first crop while outdoor temperatures were still too cold



FIG. 1.8 Commercial shrimp nursery in Texas using biofloc. The eight concrete raceways are modeled on the 100-m³ Texas A&M-ARML raceways. (Tim Morris, Bowers Shrimp. Used with permission.)

- head-starting the second crop while extending the culture period of the first
- stocking any pond on the farm from the centralized nursery
- securely storing juveniles in the indoor nursery until ponds are ready to be stocked

The biofloc nursery is credited with other advantages, including lower electricity expenses in the smaller, insulated nursery space; an extended production period with increased shrimp size at harvest; reduced need for nitrogen fertilizers to stimulate plankton blooms in ponds; increases in farm yield; and a more efficient use of capital assets.

There are indoor biofloc facilities in other countries, but owing to the generally proprietary nature of commercial operations, few published details are available. One, “Eco-Farming,” near Padua, Italy, recently began production of Pacific White Shrimp. Another, located in Medina del Campo outside of Madrid, Spain, is a joint venture with the Natural Shrimp Company and also cultures Pacific White Shrimp. Fig. 1.9 illustrates the layout of their facility.

Large-scale projects also are underway in China. The stated goal of the South China Sea Fisheries Research Institute (Guangdong

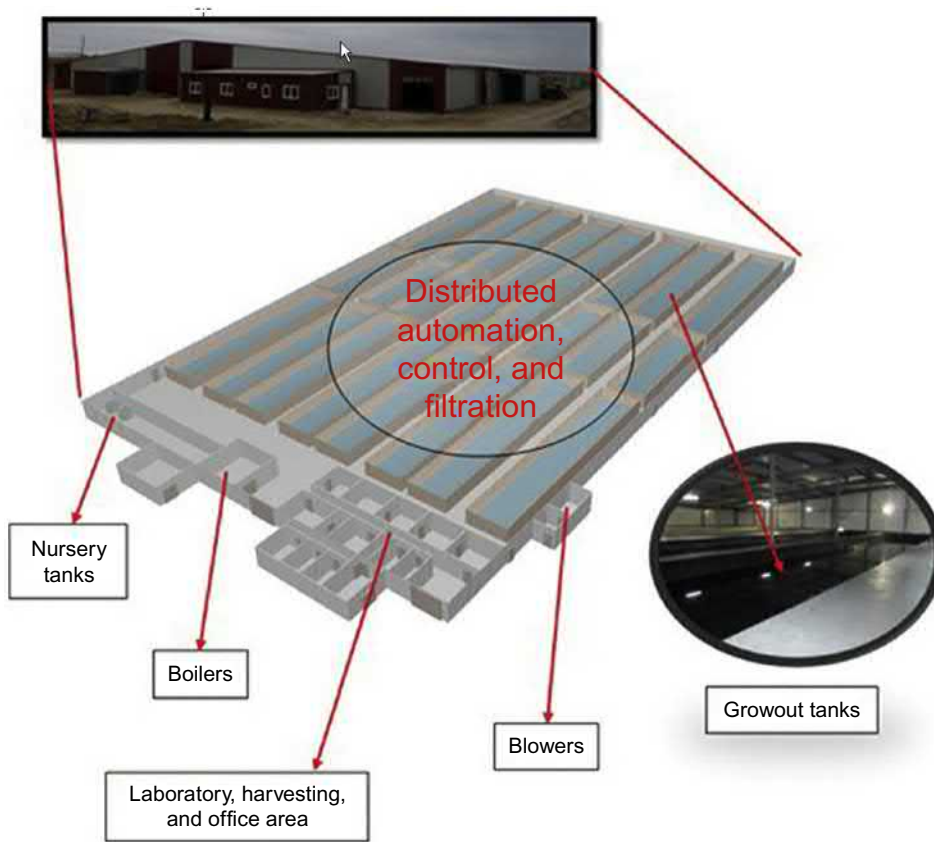


FIG. 1.9 Indoor shrimp production facility in Medina del Campo, Spain. (Source: Natural Shrimp International, www.naturalshrimp.com. Accessed 26 September 2018.)

Province) is to be the world's largest producer of indoor biofloc shrimp (Bee Teo, personal communication). Fig. 1.10 shows about a one-quarter of their present facility.

As is the case in any sector in which new production technology is introduced, several US indoor closed-system shrimp culture operations have failed over the years. Among these are several clearwater systems, including King James Shrimp/Aquabiotics (near Chicago, Illinois), Penbur Farms (Buda, Texas), A&P Aquaculture (Rockport, Texas), and Ganix (Las Vegas, Nevada; Fig. 1.11). Another was a biofloc



FIG. 1.10 Indoor production facility for *L. vannamei* in China. (Bee Teo, Austin, Texas, USA. Used with permission.)



FIG. 1.11 The Ganix Blue Oasis farm in Las Vegas, Nevada, USA was very short lived. (Photo by Adrian Zettell, Newburg, North Dakota, USA. Used with permission.)

facility, Magnolia Shrimp, in Kentucky. Natural Shrimp International (La Coste, Texas) has started and terminated production several times over the past 10 years using clearwater and bio-floc. Biofloc production was stopped after bacterial (*Vibrio*) problems.

1.4.4 Economics of RAS and Biofloc Systems

The economics of the biofloc system described in this manual is discussed in detail in Chapter 13. A broader comparison with

other systems is briefly summarized here. Table 1.3 compares production costs in earthen ponds and RAS using data from the USDA-funded US Marine Shrimp Farming Program. Pond data are from an intensive farm in Arroyo City, Texas while data for the RAS systems was obtained from trials conducted at the Oceanic Institute, Hawaii over 4 years (2005 to 2007, 2009).

Production costs per unit shrimp were less in RAS than in earthen ponds. Even at higher stocking densities, survival and growth in the RAS trials were better. At harvest, shrimp produced in RAS were just as large and, in some cases, even larger than those from ponds.

Closed, indoor super-intensive RAS can be operated for less than earthen ponds (Moss and Leung, 2006). See cost comparison in Table 1.3 between a farm and a closed, indoor super-intensive RAS system. The cumulative distribution of total cost for ponds and RAS (Fig. 1.12) indicates that RAS has a lower cost per unit weight than ponds.

The Texas A&M-ARML has reduced indoor biofloc operating costs from \$11.00/kg, the US average for super-intensive systems, to about \$4.53/kg. That work also suggests the feasibility of extending the number of annual crops from 3.5 to 5.5. Economic projections suggest that these

TABLE 1.3 Grow-Out Trial Comparison

	Texas Farm 2001–2002	Hypothetical 1999	USMSFP 2005	USMSFP 2006	USMSFP 2007	USMSFP 2009
Stocking (shrimp/m ²)	50	140	705	401	828	450
System size (m ²)	20,234	n/a	58.4	75	337	40
Survival (%)	50.0	80.0	70.3	90.6	67.9	96.3
Harvest weight (g)	18.0	23.0	17.9	21.0	18.3	23.1
Growth (g/wk)	1.00	1.50	1.37	1.49	1.50	1.39
Production (kg/m ²)	0.45	2.60	8.90	7.60	10.30	9.75
Cost (\$/kg)	6.72	13.05	4.96	4.85	3.66	5.51

Note cost difference in farm and indoor RAS in bold.

(USMSFP at USDA review panel, Shaun Moss, personal communication.)

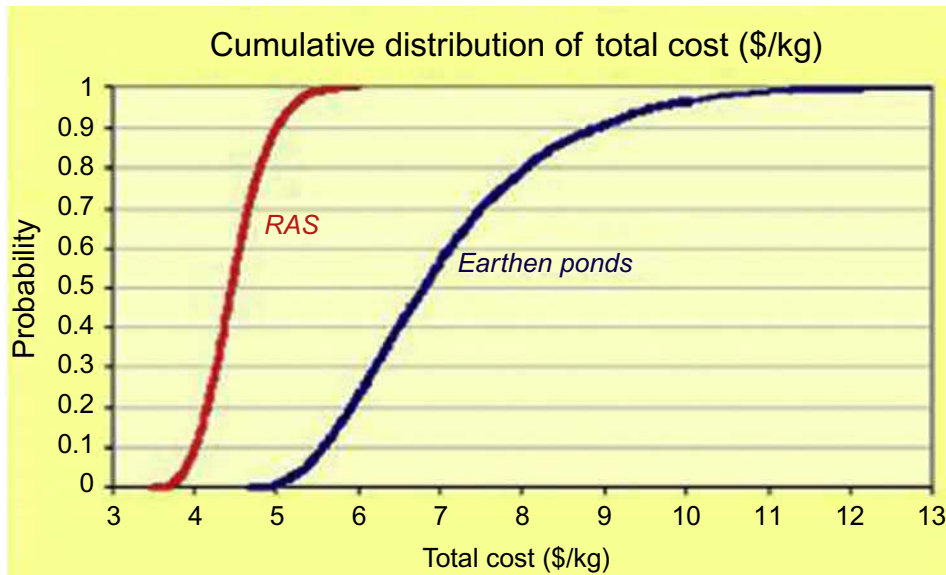


FIG. 1.12 Cumulative distribution of total cost (\$/kg) for earthen ponds vs. RAS. (From Moss, S.M., Leung, P.S., 2006. Comparative cost of shrimp production: earthen ponds vs. recirculating aquaculture systems. In: Leung, P.S., Engle, C. (Eds.), *Shrimp Culture: Economics, Market, and Trade*. Blackwell Publishing, Ames, Iowa, USA, pp. 291–300.)

systems can be profitable when targeting niche markets for live or fresh (never frozen) shrimp (Hanson and Posadas, 2004; Hanson et al., 2013).

1.4.5 Current Issues With Indoor Biofloc Shrimp Culture

Advances in indoor biofloc systems have been impressive, but current knowledge certainly is not complete. For example, the failure of some indoor biofloc projects can be traced to the complex interrelationships that characterize the diverse and difficult-to-control microbial biofloc community. This assemblage can be unstable in relatively small tanks stocked at high densities and driven by the large input of feed required for good shrimp growth. If the microbial community of the biofloc system is not balanced properly, harmful chemicals can accumulate, particularly ammonia, nitrite, and nitrate. Water quality changes are exacerbated when water is reused over multiple crop cycles.

Biofloc systems also are susceptible to outbreaks of noxious organisms, such as *Fusarium*

solani (responsible for closure of a commercial facility in Kentucky) and *Vibrio* sp. (which caused a commercial operation in Texas to abandon biofloc). The Waddell Center research system has experienced outbreaks of the cyanobacterium *Synechococcus* sp. and the dinoflagellates *Gymnodinium* sp. and *Pfiesteria piscicida*, each with an unpredictable and decidedly negative impact on production.

The Texas A&M AgriLife Research indoor biofloc system also has experienced crop-threatening outbreaks of *Vibrio*. Along with many other relevant topics, ways to avoid such diseases (and to treat them if they arise) are addressed in detail in this manual.

1.4.6 The Manual

The principal author of this manual has worked for more than a decade at Texas A&M AgriLife Research to advance the concept of high-density indoor, year-round production of shrimp using biofloc technology. His research on biofloc design and operation has resulted in

yields of marketable Pacific White Shrimp greater than 9.7 kg/m³/crop (Braga et al., 2016; Magalhães et al., 2013; Samocha, 2010). This is nearly 10 times greater than typical yields from the pond culture methods that supply most of the \$4.5 billion of shrimp imported annually to the US (USDA, 2013). The biofloc approach dominates current development of indoor shrimp cultivation.

This Texas A&M-supported R&D has reached a point at which a detailed description of the design and operation of this system are ready to be communicated beyond the research community to the US commercial aquaculture sector. As such, this manual is intended to provide a comprehensive description of the Texas A&M-ARML's indoor biofloc production system.

The manual is divided into 15 chapters and an Appendix. It begins with a very brief introduction to Shrimp Biology for readers coming to this subject without a background in shrimp aquaculture.

A general introduction to the composition and function of Biofloc comes next, with particular attention paid to concepts needed in the following chapters. References are provided for more detailed discussions. Of particular note, the biofloc production technology described in this manual differs significantly from the approach of Avnimelech (2015) in that it does not require continuous organic carbon supplementation to sustain heterotrophic microbial communities. Rather, as explained in the manual, the system can be described as mixotrophic, biofloc-dominated.

The sources and treatment of Water for indoor biofloc aquaculture are considered next. Disinfection, a key first-step in ensuring a biosecure culture environment, is explained, along with details of protocols used at the Texas A&M-ARML facility.

The Site Selection and Production System Requirements chapter discusses the main

considerations in choosing a production site and lists the equipment needed to outfit a biofloc production facility. An addendum to this chapter describes the Texas A&M-ARML Systems, providing detailed descriptions of the two main experimental production systems.

Once a site has been chosen and the necessary equipment installed, it must be prepared for production. This is the subject of the next chapter, System Treatment and Preparation.

The following chapter, Water Quality Management, explains the fundamentals of this very important aspect of any form of successful aquaculture, with particular attention to the control of water quality in indoor biofloc systems.

The Nursery Phase of indoor shrimp biofloc production, as developed at the Texas A&M-ARML, is described in detail.

The Grow-out Phase developed at the Texas A&M-ARML, which has produced up to 10 kg/m³, is detailed next. As with the previous chapter, this narrative outlines the lessons learned as the system evolved over more than ten years. This is followed by a chapter on Shrimp Harvest.

Waste Treatment and Disposal protocols that satisfy environmental regulations are an essential part of the indoor biofloc work-flow. This chapter outlines general considerations and presents those practices implemented at the Texas A&M-ARML.

Disease and Biosecurity issues are raised as needed in previous chapters, but they have become so critical to successful aquaculture that important considerations are collected in their own chapter.

The very important topic of the Economics of Super-intensive, Recirculating Shrimp Production Systems is presented in this chapter. It summarizes the results of simulations of various production scenarios using data derived from the indoor biofloc production runs conducted at the Texas A&M-ARML and described in earlier sections of this manual.

One chapter details the research conducted at the Texas A&M-AMRL since 1998, current and future research directions, and perspectives.

The final chapter contains a Troubleshooting Table listing potential problems that may be encountered when operating these systems, along with possible causes, potential solutions, and links to the relevant section of the manual for further detail.

A set of relevant topics has been assembled in the Appendix. They range from explanations on performing certain calculations to additional background on water quality and relevant technical sheets. Excel sheets are attached to provide example forms and templates for data recording and calculations, and a series of short videos supplement explanations in the manual.

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Shrimp Biology

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2.1 MORPHOLOGY

2.1.1 External Morphology

A basic understanding of shrimp morphology and physiology is important for monitoring development, and identifying and communicating problems during culture. An annotated view of external shrimp morphology is shown in Fig. 2.1 and Fig. 2.2.

2.1.2 Internal Morphology

An annotated view of internal shrimp morphology is shown in Fig. 2.3.

2.2 LIFE CYCLE

Pacific White Shrimp spawn in the open ocean in salinity of about 35 ppt and eggs hatch after 14–16 h at 28°C (Juarez et al., 2010). The planktonic larvae then progress through five naupliar sub-stages over 48 h, three protozoal (also called zoea) substages over 120 h, and three mysis substages

over 72 h (Kitani, 1986). They subsequently become postlarvae (PL) and assume a benthic lifestyle. Postlarvae are designated by the number of days after their metamorphosis, that is, PL1 for one-day-old postlarva, PL2 for two-day-old, and so on. Postlarvae migrate inshore and grow through juvenile and subadult stages. Adults then migrate back into oceanic waters to spawn (Fig. 2.4).

In shrimp aquaculture, adults are spawned in hatchery tanks under optimal conditions. Fertilized eggs are collected and stocked in larval rearing tanks. After hatching, they are reared through their larval stages and offered live feed—microalgae and *Artemia* nauplii—and artificial feed in liquid and dry forms. Shrimp are weaned onto artificial feed as early postlarvae and typically stocked in nursery tanks at PL7–12 (1.5–4.9 mg), about 3 weeks after hatching. Shrimp then are transferred to secondary nursery or grow-out tanks/ponds. Depending on the producer's preference, this varies from a few tens of mg to a few grams per individual. Market-size shrimp are harvested in 3–6 months at 18–25 g.

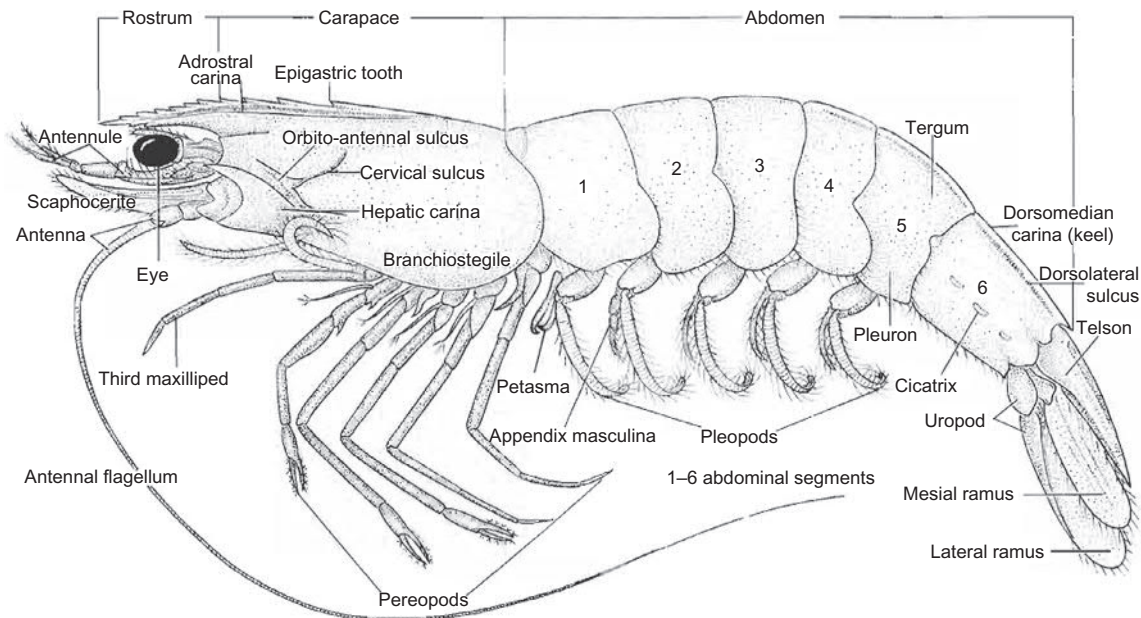


FIG. 2.1 Lateral view of the external morphology of a generalized penaeid shrimp. (Farfante, I.P., 1988. *Illustrated key to Penaeid shrimps of commerce in the Americas*. NOAA Technical Report NMFS 64, 1–33. Used with permission.)

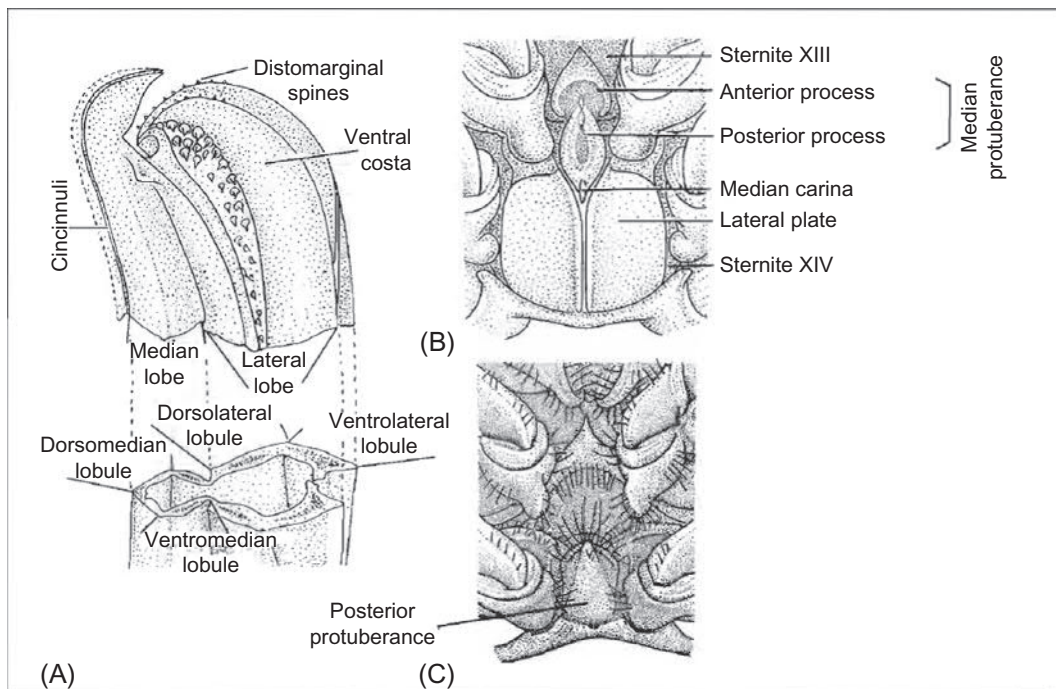


FIG. 2.2 External genitalia of generalized adult penaeid shrimp, (A) petasma (male), (B and C) thelyca (female). (Farfante, I. P., 1988. *Illustrated key to Penaeid shrimps of commerce in the Americas*. NOAA Technical Report NMFS 64, 1–33. Used with permission.)

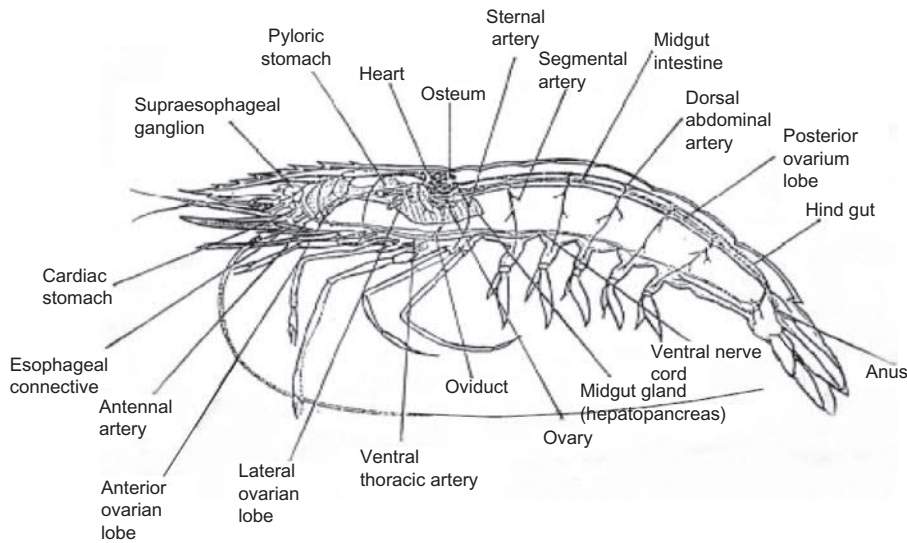
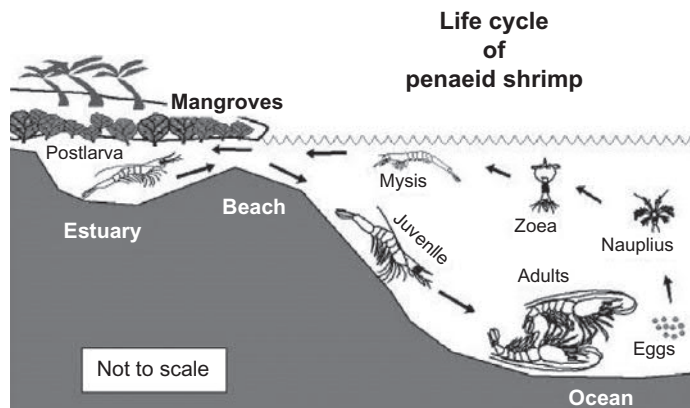


FIG. 2.3 Lateral view of the internal morphology of an adult female penaeid shrimp ("shrimp-culture.blogspot.com").

FIG. 2.4 Typical lifecycle of penaeid shrimp.
(Bob Rosenberry, personal communication. Used with permission.)



2.3 NUTRITION

Nutrition plays a key role in aquaculture as it influences growth, health, product quality, and waste generation. Development of nutritious, efficiently delivered, and cost-effective feeds depends on meeting the requirements of the target species with a well-balanced diet and optimal feed management.

Assessing shrimp nutrient requirements is challenging as a consequence of their behavior of breaking feed pellets outside of their mouth before ingestion. Feed manufacturers thus must ensure that pellets are sufficiently stable to endure long immersion in water. The particle or pellet size also must be adapted to shrimp size because this influences consumption and growth.

Protein is the most expensive component of shrimp diets. Hence nutrition studies often start by estimating the optimal dietary protein level. Protein requirements are determined in dose-response studies in which diets with graded levels of protein are fed and the resulting growth is measured. Protein requirement then is estimated as the level below which growth will be depressed, or above which it will not increase. A disadvantage of such studies is that protein intake is defined only as the dietary inclusion level, with limited information on feed intake. Expressing protein requirement in this way is incomplete. Also, because protein can function as an energy source, the optimal ratio of dietary energy to protein is a critical consideration.

Pacific White Shrimp have a dietary protein requirement of 15% when fed *ad libitum* 15 times/day in the presence of biofloc, which may mask their true requirement (Aranyakananda and Lawrence, 1993). The optimal crude protein level is between 20% and 24% for postlarvae of 0.9 and 1.0 mg weight fed *ad libitum* with continuous feeders (Velasco et al., 2000). Using more controlled inputs, a protein requirement of 32% for juveniles and subadults is recommended (Kureshy and Davis, 2002). Such broad variation is not surprising given that protein requirement varies with size, physiological state, water temperature, growth rate, access to other nutrient sources such as biofloc, and of course, feed intake.

In view of these difficulties, Lupatsch et al. (2008) have proposed a different approach to determining shrimp protein and energy requirements. This approach sums requirements for maintenance and growth. The metabolic expenditure for maintenance at a given temperature is mainly a function of body weight, and the requirement for growth is dependent on the amount and composition of the weight gain, including the energy cost to deposit the new growth.

The daily digestible energy requirement for maintenance was estimated to be 345 J and, for digestible protein, 7.5 mg/g shrimp biomass.

The energy and protein contents per gram of weight gain averaged 4.844 kJ and 172 mg, respectively. With retention efficiencies of 0.31 and 0.44 for digestible energy and protein, respectively, absolute energy and protein demands can be estimated (Table 2.1).

TABLE 2.1 Calculations of Daily Energy and Protein Requirements for Pacific White Shrimp

Body Weight (g/shrimp)	2	10		
Weight gain ^a (g/shrimp per day)	0.075	0.191		
Energy requirement (kJ/shrimp per day)				
DE _{maint} ^b	0.690	3.450		
DE _{growth} ^c	1.171	2.988		
DE _{maint+growth} ^d	1.861	6.438		
Protein requirement (g/shrimp per day)				
DP _{maint} ^e	0.015	0.075		
DP _{growth} ^f	0.029	0.075		
DP _{maint+growth} ^g	0.044	0.150		
Feed formulation				
GE content of feed ^h (kJ/g)	15.0	17.5	15.0	17.5
Feed intake (g/shrimp per day)	0.155	0.133	0.536	0.460
CP content of feed ⁱ (mg/g)	335	391	328	383
FCR	2.07	1.78	2.81	2.41
DP/DE ratio (mg/kJ)	23.8	23.8	23.2	23.2

^a Anticipated weight gain at 27°C.

^b Digestible energy (DE) required for maintenance = 345 J/g BW per day.

^c Digestible energy required for growth = expected weight gain × energy content of gain (4.844 kJ/g) × 3.23 (cost in units of DE to deposit one unit of energy as growth).

^d Total DE required for maintenance and growth.

^e Digestible protein (DP) required for maintenance = 7.5 mg/g BW per day.

^f Digestible protein required for growth = expected weight gain × protein content of gain (172 mg/g) × 2.27 (cost in units of DP to deposit one unit of protein as growth).

^g Total DP required for maintenance and growth.

^h Assumes energy digestibility of 80%.

ⁱ Assumes protein digestibility of 85%.

(After Lupatsch et al. (2008))

The energy and protein consumed by shrimp naturally depends on the energy and protein content of the feed. Therefore the feed protein level will change according to the selected energy density of 15 or 17.5 kJ/g (Table 2.1). Shrimp thus could be fed lower energy and protein diets, provided that they consume sufficient feed to acquire the energy and protein needed for maximum growth. In this case, the Feed Conversion Ratio (FCR) would be higher (Table 2.1).

Using this approach to quantify energy and protein demands, it is possible to estimate the biological and economic efficiency of different feeds and culture systems.

In addition to protein and nonprotein energy such as carbohydrates and lipids, formulated feeds must supply minimum levels of vitamins and minerals for optimal growth. A summary of dietary requirements for several shrimp species is presented in Table 2.2.

TABLE 2.2 Recommended Dietary Vitamin and Mineral Requirements for Shrimp

	Requirement (mg/kg diet)	References
Vitamins		
Vitamin A (<i>L. vannamei</i>)	1.44	He et al. (1992)
Vitamin D (<i>P. monodon</i>)	0.1	Shiau and Hwang (1994)
Vitamin E (<i>L. vannamei</i>)	99	He and Lawrence (1993a)
Vitamin K (<i>P. monodon</i>)	30–40	Shiau and Liu (1994)
Thiamine, B1 (<i>M. japonicus</i>)	60–120	Deshimaru and Kuroki (1979)
Riboflavin, B2 (<i>P. monodon</i>)	25	Chen and Hwang (1992)
Pyridoxine, B6 (<i>L. vannamei</i>)	80–100	He and Lawrence (1991)

Continued

TABLE 2.2 Recommended Dietary Vitamin and Mineral Requirements for Shrimp—cont'd

	Requirement (mg/kg diet)	References
Pantothenic Acid, B5 (<i>P. monodon</i>)	100–140	Shiau and Hsu (1999)
Niacin, B3 (<i>P. monodon</i>)	7.2	Shiau and Suen (1994)
Biotin, B7 (<i>P. monodon</i>)	2.0–2.4	Shiau and Chin (1998)
Inositol (<i>P. monodon</i>)	3400	Shiau and Su (2004)
Folic Acid, B9 (<i>P. monodon</i>)	1.9–2.1	Shiau and Huang (2001)
Cyanocobalamin, B12 (<i>P. monodon</i>)	0.2	Shiau and Lung (1993)
Choline (<i>P. monodon</i>)	6200	Shiau and Lo (2001)
Vitamin C (<i>L. vannamei</i>)	90–120	He and Lawrence (1993b)
Micro Minerals		
Manganese (<i>L. vannamei</i>)	required	Koshio and Davis (2010)
Iron (<i>L. vannamei</i>)	dispensable	Koshio and Davis (2010)
Zinc (<i>L. vannamei</i>)	15	Davis et al. (1993a)
Copper (<i>L. vannamei</i>)	35	Davis et al. (1993b)
Selenium (<i>L. vannamei</i>)	0.2–0.4	Davis (1990)

Postlarval feeds are typically nutrient dense with high lipid and protein levels. These decrease as shrimp grow. Although low-protein diets or those of low nutrient density can be used to rear shrimp, they are not recommended for intensive systems. Biofloc is a source of nutrients, but the levels generally are not adequate to meet all nutritional requirements and nutrient content varies significantly over time and culture conditions.

Hence it is critical that a nutritionally complete feed is utilized. In addition to protein and lipid levels, one of the primary considerations in choosing a feed is digestibility. The protein or nutrient density of the diet will directly affect growth and nutrient release into the culture system. Nitrogen loading is relatively easy to deal with, but organic loading is often more difficult. The feed digestibility and nutrient density thus have a profound effect on growth and nutrient loading of the culture system.

2.4 CHOICE OF SPECIES FOR BIOFLOC SYSTEMS

Biofloc systems provide a stable and environmentally sustainable environment with many advantages over traditional systems with high water exchange. Not all species, however, are well suited for biofloc culture. Those that are share certain characteristics (Emerenciano et al., 2013; Hargreaves, 2013), including tolerance of:

- high suspended solids (>200 mg/L).
- moderate dissolved oxygen (3–6 mg/L).
- high concentrations of dissolved nitrogen compounds (TAN and $\text{NO}_2\text{-N} > 1 \text{ mg/L}$, $\text{NO}_3\text{-N} > 50 \text{ mg/L}$).
- high stocking density

Additionally, suitable biofloc species should have

- omnivorous feeding habits
- presence of suitable filtering structures
- an adaptable digestive system

Tilapia and certain penaeid shrimp have been cultured successfully in biofloc. *Macrobrachium* also has been cultured in biofloc, but its commercial potential has not yet been realized.

Litopenaeus vannamei juveniles are better adapted to collecting and consuming biofloc than those of *Fenneropenaeus chinensis* and *Mar-supenaeus japonicus* (Jang and Kim, 2014).

Marine shrimp species that have been cultured in biofloc with different degrees of success include *F. brasiliensis*, *F. chinensis*, *F. duorarum*, *F. indicus*, *F. merguensis*, *F. paulensis*, *F. setiferus*, *L. stylirostris*, *L. vannamei*, *M. japonicus*, *Melicer-tus kerathurus*, *Penaeus esculentus*, and *P. monodon* (Emerenciano et al., 2013; Ghanekar, 2009; Jang and Kim, 2014).

Because shrimp aquaculture is dominated by the Black Tiger Prawns and the Pacific White Shrimp, most interest in biofloc naturally has focused on these two species, particularly Pacific White Shrimp.

2.4.1 Black Tiger Prawn vs. Pacific White Shrimp

Whether in traditional outdoor ponds or biofloc systems, Pacific White Shrimp has become the dominant cultured shrimp species worldwide. Until the late 1990s, Black Tiger Prawns were the main species cultured in Asia and Pacific White Shrimp was the principal species in Central and South America. As viral diseases reduced Black Tiger Prawn production, the high performance and economic characteristics of Pacific White Shrimp became apparent when specific pathogen free (SPF) broodstock became available. Most Asian countries—China, Indonesia, Vietnam, the Philippines, Thailand, Malaysia, and India—began the shift to Pacific White Shrimp. As a result, the Black Tiger Prawns market declined rapidly.

Black Tiger Prawns are also more expensive to raise than Pacific White Shrimp. The latter grows faster to the 18–20 g market size (Wyban, 2008) whereas it generally is not economical to grow Black Tiger Prawns to 30 g, despite the higher market price. Selectively bred Pacific White Shrimp grow to 30 g just as fast as Black Tiger Prawns.

Comparing the two species, Pacific White Shrimp are more omnivorous and Black Tiger Prawns are more carnivorous. Black Tigers thus have a higher dietary protein requirement,

resulting in higher feed costs and higher release of dissolved nitrogenous compounds (mainly ammonia) into the culture environment. Pacific White Shrimp are better equipped to utilize natural productivity, including biofloc, and tolerate high stocking density much better. Combined with its faster growth and greater disease resistance, the productivity and economic returns of Pacific White Shrimp culture has proven to be superior.

These factors are particularly advantageous in intensively managed, super-intensive biofloc systems, for which input costs are comparatively high. Conventional biofloc technology with high heterotrophic bacterial biomass is unsuitable for the Black Tiger Prawns (Conn and West, 2012). This mainly is attributed to differences in behavior of the two species, stocking-density limitations, and existing infrastructure limitations (e.g., aeration capacity).

The superior growth and disease-resistance advantages of Pacific White Shrimp are an outcome of genetic improvement programs (Wyban, 2009). “Domestication” refers to selective breeding (e.g., Specific Pathogen Resistant or SPR), whereas “high health” refers to pathogen status (e.g., Specific Pathogen Free or SPF) (Wyban, 1992; Wyban et al., 1992). SPF shrimp are certified “clean” animals produced in a certified clean facility. “High health” animals, on the other hand, are produced in a facility that is not necessarily certified as “disease-free,” but which received SPF animals (usually broodstock or nauplii) from a certified SPF facility.

For example, Shrimp Improvement Systems (SIS, FL, US), a shrimp breeding and broodstock supply company with a 12-year genetic improvement program specializing in Pacific White Shrimp (Table 2.3), sold their SPF broodstock to a Texas hatchery, Harlingen Shrimp Farms, Ltd (now KAAPA Aqua Farms, Harlingen, TX, US). As soon as those broodstock arrived at the Texas hatchery they no longer were SPF, but properly designated as “high health.”

TABLE 2.3 Summary of Progress in the Genetic Improvement of Pacific White Shrimp by Shrimp Improvement Systems (SIS)

45 Families at Last Cross	
<i>Growth lines</i>	<i>TSV-resistant line</i>
1.9 g/week in raceways at 5100 kg/ha 2.1 g/week @ 21.5/m² in ponds in Belize 3.2 g/week in extensive ponds	TSV laboratory challenge: 63%–74% survival vs 13% (control)
	<i>WSSV-resistant lines</i>
65% faster growth than Panamanian stocks - Compact size distribution (4 size classes vs. 8–10 for Panamanian stocks)	Under development, survivals of 15%–57% in laboratory WSSV challenges, average of 30.3%
	<i>Disease-free status</i>
	A >4-year history of SPF certification

Some US companies working on domestication of Black Tiger Prawns claim to possess a high-health line. One, High Health Shrimp, Inc. is owned by CP, who also owns the company in Hawaii and in Florida, working on breeding Black Tiger Prawns. The emphasis thus far has been more on producing SPF Black Tiger Prawns, rather than selective breeding. Moana Technologies in Hawaii, George Chamberlain’s operation in Brunei (assisted by Chris Howell), and CSIRO in Australia, all have genetic programs for SPF Black Tiger Prawns. Most claim to be working on selective breeding. Many groups are finding it hard to keep their animals at SPF status. With time, this work may improve the suitability of Black Tiger Prawns for biofloc culture.

Although intensive production of Black Tiger Prawns in biofloc currently is limited, successful production at lower densities is possible. Several Australian farms produce Black Tiger Prawns in

biofloc ponds operated with low water exchange rates (Smith and West, 2011). With stocking densities of 35–60 PL/m² in 1 ha ponds (1.5–2 m depth), productivity was increased 50% to 12 t/ha (1.2 kg/m² or 0.6–0.8 kg/m³). Nitrogen discharge, water exchange, and feed costs per unit weight were reduced by 77%, 70%, and 30%, respectively, compared to conventional flow-through ponds (Conn and West, 2012; Smith and West, 2011). In comparison with the 0.6–0.8 kg/m³ harvested in those Black Tiger Prawn ponds, Pacific White Shrimp yields as high as 9.8 kg/m³ have been achieved in the indoor raceway systems at the Texas A&M Agri-Life Research Mariculture Lab (ARML) described in detail in this manual.

Ghanekar (2009) reported that Black Tiger Prawns and *Farfantepenaeus indicus* did well in a biofloc nursery. The biofloc helped remove nitrogenous waste, which reduced the size and cost of the filtration system and provided supplemental food that lowered feed costs without compromising shrimp performance (growth and survival) or health. The FCR for *F. indicus* ranged from 0.68 to 0.89 and that of Black Tiger Prawns dropped to 1.25 with good crop growth.

In another application of biofloc technology, dietary supplementation with dried biofloc to reduce fishmeal content improved growth and digestive enzyme activity of Black Tiger Prawns (Anand et al., 2013; Glencross et al., 2014).

Thus although biofloc technology may be applied to Black Tiger Prawns and other penaeid species, it thus far has been most successful with the Pacific White Shrimp.

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Biofloc

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3.1 COMPOSITION AND STRUCTURE

Biofloc aggregates span a wide range of particle size, from the microscopic to those greater than 1 mm. Even larger organisms—copepods and nematodes, for example—may graze floc and become an integral part of some aggregates (Hargreaves, 2013; Ray et al., 2010). Aggregates are irregularly shaped and rather fragile. They are held together by bacterial secretions, a tangle of filamentous microorganisms, and electrostatic forces (De Schryver et al., 2008).

The wet-weight density of floc usually is only slightly greater than 1 g/mL, so aggregates sink slowly and are relatively easy to maintain in suspension (De Schryver et al., 2008; Sears et al., 2006). With up to 99% porosity (empty space), nutrients, oxygen, and waste products are readily exchanged between the floc interior and surrounding water, and this is enhanced by the mixing common in biofloc systems (Chu and Lee, 2004; Crab et al., 2012) (Fig. 3.1).

Biofloc microorganisms vary among systems and also within the same system over time (Leffler and Brunson, 2014). Monroy-Dosta et al. (2013) identified fluctuations in the

taxonomic composition of bacteria, microalgae, yeast, and other microorganisms in floc from a tilapia system. Among the bacteria and yeast taxa were *Aeromonas* spp., *Vibrio* spp., *Enterobacter* sp., *Nitrospira* sp., *Bacillus* spp., *Sphingomonas* sp., *Pseudomonas* spp., *Microthrix* sp., *Nitrobacter* sp., *Micrococcus* sp., *Alcaligenes* sp., and *Rhodotorula* sp.

Bacteria typically dominate the biofloc in aquaculture systems. Not only are they abundant (up to 100 million bacteria/mL), but they exhibit high diversity. Jang and Kim (2014) identified 1265 genera in samples from ten different aquaculture sites, with 351–773 operational taxonomic units (roughly equal to the number of species). Bacteroidetes, common in wastewater treatment tanks, was the most dominant (26.5% of total taxa). *Chloroflexi*, another common wastewater bacterium, made up about 66.3% of the bacteria in four biofloc systems studied by Kim et al. (2015b).

Emerenciano et al. (2013) provide a thorough discussion of the many factors that determine floc composition, among which are temperature, salinity, pH, photoperiod, the intensity of vertical mixing, and the type of organic carbon available for bacterial metabolism.



FIG. 3.1 Appearance of the water surface (*left*) and a microscopic view of a biofloc aggregate (*right*) from an indoor, biofloc-dominated production system. (Photos by Leandro Castro. Used with permission.)

3.2 BIOFLOC DEVELOPMENT

Biofloc develops in newly filled systems soon after a suitable source of organic matter—such as uneaten feed, shrimp waste, or an added organic compound—has accumulated to a sufficiently high level. Aggregates usually become sufficiently dense to color the water during the third week after stocking (Monroy-Dosta et al., 2013). The rate of floc development can be advanced by “boosting,” that is, adding organic carbon to stimulate floc formation (Avnimelech, 1999; De Schryver et al., 2008).

Development is affected by a range of factors, foremost of which are temperature, dissolved oxygen, pH, organic load, light, and mixing (De Schryver et al., 2008; Ogello et al., 2014). In general:

- Aggregates are larger and denser at higher temperatures (Krishna and Van Loosdrecht, 1999) and higher dissolved oxygen (Wilen and Balmer, 1999).
- Intense mixing disrupts aggregates, reducing average floc size (De Schryver et al., 2008).
- High pumping rates through small orifices reduce floc size (Samocha, unpublished).
- Lower dissolved oxygen favors filamentous bacteria (Martins et al., 2003), likely because of their high surface-to-volume ratio.
- pH affects floc directly—each species has its optimal range—and indirectly through its relationships with alkalinity, inorganic carbon, and ammonia.
- High organic loads promote faster development (until other factors become limiting).
- Light affects the abundance of photoautotrophic organisms (i.e., cyanobacteria, green algae, diatoms, dinoflagellates, rhodophytes, etc.) in floc. Only biofloc maintained in the dark lacks photoautotrophs (John Leffler, personal communication). Photoautotrophic abundance typically declines over time in light-exposed systems as increasing floc concentrations reduce light penetration (Hargreaves, 2006, 2013; Prangnell et al., 2016).

Floc has been well studied in wastewater treatment, so insights from that field are useful in understanding biofloc aquaculture systems. Of note, about 60%–70% of floc in wastewater

systems is made up of organic matter, with 2%–20% of that found in living cells (De Schryver et al., 2008; Wilen et al., 2003). This seems a reasonable range for aquaculture biofloc too.

3.3 ADVANTAGES OF BIOFLOC

Among biofloc's advantages in aquaculture are its high nutritional value, its role in improving water quality, and its probiotic effect on shrimp.

3.3.1 Biofloc as Feed

Biofloc is similar in nutritional quality to the food that wild shrimp graze in their natural habitat. Maintaining suitably dense floc throughout the crop cycle thus reduces the need for formulated feed (Avnimelech, 2009; Tacon et al., 2002) that typically accounts for at least half of production expenses in traditional aquaculture.

Biofloc proteins trigger digestive enzymes that make them more easily metabolized than protein in manufactured feed (Xu et al., 2012). Floc's probiotic effect also stimulates parts of the shrimp immune system (Emerenciano et al., 2013; Kim et al., 2014).

Dried biofloc incorporated into a formulated feed low in the essential fatty acid DHA stimulates shrimp feeding (John Leffler and Andrew Ray, personal communication). Although the dried floc does not provide DHA itself, it stimulates feed intake and thereby contributes to reducing the DHA deficit.

The nutritional quality of biofloc is related to the carbon-to-nitrogen ratio of culture water, the dietary protein level, and light intensity. These and other factors are discussed in detail by Crab et al. (2012), De Schryver et al. (2008), Ekasari et al. (2014), Emerenciano et al. (2013), Martins et al. (2016), Xu and Pan (2014), and Yun et al. (2016).

Based on Kuhn et al. (2010), Richardson et al. (2011), Crab et al. (2012), Taw (2012), Xu et al.

(2012), Emerenciano et al. (2012, 2013), Hargreaves (2013), Ekasari et al. (2014), Ogello et al. (2014), and Xu and Pan (2014), the proximate analysis of biofloc (dry weight) is as follows:

- 12%–50% protein, but typically 30%–45%, similar to most manufactured feeds
- 0.5%–41.0% lipids, but usually 1%–5%
- 14%–59% carbohydrates
- 3.0%–61.4% ash

The wide variation is owed to differences in composition between young and mature floc aggregates and also to different culture conditions. Regarding the latter, Ogello et al. (2014) reported that biofloc grown on one of glucose, starch, or acetate had protein levels of 40%, 21%, and 19%, respectively. There were similar substrate-related variations in lipid, carbohydrate, and energy content. At 30 ppt salinity and no supplemental carbon, biofloc protein was less than 20% (Richardson et al., 2011). Protein in biofloc at Texas A&M-AgriLife Research Mariculture Lab (ARML) varied between 19% and 20% (dry weight).

The quantity and quality of organic matter stored by bacteria ultimately determine the nutritional value of floc. This stored organic matter depends on the amount and type of organic carbon available for bacterial growth (Crab et al., 2012; De Schryver et al., 2008). As an example, biofloc grown on acetate (a 2-carbon organic compound) stored poly- β -hydroxybutyrate, while floc raised on propionate (a 3-carbon organic) stored 3-hydroxy-2-methylvalerate and polyhydroxyvalerate (Yagci et al., 2007). Those are tongue-twisting chemical names, but the point is simply that “biofloc is what it eats.” If the proper organic substrates are provided, then floc will store high-quality compounds that contribute to the nutritional needs of the shrimp.

This raises the question: How does a manager ensure that the proper organic substrates are present? The answer developed at the Texas A&M-ARML facility is described in Chapter 6.

Beyond its proximate analysis, marine biofloc typically is rich in the amino acids valine, lysine, leucine, phenylalanine, and threonine, but it can be deficient in the essential amino acids arginine, methionine, and cysteine, as well as deficient in Vitamin C (Crab et al., 2012; Ekasari et al., 2014; Taw, 2012).

Biofloc alone, therefore, is insufficient to guarantee the growth and survival required by high-density shrimp culture. This is the rationale for a biofloc-dominated approach—the subject of this manual—instead of a biofloc-exclusive approach. The former relies on both biofloc and formulated feed, with feed supplying nutrients that are missing in typical floc aggregates.

Despite its nutritional benefits, and as discussed in Chapter 2, not all species are equipped to ingest biofloc efficiently. The degree to which a species is able to consume floc depends on the morphology and size of its feeding appendages. This varies among species and with life-history stage (Kim et al., 2015a).

As noted earlier, Pacific White Shrimp are suited for biofloc-dominated production. Their postlarvae are better able to eat biofloc than those of either *Fenneropenaeus chinensis* or *Marsupenaeus japonicus* (Jang and Kim, 2014) and their juveniles satisfy up to 30% of their requirements with floc (Burford et al., 2004).

The difference in setal (hair) structure of the third maxilliped (Fig. 3.2) appears to be key. Pacific White Shrimp have a greater number of setae on their longer third maxilliped than either of the other two species, and this confers an advantage in filtering fine particles. Their postlarvae thus can grow faster and survive better in biofloc.

Fig. 3.3 shows a scanning electron micrograph of the net-like structure of the third maxilliped, which is used in a sweeping motion to capture particles above a certain size.

Finally, because of its nutritional value, dried floc might replace some portion of the fishmeal

now used in formulated feeds (Kuhn et al., 2010; Richardson et al., 2011). This would contribute to environmental sustainability on a broader scale by reducing demand for the pelagic fish stocks that currently are an important source of protein in many feeds.

3.3.2 Biofloc and Water Quality

Beyond its nutritional value, biofloc bacteria can be managed to improve water quality. These can be classified according to the way they obtain nourishment. Broadly, these are autotrophs and heterotrophs.

All organic matter in the food web originates from autotrophs. They synthesize organic carbon compounds from inorganic carbon sources, such as carbon dioxide and bicarbonate. This group includes *photoautotrophs* that derive energy from sunlight and *chemoautotrophs* that derive energy from inorganic chemical compounds. The former include the familiar algae and the latter nitrifying bacteria.

Unlike autotrophs, heterotrophs must ingest organic compounds to meet their nutritional needs. Heterotrophs thus must consume other heterotrophs, autotrophs, or organic material derived from them. All animals (including shrimp) and many important biofloc bacteria are heterotrophs.

Both autotrophic and heterotrophic organisms that populate biofloc aggregates improve water quality by assimilating or transforming dissolved inorganic nitrogen compounds (ammonia, nitrite, nitrate) that, to differing degrees, are harmful to shrimp. To this end, a biofloc-dominated system can be managed to favor autotrophic bacteria, heterotrophic bacteria, or some combination of the two in a mixotrophic system. Each choice has different implications for water quality. This topic is explored in more detail in Chapter 4 as part of the discussion of the nitrogen cycle.



FIG. 3.2 Morphology of the third maxilliped in three penaeid species: (A) *Litopenaeus vannamei*, (B) *Fenneropenaeus chinensis*, (C) *Marsupenaeus japonicus*. Scale Bar: 0.5mm. (Jang, I.-K., Kim, S.-K., 2014. Evaluation of immune enhancement in shrimp growth in biofloc systems. In: Browdy, C.L., Hargreaves, J., Tung, H., Avnimelech, Y. (Eds.), Workshop on Biofloc Technology and Shrimp Diseases. Ho Chi Minh City, Vietnam, 9–10 December 2013. In Kwon Jang. Used with permission.)

3.3.3 Biofloc and Immune Response

Shrimp have a nonspecific, labile (no long-term memory) immune system. This means that they have no specific antibody-antigen

mechanism to respond to new pathogens (Roch, 1999; Söderhäll and Cerenius, 1992). The microbial population in biofloc systems, however, may play a role in activating their non-specific immune system, resulting in a defense

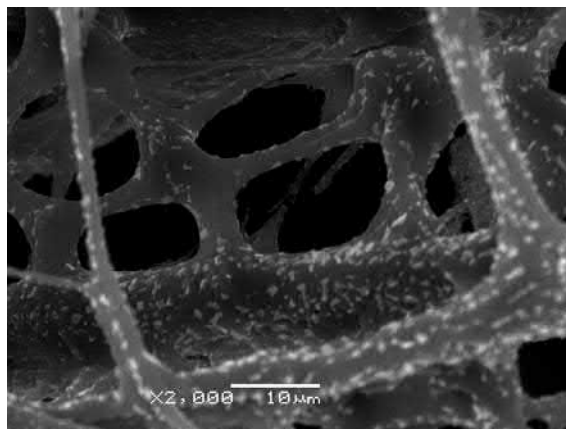


FIG. 3.3 A scanning electron micrograph showing the net-like structure of the third maxilliped of Pacific White Shrimp. (Photo by Megan Kent Pollock. Used with permission.)

that responds quickly to fight bacterial infections (Kim et al., 2014).

Biofloc's probiotic effect, mentioned earlier, involves short-chain fatty acids (lipopolysaccharides, peptidoglycans, and β -1,3-glucans) in bacterial and fungal cell walls that also play a role in the immune response (Crab et al., 2012; De Schryver et al., 2008; Emerenciano et al., 2013). When encountered, these fatty acids activate the nonspecific immune system, as evidenced by increased expression of genes related to the immune response, thereby enhancing resistance to infections (Chang et al., 1999; Kim et al., 2014; Söderhäll and Cerenius, 1992; Song et al., 1997). Biofloc microorganisms also suppress pathogen growth by competing for space, substrate, and nutrients, as well as by excreting inhibiting compounds (Emerenciano et al., 2013).

It is important to note that, although most of the earlier reports attributed positive immunity impact stemming from bioflocs, none of these publications demonstrated evidence for these activities.

On the other hand, only Kim et al. (2014) were able to show significantly better growth, survival, and immune-related gene expressions, through mRNA of six immune-related genes,

in postlarvae of *L. vannamei* reared in biofloc-rich water compare with those raised in clear water.

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Water

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4.1 SOURCE

A supply of high-quality water is an obvious consideration when choosing a marine aquaculture site. Clean seawater is best, but estuarine water, saline groundwater, and even freshwater may be suitable after treatment.

Sodium and chloride ions make up about 86% of the total dissolved solids in seawater. Even when salinity varies from its ocean-wide average of about 35ppt—whether lowered by precipitation (~30ppt) or raised by evaporation (~43ppt)—the ratio of these elements, as well as the ratios of all of seawater's major components, remains essentially the same.

The salinity of river water generally is less than 1 ppt. Unlike seawater, its top two dissolved components are not sodium and chloride, but usually calcium and bicarbonate. Also unlike seawater, the ionic composition of river and lake water varies widely from region to region, depending on the geology of the drainage basin. Thus even if river water is evaporated to raise the salinity to that of average seawater, it never will match seawater's ionic composition. Without proper adjustment, it will be unsuitable for marine aquaculture.

4.1.1 Seawater and Estuarine Water

Unpolluted seawater naturally has a chemical profile suitable for shrimp culture, although it may have a less than desirable temperature, salinity, and/or turbidity. This is especially true of water drawn from estuaries and shallow, semienclosed bays. The salinity of estuarine water varies from full-strength seawater (~35ppt) to freshwater (<0.5ppt), with the highest salinities at depth near the estuary mouth. This is the region where higher density seawater forms a wedge below less dense, fresher surface water. Farther up the estuary, mixing gradually reduces salinity from brackish to freshwater.

Shallow marine bays, such as those along coastal Texas, are at the other extreme. During summer, evaporation rates are high and the bays become hypersaline, reaching salinity in excess of 65ppt. In the rainy season, precipitation and runoff reduce salinity well below 25ppt. Intake salinity for the Texas A&M-AgriLife Research Mariculture Lab (ARML), drawn from such a source, ranges from 20ppt in the rainy season to as high as 65ppt in late summer (Fig. 4.1).



FIG. 4.1 Supply canal linked to the coastal lagoon from which the Texas A&M-ARML and Texas Parks and Wildlife Laboratory draw water.

When salinity is too high, it can be diluted with clean freshwater. When it is too low, salinity can be increased by evaporation, although this is impractical for large volumes. Alternatively the proper combination of salts can be added to increase salinity and assure that the resulting water matches the ionic profile of seawater. This procedure is outlined in [Section 6.3](#).

Water drawn from sources influenced by population centers or agricultural activities may contain nutrients, pathogens, toxic chemicals, or municipal waste. Raw seawater also carries a variety of fouling organisms (e.g., algae, barnacle larvae) and the larvae of predators. Incoming water thus must be filtered and disinfected prior to use.

The high cost of coastal land often forces aquaculture facilities to seek inland sites. Water then must be pumped or transported over a sometimes considerable distance. This leads to higher investment in infrastructure, higher maintenance expenses, and additional regulatory permissions. Transport costs nevertheless may be justified by the much lower inland land cost.

4.1.2 Saline Surface Water and Groundwater

These sources include tidally influenced coastal wells, inland saline wells, and inland saline surface water. Groundwater has a more stable temperature than surface water. It also is typically pathogen- and predator-free, so it offers greater biosecurity than other sources. Its lower turbidity also reduces the need for filtration.

The salinity and chemical composition of groundwater is stable on a timescale of years to decades, but it varies from site to site and even at different depths of the same site. Of special importance, the ionic composition of inland saline water is almost never the same as that of seawater. The Great Salt Lake, for example, has a salinity of about 254ppt (compared to 35ppt for seawater) and has an ionic profile with much more magnesium and sulfate than seawater.

Underground sources with overlying porous soils and rock are subject to surface seepage that may contaminate it with municipal, residential, or agricultural waste. Depending on the duration that water has been underground, it also may contain high levels of heavy metals, especially iron and copper, leached from the surrounding rocks. Heavy metal tolerance of shrimp increases as they grow ([Chien, 1992](#)), but even moderately high levels are unsuitable for aquaculture production. High levels of heavy metals in shrimp flesh also are hazardous to consumers.

The best way to determine the suitability of these sources for aquaculture is to conduct a bioassay. This involves exposing test organisms, such as the shrimp species to be cultured, to the source water and then observing any detrimental effects on growth and survival that may arise. Alternative bioassay organisms are phosphorescent bacteria, protozoans, aquatic invertebrates, zebrafish, and fathead minnows.

Commercial and university laboratories that perform these tests according to accepted protocols can be contracted for this purpose. If a water source does prove suitable, the next step is to determine if it can provide the flow required by the project.

Groundwater is subject to local regulations, especially where a freshwater aquifer sits atop a saline source, so regulatory agencies must be contacted to assure compliance with standing laws.

4.1.3 Freshwater

Freshwater may be drawn from a groundwater well, a surface source (river or lake), or a municipal water supply. Sea salts then must be added to produce artificial seawater. Commercial sea salts that have been used successfully in aquaculture include Crystal Sea Marinemix (Marine Enterprises International, Baltimore, MD, USA), Instant Ocean (Aquarium Systems Inc., Mentor, OH, USA), and Red Sea Salt (Red Sea USA, Houston, TX, USA).

Different brands yield solutions with different ionic compositions, nutrient levels, pH, and alkalinity. Artificial seawater mixtures designed for home aquaria have been used in aquaculture but can have higher concentrations of trace elements (Atkinson and Bingman, 1997); some also have high heavy metal levels (Hovanec and Coshland, 2004). A brand's composition also may change from batch to batch, so its ionic profile should be confirmed prior to use.

The water of hydration of certain salts must be considered when preparing artificial seawater. Water of hydration (or water of crystallization) refers to water molecules bound to some crystalline salts. Magnesium chloride, for example, commonly is sold as magnesium chloride hexahydrate, with the formula on the label written either as $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ or $\text{MgCl}_2(\text{H}_2\text{O})_6$. This indicates that six water molecules are associated with each magnesium chloride molecule.

Magnesium chloride without any bound water—*anhydrous magnesium chloride* (MgCl_2)—has a molecular mass of about 95.2 g/mole; magnesium chloride hexahydrate, however, has a molecular mass of 203.3 g/mole owing to the six water molecules. Failure to account for this difference leads to an unacceptable error in the salinity of the final solution. For example, 35 g of sea salt added to 1 L of deionized water would yield a salinity 2–6 ppt below 35 ppt if the chemically bound water of the hydrated salts is ignored (Atkinson and Bingman, 1997).

The freshwater in which the salts are mixed must be tested for heavy metals and other contaminants. A bioassay will detect the presence, if not always the exact identity, of toxic agents. Table 4.1 is a very general summary of the characteristics of potential water sources.

4.2 IONIC COMPOSITION

Table 4.2 compares the ionic composition of standard surface seawater with a sea salt mix and two inland saline sources. Compared to seawater, saline groundwater often is deficient in potassium and, depending on local geology, has concentrations of calcium, magnesium, and sulfate that are either higher or lower (Prangnell and Fotedar, 2006; Samocha et al., 2004).

Ionic composition generally has a greater impact on shrimp health than salinity (Davis et al., 2004). Although many species tolerate a wide range of salinity, they are adapted to grow and survive best at the ratios of major ions in standard seawater (Table 4.2). This is particularly true for sodium and potassium, which affect the ability of shrimp to manage their internal water and ion balances. As sodium increases relative to potassium, above a certain point shrimp growth and survival declines substantially. The critical point that triggers this decline

TABLE 4.1 General Characteristics of Water Sources for Shrimp Culture (Chien, 1992; Davis et al., 2004; Prangnell and Fotedar, 2006)

Characteristic	Marine Water			Inland Saline Water		Freshwater	
	Coast	Estuary	Ground	Surface	Ground	Surface	Ground
Water volume	1	1	3	Varies	3	Varies	3
Water stability	1	1	1	1	1	3	1
Salinity stability	1	3	1	3	1	1	1
Dissolved oxygen	1	1	3	1	3	1	3
Turbidity	1	1	3	1	3	1	3
Heavy metals	3	3	1	3	1	3	1
Conditioning requirement	3	3	1	3	1	3	1
Ionic composition adjustment requirement	3	3	3	1	1	1	1
Sea salt addition required	No	No	No	No	No	Yes	Yes
Pollution risk	1	1	3	2	3	1	Low
Predators	1	1	3	2	3	2	3
Fouling organisms	1	1	3	2	3	2	3
Pathogen risk	1	1	3	2	3	2	3
Bioassay advised	No	No	Yes	Yes	Yes	Yes	Yes
Procurement cost	3	3	1	3	1	3	1

1: High; 2: Moderate; 3: Low.

TABLE 4.2 Ionic Composition of Seawater Compared to a Sea Salt Mix and Two Inland Saline Waters

Constituent	Marine Water ^a	Sea Salt Mix ^b	ISW 1 ^c	ISW 2 ^d
Salinity	34	34	32	5.1
pH	8.2–8.4	8.35	8.21	7.31
Alkalinity (mg/L CaCO ₃)	116–160	132	–	47
Cl [−] (mg/L)	19,000	21,179	15,800	1900
Na ⁺ (mg/L)	10,500	12,185	8026	1500
SO ₄ ^{2−} (mg/L)	2700	2534	1614	1857
Mg ²⁺ (mg/L)	1350	1449	1,537	36
Ca ²⁺ (mg/L)	400	432	592	520
K ⁺ (mg/L)	380	421	80	10
Na:K	28:1	29:1	100:1	150:1

TABLE 4.2 Ionic Composition of Seawater Compared to a Sea Salt Mix and Two Inland Saline Waters—cont'd

Constituent	Marine Water ^a	Sea Salt Mix ^b	ISW 1 ^c	ISW 2 ^d
Mg:Ca	3.4:1	3.3:1	2.6:1	1:14
Mg:Ca:K	3.38:1:0.95	3.35:1:0.97	2.60:1:0.14	0.07:1:0.02
Cl:Na:Mg	14.1:7.8:1	14.6:8.4:1	10.3:5.2:1	52.8:41.7:1

^a Goldberg (1963).^b Sea salt mix—Instant Ocean (Modified from Atkinson and Bingman, 1997).^c Inland saline surface water from Wannamal, Western Australia (Prangnell and Fotedar, 2006).^d Inland saline well water from AZ, US (Gong et al., 2004).

depends on the species, but aquaculturists should maintain a sodium-to-potassium ratio close to that of seawater, about 28:1 in terms of mass.

To avoid confusion when dealing with this important ratio, be aware that it may be computed either in terms of mass or moles. That is, sodium to potassium is about 28:1 when figured as a mass ratio (both sodium and potassium expressed as g/L or mg/L) and about 45.9:1 when figured as a molar ratio (both expressed as mol/kg or mmol/kg). Both figures are found in the literature (e.g., Gong et al., 2004; Prangnell and Fotedar, 2006; Zhu et al., 2004).

Similarly, Mg:Ca:K should be near 3:1:1 (mass ratio) and Cl:Na:Mg close to 14:8:1 (mass ratio). When these ionic ratios are respected, low-salinity water (<0.5) is suitable for culture of Pacific White Shrimp as long as calcium is high (>30 mg/L) and alkalinity is above 75 mg/L (Boyd and Thunjai, 2003; Davis et al., 2004). Low salinity water can be supplemented with potassium and magnesium to allow inland cultivation of Pacific White Shrimp.

4.3 THE NITROGEN CYCLE

Much of routine aquaculture management is dedicated to controlling the forms and concentrations of nitrogen in culture water. Understanding the basics of the nitrogen cycle in

natural aquatic ecosystems (Fig. 4.2A) thus provides insight into the design and operation of aquaculture systems in general, and biofloc systems in particular.

The following is a brief introduction to the marine nitrogen cycle. In-depth technical accounts are available in Capone et al. (2008) and in Zehr and Kudela (2011).

4.3.1 Forms of Nitrogen

The aquatic nitrogen cycle comprises five important inorganic compounds plus a variety of organic compounds.

The inorganic compounds are nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_3), nitrogen gas (N_2), and nitrous oxide (N_2O). (Distinguishing between un-ionized ammonia, NH_3 , and the ammonium ion, NH_4^+ , is not necessary for this overview. The toxicity of the un-ionized form is discussed in Section 4.4.5.1).

Among the organic nitrogen compounds are proteins (about 16% N), amino acids (the building blocks of proteins), and nucleic acids (RNA and DNA). There is no loss of detail below by grouping all of these as “organic nitrogen.”

4.3.2 Nitrogen Transformation Processes

Six key processes compose the nitrogen cycle: assimilation, ammonification, ammonium oxidation, nitrite oxidation, denitrification, and

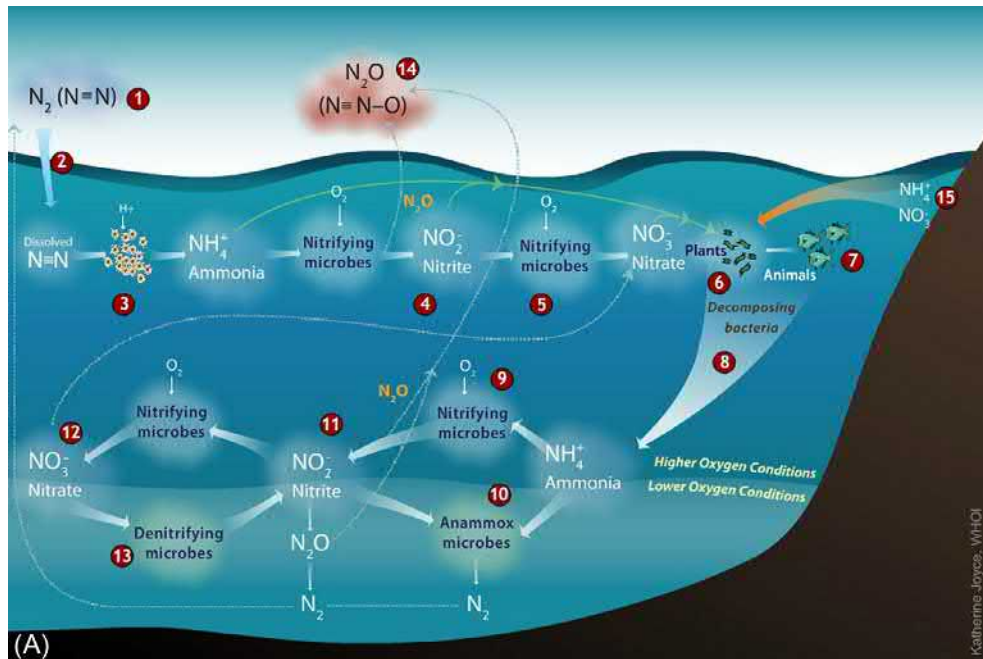


FIG. 4.2A The Marine Nitrogen Cycle. Features of particular importance to aquaculture that are discussed in the text. Ammonia produced by shrimp and some biofloc bacteria (8) is converted by ammonia-oxidizing bacteria (4 & 9) into nitrite. Nitrite-oxidizing bacteria (5 & 11) convert nitrite to nitrate. Together, these processes are referred to as *nitrification* and occur in oxygenated environments. Under anoxic conditions, denitrifiers (13) and anammox microbes (10) follow different pathways to produce nitrogen gas that is lost to the atmosphere, thus removing nitrogen from the system. (Illustration courtesy of the Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, US).

nitrogen fixation. Each transforms one form of nitrogen into another through biological activity.

Assimilation is a general term for the uptake of dissolved substances. In the nitrogen cycle, algae and certain bacteria assimilate one or all of ammonia, nitrate, and nitrite to build organic nitrogen compounds essential for life, such as proteins.

Ammonification produces ammonia from the breakdown of organic nitrogen (Fig. 4.2A, #8). There are two main pathways: bacterial decomposition of dead shrimp, uneaten feed, and feces; and ammonia excretion (by shrimp) after metabolizing feed protein.

Ammonium oxidation converts ammonia to nitrite, NO_2^- (Fig. 4.2A, #4 & 9). This is carried

out by specialized bacteria that use ammonia as an energy source. They require an aerobic environment (i.e., free oxygen, O_2), bicarbonate ion (HCO_3^-) as a carbon source (this is one reason to maintain adequate alkalinity in culture water), and are more efficient between pH 7.8 and 8.0. This process lowers pH and alkalinity. The most studied of these bacteria belong to the genus *Nitrosomonas*.

Nitrite oxidation converts nitrite to nitrate, NO_3^- (Fig. 4.2A, #5 & 11). This is performed by specialized bacteria that use nitrite as an energy source. They require an aerobic environment and function better between pH 7.3 and 7.5. This process has no effect on pH or alkalinity. The most prominent of these bacteria belong to the genus *Nitrobacter*.

Together, ammonium oxidation and nitrite oxidation are referred to as nitrification. The net effect of nitrification is removal of ammonia and production of nitrate (NO_3^-).

Denitrification produces nitrogen gas (N_2) from inorganic nitrogen compounds. Some N_2 escapes to the atmosphere, thereby removing nitrogen from the aquatic system.

Denitrifying bacteria require an anoxic environment (i.e., no free O_2 , but oxygen bound in NO_3^- or NO_2^-). Some denitrifiers are heterotrophs that require organic matter and nitrate, NO_3^- (Fig. 4.2A, #13). Most denitrifying reactors currently used in aquaculture and wastewater treatment are designed for heterotrophic denitrification.

Mulder et al. (1995) discovered autotrophic denitrifying bacteria that produce N_2 by combining ammonium and nitrite, NO_2^- (Fig. 4.2A, #10). They named this reaction anammox, which stands for **anaerobic ammonium oxidation**. Its contribution to the marine nitrogen cycle and its potential for regulating water quality in closed-system aquaculture (Francis et al., 2007) are ongoing topics of research.

Both heterotrophic and anammox denitrification raise pH and increase alkalinity, but they differ in important ways. Of particular note, unlike anammox, heterotrophic denitrification produces nitrous oxide (N_2O) as an intermediate product (Fig. 4.2A, #14). N_2O is a much more potent greenhouse gas than carbon dioxide, so its production reduces a system's overall claim of environmental sustainability.

Other processes may prove useful in aquaculture. One of these is *OLAND*, short for **Oxygen-Limited Autotrophic Nitrification-Denitrification**. This is an autotrophic process that transforms ammonium directly into nitrogen gas (Kuai and Verstraete, 1998). Its application to aquaculture has yet to be thoroughly investigated.

Finally, *nitrogen fixation* completes the cycle by converting atmospheric or dissolved N_2 gas into ammonia (Fig. 4.2A, #3). That ammonia

again becomes available for assimilation and the biological transformations described before.

In natural ecosystems, this critical step is performed by highly specialized nitrogen-fixing microorganisms equipped to break the triple bond that tightly binds the two nitrogen atoms in nitrogen gas. Cyanobacteria of the genus *Trichodesmium* are responsible for much of the nitrogen fixation in the sea. These are photosynthetic bacteria often referred to as “blue-green algae”—a misnomer, as they are not algae, but bacteria.

4.3.3 Implications for Aquaculture

The aquaculture nitrogen cycle involves the same players and processes found in natural aquatic ecosystems, but there are two noteworthy exceptions:

- 1) nitrogen fixation does not play a significant role in adding ‘new’ nitrogen over the course of a typical crop. Instead, nitrogen enters aquaculture systems almost exclusively through the addition of high-protein feed.
- 2) few recirculating systems yet are designed to operate with denitrification units that balance the high input of feed nitrogen.

The general features of the nitrogen cycle in the mixotrophic biofloc-dominated system described in this manual can be simplified as in Fig. 4.2B. As illustrated in that figure, feed is the main nitrogen source and, without a denitrification step, nitrate builds up in the system.

Nitrate is less toxic than ammonia and can reach relatively high levels (several times its concentration in seawater) without any apparent negative effect on shrimp growth or survival (Correia et al., 2014).

Even low concentrations of the un-ionized form of ammonia (NH_3), however, are deleterious to shrimp health. As a result, much of routine water-quality management focuses on controlling ammonia levels.

Flow-through systems, whether land-based ponds or open-ocean cages, deal with these

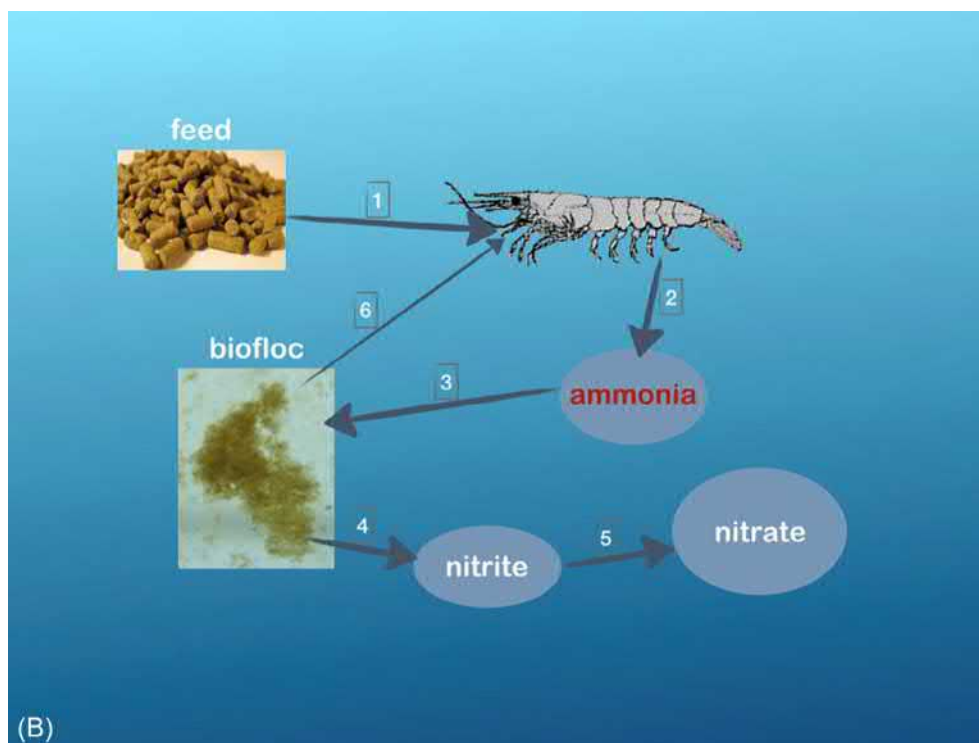


FIG. 4.2B The Basic Nitrogen Cycle in a Mixotrophic Biofloc-Dominated System. Shrimp ingest protein-nitrogen from formulated feed (1) and biofloc (6) to support growth and build biomass. They excrete mainly ammonia (2) that is assimilated by both heterotrophic and autotrophic floc bacteria (3). The heterotrophs build bacterial biomass and the autotrophs nitrify ammonia in two steps: first to nitrite (4) and then to nitrate (5). The autotrophic nitrifiers produce far less bacterial biomass. Without a denitrifying process, nitrate accumulates in the system. (Illustration by author.)

problems by discharging nitrogen-rich water directly to the environment. This violates environmental sustainability and is illegal where wastewater standards are enforced.

Integrated Multi-Trophic Aquaculture (IMTA) systems (Neori et al., 2004; Samocha et al., 2015) are cleverly designed to rely on micro- or macro-algae to assimilate excess ammonia and nitrate. The algae then are harvested for high-end (pharmaceutical and nutraceutical) or low-end (fertilizer and feed) markets.

Traditional RAS incorporates a biofilter to oxidize ammonia to nitrate. Those that also use denitrifiers remove the nitrate by transforming it into N_2 (Van Rijn et al., 2006). Those

without a denitrification stage eventually must replace culture water with new water to lower the nitrate load.

Biofloc systems depend on microbial activity in floc aggregates to remove ammonia. Some rely primarily (or exclusively) on heterotrophic bacteria to transform ammonia into bacterial biomass that subsequently becomes supplemental feed for the shrimp (Fig. 4.2B, #6).

The Texas A&M-ARML biofloc-dominated system described in this manual is different: It discourages complete dominance of heterotrophs and encourages development of a mix of hetero- and chemoautotrophic floc bacteria. By relying on both, it correctly is termed a *mixotrophic* biofloc system. This mixotrophic

approach has been found to be a more efficient way to ensure high water quality and, at the same time, provide a supplemental natural feed for the shrimp. [Section 4.3.1](#) explains this beneficial effect in more detail.

Both types of biofloc are aerobic, so they have no inherent ability to restrict nitrate accumulation, although nitrate accumulates more slowly in heterotrophic systems. Thus as in traditional RAS, these systems either must add a denitrification stage or eventually exchange unsuitable culture water.

The next section expands on this topic with a quantitative explanation of the advantages of producing shrimp in a biofloc-dominated mixotrophic system.

4.3.4 Autotrophic, Heterotrophic, and Mixotrophic Systems

As noted in [Chapter 3](#), one way to classify organisms is according to the type of energy they use to fuel their life activities. That distinction plays a role in the following discussion, so it is worth briefly restating that there are two broad categories: autotrophs that get energy from nonorganic sources and heterotrophs that derive energy from organic matter ([Hagopian and Riley, 1998](#); [Ebeling et al., 2006](#)).

Autotrophs are further divided into photoautotrophs powered by light energy and chemoautotrophs that use energy stored in inorganic compounds.

Photoautotrophs comprise plants, algae, and photosynthetic bacteria (the so-called blue-green algae). Chemoautotrophs include the bacteria that play critical roles in the nitrogen cycle, such as those that transform ammonia to nitrite, nitrite to nitrate, and ammonium + nitrite to nitrogen gas via the anammox reaction.

Heterotrophs, whether bacteria or animals, derive energy from organic compounds. Shrimp are heterotrophs, as are the bacteria that decompose nonliving organic matter to produce ammonia and those that denitrify nitrate to nitrogen gas.

Certain microorganisms have evolved the ability to function as both auto- and heterotrophs, depending on the environmental conditions to which they are exposed. These organisms are termed mixotrophs.

This distinction can be extended to describe production systems such as auto-, hetero-, or mixotrophic. For example, systems in which algae dominate are referred to as autotrophic and those in which animals (such as shrimp) dominate are heterotrophic.

In the strictest sense, all practical aquaculture systems naturally contain a mix of autotrophic and heterotrophic organisms. Such systems are, therefore, functionally mixotrophic. (It is, in fact, very difficult to maintain any pure culture of autotrophs or heterotrophs at anything other than laboratory scale.)

As a side note, the terms autotroph, heterotroph, and mixotroph understandably will be new to anyone unfamiliar with microbial ecology. They were coined more than 120 years ago by the German plant physiologist Wilhelm [Pfeffer \(1897\)](#). They first found their way into scientific English soon after in the translation of Pfeffer's seminal work by the English-Australian botanist Alfred [Ewart \(1900\)](#).

The term mixotrophic is perhaps the least familiar of the three, but a Google Scholar search confirms that, since it was coined, it has appeared in the title or text of open publications over 20,000 times. The term "mixotrophic" and its related grammatical forms thus are very common in scientific English.

All three terms—including "mixotrophic"—are used in this manual only in their widely accepted and long-standing generic sense.

Autotrophs and heterotrophs affect water quality differently and, therefore, influence the design and management of aquaculture production systems. For example although both photoautotrophs and heterotrophic bacteria assimilate ammonia, their metabolic activities have different consequences for aquaculture water quality ([Table 4.3](#)).

Biofloc systems favor development of heterotrophic bacteria when shrimp are fed a low-protein (~12%) feed, even without supplemental organic carbon, that is, the amount of carbon in the feed is adequate for the heterotrophic bacteria to process all generated ammonia. Heterotrophic bacteria become dominant because of their aggressive metabolism and the availability of enough organic carbon (from feed). They can rapidly remove ammonia from culture water. The biomass production per unit nitrogen of heterotrophs is about 40 times greater than that of nitrifiers, thus providing more supplemental feed for shrimp, but this is at the expense of greater oxygen consumption and carbon dioxide production per unit nitrogen than nitrifiers (Table 4.3).

In contrast, applying a higher protein feed with no supplemental organic carbon lowers the C:N ratio and results in organic carbon deficiencies. This favors development of chemoautotrophic bacteria, including nitrifying bacteria that oxidize ammonia to nitrate and reduce

alkalinity (Table 4.3). Removing the nitrate that results from this process is one of the main motivations for periodic water exchange in closed systems.

A fully heterotrophic system thus requires greater management effort, including additional resources to control bacterial biomass (harvest and suspension), much more aeration (or oxygenation), and regular addition of dissolved organic carbon (Avnimelech, 1999) to maintain a favorable carbon-to-nitrogen ratio. Table 4.4 compares heterotrophic and autotrophic systems.

Biofloc systems that contain both chemoautotrophic and heterotrophic bacteria and, when exposed to light, photoautotrophic microorganisms, have been named “mixotrophic” because they are a mix of these different metabolic types.

Table 4.5 summarizes some of the water-quality consequences in a well-balanced mixotrophic system with 50.4 g of $\text{NH}_4^+\text{-N}$ produced for every 1 kg of 35% protein feed. The TAN generated by marine shrimp in no-exchange biofloc

TABLE 4.3 Consequences of Chemoautotrophic, Heterotrophic Bacterial, and Algal Metabolism for 1 g of Ammonia-Nitrogen (Ebeling et al., 2006; Leffler and Brunson, 2014)

Per g of $\text{NH}_4^+\text{-N}$ Consumed	Autotrophic Nitrification (g)	Heterotrophic Assimilation (g)	Photoautotrophic Biosynthesis (g)
CONSUMABLES			
Carbohydrate ($\text{C}_6\text{H}_{12}\text{O}_6$)	0	15.17	0
Alkalinity (as CaCO_3)	7.05	3.57	3.13
O_2	4.18	4.71	0
CO_2	0	0	18.07
PRODUCTS			
Bacterial biomass (VSS)	0.20	8.07	15.85
O_2	0	0	15.14
CO_2	5.85	9.65	0
$\text{NO}_3\text{-N}$	0.976	0	0

TABLE 4.4 The Main Characteristics of Heterotrophic and Autotrophic Systems

Heterotrophic vs. Autotrophic Systems	
Heterotrophic	Autotrophic
• High bacterial biomass production = Supplemental nutrition to shrimp, but solids control and suspension required • Requires regular organic C supplementation • Higher O₂ consumption = More oxygenation required • Higher CO₂ production	• Slower growing with smaller production of bacterial biomass • Greater loss of alkalinity = Requires inorganic C supplementation (e.g., bicarbonate) or denitrification • NO₃ accumulation

TABLE 4.5 Consequences of Chemoautotrophic and Heterotrophic Bacterial Metabolism in a Mixotrophic System With 1 kg of 35% Protein Feed, No Supplemental Organic Carbon, and 50.4 g NH₄⁺-N (Ebeling et al., 2006)

per 50.4 g of NH ₄ ⁺ -N Consumed	Autotrophic Nitrification (g)	Heterotrophic Assimilation (g)	Total (g)
CONSUMABLES			
NH ₄ ⁺ -N	32.5	17.9	50.4
Carbohydrate (from feed)	0	272	272
Alkalinity (as CaCO ₃)	229.1	63.9	293.0
O ₂	135.9	84.3	220.2
PRODUCTS			
Bacterial biomass (VSS)	6.5	144.0	150.5
CO ₂	189.5	173.9	363.4
NO ₃ -N	31.7	0	31.7

systems can be calculated with the following formula (Ebeling et al., 2006): TAN production (kg/day) = Feed rate (kg /day) × Protein concentration in feed (decimal value) × 0.144.

Assuming no solids removal, sufficient organic carbon is available from feed and shrimp waste for heterotrophic bacteria to metabolize approximately one-third of the ammonia. The remaining two-thirds is available for nitrification by chemoautotrophs (Ebeling et al., 2006).

Compared to a purely heterotrophic biofloc system, the mixotrophic system demands less

oxygen, requires fewer carbohydrate additions, generates less CO₂, and produces lower microbial biomass. Compared to a purely chemoautotrophic biofloc system, the heterotrophic system requires less alkalinity supplementation and produces less nitrate.

If supplemental organic carbon is added to the culture or otherwise accumulates owing to shrimp mortality, the system would shift toward a more heterotrophic regime. Thus the management goal is to maintain the proper balance of auto- and heterotrophic processes throughout the production cycle (see Section 4.3.4).

4.4 PARAMETERS

4.4.1 Dissolved Oxygen Concentration

Dissolved oxygen (DO) is the most important water-quality parameter to monitor in aquaculture systems. It affects short-term survival, long-term growth, bacterial performance, and system carrying capacity. It typically is expressed in mg/L or percent saturation. Maintain DO concentration at 4–8 mg/L (52%–105% saturation at sea level and 30°C) and preferably above 5 mg/L (65% saturation).

Oxygen solubility decreases as water temperature and salinity increase (Table 4.6) and as atmospheric pressure decreases (e.g., at higher elevations).

Low DO reduces the performance of shrimp and aerobic bacteria. It also influences the structure and composition of biofloc (De Schryver et al., 2008). Dissolved oxygen rarely is uniform throughout a culture system and tends to be higher at the surface and nearer

aeration devices, especially when mixing is weak. This must be taken into account when determining where to measure DO within a culture tank.

Organic matter that accumulates in “dead zones” (tank corners and the bottom) can be responsible for local DO lows or even anoxic zones that produce highly toxic hydrogen sulfide, methane, and ammonia. They also encourage pathogen development (see Section 7.13).

Dissolved oxygen in nursery tanks, filled with virgin seawater, should be high during the first few weeks after stocking, even under conditions of no water exchange when shrimp biomass and bacterial loads are low. In systems with high phytoplankton concentrations and exposed to natural sunlight, DO typically is lowest in the early morning, just before sunrise. Dissolved oxygen is more difficult to maintain at levels suitable for good growth and production as biomass and temperature increase. Oxygen consumption by shrimp and bacteria also increases as temperature increases.

Dissolved oxygen is strongly influenced by Biochemical Oxygen Demand (BOD), a measure of the amount of oxygen consumed by aerobic organisms to oxidize organic matter and inorganic chemical compounds, such as sulfides and ferrous iron (Eaton et al., 1995). It measures the oxygen consumption by microorganisms and, for historical reasons, usually is measured over a 5-day period and referred to five-day carbonaceous biochemical oxygen demand (symbolized as cBOD_5).

BOD increases as the concentration of bacteria increases. This puts additional pressure on DO concentration owing to increased respiration. Response times can be very short in intensive biofloc systems because of high BOD. Events such as the untimely failure of a pump thus can be critical if an adequate backup is not quickly activated. BOD is reduced if biofloc, with its associated bacterial load, is removed by sedimentation or filtration. It is not necessary to routinely measure BOD in commercial systems,

TABLE 4.6 Oxygen Solubility at Atmospheric Pressure (101.3 kPa)

Temperature (°C)	Oxygen solubility (mg/L)					
	Chlorinity: 0	5	10	15	20	25
	Salinity: 0	9.0	18.1	27.1	36.1	45.2
20	9.09	8.62	8.17	7.75	7.35	6.96
22	8.74	8.30	7.87	7.47	7.09	6.72
24	8.42	7.99	7.59	7.21	6.84	6.50
26	8.11	7.71	7.33	6.96	6.62	6.29
28	7.83	7.44	7.08	6.73	6.40	6.09
30	7.56	7.19	6.85	6.51	6.20	5.90
32	7.31	6.96	6.62	6.31	6.01	5.72
34	7.07	6.73	6.42	6.11	5.82	5.55

(Based on Eaton, D.E., Clesceri, L.S., Greenberg, A.E. (Eds.), 1995. *Standard Methods for the Examination of Water and Wastewater*. nineteenth ed. Publication Office, American Public Health Association, Washington, DC.)

but an understanding of the concept is beneficial. Methods for measuring BOD are found in Eaton et al. (1995).

4.4.2 Temperature

Temperature is a vital parameter in shrimp growth, feeding behavior, and survival, as well as in ammonia toxicity, the concentration of dissolved oxygen, and evaporation rate.

The optimum temperature range for Pacific White Shrimp is 28°C to 30°C, with an outer range of 26°C to 31°C. Below this range, growth is limited and biofloc activity slows. Shrimp also become more susceptible to fungal infections (e.g., *Fusarium* spp.). Above this range, oxygen saturation is too low, BOD and ammonia toxicity increase, and shrimp are more susceptible to disease.

4.4.3 pH

pH measures a solution's acidity on a scale from 0 to 14. Lower values imply greater acidity. The neutral pH of pure water (i.e., no dissolved substances) at 25°C is 7. (Neutral pH is higher or lower than 7 at different temperatures, salinities, and pressures). The pH of marine waters inhabited by commercial shrimp typically is between 8.0 and 8.3.

pH strongly influences other water-quality parameters, such as ammonia, that affect the performance of bacteria and culture animals. It decreases in closed culture systems owing to CO₂ produced by respiration. Dense algae concentrations cause large diel pH variations, with higher pH during the day when they are photosynthetically active and lower pH at night. pH in blooms can exceed 9.5.

A pH that is too low (<7) can be detrimental to shrimp and bacteria (Ebeling et al., 2006). It also stresses shrimp and causes soft shells, poor growth and survival, and increased risk of nitrite and hydrogen sulfide toxicity (Chien, 1992). Growth and survival in biofloc systems

is lower at pH 7 than at pH 8 (Wasielesky et al., 2015), and the functioning of nitrifying bacteria declines below pH 6.8 (DeLong et al., 2009).

At higher pH, the proportion of toxic unionized ammonia increases (see Section 4.4.5.1). Resistance to pathogens also declines at suboptimal pH (Mikulski et al., 2000). Chen et al. (2015) reported that long-term exposure to low pH (6.8) lowers the immune response of juvenile Pacific White Shrimp and its resistance to *Vibrio alginolyticus*. The general influence of pH on shrimp is summarized in Table 4.7.

Wasielesky et al. (2015) suggest maintaining pH between 7.4 and 8.2. Note, however, that maintaining pH above 7.5 can be difficult at the high biomass of intensive biofloc systems because of the high production of CO₂ by shrimp and bacteria. A more realistic range is 7.0–7.5.

4.4.4 Alkalinity

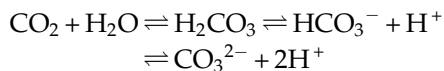
Alkalinity is the capacity of water to neutralize a strong acid or base. It is better expressed as milliequivalents/L (meq/L)—which is the same as millimoles/L (mmol/L)—but most aquaculturists still use the legacy engineering units of mg/L CaCO₃ (ppm CaCO₃).

TABLE 4.7 The Influence of pH Directly on Shrimp

pH	Effect
4	Acid death point
4–5	No reproduction
4–6	Slow growth
6–9	Best growth
9–11	Slow growth
11	Alkaline death point

(Whetstone, J.M., Treece, G.D., Browdy, C.L., Stokes, A.D., 2002. Opportunities and constraints in marine shrimp farming. Southern Regional Aquaculture Center Publication No. 2600. Southern Regional Aquaculture Center. Used with permission.)

In most aquaculture systems, alkalinity is composed predominantly of bicarbonate ions (Eaton et al., 1995). Borates, phosphates, silicates, ammonia, and organic acids contribute to total alkalinity, but their contribution generally is much smaller. The components of carbonate alkalinity are related by the following equations (Gerardi, 2003):



The components shift to the right as pH increases, yielding a higher amount of carbonate; and to the left as pH decreases, resulting in a higher carbonic acid.

Bicarbonate is the major component of alkalinity between about pH 6.5 and 10.5. In seawater at pH 8, alkalinity consists of 89.8% bicarbonate (HCO_3^-), 6.7% carbonate (CO_3^{2-}), 2.9% borate (B(OH)_4), 0.2% silicate (SiO(OH)_3), and 0.1% each of magnesium monohydroxylate (MgOH^+), hydroxide (OH^-), and phosphate (HPO_4/PO_4) (Millero, 1996).

Alkalinity stabilizes pH. It declines in closed systems partly owing to the action of nitrifying bacteria that use it as a carbon source (Ebeling et al., 2006). Heterotrophic bacteria consume about half as much alkalinity as nitrifying bacteria to metabolize the same quantity of ammonia (Ebeling et al., 2006).

Microalgae in the system consume alkalinity (HCO_3^-) when metabolizing ammonia, usually in the early stages of production; and they produce alkalinity (HCO_3^-) when denitrifying nitrate, usually in the latter stages of production, when nitrifying bacteria are well established (Ebeling et al., 2006) (see Section 6.4 for more detail). The overall effect of microalgae in mature indoor systems generally is minimal compared to that of bacteria, barring a heavy algae bloom.

High-protein feed contains more nitrogen that results in increased nitrification and alkalinity consumption. For example, alkalinity in a

system fed with 40% protein feed declines faster than one fed 35% protein feed (Prangnell et al., 2015).

Loss of alkalinity limits the amount of inorganic carbon available for bacterial nitrification and results in pH declining to a point that limits bacterial activity, resulting in an accumulation of ammonia. This deterioration in water quality reduces shrimp performance.

4.4.5 Nitrogenous Compounds

Dissolved nitrogenous waste includes ammonia, nitrite, and nitrate. When a system is managed correctly, ammonia and nitrite should remain close to zero and certainly below 2mg/L. Nitrate, however, will accumulate (Fig. 4.3). Shrimp cultured in limited-exchange biofloc systems can tolerate high ammonia and nitrite (Krummenauer et al., 2014), but the toxicity of nitrogenous wastes is greater at lower salinity.

4.4.5.1 Ammonia

Ammonia enters the system as the principal metabolic waste of shrimp and from decomposition of uneaten feed by bacteria. High ammonia increases shrimp oxygen consumption, damages gills, lowers immunity, and reduces both growth and survival.

The amount of ionized ammonium (NH_4^+) and un-ionized (free) ammonia (NH_3) depends especially on pH, as well as on temperature and salinity (Appendix I). Un-ionized ammonia is significantly more toxic because it moves more easily across gill membranes.

Total ammonia is the sum of the concentrations of NH_3 and NH_4^+ . Total ammonia nitrogen (TAN) is the concentration of nitrogen in total ammonia and is the value measured by the most commonly used commercial test kits. Multiplying TAN by 1.216 converts it to NH_3 ; multiplying it by 1.288 converts it to NH_4^+ . pH, temperature, and salinity are required to calculate the concentration of each (Table 4.8).

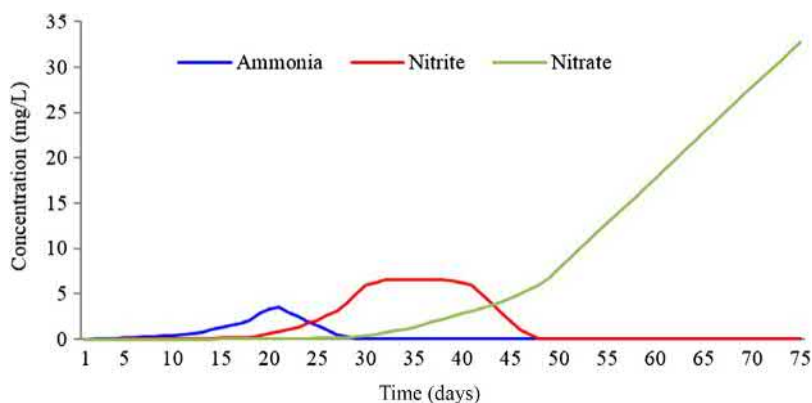


FIG. 4.3 The typical pattern of ammonia, nitrite, and nitrate concentrations in a newly started system, demonstrating how ammonia-oxidizing bacteria develop sooner than nitrite-oxidizing bacteria (leading to nitrite buildup), and the accumulation of nitrate when there is insufficient denitrification or water exchange. (Data from observations at Texas A&M-ARML. Correia, E.S., Wilkenfeld, J.S., Morris, T.C., Wei, L., Prangnell, D.I., Samocha, T.M., 2014. Intensive nursery production of the Pacific white shrimp *Litopenaeus vannamei* using two commercial feeds with high and low protein content in a biofloc-dominated system. *Aquac. Eng.* 59, 48–54; Prangnell, D.I., Castro, L.F., Ali, A.S., Browdy, C.L., Zimba, P.V., Laramore, S.E., Samocha, T.M., 2016. Some limiting factors in super-intensive production of juvenile Pacific White Shrimp, *Litopenaeus vannamei*, in no water exchange, biofloc-dominated systems. *J. World Aquacult. Soc.* 47 (3), 396–413; Samocha, T.M., Patnaik, S., Speed, M., Ali, A.M., Burger, J.M., Almeida, R.V., Ayub, Z., Harisanto, M., Horowitz, A., Brock, D.L., 2007. Use of molasses as carbon source in limited discharge nursery and grow-out systems for *L. vannamei*. *Aquac. Eng.* 36, 184–191.)

TABLE 4.8 Percentage of Total Ammonia in the More Toxic Un-Ionized Ammonia Form in 32–40 ppt Salinity Seawater at Different Temperatures and pH

pH	Temp (°C)													
	20	21	22	23	24	25	26	27	28	29	30	31	32	33
7.0	0.31	0.33	0.36	0.38	0.41	0.45	0.48	0.52	0.56	0.60	0.65	0.70	0.75	0.81
7.1	0.39	0.42	0.45	0.48	0.52	0.56	0.61	0.65	0.70	0.76	0.81	0.88	0.95	1.02
7.2	0.49	0.52	0.56	0.61	0.65	0.71	0.76	0.82	0.88	0.95	1.02	1.11	1.19	1.28
7.3	0.61	0.66	0.71	0.76	0.82	0.88	0.95	1.03	1.11	1.19	1.28	1.38	1.49	1.61
7.4	0.77	0.83	0.89	0.95	1.03	1.11	1.20	1.29	1.39	1.49	1.61	1.74	1.87	2.01
7.5	0.96	1.04	1.12	1.20	1.29	1.39	1.50	1.61	1.74	1.87	2.02	2.17	2.33	2.51
7.6	1.21	1.30	1.40	1.51	1.62	1.75	1.88	2.02	2.18	2.34	2.52	2.71	2.92	3.14
7.7	1.52	1.63	1.76	1.89	2.04	2.19	2.36	2.54	2.73	2.94	3.16	3.40	3.66	3.94
7.8	1.90	2.05	2.20	2.37	2.55	2.74	2.91	3.12	3.33	3.56	3.94	4.01	4.35	4.63
7.9	2.39	2.57	2.76	2.97	3.19	3.43	3.64	3.89	4.17	4.44	4.91	5.08	5.41	5.78
8.0	2.98	3.21	3.45	3.71	3.98	4.28	4.53	4.85	5.18	5.52	6.11	6.29	6.71	7.14

Continued

TABLE 4.8 Percentage of Total Ammonia in the More Toxic Un-Ionized Ammonia Form in 32–40 ppt Salinity Seawater at Different Temperatures and pH—cont'd

pH	Temp (°C)													
	20	21	22	23	24	25	26	27	28	29	30	31	32	33
8.1	3.73	4.01	4.30	4.62	4.96	5.32	5.65	6.02	6.45	6.85	7.57	7.81	8.33	8.85
8.2	4.65	4.99	5.36	5.75	6.17	6.61	6.99	7.46	8.00	8.48	9.35	9.62	10.20	10.87
8.3	5.78	6.20	6.65	7.13	7.64	8.18	8.62	9.26	9.80	10.53	11.49	11.77	12.50	13.33
8.4	7.17	7.69	8.23	8.81	9.43	10.10	10.75	11.50	12.30	13.15	14.05	15.04	16.09	17.21
8.5	8.87	9.49	10.10	10.80	11.60	12.40	13.16	14.06	15.01	16.03	17.06	18.27	19.51	20.84

(Based on Bower, C.E., Bidwell, J.P., 1978. Ionization of ammonia in seawater: effects of temperature, pH, and salinity. *Fish. Res. Board Canada* 35 (7), 1012–1016; EIFAC, 1986. Report of the working group on terminology, format, and units of measurement as related to flow-through and recirculation systems. European Inland Fisheries Advisory Commission (EIFAC) Tech. Pap. No. 49. Rome, Italy; FDEP, 2001. Calculation of un-ionized ammonia in fresh water. Florida Department of Environmental Protection Chemistry Laboratory Methods Manual, Tallahassee, FL. https://floridadep.gov/sites/default/files/5-Unionized-Ammonia-SOP_1.pdf (Accessed 03 September 2018).)

To calculate NH_3 using Table 4.8, first find the percentage of total ammonia as NH_3 at a set temperature and pH in the table, and then multiply this value by the measured total ammonia concentration. For example, the percentage of NH_3 in seawater at pH 7.8 and 25°C is 2.74%. If total ammonia is 2 mg/L, then the concentration of NH_3 is $2 \times 0.0274 = 0.055$ mg/L.

For simplicity, apps such as Blue Aqua (Owamo Company Ltd., Bangkok, Thailand) calculate the concentration of un-ionized ammonia once TAN, pH, and temperature are entered. Equivalent tables showing the proportion of un-ionized ammonia in freshwater and at salinities of 18–22 ppt and 23–27 ppt are in Appendix I.

4.4.5.2 Nitrite

Nitrite is formed by the oxidation of ammonia by ammonia-oxidizing bacteria (AOB). It is toxic to shrimp, although less so than ammonia, especially in saline water. Nitrite toxicity nevertheless can become a serious problem in newly started systems because populations of nitrite oxidizers (NOB) develop later than ammonia oxidizers. Nitrite usually peaks and remains high for a longer duration than ammonia (Fig. 4.3).

Nitrite toxicity increases with pH and decreases with salinity. Nitrite tolerance increases as shrimp grow (Chien, 1992). High nitrite disrupts oxygen transport, reduces growth, suppresses the immune response, and increases mortality. Nitrite should be near 0 mg/L once NOB are established, usually within 6–8 weeks in a new system. Nitrite-nitrogen ($\text{NO}_2\text{-N}$), the concentration of nitrogen in nitrite, is measured by most available test kits. To convert $\text{NO}_2\text{-N}$ to NO_2 , multiply by 3.284.

4.4.5.3 Nitrate

Nitrate, the least toxic of the three main inorganic nitrogen forms, is produced by oxidation of nitrite by NOB. Heterotrophic bacteria do not produce nitrate. Nitrate-nitrogen ($\text{NO}_3\text{-N}$), the concentration of nitrogen in nitrate, is measured by most test kits. Convert $\text{NO}_3\text{-N}$ to NO_3 by multiplying by 4.427.

Autotrophic nitrification causes nitrate to accumulate in closed systems in the absence of water exchange or denitrification. Untreated, it may exceed 450 mg/L (Kuhn et al., 2009; Samocha et al., 2010). At 30 ppt, nitrate has no discernible impact on shrimp until $\text{NO}_3\text{-N}$ exceeds 400 mg/L. Beyond that point, feed

consumption declines (Hargreaves, 2013; Leffler and Brunson, 2014) and other detrimental effects are apparent, including damage to the hepatopancreas, shortening of antennae, gill abnormalities, growth suppression, and poor survival (Kuhn et al., 2010).

Nitrate toxicity is greater at lower salinity (Tsai and Chen, 2002; Kuhn et al., 2010). For example, Kuhn et al. (2010) reported that 220 $\text{NO}_3\text{-N}$ mg/L negatively affected survival, growth, and biomass at 11ppt, compared to 400mg/L at 30 ppt. At salinities below the isosmotic point, shrimp use more energy maintaining osmotic balance between their hemolymph and the surrounding environment (see Section 4.4.7); less energy thus is available to osmoregulate in response to toxic chemicals (Kuhn et al., 2010; Pequeux, 1995). When osmoregulating at low salinity (hypo-osmotic conditions), shrimp absorb more nitrate and other toxic compounds (Kir and Kumlu, 2006; Mantel and Farmer, 1983).

4.4.6 Solids

Total solids include Settleable or Suspended Solids (SS) and Total Suspended Solids (TSS). If the concentration of solids becomes too high,

pathogenic organisms may proliferate, the gills of shrimp may become fouled, more mixing will be needed to keep solids in suspension, and oxygen consumption (BOD) will increase (Hargreaves, 2013).

On the other hand, if too many floc solids are removed (Fig. 4.4), then floc bacteria may be reduced to the point that ammonia and nitrite increase and cannot be managed (Ebeling et al., 2006). This is especially the case for nitrifying bacteria because they grow much more slowly than heterotrophic bacteria and generally are outnumbered by them by about 40:1. Low solids also allow greater light penetration, and this encourages phytoplankton, which increases the risk of an algal bloom.

Solids increase over the production cycle from excess feed, shrimp fecal matter and exuviae, and growth of biofloc. Removing suspended solids from biofloc systems with settling tanks significantly reduces nematodes, rotifers, cyanobacteria, and bacteria without significantly affecting chlorophytes, diatoms, or dinoflagellates (Ray et al., 2010). Regular solids removal is recommended to maintain a younger biofloc with a lower heavy metal load (Kuhn et al., 2015).



FIG. 4.4 Organic matter (biofloc) removed from a system by a foam fractionator.

4.4.6.1 *Settleable/Suspended Solids*

Settleable/suspended solids (SS), measured in mL/L, are the portion of solids that settle if not actively kept in suspension by mixing. Measuring SS is quick, easy, and is the most practical way to estimate biofloc concentration. Nevertheless, total suspended solids (TSS), although requiring extra effort, is a more accurate and highly recommended way of measuring biofloc concentration.

4.4.6.2 *Total Suspended Solids*

Total Suspended Solids (TSS), measured in mg/L, are the suspended solids that do not pass through a filter of a specific pore size, usually 2 μ m according to standard laboratory methods (Eaton et al., 1995). TSS in biofloc systems should be sufficiently high to provide surface area for bacterial growth, but not so high that they clog the gills of culture animals or encourage disease.

4.4.6.3 *Turbidity*

Turbidity measures water clarity in Nephelometric Turbidity Units (NTU), Jackson Turbidity Units (JTU), or Formazin Turbidity Units (FTU). Turbidity is less expensive and time consuming to measure than TSS. Turbidity and TSS are strongly correlated ($r^2=0.916$) in biofloc systems, suggesting that TSS can be estimated confidently from turbidity. The exact relationship varies according to the grow-out situation. It is different in systems with a dense algal bloom. Regular recalibration of the turbidity-TSS relationship is recommended.

4.4.7 *Salinity*

Salinity is the total concentration of dissolved salts in a solution. The current oceanographic standard for describing seawater salinity is Absolute Salinity (S_A). This is expressed as g/kg of solution or parts per thousand by mass (IOC et al., 2010). Salinity measurements reported in this manual, however, were made with a conductivity meter, for which the previous standard, Practical Salinity Units (S_P or psu), is appropriate.

There is a conversion between S_P and S_A , but the difference generally is too small to be of critical importance in routine commercial aquaculture. As such, salinity measurements reported herein are expressed according to the earlier standard (ppt).

Salinity in closed, indoor systems increases over time owing to evaporation. Periodic additions of freshwater thus are required to maintain the desired salinity.

Euryhaline species, such as Pacific White Shrimp, grow well over a wide salinity range, from near freshwater to 55 ppt. They commonly are reared between 20 and 35 ppt. When using natural seawater, the preference is for salinity of about 30 ppt. Salinity in many inland systems is much lower—between 10 and 15 ppt—mainly to save money on artificial salt mixtures.

Salinity should not change appreciably over a production cycle. In most cases, the change in the Texas A&M-ARML systems is no more than ± 4 ppt. Postlarvae shipped from commercial hatcheries are reared at 30 ppt or higher, thus if feasible, acclimation to local salinity should start at about 30 ppt to reduce stress.

Shrimp expend metabolic energy to maintain a balance of total salts (osmoregulation) and individual ions (ion regulation) between the external environment and their hemolymph (Pequeux, 1995). Ignoring other factors, the optimum for a species is the salinity at which the osmolality (salinity) of the external medium and that of the internal fluids (hemolymph) are equal. This is the isosmotic point, and it varies among species. The isosmotic point of several different Western Hemisphere shrimp species ranges from 23.3 to 26.3 ppt at 23°C, and is 24.7 ppt for Pacific White Shrimp (Castille and Lawrence, 1981).

The more salinity departs from a shrimp's isosmotic point, the greater the energy that must be expended to maintain its preferred internal state. If the difference is too great, the shrimp may become more vulnerable to cannibalism and have less energy to forage for food (Chien, 1992).

The optimum salinity range (and isosmotic point) for many species changes with life history stage and temperature. For example, the isosmotic points of Pacific White Shrimp juveniles at 20, 24, 28, and 32°C are 26.4, 25.2, 28.3, and 26.7ppt, respectively (Buckle et al., 2006). Species with wide salinity tolerance generally have a lower isosmotic point.

Oxygen saturation decreases at higher salinity and this affects the physical transfer of oxygen by aeration. The a³ injectors used at the Texas A&M-ARML system (see Sections 5.3.2 and 5.9.2.3) produce finer bubbles in saltwater than in freshwater, and so improve oxygen transfer.

Salinity also affects microorganism composition and density. Decamp et al. (2003) reported lower chlorophyll *a* with increasing salinity (9–36ppt) in closed shrimp systems.

4.4.8 Phosphate

Phosphate enters aquaculture systems through feed and accumulates over time. Phosphorus is present as reactive orthophosphates and organically bound phosphates (Eaton et al., 1995). Together, they are termed total phosphorus.

No data are available regarding the toxicity of phosphate to shrimp. Concentrations greater than 32mg/L have been reported in grow-out systems without any apparent impact on growth or survival. High concentrations do encourage algae growth, but this is not a problem in indoor biofloc systems without exposure to intense natural light. Algal blooms can occur when the concentration of biofloc is low, such as during the first weeks after stocking or when too much biofloc is removed (see Section 7.12); but even in the presence of sunlight and vigorous mixing, high biofloc concentrations reduce light sufficiently to inhibit blooms.

High phosphate in discharge water or in solid effluent can pollute natural aquatic systems.

There are no limits on the phosphate allowed in aquaculture effluent in Texas and some other states, but some states and municipalities do set limits. There also are federal effluent guidelines for aquaculture, although most facilities are too small to fall under the Clean Water Act.

4.4.9 Other Ions, Trace Elements, and Heavy Metals

The ionic composition of water in closed aquaculture systems changes over time, and this influences the performance of cultured animals and bacteria, especially if the concentration of any trace element or heavy metal is very high.

For example, over 6 weeks of grow-out, strontium decreased from 3.37 to 2.80mg/L and potassium increased from 405 to 456mg/L in biofloc raceways at Texas A&M-ARML. Leffler and Brunson (2014) found increases in several elements in culture water after 18 weeks of grow-out: arsenic (1–29µg/L), copper (32–104µg/L), iron (2–38 µg/L), manganese (13–189µg/L), molybdenum (3–20µg/L), selenium (2–8µg/L), and zinc (8–136µg/L). They reported heavy metal accumulations in both biofloc water (arsenic, cadmium, chromium, iron, molybdenum, lead, selenium, and zinc) and shrimp (arsenic, cadmium, lead, manganese, molybdenum, and selenium). Kuhn et al. (2015) reported manganese accumulation in 60-day-old biofloc reactors. Shrimp growth was suppressed by 30% when this floc was used as a feed supplement.

Table 4.9 displays the maximum concentration of some heavy metals and pesticides allowed by the FDA in the edible portion of seafood (i.e., shrimp tail muscle).

Heavy metals may continue to accumulate in water, biofloc, and shrimp if culture water is reused over several production cycles. This process and remediation techniques are the subject of ongoing research. Preliminary observations suggest safe reuse of culture water for two or

TABLE 4.9 Maximum Concentrations of Heavy Metals, Pesticides, and PCBs Permitted by the FDA in Farmed Shrimp (Aquaculture Certification Council, 2009; Drazba, 2004; FDA, 2011)

Chemical	Maximum Concentration (mg/L)
Arsenic	76
Cadmium	3
Chromium	12
Lead	1.5
Nickel	70
Methylmercury	1
PESTICIDES AND PCB'S	
Aldrin and dieldrin	0.3
Chlordane	0.3
Chlordecone (Kepone)	0.3
DDT, TDE and DDE	5
2,4 Dichlorophenoxyacetic acid (2,4-D)	1
Diquat	3
Endothall	0.1
Heptachlor and heptachlor epoxide	0.3
Mirex	0.1
Polychlorinated Biphenyls (PCB's)	2

Note that these values do not represent toxicity to shrimp.

three cycles, but more data are needed to determine longer term changes in water, biofloc, and shrimp. If pesticides and PCBs are present, these also may accumulate in shrimp tissues, especially the hepatopancreas.

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Site Selection and Production System Requirements

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5.1 SITE SELECTION

Choosing a suitable site is critical to the viability of any aquaculture project. Among considerations are environmental factors, such as water source and climate; physical factors, such as topography and geology; and socioeconomic factors, such as local regulations and availability of a competent workforce.

High-density systems require much less production area than conventional pond systems. They thus offer the possibility of year-round production in seasonally cold climates in indoor facilities that supply locally produced, ultra-fresh seafood to major urban markets. This advantage must be balanced against the higher construction costs and heating expenses incurred in these climates.

Two determining factors in site selection are water supply (covered [Section 4.1](#)) and local regulations. Regulations may differ significantly among countries and even within a country, so they must be addressed on a case-by-case basis.

In the United States, state-level Fish and Wildlife Commissions and Agriculture Departments are good places to begin researching regulatory requirements.

Site selection factors for aquaculture facilities have been covered extensively elsewhere ([Huguenin and Colt, 2002](#); [Lawson, 1995](#); [Lekang, 2013](#)). Some of that information is summarized in [Table 5.1](#).

5.2 INFRASTRUCTURE

5.2.1 Buildings

Structures dedicated to indoor shrimp culture add considerable capital costs and maintenance expenses to a project's budget. They do, however, offer distinct advantages, among which are as follows:

- Temperature control
- Lighting control (photoperiod manipulation)
- Environmental stability (e.g., less diel fluctuation, lower evaporation)

TABLE 5.1 Site Selection Factors for an Indoor Shrimp Production Facility

Aspect	Considerations
ENVIRONMENTAL	
Seawater source	• Quantity—can culture water be exchanged in 24h • Quality—salinity, turbidity, pH, ionic composition, etc. • Variability • Ease of access • Risk of pollution—fertilizers, detergents, heavy metals, phenols, hydrocarbons, etc. • Risk of disease and parasites • Tidal range • Storage capacity
Freshwater source	• Quantity • Quality • Variability • Ease of access • Risk of pollution • Risk of disease and parasites
Climate	• Temperature range • Prevailing wind • Rainfall • Evaporation • Sunlight hours • Flooding, severe storm, and hurricane risks
PHYSICAL	
Waste discharge options	• Discharge site separate from intake • Area for water treatment facilities, such as artificial wetlands, evaporation basins, or secondary crops
Soil type	• Does soil permit easy digging to install lined tanks? • Solid foundation for heavy infrastructure, such as culture tanks
Water table	• Potential groundwater use • Interference with infrastructure
Topography	• Flooding/storm surge risk • Shading effect of hills • Gradient for discharge • Construction cost • Distance and height of pumping required • Room to expand
Translocation	• Can exotic species be legally cultured? • Distance to reliable hatchery • Room for on-site hatchery
SOCIOECONOMIC	
Local laws and regulations	• Land ownership • Leasehold limitations • Aquaculture and environmental legislation

TABLE 5.1 Site Selection Factors for an Indoor Shrimp Production Facility—cont'd

Aspect	Considerations
	• Taxation • Building limitations • Water supply and discharge regulations • Proximity to marine parks
Electricity source	• Reliability of supply • Cost of supply • Backup options • Cost of fuel
Labor	• Locally available skilled and unskilled labor • Options for further training • Distance to amenities, such as housing, schools, hospitals, etc.
Construction materials	• Availability • Cost
Communications	• Reliable telephone and internet services
Transport	• Reliable all-weather transport routes and infrastructure • Transport cost
Market	• Distance to market • Potential for farm gate sales • Marketing options
Conflict with other stakeholders	• Neighbors • Local environment • Local cultural heritage • Waterfront access • Potential for theft and poaching • Shrimp fishery • Distance to other shrimp producers
Research infrastructure/ Technical support	• Availability of technical support from government research & extension service, and private consultants • Disease diagnostic services
Suppliers	• Availability and cost of consumables (feed, postlarvae, chemicals, ice, packing materials, etc.) and equipment (pumps, blowers, water quality monitoring, vehicles, etc.) • Supplier support, flexibility and reputation
Political environment	• Stability • Corruption
Zoning	• Restrictions and covenants • Future plans for surrounding area

- Independence from weather events (rainfall, wind, storms)
- Greatly improved biosecurity
- Greatly reduced predation and poaching
- Improved working environment for staff

Indoor systems have been installed in newly constructed and retrofitted buildings previously used for other purposes. In addition to space for nursery and grow-out (and, in some cases, hatchery operations), plans must include a water quality laboratory; storage areas for feed, chemicals, harvested product, and equipment; workshop; staff facilities (eating area, shower, toilets); and office space. Some projects find it necessary to include a retail shop and overnight staff accommodations. These areas can be housed in a single building or, more typically, in separate units.

Some parts of the facility can be either indoors or outdoors: harvest basins, recirculation and filtration equipment, water storage tanks, waste-treatment systems, backup generators, loading areas, a vehicle wash area, and some equipment storage. Other factors to consider include:

- Expansion of production
- Projected timeframe of operations (short- or long-term structures)
- Local building codes
- Topography: slope, soil, water supply, road access, and discharge channels
- Cost of construction materials
- Durability of materials in a humid, salty environment
- Vulnerability to environmental hazards (e.g., hurricane, flood, earthquake, etc.)
- Local climate (e.g., what degree of insulation is required?)
- Reliability of electrical grid

Several building types are used for super-intensive production: open-walled structures, greenhouses, barns, frame buildings, and inflated buildings. Aquaculture building design



FIG. 5.1A Open-walled tank.

is covered extensively by [Lekang \(2013\)](#). A summary of some common structures is given as follows.

5.2.1.1 Open-Walled

- Typically a steel frame covered by canvas or plastic ([Fig. 5.1A](#) shows a tank at the Marine Farms Pty. Ltd., Western Australia).
- Provide shading and some predator control
- Easy to assemble
- Easy to dismantle and secure in a storm
- Limited environmental control
- Only suited to tropics with year-round warm temperatures

5.2.1.2 Greenhouse

- Fully enclosed units often used for horticulture. [Fig. 5.1B](#) shows greenhouse at Texas A&M AgriLife Research Mariculture Lab (ARML) housing two 100m³ raceways
- Generally covered with plastic (single- or double-layer with inflated gap) or fiberglass; glass greenhouses are too expensive for aquaculture ([Fowler et al., 2002](#))
- Relatively inexpensive, easy to assemble
- Short lifespan, need regular upkeep, vulnerable to severe weather



FIG. 5.1B Greenhouse used at the Texas A&M-AMRL.



FIG. 5.1C Inflated air-supported structure (Photo by Wikipedia. Used with permission)



FIG. 5.1D A large wooden structure used by Florida Organic Aquaculture, Fellsmere, FL.

- Artificial lighting not required during daytime
- Sides can be rolled up for cooling
- Often need a cooling system in summer
- Difficult and expensive to heat in winter (Fowler et al., 2002)

5.2.1.3 Inflated Structures

- Structure or only roof (canvas or plastic) inflated with air blowers, see example in Fig. 5.1C.
- Requires expensive concrete slab
- High humidity favors bacteria and fungi
- Similar pros and cons as greenhouses

5.2.1.4 Framed Buildings

- Including barns, sheds, and other covered buildings (Fig. 5.1D).
- Wood- or metal-framed, covered with fiberglass, plastic, metal, wood, bricks, or other materials
- More expensive to construct than greenhouse structures but much longer lifespan (>20 years), more versatile, and

better able to withstand severe weather (Fowler et al., 2002)

- Easy to insulate, allowing temperature control and lower heating costs (Fowler et al., 2002)
- Require artificial lighting, unless part of the roof has clear panels
- More permanent than greenhouses, thus more difficult to relocate, if desired
- Corrosion of metal supports from high humidity in a closed environment
- Suited to any climate

5.2.1.5 Reservoir and Mixing Tank

Operations can include a reservoir tank or a pond for water storage and mixing (Fig. 5.1). The reservoir can be used for storing water during harvest, adjusting salinity, or disinfecting.

5.2.1.6 Harvest Basins

A basin that collects water drained from culture tanks (preferably by gravity) simplifies harvesting. It can be constructed of concrete



FIG. 5.1 A 2500-m³ reservoir pond (left) and 36-m³ mixing tank (right) at the Texas A&M-ARML.

fiberglass, wood, or lined plastic sheeting. Following are pictures of concrete harvest basins (Fig. 5.2).

5.2.2 Temperature Control

A major advantage of indoor facilities is the ability to control water temperature. In principle, this permits year-round production.

Heating and cooling water can impose a significant expense on a project, so temperature control should incorporate both passive and active methods where possible. Passive measures include building design (site selection, structure orientation, insulation, ventilation, materials) and heat capture. Active measures, such as heat exchangers and air conditioners, consume energy.



FIG. 5.2 Concrete harvest basins at the Texas A&M-ARML (A) and at Bowers Shrimp Farm, Palacios, Texas, US (B). (Photo by Tim Morris, Bowers Shrimp. Used with permission.)

Water has a relatively high heat capacity, so it heats and cools rather slowly. Once heated to the target, water thus requires a relatively small amount of energy to maintain the desired temperature in a well-insulated building (Helfrich and Libey, 1991).

The temperature of the culture water and the building's air temperature must be controlled (Malone, 2013). In biofloc systems, care must be taken not to heat water above the tolerance limits of either the shrimp or the microorganisms in floc aggregates (Hoque et al., 2012). Systems in which culture water does not pass directly through a heating unit thus are preferable.

A few observations about passive temperature control methods follow:

- *Site selection*

Heating requirements obviously are lower in warmer climates, but this natural advantage must be weighed against the distance to high-latitude markets when considering a warm-weather site.

- *Building orientation*

Orientation to the sun and prevailing winds affects heating and cooling. In cooler climates, maximize southern exposure (in the northern hemisphere). Ventilation can be opened to the prevailing winds for summer cooling.

- *Insulation*

Floors, roofs, and walls should be insulated to limit conductive heat loss. Materials include mineral wool, expanded polystyrene, cellulose, polyester, paper, and high density blown-in fiberglass (Klingenberg, 2012; Lekang, 2013). Insulating material is rated by its thermal resistance (R) that increases with thickness. Table 5.2 lists R for some common materials. The recommended R for framed aquaculture buildings is 11 ft² °F h/BTU (1.94 m² K/W) (Fowler et al., 2002).

Rigid, foil-faced, polystyrene insulation board and other nonfibrous materials have

proven effective in aquaculture facilities. These materials lose thermal resistance and deteriorate with exposure to water, so a waterproof outer layer and humidity control should be used to limit moisture exposure.

A thermal bridge is an area of a building with higher thermal conductivity than its surroundings (Klingenberg, 2012). Examples include gaps in insulation, steel frames connecting inside and outside faces of a wall, and wall studs. Correct installation of insulation reduces thermal bridge heat loss and the resulting condensation.

Greenhouses generally are not insulated, but many two-layer polyethylene greenhouses include an inflated space filled with air (Fig. 5.3) or glass wool. This increases thermal resistance by about 50% compared to single-layer covers (Fowler et al., 2002). Moisture trapped between layers is reduced by sucking dry air from outside the greenhouse.

Double-layer greenhouses have less than 12% of the thermal resistance of insulated frame buildings (Fowler et al., 2002). Note also that pumps used to inflate the layers rust rapidly in the humid greenhouse environment and require conscientious antirust treatment or external installation.

Shade cloth, aluminum sheets, and spray-on products such as Kool Ray (Continental Products Company, Euclid, OH, US) have been used to cool facilities by reducing direct sunlight.

- *Ventilation*

Simple forms of ventilation include adjustable slats, roll-up side walls (Fig. 5.21A(J)), and turbine vents. These promote summer cooling and reduce internal humidity, but also risk introducing air-borne debris that may compromise biosecurity.

- *Greenhouses*

Greenhouses reduce winter heating in temperate and subtropical climates (Malone, 2013). Fans and shading (such as aluminum blankets that reflect sunlight) limit solar heating in warmer months.

TABLE 5.2 Thermal Resistance (R) of Common Materials ([Fowler et al., 2002](#); [InspectAPedia, 2015](#))

General Materials	R-Value (ft ² °F h (BTU [m ² /kw]))
Air space (1.25–10cm)	1.00 (0.176)
Brick (10cm)	0.44 (0.078)
Concrete block 10-cm hollow core	1.11 (0.196)
Concrete block 20-cm hollow core	1.90 (0.335)
Drywall (1.25cm)	0.45 (0.079)
Fiberglass sheet	0.85 (0.150)
Glass	0.14 (0.025)
Plywood (1.25cm)	0.63 (0.111)
Polyethylene film	0.85 (0.150)
Shingles—asphalt (roofing)	0.44 (0.078)
Shingles—wood (roofing)	0.94 (0.166)
Straw bale	1.45 (0.255)
Timber—Hardwood (2.5cm)	0.71 (0.125)
Timber—Softwood (2.5cm)	1.01–1.41 (0.178–0.248)
INSULATION MATERIALS	
Cardboard insulation (2.5cm)	3.0–4.0 (0.528–0.704)
Cellulose insulation (loose fill)/inch (2.5cm)	2.8–3.5 (0.493–0.616)
Cotton batts	3.7 (0.652)
Extruded polystyrene board (2.5cm)	5.0 (0.881)
Fiberglass blown insulation (2.5cm)	3.6–4.4 (0.634–0.775)
Fiberglass batt insulation (2.5cm)	3.1–5.0 (0.546–0.881)
Mineral wool insulation (2.5cm)	3.2–3.7 (0.564–0.652)
Polyethylene foam (2.5cm)	3.0 (0.528)
Polyurethane foam rigid panels (2.5cm)	5.5–8.0 (0.969–1.409)

- *Solar Thermal Heating*

Roof-mounted solar heaters supplement heated water needs. Photovoltaic cells may be more cost effective under some circumstances. In-tank plates or pipes transfer heat to culture water. An insulated storage tank holds heated water to moderate night-time cooling ([Hoque](#)

[et al., 2012](#)). A solar contractor should be contacted to perform the necessary design calculations.

- *Water Storage*

Placing water storage, mixing, and digestion tanks in the same building as culture tanks helps



FIG. 5.3 Air blowers inflate double-layer polyethylene greenhouse roofs at the Texas A&M-ARML.

heat the building (Hoque et al., 2012). The temperatures of stored and culture water also should be close enough that little adjustment is required when water is transferred. Bacterial digestion of waste generates some heat that offsets a small portion of requirements (Hoque et al., 2012).

5.2.2.1 Active Temperature Control Methods Include

- *Fans*

Fans (Figs. 5.21A(D) and 5.35D) ventilate and reduce humidity. This increases evaporative cooling of culture water. To retain heat, ventilation slats are closed when fans are not running.

- *Gas/Electric Air-heating Units*

Space heaters (propane or electric) can maintain air temperature 1–2°C higher than that of culture water. This reduces condensation (Helfrich and Libey, 1991). Fossil fuel heaters require exhaust vents at least 1.2m above the roof peak (Buffington et al., 1992).

- *Heat Exchangers*

Plate, shell/tube, coil, bayonet, and panel heat exchangers are made of carbon glass, Teflon, titanium, or stainless steel (Huguenin and

Colt, 2002). Titanium and carbon last longer in saltwater. Counter-flow exchangers are the most efficient (Huguenin and Colt, 2002).

- *Heat Pumps*

The most efficient way to heat RAS water is with a heat pump and heat exchangers. Heat pumps operate like a refrigerator, moving heat from one location to another (Baird et al., 1993). Air-source heat pumps are cheaper to install, but a backup may be needed in colder climates. Water-source pumps are more efficient and, where possible, tap geothermal heat from a circulation loop that may contain antifreeze. This yields a more stable temperature independent of external air temperature. Water-source pumps with a ground loop are more expensive to install, but they are 2–6 times more energy efficient and can cool water when required (Baird et al., 1993).

- *Environmental Sources*

Pumping culture water in a closed loop through pipe sunk into the water table will warm or chill water, depending on groundwater temperature. Water also can be pumped through black pipes that collect heat from solar radiation (Lee, 2009).

- *Heating Coils*

Many indoor facilities utilize thermostat-controlled boilers to heat water piped through heating coils (polypropylene or other material) in culture tanks, a sump, or around the building (Buffington et al., 1992; Malone, 2013). The heated water also may flow through flattened metallic plates in the tanks. There is no exchange of water with the culture system, so scaling is avoided (Malone, 2013). Any pipe sections outside of the building should be properly insulated.

- *Submersible Heaters*

Submersible heaters (electrical immersion heaters or counter-flow immersion coils) heat water directly in culture tanks, sumps, or in-line. They are designed for underwater use, but submerging any electrical device always requires safety precautions. In practice, these heaters are mainly for small-scale work. Calcium deposits reduce transfer efficiency and require frequent cleaning.

- *Other Active Heating Methods*

Large greenhouses often are heated with steam from external boilers that is circulated through a network of internal pipes (Buffington et al., 1992). Such systems are expensive but long-lasting.

- *Other Active Cooling Methods*

In dry climate, porous absorbent material, such as burlap, can be hung from the roof to cover open walls. Freshwater then is dripped or sprayed over this material and air flow through the damp material provides evaporative cooling. These “swamp coolers” are commercially available. Freshwater sprinkler system installed on the roof of a building has the same cooling effect.

Automatic control systems linked to thermostats provide stable temperatures. These systems must be inspected regularly and calibrated to ensure accurate functioning. See

Huguenin and Colt (2002) and Lekang (2013) for detailed descriptions system design and equipment.

Condensation occurs when warm, moist air meets cooler air. Condensate often is acidic in indoor aquaculture facilities owing to higher atmospheric carbon dioxide. Condensation on walls and ceilings promotes growth of mold and bacteria. This requires periodic disinfection. It also damages equipment and structures. Equipment thus should be water resistant, with a water-vapor barrier to divert condensation away from vulnerable areas (Malone, 2013). Fixed electrical equipment should be housed in NEMA (National Electrical Manufacturers Association) enclosures with positive air pressure.

5.2.3 Culture Tanks

5.2.3.1 General Design

VOLUME

Culture tanks come in a variety of shapes and sizes. Sizes range from 20 to 2000 m³. Smaller tanks are less cost effective but more easily managed and appropriate for testing new procedures. Size is determined partly by marketing goals: Tanks typically are harvested completely at the end of a crop, so the harvest should closely match the demand of the market being supplied.

SHAPE

The three most common shapes are circular, rectangular, and raceway (RW). Water depth varies from 0.5 to 3.0 m, with 0.5 to 1.0 m more common. Tank bottoms slope to a drain—at the center of circular tanks and at one end of rectangular tanks and raceways.

CIRCULAR

- Constructed of thin plastic or fiberglass, as tank walls are self-supporting through internal water pressure
- Good mixing, few “dead zones.” Uniform DO and food distribution

- Circulation directs solids to tank center for removal via central drain
- Larger tanks more difficult to access, and thus to manage
- Oxygen consumption per unit weight of culture animals often greater
- Less efficient use of floor area (more wasted space “packing” circles)
- Generally more suited for small systems

RECTANGULAR

- Corners often rounded to improve water flow
- More efficient use of floor area (less wasted space “packing” rectangles)
- Easier to access all parts of tank
- Easier to harvest than circular tanks
- Require stronger construction materials, extra reinforcement, or partial burial
- Poor solids movement, prone to accumulate solids in “dead zones”
- Prone to inefficient, uneven DO distribution
- More suited for larger volume systems

RACEWAY WITH CENTER PARTITION

- Center partition along most of length improves circulation (see [Sections 5.9.1.3](#) and [5.9.2.3](#) describing Texas A&M-ARML raceway systems)
- Partition increases construction cost
- Partition complicates harvesting unless easily detachable
- Superior solids movement and collection compared to rectangular tanks
- Most common design for indoor, super-intensive biofloc shrimp culture

5.2.3.2 Construction

Tanks can be installed in or above ground level. The former take advantage of structural support from the surrounding soil plus side-wall and bottom insulation can help stabilize water temperature. These usually are constructed of concrete or plastic liners.

It may be difficult to engineer quick and complete drainage of in-ground tanks, and some

sites may be so rocky that digging is impractical. In areas with a shallow water table, the liners of in-ground tanks may deform (float), making routine production operations challenging.

Above-ground tanks require additional support, but can be installed at ground level makes construction cheaper. Their shape, design, and plumbing also are more easily changed after installation.

Tank materials must be sufficiently durable to support the hydrostatic pressure of the water column and withstand the corrosive effects of salt water over long-term use. They also must not leach toxic substances that contaminate shrimp or bacterial floc. Further, the materials must be smooth to facilitate water flow, simplify cleaning, and not damage shrimp that come into contact with it. Finally, of course, the construction material must be affordable ([Lawson, 1995](#)).

Some commonly used materials are described as follows:

5.2.3.3 Concrete

- Often alkaline, potentially increasing culture water pH unless lined
- A rough finish compromises cleaning, disinfection, and will abrade shrimp
- Sealing with epoxy paint reduces the effects on water quality, smooths the surface, and improves its water-holding capacity; expensive and requires renewal every few years
- Sealing with rubber spray coating can be very effective, but expensive
- Expensive compared to other materials and requires more form-work/footings
- Impractical to alter once set in place
- Gunite is more durable and versatile than poured concrete, but more expensive

5.2.3.4 Fiberglass

- Versatile, strong, lightweight, and durable (see [Fig. 5.3A](#)—fiberglass tanks at Texas A&M-ARML)
- Nontoxic and inert when cured



FIG. 5.3A Round fiberglass tanks used at the Texas A&M-ARML.



FIG. 5.3B Rigid polyethylene tanks.

- Easy to clean and disinfect
- Minimal, easy-to-perform repairs
- Finishing with “gel coat” provides a smooth finish
- More expensive than some other construction materials

5.2.3.5 Galvanized Steel/Zinc

- Corrugated circular tanks made of this material are cheap, strong, and simple to construct
- May leach zinc into the culture water and corrode rapidly in saltwater
- Generally only used as a frame for HDPE, EDPM, or PVC liners

5.2.3.6 Plastic

- Rigid polyethylene, polypropylene, polybutylene, PVC, or other plastic (Fig. 5.3B of rigid polyethylene at the Marine Farms Pty. Ltd., Western Australia).
- Ensure that the particular plastic is nontoxic
- Lightweight, versatile, and durable
- Easy to repair with a plastic welder
- Larger tanks require extra reinforcement to prevent buckling under high water pressure

- Swimming pool kits for small-scale culture, often aluminum/steel frame supporting thin PVC liner
- Some liners with fungicides that may be toxic to bacteria and shrimp. Contact manufacturer regarding this issue and conduct bioassays before use

5.2.3.7 Wood

- Light, easy to work with, and comparatively inexpensive for smaller tanks
- Marine plywood (minimum 19-mm thick) often used (Lawson, 1995), but pressure-treated lumber is cheaper and effective
- Do not use chemically treated wood for tanks or surrounding framework
- Seal wood with epoxy or fiberglass resin, or cover with liner (Lawson, 1995)

5.2.3.8 Flexible Liner

- Usually composed of EPDM (Ethylene-Propylene-Diene-Monomer), HDPE (High Density Polyethylene), PVC (Polyvinyl Chloride), polyurethane, or butyl rubber. Table 5.3 lists characteristics of liners used in aquaculture.

- High versatility, fitted to any tank size or shape (Fig. 5.3C shows an EPDM-lined raceway at Texas A&M-ARML).
- Cost effective and very common in large culture systems
- US liner thickness measured as “mil”:
1 mil = 1/1000 in = 1/40 mm. Industry standard is 45 mil (1.125 mm), 20–60 mil (0.5–1.5 mm) typical. Thicker liners are more puncture resistant and last longer, but are more expensive and difficult to install
- Prone to punctures (e.g., from shrimp rostra), but easy to repair
- Not as durable as fiberglass or rigid polyethylene (generally preferred over liners for small tanks)

TABLE 5.3 Characteristics of Three Liners Commonly Used by in Aquaculture

	EPDM	PVC	HDPE
	Ranking (1 = Highest, 3 = Lowest)		
Cost per unit	1	2	3
Ease of installation	1	1	2
	Easy to join with solvent cement and seam tape	Requires expensive equipment (fusion welder) to weld seams that can be difficult to use	
Ease of repair	1	1	2
	Easy to join and repair with solvent cement and seam tape	Glue will not bond permanently to HDPE	
Flexibility/Elasticity	1	2	3
			Can be damaged by

TABLE 5.3 Characteristics of Three Liners Commonly Used by in Aquaculture—cont’d

	EPDM	PVC	HDPE
			stretching (>12%) and tends to wrinkle.
Strength	2	2	1
Durability	1	2	1
Puncture resistance	1	1	2
Surface friction	1	1	2
			Slippery material, prone to slipping on soil
UV Resistance	1	2	1
		Indoor or shaded location recommended	
Chemical resistance	1	1	1
High temperature tolerance	1	1	1
Low temperature tolerance	1	2	2
Lifespan	1	3	2
	20 years	10 years	10–20 years
Other			Some HDPE liners may limit shrimp growth compared to EPDM liners



FIG. 5.3C Raceway lined with EPDM membrane.

- A protective layer, such as carpet/geotextile/canvas, may be placed between the liner and support structure to reduce abrasive damage
- Some EPDM and butyl rubber liners are toxic to nitrifying bacteria and shrimp. Examples and simple toxicity tests are found in [Horowitz et al. \(2001\)](#) and Rosenberry (personal communication). Some liners are not fatally toxic but may lower growth. Washing removes some toxins; others (small molecules used in the manufacturing process) leach over time ([Horowitz et al., 2001](#)). Good practice: rinse and “weather” new liners before use (Bob Rosenberry, personal communication). Always confirm with the manufacturer that the liner is

aquaculture grade (nontoxic). A bioassay before purchase is highly recommended

- Most liners have a 5- to 20-year lifespan, depending on grade and use
- Liner support can be constructed from concrete, cinder blocks, plywood, fiberglass, PVC, corrugated iron, sand-filled bags, steel, or soil (burial)
- Corrugated iron- or zinc-framed tanks lined with food-grade polyethylene (designed for rainwater tanks) come in a kit, are durable and are easy to construct ([Fig. 5.3D](#) shows a photo of a tank at the Marine Farms Pty. Ltd., Western Australia).

5.2.3.9 Other Tank Design Considerations

FREEBOARD AND ANTIJUMP NETTING

Subadult shrimp often jump as high as 1 m or more when agitated by a sharp noise, growth sampling, or during batch feeding. To prevent escape, allow a minimum freeboard (the distance between the water surface and the top of the tank) of 5 cm in nursery tanks and 20 cm in grow-out tanks. Antijump netting (minimum height: 85 cm) is recommended (see [Figs. 5.22A](#) and [Fig. 5.37A](#)).



FIG. 5.3D Corrugated round tank lined with polyethylene.

5.2.3.10 Access

Allow sufficient space around the tank for unobstructed observation, manual mixing, sampling, equipment adjustments, and recovery of dead shrimp. Regularly spaced boardwalks across raceways improve access to the center. When antijump netting is used, simple gates allow access to the tank.

5.2.4 Plumbing and Drainage

Plumbing includes pipes, valves, fittings, flow meters, and distribution devices for both water and air (Bankston Jr and Baker, 2013). A system designed for efficient flow minimizes pumping costs and reduces fluid losses. A plumbing professional will help greatly in designing a correctly sized system.

5.2.5 Materials

As with tank construction, plumbing material must be durable, nontoxic, inert, smooth, and affordable. It must withstand significant internal pressure (from water or air) and possibly external pressure from vehicles driving over buried pipes.

Most facilities use PVC for air and water distribution because it is relatively cheap, available in a wide range of sizes, durable, nontoxic, and easy to work with (PVC is lightweight and can be glued or welded together). Polyethylene is also used for air and water supply.

Other materials—concrete, fiberglass, rubber—can be used for water supply and drainage. Avoid copper or brass because these are toxic to crustaceans and biofloc microorganisms. External pipes must tolerate the local temperature regime and be UV resistant if not protected from the sun.

5.2.5.1 Sizes

Pipes and fittings are available in different pressure classes, identified by a PN or “schedule” code. Thicker pipe has a higher schedule number and is more expensive. PN9/Schedule 40PVC is common in aquaculture. Typical sizes

are 2.5 cm (1 in), 3.8 cm (1.5 in), 5 cm (2 in), and 10 cm (4 in). Polyethylene, particularly 1.3–1.9 cm ($\frac{1}{2}$ – $\frac{3}{4}$ in), often is used for air distribution. Using standard pipe sizes simplifies planning, construction, and maintenance.

5.2.6 Pressure Loss

Pressure (head) loss arises from friction induced by contact of flowing water (or air) with the internal walls of the pipe and its fittings. Friction increases as flow velocity increases.

“Rough” flow that occurs when a fluid abruptly changes direction (as in a 90-degree elbow joint) or when the pipe diameter narrows (as in a Venturi injector) results in substantial head losses (Lekang, 2013); and the greater the head loss, the more energy is needed to deliver a particular pressure and flow rate.

Factors that reduce head loss and improve pump efficiency include:

- Pumping height (or lift). Install the pump and distribution network near or below water level. Flooded suction is best.
- Pipe material. Different materials have different head losses based on a “roughness coefficient.” Use material with low head loss. The coefficient of PVC is 150.
- Pipe diameter. At a given flow rate, head loss decreases with increasing pipe diameter. For example, at 189 Lpm, head loss is 19.2 m/100 m in 40-mm PVC pipe. It is only one-fourth of this (4.7 m/100 m) in 50-mm pipe. Where possible, use wider diameter pipe at least as large as the pump inlet. The intake diameter always should be at least as large as the discharge diameter.
- Pipe length. Head loss increases with pipe length, so design plumbing to limit pipe length, particularly at the pump intake.
- Fittings. Minimize the number of fittings (elbows, sockets, unions, tees) and flow restrictions (valves, Venturis), particularly on the suction side of the pump.

There is abundant information on aquaculture plumbing systems, including calculation of head losses (Bankston Jr and Baker, 2013; Lawson, 1995; Lekang, 2013; Timmons and Ebeling, 2013). Friction loss tables for different materials and fittings also are available, particularly from manufacturers.

5.2.6.1 Tank Drainage

Culture tanks can be drained by gravity, pumping, or a combination of the two. The most efficient systems have above-ground tanks that drain by gravity. When using pumps for circulation/aeration, plumbing design should plan for draining with the system's recirculation pumps.

The drain should be at the deepest point of the tank: at the center of circular tanks and the end of raceways. Wide drainage pipes (15–25-cm diameter) reduce the chance of blockage and increase drainage rates, both critical when harvesting. Dual drains with low-volume/high-solids flow and a high-volume/low solids flow are recommended for larger tanks.

Drainage is controlled with an internal or external standpipe that is higher than the maximum water level. External swivel standpipes, standpipes of different heights, pumps, and valves are used to lower tank water in stages. When using an external standpipe, pump, or valve, a filter pipe inside the tank is required to prevent animal escape. Our experience showed that in-tank vertical filter pipes more effectively excluded shrimp than horizontal filter pipes when drawing water from the tanks with a pump. Because of jumping, it is highly recommended to install netting on top of vertical filter pipes and outlets whenever the shrimp are large enough to jump to the top of these outlets (see Figs. 5.39C and 5.44B).

5.2.6.2 Electrical Supply

In addition to running all equipment at full capacity, the main electricity supply line should accommodate expansion. The power needed to

run a facility is calculated by adding the demand of all equipment and increasing the allowance in consideration of the start-up surge required by motor-driven equipment, such as blowers and pumps. Installing "soft starters" reduces surge. A licensed electrical contractor should be consulted for electrical design, installation, and repair.

Some basic safety guidelines include:

- Install ground-fault circuit interrupters (GFCI) on all circuits to protect staff and equipment
- Regularly inspect, test, and tag all portable electrical equipment and GFCIs to ensure that they operate correctly. (This is a code requirement in many areas).
- Never use faulty electrical equipment to operate an intensive shrimp production system
- Attach a danger tag close to the plug of faulty electrical equipment. Dispose of or repair such items as soon as possible; do not leave them "tagged out"
- All electrical fixtures should be rated for use in wet locations (outdoor standard) and mounted flush to surfaces, rather than recessed
- Fixed electrical equipment should be housed in NEMA boxes with positive air pressure to limit corrosion from humidity and condensation
- Use extension cords only for temporary equipment, not permanent fixtures
- Dry hands before touching switches or electrical equipment

5.2.6.3 Generators

A backup generator is essential when the main power supply fails. A commercial facility needs a stand-by generator large enough to run all pumps, blowers, freezers, and monitoring equipment for at least 24h, depending on the facility's isolation. Enough fuel must be stored for the crop to survive such events on

backup power only. As a general rule, generators should be twice as large as needed to run all essential equipment and should run at between 25% and 75% of maximum load.

The generator ideally should include an Automatic Bus Transfer (ABT) switch that turns on when main power is lost and shuts down when it is restored. The generator and associated power-failure alarm must be tested at least weekly and serviced regularly according to recommended maintenance schedules to ensure system readiness. This includes manually switching off the main power to check the alarm response and automatic startup. A smaller portable generator should be available to maintain individual systems in the event of a localized on-site power problem.

Diesel-fueled generators (Fig. 5.4) are the most common type used in aquaculture facilities. They are more powerful and last longer than others, although they are more difficult to start in cold weather. Gasoline generators are quieter, but gasoline has a shorter shelf life and is not as safe to store as diesel. Natural gas or propane generators are cleaner and also quieter. Their fuel has a much longer shelf life. Stored propane, however, loses pressure in cold weather. Methane generated on-site in

anaerobic digesters potentially can be used as a generator fuel, but this demands installation and maintenance of another engineering system. Finally, advances in renewable energy, such as wind and solar, are increasingly viable in certain areas and eventually may eliminate the need to be connected to the grid.

5.3 AERATION AND WATER CIRCULATION EQUIPMENT

This equipment supplies DO, degasses CO₂, and keeps biofloc in suspension.

Mechanical aerators—vertical pump sprayers, propeller-aspirator pumps, and paddlewheels—are widely used in outdoor ponds (Boyd, 1998; Kumar et al., 2013) but generally unsuitable for most indoor systems. Even scaled down, they risk inflicting serious damage to stock in super-intensive systems.

Indoor biofloc systems typically aerate and circulate water with blowers, diffusers, and air-lift pumps or by mechanically pumping water through Venturi injectors. Either can be the sole method of aeration and mixing or both may be combined in the same system. Some characteristics of each are listed in Table 5.4.



FIG. 5.4 Backup diesel generators (30kW and 250kW) installed at aquaculture facilities.

TABLE 5.4 Characteristics of Blower-Driven, Pump-Driven, and Combined Methods for Indoor Biofloc*Blower-driven*

- May not provide enough DO to support shrimp biomass $>3\text{ kg/m}^3$
- Delivered through diffusers and air stones
- Airlift pumps provide water circulation and oxygenation

Pump-driven

- Can include Venturi injectors to add pure oxygen and chemical treatments. Note that the Venturi setup used in the Texas A&M-ARML 40m³ raceway supported the oxygen demand of up to 9.75kg/m³ biomass when supplemented with pure oxygen.
- a³ injectors (produced by All-Aqua Aeration, Orlando, FL, US) have excellent aeration and mixing capacity and support the oxygen demand of biomass $>9\text{ kg/m}^3$ using atmospheric air.
- Preliminary data suggest that the a³ system is more power efficient than combined systems, particularly when operated with variable speed pump.

Combined

- If either system fails (unrelated to power loss), oxygenation is maintained by the other

5.3.1 Blower-Driven Systems

Air blowers produce a high volume of air low pressure ($<27.5\text{ kPa}$); compressors produce low flow at high pressure. Flow rate (as cubic meters per min, cmm, or cubic feet per min, cfm) and pressure (as kilopascals, kPa, or pounds per square inch, psi) are important design factors.

Air pressure typically is in the range of 20.7–34.5kPa (3–5psi) and must be filtered to prevent damage to the blower. Table 5.5 relates blower pressure to the depth at which air is delivered.

The data in Table 5.5 indicate that delivery of air to a column of water 1.2m (48in) deep takes less than 13.8kPa (2psi) because it can reach 1.4m (55in) at that pressure. This assumes that pressure (head) loss from pipe fittings and restrictions is not greater than 0.2mWg (7IWG).

The total head that a device delivers depends on the depth of the diffusers plus head losses through the diffusers and the distribution system. That is,

$$\text{Total Head} = \text{Submergence} + \text{HL}_{\text{pipe}} + \text{HL}_{\text{diffuser}}$$

where Submergence = depth of diffuser, HL_{pipe} = head losses in pipes, and $\text{HL}_{\text{diffuser}}$ = head losses in diffuser. HL_{pipe} depends on air flow, pipe size, pipe roughness, the type and number of fittings, and pipe length. $\text{HL}_{\text{diffuser}}$ depends on the type of diffuser (smaller bubbles mean greater head loss), the number of diffusers, and air flow rate. Diffuser manufacturers

TABLE 5.5 Water Depth to Which Air Can Be Pumped at Different Air Pressures

Pressure	Depth
6.9kPa ^a (1psi ^b)	0.703mWg ^c (27.7IWG ^d)
13.8kPa (2psi)	1.406mWg (55.4IWG)
20.7kPa (3psi)	2.110mWg (83.1IWG)
27.6kPa (4psi)	2.813mWg (110.7IWG)
34.5kPa (5psi)	3.516mWg (138.4IWG)

^a kPa = kilopascals.

^b psi = pounds per square inch.

^c mWg = meters of water gauge or pressure.

^d IWG = inches of water gauge or pressure.

publish tables of frictional loss at rated flow capacities. Once head loss is computed for different flow rates, a blower or compressor can be selected.

Note that head is expressed in units of length (meters, feet). Converting head to pressure requires the specific gravity of water. This varies with temperature, salinity, pressure, and reference temperature. At the reference temperature of 4°C, the specific gravity of pure water at 20°C is 0.998; for mean surface seawater, it is about 1.020. These differences are important in studies of vertical circulation in the sea or lakes, but small enough under aquaculture conditions that no serious error is made by taking specific gravity as 1.0 for both freshwater and seawater. To calculate pressure in psi from head in feet, multiply head times specific gravity times 0.433 (the factor that accounts for pressure increase with water depth).

The system must provide air to the deepest unit. Valves installed on distribution lines ensure uniform supply to shallower units, but this increases head loss and decreases blower output. The greatest source of head loss is clogging of pipes and growth on diffusers. Other factors that influence blower capacity are the elevation difference between blowers and tanks, site elevation, salinity, and temperature.

Friction loss is minimized by ensuring that the main distribution pipe is at least as large as the blower outlet port. The total length of the pipes should be minimized and the number of fittings should be limited, with few 90 degree elbow joints. Air discharged by a blower can be very hot and may require a heat dissipation pipe on the outlet to cool air before it enters the plastic piping (Rogers, 2010).

Large networks should have a pressure gauge and bleeder valve (Fig. 5.5) to ensure that pressure does not build to a level that damages the blower. Higher pressure can be achieved by reducing discharge, but it is extremely



FIG. 5.5 Air pressure gauge. Note installation of a 5-cm PVC valve for pressure regulation.

important that the blower be operated within the rated pressure range.

5.3.1.1 Blowers

Three common blower types are rotary vane, positive displacement (rotary lobe), and regenerative (centrifugal). Rotary vane blowers produce high pressure and low volume. They are less energy efficient than regenerative blowers: A ½-hp regenerative blower provides as much volume (1.7 cmm/60 cfm) as a 5 hp rotary vane. Rotary vane blowers also are more expensive and rarely are used in aquaculture.

Positive displacement, lobe-type blowers (Fig. 5.6A) produce moderate to high pressure more efficiently but require more maintenance and are noisier than regenerative blowers.

Capacities range from 10 to 10,000 m³/h at pressures up to 98 kPa (14.2 psi). They are suitable for systems that need extra pressure to overcome high head loss, such as those with an extensive delivery network and many outlets. A pressure relief valve should be installed on the discharge line near the blower to prevent overheating and failing if flow is restricted to the point that excessive pressure develops. Thermal protection prevents motor damage from overheating and is recommended. Air intake filters should be cleaned or replaced regularly to prevent clogging or suction of sand particles that can reduce performance and cause premature failure.

Regenerative blowers (Fig. 5.6B) are energy efficient, reliable, and require little maintenance because the impeller is the only moving part. They are available in a wide range of sizes (0.09 kW [1/8 hp] to 22.4 kW [30 hp]), flow rates (42 to over 1700 m³/h), and pressure ratings (<75 kPa [10.9 psi]). Because of their low pressure output, they are suited for water depths less than 4 ft (1.2 m) (Rogers, 2010). Regenerative and lobe blowers have air filters on the inlet (Fig. 5.6) that must be cleaned periodically to prevent

damage and save energy. A backup blower should be ready if the primary blower fails.

5.3.1.2 Compressors

In contrast to blowers, compressors deliver low volume at high pressure, require more maintenance, and have a shorter lifespan. Oil-less rotary vane compressors are better suited for pumping air over long distances and to greater depths.

Compressors consume more power than blowers. A 690-kPa (100-psi) compressor needs about 25 bhp (brake horsepower) per 2.83 m³/min (cmm) or 100 ft³/min (cfm). A 0.4-hp regenerative blower provides 0.85 cmm (30 cfm) at 4.8 kPa (0.7 PSI) for \$340/year; a compressor would cost \$6510/year.

High-pressure compressors are not rated for continuous duty. The aeration demand of aquaculture likely would lead to early failure of the unit. Piston/membrane compressors are quiet and energy efficient but produce only small air volumes. They are more suited for small-scale applications, such as transport tanks. Blowers thus are preferred to compressors in most aquaculture applications.



FIG. 5.6 Positive displacement blower with belt drive (A) and regenerative blowers (B) driving diffusers and airlifts in the Texas A&M-ARML 40 m³ raceways. Blowers have inlet filters.

5.3.1.3 Diffusers

Air is transferred to the water through submerged diffusers and/or airlift pumps. Rising bubbles also mix the water, thereby improving solids suspension and reducing stagnant regions. Commonly used diffusers are air stones, porous hose, and micro-bubble diffuser pads (Table 5.6 and Fig. 5.7).

Bubble size produced by typical air stones ranges from 0.5 to 3.0 mm. Smaller bubbles enhance gas transfer because of their higher surface area to volume. They also rise more slowly, which increases air–water contact time. Diffusers with smaller pores clog much more readily, especially in biofloc systems, and need higher pressure to operate. They also are more

expensive. Small-pore diffusers are best in applications that use pure oxygen.

Air stones are suitable for small-volume applications, such as aquaria, small culture tanks, and shrimp acclimation tanks. They are made of silica, plastic, glass, wood, or ceramic and are available in a variety of pore sizes. Price is based on size, performance, and composition.

Hose diffusers are tubes of porous polyester or rubber. They produce medium-sized bubbles (0.3–3.0 mm) and support relatively good air flow rates with modest head loss. They do not require high pressure. They are flexible, can be cut to desired lengths, and are easier to clean than air stones. This makes them suitable for large systems. One type, Aero-Tube

TABLE 5.6 General Characteristics of Different Diffusers

	Air Stones ^a (Silica)	Micro-Bubble Diffusers ^a (Ceramic)	Hose Diffusers ^a (Polyester)
Bubble size (mm)	0.5–3.0	0.01–0.50	0.3–3.0
Air supply (Lpm)	1.42–25.50	1.13–78.00	5.67–17.00
Water depth (m)	<0.9	<0.9	<0.9–3.0
Flow rate	94.4 L/min per m ²	0.15 L/min per cm ²	18.9–56.7 L/min per m ²
Price ^b (\$US per L/m)	0.13–2.22	14.00–33.00	24.43–185.00

^a Characteristics differ with design and brand.

^b Price estimated from 2016 catalogs and specialist websites.

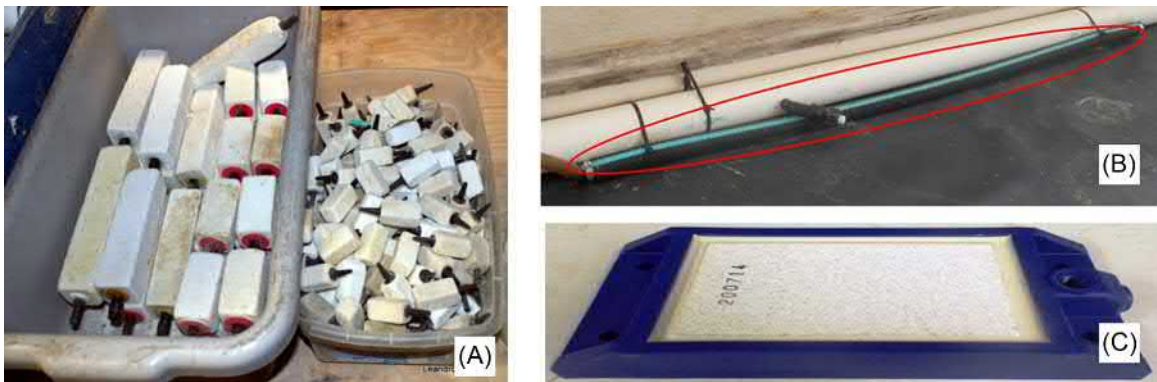


FIG. 5.7 Silica air stones (A), diffuser hose (B) (black hose with blue line) (light gray line in print version), and micro-bubble diffuser (ceramic plate) (C).

(Colorite Plastics, Ridgefield, NJ, US) includes an FDA-approved antifouling compound that reduces biofouling. Stone and hose diffusers must be weighted (stainless steel rod, ceramic weights) or attached to the tank bottom to stay at the desired depth.

Micro-bubble diffusers (ceramic plate diffusers) have a pore size around $0.3\mu\text{m}$ and produce very small bubbles ($0.01\text{--}0.5\text{mm}$). These are the most expensive diffusers and require high pressure in the range of $172\text{--}241\text{ kPa}$ ($25\text{--}35\text{ psi}$) to operate correctly. They are heavy enough that they do not require added weight. They are suitable for delivering pure oxygen to small units such as small culture tanks, acclimation tanks, and transport tanks. Micro-bubble diffusers that operate at low air pressure are becoming available and may prove useful in larger systems.

Biofouling is common in intensive culture systems and leads to reduced flow through diffusers. Regular inspection and cleaning ensures good performance. Diffuser hose is easily cleaned with a brush and freshwater but periodically requires more thorough cleaning with muriatic acid (dilute hydrochloric acid) or bleach. Air stones and micro-bubble air diffusers are treated the same way to remove fouling. The latter also can be dried and lightly sanded. Despite diligent upkeep, performance declines over time. This increases energy consumption, so diffusers must be replaced periodically.

5.3.1.4 Airlift Pumps

Airlifts aerate, mix, and circulate culture water. Their main role in biofloc systems is to provide enough mixing to keep floc aggregates in suspension. Their operating principle is simple: Air introduced near the base of a vertical section of pipe creates an air-water slurry with a lower bulk density than that of the water below the injection point. This density difference drives the air-water mixture to the surface where it is ejected from the pipe. Tank water near the lowest part of the pipe subsequently is entrained and follows the same path, thereby

setting up vertical circulation through the airlift (Lawson, 1995).

Introducing air through small holes around the perimeter of the pipe base increases flow and oxygenation, but at the expense of increased head loss and power consumption. An airlift's flow is enhanced by smaller bubbles, larger pipe diameter, and higher flow rates (Wurts et al., 1994). Airlifts often are used with diffusers in the same tank. In this case, the airlifts are installed at an angle to the water surface to drive a horizontal flow that circulates water within the tank.

Two airlift pumps used in biofloc systems are shown in Fig. 5.8. These are not the standard types made of a whole section of pipe. Instead, the pipe is cut in half length-wise, opening it to water along its entire length. These "half-pipe" airlifts operate at a lower pumping rate than the whole-pipe airlifts.

5.3.2 Mechanical Pump Systems

Some systems use mechanical pumps to circulate water and drive aeration devices, such as Venturi injectors that deliver air, pure oxygen, or a mixture of the two.

5.3.2.1 Pump Types

Centrifugal (radial flow/impeller) and axial flow (propeller) pumps are popular in RAS. Centrifugal pumps, the most common, are available in a variety of flow and head ratings. Smaller submersible pumps are used to drain reservoirs and sometimes tanks after harvest.

Those with high flow rates and low head minimize energy consumption (Malone, 2013). Most indoor biofloc systems use external (dry) centrifugal pumps with inlet filter baskets designed for swimming pools. Axial flow pumps are durable and designed for low-head/high-flow work. They are more efficient than centrifugal pumps in low-head applications, resistant to clogging, but more expensive (Malone, 2013). They are more popular in larger systems.

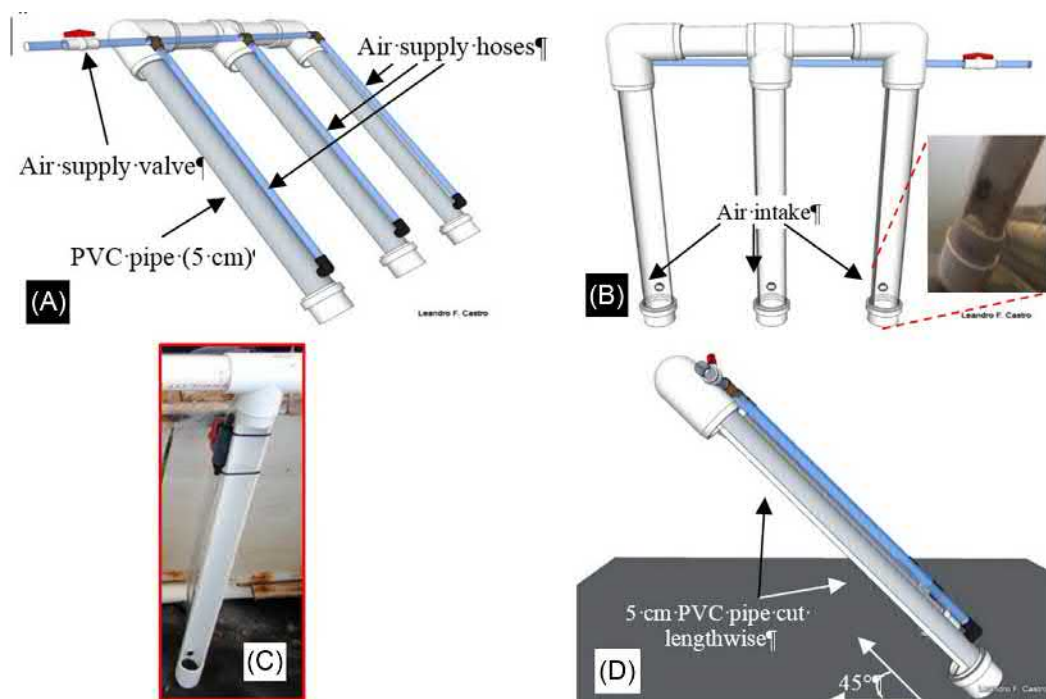


FIG. 5.8 Schematics (A, B, D) and photo (C) of an airlift in the Texas A&M-ARML 40m³ raceways. Air is injected via a polyethylene hose at the base of a 5-cm PVC pipe cut in half length-wise.

In a system fitted with pump-driven injectors, variable-speed pumps can be programmed to adjust flow rate to suit particular phases of production. For example, full flow is unlikely to be needed in the early phase of nursery production, so it can be set to a lower speed. Speed is increased to satisfy DO demand as biomass and feeding rate increase. Variable-speed pumps currently are about twice as expensive as equivalent standard pumps, but energy savings reduce operating expenses.

When choosing a pump, total system head, suction lift (height of the pump above water level), fluid characteristics (salinity, TSS), flow rate, power source (single- or three-phase power), and pumping regime (continuous or intermittent) must be considered (Bankston Jr and Baker, 2013; Timmons and Ebeling, 2013). Suppliers provide performance curves at different head and flow rates to assist selection.

5.3.2.2 Venturi

Venturi injectors (Fig. 5.9) use air, pure oxygen, or a mixture of the two to increase DO and mix the culture environment. They also can be used to deliver chemical treatments (see Section 6.2, Fig. 6.3 and Video # 28).

Because they restrict water flow (and thus increases flow velocity), moderate to high-head pumps are needed to overcome friction-induced

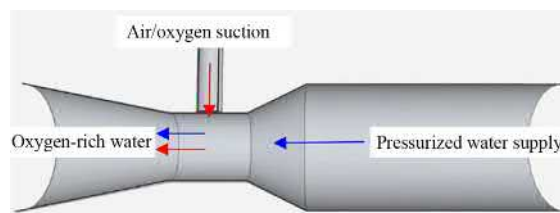


FIG. 5.9 Schematic of a Venturi injector. Air-oxygen is drawn into the flow at the point of restriction.

head losses. Venturis are installed in the water recirculation line outside of a culture tank or in a tank on individual outlets. Fig. 5.28 details the Venturi setup for the 40 m³ raceways at the Texas A&M-ARML.

The volume of air mixed into the water by a Venturi injector depends on flow rate, water pressure, tube size, and water depth. Higher pressure is needed for deeper delivery. Venturis theoretically mix air and water at a 1:1 ratio, but in practice they are much less efficient. A system using traditional Venturi devices thus may need blowers and diffusers to ensure sufficient supply of aeration.

5.3.2.3 a³ Injector

The a³ (“a-three”) injectors are an alternative to Venturis (Fig. 5.10). They operate in a similar way but produce a much higher air-to-water ratio—up to 3:1. This greatly increases aeration and provide excellent water mixing. Biofloc systems thus can be pump driven using only air, without the

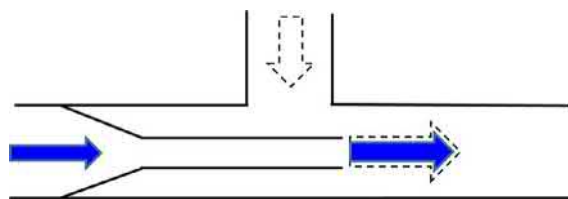


FIG. 5.10 Schematic of a³ injector. 45-psi water (blue arrow) (dark gray arrow in print version) mixes with air (dashed-line arrow).

expense of supplemental oxygen. They have been used successfully in the Texas A&M-ARML 100 m³ raceways (see Section 5.9.2.3) and commercially in Texas, Mexico, and several other facilities in Latin America and Asia.

5.3.2.4 Spray Nozzles

Spray nozzles attached to a bottom PVC pipe enhance mixing and deliver oxygen-rich water. These were installed on both sides of a 5-cm PVC pipe set under the center partition of the 40 m³ raceways at the Texas A&M-ARML to stir the bottom and uniformly distribute oxygen-rich water (see Section 5.9.1.3).

5.3.3 Pure Oxygen

High-density biofloc systems (shrimp biomass >5 kg/m³) have high oxygen demands from shrimp and floc microorganisms. With proper management (no overfeeding, control of biofloc levels), our experience is that only minimal pure oxygen is needed to support high yields. Having pure oxygen on-site nevertheless is highly recommended as a form of insurance in an emergency, such as power failure, an algal bloom crash, overfeeding, an excessive dose of organic carbon, or crop mortality.

Pure oxygen is supplied from compressed cylinders, liquid oxygen (LOX) cylinders, or an on-site generator (Fig. 5.11, Table 5.7). LOX is one-third the cost of compressed oxygen, but it

TABLE 5.7 Comparison of Pure Oxygen Sources

Equipment	Advantages	Disadvantages
Oxygen gas cylinders	Unlimited shelf life No electricity requirement	Expensive Bulky, Potentially dangerous
Liquid oxygen cylinders (LOX)	Generally cheaper than equivalent gas cylinders No electricity required	~5%/day oxygen loss
Oxygen generator	On-site supply More efficient for high volumes For remote locations	Expensive to purchase and run Vulnerable to power failure (backup recommended)



FIG. 5.11 Pure oxygen supply; (A) Liquid oxygen bottle (LOX), (B) Compressed oxygen cylinders, (C) Oxygen generator.

is lost to evaporation at about 5%/day and so is advisable only when daily supplementation is needed.

Some industrial-sized cylinders have telemetry that alerts the supplier when a tank is so low that it must be recharged. This does not release the user from periodically checking a cylinder's pressure to guard against telemetry failure.

On-site generators/concentrators provide a reliable and continuous oxygen supply. They often are employed at remote sites where commercial delivery is prohibitively expensive. Generators come in a variety of capacities: 2.7–60 kW and 1.7–59.2 m³ O₂/h. Operating and maintenance expenses (electricity, filter, oil changes) can be high, so this option naturally must be compared with others.

Pure oxygen is expensive, so it must be transferred very efficiently to culture water. Simply adding it through diffusers is very inefficient: Less than 40% of the oxygen is transferred, with the rest escaping to the atmosphere (Losordo et al., 1999). Absorption efficiency is increased by incorporating one of the following devices (Helfrich and Libey, 1991; Lekang, 2013; Losordo et al., 1999; Malone, 2013):

- An in-line, pump-driven Venturi injector with dispersal through existing nozzles or injectors. Increased pressure increases

oxygen diffusion. (This is used at the Texas A&M-ARML).

- Speece cones (down-flow bubble contactors) (Fig. 5.12), usually made of fiberglass. Water enters the narrow top at a controlled rate and leaves through an outlet at the base. Oxygen is injected at the base, middle, or top. The downward flow rate decreases as the cone widens and, at some point, equals the speed of the rising oxygen bubbles. This traps them, increasing contact time and oxygen absorption. Speece cones are simple and effective, but have a high head loss.
- U-tubes implement a clever design to increase oxygen dissolution by raising ambient pressure. In one form, a pipe configured in the shape of the letter “U” is buried to a depth of at least 10 m (~30 ft). Water flowing down one leg (the down-leg) of the U is directed to culture tanks when exiting the other (the up-leg). Oxygen is injected at the top of the down-leg and the increased pressure at the bottom—about 10 m underground—forces more oxygen into solution than it would at ground level. Outflow to the tank thus contains higher DO. A simpler design uses a straight section of outer pipe sealed at the bottom as the up-leg and a smaller-diameter inner pipe for the



FIG. 5.12 Speece cone.

down-leg and oxygen injection. Returning high-DO water then spills over the sides of the outer pipe. This is an energy efficient process, but oxygen absorption is less than with a Speece cone.

- Packed columns are sealed tubes (pressurized or not) filled with a medium—much like media used in biofilters—that increases contact between water and oxygen. Water flows in at the top and oxygen bubbles up from the bottom. Absorption efficiency is 50%–90%. A spray box operates in a similar fashion, with a nozzle at the top of the column trickling water through the medium in an oxygen-rich environment. Both columns are subject to fouling and require periodic backwashing.

Consult [Losordo et al. \(1999\)](#), [Lekang \(2013\)](#), [Malone \(2013\)](#), and [Timmons and Ebeling](#)

[\(2013\)](#) for further details on the design and use of oxygen diffusion equipment.

5.3.4 Online Oxygen Monitoring Systems

Maintaining optimal DO in any aquaculture system minimizes animal stress and crop losses. This is especially true for no-exchange, high-density biofloc systems where, in addition to shrimp, floc microorganisms consume large amounts of oxygen. A dependable system that alerts operators of low DO and implements corrective measures thus is an invaluable management tool. DO probes generally are sold with a temperature sensor. Multiple-parameter probes for pH, salinity, and turbidity also are available.

DO probes are exposed to heavy fouling in biofloc systems, so it is important to select models with proven performance and minimal maintenance requirements. Trials at the Texas A&M-ARML with YSI Inc. (Yellow Springs, OH, US) probes identified optical DO sensors with the 5500D Multi-parameter monitoring system as the best choice. They resist fouling and require less frequent calibration than either polarographic or galvanic probes under biofloc conditions.

The monitoring system software can be programmed to set DO levels that trigger corrective actions. In addition to on-site alarms, the systems can send its data to multiple offsite locations via land line, cellphone, or the internet.

Our trials have shown that the online monitoring system is very valuable in preventing water quality deterioration caused by overfeeding and DO fluctuations (see [Section 5.9.1.3](#) and [Section 14.2.1](#) and [Fig. 5.29](#)) for more information).

5.4 SOLIDS CONTROL

A variety of equipment is available to manage solids that accumulate over a biofloc run ([Table 5.8](#)). Several popular types, including those used at the Texas A&M-ARML, are discussed in more detail as follows.

TABLE 5.8 Comparison of Equipment for Solids Control in Indoor Biofloc Systems

Equipment	Filtration Size (μm)	Advantages	Disadvantages
Settling tank—baffle	>60	Cheap to construct; simple to operate; No head loss	Regular cleaning required; Poor space efficiency
Settling tank—vertical	>60		Does not remove particles <60 μm
Swirl separator	>60	Simple to operate; No head loss; Compact size	Does not remove particles <60 μm
Foam fractionator	<30	No head loss	Difficult to set flow to optimize foam production
Cyclone filter	1–75	No moving parts or filter media	High head loss
Drum filter	20–60	Space efficient; low head loss; low maintenance	High cost and power consumption; Water needed to backwash
Sand filter	>40	Easy to backwash	Moderate head loss; Frequent backwashing when high solids
Bead filter	>5	Self-cleaning; Runs at high flow; Space efficient	Not suitable for high solids concentrations

Solids removal is not likely to be needed in the early stages of production if new water is being used, but when a crop is stocked in reused (aged) water with well-established biofloc, close attention must be paid to particulate matter load from the beginning. It is important not to remove too many floc aggregates, as this defeats the purpose of using biofloc.

5.4.1 Settling Tanks

External settling (sedimentation) tanks remove settleable solids by gravity. They can be conical (Figs. 5.13, 5.30, and Fig. 5.45), rectangular, or circular and may be fitted with baffles to minimize turbulence, thus enhancing particle settling (Timmons and Ebeling, 2013). They are cheap to construct, simple to operate, and effective at removing particles

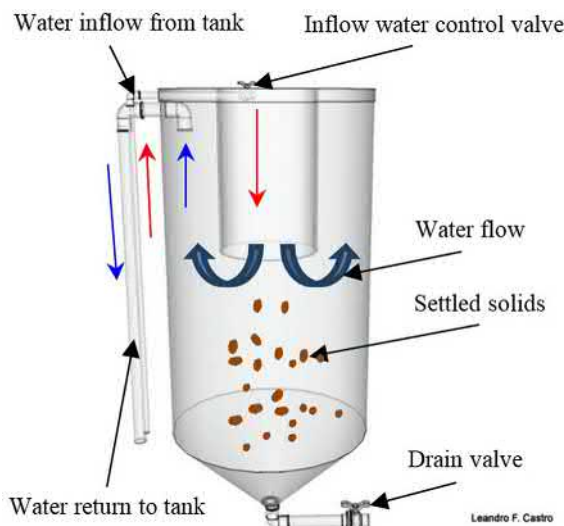


FIG. 5.13 Diagram of a simple conical settling tank. Red arrows (light gray in print version): water from culture tank. Blue arrows (dark arrow in print version): water return to tank.

>60 μm . They require regular cleaning to remove accumulated solids, particularly as system biomass increases.

Settling tanks can be fed by a portion of the water flowing through a side loop of the return line to the culture tank. Water depth should be at least 1.2 m (~4 ft) and have a retention time of at least 15–30 min (Malone, 2013). Settling times at different stages of production are estimated with Imhoff cones see Fig. AI.1, Video # 31, and Malone (2013). Sedimentation rates are influenced by tank design, solids characteristics (density, geometry), and by adjusting flow to regulate retention time.

All facilities must have procedures to dispose of solids captured from the culture tanks. Reducing the high water content in the settled solids facilitates disposal (see Fig. 5.33 and Section 5.9.1.3 for details). A larger filtration surface area is necessary when a high volume of solids is collected. Large-scale dewatering methods include Geotube, evaporation basins, and sand drying beds.

Denitrification may occur in areas of the settling tank that become anoxic (see Section 11.1 and Fig. 11.1). Some decomposition also occurs in settling tanks. This can increase ammonia and lower DO in return water (Losordo et al., 1999). Hydrogen sulfide may also be produced by sludge collected on the tank bottom. To avoid this, remove sludge at least weekly and dispose or digest in a separate treatment unit. Increase the frequency of removal as biomass and sludge accumulation increase. Water returned to the tank must be tested regularly to ensure that it is free of hydrogen sulfide.

Sections 5.9.1.3 and 5.9.2.3 describe the settling tanks used at Texas A&M-ARML.

5.4.2 Foam Fractionators

A foam fractionator, also called a protein skimmer, is an effective and inexpensive device for controlling the concentration of small (<30 μm) suspended particles. “Foam

fractionator” is the more appropriate term because more than protein is removed. They can be purchased from commercial suppliers or, owing to their very simple design, constructed by the production staff.

The operating principle is simple: a constant supply of small air bubbles captures fine particles and some colloidal material from the tank by adsorption. The thick foam that results is collected, dewatered, and disposed. A foam fractionator operating at maximum efficiency removes about 30 g of fine solids for every 20 Lpm air flow and 90 cm² of column cross-sectional area (Timmons and Ebeling, 2013).

Transfer is more effective with smaller bubbles, longer contact times, and higher pH (Losordo et al., 1999; Timmons and Ebeling, 2013). Most foam fractionators have Venturi injectors because of their finer bubbles (see Section 5.9.1.3). Ozone may be used, but care must be taken so that residual ozone is not returned to the tank where it can harm biofloc microorganisms and shrimp (see Section 6.2.8).

5.4.3 Cyclone Filter

Hydrocyclones (Fig. 5.14) remove solids by spinning them out of suspension. They are simple to operate and effective if flushed frequently. The Waterco Multicyclone 16 operates best within 50–500 Lpm. They often are used as prefilters to reduce the particle load on downstream filtration gear.

5.4.4 Other Solids Filtration

5.4.4.1 Swirl Separator

Radial flow (swirl) separators (Fig. 5.15) are popular alternatives to settling tanks. These devices produce a vortex (whirlpool) that spins heavier solids outward toward the walls of a conical tank. Particles then settle through this less turbulent boundary, collect at the base of the cone, and are removed.



FIG. 5.14 Hydrocyclone filter.

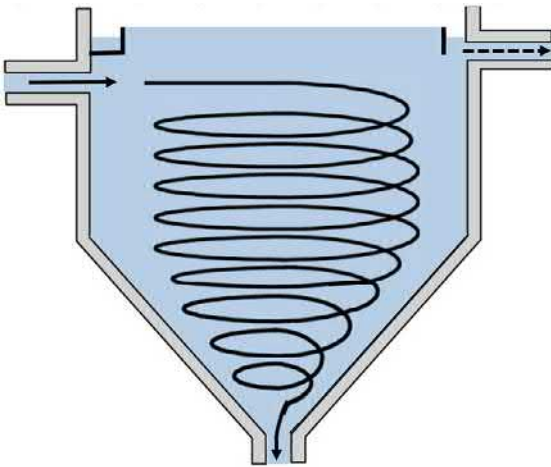


FIG. 5.15 A swirl separator.

The swirling shortens retention time and thus permits higher flow rates without sacrificing solids removal. These units are compact

(Losordo et al., 1999) and more efficient than traditional settling tanks.

5.4.4.2 Sand Filter/Floating Bead Filter

Pressurized sand filters (Fig. 5.16) force water through a volume of sand. Depending upon the sand's grade (grain size), particles as small as $40\mu\text{m}$ are removed from the flow stream. Flow capacities range from 110 to 150 Lpm to more than 11,500 to 15,000 Lpm for units used in large commercial systems.

Sand filters are cleaned by reversing flow (back-washing) to free captured particles. Bacterial growth clogs long-running filters. This causes head loss and requires more labor-intensive cleaning.

Floating-bead filters, such as PolyGeyser filters (Fig. 5.16), trap solids in a similar manner to sand filters, but plastic beads are the filter medium and water is pumped up through the beads. Collected solids settle to the base for manual or automatic removal through a purge valve (Malone, 2013).

These units filter particles in the $5\text{--}30\mu\text{m}$ range and are available in a variety of sizes and flow capacities. They clog less frequently than sand filters and self-backwash with propellers or air bubbles.

Both sand and bead filters are space efficient, operate at high flow rates, and are suitable for treating incoming water. They do, however, clog too quickly for effective filtering of biofloc systems.

5.4.4.3 Drum Filters

Drum filters process water through a rotating steel or polyester screen with a mesh size usually from 20 to $60\mu\text{m}$ (Fig. 5.17). Solids caught on the screen are removed continually or periodically by high-pressure water jets and collected in a waste drain. Flow rates range from 120 to 75,000 Lpm.

They are space efficient, require minimal maintenance, and operate at low head (Losordo et al., 1999; Malone, 2013). They are,



FIG. 5.16 Left photo—Pressurized Sand Filter with sand used for filtration; Right photo—Poly Geyser bead filter with bead media.



FIG. 5.17 Drum filter.

however, more expensive to install and operate than passive filtration methods and rarely are used in biofloc systems. See [Losordo et al. \(1999\)](#) and [Timmons and Ebeling \(2013\)](#) for more details on drum filter design and operation.

5.5 AUTOMATIC FEEDERS

Automatic feeders deliver feed on a set schedule with minimal labor. They improve growth and FCR ([Limsuwan and Ching, 2013](#)) and also reduce production expenses, cannibalism, accumulation of uneaten feed, postfeeding ammonia spikes, and DO drops. The same equipment can dispense probiotics and medications. Automatic feeders employed in indoor shrimp production include:

5.5.1 Belt Feeders

Spring-wound belt feeders ([Fig. 5.18](#)) are common, especially with relatively small tanks. They have a clock mechanism that can be set to deliver 3–5 kg of feed over 12 or 24 h. Belt feeders typically cost \$230–\$300. A 30 × 5 m raceway requires feeders spaced 15 m apart for a total cost of \$1200. For eight raceways (and two spare feeders), the cost is more than \$10,000. They thus can represent a significant cost.



FIG. 5.18 Belt feeders placed over shrimp production raceways.

Some producers prefer 12-h feeding cycles because problems are identified more quickly if a unit fails, but this rarely happens when feeders are maintained in good condition with regular cleaning and lubrication. Twelve-hour feeders require more staff attention but have a higher daily capacity. Four 24-h feeders loaded once per day deliver a maximum ration of 20 kg/day. Four 12-h feeders loaded twice per day deliver 40 kg/day.

Twelve-hour feeders make it easier to adjust ration based on observed consumption. For example, if there is uneaten feed in the tank before refilling the feeders, smaller rations are loaded. When algae dominate the system, DO will be lower at night, so 12-h feeders make it easier to manage feed portions. Electric models can be connected to a DO monitoring system.

5.5.2 Electric Feeders

Electric and battery-powered feeders are driven by a magnet with an alternating movement, a motor with a revolving movement, or vibration (Varadi, 1984). The distributor may be an electromagnet sliding over an outlet, rotating disks, screws (auger), revolving spikes that tip over feed containers, or belts (Lekang, 2013; New, 1987; Pillay and Kutty, 2005). A container (hopper) sits above the distribution mechanism. Electric feeders are more expensive than belt feeders but provide more control. For example, a timer or computer can set the amount, interval, and duration of feed delivery. Distribution also

can be linked to monitoring equipment that adjusts feeding if DO drops below a set threshold.

5.5.3 Pneumatic (Compressed Air) Feeders

Pneumatic feeders use compressed air to dispense feed according to a timer, a computer, video- or acoustic-sensors to adjust the feeding interval. These are widely used in salmon farming net pens and more recently in shrimp ponds.

Blowers mounted on a vehicle broadcast feed over wide areas (Pillay and Kutty, 2005), but these are more suited for outdoor ponds, extensive arrays of wide raceways, or very large indoor tanks.

5.5.4 Peristaltic Pumps

Shrimp may benefit from live or wet feed, such as *Artemia* or a microencapsulated *Artemia* replacement, during early nursery stages. This may be added manually, but wet diets can be kept in aerated containers and delivered with a variable-speed peristaltic pump.

Automatic feeders are spaced around culture tanks to ensure even feed distribution. They can be mounted on tank sides, walkways (Fig. 5.19), or over tanks. In 30×5 m and 50×6 m raceways, spacing of 15 and 16.7 m, respectively, has proven adequate. Spacing of 25 m was ineffective in nursery systems where water flow is purposefully low. It led to poor feed distribution



FIG. 5.19 Evenly spaced belt feeders mounted on walkways over a raceway, and a single belt feeder mounted on the side of a culture tank.

that increased the energy postlarvae had to expend foraging for food.

Avoid placing feeders near pump intake screens so that newly added feed is not quickly washed out of the tank. Install feeders at least 30–45 cm above the water surface to avoid splashing the feeder outlet: Wet feed forms clumps that block smooth delivery and wastes feed. Distributing small or powdered feeds along the midline of belt feeders also reduces sticking. There is, in fact, no need to cover the whole width of the belt with feed because it disperses sufficiently when dropped into a well-mixed tank.

Mounting feeders between waist- and chest-level provides easier access for workers. This pays off with fewer feed spills and more thorough routine maintenance and cleaning. Periodically

inspect all feeders to ensure that they are operating correctly and have not become blocked by feed clumps. Clean feeders before refilling to maintain good hygiene and performance. Conduct regular servicing, such as lubricating gears, according to the manufacturer's recommendations.

5.6 SAFETY SYSTEMS

5.6.1 Theft and Predator Control

Standard security measures prevent entry of unauthorized personnel and predators, both of which can damage facilities, take shrimp, and introduce pathogens. Specific measures depend on the facility's remoteness and local predators. Defensive responses include (Fig. 5.20):

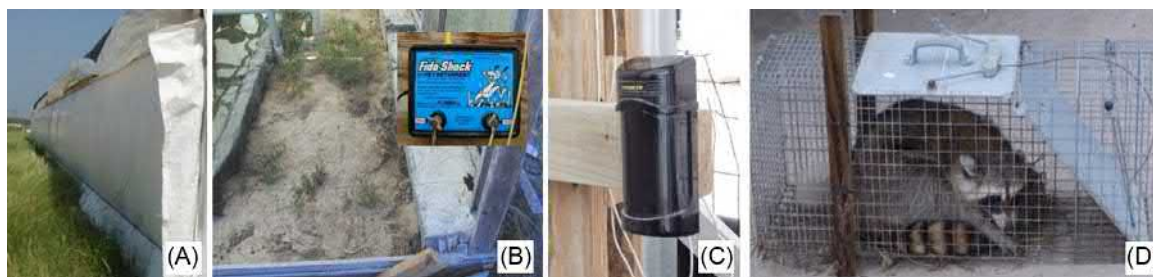


FIG. 5.20 Some measures to prevent entry of unauthorized personnel and predators: (A) walls, (B) electrified wire, (C) motion detector, (D) predator trap.

- Perimeter fencing with lockable gates
- Alarm systems and motion detectors linked to sirens and lights to alert staff and guards
- Security lighting (fixed and motion-activated)
- Security cameras
- Workers living on-site or 24-h work shifts
- Solid walls around culture tanks and lockable individual buildings
- Electrified wire around the perimeter
- Predator traps

5.6.2 Backup Power

Reliable backup power is vital for super-intensive systems. Maintain both a fixed standby generator of sufficient capacity to operate all essential equipment and a smaller portable generator to serve individual systems in the event of a power outage. See [Section 5.2.6.3](#) for generator requirements.

5.6.3 Backup Equipment

All backup equipment must be in good working condition and with compatible fittings so that a unit is ready for installation in case of an equipment failure. Time is at a premium when switching a pump that supplies DO or maintains floc in suspension; staff should not have to search for a spare or its fittings in the middle of the night when a raceway filled with shrimp is without water or air flow.

Alternatively, spare pumps and blowers can be permanently connected to the system and quickly turned on when needed simply by opening a few valves. Likewise, backup oxygen cylinders should be close to tanks, ideally with a bottle connected to each tank's recirculation line. Other recommended backup equipment includes nets, DO meter, and water quality test kits (ammonia, nitrite, and pH).

5.6.4 Water Quality Monitoring

Water quality can deteriorate rapidly in super-intensive systems. Without quick action, this can lead to significant crop loss. Modern

DO monitoring systems inform staff via alarm, text message (SMS), and/or phone when DO drops below a set limit. These systems output real-time DO over an internet connection and some can be programmed to start pure oxygen supply or stop automatic feeders. Temperature control systems also can be programmed to start, open, or close ventilation, and control heat exchangers.

5.6.5 Alarm Systems

Alarm systems are recommended to monitor the following properties:

- Low DO
- Power loss
- Low or high temperature
- Water, air, or oxygen flow (e.g., through flow switches)
- Low or high water level (e.g., through float switches)
- Theft and predation (e.g., motion sensors)

Systems should call managers when an incident occurs and repeat until the alarm is acknowledged.

5.7 WATER QUALITY LABORATORY

The water quality laboratory is a core part of an intensive production facility. Its analytical equipment depends on the facility's scale and budget. A large facility might use an expensive flow-injection auto-analyzer ([Fig. 5.21](#)) to measure dissolved inorganic compounds, but a small facility can manage well with commercial test kits.

[Appendix IV](#) provides recommended equipment, and supplies for an aquaculture water quality lab, and basic safety guidelines. For more detail on general laboratory setup and operation, see [Barker \(1998\)](#).



FIG. 5.21 Flow-injection analyzer used to measure ammonia, nitrite, nitrate, and phosphate at the Texas A&M-ARML.

5.8 RECOMMENDED EQUIPMENT SUMMARY

A list of recommended equipment for biofloc shrimp production as practiced at Texas A&M-ARML is compiled in [Table 5.9](#).

5.9 THE TEXAS A&M-ARML SYSTEMS

Nursery and the grow-out trials at Texas A&M-ARML were conducted in two systems. The older one had six 40m³ raceways built in 1979 for over-wintering shrimp broodstock. Budget constraints limited modifications that would make these tanks suitable for nursery and grow-out, so the design described as follows reflects cost-effective adaptations.

In 2010 the USDA Marine Shrimp Farming Program funded construction of two 100m³ raceways in a greenhouse. The system had most desired features except active temperature control.

5.9.1 40m³ Raceway System

5.9.1.1 Greenhouse

Tanks are protected by a 1000m² Quonset greenhouse (34 × 30m) with no active heating. The structure has semitranslucent woven

polyethylene folding side walls and a roof of translucent corrugated fiberglass panels ([Fig. 5.21A](#)) covered by 73% light-reduction knitted black shade cloth (DeWitt, Sikeston, MS, US). It is sprayed with white greenhouse shade paint (Kool Ray, Growers Supply, Dyersville, IW, US).

The greenhouse extends the growing season from early spring to late fall, periods when air temperatures usually are too low for outdoor culture in south Texas.

Three two-speed, ¼ hp, 76cm exhaust ventilation fans (item # 294498A, Global Equipment Company, Charlotte, NC, US) mounted at the front of the greenhouse plus shutters at the back ([Fig. 5.21A](#)) circulate air and lower water temperatures, which can exceed 34°C during summer.

More expensive shading and light-deflecting materials have become available and can lower inside air temperatures by 3–4°C more than shade cloth.

The structure has a small side door and three garage doors at the front that facilitate stocking and harvest activities. Three small doors at the back provide easy access to air blowers and backup power. Internal light fixtures allow for nighttime activities.

Side walls are protected by an electric-wire shocker to exclude predators when side walls are up [[Fig. 5.21A\(H\)](#)]. The structure has a

TABLE 5.9 Recommended Equipment for Indoor Super-Intensive Biofloc Shrimp Production

Equipment Culture System	Purpose
• Acclimation tanks	Acclimating newly arrived postlarvae
• Culture tanks (nursery)	Postlarvae to juvenile shrimp rearing
• Culture tanks (grow-out)	Juvenile shrimp rearing
• Recirculation equipment (i.e., pumps) and associated plumbing and gauges	Water circulation, mixing and aeration
• Aeration equipment (i.e., blowers, diffusers, airlift pumps, Venturi nozzles, injectors) and associated plumbing and gauges	Water circulation, mixing and aeration
• Solids removal equipment (e.g., settling tanks, foam fractionators, sludge separator tanks, etc.)	Solids control
• Pure oxygen supply equipment (e.g., oxygen generator or cylinders) and plumbing	Pure oxygen supply
• Temperature control equipment (e.g., heat exchanger, heat pump, fans, etc.)	Maintaining optimum temperature
• Manual mixing tool	Mixing culture tanks
WATER SUPPLY AND WASTE TREATMENT	
• Water supply pump and associated plumbing	Water supply
• Intake screens/filters	Prevent predator, parasite, and disease carrier entry; Filter particulate matter
• Reservoir	Water storage and treatment (volume at least that of all culture tanks combined)
• Mixing tank	Water treatment and preparation
• Denitrification/Digestion tank/system	Water quality restoration and solids digestion
• Water/solids disposal system (e.g., artificial wetland, evaporation basin, aquifer, etc.)	Disposing wastewater and solids
• Sludge pumps/submersible pumps	Wastewater/solids transport
• Assorted hoses	Water transport
FEED	
• Storage bins (silos) or temperature-controlled room for feed bags	Feed storage
• Belt feeders	Gradual feed distribution
• Buckets	Feed transport and distribution
• Electronic balance (1g readability for nursery, 5–10g for grow-out)	Weighing feed rations
• Feed scoops	Feed ration handling

Continued

TABLE 5.9 Recommended Equipment for Indoor Super-Intensive Biofloc Shrimp Production—cont'd

Equipment Culture System	Purpose
SAMPLING AND EXAMINATION	
• Nets (various size scoop nets and cast nets)	Shrimp sampling
• Sample jars or beakers	Sample transport and storage
• Microscopes (dissecting and compound) and related supplies	Shrimp examination
• Electronic balances (0.1–1.0-mg readability)	Weighing shrimp
• Dissecting kits	Shrimp examination and sample preparation
• Refrigerator and freezer	Sample storage
WATER QUALITY MONITORING^a	
• Multiprobe (DO, salinity, temperature, pH)	Measure DO, salinity, temperature, and pH
• Refractometer	Measure Salinity
• Imhoff cones	Measure Settleable solids
• Meter to measure dissolved nutrients and TSS (e.g., spectrophotometer)	Measure NH ₃ , NO ₂ , NO ₃ , PO ₄ , other ions, alkalinity, and TSS
• Glassware for analyzing alkalinity (burette, stand, Erlenmeyer flasks, graduated cylinders)	Measure alkalinity
• TSS probe	Real-time TSS monitoring
• DO monitoring system with remote access	Continuous DO monitoring in each tank
• Electronic balance (0.1-mg readability)	Weighing reagents
• Refrigerator	Sample and chemical storage
• Micropipettes (electronic or manual)	Sample handling
• Assorted glassware	Sample handling
• Plastic sample jars	Transport and storage of water samples
• Safety equipment (e.g., gloves, safety glasses, respirators, lab coats, eye wash station, etc.)	Reagent handling
WATER TREATMENT AND DISINFECTION	
• Chemical storage bins	Chemical storage (sugar, sodium bicarbonate, sodium hydroxide, etc.)
• Liquid chemical containment trays	Contain stored chemicals
• Electronic balance (1-g readability)	Weighing chemicals
• Chemical/inoculant handling and transport gear (measuring cylinders, buckets, scoops, etc.)	Chemical/inoculant (e.g., probiotics, nitrifying bacteria) handling and transport
• Pressure cleaner	Cleaning culture tanks and equipment
• Pressure sprayer	Disinfecting tanks and equipment

TABLE 5.9 Recommended Equipment for Indoor Super-Intensive Biofloc Shrimp Production—cont'd

Equipment Culture System	Purpose
• Safety equipment (e.g., gloves, safety glasses, respirators, lab coats, eyewash station, etc.)	Chemical handling
HARVEST AND POSTHARVEST	
• Dip, seine, and cast nets	Harvesting shrimp
• Live hauling tanks	Live harvest
• Harvest basin	Harvesting shrimp
• Harvesting machinery (Fish pump, pneumatic pump, submersible pump, dewatering device, grader, conveyer, and associated hoses)	Harvesting shrimp
• Harvest baskets with lids	Collecting, weighing, moving harvest
• Tallies (hand held counters)	Counting harvested shrimp samples
• Counting bowl/frame	Counting and inspecting harvested shrimp
• Buckets	Handling shrimp samples and ice
• Electronic balance (top load, washable)	Weighing harvested shrimp
• Ice maker	Ice supply for harvest and transport
• Insulated bulk containers (harvest bins)	Ice storage; shrimp storage and transport
• IQF machinery	Freezing shrimp
• Cold storage facilities	Harvested shrimp storage
SAFETY SYSTEMS	
• Main backup generator	Backup power for all essential equipment
• Portable generator	Backup power for smaller electrical units
• Alarm system (Water quality and security)	Notify workers and managers of water quality problem such as low DO, power failure, or unauthorized access to the facility
• Security lighting	Security
OFFICE AND WORKSHOP	
• Computer (spreadsheet, word processing, water quality software) internet access	General office functions: record-keeping, data analysis, communications, marketing
• Printer/Scanner/Photocopier	General office functions
• Laminator	Producing signs and information sheets
• Telephone	Communications and sales
• Reference material, such as manuals and related texts (electronic or hard-copy)	Information sources

Continued

TABLE 5.9 Recommended Equipment for Indoor Super-Intensive Biofloc Shrimp Production—cont'd

Equipment Culture System	Purpose
Workshop supplies and tools (cordless drill, grinder, circular saw, hack saw, heat gun, staple gun, pipe wrench, plumbing materials, shovel, extension cords, safety equipment, etc.)	General construction, maintenance and repair
First aid kit	Staff first aid needs
LIFTING AND TRANSPORT	
Vehicles (on-road and off road)	Transport personnel, gear, materials, shrimp
Forklift	On-site lifting and transport
Hand-transport equipment (e.g., wheelbarrows, pallet jacks, and hand trucks)	Small-scale lifting and transport

^a See [Appendix IV](#) for a more detailed list for a water quality lab.

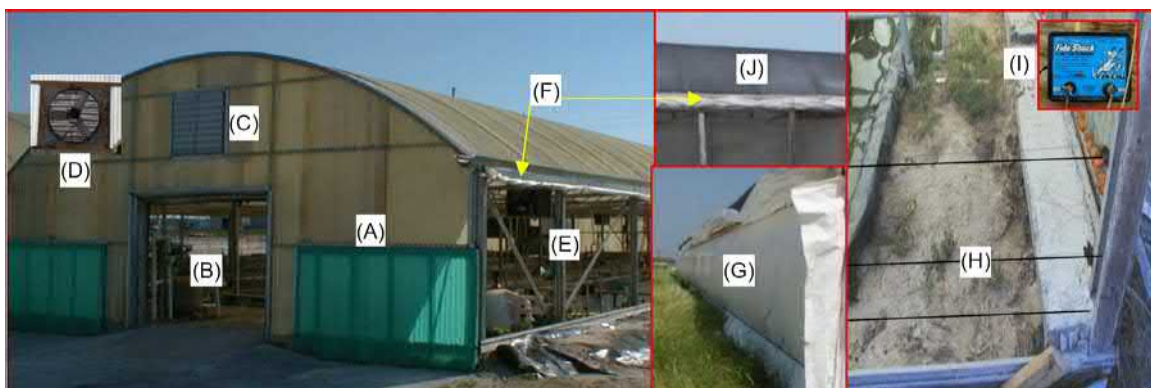


FIG. 5.21A A greenhouse with six 40m³ raceways at Texas A&M-ARML. Corrugated fiberglass on front wall (A), one of three garage doors (B), outside view of fan-shutter (C), inside view of fan (D), open side wall (E) rolled-up (F) and rolled-down (G), electrified wires on the side wall (H) with a controller (I), and shade cloth covering the roof (J).

concrete footing to prevent raccoons from digging tunnels to enter into the greenhouse.

The greenhouse is equipped with a dial-out remote monitoring system (Sensaphone 400, Aston, PA, US) with power outage, air pressure, loud noise, and air temperature sensors. The facility is protected by motion sensors to discourage theft.

5.9.1.2 Culture Tanks

The culture system has six shallow (45cm) rectangular tanks constructed of excavated trenches reinforced with pneumatically sprayed concrete side walls. Each raceway is lined with 1 mm EPDM (Firestone Specialty Products Company, Indianapolis, IN, US).

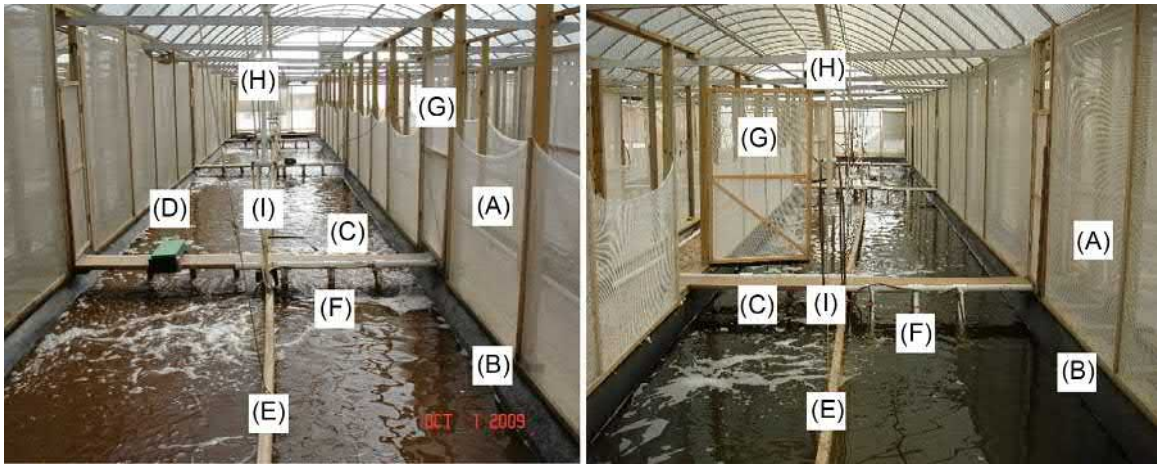


FIG. 5.22 Photos of 40m³ raceways and support systems: (A) antijump netting, (B) freeboard, (C) boardwalk, (D) belt feeder, (E) center partition, (F) three 5-cm airlifts, (G) access door, (H) 2.5-cm PVC air distribution pipe, (I) ropes for positioning center partition.

The elongated shape and center partition resemble a racetrack, thus the name “raceway.”

All but one raceway (which has a concrete bottom) have sand under the liner with a 0.5% slope. Each has a surface area of $30.5 \times 3.4 \text{ m} = 103.7 \text{ m}^2$ ($100.1 \times 11.15 \text{ ft} = 1116.1 \text{ ft}^2$) with bottom area of $28.0 \times 2.4 \text{ m} = 67.2 \text{ m}^2$ ($91.86 \times 7.87 \text{ ft} = 723 \text{ ft}^2$). The working water volume is 40m³ (10,570 gal) with a 45-cm average water depth.

Each raceway is fitted with five wooden planks— $30.5 \times 5 \text{ cm} \times 3.65 \text{ m}$ ($12 \times 2 \text{ in} \times 12 \text{ ft}$). Two are placed about 1 m from each end and the other three are placed equidistantly across the width to support airlift pumps and belt feeders (see Fig. 5.22 and Fig. 5.23). These boardwalks also facilitate bottom inspection and feed consumption monitoring.

FREEBOARD AND ANTIJUMP NETTING

Each raceway is surrounded by a wooden frame with five access doors above the boardwalks. Doors were made from untreated $5 \times 10.2 \text{ cm}$ ($2 \times 4 \text{ in}$) planks and covered by 1-m (39-in) high white knitted shade cloth (Dewitt, Sikeston, MS, US) to prevent jumping.

Fig. 5.22 shows the 40m³ raceways surrounded by wooden structures with access doors and antijump netting (see Video # 27). Fig. 5.23 shows a top-view schematic of the support systems.

These raceways were not designed for shrimp production, so many modifications were made over the course of our studies. The experience gained in that work was essential in designing larger scale commercial systems with six or more 300–500m³ raceways in South Korea.

Observations made over three decades of work with this system are summarized as follows.

- Avoid greenhouse-enclosed shallow tanks, especially in temperate climates. Stocking densities in no-exchange, biofloc-dominated systems are determined by volume rather than surface area, so a tank with volume-to-surface ratio of 1:1 produces twice the yield of a tank of the same surface area but half the volume. Lower temperature fluctuations in deeper tanks improve stability of the culture environment and shrimp performance.
- Avoid membrane-lined, in-ground tanks in areas with a shallow water table. In addition

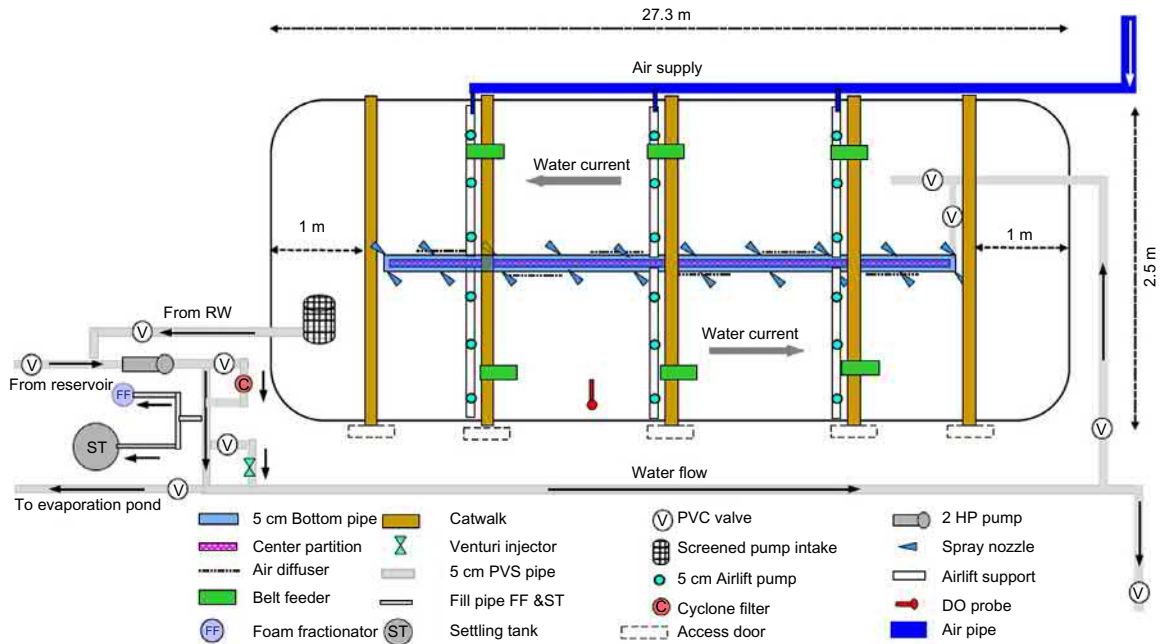


FIG. 5.23 Top-view schematic drawing of 40m³ raceway with support systems.

to potential contamination from groundwater, the liner may float after a heavy rain, interfering with stocking, harvesting, and routine husbandry.

- Avoid tanks that cannot be gravity drained for harvest.
- Use separate pumps for oxygen enrichment and draining.

ACCESS

Allow space around the tank for access by staff to observe, mix, sample, adjust equipment, and recover dead shrimp. Regularly spaced boards across raceways improve access to the center (Fig. 5.22 and Fig. 5.23). Entrance gates are needed when using antijump netting.

5.9.1.3 Raceway Support and Management Tools

PUMPS AND WATER MOVEMENT

The 40m³ raceways do not drain by gravity. Shrimp are harvested with dip nets after pumping out at least two-third of the volume with a

2-hp centrifugal pump (Hydrostorm, Waterco Inc., Augusta, GA, US). Except for limited use during filling and draining, the pump mainly serves to circulate and oxygenate culture water.

WATER INTAKE AND PIPING NETWORK

All PVC piping is Schedule 40. The pump is fed via a 15-cm (6-in) PVC filter pipe nested in a concrete-embedded 90° PVC elbow. The outlet is a few centimeters below grade to facilitate draining. The nested filter pipe has a perforated air ring made from 1.6-cm (5/8-in) clear flexible polyethylene tubing to minimize clogging (Fig. 7.7 and Fig. 9.1 and Video # 1).

The 2-hp pump has several functions:

- (1) Circulate and oxygenate culture water via a Venturi injector
- (2) Enhance bottom circulation using a bottom spray pipe
- (3) Fill the raceway from a 2200-m³ lined pond or outdoor 36-m³ fiberglass storage tank
- (4) Add freshwater or seawater from an outdoor fiberglass storage tank

- (5) Pump water from raceways to an evaporation pond for disposal
- (6) Transfer water from a raceway with high nitrifying activity or start a controlled study with uniform-quality water in all raceways

CENTER PARTITION

Each raceway has a fiberglass partition (25 m long $\times$ 0.6 m high $\times$ 3 mm thick) running longitudinally along the center, 1.5 m from each wall. The bottom and top of the fiberglass are fitted with wooden slats. Ropes hanging from the greenhouse are attached to the top of the partition keep it centered. Air diffusers, weight stabilizers, and a bottom spray pipe are fastened with zip-ties (tie-wraps or cable ties). Weight stabilizers are made from capped 3.8-cm (1.5-in) PVC pipe filled with sand and fixed directly below the partition and above the bottom spray pipe. They help keep the partition in place by preventing it from floating (Fig. 5.24).

BOTTOM SPRAY PIPE

Spray nozzles attached to a bottom PVC pipe enhance mixing and deliver oxygen-rich water. They are on both sides of a 5-cm (2-in) PVC pipe under the center partition. This pipe has 45 degree 1.6-cm (5/8-in) street-spray nozzle assemblies (Remcor Inc., Howe, TX, US) every 0.9 m. The first one-third of the pipe from the shallow end has the complete nozzle assembly, the next one-third has the nozzle assembly without the sprayer tips, and the last segment has only the 45 degree street adapter (Fig. 5.25A–C and Video # 2).

This arrangement facilitates uniform delivery of oxygen-rich water along the length of the raceway, preventing release of oxygen-rich water only at the shallow end. The deep end of the pipe has a 5-cm (2-in) PVC threaded cap for removal of any accumulated solids (Fig. 9.2).

The pump discharge is connected to two PVC ball valves that regulate water flow: One controls supply to the raceway (Fig. 5.26C) and

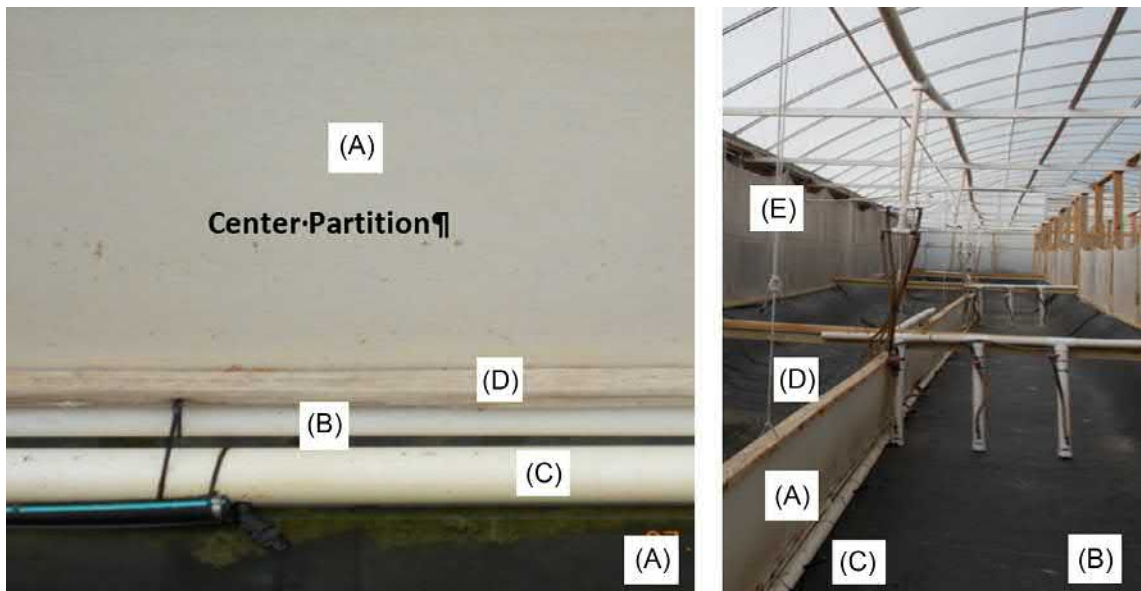


FIG. 5.24 Close-up (A) and general layout of the raceway's center partition (B); center partition (a), weight made of 3.8-cm PVC pipe above spray pipe (b), 5-cm PVC spray pipe (c), partition support (d), rope holding the partition (e).

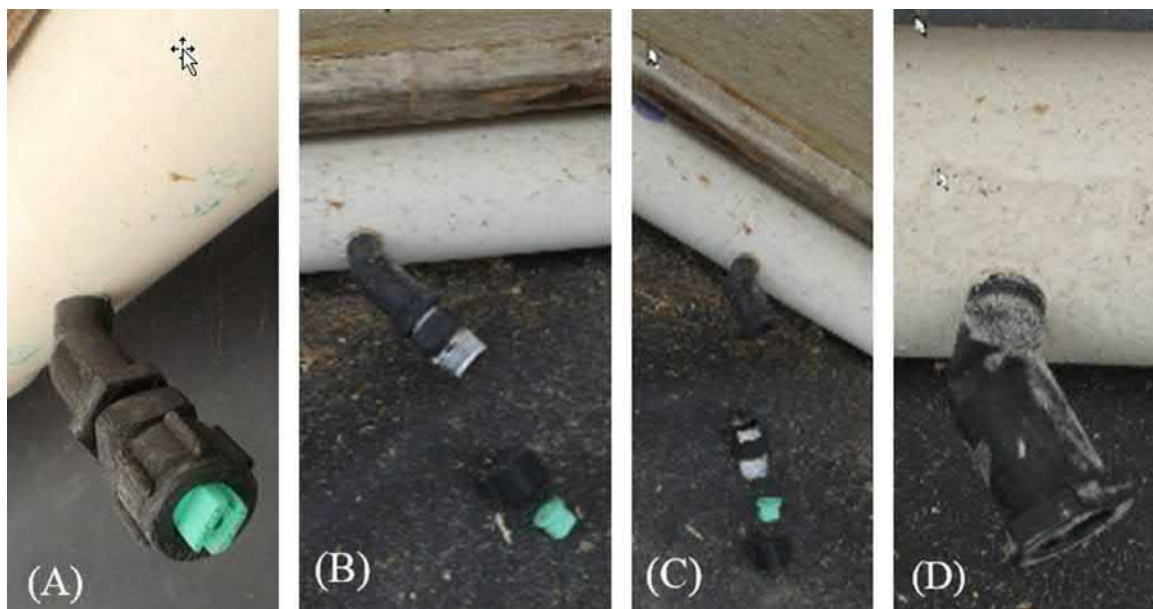


FIG. 5.25 Spray nozzle in bottom spray pipe: (A) complete set, (B) assembly without spray tip, (C) street adapter.

the other diverts water to an evaporation pond (Fig. 5.26D). Two valves at the shallow end of each raceway regulate flow to the bottom spray pipe and the raceway (Fig. 9.4). Adjusting flow through the valve and the Venturis (Fig. 9.3) ensures delivery of fine bubbles through both outlets.

AIR-DELIVERY SYSTEM

Six banks of three 5-cm (2-in) slotted-type airlift pumps mix and aerate the tank (Fig. 5.8 and Video # 2). Each raceway has six 0.92-m air diffusers (1.9-cm outside diameter Aero-Tube) attached to the bottom of the spray pipe, adjacent to the airlift pump banks.

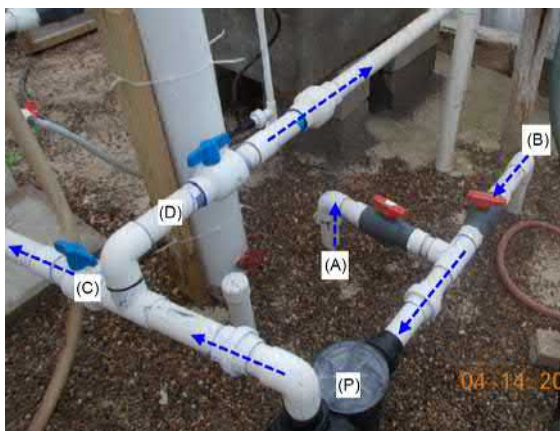


FIG. 5.26 Two-hp pump with 5-cm PVC pipe network and valves of 40m³ raceway; (A) water from raceway, (B) water from reservoir, (C) water to raceway, (D) water to evaporation pond, (P) pump. Blue lines (dotted dark gray line in print version) show direction of flow.

Airlift pumps and diffusers are set equidistant on both sides of the partition and supplied by either a 3.5-hp regenerative blower (S63 Sweetwater, Pentair, Aquatic-Eco System, Apopka, FL, US) or a 7.5-hp positive displacement blower (see Chapter 7). The larger blower is used when biomass exceeds 2 kg/m^3 .

Air is delivered via a 7.5-cm (3-in) PVC pipe connected to a 5-cm (2-in) PVC distribution network that can isolate the air supply in any raceway. The pipe network is interconnected to equalize air pressure throughout the distribution system. Air is delivered from the network to the banks of airlift pumps and diffusers through 2.5-cm (1-in) PVC pipes and then to each unit through a 1.6-cm (5/8-in) clear polyethylene hose connected to a PVC ball valve (Fig. 5.27C–D).

PURE OXYGEN DELIVERY

Each raceway has an oxygen mixing and delivery system. Oxygen enrichment and tank draining use the same pump. It is better to separate oxygenation and draining to expedite harvests and prevent low DO when shrimp are concentrated in a small volume of water during harvest.

Unlike the 100 m^3 raceways, in which biomass can exceed 9 kg/m^3 when using a^3 injectors and air, Venturis in the 40 m^3 system sustained only $5\text{--}6\text{ kg/m}^3$ with air. Trials showed that enriching air with oxygen (from compressed oxygen or LOX cylinders) at 0.35 L/min supported up to yield of 9.75 kg/m^3 . The Venturi manufacturer (Mazzei Injector Co., Bakersfield, CA, US) suggested that oxygen consumption could be reduced by modifying the existing oxygen

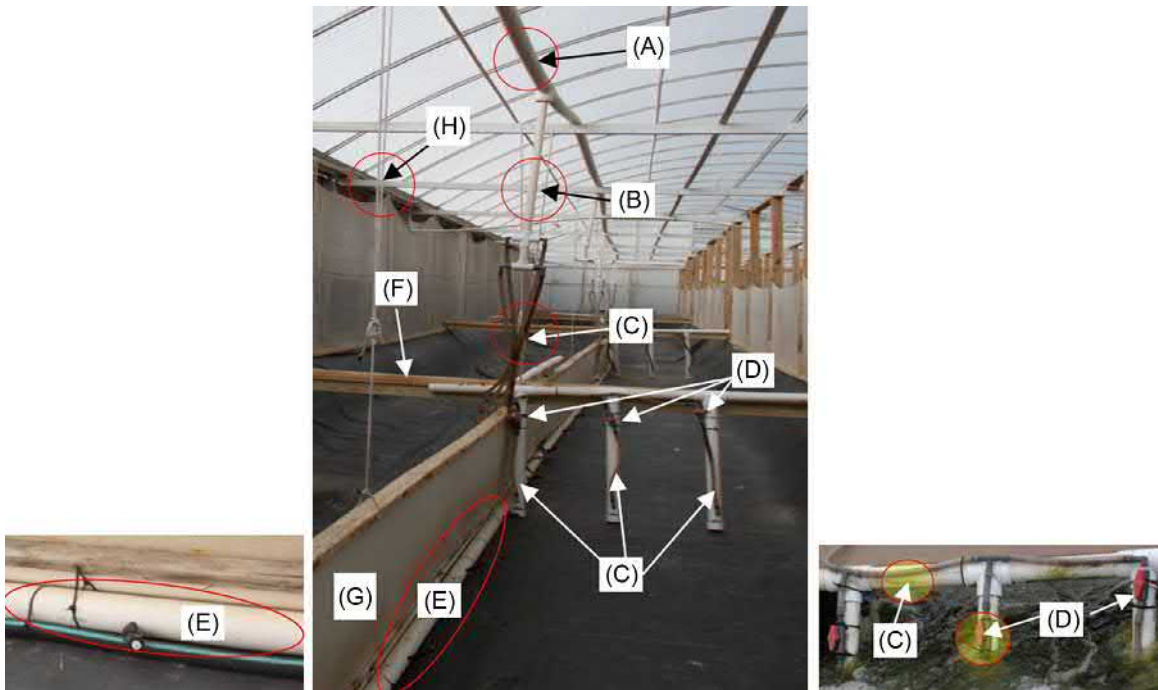


FIG. 5.27 A photo of 40 m^3 raceway showing (A) 5-cm PVC air distribution pipe, (B) 2.5-cm PVC air delivery pipe, (C) 1.6-cm flexible air supply hoses to airlift pumps and diffusers, (D) 1.6-cm PVC ball valve controlling air supply to airlift and diffusers, (E) bottom spray pipe with spray nozzle and diffuser, (F) boardwalk, (G) center partition, (H) rope holding partition in place.

mixing and the delivery system of the oxygenated water by including Flash Reactors and MTM nozzle on the bottom spray pipe.

VENTURI INJECTOR

The Venturi injector is designed to increase DO by mixing raceway water with atmospheric air, pure oxygen, or a mixture of the two. In our case, atmospheric air and oxygen were mixed through a 5-cm (2-in) Venturi (Model MIC-1583 A, Mazzei Injector Co.).

The injector is connected to a 5-cm (2-in) PVC discharge pipe from the pump that has a 5-cm (2-in) ball valve to regulate water flow (Fig. 9.3). This adjusts the gas intake and the injector's capacity to generate fine bubbles. Forcing all of the flow through the injector creates large bubbles that lower oxygen transfer efficiency.

A check valve (Fig. 5.28D) prevents backflow from the pump into the injector suction point. A

6-mm (¼-in) T-shaped adapter connected to the back of the check valve takes in air, an air-oxygen mixture, or oxygen when the ambient air intake is plugged. Oxygen supply is regulated by a flow meter (Fig. 5.28A). Typical flow rates are 0.3–1.0 Lpm.

ONLINE OXYGEN MONITORING SYSTEMS

Each raceway is equipped with an optical oxygen probe (Fig. 5.29C) connected to an online monitoring and alarm system (YSI 5500D multiparameter monitoring system, Yellow Springs Instruments, Yellow Springs, OH, US) with an LCD display in the greenhouse (Fig. 5.29A–B). The system is wired to a lab computer that displays real-time numeric and graphical data. Companion software (AquaManager) can be programmed to trigger local and remote (via cellphone or Internet) alerts whenever a preset boundary has been reached. See Video #30 for details.

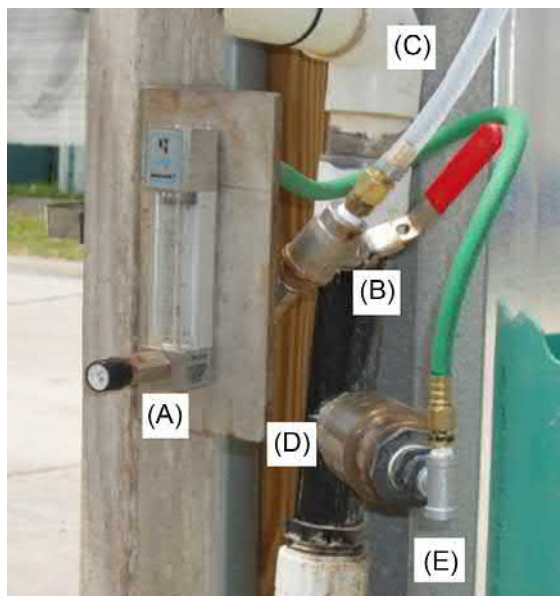


FIG. 5.28 Venturi injector assembly: (A) oxygen flow meter, (B) oxygen supply valve, (C) oxygen supply hoses, (D) check valve, (E) air intake.



FIG. 5.29 YSI 5500D DO monitoring system: (A) on-site display, (B) computer display with audio, (C) optical probe, (D) programming and screenshot of alarm-setting software.

PARTICULATE MATTER CONTROL

One of the most important practices in no-exchange systems is control of biofloc concentration. Foam fractionators, settling tanks, and multicyclone filters are used to regulate suspended particulate matter concentration in the 40 m³ raceways. Foam fractionators and the multicyclone are inexpensive off-the-shelf items. The settling tanks are homemade.

More efficient and more expensive equipment is available (drum filters, self-cleaning foam fractionators with oxygen/ozone supplementation, etc.), but the three devices mentioned before were suitable for solids control in our intensive biofloc systems.

SETTLING TANKS

Each raceway has a separate settling tank outside of the greenhouse (Fig. 5.30). They are made of 550-L HDPE cylindro-conical tanks (PT308 Polytank, Litchfield, MN, US) with a nested internal sleeve to enhance particle settling (Fig. 5.30A). The tanks are mounted on a wooden rack that permits return flow to the raceway by gravity (Fig. 5.30B). A black lid reduces accumulation of floating particulate matter and surface microalgae growth (Fig. 5.30C). Water is supplied via a 1.6-cm (5/8-in) clear hose fed by a side loop on the pump discharge (Fig. 5.30D). A large-diameter (5-cm) PVC pipe returns water from just below the tank surface to

prevent floating material from flowing back to the raceway (Fig. 5.30F).

Flow rates between 3 and 6 Lpm are regulated by a 1.6-cm (5/8-in) valve at the tank inlet (Fig. 5.30E). Our data showed a 32% ± 11% TSS removal rate (settling area: 0.4 m²; settling height: 0.9 m; flow rate: 5 Lpm; initial TSS: 221–320 mg/L).

FOAM FRACTIONATOR

Each raceway has a small commercial foam fractionator (VL65, Pentair Aquatic Eco-Systems, Apopka, FL, US). Two 2-cm Venturi injectors (model 484, Mazzei Injector Co. Bakersfield, CA, US) produce the fine air bubbles needed by the foam fractionator.

A 1.6-cm (5/8-in) ball valve on the raceway pump discharge pipe controls water flow to the foam fractionator. Diverting 3–6 Lpm to the foam fractionator, depending on the targeted TSS, has no noticeable impact on oxygenation or mixing in the raceway.

A 2.5-cm PVC gate valve controls return flow to the raceway. Clear acrylic pipe on top of the device adjusts flow to produce a thick foam with low water content (Fig. 5.31G and Video # 2 and Video # 3). Concentrated foam is collected in a separation tank, similar to the one described later for drying the sludge collected by the settling tank, and water collected during the drying process from these separation tanks is returned to the raceways (see Fig. 5.33G).



FIG. 5.30 Settling tanks for 40 m³ raceway system: (1) side view, (2) top view, (3) all six settling tanks: (A) sleeve preventing mixing of water entering and leaving the tank, (B) wooden support, (C) tank lid, (D) 1.6-cm supply hose, (E) 1.6-cm PVC supply valve, (F) 5-cm PVC return pipe, (G) 5-cm PVC drain valve.

MULTICYCLONE FILTER

The multicyclone filters, also called hydrocyclone filters, spin solids out of suspension. The Waterco Multi-cyclone 16 (Waterco, Inc., Augusta, GA, US) operated in our raceways at a flow of 50–500 Lpm. Maintenance is low because there are no moving parts or screens to clean. The device is mounted on the pump discharge pipe as a side loop on each raceway (Fig. 5.32).

At the Texas A&M-ARML, cyclone filters removed 14%–19% of TSS (initial TSS: 333–433 mg/L) when starting with empty sediment chambers. If more than half full, however, they had no effect on TSS or even increased TSS slightly. Sediment chambers thus must be emptied regularly to maintain removal efficiency. Alternatively, a solenoid can be timed to drain chambers at set intervals. Backwash water from the filter is directed to the same collection/

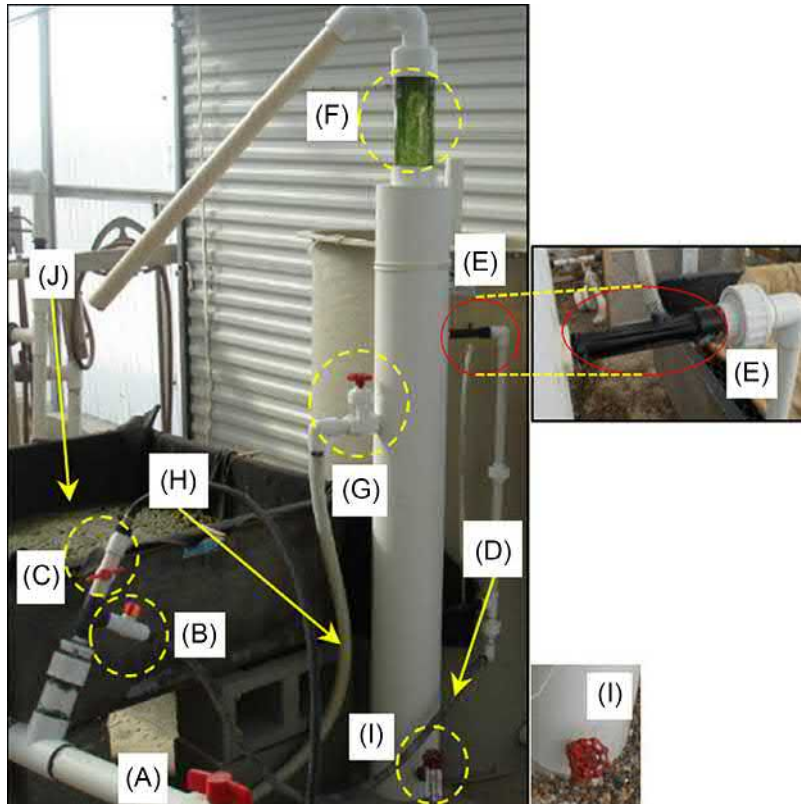


FIG. 5.31 Foam fractionator in the 40 m³ raceway: (A) 5-cm PVC valve on pump discharge pipe, (B) 1.6-cm PVC valve controlling water supply to foam fractionator, (C) 1.6-cm PVC valve controlling water supply to settling tank, (D) 1.6-cm hose connecting valve and foam fractionator, (E) one of two 2-cm Venturi injectors, (F) clear acrylic tube, (G) 2.5-cm PVC gate-valve controlling flow from foam fractionator to raceway via 2.5-cm flexible hose (H), (I) foam fractionator drain valve, (J) separation tank.

separation tank of the foam fractionator. Although these filters impose a head loss, operating this device did not interfere with other pump-driven tasks.

WASTE DISPOSAL

Each raceway has one false-bottom separation/collection tank (1 m² × 0.55-m deep) inside the greenhouse that receives sludge from the foam fractionators and multicyclone filter. The false-bottom is made of wire mesh on a 5 × 10 cm wooden frame. A porous geotextile fabric (Mirafi 180N, TenCate Geosynthetics

Americas, Pendergrass, GA, US) or a few layers of burlap cloth placed on the wire mesh separates water from solids. As the retained solids dry, the water drains back into the raceway via a small hole in the tank bottom (Fig. 5.33G and Video # 3). Additional tanks per raceway would allow more thorough drying, but space limitations dictated use of only one per raceway. Sludge was removed every other day to avoid H₂S formation.

Two other separation tanks were used to dewater solids from the settling tanks. These were about 50 m from the settling tanks, so a

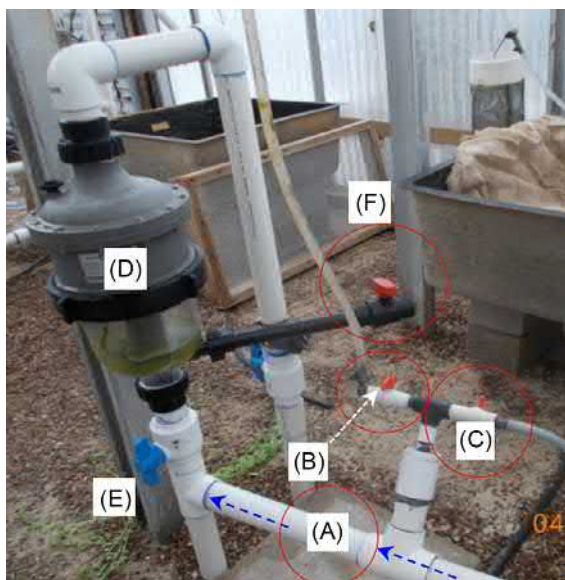


FIG. 5.32 Multicyclone mounting and valve arrangement in 40m³ raceway: (A) 5-cm PVC discharge pipe, (B) 1.6-cm PVC valve controlling supply to foam fractionator, (C) 1.6-cm PVC valve controlling supply to settling tank, (D) multicyclone filter, (E) 5-cm PVC valve controlling supply to multicyclone filter, (F) waste drain valve.

pump was used to transfer the solids stream. Alternate use of the separation/drying tanks allowed wet organic material to dry in one tank while the other received a new load of solids. Increasing the drying period facilitated disposal

into a landfill and enabled multiple use of the separation membranes (Fig. 5.34). The small volume of decanted water from these tanks was allowed to seep into the ground or evaporate and was not returned to the raceways to avoid potential introduction of H₂S-rich water.

5.9.2 100m³ Raceway System

5.9.2.1 Greenhouse

The two 100m³ raceways are covered by a 9.1-m × 39.6-m Bowhouse (American Plant Products and Services, Oklahoma City, OK, US) with two side doors on each end wall, one garage door, and two 1.22-m diameter, 1-hp exhaust fans on the leeward side. Shutters on the far end are synchronized to open whenever exhaust fans are operating. The greenhouse (Fig. 5.35) has inflated double-layer woven polyethylene end- and side-walls to improve insulation. The roof is covered by inflated double-layer clear 0.15-mm polyethylene and a 73% light reduction shade cloth similar to the one described for the other system.

In addition to shade cloth and fans, raceway water temperature is manipulated by placement of air intakes of the a³ injectors. Raceway water temperature can be increased by 2–3°C during the fall simply by drawing hot air that collects

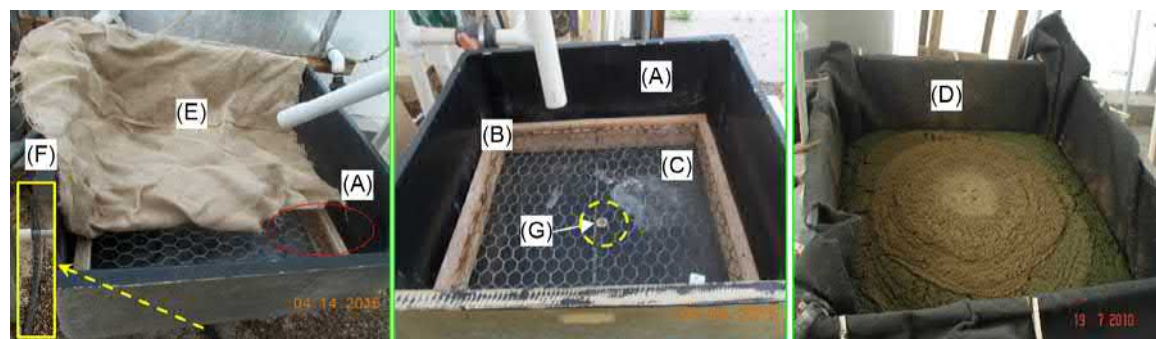


FIG. 5.33 Separation tanks with drying biofloc (A), a false-bottom is created by placing a wooden frame (B), covered with chicken wire (C), and covered by a geotextile membrane (D), or burlap cloth (E) for water separation, with hose returning water back to the raceway (F) via an outlet at the bottom of the tank (G).



FIG. 5.34 Dry biofloc in a separation tank.

below the roof rather than from near the water surface. Thus installing an air intake manifold that controls the air source to the a^3 injectors—near the roof, close to water surface, or from outside the greenhouse—is one way to improve control over water temperature. [Video #14](#) shows how the injector been used to increase water temperature.

The monitoring system described for the 40m^3 system is used to report power outages.

5.9.2.2 Culture Tanks

The 100m^3 raceways were designed to avoid problems experienced with the 40m^3 system. Each raceway is 33.5m long $\times$ 3m wide and lined with a 1-mm (40-mil) EPDM membrane. The working water volume is 100m^3 and surface area is 100m^2 .

Raceway modifications include:

- Water is deeper for higher yields and less temperature fluctuations. It is 90 cm (34.5 in) at the shallow end and 112 cm (44 in) at the deep end.
- The center partition was glued to the bottom and held in place by ropes attached to the greenhouse structure (described later). The partition was made in one piece of EPDM membrane with 5 cm pipe inserted in a sleeve at its top for improved water flow. This also made it easier to clean than the sectioned fiberglass partitions in the 40m^3 raceways.
- Building above the natural soil elevation, raceway walls were buried 40 cm (16 in) and 65 cm (25.5 in) in the soil at shallow- and deep-end, respectively. A thermoplastic membrane thus was enough to retain water while avoiding problems with a high water table. (Additional soil was needed to increase the

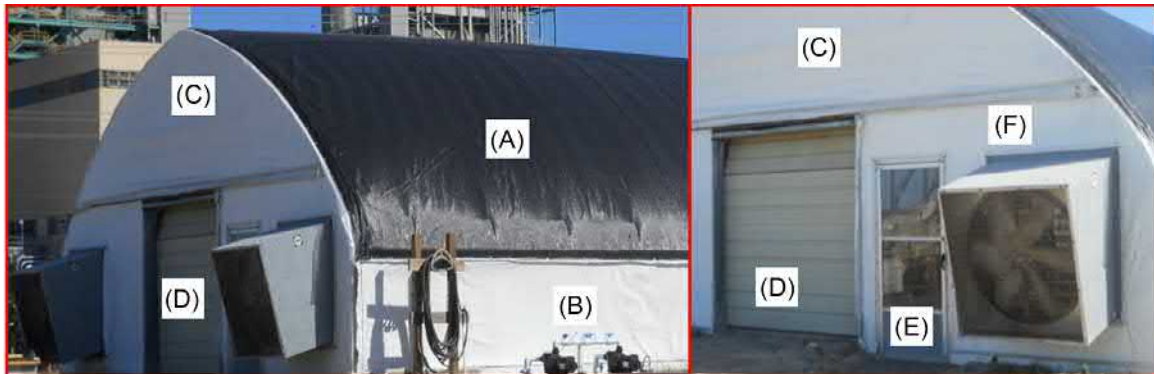


FIG. 5.35 Greenhouse for two 100m^3 raceways with double-layer inflated roof covered by black shade cloth (A), inflated double-layer woven polyethylene side- (B) and end-walls (C), garage door (D), side door (E), exhaust fan (F).

base elevation by 1.2 m). This also reduced the wall reinforcement needed for above-ground tanks and provided sufficient elevation to construct a common harvest basin.

- The harvest basin drained raceways by gravity and harvesting was by fish pump.

Raceway side walls were constructed of treated wooden planks ($5.1 \times 25.4 \text{ cm} \times 3.66 \text{ m}$, or $2 \times 10 \text{ in} \times 12 \text{ ft}$) attached to concrete-anchored wooden posts ($10 \times 10 \text{ cm}$ or $4 \times 4 \text{ in}$) placed 1.1 m (43.4 in) apart. End walls were constructed in a half-hexagonal shape for smoother circulation around the center partition. To reduce damage to the membrane, walls were padded with a nonwoven geotextile membrane made of polypropylene fibers (Mirafi 180N, TenCate Geosynthetics Americas, Pendergrass, GA, US). The substrate beneath the liner consisted of a 10 cm layer of beach sand and had 1% slope for harvesting and draining.

Four $30.5\text{-cm} \times 5\text{-cm} \times 3.1\text{-m}$ ($12\text{-in} \times 2\text{-in} \times 11\text{-ft}$) wooden boardwalks (Fig. 5.36 and Fig. 5.37) span the width of the tank at equidistant points along its length to facilitate bottom inspection and as a platform for belt feeders.

FREEBOARD AND ANTIJUMP NETTING

Raceways have 20 cm freeboard all around and footings made of $5 \times 15\text{-cm}$ wooden planks mounted on the top of the raceway walls.

Antijump netting (the same net as in the other system, see [Video # 27](#)) is attached to vertical $5 \times 10\text{-cm}$ planks connected to the raceway footings and greenhouse. Each raceway has four $1 \times 1\text{-m}$ access doors above the boardwalks (Fig. 5.37).

ACCESS

To allow sufficient space around the tank perimeter for ready access by staff, raceways were positioned 1 m apart and 0.75 m from the greenhouse walls. This space allows unobstructed observation, manual mixing, collecting samples, valve adjustment, and mortality recovery. Four boardwalks across raceways improve access to the center (Figs. 5.36 and 5.37D).

5.9.2.3 Raceway Support and Management Tools

PUMPS, PIPE NETWORK, AND WATER MOVEMENT

Each 100 m^3 raceway has two Hydrostorm 2-hp centrifugal pumps (Fig. 5.38) identical to those in the 40 m^3 raceways. The pumps drive water through 14 a^3 injectors (All-Aqua Aeration, Orlando, FL, US) at 310 kPa (45 psi) and 28.4 Lpm (7.5 gpm) per injector. This amounts to approximately 24,000 L/h or a 24% hourly

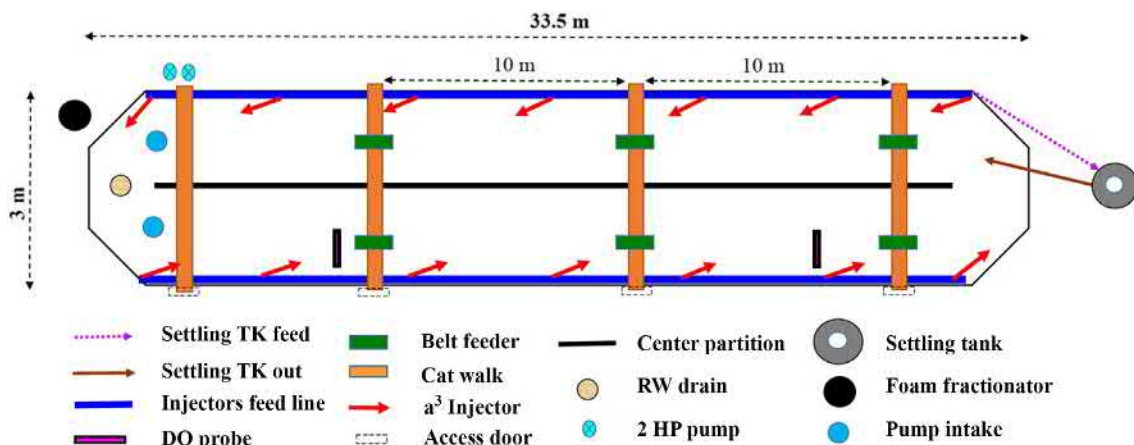


FIG. 5.36 Schematic top view of the 100 m^3 raceway.



FIG. 5.37 100m³ raceway: Antijump netting (A), 5-cm PVC distribution pipes (B), 2.5-cm PVC a³ water supply pipe (C), boardwalk (D), center partition (E), access door (F), belt feeders (G).



FIG. 5.38 Two 2-hp centrifugal pumps for a 100m³ raceway. The 5-cm PVC valve manifold controls single or dual pump use. Valve handles are painted to reduce UV degradation.

turnover. This is much lower than in standard clearwater RAS, but it provides sufficient DO and mixing to support the very high biomass in the Texas A&M-ARML biofloc system.

Beside the water supply for the a³ injectors, the pumps also supply water to the settling tank and foam fractionator (which uses one additional injector). The pumps also fill and drain raceway water when needed. Drained water is diverted to the harvest basin through a 5-cm outlet at the top of the basin's end wall (Fig. 5.47A).

A raceway can be operated with one or two pumps, depending on oxygen demand.

Although each pump only receives water from one of the two 20-cm (8-in) filter pipes (Video # 18), the returned water is sent into two 5-cm distribution pipes that supply the a³ injectors (Figs. 5.36 and 5.37B).

AERATION AND MIXING SYSTEMS

The a³ injectors (using only air) satisfy the high oxygen demand of the biofloc and shrimp. Water flow to each distribution pipe is controlled by two 5-cm (2-in) PVC ball valves at the deep end of the raceway (Fig. 9.6). Each valve controls supply to a 5-cm distribution pipe on the raceway footing above the boardwalks. A 5-cm ball valve at the shallow end is for filling the raceway (Fig. 5.40I).

Each raceway has a pressure gauge on one of the distribution pipes at its shallow end to ensure optimal water pressure (45 psi) (Fig. 9.6E). The other distribution pipe has a saddle for attaching a paddlewheel flow meter (Fig. 5.39A) at the deep end. A 1.6-cm PVC ball valve at the shallow end regulates flow to the settling tank (Fig. 5.46C).

Water supply to the a³ injector of the foam fractionator is controlled by a 2.5-cm ball valve on the pump discharge pipe just before it connects to the two distribution pipes (Fig. 5.47E).

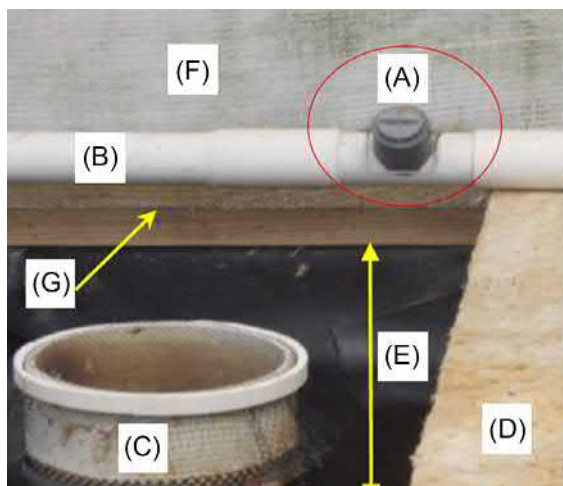


FIG. 5.39 A saddle for a paddlewheel flow meter (A), one of two 5-cm PVC distribution pipes feeding seven a^3 injectors in each raceway (B), screened pump intake (one of two) note guard net on top of the filter pipe (C), boardwalk (D), free-board (E), antijump netting (F), and raceway footing supporting antijump netting (G).

Each distribution pipe supplies water to seven a^3 injectors on each side of the raceway via 2.5-cm PVC ball valves connected to 2.5-cm pipes with barrel union adapters (Fig. 5.40C). This setup allows for isolation and removal of any injector without interfering with operation of the others. Every 2.5-cm pipe runs from the top to the bottom of the raceway (Fig. 5.40D) and is connected by an elbow to a 2.5-cm PVC Schedule 80 T-joint with a built-in a^3 injector (see Figs. 5.40F, 9.6 and Video # 15, Video # 16, and Video # 17).

A^3 INJECTOR

As mentioned earlier, a^3 injectors require high pressure water and a certain flow to operate at full efficiency. The vacuum created by the water flow through the orifice draws in air and mixes it with the water to produce very fine bubbles (Fig. 5.40G, Fig. 9.6C, and Video # 22).

The 5cm ball valves located outside of the raceway control water supply to the distribution pipes. They also can be used to regulate water flow to the injectors. This is useful for adjusting the amount and size of bubbles and water

mixing. The 2.5-cm valves of each injector achieve the same end, but this is more time consuming than adjusting all injector flows at once.

Controlling water flow is extremely important when raceways are first stocked because too much turbulence damages fragile young postlarvae. Depending on oxygen demand and shrimp needs, 2–28 Lpm to each injector is suitable. Rates are lower for young postlarvae and are increased as the shrimp grow and oxygen demand increases.

ON-SITE OXYGEN AVAILABILITY

Supplemental oxygen was not required to produce high yields, but a backup system is highly recommended for emergencies that might arise because of pump malfunction or overfeeding. The oxygen system is easily constructed using 4-mm aquarium air hose and air valves (Fig. 5.41) to deliver pure oxygen into the air intake of every other injector.

CENTER PARTITION

Each raceway has a collapsible 30-m center partition of EPDM membrane 1.75m from each end wall. Partitions are glued to the bottom and supported by ropes hanging from the greenhouse. Their height is 0.83m at the shallow end and 1.07m at the deep end. A capped 5-cm (2-in) pipe is inserted into a sleeve at the top of the partition for flotation (Figs. 5.42 and 5.43).

OUTLETS

The deep end of each raceway has three bottom-flashed, concrete-embedded, 20-cm (8-in) PVC elbows. Two are positioned about 40cm from the side walls and 1.46m from the end walls. A 5-cm (2-in) PVC pipe network connects these outlets to two 2-hp pumps. Each outlet has a nested 20-cm PVC filter screen to prevent sucking shrimp into pumps (see Fig. 7.7 and Fig. 10.3, and Video # 18).

The third outlet, located along the raceway centerline, 0.6m from the end wall, has a nested 20-cm PVC standpipe that connects to the concrete harvest basin. This standpipe, which is

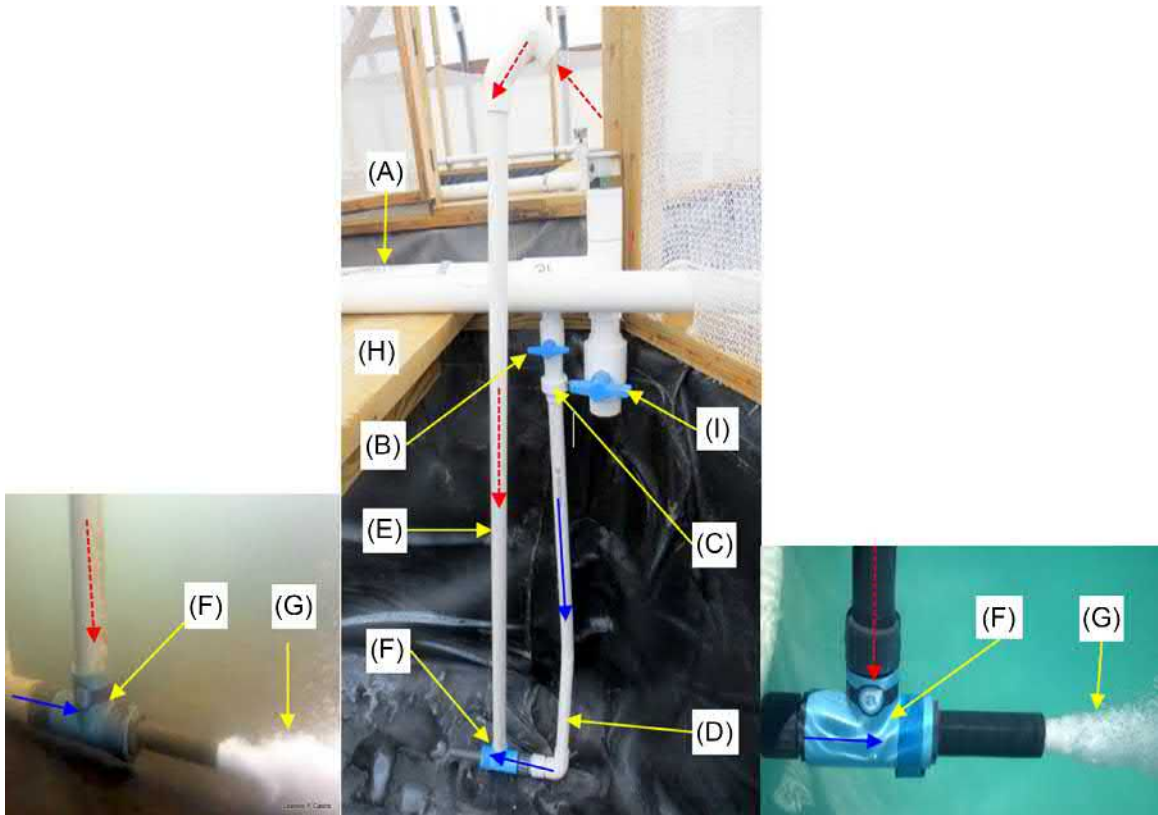


FIG. 5.40 Water and air flow of a³ injector for aeration and mixing in the 100m³ raceway: One of two 5-cm PVC distribution pipes (A), 2.5-cm PVC ball valve controlling water to injector (B), 2.5-cm PVC barrel union adapter (C), 2.5-cm water supply pipe (D), 2.5-cm air suction pipe (E), a³ injector (F), air bubble and water mixture streaming out of injector (G), boardwalk (H), 5-cm ball valve for quick fill of raceway (I). Blue arrows (dark gray arrows in print version): high pressure water supply; Red arrows (dotted light gray arrows in print version): atmospheric air suction.

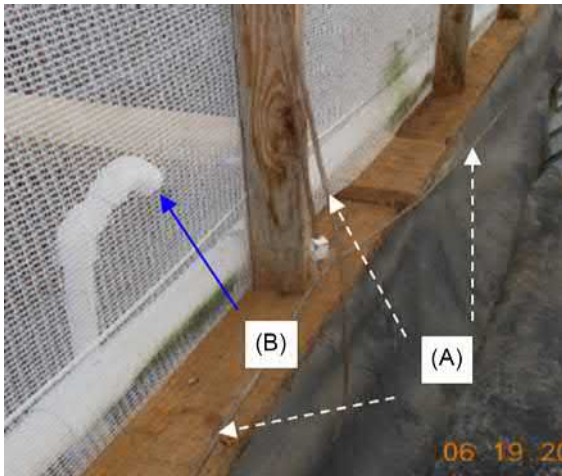


FIG. 5.41 Oxygen backup system: aquarium hose (A) delivers oxygen to a³ suction pipe (B).

higher than the maximum water level during production, is removed to drain the raceway and allow shrimp to pass to the harvest basin (see [Section 10.3](#), and [Fig. 10.3](#)). [Fig. 5.44](#) shows the standpipe in place when a raceway is filled to working water depth. When shrimp are larger than 1 g, a net is placed over the standpipe opening to exclude shrimp from the drain line.

ONLINE OXYGEN MONITORING SYSTEMS

The online oxygen monitoring system was identical to the one in the 40m³ raceways (see [Section 5.9.1.3](#) and [Fig. 5.29](#)) except that each 100m³ raceway had two optical probes. One was fixed about 5m from the deep end and the other about 5m from the shallow end. Both



FIG. 5.42 Center partition: EPDM glued to bottom and supported by ropes connected to 5-cm capped flotation pipe. 20-cm PVC concrete-embedded elbow connected to harvest basin (A), bolting EPDM membrane into concrete with stainless-steel frame (B).



FIG.5.43 A full and empty raceway. Notice freeboard in the full raceway.

were easily accessed from the central walkway for daily calibration. Having two probes contributed to informing management decisions that kept DO relatively uniformly distributed throughout these larger raceways.

PARTICULATE MATTER CONTROL

Particulate matter concentrations were managed with only a settling tank and foam fractionator. Each tank also had a multicyclone filter used successfully in the smaller system, but



FIG. 5.44 Raceway filled to working depth with 20-cm PVC standpipe extending above the surface (A). Net prevents shrimp larger than 1 g from entering the drain line (B).

the high pressure and flow requirements of the a^3 injectors precluded its use.

Settling tanks and foam fractionators were homemade with few off-the-shelf components.

SETTLING TANK

Settling tanks were built from 2-m³ conical-bottom self-supported fiberglass tanks (height: 2.21 m; top diameter: 1.35 m; settling area: 1.2 m²; settling height: 1.7 m). A nested sleeve (Fig. 5.45A) improved settling.

Water supply to the tank was provided by a 1.6-cm hose (Fig. 5.45B) with a 1.6-cm PVC valve (Fig. 5.45C) at the end of one of the two 5-cm water distribution pipes (Fig. 5.28D). After passing through the settling tank, water returned to

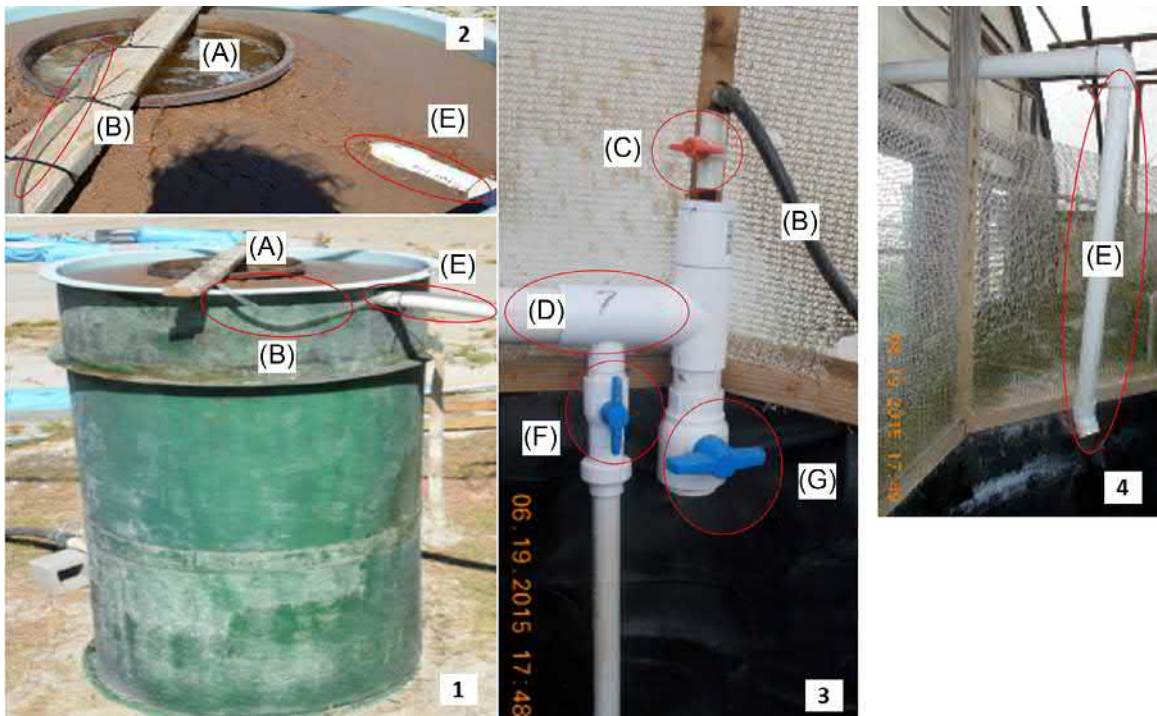


FIG. 5.45 (1) 2-m³ outdoor fiberglass settling for one raceway; (2) top view of settling tank; (3) piping system at shallow end of raceway; (4) 5-cm PVC pipe returning water from settling tank to raceway: (A) sleeve to prevent mixing of water entering and leaving settling tank, (B) 1.6-cm hose delivering water from raceway to settling tank, (C) 1.6-cm valve controlling flow to settling tank, (D) 5-cm PVC distribution pipe, (E) 5-cm PVC pipe returning water from settling tank to raceway, (F) 2.5-cm PVC valve feeding a^3 injector, (G) 5-cm PVC valve to quickly fill raceway.

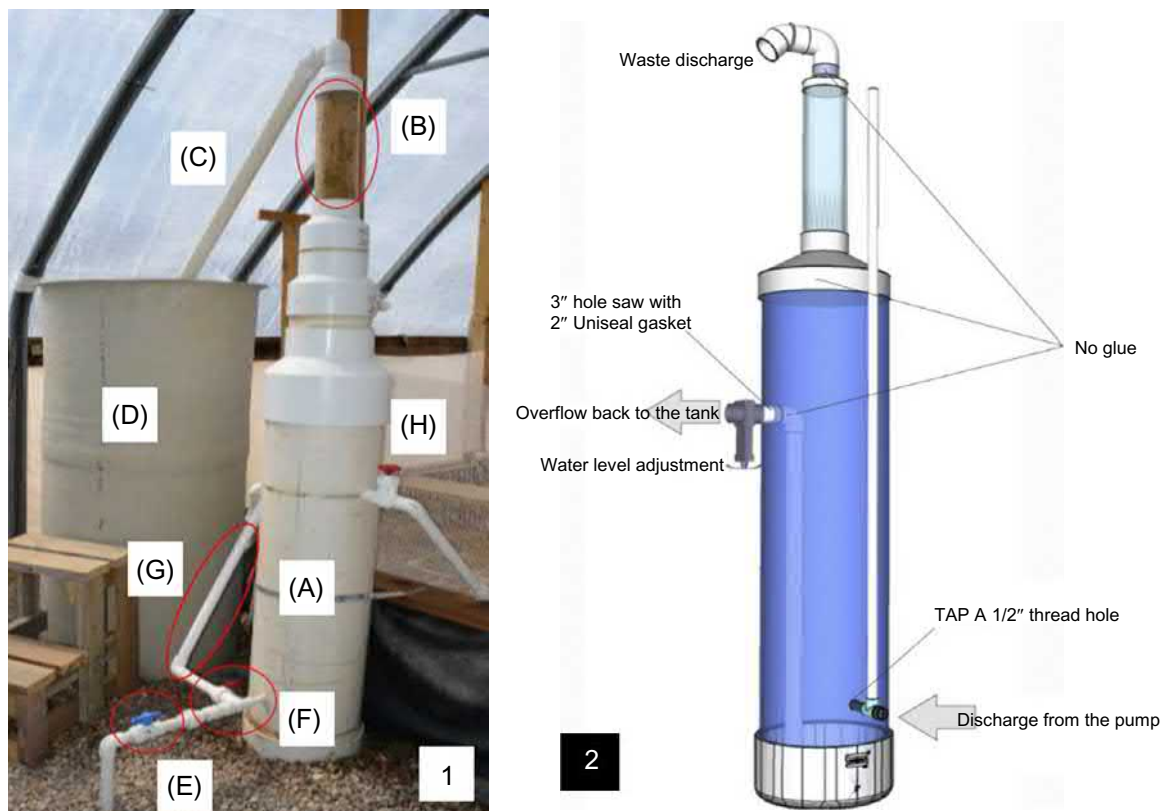


FIG. 5.46 (1) Homemade foam fractionator, (2) schematic of foam fractionator: (A) 30-cm PVC pipe, (B) 10-cm acrylic pipe, (C) 5-cm PVC foam delivery pipe, (D) temporary foam storage tank, (E) 2.5-cm PVC ball valve controlling flow to foam fractionator, (F) a^3 injector, (G) 2.5-cm PVC air intake pipe, (H) 2.5-cm PVC gate valve controlling return flow to raceway.

the raceway by gravity (Fig. 5.45E). Flow to the tanks varied from 6 to 20 Lpm, depending on particulate load. Sludge accumulated on the bottom was drained through a 5-cm PVC valve every few days.

With flow of 20 Lpm and TSS of 200–350 mg/L, the tank had a removal efficiency of $71\% \pm 7\%$. The ratio of the flow rate to the settling area is between 12 and 16 Lpm/m². This is considerably less than the recommendation for clearwater RAS: 40 Lpm/m² (Timmons and Ebeling, 2013). The reason is that the solids concentration

in biofloc systems is much higher and settling tanks are operated only intermittently.

FOAM FRACTIONATOR

Each raceway has a 2-m tall homemade foam fractionator made of 30-cm (12-in) pipe (Fig. 5.46A). A 10-cm (4-in) clear acrylic pipe (Fig. 5.46B) is connected near the foam discharge. Foam rising in the acrylic pipe is directed via a 5-cm PVC pipe (Fig. 5.46C) to a temporary storage tank (Fig. 5.46D), then to outside separation tanks.

As described earlier, water to the foam fractionator is delivered via a side loop on the 5-cm pump discharge pipe. Flow is controlled by a 2.5-cm PVC ball valve (Fig. 5.46E) connected to an a³ injector (Fig. 5.46F) that has the same 2.5-cm air suction pipe (Fig. 5.46G) used in the other injectors. Flow rate leaving the foam fractionator is regulated by a 2.5-cm PVC gate valve on the 30-cm pipe (Fig. 5.46H). Adjustment of the water entering and leaving the foam fractionator is needed to produce foam of the right consistency (see Video # 16 and Video # 17). Although flow into the injector feeding the foam fractionator can be as high as 28 Lpm, thick foam is produced at 14 Lpm.

MULTICYCLONE FILTER (MCF)

Each raceway has one MCF on a side loop from the pump discharge pipe before it connects to the two distribution pipes. These filters were not used for cropping biofloc because this would have resulted in high head loss in the distribution pipes and significant DO reduction.

WASTE DISPOSAL

Space limitations dictated placing separation/foam collecting tanks outside of the greenhouse. Biofloc removed by each foam fractionator was diverted to a temporary storage tank (Fig. 5.46D). This watery foam was drained by gravity into two outdoor separation/collection tanks. These tanks were identical to the separation tanks used for biofloc drying in the 40 m³ raceways (see Section 5.9.2.1 and Figs. 5.33 and 5.34). Unlike the limited drying time available with only one separation tank, alternately using two tanks produced much drier material for disposal.

Water decanted from the separation tanks drained by gravity into a reservoir tank that was covered to avoid collecting debris. This water was returned to the raceway by a submersible pump with a water level activated switch.

Solids collected in the settling tank were drained by gravity into two common separation tanks. Alternating use of the tanks allowed for more complete drying. Water from these tanks was not pumped back to the raceways to minimize risk of introducing H₂S and other inimical substances.

USE OF A DIGESTER

A digester tank reduced solid waste from the settling tanks, removed nitrate and phosphate, and contributed to alkalinity and pH in the culture water. This consisted of a single open-air 14 m³ (height: 3.05 m, diameter: 2.45 m) flat-bottomed cylindrical fiberglass tank into which waste solids and culture water were pumped. A 1 hp sludge pump circulated the slurry in the tank under aerobic and anaerobic conditions, and then returned the supernatant to the raceways.

HARVEST BASIN

The two 100 m³ raceways have a common concrete harvest basin (4 × 2 m × 1.8-m deep) with 20-cm thick, reinforced walls 2 m in front of the greenhouse. A 15-cm (6-in) PVC schedule 80 threaded elbow is 38 cm from the bottom and flush with the basin side wall (Fig. 5.47B). The threaded outlet was designed to accept a valve, swivel standpipe, or a fish pump adapter for draining or harvest (Section 10.3).

The bottom of the harvest basin has 1% slope and a 20-cm (8-in) outlet fitted with a nested filter pipe at the deepest corner for complete drainage. The outlet has a filter pipe to avoid suction of material that might damage the pump (Fig. 5.47C). The outlet, made of a PVC elbow imbedded and leveled at the bottom, is connected to a 7-hp pump that transfers raceway water to an evaporation pond. This drain pipe also had a gate valve for gravity draining of the raceway into a discharge canal.

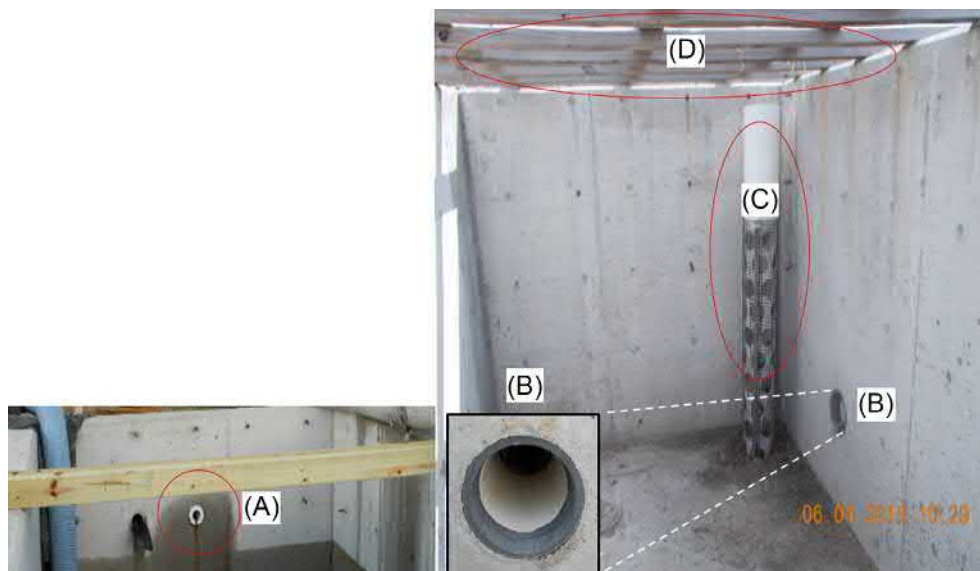


FIG. 5.47 Concrete harvest basin. (A) 5-cm PVC outlet for draining the raceway by pump, (B) 15-cm PVC threaded outlet (one on each side wall) for connecting a fish pump, (C) nested 20-cm PVC filter pipes prevent clogging the discharge line with foreign objects, (D) safety wooden grid on top of the structure.

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System Treatment and Preparation

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6.1 PREFILTRATION

Water drawn from a natural source must be filtered to remove any macroscopic organisms, eggs, and cysts prior to disinfection and stocking. This increases the efficacy of disinfection and reduces the chance of predators, fouling organisms, and pathogens entering the culture system. Primary filtration methods include pumping water through a filter screen or bag (50–350- μm mesh) (Fig. 6.1) or passing incoming water through a sand filter.

Water with high turbidity may require additional filtration, such as filter bags with 1–5- μm mesh, a settling tank, or a settling pond before filling the culture tank.

6.2 DISINFECTION

Strict sanitary practices that prevent introduction of harmful viruses, bacteria, fungi, protozoans, and predators are essential in establishing a biosecure facility. Foremost among these practices is disinfection of all components directly involved in shrimp production. Disinfection reduces harmful microorganisms and sterilization, a much more thorough

procedure, eliminates all microorganisms, including bacterial spores. Sterilization, however, is impractical and typically unnecessary in commercial aquaculture.

Disinfectants are classified as chemical or physical. Popular chemical disinfectants include chlorine (bleach), chloramine, ozone, quaternary ammonium, phenols, iodine (as iodine-containing compounds plus a detergent), formaldehyde, weak acids, and strong bases. Physical disinfectants include heat, desiccation (drying), and ultraviolet (UV) light from direct sunlight or UV lamps.

Chlorine and iodine are readily available, easy to use, relatively inexpensive, and effective. FDA approval of disinfection chemicals is not required for pretreatment of culture water and equipment as long as no residue remains.

The disinfection procedure used at the Flour Bluff facility is outlined as follows.

6.2.1 Culture Tanks

1. After harvest or before adding water, pressure-wash the tank to remove material attached to the sides and bottom (Fig. 6.2). This improves the effectiveness of disinfectants.



FIG. 6.1 Filter bag on seawater inlet of Texas A&M-AgriLife Research Mariculture Lab.



FIG. 6.2 Pressure spraying raceways with freshwater to remove organic matter.

2. Fill the tank to capacity with freshwater and add bleach or sodium hypochlorite to a concentration of 10ppm.
3. Start all pumps to circulate the disinfection solution through pipes and particulate control equipment (e.g., foam fractionators, settling tanks, and cyclone filters).
4. Simultaneously run all airlifts and diffusers at full capacity for 24h (40m³ raceway system only).

5. Shut down all pumps and air diffusers (40m³ RW system only), drain the tank, and allow it to dry for one day.
6. To conserve freshwater, pump the disinfectant solution into the next tank to be disinfected and readjust chlorine concentration.

6.2.2 Culture Water

All new culture water must be disinfected prior to being used to grow shrimp. This is done either in the culture tanks before stocking or in a storage tank. Prefiltering removes some potential pathogens, thereby reducing the volume of disinfectants that must be applied (see [Section 6.1](#)). To improve mixing, a Venturi injector can be used to add disinfectants, such as chlorine, to culture water. The Venturi is installed on the tank's recirculation pipe at a location where a bucket containing the disinfectant can be placed under, or next to, the pipe ([Fig. 6.3](#) and [Video # 28](#)). The suction line draws the chemical from the bucket, reducing handling and spillage.

6.2.3 Tank Components and Equipment

If water is discarded after a production cycle or if there is a disease outbreak, all tank components and equipment must be cleaned and disinfected. The system is first washed with freshwater, preferably with a pressure washer, to remove attached material ([Fig. 6.2](#)). The tank and its components then are sprayed with a pressure sprayer (hand held or backpack) containing a disinfectant such as chlorine. This includes structures, netting around the tank, air delivery pipes, hoses, walkways, and other equipment. Tanks then are allowed to dry for a day before refilling.

Smaller equipment used during production—nets, buckets, and so on—are washed with a liquid detergent and soaked in a chlorine (10ppm for 5min) or iodine solution (at least



FIG. 6.3 Venturi injector for adding disinfectants to a reservoir. As the middle 5-cm valve is closed, the suction pressure through the Venturi increases.

200ppm for a few seconds). Air stones and air lines can be disinfected in 3% hydrogen peroxide for 30min or by soaking in 10% muriatic acid. This is followed by a freshwater rinse and drying in the sun.

Equipment that regularly comes in contact with culture water over short periods of time—such as water-quality probes, dip nets, and mixing tools—are rinsed in clean freshwater between each use and disinfected at the end of each session. Table 6.1 outlines the cleaning and disinfecting protocol for aquaculture facilities.

6.2.4 Chlorine

Chlorine is a general-purpose disinfectant used routinely in households, the food service industry, public swimming pools, and aquaculture to kill common parasites, fungi, bacteria, and especially viruses. It is readily available in liquid (sodium hypochlorite or bleach) and powdered (calcium hypochlorite) forms.

TABLE 6.1 Cleaning and Disinfection Protocol (Yanong and Erlacher-Reid, 2012)

Recommended routine to clean and disinfect equipment:

1. Remove any attached material with a pressure washer.
2. Scrub vigorously with a detergent.
3. Rinse with freshwater.
4. Apply a disinfectant for an appropriate contact time (e.g., 10ppm chlorine/5min).
5. Rinse again with freshwater to remove the disinfectant.
6. Dry in a clean area before storing or reusing.

Chlorine gas is used in municipal water treatment and some public swimming pools, but it is highly toxic and not recommended for aquaculture (Boyd, 2008).

Liquid chlorine products generally are cheaper than granular products and are easier to apply. Sodium hypochlorite (bleach) is cheaper than calcium hypochlorite, but because it usually has a lower concentration, it must be applied at a higher dosage (Tonguthai, 2000).

Several bleach concentrations are available in the United States: common household bleach (5.25% to 6% NaClO), commercial bleach (18% NaClO), and the commercial grades (10, 10.5, or 12.5% NaClO) used to treat swimming pools. It can be purchased in 3.8-L (1-gal.) jugs, 20-L (5.3-gal.) or 200-L (53-gal.) drums, and in 950-L (251-gal.) tanks (Fig. 6.4). The concentration of active chlorine in stored bleach declines over time. Its useful shelf life generally is less than one year. Chlorine should be stored out of direct sunlight and at lower temperature in an airtight container to maximize shelf life.

Avoid chlorine products that contain additives, such as fragrances (for household use) and reagents for pH adjustment (for swimming pools). As with other hazardous materials, chlorine must be stored in containment trays or vessels that limit the extent of spills (Fig. 6.5).

Chlorine is used to disinfect culture water and tanks prior to stocking. A dose of 10ppm active chlorine for 30min is common. For a 12.5% solution, adding 0.168mL of bleach per 1L of water (e.g., 0.08mL/L of 12.5% NaClO (47.62% Cl^-)) results in 0.168mL/L Cl^- . This must be increased if the organic load is high, which is why filtering before disinfection always is recommended. Test the addition of an extra 5ppm chlorine in a 1-L sample of culture water if the organic load is high.



FIG. 6.4 Liquid (12.5%) sodium hypochlorite in a 200-L (55-gal.) drum with a siphon pump.

If the chlorine concentration is still >10 ppm after 30 min, then apply this higher dose. There is a reduced disinfection capacity at lower temperature; this requires a longer contact time and/or a higher concentration. Chlorine is added while running all pumps so that it mixes



FIG. 6.5 Chemical storage in containment trays to limit spills.

thoroughly throughout the tank and all other equipment (filtration and aeration devices) plumbed into the system.

Free chlorine in water exists as chlorine gas, hypochlorous acid (HOCl), and hypochlorite ions (OCl⁻). Chlorine gas and HOCl are 100 times more toxic than OCl⁻. The proportion of each depends on pH (Boyd, 2008). Chlorine gas occurs below pH 2, only HOCl between pH 2 and 6, HOCl and OCl⁻ are about equal at pH 7.5, and OCl⁻ dominates as pH increases above 7.5, resulting in more oxidation than disinfection. The application rate thus must be increased at higher pH (Boyd, 2008). When disinfecting with chlorine spray, the target concentration is 500 ppm with pH adjusted to 6.

The optimal pH for chlorine disinfection is between 6 and 8, so chlorine effectiveness can be increased by lowering pH with muriatic acid prior to chlorination. Muriatic acid and chlorine should not be added to the culture tank at the same time, and do not mix them together beforehand because they will combine to produce dangerously toxic chlorine gas.

Bromide reacts with HOCl in seawater to form hypobromous acid, a disinfectant that degrades in the same manner as HOCl. Because this is more effective at higher pH (8.0), the overall process of chlorine disinfection is more effective at higher pH when salinity is higher.

Chlorine is extremely toxic to aquatic organisms—including the microorganisms in bio-floc—so it must be neutralized completely before beginning culture operations. Because it is volatile, it can be removed with vigorous aeration.

Table 6.2 presents suggested concentrations and exposure times for chlorine disinfection of tools, equipment, and tanks.

Operating Venturi injectors, airlift pumps, and diffusers at full capacity in the 40m³ race-way system for 24h reduces chlorine to less than 1 ppm. When residual chlorine concentration is higher than this and there is not enough time to allow chlorine to dissipate naturally, sodium thiosulfate (Na₂S₂O₃), vitamin C, or hydrogen

TABLE 6.2 Recommended Concentrations and Exposure Times for Chlorine Disinfection (Huguenin and Colt, 2002; Lawson, 1995)

Item	Time (min)	Requirements (mL/L)	
		Bleach ^a	Calcium Hypochlorite ^b
Nets, buckets	5	0.7	40
Transport equipment	30	2.64	150
Rearing tanks	60	3.51	285

^a 3.7% available chlorine.

^b 65% available chlorine.

peroxide may be added to neutralize the chlorine before stocking. Guidelines for each treatment are as follows:

- Sodium thiosulfate. Add 2 parts thiosulfate per 1 part chlorine. For example, add 0.02 g/L of thiosulfate to neutralize 0.01 g/L (10 ppm) of chlorine.
- Vitamin C in the form of ascorbic acid or sodium ascorbate. Add 2.5 parts ascorbic acid to 1 part chlorine; or 2.8 parts of sodium ascorbate to 1 part chlorine (Land, 2005). This will not lower DO as much as thiosulfate and it breaks down in 1 to 2 days.
- Hydrogen peroxide. Add 0.5 part hydrogen peroxide for every 1 part chlorine. Use peroxide only at higher pH because it rapidly neutralizes hypochlorite, but it reacts slowly with hypochlorous acid.

Before stocking, a commercial test kit (Appendix I) can be used to ensure that all free chlorine has been neutralized. These kits are also used to confirm the target concentration for disinfection.

Workers who handle chlorine must take special care to avoid potential health hazards. This includes wearing protective gear, especially chemical gloves and eye protection. When working in a confined area or using a disinfectant mist, wearing a respirator (Fig. 6.6) is essential.



FIG. 6.6 Disinfecting a raceway with chlorine solution spray while wearing protective equipment.

6.2.5 Formaldehyde

Formaldehyde (CH_2O) is commonly used for disinfecting intensive shrimp culture facilities. As a gas, formaldehyde is a powerful fumigant used to sterilize contaminated buildings (Bell and Lightner, 1992). In aqueous form, it is known as formalin. Solutions of 37% formaldehyde in water and 8% formalin in 70% ethanol are very effective against the most difficult pathogens, including bacteria, bacterial spores, viruses, and parasites (Yanong and Erlacher-Reid, 2012). Nodaviruses, however, are resistant to formalin.

A dose of 10 to 15 ppm of 37% to 40% formalin is typical to disinfect water or surfaces, and solutions as high as 200 ppm are common in shrimp hatcheries (Tonguthai, 2000). Yanong and Erlacher-Reid (2012) report dosage rates of 1% to 8% formaldehyde (~3% to 20% formalin) for 20 min to 16 h.

Care must be taken with formalin because it produces an irritating vapor and is carcinogenic. Always use a respirator and wear chemical gloves when handling it and avoid lingering near areas that are under treatment. Its use should be minimized, as safer alternatives exist.

6.2.6 Iodine

Iodine (I_2) is available as an iodophor, which is a combination of an iodine-containing compound and a detergent. Iodine is one of the least toxic disinfectants, but it readily is rendered inactive by excess organic material. It nevertheless is effective against a variety of pathogens and parasites.

The following reaction describes the equilibrium of iodine in water from pH 5 to 8 (NAS, 1980):



This equilibrium shifts to the left (more iodine, I_2) as pH decreases and the initial iodine concentration increases; and to the right (more hypiodous acid, HOI) as pH increases and the initial iodine concentration decreases. Iodine solutions are more effective against cysts and spores, and less effective against bacteria and viruses when pH is below 7 (i.e., when I_2 is dominant). The reverse is true above pH 7, when HOI dominates. Iodine's disinfection capacity declines significantly above pH 8 where hypiodous acid degrades to iodate (HIO_3) and iodide (I^-). The optimum pH for iodine disinfection, therefore, is near or just above pH 7, a level at which both iodine and hypiodous acid are present (USPHC, 2011).

Iodophor has an amber (brownish-yellow) color that indicates its effectiveness as a disinfectant. It no longer is effective when yellow or colorless. Low temperatures reduce the disinfection capacity of many chemical disinfectants, including iodine (NAS, 1980). Iodine's effectiveness is approximately halved for every 10°C decrease in water temperature. Lower temperatures thus require a higher iodine concentration or a longer contact time for useful disinfection (USPHC, 2011).

Iodophor concentrations from 50 to 100 mg/L for 10 to 30 min are sufficient to disinfect culture equipment. Alternatively, exposure to 200–250 mg/L for a few seconds effectively disinfects

hands, hard surfaces, and is appropriate for foot baths at the entrance of culture spaces (Yanong and Erlacher-Reid, 2012). Check the specifications of the particular brand to calculate the required dose.

6.2.7 Hydrogen Peroxide

Large commercial operations are reducing their use of chlorine. Hydrogen peroxide (H_2O_2), a strong oxidizer that eradicates some bacterial and nonbacterial pathogens, is one popular alternative. Information on the precise toxicity of hydrogen peroxide to common pathogens, parasites, and culture species is limited, but a dose of 75 ppm is recommended for disinfecting water. A 75 ppm solution can be made from 35% H_2O_2 by mixing 214 mL of peroxide per 1 m^3 of water. Hydrogen peroxide is also an effective general-purpose surface disinfectant. Exposing a surface to a concentration of 3% for 5–30 min is recommended (Yanong and Erlacher-Reid, 2012).

Peroxide generally is sold in concentrations of 35% and 50%. It is roughly the same price as, or sometimes even cheaper than, sodium hypochlorite. Hydrogen peroxide does not produce harmful residues because it quickly degrades to oxygen and water, usually within 24 h. This is an especially attractive feature that eliminates the need for a protocol to remove toxic end-products. Light oxidizes hydrogen peroxide, so it must be stored in an opaque container. As a standard precaution, gloves and eye protection should be used when handling it or other disinfectants.

6.2.8 Ozone

Ozone (O_3) has seven times the oxidizing capacity of free chlorine and is very effective in eliminating bacteria and viruses in potable water. It is also used in aquaculture. Ozone degrades to oxygen and the resulting free radicals are responsible for its effectiveness as a disinfectant.

The level of free radicals present in ozonated water is indicated by the redox potential or ORP (Oxidation Reduction Potential). Higher ORP means a higher concentration of free radicals. The optimum ORP is between about 300 and 330 mV. If it is greater than 400 mV, then physical oxidation starts to occur and a higher percentage of bacteria are eradicated. Beyond 500 mV, a yet greater percentage of the water is disinfected, but then animals such as shrimp are at risk for serious damage. There is complete disinfection at 750 mV, a concentration used to sterilize swimming-pool water, but this is entirely unsuitable for shrimp, biofloc organisms, and other aquatic life. Even if the cultured organisms are isolated from water exposed to this high ozone level, additional treatment is needed to reduce ORP to a safe value ($\sim 300\text{ mV}$) before returning ozonated water to the culture tank.

A residual concentration must be maintained for a certain period to achieve sufficient disinfection (Goncalves and Gagnon, 2011). A dose of 0.56–1.00 mg/L with a contact time of 1 to 5 min is sufficient for most aquaculture systems, although higher residual concentrations may be required to eliminate some pathogens (Treece and Fox, 1993). Residual ozone is measured with colorimetric test kits, spectrophotometers, photometers, or indirectly with an ORP (oxidation redox potential) meter. Colorimetric procedures and calculations to determine residual ozone in seawater are found in Treece and Fox (1993) and Eaton et al. (1995).

Ozone is highly unstable and degrades too rapidly to be transported, so it must be generated on-site. The efficiency of ozone generation is increased by using pure oxygen instead of air, but this increases equipment costs and operation expenses.

The required ozone production (mg/min) is determined by multiplying water flow rate (L/min) by the desired dosage (mg/L). For example, to introduce a dose of 0.75 mg/L ozone into

a water flow of 100L/min water requires $(100 \text{ L/min}) \times (0.75 \text{ mg/L}) = 75 \text{ mg/min}$.

As noted before for chlorination, ozone also reacts with bromide in seawater to form bromine disinfectants that are toxic to aquatic animals (especially molluscs) and perhaps carcinogenic (Goncalves and Gagnon, 2011). Post-ozone treatment thus may be required in seawater to ensure complete breakdown of these compounds prior to stocking culture tanks. See Goncalves and Gagnon (2011) for further details on ozone treatment and the related chemistry of bromide reactions.

Ozone is highly toxic to humans, so disinfection should take place in a sealed reactor made of ozone-resistant materials and situated in a well-ventilated area, rather than in the culture tanks themselves. Workers should be trained in ozone safety protocols.

Ozone decomposes within a few minutes of treatment, but de-ozoneation nevertheless may be needed in some cases to ensure that no residual ozone is present before beginning production. This can be accomplished with an inline UV lamp, foam fractionators, activated carbon filters, aeration, or hydrogen peroxide.

Disinfecting with ozone or UV light (see next section) involves much higher setup costs than disinfecting with chlorine or hydrogen peroxide. Operating expenses (mainly electricity), however, generally are lower and the effectiveness of ozone and UV light is potentially greater.

6.2.9 Ultraviolet (UV) Light

Ultraviolet (UV) light disinfection involves exposing flowing water to UV light from a lamp enclosed in a quartz or glass sleeve. The high-energy, short-wave radiation to which the water is exposed kills microorganisms by disrupting their DNA. No harmful residues are produced. The effectiveness of UV depends on its intensity and exposure time. Water flow rate, UV lamp rating, water clarity, and thickness of the water stream passed by the lamp are important design factors.

Data provided by manufacturers can be used to determine the lamp size needed to achieve adequate disinfection for a particular situation. The UV dose required to eliminate different microorganisms varies. Gram-negative bacteria are the most susceptible to UV and require the lowest dose, followed by gram-positive bacteria, viruses, spore-forming bacteria, and protozoans (Yanong and Erlacher-Reid, 2012). For example, inactivation of 99.9% of common bacteria requires 3–22 mW-sec/cm²; viruses, 1–900 mW-sec/cm²; fungi, 10–40 mW-sec/cm²; and parasites, 27–318 mW-sec/cm² (Liltved and Cripps, 1999; Summerfelt and Vinci, 2013; Wedemeyer, 1996; Yanong and Erlacher-Reid, 2012). As with other disinfectants, prefiltering water reduces turbidity and increases effectiveness (Treece and Fox, 1993).

Periodic cleaning of the quartz sleeves and bulb replacement should follow the manufacturer's recommendations because the intensity of UV light emitted by bulbs diminishes over time. Special care must be taken when handling these expensive items. Both are easily damaged and, if not isolated from the main flow, can break from the water hammer effect of pump startup.

UV disinfection may be prohibitively expensive for large volumes of water intended for aquaculture grow-out. It is, in fact, more commonly used in clear-water RAS, hatcheries, and sterile algae production than for biofloc systems. A more complete description of chemical disinfectants and dosage protocols in aquaculture is found in Yanong and Erlacher-Reid (2012).

6.3 IONIC AND HEAVY METAL COMPOSITION

As noted in Section 4.2, culture water drawn from nonmarine sources generally has an ionic composition very different from that of seawater. Groundwater also may contain high levels of heavy metals. Additionally, when water is reused from a previous shrimp crop, some

elements may have been depleted and others may have accumulated to problematic levels. In either case, water requires treatment to ensure a composition similar to that of natural seawater before stocking.

If major constituents are deficient, these can be restored with common products such as potassium chloride for potassium, lime or calcium chloride for calcium, and dolomite or Epsom salts for magnesium (Table 6.3). Various commercial products are available to restore specific trace element concentrations, such as iodine and strontium. Products such as Azomite are a source of a broad spectrum of over 70 minerals and trace minerals.

These chemicals have different solubilities and different effects on other water-quality properties. Chloride salts, such as CaCl_2 , KCl , MgCl_2 , for example, generally are highly soluble compared to sulfate salts, such as $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, K_2SO_4 , and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$.

Here is an example of how to calculate the amount of a chemical to add to increase a particular ion. For a salinity of 32 ppt and a potassium concentration of 80 mg/L, potassium chloride (KCl) will be added to increase the potassium concentration to 286 mg/L.

1. Calculate the amount by which potassium must be increased: $286 \text{ mg/L} - 80 \text{ mg/L} = 206 \text{ mg/L}$
2. From Table 6.3, the percentage of potassium (K) in KCl is 52%
3. The amount of KCl to add is calculated as $(206 \text{ mg/L}) \div (0.52) = 396 \text{ mg/L KCl}$

Thus adding 396 mg/L potassium chloride raises potassium to the desired level. In a 100-m^3 (i.e., 100,000-L) tank, this requires $396 \text{ mg/L} \times 100,000 \text{ L} = 39.6 \text{ kg}$ potassium chloride

Rather than adding these compounds directly to the water, research has been conducted on adding them to shrimp feed (Gong et al., 2004; Roy and Davis, 2010). Results thus far have been mixed.

There are many methods to remove excess heavy metals from water, the simplest of which

TABLE 6.3 Products to Increase the Concentration of Major Cations in Culture Water

Element	Mineral Supplement	% Target Ion ^a (max)
Ca	Agricultural lime (CaCO_3)	40
	Burnt lime (CaO)	71
	Hydrated lime (Ca(OH)_2)	54
	Calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$)	27
	Gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$)	24
K	Muriate of potash (KCl)	52
	Potassium sulfate (K_2SO_4)	45
	Potassium nitrate (KNO_3)	39
	Potassium hydroxide (KOH)	70
	Potassium carbonate (K_2CO_3)	57
Mg	Sulfate of potash magnesia ($\text{K}_2\text{SO}_4 \cdot 2\text{MgSO}_4$)	18
	Dolomite ($\text{CaMg(CO}_3)_2$)	13
	Epsom salts ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	10
	Magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$)	12
	Magnesium oxide (MgO)	60
Na	Sodium chloride (NaCl)	39
	Sodium bicarbonate (NaHCO_3)	26

^a The percentage of the target ion in each product varies with the quality and purity of the specific product. Check the product label for actual contents. (Based on Boyd, C.E., Thunjai, T., 2003. Concentrations of major ions in waters of inland shrimp farms in China, Ecuador, Thailand, and the United States. *J. World Aquacult. Soc.* 34 (4), 524–532; Davis, D.A., Samocha, T.M., Boyd, C.E., 2004. Acclimating Pacific White Shrimp, *Litopenaeus vannamei*, to inland, low-salinity waters. Southern Regional Aquaculture Center Publication No. 2601.)

is to let the water settle for a few days. Other options include a combination of aeration and filtration (for iron); charcoal filtration; applying chelators, such as ethylenediaminetetraacetic acid (EDTA); and dosing with ozone to oxidize and then precipitate metals (Treece and Fox, 1993).

6.4 NITRIFYING BACTERIA

Aerobic nitrifying bacteria are vital in mixotrophic biofloc systems for oxidizing potentially toxic ammonia produced by shrimp metabolism (see Section 4.3). Ammonia-oxidizing bacteria (AOB: *Nitrosomonas* spp., *Nitrosococcus* spp., *Nitrosospira* spp., *Nitrosolobus* spp., and *Nitrosovibrio* spp.) catabolize ammonia to nitrite; and nitrite-oxidizing bacteria (NOB: *Nitrobacter* spp., *Nitrococcus* spp., *Nitrospira* spp., and *Nitrospina* spp.) transform nitrite into nitrate (Hagopian and Riley, 1998).

These bacteria naturally develop in biofloc systems, depending on nutrient availability, attachment area, and factors such as temperature, pH, and DO. AOB develop sooner than NOB, resulting in a temporary accumulation of nitrite.

When sufficient carbon is available, faster growing heterotrophic bacteria outcompete nitrifying bacteria for ammonia. Development of nitrifiers in culture water can be accelerated by inoculating new water with bacteria from established nitrifying populations. For optimal results, 10% of the total tank volume should be added as inoculum. If, however, inoculum is limited, good results can be obtained with only 5% of the tank volume. To respect biosecurity, the inoculum should come from disease-free tanks.

Another method to develop nitrifying bacteria is to add a commercially available source of bacteria. Numerous products are available, each with different recommended dosages and specific applications. Examples include KI-Nitrifier (Keeton Industries, Inc., Wellington, CO, US), Fritz-Zyme 9 and Fritz-Zyme Turbostart (Fritz Industries, Mesquite, TX, US), Microtack 22L (Microtack Organic Aquaculture & Wastewater Treatment Supplies, Bangkok, Thailand), Pond-Protect (Novozymes Biologicals, Inc., Salem, VA, US), and Bacta-Pur N3000 for fresh and salt water (ET-Aqua Research Ltd., North Hatley QC, Canada).

Treatment ideally should occur 2 to 3 weeks prior to stocking or in the very early stages of production. This ensures that nitrifying bacteria are well established to prevent exposing shrimp postlarvae (PL) to high concentrations of ammonia and nitrite. If inoculation occurs prior to stocking, an ammonia source such as ammonium chloride or formulated feed (crumble) may be added to tanks at the same time to stimulate bacterial growth.

Nitrification in shrimp biofloc raceways at the Texas A&M-AgriLife Research Mariculture lab (ARML) was established during one season by inoculating disinfected seawater in aerated 10-m³ tanks with 10 mg/L NO₂ (KNO₂ 97%) and a commercial nitrifying bacteria product four weeks before stocking raceways. Once NO₂ concentrations declined to 0 mg/L, an additional 5 mg/L NO₂ was added. The extended water preparation period of four weeks was related to low water temperature during early spring. After the inoculation period, water containing active nitrifiers was pumped into raceways containing filtered water, constituting 10% of the total raceway volume. Raceways were stocked with PL the following day. Nitrite concentrations were substantially lower than observed during previous nursery cycles for which nitrifying bacteria were not inoculated.

6.5 PROBIOTICS AND VIBRIO CONTROL

Beneficial microorganisms may be encouraged in the culture system by adding probiotics that:

- a) suppress development of pathogenic viruses and bacteria (such as *Vibrio* spp.)
- b) improve shrimp health and nutrient digestibility
- c) improve water quality
- d) break down organic matter

The most common genera in probiotic formulations for shrimp aquaculture are gram-positive bacteria (*Bacillus*, *Lactobacillus*, and *Pseudomonas*), gram-negative bacteria (*Vibrio*), yeast (*Saccharomyces* and *Phaffia*), and microalgae (*Tetraselmis*) (Farzanfar, 2006; Hai and Fotedar, 2010). Many probiotic products also contain nitrifying and denitrifying bacteria.

Probiotics can help control *Vibrio* outbreaks by adding “good” species that increase competition for chemicals, nutrients, and adhesion sites; produce inhibitory compounds or antibiotics; enhance shrimp immune response; and supply nutrients and digestive enzymes (Cruz et al., 2012; Hai and Fotedar, 2010; Lakshmi et al., 2013).

In biofloc culture systems infected with *V. parahaemolyticus*, inoculation with a probiotic suppressed this pathogen and significantly improved growth, survival, and FCR of Pacific White Shrimp (Krummenauer et al., 2014).

Probiotics are sprayed onto feed to facilitate rapid ingestion—many commercial shrimp feeds contain probiotics—or added directly to the culture water. Feed, whether pelletized or live, is the more effective way to introduce probiotics into shrimp (Hai and Fotedar, 2010).

Probiotics available for marine aquaculture systems vary in effectiveness. Manufacturers should be contacted for details on the applicability of each brand to shrimp biofloc systems. Adding a cocktail of probiotics generally is more effective for controlling pathogens and boosting shrimp performance than any single probiotic. Effectiveness should be assessed regularly by monitoring *Vibrio* spp. populations and shrimp performance (growth, survival, FCR) so that changes in type and dosage can be made, if necessary.

Commercial probiotics and bacterial amendments used for shrimp include ShrimpShield (Keeton Industries, Inc., Wellington, CO, US), Macrogard (Orffa International BV, Werkendam, The Netherlands) which contains purified

beta 1,3/1,6-glucans, Aquastar (Biomim Holding GmbH, Herzogenburg, Austria), Sanolife MIC (INVE Aquaculture, Breda, The Netherlands), and Alken Clear-Flo 1006 (Alken-Murray Corp., Flint Hill, VA, US).

A probiotic that was used during one season in our shrimp biofloc systems to control pathogenic *Vibrio* spp. is EcoPro (EcoMicrobials, LLC, Miami, FL, US). This product is incubated and aerated in disinfected freshwater for 18 to 24h at 10 g/L prior to direct application to production tanks at 200 mg/m³ (20 mL of incubated EcoPro/m³). The rate and application frequencies were adjusted according to the prevalence of pathogenic bacteria and the organic load. For example, in response to increasing pathogenic *Vibrio* counts (GCFU) in culture water, EcoPro application frequency was increased from every three days to daily, and the dose was increased periodically up to 400 mg/m³. Furthermore, a 62-day nursery trial using this probiotic resulted in production of juvenile shrimp (>6.4 g) with very low FCR (0.81) and high survival (>94%). Nevertheless, for lack of control, these results suggest further trials are needed to determine the full benefit from using this product.

Probiotics are often confused with prebiotics. Probiotics add beneficial microorganisms to a culture system or feed to prevent establishment of pathogenic viruses and bacteria, improve shrimp health and nutrient digestibility, improve water quality, and break down organic matter. Prebiotics are indigestible feed additives that stimulate growth and functioning of beneficial digestive tract bacteria (gut flora) that improve shrimp growth rate, immune response, and stress resistance.

Probiotics and prebiotics thus serve similar functions, but probiotics are live microorganisms while prebiotics stimulate the growth of microorganisms. In other words, prebiotics are feed for probiotics and other beneficial gut microflora. See Gatlin and Peredo (2012) for more details.

6.6 ORGANIC CARBON SUPPLEMENTATION

Organic carbon can be added to a new system to stimulate heterotrophic bacterial control of ammonia while nitrifying bacteria develop. Additional carbon should not be necessary once nitrifying bacteria are established unless ammonia and nitrite concentrations continue to rise. At this stage, heterotrophic bacteria consume about one-third of available ammonia by using organic carbon in waste from feeding. The remaining two-thirds is consumed by nitrifying bacteria.

The main considerations when adding organic carbon in the early stages of a newly started system are as follows:

1. maintaining ammonia and nitrite within a safe range for shrimp PL
2. ensuring enough ammonia and nitrite to support populations of nitrifying bacteria
3. limiting ammonia for phytoplankton to prevent an algal bloom while turbidity is low
4. ensuring dissolved oxygen does not drop too low (usually not a problem in new systems)

These factors must be balanced when determining the amount and duration of organic carbon supplementation. See [Section 7.5.4](#) and [Section 4.3.1](#) for further details.

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Water Quality Management

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7.1 DISSOLVED OXYGEN

7.1.1 Maintenance

Dissolved oxygen is routinely maintained within the desired range by adjusting aeration rate or water flow, depending on system design. As mentioned in [Section 5.6](#) the six 40m³ raceways were equipped with two types of air blower. During the first few weeks of the nursery when biomass was less than 20 kg/raceway (0.5 kg/m³), air was provided by one 3.5-hp regenerative blower capable of producing 190 CFM of air at 0.72 psig at 3450 RPM (S63 Sweetwater, Pentair Aquatic Eco-Systems, Apopka, FL, US). This air blower kept DO above 4 mg/L when the daily ration was as much as 2 kg feed/raceway (about 0.05 kg/m³ per day).

When this blower could not maintain the required minimum DO, a stronger 7.5-hp, lobe-type blower, capable of producing up to 500 CFM at 7 psig operated at 1800 RPM (4007 21L2 Tuthill, Springfield, MO, US) was used. This blower maintained the required DO with biomass of about 120 kg/raceway (3 kg/m³) and daily feed of 3–4 kg/raceway. A pump-driven (2hp) 5-cm Venturi injector sent oxygen-rich water into each raceway (see [Sections 5.3.2](#)

and [5.3.3](#)) to help maintain DO at a biomass of up to 6 kg/m³ and a daily ration of 5–6 kg of feed per raceway. In most cases, the Venturi was operated with atmospheric air, but from time to time oxygen enrichment was required to maintain DO above 4 mg/L. This enrichment generally was needed for biomass between 240 and 380 kg/raceway (6–9.5 kg/m³) and daily feed of up to 8.5 kg/raceway.

For the two 100m³ raceways, nursery observations demonstrated that one 2hp pump maintained DO above 4 mg/L when biomass was over 340 kg/raceway (3.4 kg/m³) and daily feed was about 12 kg/raceway. In grow-out trials, DO was maintained by the same 2-hp pump with biomass as high as 650 kg/raceway (6.5 kg/m³) and daily feed of about 16 kg/raceway. Two of these pumps per raceway could maintain DO when biomass was above 900 kg/raceway (9 kg/m³) with daily feed up to 22 kg/raceway.

An on-site oxygen source can be used in emergencies, when the existing aeration system is insufficient for maintaining DO above 4 mg/L at high biomass, or when DO is low owing to leftover feed, excessive application of organic carbon, or high microbial and shrimp biomass. This can be delivered as liquid oxygen (LOX),

compressed oxygen cylinders, or an oxygen generator. Pure oxygen can be supplied through the Venturi injectors. See [Sections 5.2.3, 5.3.2, and 5.3.3](#) for further details on aeration and oxygenation systems.

In an emergency when pure oxygen is not available, hydrogen peroxide (H_2O_2) can be used to increase DO because it degrades to O_2 and water, with organic matter acting as a catalyst ([Furtado et al., 2014](#)). Adding 0.3 mL of 6% H_2O_2 increases the DO of 1 L of water by approximately 1 mg/L. For example, if the DO of a 1000-L nursery transport tank has decreased to 3.5 mg/L and there is no compressed oxygen, raise the DO to a safe concentration (5 mg/L) by slowly adding about 450 mL of 6% H_2O_2 . Adjust this rate depending on the concentration of H_2O_2 on hand and the desired DO increase. Avoid H_2O_2 concentrations above 5 mg/L for more than a few hours ([Boyd, 2013](#)). Hydrogen peroxide can be used as a safe source of oxygen for Pacific White Shrimp juveniles in biofloc systems up to 14.3 μL H_2O_2 /L ([Furtado et al., 2014](#)).

Closely monitor DO when adding organic carbon to control ammonia and nitrite levels. Depending on the amount added, DO is likely to decrease within 30 min of adding organic carbon. If carbon is added several times throughout the day, DO may become progressively lower after each addition and take several hours to recover without oxygen supplementation. Having oxygen on-site thus is strongly recommended to avoid low DO and/or fluctuations. Other ways to manage low DO include:

- reduction or short-term cessation of feeding
- removal of uneaten feed
- reducing solids (TSS/SS) to lower bacterial oxygen demand
- using foam fractionators to decrease dissolved organic matter
- increasing water flow rate in injector-equipped tanks

- partial harvest to decrease shrimp biomass
- reducing culture water temperature
- exchanging water

7.1.2 Monitoring

Ideally, each culture tank would have a monitoring system to track DO changes. These can be expensive (e.g., \$2000 for a two-channel DO monitoring system; \$6000 for a four-channel system with optical probes), so it is important to select one that performs well in biofloc-rich water. DO monitoring systems with optical probes have performed very well for five years in our systems. The data reveal short- and long-term changes that help manage the culture systems more efficiently. The software which comes with the monitoring system enables programming to alert operators when DO drops below a critical level and automatically activates a backup protocol. We set the minimum DO level at 4 mg/L and the maximum at 5.5 mg/L for the 40 m³ raceways. The “low” alarm was set at 4 mg/L to avoid DO levels that would stress the shrimp; the “high” alert was designed to prevent the unnecessary use of oxygen. The same low DO alert was used for the 100 m³ raceways, but no upper limit was set because maintaining DO above 5.5 mg/L did not require pure oxygen. When linked with the automatic feeders, the unit can be programmed to enable feed delivery only when DO is greater than a concentration deemed safe by the production manager.

In addition to continuous monitoring of DO and temperature, DO should be measured manually in each tank at least twice daily (morning and afternoon) to ensure that there are no discrepancies between continuous and manual measurements. A handheld meter that uploads data to a computer (remotely or via cable connection) streamlines data collection and management.

7.2 TEMPERATURE

7.2.1 Maintenance and Monitoring

Shrimp feed consumption varies considerably with temperature, so water temperature is monitored to adjust daily rations appropriately. Below 28°C, feed consumption, metabolism, and growth decline, so rations must be reduced to avoid adverse effects on water quality and needless expense. Microbial activities also decrease at lower temperatures.

Shrimp are stressed at temperatures higher than 31°C. Adequate procedures to lower water temperature thus must be available to deal with such conditions in hot climates. These may include covering the greenhouse roof with sunlight reflecting material, removing the sidewalls, and promoting evaporative cooling with fans.

Systems with temperature control (e.g., heat exchangers, space or submersible heaters) can link to an alarm that alerts managers when temperatures are outside the target range. Monitor local weather forecasts for unusual changes (cold fronts, extreme heat) and prepare accordingly (e.g., add extra insulation or shade cloth). Building design significantly impacts energy consumption, so an experienced engineering firm should design the building and temperature control system (see [Section 5.2.2](#) for more details).

7.3 pH

7.3.1 Maintenance

pH is stabilized by maintaining adequate alkalinity (see the following section). This is done in the Texas A&M-AgriLife Research Mariculture Lab (ARML) systems by adding sodium bicarbonate, which raises alkalinity and also raises pH when it is much lower than 7. Near pH 7, however, the effect of bicarbonate on pH

generally is small. (This somewhat counterintuitive result is explained graphically in [Appendix V](#)). Adding sodium hydroxide (caustic soda) or calcium hydroxide (hydrated lime) raises pH dramatically and must be used with caution for the safety of both the technician and the shrimp crop. A combination of sodium bicarbonate and sodium hydroxide has been used to control both pH and alkalinity successfully in the Texas A&M-ARML biofloc raceways.

All pH adjustments should be made gradually to avoid stressing shrimp and nitrifying bacteria. Wear appropriate protective gear when handling liquid/powder caustic soda or lime.

Only limited intervention is needed to ensure optimal pH during the nursery and early grow-out phases. With 30ppt natural seawater and in the absence of an algal bloom, pH in the nursery typically declines from about 8.2 to 7.4 as biomass increases to 5–6 kg/m³. This is owed primarily to the activities of nitrifying bacteria and CO₂ production by shrimp and the floc bacteria (CO₂ forms carbonic acid in water, depressing pH when it dissociates).

The pH of some saline ground waters is less than 6.5. Degassing CO₂ with a column or degassing tower will raise pH to a value acceptable for shrimp culture.

At the beginning of a nursery run using virgin water, when biofloc concentration is low, an algal bloom can raise pH well above 9. In such a case, pH can be lowered to an acceptable level in the 100-m³ tanks in less than 20 min by injecting bottled CO₂ through air diffusers. Our experience with the 40 m³ raceways shows that this treatment is very effective during the first two weeks of a nursery cycle and rarely is required for more than two consecutive days to stabilize pH.

pH should be monitored constantly in grow-out tanks, especially when biomass is high and alkalinity is low, because it can vary significantly over 24 h and drop below 7.0.

7.3.2 Monitoring

pH is measured at least once per day throughout production and more frequently when a bloom or unusual mortality is detected. Until a manager becomes familiar with the system, it is worthwhile to measure pH at more frequent intervals to develop insight into how it changes over a typical diel (day-night) cycle.

7.4 ALKALINITY

7.4.1 Maintenance

Numerous observations suggest that shrimp can be raised successfully in biofloc-dominated systems with alkalinity above 400 mg/L. [Timmons and Ebeling \(2013\)](#) recommended the 100–150 mg/L range for optimal nitrification. Our results from grow-out trials showed very good shrimp performance when alkalinity was between 140 and 180 mg/L CaCO_3 .

Alkalinity is continuously consumed in mixotrophic biofloc systems, so monitoring and adjustment (2–3 times a week) are required. It is restored by adding bicarbonate or other chemical reagents. Less chemical adjustment is needed in systems with denitrification, as this process increases alkalinity.

The following chemicals are commonly used to increase alkalinity: sodium bicarbonate (NaHCO_3), potassium bicarbonate (KHCO_3), sodium carbonate (Na_2CO_3) (soda ash), potassium carbonate (K_2CO_3), and calcium carbonate (CaCO_3) (agricultural lime) ([Table 7.1](#)). The most effective, safe, and easy to dissolve are the bicarbonates ([Wasielesky et al., 2015](#)), followed by soda ash. All are readily available and have a long shelf life. Soda ash is generally cheaper and more efficient (less is required to raise alkalinity) than sodium bicarbonate, but is more likely to form a precipitate in the water (difficult to dissolve). Some liming materials, such as CaO , Ca(OH)_2 , and CaMg(OH)_4 , cause large

TABLE 7.1 Common Reagents Used to Increase Alkalinity and Their Characteristics

BICARBONATES VS. CARBONATES TO INCREASE ALKALINITY	
Bicarbonates	Carbonates
Sodium bicarbonate (NaHCO_3), Potassium bicarbonate (KHCO_3)	Sodium carbonate (Na_2CO_3) (soda ash), Potassium carbonate (K_2CO_3), Calcium carbonate (CaCO_3)
• More effective	• Cheaper (soda ash)
• Safer	• More efficient (soda ash)
• Ease of use	• Lower solubility

and abrupt increases in pH, are caustic and so require care in handling, and are difficult to dissolve ([Gerardi, 2003](#)). They often are, however, cheaper than bicarbonates and carbonates ([Wasielesky et al., 2015](#)).

Operators using CaCO_3 to maintain alkalinity in a biofloc-dominated system reported much higher and stable pH (around 7.4) than achieved with either sodium bicarbonate or sodium carbonate ([Dariano Krummenauer](#), personal communication).

Even though sodium compounds were used for alkalinity and pH control at the Texas A&M-ARML, no sodium accumulation was observed over a single production cycle ([Prangnell et al., 2016](#)). If sodium does accumulate over multiple cycles, calcium salts could be used for alkalinity maintenance.

Any of these chemicals should be added slowly to avoid settling on the tank bottom and to prevent sudden changes in pH, alkalinity, or oxidation-redox potential (ORP) that may adversely affect shrimp or floc bacteria ([Gerardi, 2003](#)). This is accomplished by dripping a concentrated solution of the dissolved chemical from a valved container ([Fig. 7.1](#)) or spreading the required dose periodically throughout the day. This method also is used to add an organic carbon source (e.g.,



FIG. 7.1 A modified container used to drip a chemical solution into a culture tank.

sugar solution, molasses) in liquid form. Regularly monitor the flow rate, as the outlet valve may clog with inadequate mixing or precipitates.

The amount of bicarbonate needed to compensate alkalinity loss can be estimated from measured alkalinity and online alkalinity calculators or simple equations (Skinner and Hales, 1995). As an example of the latter, consider a 100,000-L tank with an alkalinity of 140 mg/L CaCO_3 . The amount of sodium bicarbonate required to increase alkalinity to 160 mg/L CaCO_3 (i.e., by 20 mg/L) is $(100,000 \div 596,005) \times 20 = 3.36$ kg. The amount of sodium carbonate (soda ash) required to increase alkalinity to 160 mg/L CaCO_3 (increase of 20 mg/L) is: $(100,000 \div 944,855) \times 20 = 2.12$ kg (Skinner and Hales, 1995).

Based on the expected decline in alkalinity from nitrification of ammonia originating from feed protein, every kilogram of 35% protein feed (assuming no supplemental carbon and $\sim 2/3$ of

ammonia oxidized by nitrifiers) should be supplemented with 0.25 kg of sodium bicarbonate (Timmons and Ebeling, 2013). For example, if 8 kg of 35% feed is added, then also add $8 \times 0.25 = 2$ kg of sodium bicarbonate to maintain alkalinity. More sodium bicarbonate is needed for feed with higher protein content.

Alkalinity decreases during nitrification by about 7.14 mg CaCO_3 for every mg of ammonia-N oxidized to nitrate-N (2 meq of alkalinity per mole NH_4^+) (Van Rijn et al., 2006). Part of this loss (3.57 mg CaCO_3 for every mg of nitrate-N converted to N_2) can be restored if denitrification is part of the culture system (see Section 11.1). This also increases pH and removes nitrate and phosphate (Sedlack, 1991; Van Rijn et al., 2006).

Alkalinity rarely is too high (>250 mg/L CaCO_3) unless an excessive amount of bicarbonate is added. High alkalinity in groundwater, however, may necessitate remediation prior to use. Alum (aluminum sulfate: $\text{Al}_2(\text{SO}_4)_3 \cdot 14\text{H}_2\text{O}$) reduces alkalinity and pH by neutralizing carbonate and bicarbonate compounds (Barkoh et al., 2013; Wilkinson, 2002). Hydrogen ions react with carbonates and bicarbonates to form carbon dioxide and water. One mg/L of alum reduces alkalinity by about 0.5 mg/L and pH by 0.03–0.06 units (depending upon the initial alkalinity) (Boyd, 1979).

Alum also acts as a precipitant that reduces turbidity, inorganic phosphate, and inorganic nitrogen (Barkoh et al., 2013; Wilkinson, 2002). High aluminum concentrations may restrict bacterial functioning, so alum treatment generally is performed outside of culture tanks, usually pre- or post-culture, and includes a settling stage to remove aluminum precipitates.

7.4.2 Monitoring

When stocking postlarvae (PL) into new water, measure alkalinity twice weekly during the first month. Increase monitoring frequency to every 1–2 days when nitrifying bacteria are

fully established and large daily declines in alkalinity ($>5\text{ mg/L CaCO}_3/\text{day}$) are observed. If a calculated amount of bicarbonate/carbonate is added regularly with the feed to avoid fluctuations in alkalinity, regular testing should be done to avoid large discrepancies between expected and actual alkalinity.

7.5 INORGANIC NITROGEN COMPOUNDS

When a nursery run begins with new seawater and without a sufficiently mature population of nitrifying bacteria, careful monitoring is needed to prevent the accumulation of toxic concentrations of ammonia and nitrite.

7.5.1 Ammonia

Ammonia concentration should be near zero once nitrifying bacteria (AOB—Ammonia-Oxidizing Bacteria) are established in the system, usually within 4–6 weeks in new water. To be safe, maintain Total Ammonia Nitrogen (TAN) below 3 mg/L , although shrimp have survived in higher concentrations in our raceway systems when operated at about 30 ppt. In low salinity water (2–4 ppt), keep ammonia below 1 mg/L .

7.5.2 Monitoring

Weekly monitoring is sufficient when shrimp are stocked in a nursery with well-established nitrifying bacteria. This should continue for 3 weeks. Daily monitoring is recommended when ammonia exceeds 2 mg/L . The increase in monitoring frequency is done in conjunction with careful management of organic carbon supplementation to help development of a healthy nitrifying bacterial population while preventing high ammonia (see [Section 7.5.4](#) and [Excel Sheet # 18](#)).

Ammonia below 1 mg/L for 3–4 consecutive days, along with an increase in nitrate, indicates

a healthy AOB population. Weekly monitoring then is sufficient.

7.5.3 Nitrite

Maintain $\text{NO}_2\text{-N}$ below 10 mg/L , although shrimp have demonstrated good survival when exposed to concentrations between 21.5 and 34.3 mg/L (at pH 6.9–7.1, salinity 30.8–32.0 ppt, and temperature $29.6\text{--}31.2^\circ\text{C}$) for 8 days in our raceways. The effect of these high concentrations on growth was not evaluated, but good survival under these conditions suggests no major negative impact. As with ammonia, when working at low salinity, do not exceed $1\text{ mg/L NO}_2\text{-N}$ to avoid shrimp stress and mortality.

7.5.4 Monitoring

As with ammonia, when shrimp are stocked into a nursery with a well-established nitrifying bacterial population, and after confirming that there is no increase in nitrite, monitoring can be done weekly. When PL are stocked, weekly sampling is extended for a few more weeks because of NOB's slower development. When $\text{NO}_2\text{-N}$ exceeds 5 mg/L (16.5 mg/L NO_2), daily monitoring is recommended. When $\text{NO}_2\text{-N}$ remains below 1 mg/L for 3–4 consecutive days, weekly monitoring is sufficient.

7.5.5 Nitrate

Keep $\text{NO}_3\text{-N}$ below 220 mg/L at a salinity of 11 ppt, and 400 mg/L at 30 ppt ([Kuhn et al., 2010](#)).

7.5.6 Monitoring

Periodically measure nitrate to make sure that concentrations are acceptable. Routine monitoring helps follow the activity of AOB and NOB. The typical pattern of ammonia, nitrite, and nitrate in systems with new water is shown in [Fig. 4.2](#). Ammonia and nitrite increase until AOB and NOB, respectively, become established.

Concentrations of ammonia and nitrite subsequently decline rapidly, while nitrate continues to accumulate throughout the culture period.

Only a moderate increase in nitrate (up to 50 mg/L $\text{NO}_3\text{-N}$) will occur by the end of the relatively short nursery phase. No adverse effects of nitrate on shrimp health, survival, or growth were observed in nursery trials at 30 ppt. Thus monitoring of nitrate during the nursery phase is mostly to determine if AOB and NOB are active.

7.5.7 Nitrogenous Waste Control

Nitrogenous waste is controlled in our nursery and grow-out systems with mixotrophic biofloc (see Section 4.3.1). These systems have a healthy population of nitrifying and heterotrophic bacteria, along with a small quantity of microalgae.

When the supply of organic carbon is not limited, heterotrophic bacteria transform the ammonia nitrogen excreted by shrimp into bacterial biomass (Avnimelech, 1999).

When dealing with new water without the use of nitrifying bacteria boost, carbon supplementation might be required to avoid increase in ammonia. Once nitrifiers are established, however, the supply of organic carbon should be limited to the amount in feed waste. As a result, only about 1/3 of the ammonia produced by the shrimp will be converted to bacterial and algal biomass, with the other 2/3 available for nitrifying bacteria (Ebeling et al., 2006).

Unlike the heterotrophic bacteria that, under optimal conditions, multiply as quickly as five times a day, the growth rate of nitrifying bacteria is only about once per day (USEPA, 1993). Other researchers (Crab et al., 2012; Eding et al., 2006; Hargreaves, 2006) report growth rate and biomass yield per unit substrate (0.5 g biomass C/g substrate C used) of heterotrophic bacteria to be ten times higher than that of nitrifying bacteria. For this reason, special attention is needed to nurture nitrifiers when culture water is not inoculated with nitrifying bacteria.

Ammonia is controlled by reducing the nitrogen supply (lowering or eliminating feed)

or adding organic carbon. The latter enables heterotrophic bacteria to convert a larger portion of ammonia (e.g., >1/3) to biomass (Hari et al., 2004). This should be done on an as-needed basis (e.g., when ammonia or nitrite is high, or there is an algal bloom) and is not intended to completely deprive nitrifying bacteria of ammonia. Keeping ammonia below 3 mg/L also limits the amount that AOB convert to nitrite.

As an example, assume shrimp in a tank with new seawater are fed 100 g dry feed with a crude protein of 50%. This adds 8 g of nitrogen to the system ($100\text{ g} \times 0.5 = 50\text{ g protein}/6.25 = 8\text{ g of N}$). If half of this nitrogen (4 g) is excreted as ammonia and there is no other source for organic carbon beside feed, heterotrophic bacteria will consume only 1/3 (or 1.33 g) of the ammonia produced from feeding. The other 2/3 (2.66 g) is left for nitrifying bacteria to oxidize.

The stock of nitrifying bacteria in new water is low, and because they grow slowly, they will not metabolize all of the ammonia present. This leads to an ammonia increase. Although this ammonia can be converted continuously to heterotrophic bacteria biomass, it is better to encourage development of the slow-growing nitrifiers, so carbon additions are restricted to metabolizing 10% to 50% of the ammonia (2.66 g).

Organic carbon is supplemented under the assumption that each unit of ammonia requires 6 units of carbon. Thus if white sugar (42% carbon w/w) is the carbon source, 9.5 g of sugar is needed to convert 25% of ammonia into heterotrophic bacteria biomass: $(2.66\text{ g} \times 0.25 / 0.42) \times 6 = 9.5\text{ g}$.

On the other hand, if the carbon source is molasses (24% carbon w/w), the amount needed is 16.625 g: $(2.66\text{ g} \times 0.25 / 0.24) \times 6 = 16.625\text{ g}$. Because molasses mostly is sold as a liquid, it is more convenient to measure it as a volume. Liquid molasses has a specific gravity of 1.3 g/mL, so the volume needed to provide 16.625 g of carbon is $16.625\text{ g} \times 3.205\text{ mL/g} = 53.283\text{ mL}$. When using liquid molasses, it is important to mix it in water before spreading it in small quantities throughout the tank.

White sugar is cleaner to work with and has much lower levels of impurities than molasses. For example, urea is added to some molasses used to supplement cattle feed. If added to shrimp culture systems, this increases the nitrogen input and negates the ammonia-removal effect of the carbon.

While both carbon sources yield similar results, white sugar does not stain (increase the turbidity of) water like molasses does. This

may increase the potential for an algal bloom in the early stages of culture (see [Section 7.12](#)). Similarly, dextrose results in greater water transparency and alters the composition of microbial communities compared to molasses ([Suita et al., 2015](#)). Other carbon sources include lactose (42% C) and various forms of starch (~43% C). The carbon source ideally should have a low nitrogen content to improve the C:N ratio. See [Table 7.2](#) for a list of carbon sources.

TABLE 7.2 Organic Carbon Sources for Biofloc Systems

Carbon Source	Formula	%Carbon	Advantages	Disadvantages
Molasses (~50% sucrose)	~50% $C_{12}H_{22}O_{11}$	24–37.5	Stains water, reducing light penetration and associated algal growth in new systems	High level of impurities; content variability between source; messy to work with; can increase PO_4 concentration
White sugar (99% sucrose)	99% $C_{12}H_{22}O_{11}$	42.1	High purity	Does not stain water
Lactose	$C_{12}H_{22}O_{11}$	42.1		
Dextrose	$C_6H_{12}O_6$	40.0	Dissolves quickly (rapid carbon availability)	Does not stain water
Glucose	$C_6H_{12}O_6$	40.0		
Acetate	$C_2H_4O_2$	40.0		
Glycerol	$C_3H_8O_3$	39.1		
Cellulose	$C_6H_{10}O_5$	44.4		
Starch	$(C_6H_{10}O_5)_n$	≤44.4		
Other forms of starch:		43.4	Can be relatively inexpensive and locally available	Some products may have a higher nitrogen content; dissolve/degrade relatively slowly
Cassava meal				
Corn flour				
Rice bran				
Sorghum meal				
Tapioca				
Wheat flour				
Wheat bran				

(Partially adapted from [Emerenciano et al., 2013](#). Biofloc technology (BFT) a review for aquaculture application and animal food industry. In: Matovic, M.D. (Ed.), *Biomass Now—Cultivation and Utilization*. pp. 301–328; [Serra et al., 2015](#). Use of different carbon sources for the biofloc system adopted during the nursery and grow-out culture of *Litopenaeus vannamei*. *Aquac. Int.* 23 (6), 1325–1339.)

TABLE 7.3 Calculation of Carbon Addition (as White Sugar) to Remove a Desired Proportion of Ammonia From a Given Amount of Feed

1. Note the daily weight of feed added to a culture tank: e.g., 1 kg/d
2. Multiply it by the feed's protein content. For 50% CP^a: $(50/100) \times (1 \text{ kg/d}) = 500 \text{ g protein/d}$
3. Multiply by 0.16 (~16% N in protein): $(500 \text{ g protein/d}) \times (16 \text{ g N}/100 \text{ g protein}) = 80 \text{ g N/d}$
4. Multiply by 0.50 (fraction of N converted to TAN): $(0.50) \times (80 \text{ g N/d}) = 40 \text{ g TAN/d}$
5. Multiply by $\frac{1}{3}$, the fraction of TAN to be processed by the heterotrophic bacteria (assuming no supplemental organic carbon): $(40 \text{ g NH}_3\text{-N/d}) \times (1/3) = 13.3 \text{ g TAN/d}$
6. Multiply by 6 (desired C:N=6:1): $(13.3 \text{ g TAN/d}) \times (6 \text{ C}/1 \text{ N}) = 80 \text{ g C/d}$
7. Divide by the carbon fraction of the source (white sugar (99% sucrose): 42% C): $(80 \text{ g C/d})/0.42 = 190.5 \text{ g white sugar for every 1 kg of feed.}$

^a Note that Ebeling et al. (2006) provides a simpler formula to calculate the amount of TAN produced by 1 kg of feed. This formula assumes the following for biofloc systems: $\text{TAN F} \times \text{PC} \times 0.144$, where F is the amount of feed, PC is the protein concentration, and 0.144 is the conversion factor. Thus in the earlier example, TAN generated from 1 kg of 50 CP feed will be only 72 g. These authors assume that 80% of nitrogen is assimilated by the shrimp, 80% of assimilated nitrogen is excreted, and 90% of excreted nitrogen is TAN + 10% as urea. Taking all of these assumptions into account yields about the same 40 g of TAN as in the earlier example: $72 \text{ g} \times 0.8 \times 0.8 \times 0.9 = 41.5 \text{ g}$.

Regardless of the source, a significant drop in DO is likely shortly after adding organic carbon, especially if all the carbon is added at once. For this reason, extra aeration or pure oxygen may be needed for 30 min or more after applying carbon. If water temperature is high during the afternoon, schedule supplementation for the early morning.

Table 7.3 provides an example calculation of carbon supplementation using white sugar.

7.6 SOLIDS CONTROL

Solids are managed in biofloc systems with settling tanks, cyclone filters, and foam fractionators. See Section 5.4 for further details of their operation and other options. The targets are 10–14 mL/L for settleable solids (SS) and 250–350 mg/L for total suspended solids (TSS). Turbidity in biofloc systems typically is maintained between 75 and 200 NTU.

Settleable solids usually are measured volumetrically in Imhoff cones (Fig. 7.2), total suspended solids by gravimetric method (Appendix I) or with a spectrophotometer, and turbidity with a turbidimeter or spectrophotometer.

**FIG. 7.2** One-liter Imhoff cones used to measure settleable solids.

Solids concentration is very low in the first few weeks after stocking new water, so SS monitoring is not necessary and TSS (or turbidity) is monitored weekly to track floc development (Fig. 7.3). SS monitoring is more frequent (weekly) as floc matures. If large quantities of organic carbon are added at stocking, daily monitoring is recommended to ensure that settleable



FIG. 7.3 Raceway filled with new water (clear) with low biofloc and low turbidity (*left*) and a raceway with matured biofloc water with high turbidity (*right*).

solids remain between 10 and 14 mL/L. Increase TSS monitoring to twice weekly when it reaches 300 mg/L. (It should not exceed 350 mg/L). If water from a previous production cycle is used, then twice weekly monitoring should begin at stocking. Many commercial growers develop biofloc in nursery tanks prior to stocking postlarvae. In this case, monitor SS daily and TSS twice weekly from stocking. An algal bloom or high concentration of colloids increases turbidity relative to TSS and SS.

TSS measurements in our lab were made with the gravimetric method ([Appendix I](#)). It is accurate, but time consuming. Spectrophotometry and turbidimeters are faster, but they require regular calibration against the gravimetric method.

Because of microscopic air bubbles, floc may rise to the surface of the culture tank. To get a representative sample, culture water thus is mixed thoroughly before sample collection (see [Section 7.13](#)). Analysis should begin as soon as possible after sampling, certainly within 24 h.

7.7 SALINITY

7.7.1 Maintenance

Salinity increases over time owing to evaporation. It is restored by adding freshwater. This may be required as frequently as twice-weekly,

particularly in the grow-out phase when flow and aeration (hence, evaporation) increase. Municipal water can be used without dechlorination when culture water has high dissolved organic matter that reacts with chlorine. No adverse effects have been observed in our system using freshwater with chlorine as high as 2 ppm.

The freshwater required to achieve a desired salinity is calculated as:

$$V_2 = \left(\frac{C_1 V_1}{C_2} \right) - V_1$$

where C_1 = current salinity, V_1 = water volume, C_2 = target salinity, and V_2 = volume of freshwater to add.

For example, consider a tank with salinity 31.58 ppt and volume 95 m³. To reduce salinity to 30 ppt, the volume of freshwater to add is $V_2 = [(31.58 \times 95) \div 30] - 95 = 5 \text{ m}^3$.

7.7.2 Monitoring

Salinity usually is measured with a refractometer, a conductivity meter, a hydrometer, or gravimetrically as TDS (Total Dissolved Solids). Electrical conductivity (generally as $\mu\text{S/cm}$ or mS/cm) increases with the ionic strength. TDS is the mass of all dissolved compounds smaller than 2 μm . TDS (mg/L) can be estimated by multiplying conductivity by an

empirical factor (between 0.55 and 0.90, depending on composition and temperature) or by gravimetric method (Eaton et al., 1995).

7.8 PHOSPHATE

7.8.1 Maintenance

Phosphate can be removed from culture water biologically or chemically (see Section 11.1). Biological treatment involves a digester with anaerobic bacteria that incorporate phosphate into their biomass. Phosphate-rich sludge settles at the base of the digester and is removed periodically. This is the recommended method for bio-floc systems because it is less expensive and produces far fewer solids than chemical treatment. In our experience, a properly sized and managed digester removes up to 87% of phosphate from culture water that initially had a concentration as high as 115 mg/L.

A common practice in municipal wastewater plants involves chemical treatment with a flocculent such as aluminum sulfate that, once added, forms an insoluble aluminum phosphate precipitate (Wilkinson, 2002). This process, however, produces some hydrogen sulfide and high aluminum concentrations that might affect microbial floc populations and shrimp growth.

7.8.2 Monitoring

Simple phosphate test kits are available, but as no active control is required, phosphate monitoring follows no set schedule, although it becomes more important when water is used to raise successive crops.

7.9 OTHER IONS, TRACE ELEMENTS, AND HEAVY METALS

7.9.1 Maintenance

Some heavy metals that accumulate in biofloc are removed with the bulk solids collected by settling tanks, foam fractionators, and digesters

(see Section 11.1). This material then must be disposed of properly. Trace elements are depleted by solids removal and assimilation by shrimp and bacteria. Supplements are added to replenish important elements, such as barium, iodine, iron, and strontium. This can be done gradually over a crop cycle or added to the water after harvest if it is to be used for the next crop. Water exchanges also partially replenish some of these elements. Table 7.4 presents recommended concentrations of some trace elements for shrimp culture.

7.9.2 Monitoring

Chemical elements, especially heavy metals, should be monitored periodically in water, bio-floc, and culture animals, for example, at the

TABLE 7.4 Recommended Concentrations of Selected Trace Elements in Water for Shrimp Culture Within a Salinity Range of 5 to 35 ppt (Whetstone et al., 2002)

Variable	Form in Water	Desired Concentration (mg/L)
Boron ^a	Borate (H_3BO_3 , H_2BO_3)	0.05–1.00
Cadmium	–	<0.1
Copper	Copper ion (Cu^{2+}) ^a	<0.0005
	Total copper	0.0005–0.01
Iron ¹	Ferrous iron (Fe^{2+})	0
	Ferric iron (Fe^{3+})	Trace
	Total iron	0.05–0.50
Manganese	Manganese ion (Mn^{2+})	0
	Manganese dioxide (MnO_2)	Trace
	Total manganese	0.05–0.20
Molybdenum	Molybdate (MoO_3)	Trace
Zinc	Zinc ion (Zn^{2+})	<0.01
	Total zinc	0.01–0.05

^a The desirable concentrations for these elements are poorly understood. Values listed are the typical concentrations found in surface waters.

beginning, middle, and end of each culture phase until site-specific patterns are established (Table 7.4). Test the heavy metal content of the edible portion of shrimp (i.e., the tail muscle) to ensure product safety (see Table 4.9 for maximum concentrations of heavy metals permitted by the FDA in farmed shrimp). If water is to be reused, testing at the end of each production cycle can be achieved by sending samples to a water quality testing lab for a full profile analysis. Note that inexpensive testing is offered by most of the Extension Service water and soil testing labs in each state. Alternatively, kits for several ion-specific tests are available from vendors such as YSI and Hach. These results, however, are less accurate.

Elements worth monitoring include major constituents: sodium, magnesium, calcium, potassium, and sulfate; trace elements and heavy metals: aluminum, arsenic, boron, barium, beryllium, cadmium, cobalt, chromium, copper, iron, lithium, manganese, mercury, molybdenum,

nickel, lead, selenium, silicon, strontium, vanadium, and zinc. This list and testing frequency may be refined as managers gain experience with their culture system. It is also strongly recommended to test the shrimp tissue for these heavy metal to ensure safe concentrations for human consumption. Fig. 7.4 shows steps in sample preparation for ionic composition analysis.

7.10 WATER QUALITY SUMMARY

Table 7.5 summarizes the water quality parameters relevant to biofloc systems. Optimum ranges, frequency of analysis, and adjustment methods are listed for quick reference.

7.11 MICROALGAE AND FILAMENTOUS BACTERIA

Coyle et al. (2011) describe the impact of artificial light on juvenile shrimp (0.4g) stocked



FIG. 7.4 Harvested shrimp being dissected, dried, and ground for ionic composition analysis.

TABLE 7.5 Optimal Ranges of Water-Quality Parameters for Pacific White Shrimp in Biofloc Systems, Frequency of Analysis, and Adjustment Methods

Parameter	Optimum Range	Frequency of Analysis	Adjustment Method
Alkalinity	140–180mg/L	Twice weekly; every other day or daily in established systems	NaHCO ₃ , KHCO ₃ , Na ₂ CO ₃ , K ₂ CO ₃ to increase; alum to decrease
Ammonia (TAN)	<3 mg/L, should be close to 0 once system is established	Daily until nitrifying bacteria established, then twice weekly	Add carbon, reduce feed ration
Chlorine	0 ppm	Whenever water is disinfected	Vigorous aeration and/or sodium thiosulfate, vitamin C, or H ₂ O ₂
Carbon dioxide (CO ₂)	<20 mg/L	Not necessary	Increase aeration, degassing to remove
Dissolved oxygen	4–8mg/L (50%–105% saturation at sea level and 30°C)	Continuously (when shrimp biomass >4kg/m ³) and spot check twice daily	Increase aeration, add O ₂ , reduce feed ration, remove uneaten feed, reduce solids and dissolved organics
Hydrogen sulfide (H ₂ S)	<0.005 mg/L	As required ^a	Maintain adequate mixing and aeration, maintain DO above 3mg/L, increase pH
Nitrate (NO ₃ -N)	<400mg/L (@ 30ppt)	Weekly	Denitrification treatment or water exchange
Nitrite (NO ₂ -N)	<10mg/L (@ 30ppt), should be close to 0 once system is established	Daily until nitrifying bacteria are established, then twice weekly	Add carbon to reduce the amount of NH ₃ available for conversion to NO ₂
Ionic Profile:			
Na:K	Close to 28:1	After each production cycle if culture water is to be reused	NaCl, KCl, K ₂ SO ₄ , KNO ₃ , KOH, K ₂ CO ₃ CaMg(CO ₃) ₂ , MgSO ₄ ·7H ₂ O, MgCl ₂ ·6H ₂ O, MgO, CaCO ₃ , CaO, Ca(OH) ₂ , CaCl ₂ , CaSO ₄ ·2H ₂ O, KCl, K ₂ SO ₄ , KNO ₃ , KOH, K ₂ CO ₃
Mg:Ca:K	Close to 3:1:1		NaCl, MgSO ₄ ·7H ₂ O, MgCl ₂ ·6H ₂ O, MgO
Cl:Na:Mg	Close to 14:8:1		
Trace Elements:		After each production cycle if culture water is to be reused	To increase: • Supplements (element-specific or broad-spectrum) • Water exchange To decrease: • Settling, • Aeration/filtration, • Chelators/flocculants (e.g., EDTA, ozone)

Continued

TABLE 7.5 Optimal Ranges of Water-Quality Parameters for Pacific White Shrimp in Biofloc Systems, Frequency of Analysis, and Adjustment Methods—cont'd

Parameter	Optimum Range	Frequency of Analysis	Adjustment Method
Boron	0.05–1.00 mg/L		
Cadmium	<0.1 mg/L		
Copper	Cu ²⁺ <0.0005 mg/L		
	Total 0.0005–0.01 mg/L		
Iron	Fe ²⁺ 0 mg/L		
	Fe ³⁺ Trace		
	Total 0.05–0.50 mg/L		
Manganese	Mn ²⁺ 0 mg/L		
	MnO ₂ Trace		
	Total 0.05–0.20 mg/L		
Molybdenum	Trace		
Zinc	Zn ²⁺ <0.01 mg/L		
	Total 0.01–0.05 mg/L		
pH	7.2–8.2 (7.0–7.5 at higher biomass)	Daily	NaOH or Ca(OH) ₂ to increase;
Phosphate	Unknown	Weekly	Digester, flocculent
Salinity	20–35 ppt (Stable)	Daily	Add freshwater to decrease
SS	10–14 mL/L	Daily—every other day, once systems are established	Filtration such as hydro-cyclones, foam fractionators, settling tanks, and so on, to decrease; add carbon or turn off filtration equipment to increase
Temperature	28–30°C (26–31°C outer range)	Continuously and spot check twice daily	Air flow, shading, heat exchange
TSS	250–350 mg/L	Twice weekly to every other day	Filtration such as hydro-cyclones, foam fractionators, settling tanks, and so on; add carbon or turn off filtration equipment to increase
Turbidity	75–200 NTU	Weekly	Filtration such as hydro-cyclones, foam fractionators, settling tanks, and so on; add carbon or turn off filtration equipment to increase

^a Well-managed systems in which solids do not accumulate on the tank bottom (causing anaerobic conditions) and DO, pH, and temperature are not low should not experience high H₂S.

at 465/m² in indoor biofloc-dominated tanks. In a 13-week study, the authors compared the effects of five different light sources: natural sunlight (718lux), a metal halide light lamp (1074lux), a fluorescent light (214lux), two fluorescent lights (428lux), and three fluorescent lights (642lux). Light had a significant impact on average weight, survival, yield (kg/m²), and FCR. Growth rates in all treatments were low (0.8–0.9 g/week.), with FCRs 2.1–5.3, and survival 31.9%–88.7%. There was an inverse linear relationship between the number of fluorescent fixtures and survival, which was related to gill fouling by filamentous bacteria. Natural light and the metal halide lights did not result in high concentrations of these bacteria. The effect of light quality on filamentous bacteria has not been reported in other studies. Low DO and limited organic carbon availability are known to encourage their growth in biofloc systems (Coyle et al., 2011; De Schryver et al., 2008).

Shrimp production was 17% greater and FCR 18% lower in a microalgae-dominated (photoautotrophic) system than in a heterotrophic system (Ray et al., 2009). Microalgae are always present in greenhouse-enclosed trials, but more study is needed to determine their contribution to growth and feed conversion. In part, good shrimp performance may be related to algal assimilation of nutrients, particularly dissolved inorganic nitrogen compounds, and certain metals (Chien, 1992).

In early trials at our facility, culture water was inoculated with diatoms, mostly *Chaetoceros muelleri*, with a target algal concentration of 40,000 cells/mL, before stocking postlarvae. In a recent short-term (30-day) nursery study with Pacific White Shrimp juveniles (0.22 g), shrimp survival improved when biofloc water was enriched with the diatom *Amphora coffeaeformis* (Martins et al., 2016). Diatom-enriched water had significantly higher eicosapentaenoic acid (EPA; 20:5n-3) and significantly lower linoleic acid (18:2n-6). It is not known if diatom-rich

biofloc water improves the shrimp's EPA content over a full grow-out cycle.

7.12 GREENWATER TO BROWN-WATER TRANSITION

Kirk (2010) provides a good description of how feeding rate drives the transition from an algae-dominated (greenwater) system to a biofloc-dominated (brown-water) system (Fig. 7.5). The specifics may vary somewhat in ponds, raceways, and tanks, but the general pattern is similar.

The sequence begins when feeding rate is increased in a shrimp system exposed to sunlight. At 100 to 200 kg/ha per day (10–20 g/m² per day), water is green with algae and algal uptake is the main mechanism for ammonia control. At a daily feeding rate of 300 kg/ha (30 g/m²) and limited (or no) water exchange, the lack of light at very high algal density restricts photosynthesis and bacterial biofloc begins to develop. This is accompanied by an increase in suspended solids (250–500 mg/L) and a rapid increase in respiration (6 mg O₂/L per h) that requires as much as a fivefold increase in aerator power (from 30 to 150 hp/ha) to match biofloc oxygen demand. Despite these changes, the water may continue to appear green and a slight O₂ surplus is produced by photosynthesis.

When the feeding rate is 400–600 kg/ha/d (40–60 g/m² per day), the water appears green-brown. Beyond 700 kg/ha/d (70 g/m² per day), the water is brown with biofloc and there is essentially no oxygen contribution from algae. Further increases require more aeration.

Prangnell et al. (2016) reported a similar transition in greenhouse-enclosed raceways at our facility. Algae abundance, as measured by the concentration of pigments, increased through the nursery phase when TSS was low, and then declined through the grow-out phase as shrimp biomass increased and bacteria became more

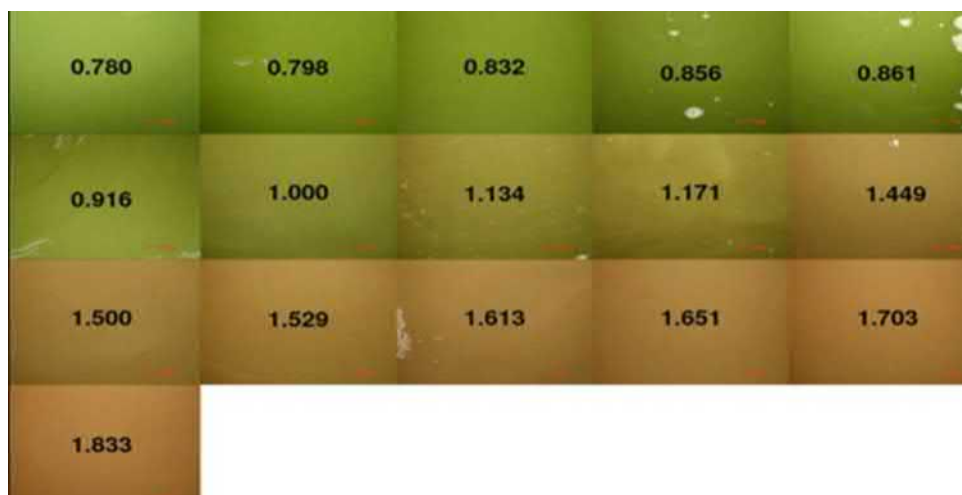


FIG. 7.5 Microbial Community Color Index (MCCI) indicating the transition from an algal to a bacterial system as feed load increases. The transition occurs at a feed rate of 300–500 kg/ha per day (30–50 g/m² per day), indicated by an MCCI between 1 and 1.2. (Kirk, K.R., 2010. *Modeling microbial and nutrient dynamics in zero-discharge aquaculture systems* Ph.D. dissertation, Clemson University, Clemson, South Carolina, USA. Used with permission.)



FIG. 7.6 Raceways with algal dominated water.

dominant. Some level of phytoplankton may be beneficial, but preventing algal blooms (Fig. 7.6) avoids wide diel fluctuations in pH and DO that characterize algal-dominated systems. This relies on management of suspended solids because microalgae blooms are more likely when TSS is less than 150 mg/L. This occurs in new water, in which biofloc is not yet well developed, and also during a production cycle if too much suspended material is removed.

Organic carbon added to the culture water during the first few weeks after stocking enhances development of heterotrophic bacteria and limits the amount of ammonia assimilated by microalgae (see Section 7.5.4). Carbon supplementation is discontinued once nitrifying bacteria are established. TSS levels above 250 mg/L limit light penetration sufficiently to inhibit microalgae blooms. If TSS drops below the 150 mg/L threshold, adding organic carbon for

few days increases heterotrophic bacteria counts and is effective in balancing the system.

7.13 FLOW CHARACTERISTICS AND MIXING

Excessive turbulence from aeration and water circulation devices during the initial weeks after stocking may result in shrimp deformities and mortalities. The goal during this period is to provide sufficient mixing and adequate DO without stressing shrimp. Circulation must be sufficient, however, to prevent accumulation of uneaten feed, feces, and other organic matter on the tank bottom. Otherwise, anoxic patches will deteriorate water quality. Uneaten feed also promotes development of pathogens such as *Vibrio* and *Aeromonas* (Yanong and Erlacher-Reid, 2012).

Bottoms should be stirred regularly to minimize the accumulation of organic matter, particularly during the first few weeks after stocking when shrimp are not large enough to stir the bottom. Two methods commonly used to suspend settled particles are short periods of increased air and/or water flow and manually stirring the bottom near dead zones.

When the 40 m³ nursery raceways are stocked with relatively large PL (>2 mg) of uniform size (5%–10% CV), air supply and water circulation (airlift pumps, air diffusers, Venturi injectors) are operated at their maximum capacity for 5–10 min in the morning during the first week. During the second week, this is done twice-daily (e.g., morning and afternoon) for about 15 min. Air and water flow then is gradually increased over time to keep organic particles in suspension and maintain adequate DO.

Curved rostra and deformed tails most often suggest infection with the IHHN (Infectious Hypothermal and Hematopoietic Necrosis Virus), but such deformities also are caused by mechanical damage to small PL (see Section 12.1). Gradual increase in mixing minimizes broken appendages, curved rostra, and deformed tails in small PL, thus improving their overall health and survival.

If tanks are stocked with PL ≤ 1 mg or if size variation is high (>30% CV), install a 500-micron sleeve on the pump intake to avoid sucking animals through filter screens (Fig. 7.7 see also Video # 22 and # 23). Gently clean these with a brush to remove molts and other particulate matter. To reduce clogging, mount an aeration ring on

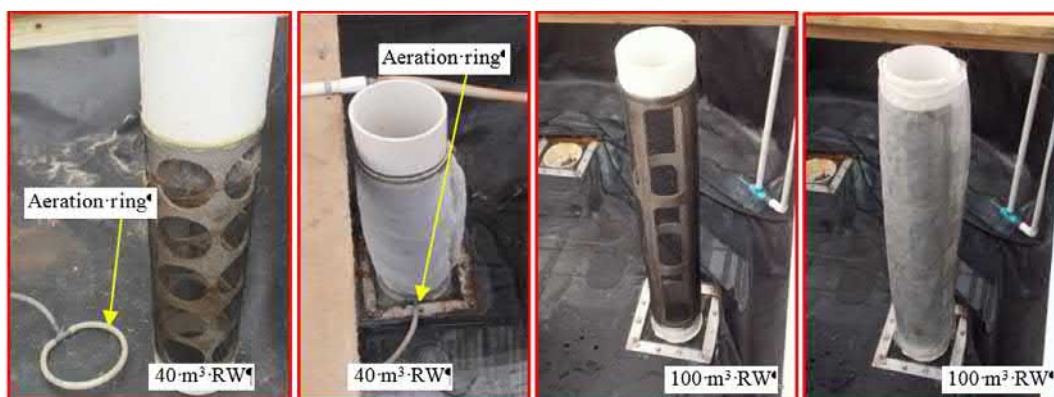


FIG. 7.7 Filter screens surrounding the pump intake standpipe of two systems to prevent entrapment of PL. An aeration ring mounted at the base of the pump intake of the 40 m³ raceway (left) aids screen cleaning (the opening at the top prevents damage to PL and cavitation).

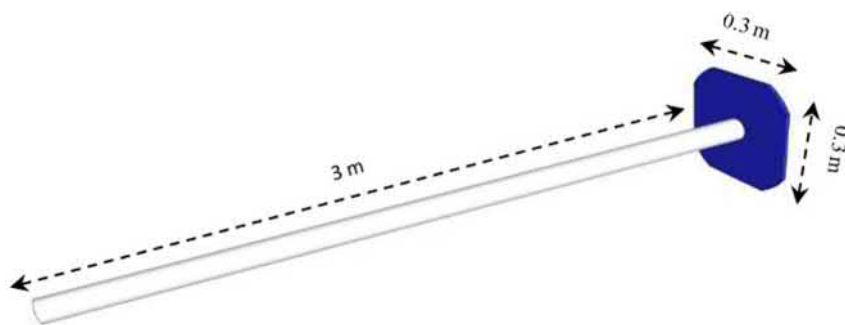


FIG. 7.8 Bottom and biofloc PVC mixing tool.



FIG. 7.9 Mixing a raceway manually. Note the uneven distribution of biofloc on the surface.

the bottom of the intake to create an air curtain. The rising bubbles help keep the screen clean.

Good tank design reduces the need to manually stir the tank bottom, but when manual

mixing is needed, a small-diameter pole with an attached plastic plate can be used (Figs. 7.8 and 7.9). The mixer is a 3-m (9.8-ft), 40-mm (1.5-in) diameter PVC Schedule 40 pipe with a square (30 × 30 cm) 0.5-cm thick PVC plate at one end. The plate has rounded corners to avoid damaging the liner (Fig. 7.8).

Manual mixing is necessary where feed and other debris tends to accumulate. Hard-to-reach areas are stirred at least twice a week. This may require entering the tank. Depending on tank design, some manual mixing may be required throughout the production cycle if dead zones continually develop.

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Nursery Phase

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8.1 BROODSTOCK AND POSTLARVAE SELECTION

Over the last two decades, most commercial hatcheries have moved away from wild shrimp breeding populations in favor of captive populations bred to be free of specific viral pathogens (SPF) and diseases. A big push to use captive breeding populations came when wild shrimp were found to carry pathogenic viruses that resulted in major financial losses. Pioneering research of the USDA—US Marine Shrimp Farming Program, of which Texas A&M-AgriLife Research Mariculture Lab (ARML) was a part, led to the development of SPF, Taura-resistant, and fast-growth breeding lines. With the increase in demand for SPF populations, more breeding centers developed their own genetic improvement programs. To have a competitive edge, commercial hatcheries will not supply their ordinary customers with seed stock from pure genetic lines. In most cases, clients will be supplied with postlarvae (PL) produced by hybridization of pure genetic lines. This practice attempts to reduce reuse by competitors of offspring as breeding populations.

The Texas A&M-ARML used PL produced from pure fast-growth lines, pure Taura-Resistant

lines, and hybrids of the two. Preliminary studies at high stocking densities with no water exchange (see [Chapter 14](#)) suggested a negative correlation in growth between Fast-Growth and Taura-Resistant lines. There was a significant difference in growth between juveniles from pure Taura-Resistant lines (1.6 g/wk.) and pure Fast-Growth lines (2.1 g/wk.) at high densities. Nevertheless, there were no such differences in growth when juveniles produced from hybrids of the two were used.

Although preliminary work at the Oceanic Institute, Hawaii, suggested a negative correlation between survival and growth in the two lines (e.g., better survival and reduced growth of the Taura-Resistant lines), later work suggested better growth in the Taura-Resistant line (Jim Wyban, personal communication). Similarly, in lab challenges, there was no significant difference in growth or survival between the two ([Wyban, 2012](#)). As growth rates significantly affect economic viability, using genetically improved PL is recommended.

Producers must comply with state regulations regarding selection of a PL supplier. For example, in Texas, production facilities close to the sea are required to use certified viral pathogen-free PL when working with Pacific White Shrimp.

Other important factors when purchasing PL are age, size, and gill-development stage. The average weight of PL at a given age is significantly affected by larval diet. For example, a well-nourished 2-mg postlarva might be 4 days old, but the same size postlarva from another hatchery might be 10 days old.

Facilities with low salinity water generally do not begin PL acclimation to low salinity water before PL12, a point at which gills are well developed. Salinity tolerance of Pacific White Shrimp larvae increases with age (Samocha et al., 1998). Producers in low-salinity areas sometimes prefer older PL because of their greater salinity tolerance and better performance.

Producers should make every effort to purchase PL of a uniform size. The coefficient of variation (CV), calculated by dividing the sample standard deviation by the average weight and expressed as a percent, should be no greater than 10%.

There are conversion tables that relate length to weight (see Shrimp PL Age and Length Page #406—Appendix VII). Measuring weight is better than measuring length because the latter requires more handling. Assuming the shipped PL are of the same age, a random sample of 100 is adequate to determine the CV. If PL are of different age groups, then determine the CV for each batch.

Commercial hatcheries often supply uniformly sized PL, grading them in rearing tanks. These PL are more expensive because of the

extra cost associated with holding the PL for longer period before the grading can be done. The following grading description is based on information from Mr. Jorge Cordova, General Manager, Naturisa, Ecuador.

A shorter production cycle (e.g., faster growth) with better profit is possible with larger juveniles of a uniform size than with smaller juveniles with high size variation. For this reason, most shrimp producers prefer to stock PL that have been graded in the hatchery. Grading generally takes place when PL reach about 4.4 mg (~230 PL/g).

The most common method for separating large from small PL involves scooping them from the larval rearing tank and placing them in a bucket (Fig. 8.1A and B) for transfer to a cage inside a larger tank with a pure oxygen supply (Fig. 8.1C). Small PL swim toward the walls and into the housing tank where they are collected (Fig. 8.1D). These are transferred to another tank (Fig. 8.1E). Separation is accelerated by slowly moving the cage. Selecting an appropriate mesh size involves trial and error. Openings of 3, 5, and 8 mm are used successfully, depending on PL sizes.

Larger PL that remain in the strainer are transferred to another tank; the smaller generally are returned to the larval rearing tank to grow for a few more days before a second grading. PL remaining after the second grading (about 10%–12% of the original population) are discarded (because of their projected poor



FIG. 8.1 Postlarvae grading from a larval rearing tank (A), transfer into a bucket (B), placement inside a cage in a tank with pure oxygen supply (C), collection of the small PL from outside the cage (D), and transfer into a new tank (E). (Photos by Jorge Cordova, Naturisa, Ecuador. Used with permission.)

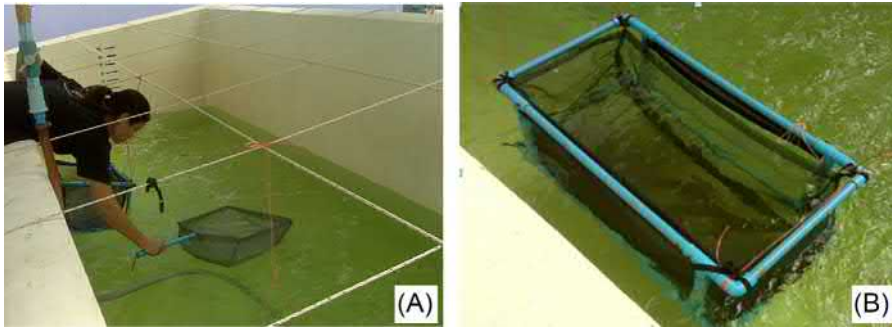


FIG. 8.2 In-tank PL separation. (A) collecting PL with a dip net from the larval rearing tank (C) and transfer into a floating cage made from netting with a mesh size that allows small PL to pass back into the tank. (Photos by Jorge Cordova, Naturisa, Ecuador. Used with permission.)

growth performance in grow-out) or sold at a reduced price.

Another commonly used separation method involves collecting PL from the larval rearing tank (Fig. 8.2A) and transferring them to a floating cage inside the same tank. The mesh is large enough to allow smaller PL to swim back into the rearing tank (Figs. 8.2B and 8.3).

Stocking PL with a large size variation reduces nursery and grow-out performance. Such cohorts require at least weekly monitoring

of their weight frequency distribution to establish the appropriate feed particle sizes in daily rations (see Section 8.4).

Uniform-sized PL improve economic return, but many US pond producers do not demand PL size uniformity. Short-term savings (lower prices and shipping) may drive this preference for nongraded PL. Owing to lower demand and the higher cost of grading, US hatcheries currently (in 2018) do not offer graded PL. To improve performance, small producers can

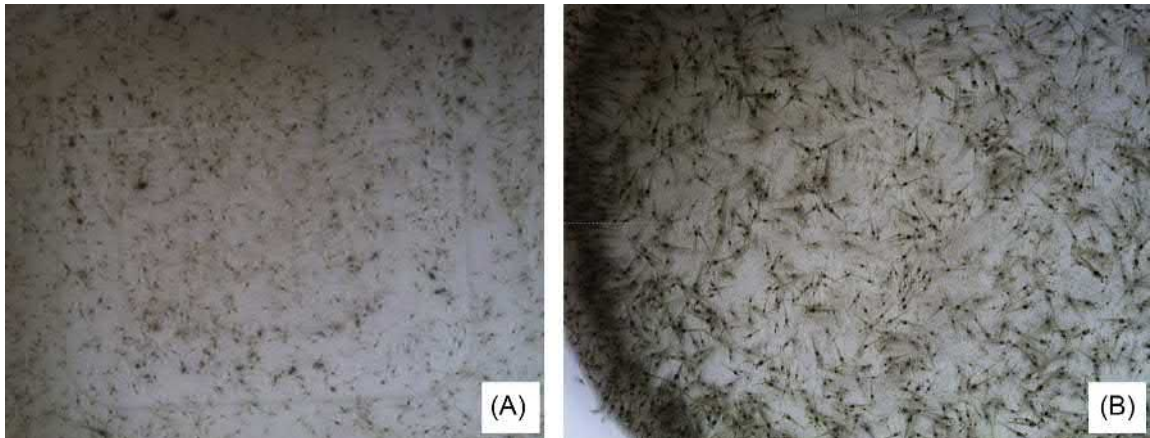


FIG. 8.3 Smaller postlarvae (A) remaining after removal of larger postlarvae (B) from the same larval rearing tank. (Photos by Jorge Cordova, Naturisa, Ecuador. Used with permission.)

hold PL shipments in small tanks for on-site grading.

8.2 POSTLARVAE TRANSPORT AND DELIVERY

Depending on distance, location, and quantity, PL are shipped in bags or hauling tanks. The most common method uses plastic bags filled with chilled water and inflated with pure oxygen. These are placed in cardboard boxes when water temperature during transport is not expected to change greatly; otherwise, they are packed in Styrofoam boxes (Fig. 8.4). To avoid low DO from oxygen leakage, most shippers use double plastic bags.

Stocking density in shipping bags is determined by water temperature, transport duration, and PL size. At 16°C to 18°C and a transport time of 24 h, bags are filled with about 10 L of seawater stocked with 1000 PL10–12/L.

The other common transport method uses hauling tanks (insulated or noninsulated) to ship large quantities (10–60 million) over long distances. Bags and hauling tanks are transported by ground or air, whichever is more cost effective and less stressful for the PL.

If water temperature is expected to increase above 23°C during transport, small quantities

of freshly hatched *Artemia* nauplii may be added to reduce cannibalism of newly molted PL. Only small quantities should be provided, however, as feeding increases ammonia. Some hatcheries accommodate customers working at low salinity by reducing shipping salinity to 2 ppt, but most hatcheries prefer to ship at around 30 ppt.

8.3 ACCLIMATION AND STOCKING

Postlarvae may be stocked directly into grow-out ponds, but many farmers report better returns when stocking nursery-reared juveniles because of compensatory growth and the hardier shrimp produced in nursery tanks. This is also true for shrimp in super-intensive systems.

Pacific White Shrimp grow well over a wide range of salinities, but a well-executed acclimation procedure that adjusts PL to local conditions reduces physiological stress. This is especially true when PL are raised in salinities beyond their optimal range. Slower acclimation also adjusts PL to local pH and temperature conditions.

Every facility must have an acclimation protocol based on its local conditions. Newly arrived PL are stocked following adequate acclimation to the salinity, ionic composition, pH, temperature, and DO of nursery tanks



FIG. 8.4 Shipping postlarvae in oxygen-inflated plastic bags (A) and packed in Styrofoam boxes (B). (Left photo, Leandro Castro. Used with permission.)

(see following for additional info). The greater the difference between shipping and nursery water, the longer the acclimation.

For PL transported in hauling tanks, a system must be in place for easy transfer to the nursery. When the salinity of the two is similar, acclimation can be done in the hauling tanks: Water is pumped from the nursery tank into the hauling tank, and water from the hauling tank is drained by gravity into the nursery tank until salinities are equal. Fig. 8.5 shows acclimation in small hauling tanks and hoses used for transferring PL to nursery tanks.

If it cannot be done in the hauling tank, then acclimation is done in dedicated acclimation

tanks (Fig. 8.6). These vary in shape and construction material (concrete, PVC, HDPE, fiberglass), with their size determined by acclimation time and the amount of water added to be processed. (A tank that is too small requires repeated draining of excess water).

Postlarvae density is 100–3000/L, depending on expected duration of the process. Positioning acclimation tanks equipped with a bottom drain above nursery tanks facilitates transfer when acclimation is completed. Acclimation tanks must have oxygen to maintain DO and air to provide adequate mixing.

The following steps are involved in processing PL shipments:

1. Check water color in the bags. Turbid or yellow-tinted water indicates poor water quality.
2. Measure DO, temperature, pH, and salinity in shipping water upon arrival.
3. Sample shipping water to measure ammonia. This is especially important when mortality or stressed PL are found.
4. Inspect shipping bags for water and oxygen leaks. Any problems, such as deflated bags, should be noted and handled promptly.
5. Inspect PL for stress (white to opaque color), mortality (white color), or limited swimming activity. Dead and/or weak PL concentrate on the bottom of the shipping container. Weak PL are easily identified by



FIG. 8.5 Acclimating PLs in hauling tanks. (Photo by Leandro Castro. Used with permission.)



FIG. 8.6 Small-tank acclimation showing a hand-held monitor with multiprobe and shipping bag with PL floating in oxygenated water (A). Bags are opened, attached to the side of the tank, and provided with an oxygen and air supply for each bag (B). Water from the acclimation tank is added gradually to a shipping bag (C).

creating a gentle circular flow in the shipping container that concentrates weak PL at the center. If mortality is observed in a small number of the containers, collect at least three samples of PL from them after thorough mixing and use these to estimate transport mortality. If mortality is similar in all containers, then only take samples from representative vessels.

6. If there are no signs of stress, thoroughly mix the contents of the shipping container and collect 3 random samples of 50 to 100 PL from each for observation with dissecting and compound microscopes. If observations cannot be done immediately, store samples in a refrigerator until they can be processed. Retain all records for future analyses. Dissecting microscope observations should record the number of PL with deformities (tail, rostrum), broken first or second antennae, broken walking legs (periopods), deformed swimming legs (pleopods), broken tail-tip (telson), damaged tail-legs (uropods), broken appendices without black tips, black spots and/or lesions on the cuticle, and any PL fouling. Also examine some whole PL under a compound microscope by placing each individual in a drop of water on a microscope slide. Use fine forceps and an eye dropper to remove a few gill lamellae, periopods, and pleopods, and place them on the slide for observation under higher magnification ($100\times$ and $400\times$). The gill examination focuses on developmental stage, presence of fouling organisms (benthic algae, sessile ciliates, filamentous bacteria), and gill color (see PL Evaluation Form Page # 402 and Excel Sheet # 2—Appendix VII).
7. Measure the weight of a random sample of 100 PL. Calculate the mean, variance, and coefficient of variation. Samples can be stored in a refrigerator for later assessment.

8.3.1 Acclimation in Shipping Bags

If PL arrive in good condition with no signs of stress, bags can be emptied into acclimation tanks, left to float in acclimation tanks filled with water from nursery tanks (Fig. 8.6), or floated directly in nursery tanks.

Acclimation of PL in shipping bags should be done in a shaded area because exposure to direct sunlight increases the risk of rapid warming the relatively small volume of shipping water. This also can occur if the temperature difference between the shipping water and acclimation/nursery tank is $>8^{\circ}\text{C}$. If this occurs, pump water from nursery tanks into small acclimation tanks and add bags of ice to reduce the temperature difference between the two sources to no more than 4°C . This will ensure a more gradual temperature change.

To avoid accidental release of PL into acclimation tanks and to facilitate adding acclimation water, attach shipping bags to the tank side walls (Fig. 8.6B). Place at least one air diffuser in each bag to mix and aerate the water as soon as it is opened. This prevents PL from aggregating on the bottom of the bag.

Based on a shipping volume of 10 L, add 1 L of water from the acclimation/nursery tanks after placing the bags in the acclimation tank. For smooth acclimation, add an additional 1 L every 15 min, or every 10 min if PL show no signs of stress. If there were no problems during transport, DO in the transport water should be supersaturated. Dissolved oxygen should near the saturation level after a few liters of water have been added.

Adding small volumes of water from acclimation tanks to shipping bags gradually exposes PL to the pH, temperature, and ionic composition of the nursery tank. Prepare a data recording sheet (see PL Acclimation Data Recording Form Page # 401 and Excel Sheet # 1—Appendix VII) ahead of time to record the volume of water added and the changes in DO, temperature, pH, and salinity in each bag before and after adding new water.

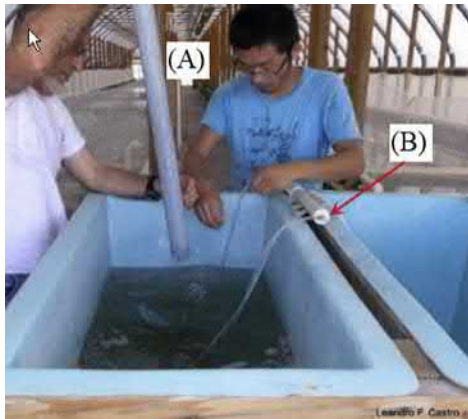


FIG. 8.7 Standpipe in acclimation tank is removed to let PL drain by gravity into the nursery tank (A), Note air supply to the acclimation tank (B).

To avoid overflow, water from the bags can be siphoned through a 350- μ m mesh strainer. Holding the strainer at an angle above the air diffuser helps prevent clogging and entry of PL into the siphon. When temperature, pH, and DO in the bag and nursery tank are similar, release PL into the nursery, preferably by gravity flow (Fig. 8.7). The acclimation time depends on the difference in water quality between the shipping and nursery water (Table 8.1).

8.3.2 Acclimation in Tanks

When the temperature difference between shipping and nursery water is $>8^{\circ}\text{C}$, acclimation is conducted in two stages. In the first stage, PL are transferred from bags to acclimation tanks. In the second, after full acclimation, they are released to the nursery. Changes in temperature of 1°C every 15–20 min, in salinity of 1 ppt every 15 to 20 min, and in pH of one unit every 1 h are suitable acclimation rates for PL10 and older (Table 8.1). Younger PL have lower osmoregulatory capacity and thus require a lower rate of change and closer observation during acclimation. Pay special attention to PL predation behavior as water temperature increases. Add

TABLE 8.1 Acclimation of Pacific White Shrimp (PL10 and Older) Based on Differences in pH, Salinity (10–40 ppt), and Temperature ($^{\circ}\text{C}$)

Differences Between Shipping and Nursery Water			Acclimation Time (min)
pH	Salinity (ppt)	Temperature ($^{\circ}\text{C}$)	
0.0	0	0	5
0.3	1	1	20
0.7	2	2	40
1.1	3	3	60
1.3	4	4	80
1.7	5	5	100
2.0	6	6	120
n/a	7	7	140
n/a	8	8	160
n/a	9	9	180
n/a	10	10	200

a small amount of feed such as live/frozen *Artemia* or crumble feed of a suitably small particle size to minimize losses.

8.3.3 Postlarvae Evaluation During Acclimation

Constantly monitor PL behavior during acclimation to identify stress factors. Place 900 mL of water from the shipping container into a 1-L glass beaker and add a 100-mL sample of PL. This should provide adequate information about the condition of PL upon arrival. Look for signs of cannibalism, molting, mortality, gut fullness, swimming activity, pigmentation, and tail muscle opacity. If stress is evident, carefully review water quality data to identify stress-causing factors (e.g., low pH or DO, high temperature, high ammonia).

An accurate shrimp count is important for subsequent water quality and feed management.

(Electronic counters are available, but these are expensive and so most likely not a good option for small producers). Our simple procedure begins by mixing the water in the acclimation tank very well before collecting a PL sample. Collect at least seven samples. Fig. 8.8 shows mixing by hand and transferring the contents of the sample cup to a larger container.

If there is reason to believe that counts provided by the hatchery are inaccurate, take samples before releasing PL into nursery tanks. In addition to the counts, review hatchery records (generally provided to customers with large orders) for the number of samples taken from PL concentration tanks and their coefficient

of variation. If the review indicates an adequate number of samples with low variation, then assume that the hatchery counts are accurate.

Because of the labor involved and the additional stress on the animals, only perform on-site counts if absolutely necessary. This is especially true because the thorough mixing required to obtain a representative samples can induce stress. Also note that water temperature impacts the accuracy of PL counts: Greater shrimp activity at higher temperature makes it more difficult to obtain a representative sample. A 20% overestimation of population size often has been observed when temperature is below 18°C compared with samples taken at 25°C (DeAnda et al., 1997). For a detailed description of the PL counting procedure, see Fig. 8.9 and the following description.

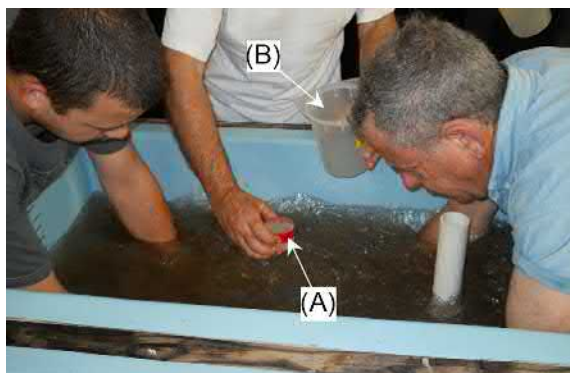


FIG. 8.8 Sampling PL in an acclimation tank. Note mixing by two people and transfer of the sample (A) to a 1-L container (B).

8.3.4 PL Sampling and Counting Method

1. After mixing, collect at least seven samples of identical volume.
2. Count PL in the first five samples, then calculate the mean, standard deviation, and coefficient of variation (CV). A white plastic teaspoon or eyedropper can be used to facilitate counts. Alternatively, PL can be counted while pouring them into a white bowl or screen (Fig. 8.9).
3. If the CV is above 10%, count the remaining two samples and calculate the new CV.

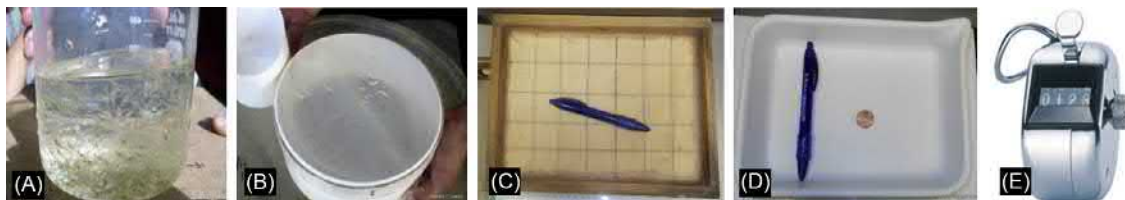


FIG. 8.9 Observation and counting of PL in samples collected from acclimation tanks or shipping bags. General observations of swimming activity, dead PL, and predation are done in a glass jar or beaker (A). Counting is done by pouring small quantities of PL on a stretched 350-μm mesh white screen (B) or framed screen with marked grid (C), or by pouring them into a flat white tray (D). Hand-held counter (E).



FIG. 8.10 Top view of PL sampling tank with bottom aeration grid.

If samples have extremely high or low counts, calculate the mean after excluding the outliers. If CV is still high, take another seven samples for better accuracy.

Postlarvae aggregate, so shipping water must be well mixed before sampling. An aeration grid (Fig. 8.10) can be used as needed to eliminate the need for manual mixing.

Use a spoutless cup (Fig. 8.11) for sampling the shipping vessel to capture 250–300 PL per sample. If PL are shipped in plastic bags with 10L of water and 1000 PL/L, the volume of the sampling cup should be about 250 mL.

Accurate measurement of the sampling cup volume reduces bias in estimating the total population. To establish the sampling cup volume, collect 10 samples from a large container filled solely with seawater in the same manner as collecting for PL counts. Submerge the cup upside down into the container and turn it over at mid-depth. As the cup fills to the top, avoid spilling the contents and transfer the sample directly into a larger cup immediately after lifting the cup from the water (Fig. 8.8). To increase accuracy, measure only the volume of samples that have intact surface tension at the time the sample is removed from the water. Measure the volume of each sample using a graduated cylinder with 1-mL increments, then calculate the average volume and SD of all ten samples.

The total number of PL in the sampled vessel is estimated from the calculated average number of PL in the collected samples, the sampling cup volume, and the total volume of the tank from which the samples were collected (see Examples 1, 2, and 3).

Scenario: A farmer ordered 50,000 PL10 to PL12 from a hatchery. Postlarvae were shipped in five plastic bags, each with 10L of water and 10,000 PL. With only five bags, samples might be taken from each bag, but because sampling is time consuming, only one is sampled.



FIG. 8.11 Spoutless sampling cups (A) compared with a regular beaker with spout (B).

EXAMPLE 1: RESULTS OF SEVEN SAMPLES WITH A 200-mL SPOUTLESS SAMPLING CUP

Sample	Count (# PL)
1	195
2	213
3	211
4	182
5	180
6 ^a	75
7 ^a	373

^aOutliers.

The average of the first five is 196.2 with a standard deviation (SD) of 15.5. The coefficient of variation (CV) is $100 \times (15.5/196.2) = 8\%$. This is less than 10%, so there is no need to count the other two samples.

The calculated average is used to estimate the number of PL in the shipping bag:

$$10,000 \times 196.2/200 = 9810$$

Assuming that the PL in all five bags were packed at the same density, the estimated total number of PL received is 49,050 (9810×5). This is in good agreement with the hatchery count.

EXAMPLE 2: RESULTS OF SEVEN SAMPLES WITH A 200-mL SPOUTLESS SAMPLING CUP

Sample	Count (# PL)
1	195
2 ^a	373
3	211
4 ^a	75
5	180
6	182
7	213

^aOutliers.

The average for the first five samples is 207 and the SD is 107, and the CV is $100 \times (107/207) = 52\%$.

Because the CV for the five first samples is greater than 10%, the other two samples are used. With all seven samples, the calculated CV is 43%. If samples 2 and 4 are discarded as outliers, the mean is 196, the standard deviation is 15.55, and the CV is only 8%.

EXAMPLE 3: SEVEN 200-mL SAMPLES WITH A SPOUTLESS SAMPLING CUP GAVE THE FOLLOWING RESULTS

Sample	Count (#PL)
1 ^a	125
2	200
3	202
4	195
5	198
6	201
7	204

^aOutlier.

The average for the first five samples is 184, the SD is 33, and the CV is $100 \times (33/184) = 18\%$. Because the CV is more than 10%, the other two samples are used. With counts from all seven samples, the calculated CV is 15%. If sample 1 is discarded as an outlier, the average is 200 and the CV is 1.6%, which is well below the 10% threshold.

8.3.5 Volumetric Method to Determine the Number of Postlarvae

The number of PL in a transport container can be estimated by volume. This may be more convenient when receiving a large shipment. The method involves passing all of the shipping water through tea strainers (Fig. 8.12) and recording the number of full strainers with PL collected from the shipping vessel. The total number of PL in a shipment then is estimated by calculating the average number per strainer (volume strained). This average should be based on counts from at least three representative strainers.



FIG. 8.12 Metal strainer for quantifying PL.

8.3.6 Stress Tests

Unless PL are obviously stressed upon arrival, perform stress tests before beginning acclimation. A simple stress test to determine hardiness consists of exposing PL (PL1 to PL7) to different concentrations of formalin and salinity (Samocha et al., 1998). The tolerance of PL to formalin increases with age, from 300 ppm at PL1 to 600 ppm at PL7.

Salinity tolerance, interpreted in terms of the salinity that results in the death of 50% of the sample population after 2 h of exposure (2 h LC50), also increases with age. Half of PL1 died at 16.8 ppt, but PL7 tolerated a much lower salinity (3 ppt) before half of them died. The 2-h LC50 increased from a salinity decrease of 11.8 ppt for PL2 to 24.9 ppt for PL7. There was no increase in 2-h LC50 between PL1 and PL2 for either low salinity or salinity decrease. Tables 8.2, 8.3, 8.4, and 8.5 present the relationship between PL age and tolerance to formalin and salinity.

These tests are appropriate for young PL (up to 7 days old). Older PL are more tolerant, so exposure times must be adjusted. Other stress tests are described in Table 8.6.

8.3.7 Microscopic Evaluation

Microscopic examination provides detailed information about the health of PL. Take a subsample of 20 to 30 PL from each acclimation tank and pour it through a 350- μ m mesh strainer to concentrate them. Dip the strainer with the PL

TABLE 8.2 Pacific White Shrimp PL Tolerance to Formalin and Low Salinity by Age

Age of PL (Days)	2-h LC50		Salinity Decrease
	Formalin (ppm)	Salinity (ppt)	
1	274	16.8	12.9
2	288	16.8	11.8
3	298	14.3	14.3
4	293	10.0	18.8
5	374	8.3	19.5
6	497	4.5	23.3
7	598	3.0	24.9

TABLE 8.3 Recommended Exposure Concentration and Expected Survival for Formalin Stress Test of PL1 to PL5 Pacific White Shrimp (n = 100)

PL Age (Days)	Recommended Exposure (ppm)	Expected Survival (%)	Confidence Interval
1	300	40	30–50
2	300	40	30–50
3	300	50	40–60
4	300	50	40–60
5	400	40	30–50
6	500	50	40–60
7	600	50	40–60

in a cold ($\pm 4^{\circ}\text{C}$) seawater bath for a few seconds to slow their swimming activity. Ice for this bath should be prepared a day or two before PL delivery by freezing nursery tank water, to reduce potential stress from reduction of salinity when using freshwater ice.

After this, PL are transferred one by one from the strainer into a single drop of cold seawater placed on a 10-cm petri dish. This is performed with an eyedropper or pipette (e.g., Pasteur pipette with large diameter tip).

TABLE 8.4 Recommended Exposure Concentration and Expected Survival for Low Salinity Stress Test of PL1 to PL5 Pacific White Shrimp ($n = 100$)

PL Age (Days)	Recommended Salinity Exposure	Expected Survival (%)	Confidence Interval
1	17	50	40–60
2	17	50	40–60
3	14	50	40–60
4	10	50	40–60
5	8	50	40–60
6	5	55	45–65
7	3	50	40–60

TABLE 8.5 Recommended Decrease and Expected Survival for Low Salinity Stress Test of PL1 to PL5 Pacific White Shrimp ($n = 100$)

PL Age (Days)	Recommended Salinity Exposure	Expected Survival (%)	Confidence Interval
1	13	50	40–60
2	13	50	40–60
3	14	50	40–60
4	19	50	40–60
5	19	55	45–65
6	23	55	45–65
7	25	50	40–60

TABLE 8.6 Pacific White Shrimp PL Stress Tests

Age	No. of PL	Stressor	Acceptable Response (% Survival)	References
PL ^a	100 in triplicate	Direct transfer into salinity of 5 ppt and water temperature of 20°C for 1 h	>60%	Villalon (1991)
PL ^a		Simultaneous drop in salinity to 20 ppt and temperature to 10°C for 4 h	80%–100%: high	Clifford (1992)
		100–150 ppm formalin for 4 h	quality; 60–79%: acceptable; <60%: reject	
PL10–12	200	Nonchlorinated drinking water for 0.5 h	>85%: strong PL; large	Nunes et al. (2004)
		Shipping water with temperature lowered by 5–8°C for 5–10 min	mortality: reject	
PL10+	300	0 ppt salinity for 0.5 h then return to original shipping salinity for 0.5 h	>75%	FAO (2003)

^a Specific PL age not given.

Postlarvae are checked individually under a dissecting scope with illumination from above and below. A dissecting needle is used to position the animals. Observations are recorded on a data sheet such as the one shown in Page # 402—Appendix VII.

Table 8.7 guides scoring of PL health based on qualitative assessment ($n \geq 20$).

Table 8.8 summarizes indications of sub-optimal conditions and suggested responses. Fig. 8.13 shows an abdomen with a half-empty gut as seen through a dissecting scope. A large

TABLE 8.7 Summary of PL Quality Assessment

Criteria	Observation	Qualitative Assessment	Score
Muscle opaqueness	Opaque muscle in tail of PL	<5%	10
		5–10%	5
		>10%	0
Deformities	Deformities in limb or head	<5%	10
		5–10%	5
		>10%	0
Gut content	Degree of fullness of digestive tract	Full	10
		Moderate	5
		Empty	0
Color of the hepatopancreas	Relative coloration of hepatopancreas	Dark	10
		Pale	5
		Transparent	0
Condition of the hepatopancreas	Relative quantity of lipid vacuoles	Abundant	10
		Moderate	5
Epibiont fouling	Degree of fouling by epibionts	<5%	10
		5–10%	5
		>10%	0
Melanization	Melanization of body or limbs	<5%	10
		5–10%	5
		>10%	0
		None	0
Intestinal peristalsis	Movement of gut muscle	High	10
		Low	5

(Modified from FAO, 2003. *Health management and biosecurity maintenance in white shrimp (Penaeus vannamei) hatcheries in Latin America*. FAO Fisheries Technical Paper no. 450. Rome, Italy, 66 pp.)

TABLE 8.8 Summary of Observations of Postlarvae and Recommended Responses

Observation	Recommended Responses
Stress signs (cannibalism, molting, mortality, gut content, limited swimming activity, pigmentation, tail muscle opaqueness)	Review water quality of transport water; improve water quality by supplying pure oxygen or increasing water exchange rate
Inaccurate count by hatchery	Review hatchery records; take aliquot samples
High size variation (CV > 10%)	Grade on-site (if feasible), adjust feed particle size, or reject shipment
Poor response to stress test	Adjust acclimation regime; or reject shipment
Poor health suggested by microscopic evaluation	Apply appropriate treatment/biosecurity measures or reject shipment
Partially or completely empty guts	Ensure adequate feed of appropriate size and attractability; review transport procedures



FIG. 8.13 Image of postlarva tail showing half-empty gut.

portion of the population of PL in a nursery tank with partially or completely empty guts indicates disease, inadequate water quality, and/or feed-related limiting factors, such as

poor attractability, inappropriate particle size, or underfeeding. Early discovery of these signs makes it easier to rectify the problem in sufficient time to save a crop.

8.4 FEED SELECTION AND FEEDING PRACTICES IN NURSERY TANKS

8.4.1 Feed and Feed Management Practices

In addition to maintaining optimal water quality (DO, salinity, pH, temperature, low ammonia and nitrite), hatchery managers also must expend significant effort on PL feed management. Feed should be formulated with high-quality ingredients, have good attractability, and be of the proper particle size. The more attention paid to these details, the higher the quality (and price) of the PL. Such PL generally are of more uniform size (length and weight), larger, and have greater stress tolerance than those produced under sub-optimal water and feed conditions. This improves growth and survival in the nursery and reduces cannibalism.

Fig. 8.14 shows two PL from a sample collected to assess growth 7 days after stocking in a nursery trial (right picture). Average weight was 4.2 mg/ind, but the high size variation illustrates the problem of selecting right particle size to accommodate PL of such different sizes.

On arrival, the average weight of a random sample of 100 of these PL was 0.94 mg/ind with

a very high CV of 60%. Individual weights varied from <0.1 to 2.3 mg.

When faced with size variation, different feed particle sizes must be provided to the same nursery cohort. This is based on the percentage of shrimp in each size category.

Determine size categories by taking up to three samples of 100 PL each from the shipment. Weigh samples individually to estimate size distribution, and divide that distribution into two or three size categories (Fig. 8.15, Page # 413 and Excel Sheet # 17—Appendix VII). This information is used with the manufacturer's feed tables to estimate the amount of each feed size to offer.

This process is repeated every two weeks; or weekly, if the size variation is high. If this is not done and only the average weight is used to determine feed size, then the feed may be too large for the smaller shrimp to consume effectively.

Individual weight sampling provides valuable information on size variability and the feed size suitable for each class, but feeding behavior also must be considered when selecting the optimal particle size.

Fig. 8.16 shows a manufacturer's feed table used to estimate daily ration based on temperature, particle size, survival, stocking density, and assumed FCR. Page # 405 (Nursery WQ



FIG. 8.14 High size variation of postlarvae in a nursery.

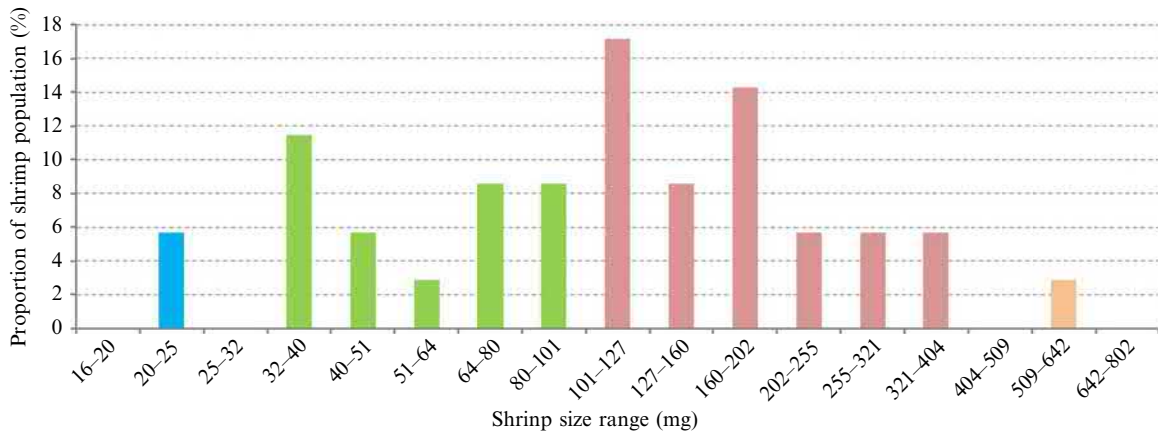


FIG. 8.15 Example of a wide size distribution in a nursery (average weight $\pm$ SD: 143 ± 118 mg/individual, CV: 83%, min: 23 mg/individual, max: 600 mg/individual). Each color represents a feed size appropriate for a size class: 6% of 0.4 to 0.6 mm, 36% of 0.6 to 8.5 mm, 56% of 1 mm, and 3% of 1.5-mm dry pellets (Zeigler Bros., Inc.).

Feed Growth FCR Electronic Data Recording Form Example & Cal and [Excel Sheet # 6](#)—Appendix VII) provides an example data recording form. The following table provides a general guideline for the transition from one particle size to another. Formulae in [Excel Sheets # 5](#) and [# 6](#) (Nursery Ration Growth FCR Survival and Nursery WQ Feed Growth FCR Electronic Data Recording Form Example & Cal—Appendix VII) can be modified to fit other densities, temperatures, survival, and FCRs.

Tags from feed bags ([Fig. 8.17](#)) provide basic feed details and traceability information that helps identify a batch's origin if a problem arises along the supply chain.

Postlarvae must be transferred from the hatchery to the nursery with as little stress as possible. Assuming that nursery water (particularly DO, temperature, pH, alkalinity, and ammonia) is satisfactory, offering newly stocked PL high-quality feed of the right size immediately will stimulate aggressive feeding. If, however, nursery conditions are suboptimal, feed consumption may not begin immediately. Any delay for more than a day, compounded by

transport and stocking stress, will have a significant negative impact on PL.

Juveniles can be transferred to outdoor ponds at different sizes (20 to 500 mg), depending on a farm's needs and the availability of nursery facilities. Stocking a grow-out system with large, healthy juveniles from a well-managed nursery enhances the likelihood of a profitable harvest ([Samocha et al., 2010](#)). Commercial producers in Ecuador documented better performance in ponds stocked with PL that have spent even a short period (a few days to a few weeks) in nurseries (Todd Blacher, personal communication). The economic benefit was far greater with PL held for a longer nursery period. Performance also was better when outdoor ponds were stocked from indoor nurseries rather than from outdoor earthen nursery ponds (Jorge Cordova, personal communication).

Juveniles were transferred to grow-out tanks at an average weight below 500 mg in a few of our nursery trials, but the average generally was above 1 g. With nursery tanks stocked at 2000–3000 PL/m³ and temperature between 28 and 30°C, PL reach about 1 g in four weeks.

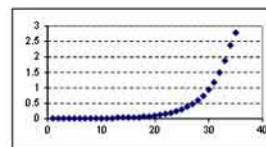
A PRODUCT OF ZEIGLER TECHNOLOGY

CHANGE ANY NUMBER IN BLUE

POND SIZE-LITERS	100,000
STOCKING DENSITY/LITER	0.540
MORTALITY/WK-%BEFORE 30 g	2.00
MORTALITY/WK-%AFTER 30 g	1.60
GAIN/WK AFTER ABOUT 4 g	2.20
FCR AFTER 3 g	1.30

MORTALITY WK-9 AFTER 30#

THIS IS ONLY A GUIDE - ADJUST TO SHRIMP BEHAVIOR



EST. TEMP.	DAY	DATE	STAGE (g)	WEIGHT (g)	GAIN (%)	GAIN (g)	SUN IV AL	NUMBER ANVLS	BIO MASS	FCR DAILY	FCR CUMULATIVE	FEED FREQUENCY	TOTAL FEED (g)	FEED D g Dry Wt.	EZ Art	Total D Wet wt	Dry Diet + (%)	Total D Dry wt (g)	TYPE	Proportion							
24.5	1	4/22/2014	PL.9	0.00094	35		1000	54,000	0.05	0.8		240	800	800	50%	148.1	50%	400	EZ ART.WR <400								
	2	4/23/2014	PL.10	0.0012	35	0.0002	970	53,800	0.06	0.8	7.09	240	1600	800	50%	148.1	50%	400	EZ ART.WR <400								
	3	4/24/2014	PL.11	0.0015	35	0.0003	967	53,200	0.08	0.8	6.002	240	2400	800	50%	150.0	50%	400	EZ ART.WR <400								
	4	4/25/2014	PL.12	0.0019	35	0.0004	964	52,001	0.10	0.8	5.26	240	3200	800	40%	159.0	41%	344	EZ ART.WR <400								
	5	4/26/2014	PL.13	0.0023	34	0.0004	962	51,992	0.12	0.8	4.46	240	4000	800	30%	159.0	41%	344	EZ ART.WR <400								
	6	4/27/2014	PL.14	0.0028	34	0.0006	959	51,784	0.14	0.8	4.14	240	4800	800	20%	99.3	30%	440	EZ ART.WR <400								
	7	4/28/2014	PL.15	0.0035	34	0.0007	956	51,636	0.16	0.8	3.66	240	5600	800	20%	99.3	30%	440	EZ ART.WR <400								
25.5	8	4/29/2014	PL.16	0.0044	34	0.0008	953	51,488	0.23	0.8	3.21	240	6400	800	10%	20.6	90%	720	EZ ART.WR <400								
	9	4/30/2014	PL.17	0.0054	34	0.0011	951	51,341	0.28	0.8	2.81	240	7200	800	0	0	10	800	EZ ART.WR <400								
	10	5/1/2014	PL.18	0.0067	34	0.0013	948	51,195	0.34	0.8	2.45	240	8000	800				900	EZ ART.WR <400								
	11	5/2/2014	PL.19	0.0082	34	0.0016	945	51,048	0.43	0.8	2.13	240	8818	818				818	EZ ART.WR <400								
	12	5/3/2014	PL.20	0.0103	34	0.0020	943	50,900	0.53	0.8	1.85	240	9828	1011	Total	981.4		1011	EZ ART.WR <400								
	13	5/4/2014	PL.21	0.0128	34	0.0023	940	50,757	0.65	0.8	1.64	240	11079	1250		EZ ART			RW 400-600	75% <400/25% 400-600							
	14	5/5/2014	PL.22	0.0159	34	0.0031	937	50,612	0.80	0.8	1.47	240	12753	1674					RW 400-600	75% <400/25% 400-600							
27.4	15	5/6/2014	PL.23	0.0200	34	0.0041	935	50,467	1.01	0.8	1.31	240	14610	2101					RW 400-600	75% <400/25% 400-600							
	16	5/7/2014	PL.24	0.0253	35	0.0052	932	50,323	1.27	0.8	1.22	240	17500	2643					RW 400-600	75% <400/25% 400-600							
	17	5/8/2014	PL.25	0.0312	35	0.0060	929	50,179	1.60	0.8	1.11	240	20002	3321					RW 400-600	75% <400/25% 400-600							
	18	5/9/2014	PL.26	0.0401	35	0.0083	927	50,036	2.01	0.8	1.06	240	24993	4172					RW 600-850	10% <400/90% 10% 400-800, 10% 600-850							
	19	5/10/2014	PL.27	0.0505	35	0.0104	924	49,898	2.52	0.8	1.01	240	30025	5242					RW 600-850	70% 400-800 30% 600-850							
	20	5/11/2014	PL.28	0.0636	35	0.0131	921	49,751	3.17	0.8	0.97	240	36821	6586					RW 600-850	70% 400-800 30% 600-850							
	21	5/12/2014	PL.29	0.0882	36	0.0165	919	49,600	3.98	0.8	0.94	240	45969	8275					RW 600-850	100% 600-850							
28.2	22	5/13/2014	PL.30	0.1103	36	0.0208	916	49,447	5.00	0.8	0.91	240	53481	10886					RW 600-850	100% 600-850 (82.7 g)							
	23	5/14/2014	PL.31	0.1373	36	0.0232	913	49,295	6.29	0.8	0.89	240	70428	14952					MP 1mm	100% 600-850 (1039 g)							
	24	5/15/2014	PL.32	0.1604	36	0.0261	911	49,194	7.89	0.9	0.90	240	88948	18462					MP 1mm								
	25	5/16/2014	PL.33	0.2001	36	0.0417	908	49,044	9.91	0.9	0.90	240	111194	23196					MP 1mm								
	26	5/17/2014	PL.34	0.2547	36	0.0524	906	48,904	12.46	0.9	0.90	240	140698	29143					MP 1mm								
	27	5/18/2014	PL.35	0.3200	36	0.0662	903	48,764	15.45	0.9	0.90	240	177803	36616					MP 1mm								
	28	5/19/2014	PL.36	0.4043	36	0.0834	900	48,623	19.66	0.9	0.91	240	223607	46004					MP 1mm								
30	29	5/20/2014	PL.37	0.5084	36	0.1091	898	48,486	24.70	0.9	0.91	240	281407	57800					MP 1mm								
	30	5/21/2014	PL.38	0.6419	36	0.1325	895	48,347	31.03	1	0.91	240	348992	75955					MP 15 mm								
	31	5/22/2014	PL.39	0.8024	35	0.1605	893	48,209	38.91	1	0.89	240	441827	92816					MP 15 mm								
	32	5/23/2014	PL.40	0.9940	34	0.1926	890	48,071	47.85	1	0.86	240	561812	116005					MP 15 mm								
	33	5/24/2014	PL.41	1.2238	32	0.2319	888	47,934	58.96	1	0.85	240	690881	129053					MP 15 mm								
	34	5/25/2014	PL.42	1.4690	22	0.2862	885	47,797	78.35	1	0.97	240	833407	142722					MP 15 mm								
	35	5/26/2014	PL.43	1.79	200	0.3664	883	47,661	95.99	1.2	0.96	240	1007600	174163					MP 15 mm								
	36	5/27/2014	PL.44	2.10	170	0.3046	880	47,524	126.12	1.01	240	1187116	173915					MP 2mm									
	37	5/28/2014	PL.45	2.41	150	0.3144	878	47,389	114.24	1.20	1.04	240	1435322	173207					MP 2mm								
	38	5/29/2014	PL.46	2.72	130	0.3134	876	47,287	128.81	1.20	1.06	240	1650807	165483					MP 2mm								
	39	5/30/2014	PL.47	3.05	120	0.3269	874	47,186	140.93	1.20	1.06	240	1728785	173958					MP 2mm								
	40	5/31/2014	PL.48	3.20	50	0.3143	872	47,085	150.33	1.30	1.15	240	1728785	-					MP 24 mm								
Total Feed (g)																				17,620							
RW <400 (g): 732																				RW 400-600 (g): 1089	RW 600-850 (g): 3,467		MP 1mm (g): 22,882	MP 1.5mm (g): 72,619	MP 2mm (g): 72,986		MP 2.4mm (g): 17,620

FIG. 8.16 Suggested daily feed rations and particle size based on water temperature, survival, stocking density, and assumed feed conversion ratio as used in a nursery trial at the Texas A&M-ARML. Suggested feeding table was provided by Zeigler Bros., Inc., Gardners, PA, US.

Average individual weight at the end of each of the first four weeks was about 80-mg, 240 mg, 500 mg, and 1 g, respectively. The 1-g size was adopted for stocking grow-out tanks partly because we found it to be a convenient standard for defining performance.

Nursery tanks at the Texas A&M-ARML were not equipped with temperature control. With shrimp stocked in early spring, production trials generally lasted six to eight weeks and juveniles ranged in size from 1 to 6 g.

Increased adoption of nursery systems by shrimp farmers over the last decade has driven

refinement of production practices. Special emphasis has been placed on selection of more nutritious and attractive feeds with optimal particle sizes for different shrimp ages and sizes. The sharp decrease in supply (and the resulting increase in price) of *Artemia* cysts over the last decade have spurred development of *Artemia* substitutes. One such product commonly used in commercial hatcheries and nurseries is EZ *Artemia* (Zeigler Bros. Inc., Gardners, PA). Commercial operators in different parts of the world report that it successfully eliminates the need for live or frozen *Artemia* nauplii in rearing shrimp larvae and PL.

ZEIGLER
SHRIMP PL 40-9
600-850 Microns with **V_{pak}**
384770-91-103

Guaranteed Analysis	Analysis	Garantizado
Crude Protein (Proteína Cruda)	Min.	40.0%
Crude Fat (Grasa Cruda)	Min.	9.0%
Crude Fiber (Fibra Cruda)	Max.	3.0%

NET WEIGHT: 20 KG. (44 LBS)

INGREDIENTS: Animal/Marine Protein Products, Processed Grain By-Products, Plant Protein Products, Grain Distillers Dried Yeast, Limestone, Soy Lecithin, Fish Oil, Dicalcium Phosphate, Magnesium Oxide, Potassium Chloride, Yeast Extract, Calcium Propionate (Preservative), Vitamin A Acetate, Vitamin D3 Supplement, dl-Alpha Tocopheryl Acetate (Vitamin E Supplement), Vitamin B12 Supplement, Riboflavin, Niacin, Calcium Pantothenate, Menadione Sodium Bisulfite Complex (source of Vitamin K Activity), Folic Acid, Thiamine Mononitrate, Pyridoxine Hydrochloride, Biotin, Manganese Protonate, Zinc Protonate, Copper Protonate, Calcium Iodate, Iron Protonate, Cobalt Protonate, Calcium Carbonate, Sodium Selenite, L-Ascorbyl-2-Polyphosphate (source of Vitamin C), Astaxanthin, Zinc Oxide.

INGREDIENTES: Productos de Proteínas de Animales y Marinos, Subproductos de Granos Procesados, Productos de Proteína de Plantas, Granos secos de Destilería, Carbonato Cálcico, Lecitina de Soya, Aceite de Pescado, Fosfato Dicalcico, Óxido de Magnesio, Cloruro de Potasio, Extracto de Levadura, Cálcico Propionato (Preservador), Acetato Vitamina A, Suplemento Vitamina D3, Suplemento Vitamina E, Suplemento de Vitamina B12, Riboflavina, Niacina, Pantotenato Cálcico, Menadiona Sódica (fuente de Vitamina K Actívada), Ácido Fólico, Tiamina Mononitrato, Clorhidrato de Piridoxina, Biotina, Protenato Manganeso, Protenato de Zinc, Protenato de Cobalto, Yodato Cálcico, Protenato de Hierro, Protenato Cobalto, Carbonato Cálcico, Sodio Selenito, Polifosfato de Ácido Ascórbico (fuente de Vitamina C), Astaxantina, Óxido de Zinc.

NO ANTIBIOTIC ADDED/NINGÚN ANTIBIÓTICO ADICIONADO

MANUFACTURED BY: ZEIGLER BROS., INC.
GARDENERS, PA 17324 (717) 677-6181 Product of USA

Lot#: 38477091-10319
Mfg. Date: 29 Apr 2015
Exp. Date: 29 Oct 2015

PLANTCODE mtime
Lot #: ~lot Bag#: ~bc

#0 **Rangen** **#0**
CONNATURAL PRODUCTS
Shrimp Production 45/10
For Cultured Crustaceans

Guaranteed Analysis	
Crude Protein (Min)	45.0%
Crude Fat (Min)	9.0%
Crude Fiber (Max)	4.0%
Phosphorous (Min)	1.0%
Ash (Max)	15.0%

Ingredient Statement

Animal Protein Products, Plant Protein Products, Processed Grain-By-Products, Squid Meal, Fish Oil, Soy Lecithin, Monosodium Phosphate, Lignin Sulfonate (binder), L-ascorbyl-2-polyphosphate (source of Vitamin C), Choline Chloride, Vitamin E Supplement, Niacin Supplement, d-Calcium Pantothenate, Riboflavin Supplement, Thiamine Mononitrate, Biotin, Pyridoxine Hydrochloride, Folic Acid, Vitamin A Supplement, Vitamin D3 Supplement, Vitamin B12 Supplement, Manganese Sulfate, Zinc Sulfate, Ferrous Sulfate, Copper Sulfate, Sodium Selenite, Potassium Iodate, Propionic Acid (Preservative), Ethoxyquin (Preservative)

Feeding Directions: Feed as the sole ration for cultured crustaceans.

Manufactured By:
Rangen Inc.
115 13th Ave. So., Buhl, Idaho 83316
Net Weight 50 LB (22.7 kg)

5311

FIG. 8.17 Typical shrimp nursery feed labels.

8.4.2 Daily Ration

The feed table in Fig. 8.16 provides recommended rations based on different water temperatures (assuming all other water-quality factors are optimal). The table provides the manufacturer's recommended feed type and sizes, along with expected shrimp growth and FCR. It is extremely important to remember that these rations are guidelines only. The actual ration should be adjusted (upward or downward) based on careful monitoring of feed consumption.

The Excel Sheets # 5 and # 6 mentioned earlier summarize data from actual nursery trials at the Texas A&M-ARML facility. Info is provided related to different feeds and particle sizes used, along with growth and FCR data. The formulae embedded in the sheet help explain the FCR calculation. An identical blank sheet is provided in which users may enter their own data to calculate FCR.

Daily nursery and grow-out rations are adjusted based on estimated population size, expected growth, FCR, water temperature, and concentrations of selected water-quality indicators (DO, ammonia, nitrite, pH, TSS, SS, and alkalinity). Rations are subject to modifications based on observations of feed consumption. Page # 403 (Excel Sheet # 3: Nursery WQ, Feed, & More_Form—Appendix VII) provides a suggested daily data recording form and a template which can be modified to fit a specific system's needs.

The amount of feed offered the first few days after stocking is purposely more than the amount consumed. This initial overfeeding ensures that there is sufficient feed to reduce PL search time and cannibalism, and also to stimulate biofloc production. The quantity of feed offered during this 2- to 3-day period generally is equal to about 100% of the total estimated biomass. At this early stage, this amount of overfeeding does

not severely deteriorate water quality in a well-mixed tank.

Based on consumption and accumulation of unconsumed feed on the tank bottom, the daily ration is reduced to 25% of estimated biomass on the third or fourth day after stocking. If uneaten feed is abundant and shrimp guts are full, daily rations are further reduced by about 1% per day to 14% per day of biomass. From that point on, the ration is reduced by 2%–3% per week until it reaches about 8% of biomass. This ration schedule is based on our experiences.

Additional adjustments are performed regularly, depending on feed consumption and uneaten feed on the bottom. This information, along with data on growth, survival, and FCR from twice-weekly sampling, is used by experienced managers to refine daily rations.

As a rule of thumb, the FCR of shrimp that weigh an average of about 0.5 g is no more than 0.5:1. That is, it requires about 0.5 kg feed to produce 1 kg of shrimp that weigh about 0.5 g each. For shrimp that weigh about 1 g, the FCR should be below 1:1, that is, a bit less than 1 kg of feed will produce about 1 kg of 1-g shrimp.

This “traditional” FCR is calculated at any point in the culture cycle as the ratio of the total amount of feed applied from stocking and the increase in biomass. This is especially true when the stocking biomass is high. Nevertheless, when stocking biomass is very low, one can calculate the FCR from the total feed offered and the harvested biomass. In addition to the overall FCR, we also use the intermittent FCR, or iFCR, which is the ratio of the amount of feed consumed since the previous sampling date to the increase in biomass from the previous sampling date. The iFCR helps determine if shrimp growth and FCR are on target (see examples in [Excel Sheets # 3–6](#)—Appendix VII).

As an example, assume that a sample of shrimp has an average weight of 0.5 g and that the immediately previous sample had an average

weight of 0.3 g. With that increase in mean individual weight of 0.2 g, and assuming that the average amount of feed consumed per shrimp between these two sampling points was 0.4 g, $iFCR = (0.4)/(0.2)$, or 2:1. This is unacceptably high. If the overall FCR is similarly high, it is assumed that the shrimp were overfed during this period and that the daily ration must be reduced. On the other hand, if the feed consumption per shrimp had been 0.1 g, the iFCR would have been 0.5:1 which is acceptable.

8.4.3 Feed Distribution and Feeding Frequencies

Besides wasting money, overfeeding has a deleterious effect on water quality and, consequently, on shrimp growth and survival. Uneaten feed consumes oxygen and leaches its nutritive value. This can occur when feeding is frequent (4 to 5 times/day) because some feed inevitably remains for an hour or two before being consumed.

This problem is reduced when feed is delivered in small portions over 24 h. This can be done with automated feeders available from a number of suppliers. Factors to consider in choosing a feeder include cost, ease of operation, capacity, and delivery interval (see [Section 5.5](#)).

The feeding regime may require modification if *Artemia* nauplii (live or frozen) or an *Artemia* replacement (such as EZ *Artemia*) are offered. Manual feeding is labor intensive, but it is preferred when water flow is so slow that it does not distribute feed uniformly. Special attention is needed to prevent accumulations of small particles. Manual feeding should be done at least four times per day.

8.4.4 Checking for Uneaten Feed and Overfeeding

When young shrimp are fed fine-particle feeds, it is difficult to distinguish between uneaten feed and feces solely by eye. As a result,

operators sometimes develop the tendency not to check carefully for uneaten feed. We use a dissecting microscope to observe settled particles more closely, but with some experience simply rubbing particles from the tank bottom between finger and thumb readily distinguishes uneaten feed from feces. Once particle size increases, this is a much quicker and easier way to identify overfeeding than using a microscope.

Because feed consumption is affected by different factors—water quality, feed quality, and molt stage, to name a few—frequent ration adjustments are important for efficient production management. When daily rations are reduced below 25% of estimated biomass, sampling the bottom at least twice a day with a fine-mesh net is needed to optimize ration sizes. When using automatic feeders, sampling focuses on areas where excess feed tends to settle: immediately below the feeders and in pockets with reduced circulation, like tank edges (see [Video # 21](#) showing the bottom of a RW after the harvest).

These daily observations, along with shrimp sampling data, help determine whether or not shrimp are overfed. Because DO decreases steadily when large amounts of uneaten feed are not quickly removed, tanks with DO monitoring systems greatly help in avoiding overfeeding.

8.5 NURSERY SHRIMP EVALUATION

8.5.1 Shrimp Sampling

Collect PL samples from the nursery tanks a few hours after stocking and place them in a clear glass/plastic container to evaluate their gut contents as a sign of active feeding. Use a small 15 × 20 cm white, fine-mesh, aquarium-type dip net, to capture and transfer PL into the observation container. [Videos # 4](#) and [# 19](#)—Appendix VIII show short underwater movie clips of PL in the 40 m³ and the 100 m³ raceways during the early nursery period.

Do this at least twice daily for the first few days. Morning observations indicate if ration adjustment is needed. For example, finding a large proportion of PL with empty guts or signs of stress requires comprehensive evaluation of feeding and water quality to identify potential problems. Because of fast gut clearance rates (on the order of a few minutes) of young PL, make these observations tank-side, rather than transferring the animals to the lab.

Collect random samples of 10 to 20 PL from different locations in the tank every day. Place them in a container with 100–200 mL of water for more thorough lab observation.

A dip net is used to concentrate collected PL into a 10-cm plastic Petri dish (we do not use the dish cover because it has shorter walls) filled to a depth of about 5 mm with water from the sampling container. Animals then are examined under a dissecting microscope. Start with the lowest magnification and proceed to higher magnifications as needed. Switching between top and bottom illumination provides better information on the condition of the PL. Adding cold water chilled with ice made from nursery water slows swimming to facilitate evaluation.

This examination observes and quantifies abnormal morphology (e.g., short or curved rostra, twisted tails, etc.), broken appendages with/without black tips (e.g., antennae, walking and swimming legs), integument fouling (e.g., attached benthic algae, filamentous bacteria, debris), black spots or lesions on the cuticle, and opaque gills.

Selected specimens are mounted on slides for additional examination under a compound microscope (see [Page # 402](#)—Appendix VII). At least weekly, observations with the compound microscope include careful examination of gill lamellae and appendages. Summarize this information for each nursery tank in terms of the proportion of PL affected by any of the indicators listed before and file these records for future reference.

8.5.2 Stress Signs

The tail tissue of healthy shrimp is clear or semitranslucent; opaque or white tails indicate severe stress. Shrimp swimming near the tank surface—especially if they appear to be lethargic—also indicate suboptimal conditions. In some cases, this indicates unfavorable water quality, such as low DO, high TSS/SS, unacceptably high water temperature, high unionized ammonia (NH_3), high nitrite (NO_2), or unsatisfactory pH (too high or too low).

Although not found in very young PL, juveniles sometimes have cramped tails. This generally takes place soon after shrimp are removed from the water. In many cases, these shrimp are so weak that they die a few hours after being returned to the tank. Although the factors responsible are not well understood, in some cases this has been associated with suboptimal growing conditions, such as temperature in excess of 31°C , nutrient deficiencies, or an unsuitable ionic composition. See [Section 12.1](#) Health Monitoring for further details.

8.6 NURSERY SHRIMP GROWTH MONITORING

Growth is evaluated twice weekly. If labor is limited, sampling may be reduced to once per week. This is essential management information, so every effort must be made to sample at least weekly. When PL are small, samples are collected with a fine-mesh rectangular dip net (home aquarium type) with a frame size of $15 \times 13\text{ cm}$. As they grow and more easily evade capture, the frame gradually is increased to $20 \times 15\text{ cm}$, and then $25 \times 18\text{ cm}$. Mesh size is increased from 1 to 2 mm, and then to 3 mm, also based on shrimp size. If size variation is high, two mesh sizes may be necessary to secure a representative sample. To further reduce bias, collect shrimp from different depths and at least three

locations in the tank. Do not include recently molted, soft-shelled shrimp because these have absorbed excess water that bias the data.

When PL are young (a few mg to 15 mg), they can be concentrated in a fine-mesh net, blotted lightly with a paper towel, and then transferred, one at a time, to a preweighed plastic container with 2–3 mm of water. For PL larger than 15 mg, it is easier to record biomass after blotting, and then counting them as they are transferred to the container. Stress is reduced by performing this procedure in an air conditioned room quickly and with minimal handling. When weighing shrimp $>0.5\text{ g}$, sampling is done adjacent to nursery tanks with a portable electronic scale. Unless air temperature in the building is controlled, this is better done during the cooler hours of the day to reduce stress.

In addition to group weights, it is important to measure individual weight. The first individual weight data are collected when PL are delivered. Weekly individual weight samples may be needed to optimize feed management in populations with high size variation. If size variation is initially low ($\text{CV} < 5\%$) but later samples show higher variation, there may be unfavorable conditions that must be corrected.

8.7 ROUTINE TASKS

Carefully observe shrimp after stocking nursery tanks and pay particular attention to water quality. Large changes in water quality are unlikely to occur until the second week poststocking, but pH, DO, and temperature must be monitored twice daily. Salinity requires only twice weekly monitoring, usually with a multiparameter meter used twice daily for other measurements.

Unless there is an algae bloom, pH will decrease gradually as biofloc develops. Salinity in biofloc systems operated with little or no water exchange increases from evaporation. Maintaining a relatively stable salinity is

important to avoid osmoregulatory stress. Temperature control is required in regions with well-defined seasons (see [Section 5.2.2](#)). Nursery DO rarely declines to low levels if the system is well designed, well managed, and its biomass capacity is not exceeded. Low DO often indicates overfeeding.

When using new seawater not seeded with nitrifying bacteria, supplemental organic carbon is added during the first few weeks. If applied in excess, this can lead to low DO. To minimize this risk, add carbon gradually and have an oxygen delivery system in place.

It is good practice to measure alkalinity, settleable solids (SS), total suspended solids (TSS), ammonia, nitrite (NO₂), and nitrate (NO₃) at least weekly. A manager's experience guides sampling frequency. Keeping a thorough record of these parameters provides useful information about the production cycle that helps troubleshoot any problems that arise. This is especially important when one is learning to manage a biofloc-dominated system.

Alkalinity is not likely to change much during the nursery phase until healthy nitrifying bacteria are established, but SS and TSS will increase noticeably because of the growing biofloc. Peaks in ammonia and nitrite often occur near the middle or the end of the nursery phase in new systems, depending on stocking density, feed supply, and the duration of the culture period.

Shrimp may be difficult to observe from outside the culture tank in the early nursery phase, even in clear water. Postlarvae samples therefore must be collected for careful observation. During the first week or two after stocking, PL tend to congregate near tank walls and at the water surface. They gradually occupy more of the water volume and the tank bottom as they grow. Daily observation of feeding and molting is easiest at these locations.

Any PL demonstrating unusual behavior or with suspect appearance are removed for

further examination. Regular microscopic examination should be done routinely, as described in [Section 8.5](#). Take pictures of normal and abnormal PL and store them for future reference. These can be done with an inexpensive camera mounted on one of the eyepieces of a dissecting or compound microscope.

Daily observations include monitoring uneaten feed and organic debris on the tank bottom. All efforts are made to identify dead zones where this material consistently collects so that they are regularly stirred to avoid anoxia (see [Section 7.13](#)).

The daily amount of feed offered is entered into an Excel spreadsheet that contains water quality and growth data. Consolidating data in one sheet provides up-to-date performance and water quality information that guides management (see examples in [Excel Sheets # 3–6—Appendix VII](#)).

[Table 8.9](#) summarizes recommended routine activities during the nursery phase of indoor super-intensive biofloc-dominated shrimp production.

TABLE 8.9 Routine Nursery Activities

Activities	Frequency			
	2/day	1/day	2/week	1/week
Measure pH, salinity, DO, temperature	X			
Measure SS, alkalinity		X	X	
Test nitrogen species, TSS		X	X	
Monitor <i>Vibrio</i>			X	
Feed consumption and adjustment	X		X	
Monitor growth			X	
Check tank bottom		X		

Continued

TABLE 8.9 Routine Nursery Activities—cont'd

Activities	Frequency			
	2/day	1/day	2/week	1/week
Manual tank mixing		X		
Increase water flow		X		
Check shrimp health		X		X
Inspect shrimp under microscope			X	
Add nitrifying bacteria ^a			X	
Add organic carbon ^b	X			
Add probiotic ^c				X
Add alkalinity and adjust pH	X			
Clean and calibrate DO probes	X			
Test backup generator			X	

^a There often is no need to add nitrifying bacteria after they have been established.

^b Continue carbon supplementation until the nitrifying bacterial population is developed. Carbon addition is based on N-input (see Section 7.5.4).

^c Probiotic additions are determined by *Vibrio* counts or manufacturer's recommendations.

Those marked with more than one frequency indicate a change as the system stabilizes and shrimp grow.

before transfer, avoid extreme changes in water quality (DO, salinity, temperature, pH, etc.) or large-volume water exchange.

8.8.1 Tank Preparations

Tank preparations are determined by harvest method. In a well-designed, large-scale operation, harvests are done with a fish pump. Otherwise, juveniles can be harvested using gravity drain, seine nets, or dip nets. Harvest can begin when the tank is filled to capacity, but most juveniles are removed when the water is lowered to about 1/3 of the working volume. Water of good quality with disease-free juveniles can be reused in other tanks.

To prevent molting or stress during harvest, DO is kept above 83% saturation (5.3 mg/L, assuming 30°C, 30 ppt, and atmospheric pressure 760 mm Hg). Depending on how the water is mixed and oxygenated, pure oxygen might be needed to maintain good DO. Uneaten feed interferes with harvest by reducing DO and making it difficult to separate feed from shrimp, so feeding should stop about 4 h before harvest. When drain harvesting, preparation includes cleaning harvest basins and making sure all valves and standpipes are in good working order.

8.8 JUVENILE TRANSFER

Prior planning ensures smooth transfer of juveniles from the nursery to grow-out tanks. The transfer should avoid mortality and minimize stress that can trigger pathogen outbreaks. For example, young shrimp molt every few days and it takes several hours after molting for soft cuticles to harden. When the cuticle is soft, shrimp are vulnerable to crowding and have a limited ability to swim. To avoid mortality of newly molted shrimp, collect samples 12 h before the transfer to determine if it can go forward or must be rescheduled. If more than 10% of the population is soft, then delay transfer for two days. To reduce the chance of mass molting

8.8.2 Equipment and Infrastructure

The number of juveniles in the nursery tank must be determined to avoid over- or understocking grow-out tanks, as both have negative effects on feed management and water quality. This is calculated from the harvested biomass and average juvenile weight. The biomass of juveniles collected in a harvest basket is measured with an electronic balance to within 10 g. Group weights are determined with an electronic balance to 0.1 g. Use a splash-proof, top-load electronic balance with remote readout for weighing plastic harvest baskets.

Weighing stations are set up near the tank before transfer (Fig. 8.18). One is for bulk



FIG. 8.18 Data recording station (A), preweighing conveyor (B) postweighing conveyor (C), and an electronic balance between the two conveyors (D) with remote display (E).

weighing and the other for weighing individuals. The electronic balance is positioned between the two 3- to 4-m long conveyors.

The first conveyor holds preweighed (tared) baskets (Fig. 8.18B); the second holds the baskets after weighing (Fig. 8.18C). All baskets are tared to the same wet weight to streamline the process. Having conveyors and balance at the same height facilitates transfer of baskets to and from the balance (Fig. 8.18D).

Every station has a table high enough to allow data recording while standing, clipboards, data recording sheets, pencils with erasers, paper towels, and two hand-held calculators. The yield-monitoring station has a sampling cup, harvest baskets, and 3-L weighing containers.

Each 3-L container has a base with a large surface area to facilitate high DO in a shallow layer of water and tall sides to prevent shrimp from jumping out. This reduces stress while shrimp wait to be counted and then are returned to the culture tank. To avoid spending too much time counting, the sampling cup holds no more than 100 juveniles. The cup size is based on the size of the harvested juveniles.

The number of baskets and weighing containers for harvest is based on expected yield, the estimated time to fill a harvest basket (assuming each basket will have no more than

6 kg of juveniles), and the basket processing time (sample collection, weighing, and emptying the basket into the grow out tank). About 10 harvest baskets are required for a 40-m³ nursery harvested with dip nets, based on a biomass of about 80 kg and 8 min to fill and process one basket. Dip-net harvesting a 100-m³ nursery with about 330 kg of juveniles, and with the same basket-processing time, requires up to 20 baskets. Video # 7 shows the use of hanging balance for weighing juveniles.

The weighing station has hand-held counters, white flat-bottom plastic bowls with a bottom area of about 300 cm² (or a wooden frame with a screen, bottom area of about 480 cm²), 20-L buckets (half numbered and half unmarked), small dip nets, and two hand-held calculators. The number of hand-held counters, dip nets, and plastic bowls/wooden frames (see Fig. 8.9) is based on the number of people available for processing samples. The numbered 20-L buckets equal the number of baskets and weighing containers required for the harvest. All 20-L buckets are filled with 500 mL of oxygenated culture water immediately before beginning the harvest.

Juveniles are transferred to the grow-out tank in perforated plastic containers that drain when lifted out of the water. Square plastic boxes can

be used, but fish baskets with lids are best because they are easily emptied (Fig. 8.19). Harvest baskets are lined with screens (Fig. 8.19A) of sufficiently small mesh (Fig. 8.19C) to prevent juveniles from passing through (Fig. 8.19B). Lining harvest baskets with 1-mm fiberglass window screening facilitates draining water during weighing without losing shrimp ranging in size from ≈ 50 mg to >3 g. All harvest baskets and weighing containers are numbered and weight calibrated. Electronic balances are used to tare the baskets or weighing containers. If juveniles can jump out of the basket, use lids when weighing (Fig. 8.19D).

8.8.3 Survival and Biomass Estimates

Average juvenile weight calculated at the beginning of a transfer often differs from averages calculated in the middle and at the end of the harvest. Adequate sampling is absolutely required to obtain a representative average of the nursery population, so a sampling cup is used to randomly sample each basket before its yield is recorded.

Numbered harvest baskets, weighing containers, and 20-L buckets are used to reduce sampling error according to the following procedure.

- A sample is collected from Basket #1 is transferred to a Weighing Container marked

#1. The weight is recorded and juveniles from that sample are placed in a 20-L bucket (marked as Bucket #1) that contains 500 mL of oxygenated water.

- A small quantity of those juveniles is captured with a dip net, moved to a counting bowl (or screened wooden frame) and counted with a hand-held counter.
- Counted juveniles then are moved to an unmarked 20-L bucket with 500 mL of oxygenated water.
- When counting is completed, the total number of juveniles from Bucket #1 is recorded and the shrimp in the unmarked bucket are transferred to the grow-out tank to be stocked.
- These two data points (sample weight and number of juveniles in the sample) are used to calculate the average weight of the shrimp in Sample #1.
- After transferring counted juveniles to the grow-out tank, both buckets (numbered and unmarked) are filled with oxygenated water in preparation for processing a new sample.

Regardless of the method used to fill the baskets, once a biomass of about 6 kg is reached, they are carried with no water and placed on the preweighing conveyor for processing. After recording biomass, the basket is removed from



FIG. 8.19 Fish basket for harvesting small juvenile shrimp (A); basket for weighing large juveniles (B); a close-up of fish basket wall lined with 1 mm net (C); a fish basket with a lid (D), and handle (E).

the balance and placed on the postweighing conveyor. When grow-out raceways are a short distance away, baskets with weighed juveniles are carried and released into the grow-out tanks. In this case, juveniles can be transferred moist. If transfer is expected to take longer (20–30 min), then harvest baskets are placed in oxygen-rich water and moved via trailer or small vehicle. To reduce stress, baskets are submerged in the water of grow-out tanks so that the juveniles can swim out.

Compute the number of juveniles collected from each nursery tank from the total harvested biomass and the average weight. Total biomass includes biomass from harvest baskets and sampling containers. Average weight is calculated from data collected from each sample (see following for details). Pages # 407 and # 408 and their templates in [Excel Sheets # 9](#) and # 10—Appendix VII are suggested forms for data recording before and during the juvenile transfer. [Table 8.10](#) presents records for a hypothetical

nursery tank with a total yield of 50.73 kg collected in 10 numbered baskets. For each of the 10 baskets, the table includes weight, number of juveniles, and computed average weight for each of the 10 samples. The overall individual average weight is 1.05 g. The total biomass in the samples was 961.5 g. This is added to the 50.73-kg yield. The estimated total number of juveniles harvested from the tank then is as follows:

$$\begin{aligned} & (50,730 \text{ g} + 961.5 \text{ g}) / 1.05 \text{ g/ind} \\ & = (51,691 \text{ g}) / 1.05 \text{ g/ind} = 49,230 \text{ individuals} \end{aligned}$$

Stocking density in the grow-out tank should be adjusted only after determining the total number of juveniles harvested from the nursery. When harvesting healthy, non- or newly molted juveniles, transfer mortality can be greatly affected by a team's experience. When juveniles show no signs of stress, our experience suggests that transfer mortality is about 5%.

TABLE 8.10 Data Sheet Recording Samples to Calculate Total Yield From a Hypothetical Nursery

Yield Recording Station					Sample Weighing and Processing Station				
Basket ID	Shrimp Weight (kg)	Cumulative Yield (kg)	Total Yield (kg)	Number of Shrimp in Tank	Weighing Container ID	Sample Weight (g)	Number of Shrimp in Sample	Sample Av. Wt. (g)	Population Av. Wt. (g)
1	5.52	5.52			1	100.5	100	1.01	
2	5.95	11.47			2	99.8	110	0.91	
3	4.73	16.2			3	89.9	95	0.95	
4	5.84	22.04			4	95.4	90	1.06	
5	5.75	27.79			5	80.5	75	1.07	
6	3.46	31.25			6	98.7	97	1.02	
7	2.73	33.98			7	105.3	99	1.06	
8	5.84	39.82			8	102.4	99	1.03	
9	4.99	44.81			9	99.1	80	1.24	
10	5.92	50.73			10	89.9	75	1.20	1.05
			51.69	49,230			961.5		

8.8.4 Transfer and Collection Options

Nursery harvests are scheduled during the cool hours of the day or at night to reduce juvenile stress from high temperature. Harvest can be done with or without a fish pump. When not used, juveniles can be collected by gravity, dip nets, seine nets, cast nets, or any combination of these.

8.8.4.1 Manual Collection and Transfer

Concentrating juveniles in 1/3 of the tank volume facilitates capture. Harvest baskets can be filled while placed on the bottom of the tank, partially submerged; or kept completely out of the water if they can be filled quickly (<4–5 min). Once filled, baskets are placed on the conveyor. Sample collection, processing, and yield recording then follow as previously described.

To avoid removing a large number of juveniles in a short period of time when drain harvesting, numbers are thinned using methods described earlier with dip nets. Tanks also are equipped with harvest basins and outlets fitted with swivel standpipes (Fig. 8.20).

Juveniles are collected by directing water from the standpipe into baskets that can be left

on the floor of the harvest basin. Baskets should be lifted above the floor at the beginning of harvest to avoid damage to the juveniles by the strong initial water flow. Adding oxygen to water in baskets should not be necessary because drained water should have adequate DO.

8.8.4.2 Harvest by Fish Pump

Fish pumps significantly reduce harvest time and juvenile stress. Because the transfer is performed in water, a dewatering device with a rack (Fig. 8.21A and B) is needed to separate juveniles for weighing and counting. Difficulty separating young juveniles from harvest water limits the individual size of juveniles that can be transferred and counted in this manner to about 1 g. Adding an electronic counter further reduces handling and provides greater accuracy in stocking.

Commercial operators can harvest more than 1200 kg of juveniles with no damage to the shrimp with an open-ended sleeve or a bag from tanks that can be drained by gravity. Ultimately, the scale of the operation dictates harvest and transfer methods.



FIG. 8.20 Harvest by swivel standpipe.



FIG. 8.21 Dewatering device (A) and close view of a dewatering rack (B) of a fish pump.

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Grow-Out Phase

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9.1 TANK PREPARATION

Removing, cleaning, and servicing belt feeders before harvest reduces tank downtime. Cleaning includes scrubbing any feed trapped on the belts. Servicing includes replacing any malfunctioning clocks, springs, and torn belts.

Grow-out preparations are similar to those for the nursery. Tanks, plumbing, and equipment must be cleaned, disinfected, and filled with suitable water. This normally begins shortly after harvest to minimize downtime. The thoroughness of cleaning and disinfection depends on the previous culture. In general,

the longer the culture period the more resistant the fouling/mineral deposits. The first step is to remove organic matter and any other growth attached to the walls and bottom, working from the shallow to the deep end. Tanks in an enclosed building can be cleaned with an electric pressure-washer. Adjust jet pressure to prevent damage to the tank liner. Scrubbing is enhanced when two people work together with one operating the pressure-washer and the other using a hard-bristle brush. The specific preparation procedures for nursery (40 m³) and grow-out systems (100 m³) at the Texas A&M-AgriLife Research Mariculture Lab (ARML) are described below.

Preparation list: 40 m³ raceways

1. Remove the pump's intake filter screen pipe (Fig. 9.1A) from the raceway and clean it using a pressure-washer and brush. Check the screen for holes and repair, if needed.
2. Remove and clean the aeration ring (Fig. 9.1C) from the bottom of the water intake filter screen pipe. If holes are clogged, submerge in 10% (v/v) muriatic acid for 10 min, rinse in freshwater, and then blow air through the ring to ensure proper functioning.

Preparation list: 100 m³ raceways

1. Remove the pump's two intake filter screen pipes (Fig. 7.7 and Fig. 10.3B) from the raceway and clean it using a pressure-washer and brush. Check the screen for holes and repair, if needed.
2. Clean water supply lines and the a³ injectors.
 - i. Turn off water supply to one of the two 5-cm PVC primary water supply pipes for the a³ injectors (Fig. 9.6A).

Continued

Preparation list: 40 m³ raceways

3. Remove the 5-cm screw cap (Fig. 9.2) from the bottom spray pipe and run clean water through it until the water coming out is clear. Completely open the bypass valve of the Venturi assembly to prevent water from going through the injector (Fig. 9.3).
4. Close water supply to the 5-cm PVC bleed valve at the shallow end of the raceway to divert maximum flow to the bottom spray pipe (Fig. 9.4).
5. Turn off the pump and replace the screw cap at the end of the bottom spray pipe. Continue to run water through the pipe at the setting described earlier until clear water flows out of all nozzles. Any blocked nozzle must be disassembled and cleaned.
6. Remove and clean all six air diffusers (Fig. 9.5) using the pressure-washer. Place air diffusers in a 0.5% bleach solution or diluted (10% v/v) muriatic acid bath for 15 min. Following treatment, transfer diffusers to a freshwater bath for another 15 min. The final step is submerging the diffusers in another freshwater bath and connecting them to a high air pressure source to remove any bleach/acid residue before reinstallation.
7. Clean the pump's filter basket.
8. Mount the aeration ring at the bottom of the pump intake filter pipe inside the raceway.

Preparation list: 100 m³ raceways

- ii. Turn off all seven valves feeding the a³ injectors receiving water from the pumps (Fig. 9.6B).
- iii. Fully open the quick-fill 5-cm PVC valve located at the end of the pipe feeding the injectors (Fig. 9.6D).
- iv. Let water flow until clear. Repeat the procedure for the valve at the end of the pipe feeding the other set of injectors. Shut both valves when done.
3. Open all valves regulating flow to the injectors on one side of the raceway. To increase pressure, close valves that control water flow to the injectors on the other wall. Check water flow; if blockage is found, disconnect and clean the injector. Repeat the same procedure for the injector on the opposite wall.
4. Clean the pumps' filter baskets.

Preparation list: 40 m³ and 100 m³ raceways

1. Clean the tank liner with the pressure-washer.
2. Check for splits and holes in the liner, and repair as needed.
3. Empty and rinse each sludge/biofloc/foam separation tank and install a new filtering cloth.
4. Empty and rinse each foam fractionator.
5. Empty and rinse each cyclone filter (Fig. 9.6A).
6. Empty and rinse each settling tank.
7. Reinstall belt feeders.
8. Remove sand or any foreign objects from the raceway drain.
9. Install the pump filter screen pipes with the required mesh size netting based on the animal size to be stocked.
10. Start refilling the tank.

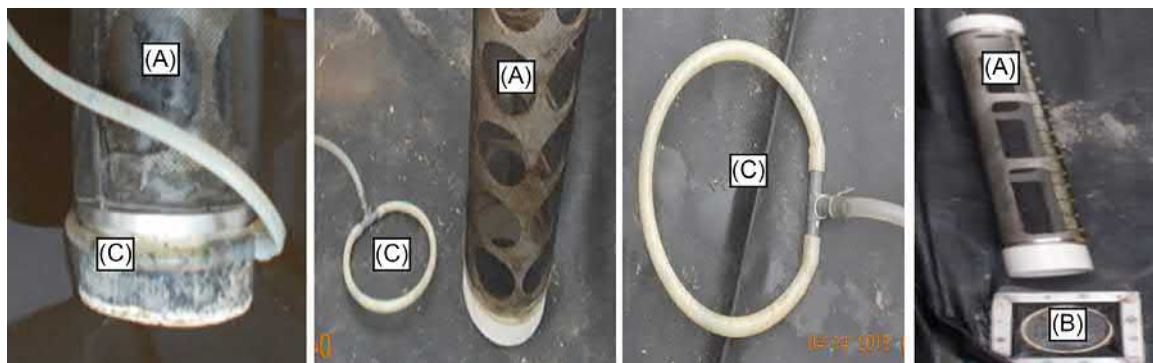


FIG. 9.1 Pump intake filter screen pipe (A), pump intake (B), and aeration ring (C).



FIG. 9.2 The 5-cm PVC screw cap of the bottom spray pipe at the raceway's deep end.

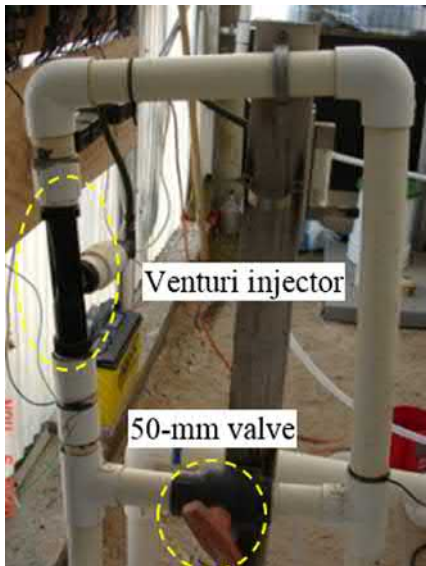


FIG. 9.3 The 5-cm PVC valve controlling water flow into the Venturi injector.

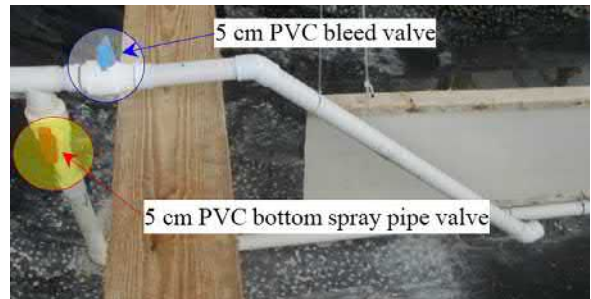


FIG. 9.4 The 5-cm bleed valve controlling water flow into the bottom spray pipe.



FIG. 9.5 An air diffuser attached to the bottom spray pipe.

9.2 STOCKING CONSIDERATIONS

Many shrimp farms have separate nursery and grow-out phases. Large farms often stock at 10–20 postlarvae (PL)/L (10,000–20,000 PL/m³) for the first phase. Smaller indoor systems stock at lower densities (2–6 PL/L or 2000–6000 PL/m³). The size at which shrimp are transferred from nursery to grow-out depends on the

producer's preference and the nursery's biomass carrying capacity.

Some producers use a three-phase system in which shrimp are harvested at a mean individual weight of 1–2 g from the primary nursery, transferred to a secondary nursery until they reach about 8–10 g, and then moved to grow-out for eventual harvest.

Stocking density is largely affected by tank carrying capacity, which depends on the ability of the system to maintain suitable water quality during production (see Table 7.5).

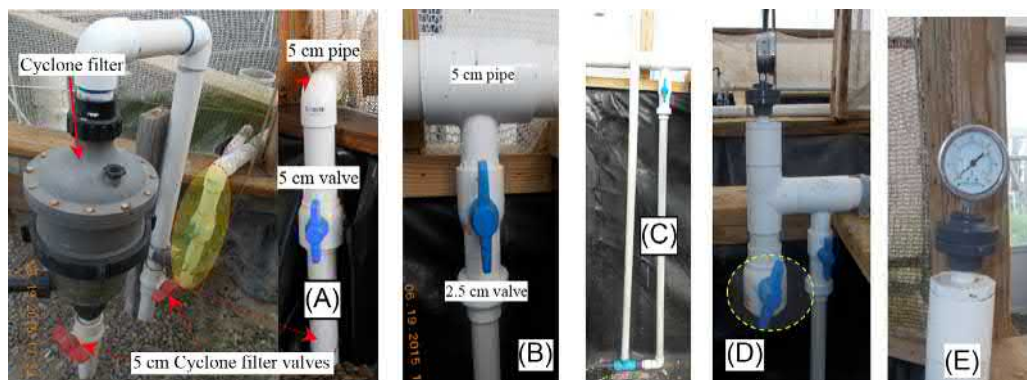


FIG. 9.6 Water supply to 100m³ raceway: 5-cm valves feeding the primary a³ injector supply pipe and the cyclone filter (A). A 2.5-cm valve controlling water flow to each a³ injector (B). The injector assembly (C). A 5-cm quick-fill valve at the end of each of the two primary water supply pipes in each raceway (D), and a pressure gage required to ensure adequate water pressure to operate the injector at maximum efficiency (E).

Following is a formula for calculating the stocking density for a grow-out tank.

$$\text{Shrimp harvest density (ind/m}^3\text{)} = \frac{\text{System carrying capacity (kg/m}^3\text{)} \times 1000}{\text{Harvest av. wt. (g/ind)}}$$

For example, assuming a tank carrying capacity of 5kg/m³ and an average of 20g/ind. at harvest, harvest density will be 250juveniles/m³. This must account for mortality during the grow-out phase. Assuming 15% mortality at harvest, the tank must be stocked at 295juveniles/m³: $(295 \text{ juveniles/m}^3) \times (1 - 0.15) \times (20 \text{ g/juvenile}) / 1000 \text{ g/kg} = 5.0 \text{ kg/m}^3$.

Juvenile stocking density also must take account of mortality during transfer from nursery to grow-out. A rule of thumb based on our experience is that if stressed juveniles are not observed during and after stocking, then transfer mortality is assumed to be 5%. This additional mortality increases the stocking density of the earlier example to 313juveniles/m³.

Knowing the number of juveniles and the stocking density in the grow-out tank is crucial for optimizing feed and water-quality

management. For relatively small, intensively managed systems, it is appropriate to report stocking density in terms of water volume rather than surface area. Studies conducted in our lab have shown that both 100 and 40m³ systems can support more than 9kg/m³. Although the 100m³ system can support this biomass with only atmospheric air, the 40m³ system requires atmospheric air enrichment with pure oxygen.

Stocking density in our grow-out studies varied between 270 and 530juveniles/m³, with growth from 1.2 to 2.3g/wk. Studies with low growth rates were, in most cases, traced to genetically predisposed slow-growing PL. Higher rates were obtained with PL from pure Fast-Growth genetic lines or hybrids of Fast-Growth and Taura-Resistant lines. Average growth rates as high as 2.3g/wk were measured even at 500juveniles/m³. Trials are needed to determine if hybrid juveniles can achieve improved growth at lower densities.

Although our systems have yielded more than 9kg/m³ of marketable shrimp, those new to managing super-intensive, no-exchange, biofloc-dominated systems initially should

target 5–6 kg/m³ or less. This can be increased after acquiring operating experience. Furthermore, the capacity of a particular system should be defined by its performance over several cycles.

If transfer of juveniles to grow-out is done properly, shrimp will not be stressed. If, however, stressed shrimp are found during or after stocking, immediate action is needed to identify and correct the problem. Because inaccurate estimation of survival negatively affects water quality and profitability, the grow-out tank must be monitored closely for mortality during the first week. Close monitoring is especially important during this period because of the difficulty in identifying dead young juveniles.

9.3 FEED SELECTION, PARTICLE SIZE, TRANSPORT, STORAGE, AND FEEDING PRACTICES

9.3.1 Feed Selection

Feed for super-intensive systems is formulated with nutrient-dense ingredients supplemented with vitamins, trace minerals, and probiotics. These feeds are more expensive than those used in shrimp ponds. Grow-out studies with commercial feeds suggest that higher nutrient density supports higher growth and lower FCRs. Even at density (500/m³), growth and FCR are better when shrimp are fed a more complete feed than one developed for extensive outdoor ponds (see [Table 14.17](#) and [Braga et al., 2016](#)).

Shrimp actively feed on biofloc (see [Video # 26](#)—Appendix VIII) as a supplemental food source. There is, however, little evidence that biofloc reduces the need for formulated feeds or that it satisfies nutritional deficiencies of a feed, especially at high density. Biofloc improves growth rates and reduces FCR when feed is properly managed. In contrast, feed cost may be reduced in high-density biofloc-dominated outdoor ponds by substituting part

of the high-protein feeds with lower protein feeds, with or without carbon supplementation.

Feed typically represents 60%–65% of production expenses. Prices are determined by nutrient quality and quantity, and the higher quality feed that drives our system is more expensive. Not all super-intensive systems are equivalent, so different feeds need to be evaluated for each system. This includes data on weekly growth, FCR, and survival, as well as an assessment of feeding higher cost feed on some days and lower cost feed on others. This information, along with prices, determines the most cost-effective feed program for a system. But because quality plays such a significant role in performance, feed selection should not be based on price alone.

[Fig. 9.7](#) demonstrates the comparatively small impact feed price has on the profitability of super-intensive biofloc-dominated shrimp production. Improving survival or growth rate, which is achieved with higher quality feed, has a much higher impact on Net Present Value than choosing a lower priced (lower quality) feed ([Hanson et al., 2009](#), see also [Chapter 13](#)).

Another factor with a significant impact on economic viability is the feed conversion ratio. The lower the FCR, the greater the economic viability. For most super-intensive systems it varies from 1.5 to 1.9, but an FCR as low as 1.2 is possible for marketable shrimp (>18 g) with good survival (>80%) at high stocking density (>500/m³). Furthermore, recent studies ([Samocha et al., 2015a,b](#)) have shown that juveniles (5.5–6.5 g) can be raised in 62 days with FCRs of 0.8–0.9. Additional improvements in feed formulations and management might lower FCR even more, reducing feed cost and improving water quality.

9.3.2 Feed Particle Metrics

Feed particle size and density are determined primarily by the ingredients and grind of the mix. The extrusion and pelleting processes also influence particle density. The number of pellets

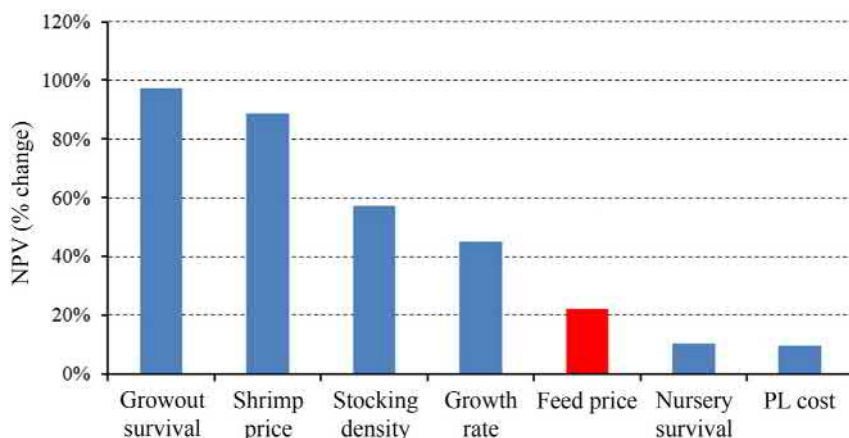


FIG. 9.7 Effect of 20% improvement in biological and price factors on 10-year Net Present Value (NPV) of a super-intensive biofloc Pacific White Shrimp production (Hanson et al., 2009).

per unit weight is influenced by pellet density and dimensions.

Pellet descriptors are not standardized. The following length criteria are used by one mill:

- Extra shortcut—less than or equal to the diameter
- Shortcut—approximately 1- to 2-times the diameter
- Regular cut—approximately 2- to 3-times the diameter
- Long cut—approximately 3- to 4-times the diameter
- Extra-long cut—5- to 6-times the diameter

The number of pellets/kg from one feed mill is as follows:

- 2.5-mm extruded regular cut: 34,000/kg
- 2.5-mm extruded long cut: 31,000/kg
- 2.5-mm extruded extra shortcut: 78,000/kg
- 2.4-mm pelleted regular cut: 27,000 pellets/kg
- 2.4-mm pelleted extra-long cut: 30,000 pellets/kg
- 2.0-mm extruded shortcut: 165,000 pellets/kg
- 1.5-mm extruded shortcut: 336,000 pellets/kg

(Tom Zeigler, Zeigler Bros., Inc., personal communication).

9.3.3 Feed Transport

Feed can be shipped in bulk containers or in bags, depending on monthly use and storage capacity. Bulk shipping is cheaper when monthly use is greater than about 20,000 kg. Facilities that use such quantities must have adequate storage silos.

Facilities with lower needs receive bags of 20 kg (44 lbs), 22.7 kg (50 lbs), or 25 kg (55 lbs) packed on pallets. Each empty pallet weighs 20 kg, with dimensions of 1.22 m × 1.02 m (48 in × 40 in). A single pallet holds a maximum of 1000 kg of bagged feed (e.g., 40 to 55 bags, depending on bag weight). When wooden pallets are used, cardboard sheets often separate the first layer of bags and the pallet to reduce potential puncturing.

Bags typically are stacked eight to ten high and covered with clear plastic shrink-wrap to prevent shifting and protect from precipitation (Fig. 9.8). A truckload (US) can carry 20,000 kg (44,000 lbs) or 800 25-kg bags. Feed mills do not recommend shipping in refrigerated containers because of condensation that occurs when bags are unloaded. Condensation, however, also occurs when hot bags are moved into a cool storage area.



FIG. 9.8 Feed bags stacked on a wooden pallet and wrapped in shrink-wrap.

9.3.4 Feed Inspection and Storage

Feed is perishable, so adequate storage and handling is required to maintain its nutritional value. Depending on ingredients and preparation, the shelf life of commercial feeds varies between 6 and 9 months when stored under optimal conditions.

Vitamin C provides an example of how ingredients can affect nutritional value. Higher levels promote better shrimp performance, but over time it degrades in storage. Its stable form (Stay C) is 80 times more stable than regular Vitamin C contained in pelleted feed stored at 23°C.

Other factors to consider are rancidity (the break-down fats or oils) and the impact of rodents, insects, and microorganisms. Any rodents or insects that infest feed not only degrade its quality but also present a biosecurity risk. Protective measures, such as close monitoring and a vigorous rodent eradication program must be in place [this is a Hazard Analysis Critical Control Point (HACCP) requirement].

Rancidity by-products can prompt shrimp to reject feed, cause off-flavor, contribute to

Vitamin E deficiency, and result in poor growth. It thus is important that manufacturers add antioxidants and use high-quality oil. Testing laboratories quantify rancidity by measuring peroxide or anisidine. Peroxide values of fresh oils are less than 10 meq/kg; at 30–40 meq/kg, rancidity is noticeable.

High temperature drives moisture out of feed and into the storage environment. If temperatures then cool, moisture condenses on the feed or the container sides. This favors mold, the mycotoxins of which are responsible for poor shrimp growth and even mortality. Feed can be checked for mycotoxin (aflatoxin and deoxynivalenol) by a testing lab or with test kits.

Secure on-site storage goes a long way in preserving feed quality. The space must be constructed to prevent ready access by birds, insects, and rodents. Feed must not be exposed to direct sunlight, high temperatures, or high humidity. If possible, store feed in a temperature-controlled room (12–18°C). This alone extends shelf life to about 6 months. When deliveries are months apart, storage in a freezer reduces major nutritional losses.

The most common and easiest storage method is to stack bags on pallets to keep them off the floor. Bags should be no more than ten layers high to prevent damage to feed in the lower bags. The space should allow easy access for a forklift to move pallets from the delivery truck.

To summarize feed storage recommendations:

1. Store feed in a cool, dry, well-ventilated area.
2. Use the oldest feed first (i.e., FIFO: first in, first out).
3. Keep at least 46 cm (18 in) between walls and stacked bags to allow air circulation and prevent wall condensation. This also facilitates cleaning and pest control.
4. Keep different feed types separated and clearly marked.
5. Remove any plastic wrapping before placing the feed in storage.
6. Rodent/insect control:
 - i. Keep storage room doors closed when not in use.

ii. Position rodent bait boxes/traps around interior and exterior walls.

iii. Collect spilled feed immediately and remove torn bags as soon as possible.

7. Minimize Handling of Bags to Reduce the Creation of Powder in the Feed

Upon receiving bagged feed:

1. Return torn, damaged, or pest-infested bags for reimbursement.
2. Spot-check tags on a representative sample of bags for any discrepancies.
3. Verify delivery of the correct feed and quantity.
4. Remove one tag from each batch (Fig. 9.9) and store in an accessible place.
5. Create a spreadsheet file with a record for each feed delivery. Enter the name of the mill, amount and number of bags, feed reference name, main ingredients (crude protein, fat, fiber, and ash), date

3/32


Rangen
CONNATURAL PRODUCTS

2.4mm

Shrimp Production 35/2.5
For Cultured Crustaceans

Guaranteed Analysis	
Crude Protein (Min).....	35.0%
Crude Fat (Min).....	8.0%
Crude Fiber (Max).....	4.0%
Phosphorus (Max).....	1.0%
Ash (Min)	15.0%

Ingredient Statement

Animal Protein Products, Plant Protein Products, Processed Grain By-Products, Squid, Fish Oil, Soy Lecithin, Poultry Fat, Monosodium Phosphate, Lignin Sulfonate (binder), L-ascorbyl-2-polyphosphate (source of Vitamin C), Choline Chloride, Vitamin E Supplement, Niacin Supplement, d-Calcium Pantothenate, Riboflavin Supplement, Thiamine Mononitrate, Biotin, Pyridoxine Hydrochloride, Folic Acid, Vitamin A Supplement, Vitamin D3 Supplement, Vitamin B12 Supplement, Manganese Sulfate, Zinc Sulfate, Ferrous Sulfate, Copper Sulfate, Sodium Selenite, Potassium Iodate, Ethoxquin (Preservative), Propionic Acid (Preservative)

Feeding Directions: Feed as the sole ration for cultured crustaceans.

Manufactured By:
Rangen Inc.
115 13th Ave. South, Buhl, Idaho 83316
Net Weight 50 LB (22.7 kg)

2513



SHRIMP GR HYPER-INTENSIVE 35
Camaron-Crecimiento Hiper-Intensivo 35
347531-71-81
EXTRA SHORT
2.4 MM (3/32")

Guaranteed Analysis		Analysis Garantizada	
Crude Protein	Min. 35.0%	Proteína Cruda	Min. 35.0%
Crude Fat	Min. 7.0%	Grasa Cruda	Min. 7.0%
Crude Fiber	Max. 2.0%	Fibra Cruda	Max. 2.0%

NET WEIGHT: 25 KG. (55 LBS)

INGREDIENTS: Grain Products, Marine Protein Products, Plant Protein Products, Processed Grain By-Products, Grain Distillers Dried Yeast, Soy Lecithin, Poultry By-Product Meal, Magnesium Oxide, Limestone, Potassium Chloride, Dicalcium Phosphate, Fish Oil, Yeast Extract, Butanoic Acid, L-Ascorbyl-2-Polyphosphate (source of Vitamin C), Calcium Propionate (Preservative), Vitamin A Acetate, Vitamin D3 Supplement, d-Alphatocopherol Acetate (Vitamin E Supplement), Vitamin B12 Supplement, Riboflavin, Niacin, Calcium Pantothenate, Manganese Sulfate, Biotin, Pyridoxine Hydrochloride (source of Vitamin B6), Folic Acid, Thiamine Mononitrate, Pyridoxine Hydrochloride, Biotin, Ascorbyl-2-Polyphosphate, L-Lysine, Cholesterol, Manganese Propionate, Zinc Propionate, Copper Propionate, Calcium Iodate, Iron Propionate, Cobalt Propionate, Calcium Carbonate, Sodium Selenite, Zinc Sulfate.

INGREDIENTES: Productos de Granos, Proteína de Productos Marinos, Productos de Proteína de Plantas, Subproductos de Granos Procesados, Granos Secos de Destilería, Lecitina de Soja, Harina de Subproductos de Pollo, Óleo de Magnesio, Carbonato Cálcico, Cloruro de Potasio, Fosfato Dicalcico, Aceite de Pescado, Extracto de Levadura, Ácido butanoico, Polifosfato de Ácido Ascórbico (fuente de Vitamina C), Calcio Propionato (preservador), Acetato de Vitamina A, Suplemento de Vitamina D3, Suplemento de Vitamina E, Suplemento de Vitamina B12, Riboflavina, Niacina, Pantoténato Cálcico, Manganese Sulfato, Biotina, Piridoxina Hidroclorada, Ácido Fólico, Tiamina Mononitrato, Carbonato de Piridoxina, Biotina, Ascorbato, L-Lisina, Colesterol, Propionato de Manganese, Propionato de Zinc, Propionato de Cobre, Yodato Cálcico, Propionato de Hierro, Propionato Cobalto, Carbonato Cálcico, Sodio Selenito, Sulfato de Zinc.

NO ANTIBIOTIC ADDED/NINGUN ANTIBIÓTICO ADICIONADO

MANUFACTURED BY: ZEIGLER BROS., INC.
GARDNERS, PA 17324 (717) 677-6181 Product of USA

Lot#: 3475317181-16119
Mfg Date: 29 Apr 2015
Exp Date: 29 Oct 2015

PLANTCODE mtime

Lot #i-lot Bag#i-bag



3475317181

FIG. 9.9 Typical feed bag labels.

manufactured, expiration date, Lot #, and code (see Fig. 9.9).

6. Open a bag from each batch and inspect for mold, rancidity, rodent feces, and insects.
7. Take a 50-g sample from one bag, label it, and store it in a cold, dry place.
8. Take a feed sample from three bags, place it in a 1-mm mesh strainer, and collect powder (fines) after sieving. If the percentage of fines is greater than 2%, report it to the feed mill for further action. Repeated high fines may suggest problems in quality control that should be discussed with the feed mill.

Indoor super-intensive facilities are unlikely to use large enough volumes of feed to require a silo, so this mode of storage is not discussed here.

9.3.5 Ration Size—Grow-Out Phase

Once shrimp are transferred to grow-out tanks, the focus is on feed management. The ration for outdoor ponds often is based on tables developed by feed manufacturers supplemented with the pond manager's experience. In most cases, rations are increased over the production cycle, but this can result in accumulation of unconsumed feed that deteriorates water quality and bottom conditions. The FCR in such ponds often is well above 2:1, indicating overfeeding. Some producers rectify this by feeding part or all of the daily ration on feed trays that are closely monitored for consumption.

Nunes (2011) provides an example of how ration changes with shrimp size (Table 9.1). Such tables provide guidelines only, as observations suggest slight overfeeding when following this schedule. It is better to calculate ration based on observed and expected shrimp performance.

Overfeeding in no-exchange, biofloc-dominated, super-intensive systems deteriorates water quality much faster and with a greater impact on shrimp performance. Changes include precipitous decrease in DO; increase in ammonia, nitrite,

TABLE 9.1 Feed Table Based on Maximum Ingestion According to Body Weight (Nunes, 2011)

Body Weight (g)	Feed Consumption (g)	Feeding Rate (% Body Weight/Day)
2	0.143	7.15
3	0.184	6.13
4	0.220	5.50
5	0.253	5.05
6	0.283	4.71
7	0.311	4.44
8	0.338	4.22
9	0.364	4.04
10	0.388	3.88
11	0.412	3.74
12	0.435	3.62
13	0.457	3.51
14	0.478	3.42
15	0.499	3.33
16	0.519	3.25
17	0.539	3.17
18	0.559	3.10
19	0.578	3.04
20	0.596	2.98

and nitrate; formation of hydrogen sulfide; growth of fungi; and proliferation of *Vibrio* and other pathogens.

Producers must optimize ration size, feeding frequency, and feed delivery to maintain a healthy growing environment. Daily ration in biofloc-dominated systems is not necessarily a simple function of shrimp size. Rations in our raceway systems are based on observed and expected performance. Among factors taken into account are expected and targeted growth rates and FCR, actual feed consumption, molt stage, observed mortality, and estimated

survival. The operator also must be aware of characteristics of the seed stock purchased from the hatchery.

The ability to predict these indicators in newly constructed systems obviously is limited by lack of performance data. If operators of new systems purchase PL from high-growth genetic lines, then these shrimp should have growth, FCR, and survival similar to those reported here. This information then can be used to calculate ration based on measured performance from several production cycles. Calculations demand good record-keeping and data, including:

1. Concentration and change of key water-quality indicators—temperature, DO, pH, salinity, alkalinity, green and yellow *Vibrio* colonies, TSS, ammonia, nitrite, and nitrate
2. Feed consumption, over- and underfeeding
3. Amount of feed provided
4. Daily and cumulative mortality
5. Molting events and intervals
6. Growth performance
7. The intermittent FCR ($iFCR = [\text{feed offered}] / [\text{biomass gained from last sampling}]$) and overall FCR ($[\text{total feed}] / [\text{biomass gained from stocking}]$).

To illustrate, assume (1) a 100-m³ grow-out raceway, (2) 50,000 juvenile shrimp of 2g average weight, (3) high-growth juveniles, (4) no transfer mortality, (5) expected individual growth of 2g/wk, (6) expected FCR of 1.4, and (7) expected mortality of 0.5%/wk. Based on these assumptions, the daily shrimp ration during the first week (100% survival) is 20 kg:

$$\begin{aligned}
 &50,000 \text{ (shrimp)} \times 2 \text{ (growth in g/wk)} \\
 &\quad \times 1.4 \text{ (iFCR g feed/g shrimp)} \\
 &\quad \times 1.00 \text{ (survival as a fraction)} \\
 &\quad / 7 \text{ (d/wk)} / 1000 \text{ (g/kg)} \\
 &= 20 \text{ kg feed/d}
 \end{aligned}$$

If those assumptions remain unchanged, the ration in the seventh week, because of the 3%

expected mortality ($0.5\% \times 6$), is 0.6 kg lower than in the first week, or 19.4 kg:

$$\begin{aligned}
 &50,000 \text{ (shrimp)} \times 2 \text{ (growth in g/wk)} \\
 &\quad \times 1.4 \text{ (iFCR in g feed/g shrimp)} \\
 &\quad \times 0.97 \text{ (survival)} / 7 \text{ (daily ration)} \\
 &\quad / 1000 \text{ (g/kg)} = 19.4 \text{ kg feed/d}
 \end{aligned}$$

If shrimp performance remains unchanged, then, based on expected survival, the ration on the thirteenth week is reduced by 6% to 18.8 kg/day. Because of these assumptions, ration calculations are not affected by average shrimp weight in this example.

The following examples demonstrate how performance indicators—such as weekly growth rates, intermittent FCR (*iFCR*), and weekly mortality—affect ration calculation.

1. *Higher than expected growth and no change in iFCR or weekly mortality.*

Assumptions: Measured growth of 2.6 g/wk and 2.8 g/wk for Weeks 1 and 2, respectively, with *iFCR* of 1.4, mortality of 0.5%/wk, and 2.7 g/wk predicted growth for Week 3.

Daily ration in Week 3 is: $50,000 \text{ (shrimp)} \times 2.7 \text{ (g/wk)} \times 1.4 \text{ (iFCR g feed/g shrimp)} \times 0.99 \text{ (survival)} / 7 \text{ (d/wk)} / 1000 \text{ (g/kg)} = 26.7 \text{ kg feed/d}$

2. *Higher than expected growth, lower iFCR, and no change in weekly mortality.*

Assumptions: For Weeks 1, 2, and 3, respectively, measured growth of 2.7, 2.8, and 2.6 g/wk; *iFCRs* of 1.3, 1.2, and 1.1; no change in weekly mortality.

Apply three-week averages for weekly growth and *iFCRs*. Daily ration in Week 4 thus is: $50,000 \text{ (shrimp)} \times 2.7 \text{ (growth in g/wk)} \times 1.2 \text{ (iFCR g feed/g shrimp)} \times 0.985 \text{ (survival)} / 7 \text{ (d/wk)} / 1000 \text{ (g/kg)} = 22.8 \text{ kg feed/d}$

3. *Lower than expected growth, an increase in iFCR, and no change in weekly mortality.*

Assumptions: For Weeks 1, 2, and 3, respectively, measured growth of 1.7, 1.6, and

1.8 g/wk; iFCRs of 1.6, 1.7, and 1.6; no change in mortality.

Daily ration in Week 4 is: $50,000 \text{ (shrimp)} \times 1.7 \text{ (growth in g/wk)} \times 1.63 \text{ (iFCR g feed/g shrimp)} \times 0.985 \text{ (survival)}/7 \text{ (d/wk)}/1000 \text{ (g/kg)} = 19.5 \text{ kg feed/d}$

4. *Lower than expected growth with increases in iFCR and weekly mortality.*

Assumptions: For Weeks 1, 2, and 3, respectively, measured growth of 1.8, 1.7, and 1.9 g/wk; iFCRs of 1.6, 1.7, and 1.6; mortality of 1, 1.5, and 1%/wk.

Daily ration in Week 4 is: $50,000 \text{ (shrimp)} \times 1.8 \text{ (growth g/wk)} \times 1.63 \text{ (iFCR g feed/g shrimp)} \times 0.965 \text{ (survival)}/7 \text{ (d/wk)}/1000 \text{ (g/kg)} = 20.2 \text{ kg feed/d}$

These examples show how rations are adjusted when performance changes from week to week. These calculations require accurate monitoring of growth, iFCR, and mortality data.

Shrimp growth (sampled twice a week), daily feed input, and estimated daily mortality from each tank are entered into a spreadsheet. Pages # 410 and # 411 and [Excel Sheets # 12–15](#) — Appendix VII provide suggested forms, templates, and electronic sheets for data entry for grow-out shrimp sampling in the two Texas A&M-ARML raceway systems. The spreadsheet files have built-in formulae that automatically compute performance indicators and daily ration. These data, along with daily mortality, form a large part of the information needed to manage production. Growth rates and iFCRs are based on data collected over two to three weeks. This extended period is needed because feed consumption and shrimp growth are greatly affected by molt stage. [Newcombe \(1945\)](#) reported that some soft crabs absorb water amounting to 70% of their total body weight prior to molting. On the other hand, anecdotal observations from our growth sampling suggest an increase of about 30% in shrimp body weight from absorbed water shortly after molting. Therefore do not include soft-shell shrimp in samples. There is a similar

bias in feed consumption data because shrimp cease feeding before molting.

A weekly mortality rate of 0.5% was assumed in ration calculations. In a population of 50,000 shrimp, this is equivalent to about 35 dead shrimp per day. Considering that some predation of newly molted shrimp will be undetected at high density ($>400/\text{m}^3$), this assumed mortality is very conservative.

Under normal conditions only a few dead shrimp are collected daily, with most going unnoticed, and expected survival at harvest generally should be greater than 90%. An increase in daily mortality or signs of *Vibrio* infection must be noted. Dead shrimp must be removed at least once or twice a day. Our experience suggests that the dead shrimp collected represent only 10–20% of the actual number.

Feed consumption also is factored into ration calculations. If uneaten feed is consistently observed during daily checks, then ration is reduced. If large numbers of shrimp gather under feeders, or if shrimp rapidly surface when feed is added, or if cannibalism are observed, then ration is increased and/or feed is distributed more uniformly.

9.3.6 Feeding

Feed management must be monitored carefully (see [Section 5.5](#) and [Section 8.4](#)) to minimize feed and water deterioration. Automatic feeders significantly improve growing conditions. In trials where shrimp were fed only four times per day, a significant DO reduction occurred shortly after feeding. Depending on temperature, TSS, dissolved organic load, and time since the previous feeding, the lowest DO occurs about an hour after feeding. In a well-designed system, DO will usually recover to near prefeeding levels after 2–3 h.

If, however, feed is added continuously with belt feeders, even at maximum daily ration (about 22 kg/d for a 100-m^3 raceway stocked at

500 shrimp/m³) there is little or no fluctuation in DO throughout the day. Beside the benefit from reduced leaching from unconsumed feed, continuous feeding also contributes to reducing predation on newly molted shrimp, and accumulation of uneaten feed. Unlike spring-loaded belt feeders, electric models can be linked to DO monitoring systems that improve management by halting feed delivery during any low-DO events.

Automatic feeders are spaced to allow uniform distribution of feed over of the tank surface. They are placed away from pump intakes to prevent fresh feed from being drawn into pumps (Fig. 9.10).

Spring-loaded 12- and 24-h belt feeders are available (see Section 5.5). The 24-h variety requires less manpower to operate, but the 12-h feeders force more frequent monitoring of feed consumption and so may reduce overfeeding. On the other hand, refilling these feeders every 12h diverts manpower from other important tasks in the early morning.

A continuous feed-delivery system connected to online DO monitoring and control sensors may be the method of choice, but their cost can be relatively high. Belt feeders and other small automatic fish feeders, on the other hand, cost only \$230–\$300 each.

Manual feeding is more labor intensive but reduces capital investment. If manual feeding is adopted, reduce the time interval between each feeding as much as possible (e.g., feed every 2–3h). Less frequent feeding requires more frequent inspection of the shrimp and tank bottom to avoid cannibalism and overfeeding. The main advantage of manual feeding is more uniform feed distribution that decreases competition for feed (Nunes and Parsons, 1999, 2000). Therefore even if automatic feeders are used, some manual feeding may be beneficial, especially when automatic feeders are being serviced.

Once the daily ration is determined, the manager must ensure that all feed is properly distributed and consumed. Periodically review the technique of workers who feed to prevent under- or overfeeding. Daily inspections should be conducted and any spilled feed removed immediately. Similarly, feed-weighing areas and feed transport routes should be kept free of spilled feed.

9.4 MONITORING SHRIMP GROWTH

9.4.1 Sample Size

Accurate growth monitoring requires representative sampling. The larger the sample, the

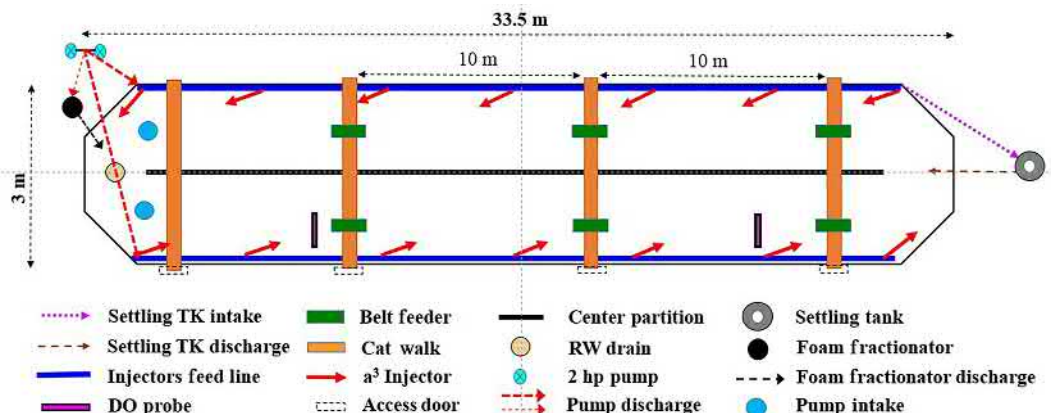


FIG. 9.10 Placement of belt feeders in a 100-m³ Texas A&M-ARML raceway.

greater the chance that it represents the population. Sampling protocol must take into account stress inflicted on the shrimp and manpower needs. In most cases, growth is determined from the group weight of a sample of 60–100 shrimp. Additional samples are taken for confirmation if results appear unreasonable, for example, if the sample indicates that shrimp have lost weight.

9.4.2 Sampling

Other than sample size, it is important to sample areas in the tank that provide an accurate representation of the overall population. Differences in depth, light intensity, flow rate, temperature, noise, feed distribution stations, among others, may result in shrimp concentrating in different areas. Sampling sometimes results in mass jumping in high-density systems. This forces a balance between minimizing shrimp stress and obtaining representative samples.

For growth sampling of juveniles (1–5 g), use a dip net with a handle long enough to reach the bottom and a mesh that minimizes sampling bias. A 1-mm mesh net underestimates 1-g juveniles because pulling it through the water

creates significant resistance that allows these larger shrimp to avoid it. Sampling the same population with 10-mm mesh overestimates them because smaller shrimp escape through the mesh.

Because of the escape response and difficulty using dip nets with very large frames, larger shrimp are sampled with cast nets, but these are difficult to use in confined spaces. [Figs. 9.11 and 9.12](#) and [Video # 9, # 10, # 11, # 24, and # 26](#)—Appendix VIII demonstrate its use in different settings. Depending on tank size, another person may be needed to facilitate sampling and recording.

Cast net mesh and diameter are selected on the basis of shrimp size and the expected number collected in a sample. Nets with 6.3-mm (2.5-in) mesh are available, but 10-mm (4-in) mesh is adequate for shrimp greater than 5 g. Density in super-intensive systems is high (>300 shrimp/m³) so, to avoid stressing a large number of shrimp in each sample, use cast nets with a diameter of 1.82–2.44 m (6–8 ft).

To minimize stress in systems without temperature control, during the hot months of the year sampling is conducted in the early morning when water and air temperatures are cooler.



FIG. 9.11 *Left and middle: Cast net used in a confined space to monitor growth in a 100-m³ tank; Right: Cast net used in an open area. (Photo by Tim Morris. Used with permission.)*



FIG. 9.12 Sampling procedure at the Texas A&M-ARML: (A) Prepare materials; (B) Tare bucket; (C) Spread the cast net.

Two people are needed to streamline the process and reduce the time required to weigh and count shrimp. To sample with a cast net (see video listed previously):

1. Level the electronic balance. Have a clean, empty bucket (with or without lid, depending on shrimp size), clipboard with data sheet, and pencil with eraser ready (Fig. 9.12A).
2. Tare empty bucket (with a lid) and place it near the sampling spot (Fig. 9.12B).
3. Prepare the net, cast it, and wait until the lead line settles on the bottom (Fig. 9.12C).
4. Pull the rope slowly and lift the net with the shrimp out of the water.
5. Empty the net into the tared bucket and cover with the lid.
6. Record the weight on a data sheet under "Total Weight" see Page # 412—Appendix VII.
7. Remove a small number of shrimp by hand or dip net (leave water, molts, debris, and dead shrimp). Count the shrimp over the tank and return to the water. Use a handheld counter to reduce errors. Record the number on the form under "Total Shrimp."
8. Weigh the residual water, molts, debris, and dead in the bucket (with the lid) and record the weight on the data sheet under "Tare."
9. Empty the bucket and tare once again.
10. Repeat the sampling process.
11. When finished, enter data into a computer spreadsheet (see Excel Sheet # 16—Appendix VII) to calculate average shrimp weight, the weight increase, and daily and weekly growth rates.

Table 9.2 provides a simplified example of data collection and processing to determine growth with samples collected from three locations. The average shrimp weight in each sample was determined after accounting for nonliving components. The average individual weight in the tank (4.73 g) is the average of the samples.

TABLE 9.2 Example of Data Collected From a Grow-Out Tank

Tank ID	Sample ID	Total Weight (g)	Total Shrimp	Tare (g)	Average Weight (g)
RW1	1	235	47	23	4.51
	2	226	41	22	4.98
	3	230	45	19	4.69
					4.73

9.5 SHRIMP EVALUATION

Counting sampled shrimp one by one provides the opportunity for detailed observation of their condition. Closely observe those in the first sample. If a large number are postmolt (soft), then delay further sampling to minimize mortality.

Also look for cramping; white or opaque tails; eyes with signs of abrasions or white spots; cuticle lesions or melanization (darkening); muscle necrosis (dead tissue); fouling (attached organisms); black/brown gills; broken or damaged antennae, walking legs, and swimming legs; or other abnormalities. [Fig. 9.13](#) shows shrimp with some of the signs described before. A large number of shrimp with any of these conditions merits a review of culture conditions.

[Fig. 9.14](#) provides an example of targeted feeding activities: The low gut content of individual (1) suggests poor feed consumption, while the full gut of individual (2) suggests aggressive feeding. [Video # 8](#) in Appendix VIII shows juveniles with full guts and intact antennae.

9.6 ROUTINE TASKS

Routine tasks must be clearly understood by staff and meticulously followed. [Table 9.3](#) lists

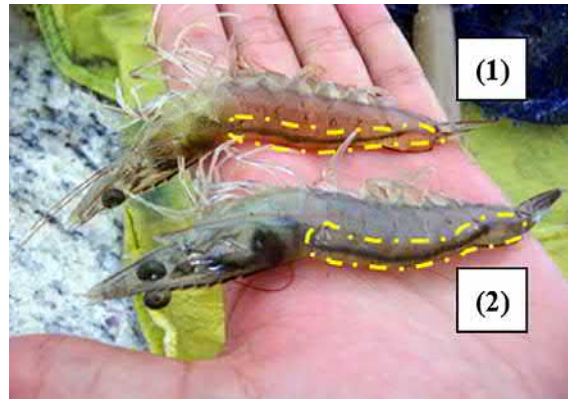


FIG. 9.14 Shrimp with suboptimal (1) and optimal (2) gut fullness.

some of these activities for the Texas A&M-ARML grow-out raceways.

For grow-out systems without inline monitoring of DO, pH, and temperature, the daily routine starts with a quick review of these parameters by the grow-out supervisor. A multiprobe with a salinity sensor costs more than the three-probe model, but a unit with all four probes saves considerable time. Because collecting water-quality data is so time consuming, a multiparameter meter that transfers data to a computer is particularly useful. Transfer usually is via cable connector, but more expensive units have wireless data transfer.

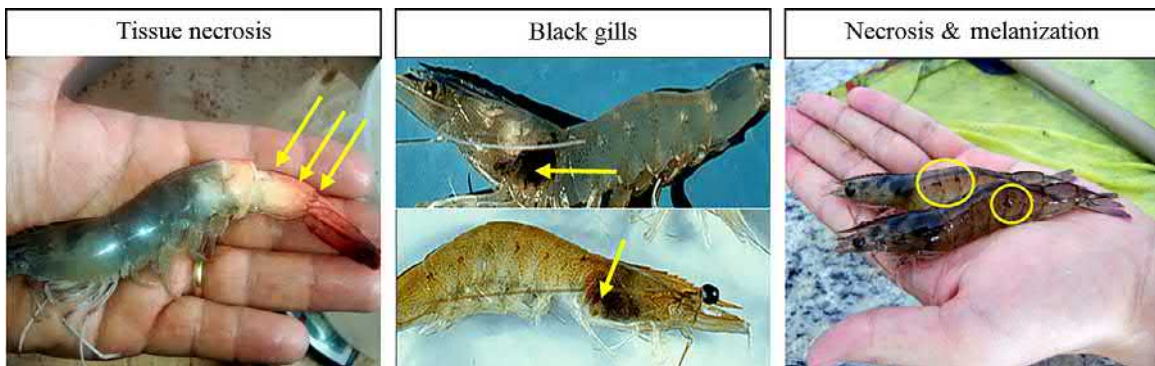


FIG. 9.13 Shrimp with signs that indicate culture problems.

TABLE 9.3 Routine Tasks Associated With Managing Grow-Out Raceways

Order	Tasks	Start Time ^a	Responsibility	Recommended Action
1	Monitor DO, pH, temperature and salinity in all tanks and upload data to a computer file	1–2h before beginning the workday (e.g., 6:00–8:00 a.m.)	Night-shift workers	Immediately notify grow-out supervisor of any alarming readings and follow emergency remediation procedures
2	Quick review of a.m. WQ data	Beginning of workday	Grow-out supervisor	Assign workers to execute preestablished protocols and long-term solutions
3	In-depth review of early a.m. and previous WQ data; focus on problem tanks	Following initial review of the WQ (e.g., about 9:00 a.m.)	Grow-out supervisor	Modify feed management for problem tanks; order additional WQ testing as needed
4	General visual inspection	Beginning of workday	Day-shift workers	Note activity, mortality, molting, floating biofloc; notify supervisor of unusual signs
5	Check for uneaten feed and unusual shrimp signs; Perform general tank husbandry	After finishing initial inspections	Assigned workers	Report tanks with uneaten feed and/or shrimp with alarming signs to supervisor; as needed, disperse biofloc mats, remove and quantify molts and dead shrimp; clean and adjust water flow to foam fractionators and settling tanks; enter data into computer; notify supervisor of anything unusual
6	Collect water early a.m. for testing; adjust WQ; clean and refill feeders	When problem tanks identified or after evaluation and feed consumption	Assigned workers	Make any adjustments based on results of WQ analyses and supervisor's instructions
7	Enter all new data into computer	2–3h before end of workday or when available	Assigned worker	Collect all written records and enter data into computer spreadsheet
8	Monitor DO, pH, temperature, and salinity and upload to computer	Mid afternoon	Assigned worker	Immediately notify supervisor of out-of-range parameters and follow remediation procedures
9	Review afternoon WQ data, identify problem tanks	Mid afternoon	Grow-out supervisor	Instruct workers to make any required adjustments
10	Visually inspect all tanks	Late afternoon	Assigned workers	Make any required modifications
11	Briefing of the night shift	Late afternoon	Supervisor and workers	Prepare list of tanks to watch

^a Assuming 8:00 a.m. to 5:00 p.m. workday.

Many management decisions depend on water quality, so this information is reviewed at the beginning of the workday on the morning shift. If a tank with out-of-range water quality

is identified, the supervisor immediately activates preestablished protocols to deal with the situation. If, for example, the review finds low DO, the response may include oxygen

supplementation and suspending feeding until the source of the problem is corrected. Data logged on a shift is reviewed by the person relieving that shift. This includes a verbal report of data collected and the shift's activities.

Following the initial review, the supervisor performs a more in-depth analysis of the latest information and data from the previous days or weeks (e.g., water quality, growth, molting, feed consumption, FCR, mortality, *Vibrio* counts, etc.). This can uncover long-term trends that inform management decisions. For example, low DO and recently high FCRs might draw attention to overfeeding as a factor in causing greater oxygen demand.

The grow-out team is assigned a list of daily tasks. Timing conflicts arise when refilling 12-h feeders (reloading takes time from other activities), so the list in Table 9.3 assumes that tanks have feeders with 24-h capacity.

The first task of grow-out workers is to inspect each tank. This is done by walking around each tank and recording swimming activity, molts, dead shrimp, any floating biofloc mats, and so on. All abnormal signs are reported immediately. Following that, tanks are checked for uneaten feed and shrimp condition. Feeding is halted in any tank with a significant amount of uneaten feed until further instructions from the supervisor. Tanks with a large number of stressed shrimp are reported to the supervisor for decisions about corrective actions. Workers then concentrate on general husbandry: removal and counting of dead shrimp and molts, dispersing biofloc mats, and adjusting flow rates of foam fractionators and settling tanks.

Water-quality work includes adding chemicals to adjust alkalinity and pH, supplementing organic carbon, adding nitrifying bacteria, adding freshwater to maintain salinity, culturing and applying probiotics, and analyzing TSS, alkalinity, ammonia, nitrite, nitrate, *Vibrio*, and so on.

The last activities include (1) cleaning and refilling automatic feeders; (2) monitoring and

uploading DO, temperature, pH, and salinity data; and (3) entering all daily data into computer spreadsheets. Before leaving for the day, employees visually inspect the tanks and alert their supervisor and the oncoming shift of any abnormalities. The supervisor reviews all data, makes any last-minute adjustments, and briefs the night shift.

When a crop is started with disease-free mature (reused) water, it will be of suitable quality to support high shrimp performance and active populations of nitrifying bacteria. In this case, monitor basic indicators (e.g., temperature and pH) twice a day; DO at least three times a day; SS and salinity once a day; alkalinity and TSS two to three times weekly; ammonia, nitrite, nitrate once a week; and *Vibrio* twice a week (Table 9.4).

Alternatively, if the tank is filled with mostly new water, a few weeks are necessary for it to mature. TSS monitoring frequency is the same as for matured water. Increased monitoring of DO (several times per day), nitrogen species, and alkalinity (up to daily) is required to ensure optimal concentrations of nitrifying bacteria. The timing and quantity of organic carbon additions affect DO monitoring, as high supplementation rates can lower DO at least in the short term.

9.7 PERSONNEL

Super-intensive biofloc-dominated production requires well-trained, attentive staff. The areas of responsibility include:

- General farm management
- Shrimp acclimation and stocking
- Water-quality and *Vibrio* monitoring (lab)
- Water-quality maintenance—preparation, flow, oxygenation and mixing adjustment, alkalinity, pH and solids control, pathogenic and nonpathogenic bacterial population monitoring and control

TABLE 9.4 Grow-Out Routine

Activities	Frequency			
	2/Day	1/Day	2/Week	1/Week
Check pH, salinity, DO, temperature	X			
Check SS, alkalinity		X	X	
Test nitrogen species, TSS		X	X	
Monitor <i>Vibrio</i>			X	
Check raceway bottom	X			
Feed consumption and adjustment	X			
Monitor growth			X	
Check shrimp health	X			X
Add nitrifying bacteria ^a			X	
Add organic carbon ^b	X			
Add probiotic ^c			X	
Add alkalinity and pH adjustments	X			
Clean and calibrate DO probes	X			
Test backup generator				X

^a Twice-weekly, according to water quality and shrimp performance until nitrifiers established.

^b Continue supplementation until nitrifiers are developed, carbon addition based on nitrogen input (see Section 7.5).

^c Application frequency determined by *Vibrio* counts or manufacturer's recommendations.

Activities with more than one frequency marked indicate changes in frequency based on the system and shrimp performance.

- Feed management—feeding, uneaten feed recovery, spilt feed removal, feed storage access and inventory
- Shrimp monitoring and evaluation—growth, feed intake, survival
- Shrimp health monitoring
- Harvesting and postharvest handling
- System preparation
- Waste management (water and solids)

- Equipment maintenance—pumps, blowers, generators, vehicles, electrical, sensors, alarms
- Construction and repairs
- Biosecurity
- Occupational health and safety
- Purchasing equipment and consumables
- Sales and marketing
- Research and development
- Security and predator control
- Office duties
- Janitorial duties

A worker might perform a single function in a larger facility and multiple functions in a smaller one. Where possible, staff should work in only one production section (hatchery, nursery, or grow-out) to foster biosecurity. An internal training program educates staff in essential procedures, such as biosecurity, shrimp health, worker hygiene, and safety.

Staffing must take into account the continuous operation of culture systems. Staff must be available to respond to emergencies, such as power outage and pump failure, as quickly as possible. Depending on the scale, production staff might work in two 12-h shifts while everyone else (mechanic, construction, WQ lab personnel) works 7:00 a.m. to 5:00 p.m.

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Shrimp Harvest

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10.1 PREPARATIONS

A manager must decide whether to reuse harvest tank water without treatment, with treatment, or to discard it. The decision to discard all or part of a tank's water requires thorough review of costs associated with hauling and treating raw seawater, purchasing artificial sea salt, treating effluent to meet regulatory requirements, and hauling old water to a disposal site. Limited-exchange facilities must keep these considerations in mind.

When a crop has been disease free and post-harvest water quality is acceptable, the water can be reused for a new crop or added to a tank already in production. If the postharvest water is satisfactory but the shrimp did show signs of disease, the water only can be used after treatment to destroy the infectious agent. In that case, water is pumped to a reservoir where it undergoes chlorination or other disinfection. (Water from the Texas A&M-AgriLife Research Mariculture Lab (ARML) grow-out trials usually was discarded in an evaporation pond because it did not meet discharge requirements).

Owing to construction constraints, the 40-m³ raceways were harvested manually. Fish pumps were used in the 100-m³ raceways (see the

following section). Preparations for the two systems were similar because both required about two-thirds of the tank volume to be pumped out before harvesting. Draining was done with the same pumps used for aeration. While the water level is lowered, belt feeders are removed to reduce the chance of damaging them and to create more space for harvest activities.

DO is monitored and carefully controlled while draining to prevent stress or mortality, especially when shrimp are destined for the live or fresh-on-ice markets. Unstressed shrimp have an appealing translucent appearance that they retain when dipped in ice water (Fig. 10.1A). The stress of low DO (2–3 mg/L), however, causes shrimp to become dull white (Fig. 10.1B), similar to dead shrimp, when placed in ice water. This translates to lower market value.

Preharvest preparation activities in both Texas A&M-ARML systems included:

1. Stop feeding 12h before harvest so shrimp empty their guts. This helps water quality.
2. Prepare lighting if harvest is at night or very early in the morning.
3. Place a portable table near the tank to be harvested.



FIG. 10.1 Vivid appearance of freshly chill-killed shrimp (A) compared to stressed or dead shrimp that have been chilled (B).

4. Prepare data recording sheets (see Page # 412 and [Excel Sheet # 16](#) – Appendix VII), clipboards, pencils, calculators, 1-L plastic sampling cup, 0.5-L plastic container, 3.7-L (1-gal.) zipper-sealed bags ([Fig. 10.2A](#)).
5. Place a top-load (for 100 m³ raceways) or hanging (40 m³ raceways) electronic balance (10-g readability, >25-kg capacity) near the raceway ([Fig. 10.2E and F](#)).
6. Prepare baskets with lids (up to 30, depending on expected yield and orders, [Fig. 10.2B](#)). Calibrate baskets to have same weight. Calibration of baskets with a hanging balance is done with the basket rope in place ([Fig. 10.2B](#) and [Fig. 8.19A](#)).
7. Assuming that some shrimp will be sold fresh-on-ice, place two 1.5-m³ shallow (60-cm deep) flat-bottom tanks ([Fig. 10.2I](#)) in a shaded area and fill with 1 m³ of a flake ice and water slurry (75:25) one hour before harvest. Position an electronic balance (25-kg capacity, 10-g readability) near tanks. Have enough zipper-sealed 3.7-L freezer bags (number based on orders for frozen

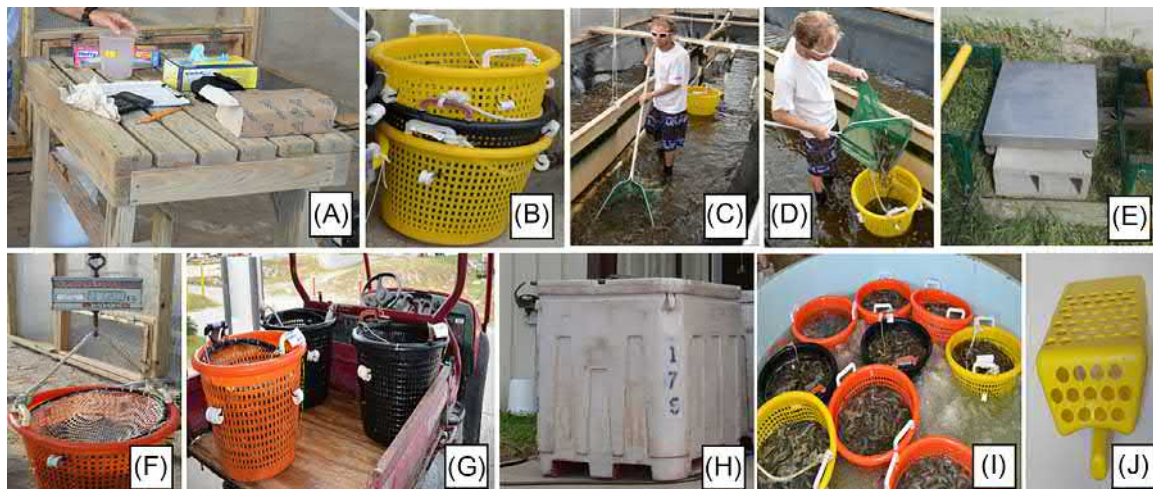


FIG. 10.2 Containers, materials, and tools for harvest at the Texas A&M-ARML: (A) table with sampling supplies, (B) tared harvest baskets, (C) harvest using a long-handle dip net, (D) harvest basket filled with shrimp, (E) splash-protected electronic balance, (F) weighing with hanging electronic balance; note lid on basket, (G) basket transfer by four-wheeler, (H) insulated harvest tote, (I) chill-kill tanks with ice water; shrimp in baskets, (J) plastic sifting scoop.

shrimp). Prepare 2-L plastic sifting scoops (4–6) (Fig. 10.2J) and a four-wheeler or truck to haul baskets to ice tanks (Fig. 10.2I).

8. Prepare 1-m³ insulated harvest totes (Fig. 10.2H), fill each with about 650 L of flake-ice with water to form a slurry (50:50).
9. Prepare plastic or wooden paddles (1–2) for mixing shrimp in harvest totes.
10. Prepare 10–15 long-handle dip nets with 0.5-cm mesh (Figs. 10.2C and D).
11. Prepare twelve 20-L plastic buckets (six empty, six full of flake ice).
12. Periodically adjust DO probe to avoid air exposure during draining.

10.2 MANUAL HARVEST, 40-M³ RACEWAY

Maintaining adequate DO in the small raceways during harvest is far more time consuming than in the large raceways. The 2-hp raceway pump has a dual purpose: maintaining DO and draining. A Venturi injector added to each raceway improves oxygenation (see Section 5.3.3). To keep up with the high oxygen demand before harvest, the injector is supplied with pure oxygen or a mixture of air and oxygen. Therefore an on-site oxygen supply (cylinders or liquid oxygen tanks) is required before draining begins.

Before draining, the Venturi injector is supplied with pure oxygen until the DO is between 6.5 and 7.0 mg/L. At that point, the pump is switched from recirculation to drain mode. Although some aeration is provided by air diffusers and the airlift pumps, DO declines quickly when the pump is used for draining because the raceway water has a high oxygen demand. When DO decreases to around 4 mg/L, the pump is switched back to supplying oxygen. Switching continues until the water level needed to harvest has been reached.

The six 40-m³ raceways are harvested with dip nets (see Video # 5 and # 6—Appendix VIII) when the volume is about 13 m³. The smaller

volume concentrates the shrimp, with more caught in each scoop.

The steps in manually harvesting and packing shrimp from the 40-m³ raceways are as follows:

1. Review existing orders to determine the quantity to be sold fresh, frozen, or processed.
2. Confirm availability of manpower for harvest, sales, and packing.
3. Turn on the balance. Select a basket with a rope connecting both handles, wet it by submerging it in water, and then tare it with the lid on.
4. Place baskets in the raceway and fill using dip nets. When full, cover with the lid, lift the basket out of the water, move it to the hanging balance, and let excess water drain.
5. Collect a 1-L sample from each basket before weighing. Fill the sampling cup to the top when the basket is full (about 23 kg) and half-fill when the basket is half-full. Once full, shrimp are transferred to the zipper-sealed 3.7-L (1-gal.) storage bag and placed in a bucket with a layer of ice. Samples then are transferred to the lab for processing.
6. Weigh and record the biomass in each basket. Move the first 20 baskets to the 1.5-m³ ice–water slurry tanks. When submerging baskets in the slurry, prevent ice from directly contacting the shrimp because ice flakes interfere with weighing. Fill orders for fresh shrimp concurrently with filling the 3.7-L storage bags. Place 2.27 kg (5 lb) in each bag, using the sifting scoop to drain excess water. For frozen shrimp, move shrimp bags to a –23°C freezer to hasten freezing, placing only single layers of bags on the shelves.
7. Measure DO every 15 min to make sure shrimp are not exposed to low DO.
8. Move shrimp to the 1-m³ totes when no more orders remain, record biomass in each tote, and keep the total below 360 kg.

9. Use the paddle to mix shrimp in the harvest tote with each added basket.
10. Check ice in the tote and add more if needed.
11. Drain more water from the raceway once the majority of shrimp have been removed.

Sampling each harvest basket can be avoided if size variation is small (CV below 10%, as determined from individual samples collected before harvest). When dealing with high size variation, sampling each harvest basket will provide a more representative average weight. The weight and the number of shrimp in each sample are recorded on a data sheet (see Group Weight Sampling Form—Page # 412—Appendix VII) and entered into an Excel file to calculate average weight (see Excel Sheet # 16—Appendix VII). Prepare data recording sheets, clipboards, pencils, erasers, two calculators, 1-L plastic sampling cup, 0.5-L plastic container, 3.7-L (1-gal.) zipper-sealed sample bags on the table (Fig. 10.2A). Because samples for average weight are collected before weighing the baskets, the total weight of shrimp removed by sampling must be accounted for in the final tabulation of yield.

Because shrimp are harvested from water of high temperature (29–30°C), after emptying each basket into the harvest tote they are

thoroughly mixed to lower their body temperature to about 4°C as rapidly as possible. Inadequate mixing results in accumulation and spoilage of shrimp near the bottom of the tote.

10.3 HARVEST BY FISH PUMP— 100-M³ RACEWAYS

Depending on the biomass, DO in the 100-m³ raceways is maintained by one or two 2-hp pumps and 14 a³ injectors using ambient air. One of the two could be used for aeration while the other is used for both aeration and draining. Close monitoring of DO during draining determines when to switch the second pump from draining to DO.

For normal operation, a 20-cm PVC standpipe is in the harvest outlet (Fig. 10.3A). A concrete harvest basin outside of the greenhouse serves for harvesting both raceways via 15-cm threaded outlets on the side walls (Fig. 10.4A).

Other devices can be used to harvest shrimp (Archimedes' pump, vacuum pump), but a submersible (Fig. 10.5A) or nonsubmersible (Fig. 10.5B) fish pump is preferred. Both are self-priming, variable speed, and hydraulic- or motor-driven. They handle shrimp very delicately, so even fragile antennae remain undamaged when passing through the impeller.

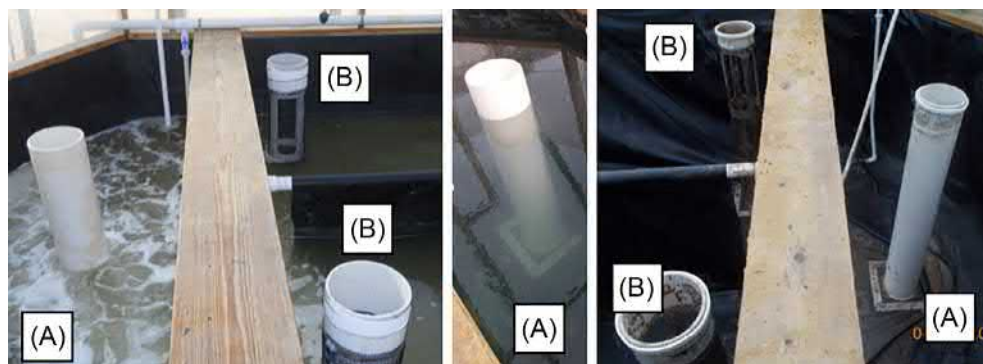


FIG. 10.3 A standpipe in the 20-cm drain outlet during normal operation (A). The standpipe is removed before operating the fish pump. Also shown are two screened pump intakes in an empty (*right picture*) and a half-full raceway (B).

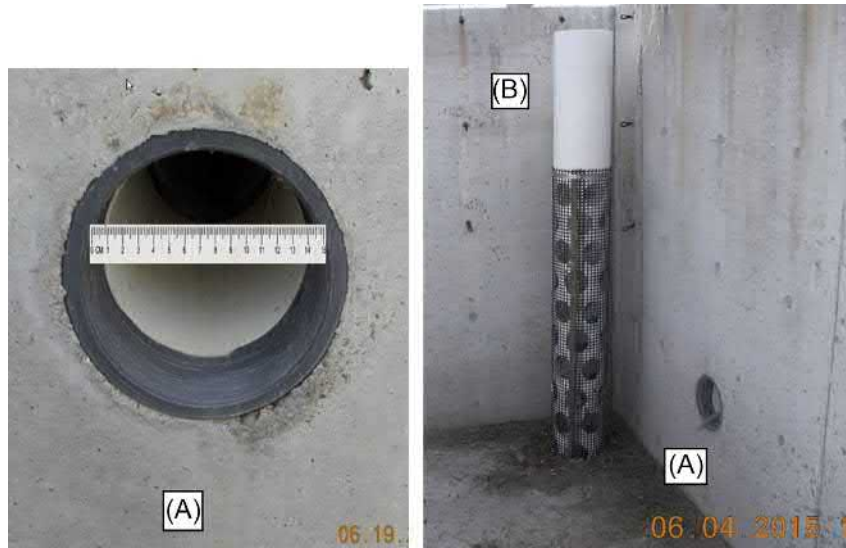


FIG. 10.4 Threaded 15-cm outlet in the harvest basin side wall above the bottom (A) and a filter pipe to prevent foreign objects from entering the drain line (B).

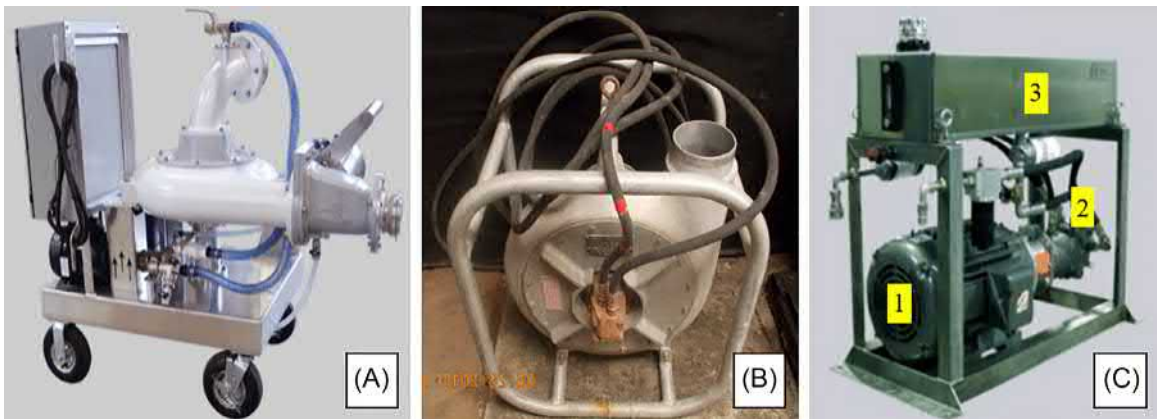


FIG. 10.5 Nonsubmersible (A) and submersible (B) fish pump with hydraulic hoses, hydraulic power pack (C) with electric motor (1), hydraulic pump (2), and hydraulic oil tank (3).

The model used at Texas A&M-ARML was a 15-cm (6-in) submersible hydraulically driven fish pump powered by a 10-hp, 230-V, 3-Phase, 60-Hz electric motor with a power pack that includes a hydraulic circuit, hydraulic oil tank, and hydraulic hoses.

When harvesting large ponds, the fish pump receives a large volume of water. To avoid excessive pumping, a screen cage is attached to the front of the pump to allow a large portion of the water to flow out while keeping the shrimp in. For the 100-m³ raceway, the pump is

connected directly to the raceway outlet because shrimp are harvested from only a relatively small volume of water (Fig. 10.4A). This results in shrimp and water being pumped into the tower where the water drops through a dewatering rack and into the harvest basin via a flexible hose (blue hose in Fig. 10.6B and C). Shrimp are separated and discharged down an incline into harvest baskets (Fig. 10.6C, see also Video # 20—Appendix VIII).

Activities carried out before, during, and after fish pump harvesting include:

1. Review existing orders to determine quantities to be sold fresh, frozen, or processed.
2. Confirm availability of manpower for harvest, sales, and packing.
3. Verify that the fish pump and hydraulic pump are working properly. Check oil and have 20 L of food-grade hydraulic oil on site. (Vegetable oil has been used in emergencies).
4. Place the pump on the bottom of the basin and carefully thread the hose connecting the pump intake into the 15-cm outlet in the side wall (Figs. 10.4A and 10.6A).
5. Place and level the dewatering tower near the harvest basin and place steps for easy access to the dewatering rack (Fig. 10.7B1).
6. Connect the discharge hose to the dewatering tower (Fig. 10.7C1). Place the flexible drain hose at the bottom of the tower inside the basin (blue hose in Fig. 10.6B).
7. Connect the high-pressure hydraulic hoses to the hydraulic circuit (Fig. 10.7D2 and 3).
8. Position a top-loading, splash-proof electronic balance between the two conveyers, with the first positioned under the outlet of the dewatering tower (Figs. 10.6C, and Fig. 8.18).
9. Turn on the balance and tare a wet basket with lid.
10. Attach an empty bottomless feed bag to the chute on the dewatering tower, place the sleeve inside an empty basket with the lid on to prevent jumping (Figs. 10.6C and 10.7A).
11. Remove the standpipe from the drain (Fig. 10.3A) and turn on the fish pump.
12. Adjust the pumping rate to fill each basket in 30–45 s using the hydraulic pump's flow control lever (Fig. 10.7D1).
13. Fill each basket to capacity, place lid on top, and slide toward the balance.
14. Collect a sample from each basket and place on ice, as described earlier.
15. Slide the basket to the balance, weigh and record the biomass on the data sheet.



FIG. 10.6 Fish pump connected directly to the raceway outlet on the side wall of the harvest basin (A). Water from the dewatering tower returns to the harvest basin via the blue hose (B) and shrimp are collected in a harvest basket (C).

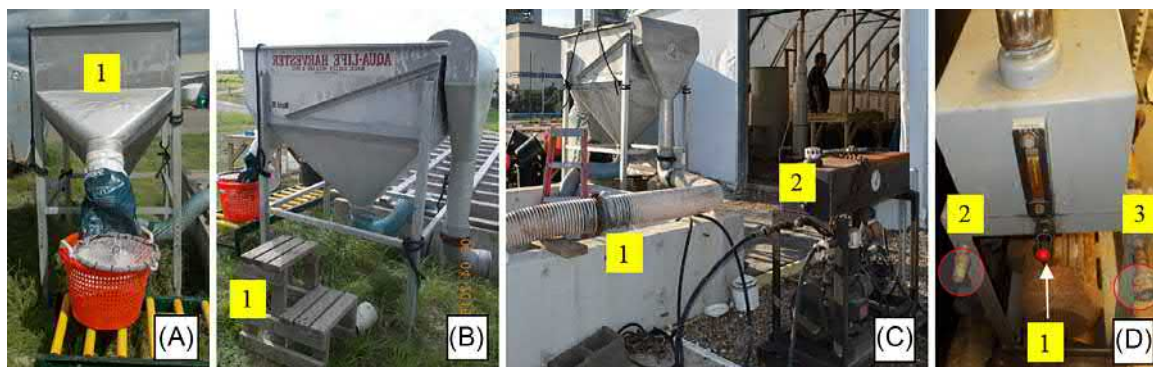


FIG. 10.7 (A) Funneling shrimp from the dewatering tower (1) into harvest basket with lid (note use of feed bag as a disposable chute), (B) dewatering tower with steps (1) for easy access, (C) hose connecting the fish pump to the dewatering tower (1) with power rack (2), (D) fish pump regulator (1) and hydraulic hose connectors (2 and 3).

16. Transfer the first 20 full baskets to the 1.5-m³ tanks filled with ice-water slurry. When submerging baskets in the slurry, prevent ice from mixing with shrimp, which interferes with weighing. Fill orders for fresh shrimp concurrently by filling 3.7-L freezer bags. Use the sifting scoop to drain excess water and fill each bag with 2.27 kg (5 lb).
17. Measure DO every 5–10 min to verify adequate levels because, at this stage, both pumps should be used for aeration only.
18. When no orders remain, begin loading shrimp to totes. Keep a record of biomass in each tote and avoid exceeding 360 kg.
19. Use the paddle to mix shrimp in the harvest tote with each added basket.
20. Check ice in the tote and add more as needed.
21. Flush remaining shrimp toward drain using fresh or seawater hose and push-brooms.

10.4 LIVE SHIPPING AND HAULING

The demand for live shrimp, especially in large metropolitan areas, presents a good marketing opportunity for year-round, super-intensive shrimp production. Anecdotal reports

from New York City suggest that subadult (12–14 g) live shrimp have been sold for \$40–\$44/kg (\$18–\$20/lb) during high-demand seasons. Extra effort associated with selling live shrimp is easily justified at such prices.

Depending on order size, live shrimp can be harvested during or before the main harvest using cast nets or traps (Fig. 10.8). Live shrimp are delivered in live-haul tanks with water or packed moist in insulated shipping boxes. Chilled shrimp can be shipped in Styrofoam boxes in an oxygen-rich atmosphere with a layer of chilled wet sawdust, although successful shipments have been made without sawdust. For the latest information on waterless shipping

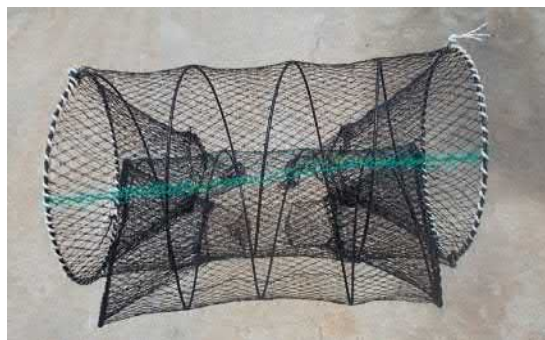


FIG. 10.8 A shrimp trap used for live harvest.

of live shrimp, see [Kuhn et al. \(2016\)](#) and [Taylor et al. \(2016\)](#).

When shipping in oxygenated water, deliver oxygen with a very-fine bubble diffuser to maintain DO well above saturation (12–14 mg/L). If needed, use a submersible pump to mix the water homogenously and prevent shrimp from concentrating in one place, as oxygen diffusers on the market today can release very fine bubbles (good oxygen transfer) but without suitable water mixing action. Carrying capacity is affected by trip duration, metabolite accumulation, pH, salinity, DO, and temperature. Our marketing of live juveniles showed high survival (>95%) at a transport density of 200 g/L for 2 h at a salinity of 35 ppt and a temperature of 17°C. Hauling tanks can be equipped with DC-powered submersible pumps that draw water from the tank bottom and spray it at the water surface to ensure adequate distribution of oxygenated water and to prevent shrimp from concentrating in one place ([Fig. 10.9](#)). Transport simulation tests with biomass loads of 50, 100, 150, 200, and 250 g/L at water temperatures from 16 to 20°C help identify the optimal conditions for the actual delivery.

Once the hauling tank is loaded, the water temperature is decreased by about 1°C every

10 min with ice in leak-free plastic bags. A protocol must be in place for acclimating shrimp to a higher temperature at the point of delivery. Fine-tuning these protocols improves survival and minimizes the risk of massive molting during or after delivery. The delivery truck must carry a sufficient number of oxygen tanks (compressed or liquid) to ensure suitable DO throughout the trip. In the case of deliveries that last a few hours at high air temperatures, carry additional bagged ice to lower water temperature, if needed. [Video #13](#) shows juveniles in hauling tank.

10.5 PRODUCT HANDLING AND COLD STORAGE

These are general recommendations to maintain product quality and marketability. Ideally move harvested shrimp in only one direction: from the culture tanks to live-haul tanks or the packing/processing facility. Do not return any live shrimp to the culture tanks. All hauling tanks and the delivery trucks must be meticulously disinfected at the end of each delivery. Institute a similar disinfection process for tanks and equipment used for moving harvested

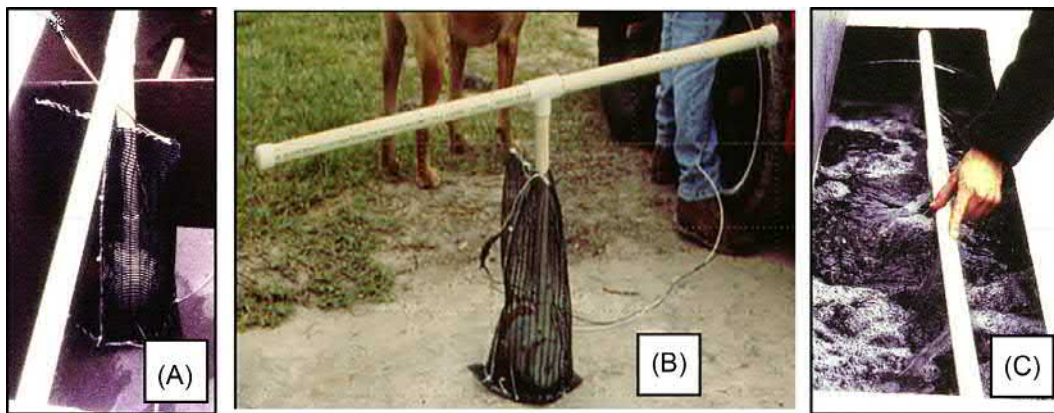


FIG. 10.9 (A) DC-powered submersible pump with protective netting and a spray bar inside a 600-L live-haul tank, (B) the pump and spray bar, (C) water mixing by pump.

shrimp to the packing/processing plant. Personnel associated with packing and processing should not have access to culture tanks.

A sufficient supply of ice is key for preserving product quality, especially because a large part of shrimp sales are expected to be fresh-on-ice. Note that using salt water to prepare the ice slurry is highly recommended as it drops water temperature to below freezing. Facilities should have equipment to produce ice on site. Use flaked (instead of crushed) ice to reduce potential damage to shrimp. Flaked ice equipment should be capable of producing enough to process the harvest of at least one raceway. Mount the ice machine on the ceiling of a well-insulated room where unused ice can be stored during low-demand periods.

Operations with high sales volumes of fresh-on-ice products should have the infrastructure to streamline processing to reduce potential for quality deterioration and spoilage. Bathrooms, showers, and locker rooms for sole use by the processing/packing personnel should be located near the processing area. Process and pack shrimp in a temperature-controlled room (12–18°C) equipped with conveyers, stainless-steel packing tables, ice bins, packing supplies, and a sanitation station for workers. Move products from the processing room into a 4°C cold storage room on conveyers. This room should have access to a ramp with conveyers for easy loading of packed shrimp with a forklift. The cold storage room should be large enough to hold all of the product to be sold fresh-on-ice. Distribute the product in refrigerated trucks that keep it at 4°C throughout the delivery.

Several factors affect the demand for ice, including existing orders for live and fresh-on-ice shrimp. If the business plan calls for weekly or twice weekly harvest, and assuming shrimp growth is on target, it is important to adhere to this schedule even when demand for fresh or live shrimp is less than the expected harvest biomass. To that end, the facility should be equipped with adequate processing, freezing,

and cold storage capacity to deal with any anticipated surplus.

The Individually Quick Frozen (IQF) process is the storage method of choice. Shrimp go through IQF as heads-on with minimal damage, so de-heading before freezing is not required. This is especially true when liquid CO₂ (–73°C or –100°F) rather than liquid N₂ (–195°C or –320°F) is used for freezing. CO₂ also extends shelf life for more than one year. In comparison, the fresh-on-ice product has a much more limited shelf life of 3–4 days.

Besides eliminating the need to deploy manpower for de-heading, heads-on IQF shrimp generate higher income than IQF tails. This is because of the higher weight of the heads-on shrimp and the perceived higher quality of the product. Facilities with on-site IQF processing and storage benefit from greater marketing flexibility when dealing with unexpected last-minute cancellations. The fact that IQF shrimp retain their quality for more than a year allows important management and marketing flexibility. Nonetheless, adding IQF processing and cold storage capacity requires a significant investment. About \$600,000–\$800,000 is required to produce IQF shrimp at 1400 kg/h, which includes cold storage capacity of about 45,000 kg. This can be reduced by about 50% if IQF shrimp are stored in a rented cold storage space (e.g., \$300 to \$400/year for 450 kg IQF, head-on). Further, because cleaning the IQF equipment at the end of a processing run takes several hours and because the minimum processing output is about 700 kg/h, the minimum recommended quantity of shrimp to be processed using this technology is 2100 kg (4600 lbs).

IQF shrimp sell for slightly lower prices than fresh-on-ice product, but their unique taste and appearance when raised in biofloc water under sustainable production practices and in compliance with HACCP regulations (Drazba, 2004) add value that fetches higher prices than other IQF shrimp (see [Section 13.6](#)). Facilities producing

IQF shrimp that have a walk-in freezer (-40°C) to store product have the added advantage of potentially providing clients with high-quality product on short notice throughout the year.

References

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- Taylor, D., Kuhn, D., Hanson, T., Lawson, L., 2016. Protocols and market opportunities for shipping live shrimp in waterless conditions. In: Aquaculture 2016, 23–26 February 2016, Las Vegas, NV, USA.

Waste Treatment and Disposal

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This chapter presents options for treatment, reuse, and disposal of water and solid waste. Waste treatment impacts biosecurity, sustainability, and profitability. It is determined to some degree by location (inland or near the coast), regulations governing aquaculture effluent releases, and treatment costs.

Water use in limited-exchange indoor biofloc systems is highly efficient. Once culture tanks are filled, very little salt water is added or discharged; only freshwater is added to compensate for losses from evaporation and removing waste. As a result, one kg of shrimp can be produced using as little as 0.098–0.169 m³ of water, compared to 20–64 m³ using traditional techniques (Krummenauer et al., 2014).

11.1 WASTEWATER AND SOLID TREATMENT OPTIONS

Reusing culture water closes the system to a great extent, saving money, improving biosecurity, and reducing environmental impact. Conserving salt also reduces expenses in inland areas where culture water is produced with artificial salt and disposal of saline effluent is restricted (Hargreaves, 2013). It only rarely is

completely closed, however, as there are occasions when water is discharged owing to accumulation of nitrate, phosphate, or heavy metals; a disease outbreak; or harvesting. High water reuse nevertheless improves a product's sustainability credentials, thereby improving its marketability.

Before reuse, water may require removing dissolved nitrogen and phosphate compounds, adjusting pH and alkalinity, reducing solids, and restoring ionic balance.

11.1.1 Digestion

Most of the main treatment requirements are met by use of an anaerobic digester or batch reactor, as is common in wastewater treatment plants. An anaerobic digester is an independent tank in which water and solids are circulated or left to settle without aeration.

Denitrifying bacteria that develop under low oxygen conditions ($\text{DO} < 2 \text{ mg/L}$) convert nitrate to nitrogen gas, which then is released to the atmosphere (Stenger et al., 2013). Denitrification is a four-step process. Nitrate (NO_3^-) is reduced to nitrite (NO_2^-), which then is reduced to nitric oxide (N_2O). The final step is reduction of N_2O to nitrogen gas (N_2). Timmons and

Ebeling (2013) note that if denitrification is not properly managed (e.g., low redox potential, $\text{DO} < 2 \text{ mg/L}$, sufficient organic carbon and nitrate, $\text{pH} 7.0\text{--}8.5$, and temperature $25\text{--}32^\circ\text{C}$) hydrogen sulfide will form. They also mention that H_2S forms when NO_3^- ranges from 10 mg/L to 50 mg/L . Some reports suggest keeping redox between -50 and $+50 \text{ mV}$.

In addition to removing nitrate, some denitrifying bacteria incorporate orthophosphate, which then can be removed from the system. Denitrification has the added advantage of increasing alkalinity (3.6 mg CaCO_3 for every 1 mg of nitrate-N removed) (Sedlack, 1991) by releasing bicarbonate (Tiedje, 1990). Solids are reduced during this process, as nitrate is used to oxidize organic matter (Hargreaves, 2013).

Ammonia and nitrite increase with the die-off of some aerobic bacteria and incomplete denitrification. Ammonia also is released from sludge. An aerobic stage, therefore, may ensure that ammonia and nitrite are converted to nitrate. This can take place within the same digester or in a separate unit called a sequencing batch reactor.

For treatment in a single digester, the wastewater-solids slurry is vigorously aerated with blower-driven diffusers or a pump with an air-fed Venturi. After 1–2 days, ammonia and nitrite will have been converted to nitrate and some solids degraded (Hargreaves, 2013). When aeration is stopped, solids settle (Fig. 11.1) and the system runs anaerobically. Denitrification then decreases nitrate and phosphate and raises pH and alkalinity (Fig. 11.2).

Ammonia may increase during the anaerobic stage, so this process is repeated to maximize nitrogen removal. Subsequent aerobic steps also encourage release of nitrogen gas produced by denitrification. Monitor the process by measuring ammonia, nitrite, nitrate, alkalinity, pH, H_2S , and redox potential daily.

Full denitrification may take several days, but it is expedited by adding a carbon source, such as sugar, molasses, methanol, ethanol, or acetate. If methanol is used, a ratio of $\text{COD}:\text{NO}_3\text{-N}$ (by weight) of 3–6, or a C:N ratio of 2–3,

facilitates conversion of 95% of nitrate to nitrogen gas (Halling-Sorensen and Jorgensen, 1993; Van Rijn et al., 2006).

Timmons and Ebeling (2013) reported that 2.47 g of methanol reduces 1 g of $\text{NO}_3\text{-N}$. Excess carbon in the absence of NO_2 and anaerobic conditions can lower redox to levels that promote H_2S production (Whitson et al., 1993). Tiedje (1990) reported that carbon limitation promotes NO_2 and N_2O production and excess carbon promotes conversion of NO_3 to NH_4 .

Methane also may be generated by heterotrophic denitrification following carbon supplementation. If methane can be recovered safely and stored, it can be used as fuel for heating, transport, or electricity generation.

Once $\text{NO}_3\text{-N} \leq 50 \text{ mg/L}$ at 30 ppt or $5\text{--}10 \text{ mg/L}$ at 10–20 ppt (0 mg/L TAN and $\text{NO}_2\text{-N}$), the slurry is allowed to settle. This separates the remaining solids, with phosphate sequestered in bacterial biomass. If nitrate has been fully depleted, volatile fatty acids may accumulate. This reduces DO when the supernatant is returned into the culture tank.

Aerate the supernatant in the tank prior to pumping it back into the culture tanks, especially if hydrogen sulfide is present, as indicated by the scent of rotten eggs. The redox potential should be above 100 mV .

When using separate tanks or independent compartments within the same tank, nitrification should take place in one volume and denitrification in another. Settling, solids removal, and aeration can take place in a third.

That configuration has a larger footprint, but it allows separate treatment steps to occur simultaneously. The design can take many forms, depending on available space and materials. Treatment can occur during a culture cycle or postharvest.

Solid and liquid waste collected by settling tanks, foam fractionators, and cyclone filters can be processed by a digester to remove nutrients, heavy metals, and reduce solids volume. If the sludge contains high levels of heavy metals, disposal options must be carefully considered.



FIG. 11.1 Settled solids level from an anaerobic digester measured with a clear sampling tube.

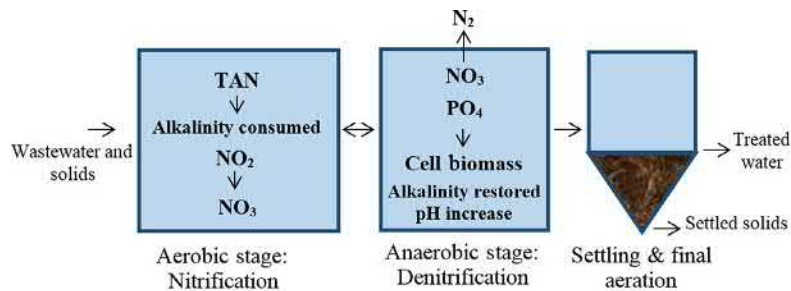


FIG. 11.2 Stages in a denitrification digester. These may be located in separate tanks or separate compartments in the same tank.

Some denitrification also may take place in settling tanks (Ray et al., 2010) especially if solids are left in the settling tanks for more than few days. Increasing retention time by reducing flow

rate or increasing tank volume expedites this process.

Timmons and Ebeling (2013) provide extensive information regarding the design and

operation of denitrification reactors. They describe a 1.89-m³ conical-bottom polyethylene tank with 1 m³ of media and up-flowed water at 10 Lpm. Carbon sources included acetic acid, refinery molasses, and starch. This reactor reduced NO₃⁻ to zero from initial levels of 11 to 57 mg/L.

Further details on denitrification and digester systems, including other designs, are found in Sedlack (1991), Whitson et al. (1993), Van Rijn et al. (2006), Neori and Mendola (2012), Hargreaves (2013), and Timmons and Ebeling (2013).

11.1.2 Other Treatment Options

11.1.2.1 Probiotics

Commercial probiotics stimulate digestion of organic sludge. They are applied in settling or digester tanks and mineralize up to 100% of the sludge.

11.1.2.2 Solids removal

Solids are managed in culture water to maintain optimum TSS and SS using equipment described in Section 5.4. Additional removal may be required at the end of a production cycle to prepare water for reuse. As described before, the digestion process also removes some solids. Depending on the sludge volume, a large settling tank, basin, pond (baffled or conical base design), or geotextile separation tubes are used to separate solids.

11.1.2.3 Solids Removal at Texas A&M-AgriLife Research Mariculture Lab (ARML)

Solids collected by foam fractionators, settling tanks, and multicyclone filters were dewatered in separation tanks. Except for the water recovered from solids removed by the settling tanks, water was returned to culture tanks (see Sections 5.9.1.3 and 5.9.2.3). Solids were dried in the separation tanks before disposal.

11.1.2.4 Disinfection

Water may have to be disinfected at the end of a nursery or grow-out cycle if pathogens are present at high levels (see Sections a & b—Appendix II for *Vibrio* Monitoring and Section 6.2 for Disinfection, respectively). This also eliminates beneficial bacteria, but it is necessary to reduce the disease risk for a new crop.

11.1.2.5 Alternative Crops

Nutrient-rich water can be used to grow alternative aquatic crops, such as seaweeds or salt-tolerant terrestrial crops (Pantanella and Bhujel, 2015). Low salinity water (e.g., 2–3 ppt) can be used for irrigation of date palms, tomatoes, various herbs, forage crops, and ornamentals like irises. The salinity tolerance of each crop must be considered (Buhmann and Papenbrock, 2013), and dilution with freshwater may be required. Care must be taken regarding salt accumulation in the soil and leaching into groundwater and surface freshwater. The intermittent nature of wastewater availability must be taken into account, unless large volumes of discharge are stored or constant partial replacement of culture water is possible.

When plants have removed nutrients and solids, any remaining water (e.g., from seaweed or hydroponic systems) can be recycled to the shrimp system or disposed. Solid waste can fertilize terrestrial crops if salts are flushed and heavy metal levels are safe. Buhmann and Papenbrock (2013) thoroughly review the use of aquaculture effluents for halophytic plants.

11.2 WATER AND SOLIDS DISPOSAL OPTIONS

Disposal of culture water and solid waste depends on the facility location, climate, salinity, cost, and local regulations. Saline effluent is restricted in many jurisdictions, particularly inland.

11.2.1 Direct Disposal

Direct disposal into the local environment, generally the cheapest option, depends on regulatory requirements and aquaculture permit conditions, such as limits on discharge volume and the allowable concentrations of water quality indicators such as DO, cBOD₅, salinity, ammonia, pH, TSS, chlorine, foam, selected heavy metals, and coliform bacteria count (Hopkins and Villalon, 1992; Samocha et al., 2004; Yoo and Boyd, 1994).

In most cases, water must pass through a filter screen to prevent shrimp escape and discharge of organic and inorganic particulate matter. Water also may have to be settled or filtered even further to reduce solids, with settled solids disposed of separately. This can be done with a settlement pond.

Digestion may be needed to reduce nitrate and phosphate. When discharging into fresh water, there may be limits on salinity or dilution requirements. Permit conditions often require regular monitoring of the environment surrounding the discharge site, in addition to monitoring the facility's effluent. All large shrimp farms in Texas that use outdoor ponds have a permit to release effluent into receiving streams, provided this water meets standards established by the Texas Commission on Environmental Quality (TCEQ).

11.2.2 Aquifer

In some cases, discharge may be pumped into an aquifer. This depends on aquifer characteristics—including salinity and recharge dynamics—and local regulations. It may not always be a viable option and, when available, is expensive compared to other options.

11.2.3 Artificial Wetland

Depending partly on the salinity of the water to be released and after solids separation, wastewater may be pumped into a purpose-built



FIG. 11.3 Artificial wetland growing *Salicornia* sp. to filter water from a shrimp system.

artificial wetland (Figs. 11.3, 11.4, and 11.5). This type of wetland usually involves a shallow clay or membrane-lined area containing salt-tolerant plants, among which are (from Buhmann and Papenbrock, 2013):

- Mangroves (in tropical regions).
- Glasswort or Pickleweed *Salicornia bigelovii*
- Cordgrass *Spartina alterniflora*
- Needle rush *Juncus roemerianus*
- Saltwort *Batis maritima*
- Seaweeds (macroalgae) such as *Gracilaria* spp.

Of these, *S. bigelovii* is often preferred (Fig. 11.3) because it is edible and an animal fodder. Its seeds may be processed to produce edible oil (Shpigel et al., 2013). Available and permitted species differ between geographic locations and local jurisdictions.

The wetland can be designed as a static pond or a stream through which wastewater passes. In addition to settling solids, plants absorb nutrients, primarily nitrate and phosphate. Nutrient removal in the wetland also occurs through denitrification. Some plants, such as water hyacinth in low-salinity water, remove heavy metals. Water can be discharged to the local environment or accumulate in the wetland and be lost by evaporation and plant evapotranspiration.

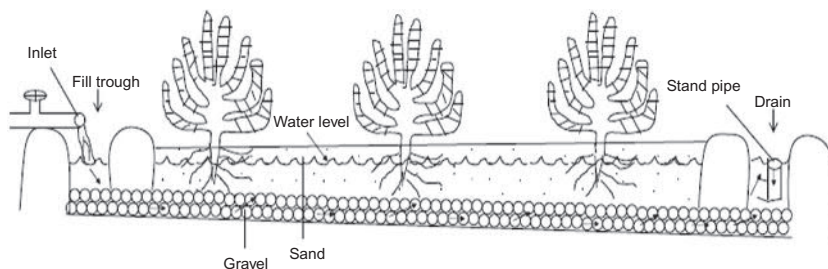


FIG. 11.4 Subsurface flow in a constructed wetland for nutrient recovery of mariculture effluent. View shows 1.5% sub-surface grade and water level with respect to surface. (Klim, B.C., 2012. *Optimization Model for the Management of a Horizontal Sub-surface Flow Constructed Wetland Planted with the Halophyte Salicornia Bigelovii in the Treatment of Shrimp Mariculture Effluent*. Master's thesis. Texas A&M University-Kingsville, Kingsville, TX. Used with permission.)

Secondary crop production with nutrient-rich effluent has been conducted on a small scale. Shpigel et al. (2013) used a constructed *Salicornia* wetland to filter aquaculture effluents. It removed nitrogen, phosphorus, and solids from the saline effluent (41 ppt) and provided an alternative agricultural crop. Costa (2011) estimated that a 1-ha plot of *Salicornia gaudichaudiana* would remove 52 kg of $\text{NH}_4\text{-N}$, 41 kg of $\text{NO}_3\text{-N}$, and 11 kg of $\text{PO}_4\text{-P}$ per year.

The design of a treatment system for a given production facility only can be done after the amount and frequency of nitrogen and phosphorus discharge has been determined. This, in turn, is based on feed protein concentration, culture tank volume, water reuse, and solids removal.

Klim (2012) described two main types of constructed wetlands: free water surface (FWS) and horizontal subsurface flow (HSSF, Figs. 11.4 and 11.5). FWS wetlands contain vegetation submerged by up to 1 m of the effluent to be treated. HSSF wetlands generally are more complex, with effluent flowing below the surface through a gravel layer and vegetation planted on the surface. FWS wetlands are better at removing high

BOD and lowering ammonia; HSSF wetlands are better at assimilating nitrate and removing tertiary BOD (Kadlec, 2008).

11.2.4 Evaporation Basin

Water and solids can be pumped to a site where water evaporates and the remaining solids are removed for disposal or alternative use. This takes the form of a shallow (<30 cm) membrane-lined pond, although it can be deeper. The basin may be lined with shade cloth to allow easier solids removal once all water has evaporated. This option depends on evaporation and so is not practical where precipitation is seasonally high or temperatures are low.

11.2.5 Geotube

Water and sludge can be filtered through Geotube (TenCate Geosynthetics, The Netherlands) containers. These have a material with small pores that trap and dewater solids, reducing the volume that must be disposed. Filtered water can be reused or disposed. The filled tube is then hauled to a solid waste disposal site.

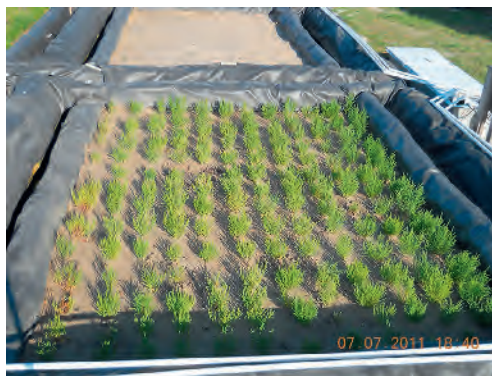
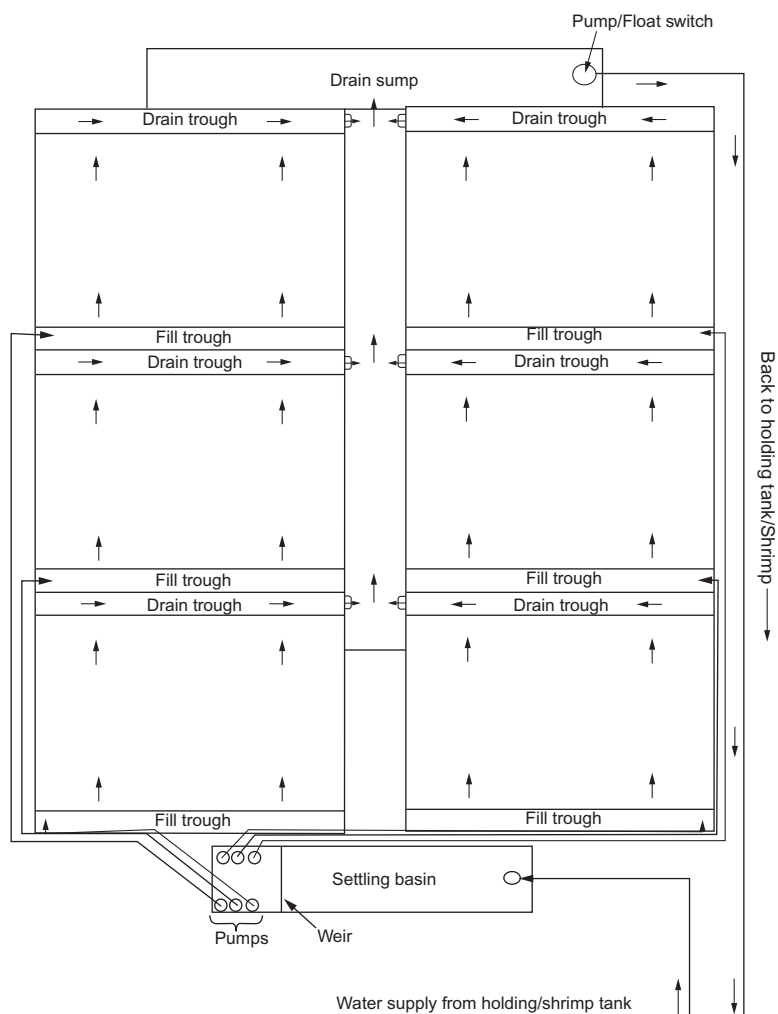


FIG. 11.5 Schematic and flow diagram with photos of HSSF constructed wetland for nutrient recovery of mariculture effluent. (Photos by Brandon Klim. Schematic drawing from Klim, B.C., 2012. *Optimization Model for the Management of a Horizontal Subsurface Flow Constructed Wetland Planted with the Halophyte *Salicornia Bigelovii* in the Treatment of Shrimp Mariculture Effluent*. Master's thesis. Texas A&M University-Kingsville, Kingsville, TX. Used with permission.)

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Disease and Biosecurity

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A disease outbreak can quickly ruin a crop, so shrimp health is a prime concern of the production manager. This chapter describes health monitoring and biosecurity, with particular attention paid to the identification and treatment of common diseases.

Shrimp health is affected by stressors (Fig. 12.1) classified as biological (nutrition, stocking density, interactions with other shrimp, pathogenic and nonpathogenic microbes, macroorganisms), chemical (poor water quality, pollution, nitrogenous waste), physical (temperature, light, sound, water turbulence, dissolved gases), and procedural (transport, acclimation, handling, harvest, treatments) (Francis-Floyd, 2015). Stress is amplified when any of these act together. For example, shrimp are more susceptible to pathogens when exposed to poor water quality and reared with inadequate nutrition.

Developmental stage (larvae, postlarvae (PL), subadult, adult) and genetics also determine the response to stress. Shrimp have a nonspecific, labile immune system, that is, they do not build up any long-term immune “memory” (Kim et al., 2014; Roch, 1999; Söderhäll and Cerenius, 1992). Monitoring and controlling each stress factor thus is a priority for

production managers. Simply put: Minimizing stress maximizes the chance of success.

Disease occurs when three factors align: poor condition of the host, presence of a virulent pathogen, and stressful environmental conditions (Karunasagar et al., 2010; Robertson, 2006). Health management and disease prevention therefore involve excluding pathogens (biosecurity) and maintaining optimal water quality and shrimp health (adequate nutrition, healthy immune response). Healthy shrimp reared under optimal environmental conditions often thrive in the presence of pathogens.

12.1 HEALTH MONITORING

Regular observations of shrimp are key to early detection of disease. This allows the culturist the time to respond with a treatment before the problem becomes uncontrollable (Clifford and Cook, 2002). Shrimp should be sampled at least weekly to check their general health (see Section 9.5). Observe shrimp behavior while conducting routine tasks, such as feeding and water quality monitoring. Remove dead and moribund shrimp immediately and examine

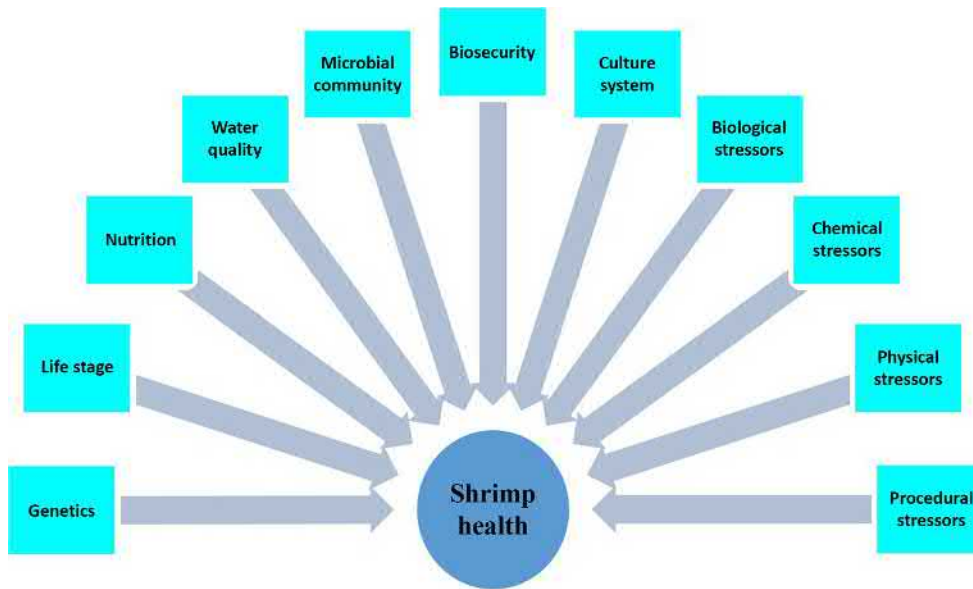


FIG. 12.1 Shrimp health in culture systems is affected by many factors that act together to determine growth, survival, and FCR.

them with dissecting and compound microscopes. Note any abnormal physical signs and behavior. This helps to determine the cause of mortality and provides a reference for future problems.

Health observations include:

1. Gut condition

- Healthy shrimp feed continuously, so guts should be full. Empty or partially full guts may indicate underfeeding, inappropriate feed size, poor quality, or loss of appetite from disease. Contents are seen clearly by holding a juvenile or adult to a light source. A dissecting microscope is used to evaluate PL guts (Fig. 12.2).
- Gut color should be similar to that of the feed and biofloc. A red/pinkish gut is likely from cannibalism of dead shrimp, indicating mortality in the system; a green gut is likely from consuming benthic algae, indicating underfeeding; and a pale white gut indicates parasite

infestation or disease (Clifford and Cook, 2002).

- Gut deformity (inflammation or epithelial damage) suggests disease, such as hemocytic enteritis (thickening) or IHNV (deformity) (Clifford and Cook, 2002).
- #### 2. Body color
- Shrimp are relatively transparent. Abnormal coloration, such as white pleopods or tails; white, red, black, or yellow discolorations; or spots on the body, hepatopancreas, or gills may indicate parasitic infestation or viral (or bacterial) infection (Fig. 12.3).
 - Red periopods, pleopods, and uropods often suggest bacterial infection (Chen, 1992). White or opaque tail muscle (necrosis) often follows periods of severe stress (Treece and Fox, 1993) and can signal *Vibrio* infection (bacterial white tail disease) (Zhou et al., 2012), microsporidiosis (microsporidian

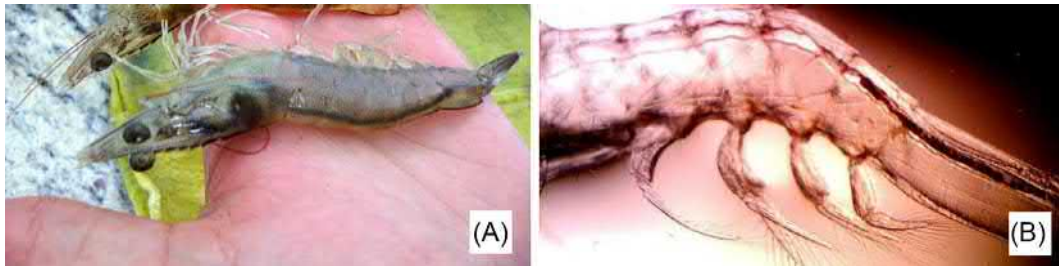


FIG. 12.2 Shrimp with full (A) and partially full (B) guts.



FIG. 12.3 Shrimp with severe discoloration of tail segments (necrosis) suggesting *Vibrio* infection, infectious myonecrosis, or microsporidiosis.

- infestation) (Johnson, 1990), or infectious myonecrosis virus (IMNV) (OIE, 2007).
- A yellowish head, caused by an enlarged hepatopancreas, can result from viruses, such as yellowhead disease (Lightner, 1996).
- Some discoloration also can result from water quality factors. For example, external white spots can be caused by

high alkalinity; red gills can be caused by low dissolved oxygen or exposure to a toxin, such as high ammonia (Robertson, 2006).

3. Physical damage (e.g., missing appendages, short antennae, or black spots) (Fig. 12.4)
 - This may indicate underfeeding, leading to cannibalism, predation, or excessive water or air flow, particularly in the



FIG. 12.4 Necrosis (dead tissue) on shrimp.

nursery phase. Hardened black spots (melanosis) are caused by production of melanin in response to injury, a foreign object, or infection (Robertson, 2006). Shrimp cultured at high density often have short antennae, but this may not indicate a problem.

- Damaged or eroded cuticle or appendages (legs, antennae, rostrum, uropods, tail) may indicate disease (Bondad-Reantaso et al., 2001).
 - Deformed rostrum and short rough antennae can indicate IHNV.
4. Gill condition (e.g., fouling or black gills)
 - Gill fouling may result from poor water quality, high TSS, or lack of grooming owing to lethargy. Discoloration may be from bacterial or viral infection, parasitic infestation, toxins, or high heavy metals (Clifford and Cook, 2002).
 5. Shell condition (fouling)
 - A large proportion of shrimp with fouling with algae or protozoans may indicate infrequent molting or inadequate grooming caused by stress-induced lethargy (Bondad-Reantaso et al., 2001).
 6. Molting
 - Sustained molting may indicate poor water quality or disease (Fig. 12.5).
 - Shrimp that are unable to molt or that die while molting (soft shell or molt partly

attached) suggest poor water quality or disease.

7. Feeding behavior

- Uneaten feed points to overfeeding or loss of appetite caused by poor water quality or disease. Large numbers gathered under belt feeders or rapidly surfacing when feed is added suggesting poor feed distribution or underfeeding. Records should note such unusual feeding patterns (Bondad-Reantaso et al., 2001).

8. Miscellaneous behavior

- Erratic swimming, such as swimming in a continuous circle at the surface, is a sign of infection, such as Vibriosis, poor water quality, or toxins.
- Surfacing is normal for shrimp, but they typically do not remain on the surface and submerge after a few moments. If more than a few appear at the surface and remain there for an extended period, this may indicate low DO, gill fouling, or disease.
- Jumping is normal evasive behavior that occurs when shrimp are startled (by light, noise, manual feeding, or other shrimp) but may indicate disease or poor water quality if it occurs continuously.
- Lethargic or unresponsive shrimp signal disease or poor water quality.



FIG. 12.5 Shrimp molts collected from a raceway.

- A large number of shrimp gathered around aeration devices likely indicates poor water quality, gill fouling, or disease.
- Tail cramping, described as Cramped Tail Syndrome (Clifford and Cook, 2002), describes the situation in which a shrimp's tail is flexed and will not relax after handling. This sometimes is associated with white tail muscle and is thought to be caused by the combined effects of handling stress at high temperature and/or salinity, although other stressors may contribute (Clifford and Cook, 2002; Johnson, 1990; Robertson, 2006; Treece and Fox, 1993). Mineral imbalances also cause tail cramping. High manganese content ($>0.02\%$) in biofloc-based feed contributes to cramping and reduced growth (Kuhn et al., 2015), as has low potassium, particularly in low-salinity culture or with repeated use of RAS water.

9. Growth and FCR

- Reduced growth or high FCR suggest poor water quality, disease, or underfeeding.



FIG. 12.6 Monitoring shrimp size variation is important in health monitoring and necessary for selecting an appropriate size feed.

- High size variation (Fig. 12.6) indicates poor PL grading, underfeeding, inadequate feed distribution, inappropriate feed sizes, genetic differences, or disease, such as hemocytic enteritis (Clifford and Cook, 2002; Robertson, 2006).

10. Hemolymph

- Disease can change hemolymph clotting time, a useful indicator of stress (Jussila et al., 2001). Establish a site-specific normal clotting time in unstressed shrimp as a baseline for comparison.

Like many organisms, shrimp are most susceptible to disease during periods of high stress, such as during stocking, sampling, and harvesting, and when exposed to poor water quality. Health monitoring receives top priority when stress is identified.

The following table is a guide to shrimp health issues and their possible

causes, with a link to the relevant section of the manual in which they are discussed (Table 12.1).

12.2 DISEASES

Disease spreads rapidly in super-intensive systems simply because of the high stocking density. Its impact varies with shrimp health,

environmental conditions, and the number and virulence of pathogens. Effects range from slowing growth and feeding to mortality.

Many pathogens, such as *Vibrio* spp., are opportunistic and become a problem only during periods of stress. Primary pathogens, such as the White Spot Syndrome virus, act independently of other stressors. Blooms of the cyanobacterium *Synechococcus* sp. suppress growth and blooms of toxic dinoflagellates, such as

TABLE 12.1 Shrimp Health Summary

	Observation	Possible Causes	Suggested Actions (Page #)
I. Morphological	Empty gut	1. Underfeeding 2. Inappropriate feed size 3. Stress (e.g., poor water quality or disease)	165 220, 297 237, 331, 335, 337, 339
	Abnormal gut coloration, Red/pink gut	1. Cannibalized mortalities	220, 339
	Green gut	1. Consumed benthic algae—underfeeding	166, 336
	Pale-white gut	1. Gregarine infestation 2. Disease	233 341
	Gut deformity or damage	1. Disease such as hemocytic enteritis	220, 223
	Abnormal body coloration or marks, Red/pink periopods and uropods (or whole body)	1. Vibriosis 2. Gill-Associated-Virus (GAV)-type disease 3. Taura syndrome 4. WSSV	231, 316, 340 165–166, 316–317, 230–231, 237–238 228, 229, 228, 238
	White spots on the cuticle	1. Water quality (e.g., high alkalinity) 2. Viral disease such as WSSV 3. Certain bacteria and fungi 4. Parasites	137 228, 229 230–232, 341 233, 340

TABLE 12.1 Shrimp Health Summary—cont'd

Observation	Possible Causes	Suggested Actions (Page #)
White (or red) opaque muscle (muscle necrosis)	1. White cotton disease (microsporidian parasite) 2. Vibriosis 3. IMNV 4. Handling during high temperature and/or salinity 5. Water quality stressor (low DO, sudden changes in parameters)	233 165–166, 316–317, 230–231, 237–239, 323, 340 340 154, 174, 219, 223, 339 133–134, 174, 195, 340
Red midgut	Hemocytic enteritis (blue green algae)	341
Yellow head (enlarged hepatopancreas)	Viral disease such as YHD	230, 340
White coloration on the outer layer of the eyeballs	Fungal infection such as <i>Fusarium</i> spp.	49, 229, 231, 233, 340
Black marks or lesions	1. Healed wound 2. Bacterial shell disease 3. Black splint disease 4. Other viral or bacterial infection 5. Parasites	230, 237–239, 340 230, 237–239, 340 237–239, 340 340
Abnormal gill coloration, Red gills	Stress caused by low DO or a toxin	133–134, 174, 195, 332, 340
Black/Brown gills	1. Fouling (high TSS/organic fouling) 2. Lack of grooming 3. Melanization following infection of filaments 4. Blue-green algae growing on filaments 5. <i>Fusarium</i> infection 6. Iron or manganese precipitation	147, 222, 321, 334, 340 222, 340 340 43, 231, 340 13, 49, 143, 227, 231–232, 334
General discoloration	Parasitic infestation, viral or bacterial infection	237–238, 340
Physical damage such as missing appendages,	1. Underfeeding leading to cannibalism	166, 339, 340

Continued

TABLE 12.1 Shrimp Health Summary—cont'd

	Observation	Possible Causes	Suggested Actions (Page #)
	short antennae, lesions, or black spots	2. Predation	160, 235, 340
		3. Excessive water or air flow, particularly in the nursery phase	110, 301, 340–341
		4. To be expected at a low level in high-density culture	222
	Erosion of cuticle or appendages	Disease	239, 340
	Deformities (e.g., bent rostrum, wrinkled antennae)	Viral disease such as IHNV	227, 238, 340
	Fouling such as algae or protozoans on body	Inadequate grooming owing to lethargy caused by disease or poor water quality.	222, 237–239, 331–334, 336–341
	Molting, Sustained increase in exuviae in system	Stress (e.g., Poor water quality or disease)	222, 237–239, 331–334, 336–341
	Shrimp unable to molt or die while molting	1. Stress (e.g., Poor water quality or disease)	222, 237–239, 331–334, 336–341
		2. Shell fouling	
II. Behavioral	Uneaten feed	1. Overfeeding	84, 173, 189, 192, 331, 334
		2. Loss of appetite owing to poor water quality or disease	222, 230, 237–239, 331–334, 336–341
	Many shrimp gathered under belt feeders or rapidly surfacing when feed is added	1. Poor feed distribution	89, 171, 192, 336
		2. Underfeeding	166, 174, 336
	Corkscrewing or Erratic Swimming	Infections such as Vibriosis	230, 237–239, 324, 336
	Extended surface swimming (Piping)	1. Poor water quality	223, 234, 331–334, 339
		2. Gill fouling	150, 222, 321, 334, 340
		3. Disease	237–239, 340
	Excessive jumping	1. Poor water quality	331–334
		2. Disease	237–239, 340–341
	Lethargy	1. Poor water quality	331–334
		2. Disease	297, 300–301, 237–239, 340–341

TABLE 12.1 Shrimp Health Summary—cont'd

	Observation	Possible Causes	Suggested Actions (Page #)
Growth	Shrimp gathered around aeration/oxygenation devices (Hanging)	1. Poor water quality	331–334
		2. Disease	237–239, 340–341
	Tail cramping	1. Stressors such as handling during periods of high temperature	156, 174, 223, 336, 338–339
		2. Mineral imbalance such as high manganese or low potassium	39–40, 54, 127, 143, 223, 334, 341
	Slow growth and high FCR	1. Poor water quality	331–334
		2. Underfeeding	165, 174, 341
		3. Disease	237–239, 336–337
	High size variation	1. Variation at stocking	153–155, 166, 167, 174, 300, 338, 341
		2. Underfeeding	
		3. Inadequate feed distribution	89, 172, 192, 339
		4. Inappropriate feed sizes	166–171, 337
		5. Genetic growth differences	153, 190, 337–338
		6. Diseases such as hemocytic enteritis or IHNV	227, 237–239, 338

(Based on Clifford, H.C., Cook, H.L., 2002. Disease management in shrimp culture ponds – Part 3. *Aquac. Mag.* 28 (4), 29–39; Robertson, C. (Ed.), 2006. *Australian Prawn Farming Manual- Health Management for Profit. The State of Queensland, Department of Primary Industries and Fisheries, Brisbane, Queensland, Australia.*)

Pfiesteria piscicida and *Gymnodinium* sp., reduce growth and are hazardous to human health (Leffler and Brunson, 2014). The Texas A&M-AgriLife Research Mariculture Lab (ARML) system has had problems at times with various *Vibrio* spp. and *Fusarium* spp.

The following are the more common diseases that may afflict a shrimp crop. They do not occur everywhere and are unlikely to be encountered in biofloc systems if biosecurity procedures are followed. Diseases observed in shrimp are based on Brock and LeaMaster (1992), Lightner (1996), Robertson (2006), Alday-Sanz (2010), Taw

(2010), and FAO (2013). For a complete description of shrimp pathogens and diagnostic procedures, see Lightner (1996).

12.2.1 Viral Diseases

- Infectious Hypodermal and Hematopoietic Necrosis (IHHN)/Runt Deformity Syndrome (RDS) (Fig. 12.7).
 - Agent: Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) (Brevidensovirus genus, Parvoviridae family).



FIG. 12.7 Preserved juvenile *L. vannamei* showing signs of IHHNV-caused runt deformity syndrome: bent rostrums (left) and deformity of the tail muscle and 6th abdominal segment (right). (Lightner, D.V. (Ed.). 1996. *A Handbook of Pathology and Diagnostic Procedures for Diseases of Penaeid Shrimp*. World Aquaculture Society, Baton Rouge, LA, USA. Used with permission.)

- Life stages affected: Larvae—early juvenile.
- Clinical signs: Bent or deformed rostrum, wrinkled antennal flagella, rough/deformed cuticle, deformed 6th abdominal segment, increased size variation (CV 30%–50%) leading to many exceptionally small individuals.
- Diagnosis: Clinical signs, historical occurrence of virus, histopathology, in situ hybridization, or identification using PCR (Polymerase Chain Reaction).
- Treatment: None available.
- Prevention and Control: Disinfection and biosecurity protocols.
- Taura Syndrome (Fig. 12.8)
 - Agent: Taura Syndrome Virus (TSV) (Aparavirus genus, Dicistroviridae family).
 - Life stages affected: Juvenile to adult.
 - Clinical signs: Pale red body and tail fan, lethargy, soft shell, melanized cuticular (buckshot) lesions.
 - Diagnosis: Clinical signs, historical occurrence of virus, microscopic evaluation, in situ hybridization, Reverse transcriptase (Rt)-PCR.
 - Treatment: None available.
 - Prevention and Control: Use Taura-resistant stock, disinfection, biosecurity.
- White Spot Disease/Syndrome (Fig. 12.9)
 - Agent: White Spot Syndrome Virus (WSSV) (Whispovirus genus, Nimaviridae family).
 - Life stages affected: Juvenile to adult.
 - Clinical signs: pink-red coloration, loose cuticle, white spots inside carapace.
 - Diagnosis: Clinical signs, historical occurrence of virus, microscopic evaluation (trypan blue/eosin wet mounts, hemolymph smears), histopathology, in situ hybridization, or PCR.
 - Treatment: None available.
 - Prevention and Control: Use SPF stock, disinfection, and biosecurity, exclude potential carriers such as crabs.



FIG. 12.8 Juvenile *L. vannamei* showing signs of Taura syndrome: red (dark gray in print version) tail fan with rough edges on the cuticular epithelium of uropods (left) and multiple melanized cuticular lesions (right). (Lightner, D.V. (Ed.). 1996. *A Handbook of Pathology and Diagnostic Procedures for Diseases of Penaeid Shrimp*. World Aquaculture Society, Baton Rouge, LA, USA. Used with permission.)



FIG. 12.9 Juvenile *L. vannamei* showing signs of white spot disease: distinctive white spots, especially on the carapace and rostrum (left and bottom right) or pink (light gray in print version) to red-brown (dark gray in print version) discoloration (top right). (Lightner, D.V. (Ed.). 1996. *A Handbook of Pathology and Diagnostic Procedures for Diseases of Penaeid Shrimp*. World Aquaculture Society, Baton Rouge, LA, USA. Used with permission.)



FIG. 12.10 *P. monodon* showing signs of yellow head disease (YHD): Yellow (light gray in print version) to yellow-brown (dark gray in print version) discoloration of the cephalothorax and gill region. Three shrimp with (left) and without (right) YHD. (Lightner, D.V. (Ed.). 1996. *A Handbook of Pathology and Diagnostic Procedures for Diseases of Penaeid Shrimp*. World Aquaculture Society, Baton Rouge, LA, USA. Used with permission.)

- Yellow Head Disease (YHD) (Fig. 12.10)

- Agent: Yellow-head virus (*Okavirus* genus, *Roniviridae* family).
- Life stages affected: Juvenile to adult.
- Clinical signs: Yellow, swollen cephalothorax, gills discolored (white/yellow/brown/ink), pale yellow enlarged hepatopancreas, pale body.
- Diagnosis: Clinical signs, historical occurrence of virus, microscopic evaluation, histopathology, Rt-PCR.
- Treatment: None available.
- Prevention and Control: Disinfection and biosecurity protocols, use SPF broodstock.

12.2.2 Bacterial

- Vibriosis (Fig. 12.11)

- Agent: The most common in shrimp are *V. anguillarum*, *V. alginolyticus*, *V. cholerae*,

V. damsela, *V. harveyi*, *V. parahaemolyticus*, *V. splendidus*, and *V. vulnificus*.

- Life stages affected: All.
- Clinical signs: Pink-red legs, uropods, and gills; extended surface swimming, corkscrewing, lethargy, loss of appetite, white/red tail muscle, and black lesions.
- Diagnosis: Clinical signs, histology, large numbers of *Vibrio* in hemolymph, growth of colonies from hemolymph or hepatopancreas samples on TCBS, RambaCHROM or general marine agar plates. Confirmation by API analysis or DNA sequencing.
- Treatment: Antibiotics (not recommended), bacteriophages (underdevelopment to target particular species), water and system disinfection postharvest.
- Prevention and Control: Hygiene and disinfection, minimize stress, probiotics and prebiotics, boost immunity, maintain good nutrition.



FIG. 12.11 *P. monodon* (left) and *L. stylirostris* (right) with signs of vibriosis. Septic hepatopancreatic necrosis caused by *Vibrio* (left). Shrimp on far right is normal, other three have pale red discoloration (especially legs), and atrophied, pale-white hepatopancreas. Bacterial shell disease caused by *Vibrio* indicated by melanized lesions (right). (Lightner, D.V. (Ed.). 1996. *A Handbook of Pathology and Diagnostic Procedures for Diseases of Penaeid Shrimp*. World Aquaculture Society, Baton Rouge, LA, USA. Used with permission.)

- Early Mortality Syndrome (EMS)/Acute Hepatopancreatic Necrosis Syndrome (AHPNS) (Fig. 12.12)
 - Agent: A strain of *V. parahaemolyticus* infected by a phage; occurs at higher pH.
 - Life stage affected: Juveniles.
 - Clinical signs: Pale and hardened hepatopancreas of reduced size, empty gut, soft or loose shell, pale coloration, lethargy.
 - Diagnosis: Clinical signs, histology of hepatopancreas, PCR.
 - Treatment: Possible phage therapy.
 - Prevention and Control: Disinfection and biosecurity, minimize stress, probiotics and prebiotics, boost immunity, feed additives to reduce gut pH.

12.2.3 Fungal

- *Fusarium* Disease/Black Gill Disease/Fusariosis (Fig. 12.13)
 - Agent: *Fusarium* spp., including *F. solani* and *F. moniliforme*.
 - Life stages affected: All, but older shrimp are more vulnerable.
 - Clinical signs: Ulcerated, raised melanized lesions; black gills and white coloration of the outer layer of the eyeball.
 - Diagnosis: Microscopic examination, histopathology, growth on mycological media.
 - Treatment: None available.
 - Prevention and control: Thorough disinfection between crops, avoid accumulation of organic matter on tank bottom, harvest at smaller size.



FIG. 12.12 Shrimp mortalities following EMS outbreak in Mexico in 2012. (Photo by Paul Frelier. Used with permission.)



FIG. 12.13 Subadult *Farfantepenaeus californiensis* (left) and *Litopenaeus vannamei* (right) showing signs of *Fusarium* disease: black, melanized lesions on the gills (left) and prominent protruding lesion (right). (Lightner, D.V. (Ed.). 1996. *A Handbook of Pathology and Diagnostic Procedures for Diseases of Penaeid Shrimp*. World Aquaculture Society, Baton Rouge, LA, USA. Used with permission.)

12.2.4 Parasites (Protozoans)

- Intestinal gregarines (Fig. 12.14)
 - Agent: *Nematopsis* sp.
 - Life stage affected: Predominantly juveniles.
 - Clinical signs: Heavy infections cause yellow discoloration of midgut, reduced growth and survival.



FIG. 12.14 *L. vannamei* postlarva with trophozoites of the gregarine *Paraophioidina scoleoides* in the midgut. (Lightner, D.V. (Ed.). 1996. *A Handbook of Pathology and Diagnostic Procedures for Diseases of Penaeid Shrimp*. World Aquaculture Society, Baton Rouge, LA, USA. Used with permission.)

- Diagnosis: Microscopic examination of the midgut intestine.
- Treatment: Some anticoccidial drugs added in feed (this treatment now questioned).
- Prevention and Control: Disinfection and biosecurity, exclude molluscs and birds.
- Microsporidiosis (cotton shrimp) (Fig. 12.15)
 - Agent: *Ameson* spp., *Agmasoma* spp., and *Pleistophora* spp.
 - Life stages affected: Juvenile to adult.
 - Clinical signs: Depending on the microsporidian species, opaque/white muscle; enlarged opaque/white gonads; dark blue to black body discoloration; and white swelling of gills, cuticle, and appendages.
 - Diagnosis: Appearance of infected organs, microscopic examination to confirm presence of microsporidian spores.
 - Treatment: None available.
 - Prevention and Control: Disinfection and biosecurity. Exclude carrier fish.



FIG. 12.15 *Litopenaeus setiferus* (left) and juvenile *L. vannamei* (right) with signs of cotton shrimp disease. Normal shrimp (bottom left) compared to "cottony" striated muscles and blue-black cuticle of shrimp infected with *Ameson* sp. (Lightner, D.V. (Ed.). 1996. *A Handbook of Pathology and Diagnostic Procedures for Diseases of Penaeid Shrimp*. World Aquaculture Society, Baton Rouge, LA, USA. Used with permission.)

12.3 DISEASE CONTROL

Preventing pathogens from entering a facility always is preferable to treating an infection. Various management practices and products are available for disease prevention and control.

12.3.1 Biosecurity

Biosecurity is defined by a set of strategies that reduce risk of aquatic pests and infectious diseases to an acceptable level in the facility and its immediate surroundings. The aim is to manage (Yanong and Erlacher-Reid, 2012):

- Stock: obtaining high quality, healthy PL and optimizing their health and immunity through good husbandry practices that minimize stress
- Pathogens: preventing, reducing, or eradicating them
- People: educating staff and controlling visitors

Farms should be constructed away from processing plants, as these often process wild and farmed shrimp from regions that may contain viruses.

There are five major pathways of pathogen introduction into a shrimp facility:

1. Infected water
2. Infected shrimp (broodstock, PL)
3. Normal host carriers (other crustaceans, such as crabs)
4. Nonhost carriers (animals such as birds, insects, raccoons, and people)
5. Nonliving objects (aerosols, wet feeds, equipment, and vehicles).

Biosecurity of high-density biofloc systems is significantly greater than standard outdoor ponds because they are indoors and operate with limited (or no) water exchange. Even so, these operations must implement a plan that protects stock and the immediate area against disease transfer, environmental degradation,

and loss of genetic diversity (Horowitz and Horowitz, 2001, 2003). Production of Pacific White Shrimp in regions where this species is not native also requires approval of a biosecurity management plan that identifies risks and details response protocols. This requires addressing the following topics for each part of the facility:

12.3.1.1 Translocation

These dictate the movement of shrimp into and within the facility. Poor PL quality and inadequate hatchery biosecurity can compromise the entire production process. Purchase PL from a hatchery with certified SPF (Specific Pathogen Free) stock, a good reputation in the industry, and ideally near the production facility. The hatchery should regularly test broodstock and PL for disease and have a health history for the major pathogens available. Prevent shrimp from escaping into the surrounding environment during transport and stocking. Upon arrival, evaluate new PL as described in Section 8.3 and acclimate them in nursery tanks apart from the main production facility. New stock, transport water, and any transport materials should not come into contact with any active culture tanks.

Carefully discard any packaging and transport water. Only move shrimp in one direction within the culture cycle: that is, from nursery to grow-out tanks, never *vice versa*. Do not allow shrimp, transport water, or related equipment (such as nets) to come into contact with other culture tanks when moving shrimp from one tank to another—a fish pump facilitates this. Design and operate the facility under the general principle that shrimp enter through one door and leave through another. Avoid mixing shrimp from different sources or cohorts during production cycles. In facilities with partial harvesting, all equipment must be disinfected after each harvest and stored for the next. Minimize stress during translocation by limiting handling and maintaining good water quality, particularly DO.

12.3.1.2 Sanitation

Sanitation procedures (Section 6.2) include removing and disposing dead shrimp, protocols for movement of staff and equipment between different sections of the facility, and types and concentrations of disinfectants. Maintaining a clean and tidy environment around the culture facility helps control pathogens and pests. This involves regularly cleaning the freeboard of culture tanks, immediately cleaning and disposing any spilled feed, rinsing equipment (such as buckets) between uses, and regularly emptying rubbish bins.

12.3.1.3 Escape Prevention

The facility must be designed and operated to prevent introduction of potentially invasive species into the surrounding environment and the possible spread of pathogens. This clearly is more important in Atlantic and Gulf coastal areas of the United States where *L. vannamei* is not native.

All discharge pipes must have screens with a mesh size that contains shrimp of all sizes. Drainage and harvest sumps for each system are advisable for managing discharge. Sump outlets are screened to limit shrimp movement. Screens should be removable for regular cleaning and inspection. In addition, the design should allow for natural events, such as flooding and tropical storms. For example, maintain sufficient freeboard (at least 30 cm) in any outdoor ponds or wetlands used for water treatment to prevent overflow during heavy rain.

All culture tanks should maintain sufficient freeboard and be surrounded by netting to prevent shrimp from jumping out of culture tanks and perhaps even jumping into an adjacent tank. Different states may have different requirements for culture of nonnative species. The Texas Parks and Wildlife Department, for example, requires *L. vannamei* farms to install three screens at the point of effluent discharge to receiving streams.

12.3.1.4 Excluding Pathogens

Probably the most important pathway for pathogen contamination is incoming water. Pathogens may be present because of natural hosts or effluent from a contaminated source. Treating water with disinfectants prior to use reduces the likelihood of contamination.

To reduce animal carriers—hosts such as crabs and scavengers on dead hosts—screen, filter, and treat incoming water with chemicals or heat (see Chapter 6). Groundwater or subsurface pumps reduce the likelihood of introducing water-borne pathogens. If possible, dry and clean water supply canals annually and exclude fish from these canals.

Other essential measures are thorough cleaning and disinfection of culture tanks and equipment, stocking only pathogen-free PL, and restricting movement through culture areas. Terrestrial predators and scavengers, such as rodents, birds, insects, and (in our area) raccoons (Fig. 12.16) also must be excluded.

Pathogen control is further enhanced by restricting use of equipment—nets, sample jars, buckets, mixing poles, water quality probes—to individual production sections (nursery, grow-out). Larger facilities should store and manage nursery and grow-out feeds separately and have



FIG. 12.16 Scavengers such as raccoons and other pests must be excluded from culture and feed storage areas to prevent predation on shrimp and disease introduction.

dedicated staff to operate each section of the facility, if possible.

Dead shrimp should be removed, recorded, and disposed daily or even more frequently. Appropriate disposal involves burial on-site or in an approved landfill, or incineration. Regulatory authorities may specify disposal requirements.

12.3.1.5 Visitors and Personnel

Movement of employees and visitors is one of the more overlooked, yet easily controlled, threats to biosecurity. Aquaculture facilities should restrict access and movement of vehicles as well as people. Clean and sanitize delivery vehicles before entry, if possible. Employees should not be allowed to visit other farms or processing plants without changing clothes and going through a disinfection process. Discourage employees from bringing live or frozen shrimp or any shrimp products onto the premises as food or bait. Similarly, any visitors, particularly if they come from another aquaculture facility, should be required to disinfect their hands and disinfect or change their footwear to reduce the risk of pathogen introduction.

Where possible, assign staff to work exclusively in specific sections of the facility (i.e., nursery or grow-out) to reduce any risk of pathogen spread between sections. Place disinfectant foot baths with chlorine or Virkon at 5–10 g/L and hand washing stations at the entrance of each culture section of the facility (Yanong, 2012).

12.3.1.6 Disease Treatment

A clearly defined Standard Operating Procedure (SOP) should be in place to address any disease outbreak. Procedures will be refined over time and tailored to deal with particular pathogens. If symptoms are observed or there is an unexplained increase in mortality, analyze the water and take shrimp samples for diagnosis (see Section 12.5). Quarantine the culture tank(s) in which the outbreak has occurred to prevent

the disease from spreading to other tanks. Quarantining individual tanks is relatively easy in limited-exchange biofloc systems because each operates independently. The following steps should be taken:

- Post signs around the affected area to alert staff that quarantine procedures are in place.
- Limit access to quarantined tank(s) to essential staff.
- Staff must use foot baths and wash hands, preferably with 70% ethanol (spray or gel), before and after contact with the affected tanks and shrimp.
- Ensure that equipment in the affected tank(s) is designated *only* for use in those tanks or is thoroughly disinfected before use in other tanks.
- Increase the frequency of water quality monitoring and observing shrimp behavior, and adjust (decrease) feeding rate. Keep thorough records.
- Control all release of solids and water from the quarantined system to ensure that nothing comes into contact with any other culture system or is released into the environment. Disinfection of water and solids may be required prior to release.
- Dead shrimp must be removed promptly (Fig. 12.17), recorded, and disposed in a prescribed manner (see Section D). A chest freezer designated for holding mortalities (in plastic bags) is appropriate prior to final disposal.
- Take samples of sick or moribund shrimp for disease identification (see Section 12.5).

Producers may be required to notify regulatory authorities of an outbreak of certain diseases. Authorities will have a disease containment protocol that may include destroying the infected crop. The World Organization for Animal Health (formerly the Office International des Epizooties) designates the following as notifiable diseases for marine shrimp (OIE, 2015):



FIG. 12.17 Molts and dead shrimp removed from a culture tank during a *Vibrio* outbreak.

- Yellowhead disease
- Infectious Hypodermal and Hematopoietic Necrosis
- Infectious Myonecrosis
- Necrotizing Hepatopancreatitis
- Taura Syndrome
- White Spot Disease

Postharvest water must be thoroughly disinfected prior to discharge or reuse, as will the culture tank and related equipment (see [Section 6.2](#)).

The biosecurity plan must be readily accessible to all staff and periodically should be reviewed and revised as needed. Train new staff in how to implement the plan. All staff should receive an annual refresher course. Install signs that detail biosecurity procedures in all areas of the facility. Meticulous record-keeping (water quality, feed consumption, growth, behavior, mortality, water treatment, inoculations, chemical use, facility access, etc.) is an essential part of biosecurity management and fosters well-informed decision-making and troubleshooting. Assign one person as the facility's biosecurity manager. A useful summary of biosecurity in aquaculture, including a template plan, is found in [Yanong \(2012\)](#) and [Yanong and Erlacher-Reid \(2012\)](#).

12.3.2 Nutrition

Nutrition has a significant impact on shrimp health. High-quality feed that meets all nutritional requirements not only improves growth and FCR, but also bolsters the immune system. Any deficiency in the feed, such as amino acids, fatty acids, vitamins, or minerals limits the ability of shrimp to combat disease ([Zhang and Mai, 2010](#)). The physical signs of specific vitamin deficiencies and toxicity are reviewed by [Zhang and Mai \(2010\)](#).

Many commercial feeds contain probiotics and immuno-stimulants (prebiotics and essential oils) that boost immune response. Research and development into additives is improving the quality and health-promoting aspects of feeds.

If storage is inadequate (open containers in a warm, humid environment) or the feed's use-by date has expired, then essential components may degrade, reducing its nutritional value. This increases susceptibility to disease and, in some cases, favor development of pathogens and parasites ([Yanong and Erlacher-Reid, 2012](#)). Stored feed should be inspected regularly for deterioration and damage to bags (see [Section 9.3](#)). Feed that is out of date, infested with vermin, rancid, or otherwise substandard must never be offered to shrimp.

Biofloc can provide a source of nutrition for shrimp and improve growth rates, but does not reduce the need for formulated feed in every case. Biofloc consumption improves shrimp immunity, particularly if probiotics are added to the system ([Crab et al., 2012](#); [Kim et al., 2014](#)).

12.3.3 Probiotics

Probiotics are beneficial microorganisms added to a tank to prevent pathogenic viruses and bacteria such as *Vibrio* spp. from becoming established ([Lakshmi et al., 2013](#); see [Section 6.5](#)). These beneficial bacteria compete

with pathogens to limit their growth, improve water quality, or improve shrimp health and immune response (Hai and Fotedar, 2010).

Probiotics are recommended in biofloc systems and are effective in controlling *Vibrio* infections in Pacific White Shrimp (Balcázar et al., 2007; Krummenauer et al., 2014). When using feeds that do not have probiotics, they can be added directly to the culture water or sprayed on feed. There are many detailed reviews of probiotics in shrimp aquaculture, including types, sources, application methods, modes of action, selection, and safety (Cruz et al., 2012; Hai and Fotedar, 2010; Lakshmi et al., 2013).

12.3.4 Prebiotics and Essential Oils

Prebiotics are indigestible feed additives that stimulate the growth and functioning of beneficial bacteria in the digestive tract (gut flora) that improve shrimp survival, growth, immune response, and stress resistance (Gatlin et al., 2006; Gatlin and Peredo, 2012; Li et al., 2009). Prebiotics can be used in conjunction with, or independent of, probiotics. They often are preferred because they are not damaged by extrusion heat during processing and require less regulatory approval than probiotics (Gatlin and Peredo, 2012). Some common prebiotics are fructooligosaccharide, transgalactooligosaccharide, 1,3 glucan, and inulin (Gatlin et al., 2006; Karunasagar et al., 2010).

Several essential oils function in a similar manner as prebiotics and have antimicrobial properties. Feed manufacturers can provide information regarding whether prebiotics and essential oils are included in their products. Reviews of prebiotics in aquaculture can be found in Gatlin et al. (2006), Yousefian and Amiri (2009), and Gatlin and Peredo (2012).

12.3.5 Vaccines

Despite having a nonspecific immune system, evidence is growing that shrimp may have some degree of immune memory (Johnson et al., 2008; Rowley and Pope, 2012). This has led to development of vaccines, particularly for WSSV and some *Vibrio* spp. strains (Lin et al., 2013).

Vibrogen-S (Aqua Health (Asia) Ltd.) is effective against Vibriosis caused by some strains of *V. parahaemolyticus* in marine shrimp. It is administered to larvae by immersion; or to broodstock and grow-out stock by injection or in feed (Tonguthai, 2000).

Aquavac Vibromax (Schering-Plough Animal Health) enhances resistance against *V. anguillarum*, *V. parahaemolyticus*, *V. vulnificus*, and *V. harveyi*. It is delivered to PL through *Artemia* nauplii (Wongtavatchai et al., 2010).

Vaccines are unlikely to prevent disease outbreaks completely and should be used in conjunction with other measures (Rowley and Pope, 2012). Johnson et al. (2008) and Rowley and Pope (2012) review vaccination theory, practice, and potential in shrimp.

12.4 DISEASE TREATMENT

Viable treatment options are limited in biofloc systems owing to cost, logistics, inadequate technology, and general ineffectiveness. Major outbreaks usually are handled with quarantine to prevent disease spread or early harvest. Prevention always is the best approach. FDA-approved aquaculture drugs, including those for treatment of disease, are found in FDA (2011).

12.4.1 Antibiotics

Several antibiotics are approved for narrow use in aquaculture to control bacterial infections, including Vibriosis, but none is approved for

shrimp in the United States. Antibiotics prohibited for use in aquaculture in some other countries include chloramphenicol, dimetridazole, ipronidazole, other nitroimidazoles, nitrofurans, fluoroquinilones, and glycopeptides (FDA, 2011).

Antibiotic use raises several issues:

- They encourage antibiotic-resistant bacterial strains
- Broad spectrum drugs (oxytetracycline) target beneficial bacteria as well as pathogens
- Antimicrobial residues may remain in shrimp, biofloc, and water, contaminating the environment and affecting human health
- Marketing is compromised if antibiotics are used at any stage of production.

We suggest avoiding antibiotics in shrimp culture. Instead, emphasize biosecurity and disease prevention (Bermúdez-Almada and Espinosa-Plascencia, 2012).

For more detailed discussion of antibiotics in aquaculture, see Bermúdez-Almada and Espinosa-Plascencia (2012) and Romero et al. (2012).

12.4.2 Phage Therapy

Phage therapy uses viruses called bacteriophages that infect only specific bacteria (Lakshmi et al., 2013). When target bacteria increase, the phages also increase (Karunasagar et al., 2010). Infecting only specific pathogenic bacteria—and not harming beneficial bacteria—thus provides a means of disease control.

Recent research has focused on controlling *V. parahaemolyticus* and *V. harveyi* with phages of the families Siphoviridae and Myoviridae (Karunasagar et al., 2010). These viral phages are very effective in the early stages of infection, before pathogenic bacteria are well established.

12.5 SAMPLE PREPARATION FOR DISEASE DIAGNOSES

Shrimp samples sent to a laboratory for disease diagnosis must be prepared according to a specific diagnostic technique. Proper sample fixation and storage are important for the preparation and accurate interpretation of microscopic slides (Lightner, 1996).

Before sending samples, contact the laboratory to learn the specific requirements for sample preservation (live, fixed, or on ice) and sample size. Communicate all background information, such as mortality patterns and chemical treatments, which may help the diagnostician.

For bacteriological analysis or when the sender is unsure about which tests should be run, a live sample is best. In this case, put shrimp in oxygenated double plastic bags, place the bags in a Styrofoam box, tape it securely, and pack it in a labeled cardboard box for overnight shipping.

For molecular identification of viruses (PCR analysis), place samples in 90%–95% ethanol, depending on lab requirements. For PL, the entire animal can be placed in ethanol. For juveniles and adults, clipped pleopods are usually sufficient. Sample containers should be tightly sealed with paraffin or tape and bagged to prevent leaking during shipping. Label the container with tank information (use a pencil, as alcohol will remove any pen or marker notes). For histological analysis, collect moribund (near death) shrimp and fix as soon as possible to obtain an accurate representation of the disease-related physical symptoms (Appendix III). Tissues such as the hepatopancreas undergo rapid deterioration after death, resulting in tissue structure being lost quickly. Sample live shrimp when possible. If recently dead shrimp are sampled, estimate the time since death (Lightner, 1996).

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Economics of Super-Intensive Recirculating Shrimp Production Systems

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This section covers several important issues related to the economic feasibility of the super-intensive, biofloc-dominated system described in previous chapters. These include:

- (1) Enterprise budgeting as a flexible tool to evaluate the economic feasibility of a super-intensive recirculating shrimp production system
- (2) Description and explanation of a bio-economic model for those considering developing a business plan or wanting to conduct an alternative scenario analysis
- (3) Capital investment examples for design, materials, construction, and economies of scale
- (4) Factors affecting cost of production and their impact on financial viability
- (5) Economic analysis of 2013 and 2014 trials at the Texas A&M-AgriLife Research Mariculture Lab (ARML)
- (6) General marketing principles and sensitivity analyses
- (7) Conclusions.

13.1 ENTERPRISE BUDGETING

Enterprise budgeting can be applied to develop future projects and analyze data from completed crops. Planning a project requires more assumptions and budgets that often are created with formulas for production, feed, and other inputs. Outputs include production quantity and the variable, fixed, and investment costs needed to analyze profit potential. In the latter case, actual quantities of production inputs and capital investment costs are used to develop the budget and economic analyses.

A combination of the two approaches can be applied to data from smaller research trials and then extrapolated to a commercial-scale. This is the approach taken over the last several years to analyze the economics of research conducted at the Texas AgriLife Mariculture Research facility (Hanson et al., 2007, 2014, 2015; Hanson and Posadas, 2004, 2005).

An enterprise budget quantifies and values all production inputs in relation to the quantity of shrimp produced and sold. Subtracting production costs from receipts provides an estimate

of net return. Investors also want to know overall capital investment and total production costs for one or multiple shrimp crops per year and over several years.

Total costs are divided into variable (operating) costs and fixed costs. Variable costs vary during the production cycle; fixed costs do not, but can change over longer time periods.

Economic measures of profitability, sensitivity analysis, and cost of production are calculated from the base enterprise budget. This provides additional information for development of multi-year cash flows used to calculate financial profitability, such as the net present value (NPV), the internal rate of return (IRR), and the payback period.

The main components of an enterprise budget are: (a) receipts, (b) variable costs, (c) income above variable costs, (d) fixed costs, (e) total costs (variable plus fixed), and (f) net returns. A breakeven price is often included to quickly see the minimum selling price at which variable and/or total costs are covered.

13.1.1 Receipts (Sales Revenue)

Quantify the value of shrimp sold. In practice, there may be multiple sales outlets and multiple shrimp sizes that are sold. In that case, there are several receipt lines, each indicating the quantity and price per outlet and product form (see [Section 13.6](#) for information on shrimp pricing).

The following formulas are used to calculate the quantity and value of shrimp produced annually when developing an enterprise budget:

$$\begin{aligned} \text{Total annual production} \\ = \text{grow} - \text{out area} \times \text{initial stocking density} \\ \times \text{survival rate} \times \text{harvest size} \\ \times \text{number of crops per year} \end{aligned} \quad (13.1)$$

$$\begin{aligned} \text{Gross receipts} &= \text{total annual production} \times \text{farm} \\ &\quad - \text{gate price} \end{aligned} \quad (13.2)$$

$$\begin{aligned} \text{Number of crops per year} \\ = (\text{weeks facility is in operation in a year} \\ \times 7 \text{ days/week}) / (\text{length of crop grow} \\ - \text{out cycle} + \text{period between} \\ \text{production cycles}) \end{aligned} \quad (13.3)$$

$$\begin{aligned} \text{Length of crop grow} - \text{out cycle} \\ = (\text{final weight} - \text{initial weight}) / \text{growth rate} \end{aligned} \quad (13.4)$$

13.1.2 Variable Costs

Represent resources expended to complete a production cycle. Typical items include postlarvae (PL), nursery and grow-out feeds, water to fill the raceway and replace losses, electricity for pumps, oxygen, fuel, sodium bicarbonate, management, labor, and short-term loans to pay for inputs until harvest.

An item's unit price times the quantity used is the variable cost for that item. Following formulas can be used to calculate the total quantity of shrimp produced, duration of the production cycle, and grow-out/nursery feed requirements. Individual costs are summed:

$$\begin{aligned} \text{Variable costs} &= \text{costs of PL} + \text{feed} \\ &\quad + \text{labor} + \text{chemicals} + \text{electricity} \\ &\quad + \text{fuel} + \text{miscellaneous} \end{aligned} \quad (13.5)$$

13.1.2.1 PL Cost

$$\begin{aligned} \text{Annual PL requirements (in 1000s)} \\ = (\text{nursery tank area} \times \text{post} \\ - \text{larvae stocking density} / 1000) \\ \times \text{number of nursery crops per year} \end{aligned} \quad (13.6)$$

$$\begin{aligned} \text{PL cost} &= \text{annual PL requirements (in 1000s)} \\ &\quad \times \text{PL cost (\$/1000)} \end{aligned} \quad (13.7)$$

$$\begin{aligned} &\text{Number of nursery crops per year} \\ &= (\text{number of operating weeks per year} \\ &\quad \times 7 \text{ days per week}) / (\text{days in a nursery crop} \\ &\quad + \text{days between crops}) \end{aligned} \quad (13.8)$$

$$\begin{aligned} &\text{Days in a nursery crop} = \\ &(\text{final weight} - \text{initial weight}) / \\ &(\text{growth weight per week} / 7 \text{ days per week}) \end{aligned} \quad (13.9)$$

13.1.2.2 Feed Costs

$$\begin{aligned} &\text{Nursery feed required per greenhouse (lb)} \\ &= (\text{PL stocking density} / \text{m}^2 \times \text{area of raceway,} \\ &\quad \text{m}^2 \times \text{juvenile harvest size, g} / 1000) \\ &\quad \times \text{feed conversion ratio} \\ &\quad \times \text{number of nursery raceways per} \\ &\quad \text{greenhouse} \times \text{number of nursery} \\ &\quad \text{crops per year} \times 2.205 \text{ lb/kg} \end{aligned} \quad (13.10)$$

$$\begin{aligned} &\text{Nursery feed cost} = \text{nursery feed required, lb} \\ &\quad \times \text{cost per lb of larval diets} \end{aligned} \quad (13.11)$$

$$\begin{aligned} &\text{Grow-out feed required per raceway per crop} \\ &= \text{initial stocking density} \times \text{survival rate} \\ &\quad \times (\text{harvest size} - \text{stocking size}) \\ &\quad \times \text{grow-out area per raceway} \\ &\quad \times \text{feed conversion ratio} \end{aligned} \quad (13.12)$$

$$\begin{aligned} &\text{Grow-out feed required per greenhouse per crop} \\ &= \text{grow-out feed required per raceway per crop} \\ &\quad \times \text{number of rearing raceways per greenhouse} \end{aligned} \quad (13.13)$$

$$\begin{aligned} &\text{Grow-out feed required per greenhouse per year} \\ &= \text{grow-out feed required per greenhouse per crop} \\ &\quad \times \text{number of crops per year} \end{aligned} \quad (13.14)$$

$$\begin{aligned} &\text{Grow-out feed cost} \\ &= (\text{grow-out feed required per greenhouse} \\ &\quad \text{per year} / 2000 \text{ lb/ton}) \times \text{feed cost per ton} \end{aligned} \quad (13.15)$$

13.1.2.3 Labor and Management Requirements

Are calculated based on the extrapolated size of the operation. An example table to determine labor and management costs would include position titles, number employed at each position, and annual salary (or wage) plus benefits. [Table 13.1](#) is a template that can be used in spreadsheets to compute labor and management expenses.

13.1.2.4 Electricity

Is a variable cost item because it is based on the number of devices using electricity (blowers, pumps, lights, fans, etc.), their horsepower, kilowatt usage, and hours of use per day. [Table 13.2](#) is a template that can be used in spreadsheets to compute electrical expenses.

13.1.2.5 Other Variable Costs

For items such as fuel, water, chemicals, and sludge removal are calculated with formulae based on the quantity used multiplied by their per-unit price. Costs of items such as hatchery supplies are figured in a like manner and then summed into one value that is entered into the enterprise budget. Telephone charges are monthly and can be estimated by contacting the service provider. General liability insurance and property taxes vary by location and must be researched by contacting insurance companies and local tax assessors.

13.1.3 Income Above Variable Cost

Is a short-term financial indicator of profitability. It is calculated by subtracting all variable costs from receipts. This value represents the

TABLE 13.1 Template for Calculating Staffing, Salary, and Wages for a Shrimp Production Facility

Position Title	Number	Annual Salary (\$)	Total (\$)	Total Salaries (\$)	Total Wages (\$)	Qualifications and Comments
Chief Operating Officer	1	75,000	75,000	75,000		
Bookkeeper	0	30,000	0		0	
Secretary/office manager	1	18,000	18,000		18,000	
Production manager	0	60,000	0	0		BA or MSc in biology; 5 years of experience in shrimp production systems desired.
Senior biologists	1	40,000	40,000	40,000		
Biologist	0	30,000	0	0		BSc in biology and some shrimp experience.
Hourly workers	2	16,640	33,280		33,280	High School diploma
Lab manager	0	40,000	0	0		MSC and 5 years of experience in quality control lab systems, water quality analysis, seafood safety or related areas.
Lab technician	0	25,000	0	0		2 year technical degree in biology or chemistry
Maintenance coordinator	0	40,000	0	0		Good hands-on person with 10 years electrical and plumbing experience.
Maintenance workers	0	25,000	0		0	Some experience and technical degree.
Fringe benefits (22.5%)			37,413	25,875	11,538	
Total production system annual salaries and wages			203,693	140,875	62,818	

cash return to the operation in the short run. The short run is the period of time when few changes can be made to production, that is, no changes can be made to the facility or the equipment being used. When income above variable costs is positive, the operation is viable in the short run; when it is negative, the operation should shut down to avoid further losses.

Any shutdown decision is, of course, tempered by the knowledge that one must allow sufficient time to correct any issues in getting the system up and running. Depending on the complexity of the operation and especially on the experience of personnel, it can take a year or more to implement the best procedures for efficient operation.

TABLE 13.2 Template for Determining Electrical Costs for Typical Machinery Items Used in a Greenhouse Shrimp Production Facility

Greenhouse Electrical Usage Component	hp	kW	Quantity	Hours Used/d	Fraction of Year (%)	kWh/d	Energy Use kWh/yr ^a
Recycle pump ^a	2	6	2	24	72.29	208	75,991
Air blower	7	2.6	1	24	96.39	60	21,953
Heat pumps	–	5.9	16	24	19.00	430	157,119
GH Lights	–	0.08	50	6	100.00	24	8760
Mechanical building lights	–	0.08	15	8	100.00	10	3504
Exhaust fans—Winter	1	0.75	41	8	30.00	74	26,937
Exhaust fans—Summer	1	0.75	41	24	70.00	517	188,559
GH inflator fans	0.25	0.1875	8	24	100.00	36	13,140
Total electrical energy use ^b						1359	495,963
Cost/kWh		\$0.08			Total Annual Energy Cost		\$39,677

^a Formula example: recycle pump energy used per year = 6 kW × 2 units × 24 h/d usage × 365 d/yr × 0.7229 = 75,991 kWh/yr.

^b Includes heating costs.

The formula is : Income above variable costs
= Gross receipts – Variable Costs
(13.16)

13.1.4 Fixed Costs

Are incurred even if there is no production. These include capital items that have been constructed or purchased and their associated expenses, such as depreciation, loan interest, repairs, taxes, and insurance. Some are cash costs and others are noncash costs that represent resource usage of a type not usually valued in cash amounts, such as depreciation. Noncash items are included in enterprise budgeting to account for all resources used in the creation and running of the facility. Depreciation of facilities, machinery, and equipment covers the value of wear and tear accumulated over a production cycle and eventual replacement.

It can be calculated many ways and is beyond the scope of this chapter. Methods can be found online or in microeconomic textbooks (Colander, 2006; Jolly and Clonts, 1993; Kay and Edwards, 1994).

The formula for total fixed costs is as follows:

$$\begin{aligned} \text{Fixed costs} = & \text{costs of depreciation} \\ & + \text{loan interest} + \text{repairs/} \\ & \text{maintenance} \\ & + \text{insurance} + \text{taxes} \end{aligned} \quad (13.17)$$

13.1.5 Total Costs

The sum of variable and fixed costs represents the true cost of producing a shrimp crop. The formula is as follows:

$$\text{Total costs} = \text{Variable costs} + \text{Fixed costs} \quad (13.18)$$

13.1.6 Net Returns Above All Costs (Variable Plus Fixed)

Is a long-term indicator of profitability calculated by subtracting total costs from receipts. It represents the true profitability of the enterprise in the long run, a time period that allows all items to be changed as needed to achieve a more profitable situation. Net returns is calculated as follows:

$$\begin{aligned} \text{Net returns above all costs} \\ = \text{Gross receipts} - \text{Total costs} \end{aligned} \quad (13.19)$$

When it is positive, the operation covers all cash and noncash costs and is profitable. A zero or positive net return is the measure for acceptance of an operational business plan; it represents a good investment. When it is negative, the operation must adapt to remain in business in the long run. The operation can, however, continue to operate in the short run if income above variable costs is positive because operating (short-term) costs are covered. In the long run, short- and long-term indicators should be positive.

Net returns above all variable and fixed costs traditionally represent the return to one or more resources, such as land, labor, capital, or management. When a net return is calculated for one or more of these resources, the value of the resource(s) is (are) not valued within the enterprise budget. For example, an enterprise budget based on net return to land does not include a charge for land. This is because all receipts and expenses are attributed to the land that supports production. Land cost is not forgotten, but is included as part of the initial investment. Also note that, in enterprise budgets, a net return to land—not a net return to land, labor, and management—is calculated. Charges for labor and management thus are included in the budget. When all land, labor, and management costs are included and noncash items are excluded, the results are a financial (not economic) measure of profitability.

13.2 BIO-ECONOMIC MODEL

Developing a detailed and realistic feasibility analysis requires a multidisciplinary team of people knowledgeable in shrimp nursery and grow-out production, system design and construction, and financial budgeting and analysis. Location-specific information is needed to find a suitable site for a commercial venture. Site-specific factors for the feasibility study include knowledge of local regulatory issues, local input availability and costs, shipping costs for nonlocal items, availability of seawater, land costs, and available infrastructure. Climatic factors affect building design, equipment, and fuel needs. A change in climate zone thus will change profitability. Even high production costs, however, can be overcome if inland sites allow for a value-added sale price in local markets.

Knowledge of historical shrimp prices and production input unit costs is needed as a basis for their variation in sensitivity analysis to determine best and worst-case scenarios. Other information required for a feasibility analysis includes land costs, sources and availability of PL, feed, energy, labor, and oxygen. The production portion of a feasibility study requires biologically realistic levels for the survival rate, nursery and grow-out stocking density, growth rates, and feed conversion efficiency. The financial portion requires sourcing greenhouse materials, equipment and machinery, local building companies for construction of the facility, and short-, intermediate-, and long-term interest rates for loans. A major determinant of feasibility is the source of capital or the mix of capital contributed by lenders and investor equity.

Spreadsheets are an excellent way to develop enterprise budgets for a business plan. One approach is to develop a detailed worksheet for each line item in the enterprise and then summarize the results in one enterprise budget worksheet. A bio-economic model developed by [Hanson and Posadas \(2004\)](#) has worksheets for biological, physical, prices/costs, and capital

investment items. Interconnected formulas automatically calculate receipts, variable and fixed costs, and measures of profitability.

13.2.1 Model Inputs

13.2.1.1 Biological Parameters

At the core of the bio-economic model are biological parameters that determine the quantity of shrimp sold and the basis for variable cost calculations. Input includes initial weight, final weight, growth rate, stocking density, survival, and FCR. [Table 13.3](#) presents this information for nursery and grow-out phases of a super-intensive recirculating shrimp production

facility. In evaluating this system, data from AgriLife trials are entered into the bio-economic model's biological parameters worksheet that drives the economic analysis.

13.2.1.2 Physical Parameters

The second set of parameters to enter into the bio-economic model are the physical parameters of the raceway and greenhouse. These include the dimensions and number of nursery and grow-out raceways per greenhouse as well as the number of greenhouses. This information is used to calculate initial investment costs and final production levels ([Hanson and Posadas, 2004](#); [McAbee et al., 2006](#)). [Table 13.4](#) presents this

TABLE 13.3 Bio-Economic Model User Input Spreadsheets, Biological Parameters to Enter

Item	Unit	Quantity
NURSERY PARAMETERS		
PL12 stocking density	PL12/m ²	405.00
Survival rate	%	80.00
Growth rate	g/wk	0.350
Stocking size	g	0.001
Desired harvest size	g	4.70
Net feed conversion	g feed/g shrimp	1.30
Length of period between cycles	d/crop	2.80
GROW-OUT PARAMETERS		
Stocking density	juveniles/m ³	324.00
Survival rate	%	93.10
Growth rate	g/wk	2.05
Stocking size	g	4.70
Desired harvest size	g	27.22
Feed conversion ratio	g feed/g shrimp	1.59
Length of grow-out crop	d	77.00
No. of grow-out crops per year	#	4.70

TABLE 13.4 Bio-Economic Model User Input Spreadsheets, Raceway and Greenhouse Physical Facility Parameters to Enter

Item	Unit	
RACEWAYS		
Rearing raceway width	ft (m)	30 (9.1)
Rearing raceway depth	ft (m)	3.7 (1.1)
Rearing raceway length	ft (m)	180 (55)
Center aisle width	ft (m)	0
Nursery raceways per greenhouse	Number	2
Grow-out raceways per greenhouse	Number	8
GREENHOUSES		
Total raceways per greenhouse	Number	10
Total greenhouses	Number	1
Greenhouse length	ft (m)	408 (124)
Greenhouse width	ft (m)	138 (42)
TOTAL REARING AREA		
Grow-out area	ft ² (m ²)	43,056 (4000)
Nursery area	ft ² (m ²)	10,764 (1000)
Subtotal	ft ² (m ²)	53,820 (5000)

information for nursery and grow-out units of the super-intensive recirculating system. These worksheets are used to determine the overall capital investment and the costs of financing all construction and capital equipment. This necessarily involves explicit consideration of intermediate- and long-term interest rates and, when applicable, the level of equity investment.

13.2.1.3 Cost-Price Parameters

The third set of parameters entered into the bio-economic model includes nursery and grow-out production inputs and their unit costs, for example, the cost per unit of all feed types, the cost per 1000 PL, the cost of specific chemicals, and so on. [Table 13.5](#) presents this information for a super-intensive recirculating shrimp production facility.

The selling price of various size categories of shrimp is also entered. The current price is easy enough to determine by probing the market or using a pricing company, such as Urner Barry, that provides this information by subscription. The best price at which to sell shrimp, however, is difficult to know and is addressed in [Section 13.6](#).

13.2.1.4 Capital Investment

A set of expenses associated with the capital investment items, including their economic life, depreciation, loan interest, and maintenance, also is required. This information, entered in [Table 13.6](#), is used to model the financing of loans, land purchase, and property tax.

After the facility's design has been determined, construction details must be addressed. Estimated costs of capital items that must be built or purchased are entered at this point. [Table 13.7](#) presents this information for the land, raceway and greenhouse systems, and equipment and machinery of a super-intensive recirculating shrimp facility.

An annual replacement spreadsheet also must be developed. The replacement values table has entries for each year of the project. The total for each year is inserted automatically into the appropriate cell of the 10-yr

TABLE 13.5 Bio-Economic Model User Input Spreadsheets, Input Unit Cost-Price Parameters to Enter

Item	Unit	Quantity
RECEIPT ITEMS		
Shrimp, whole, heads-on, selling price, avg.	\$/lb	\$3.27
VARIABLE COST ITEMS		
PL12 cost	\$/1000	\$8.00
Electricity cost	\$/kwh	\$0.08
Grow-out feed cost	\$/lb	\$0.874
NURSERY FEED COST		
Mix of larval diets	\$/lb	\$0.549
<i>Artemia</i> cysts	\$/lb	\$27.50
PL 40-9 with V-Pak 1/2 Crumble blend	\$/bag (25 kg)	\$22.89
PL 40-9 with V-Pak 2/3 Crumble blend	\$/bag (25 kg)	\$22.25
PL 40-9 with V-Pak 5/64" pellet	\$/bag (25 kg)	\$25.00
Telephone expense	\$/wk	\$50.00
Gasoline cost	\$/gal	\$3.30
Diesel cost	\$/gal	\$3.95
LIQUID OXYGEN		
Tank rental	\$/month/ 11,000 gal tank	\$1500
Liquid oxygen supply	100 ft ³ /d per greenhouse	147.84
Water, fresh	\$/1000 gallons	\$0.14
Trace minerals (water supplement)	\$/yr per greenhouse	\$10,000
Sludge removal	\$/gallon	\$15.00
Salt, Red Sea	\$/2220lb bag	\$650.00
Sodium bicarbonate	\$/lb	\$0.165

summarized cash flow statement. Net present value (NPV), internal rate of return (IRR), and payback period subsequently are calculated. [Table 13.8](#) provides information for the land,

TABLE 13.6 Bio-Economic Model User Input Spreadsheets, Capital Investment Costs

Item	Unit	Quantity
<i>CAPITAL FINANCING</i>		
Percentage of capital investment from bank	%	100
Percentage of capital from equity	%	0
<i>Investor initial operating cost contribution</i>	\$	0
<i>LOAN INFORMATION</i>		
Annual long-term capital cost	%	8.00
Length of long-term loan	yr	7
Annual intermediate-term capital cost	%	8.00
Length of intermediate-term loan	yr	7
Annual operating cost loan	%	8.00
<i>INSURANCE</i>		
Annual grow-out liability insurance	0.21% of total investment	
<i>TOTAL LAND REQUIRED FOR ENTIRE OPERATION:</i>		
Land for greenhouse	ac/operation	1.6
Land for waste treatment	ac/operation	4.0
Land for processing plant and office	ac/operation	1.0
Land cost	\$/ac	10,000
Per greenhouse	1.6 ac/ greenhouse	16,000
<i>Land preparation cost</i>	\$/ac	200
Annual property tax (a × b × c)	\$/ac	9.48
a. Land use value	\$/ac	645
b. Assessment rate	%	15
c. Millage rate	Mills	98

greenhouse, raceway, and equipment for a super-intensive recirculating shrimp production facility.

13.2.2 Model Outputs

When research data are entered, the bio-economic model calculates several useful financial tables. The first is an annualized set of intermediate- and long-term loan repayment schedules. This is presented in [Table 13.9](#) for the scenario of the preceding section. Annual payments are differentiated into interest and principal, and these are linked to the annual cash flow spreadsheets.

An enterprise budget is presented in [Table 13.10](#) for inputs from the preceding section. It provides details on calculation of receipts, variable input item costs, income above variable costs, fixed cost, total costs, and net return above all specified expenses. The cost of production and net return values are the most important and most discussed results of the enterprise budget.

The third set of tables is a ten-year annual cash flow of monthly sales and expenditures ([Table 13.11](#)). The one-year cash flow represents a single run of the ten that were generated.

Cash flow budgeting allows management to anticipate when cash surpluses and shortages may occur and this, in turn, informs decisions on paying off or acquiring debt. Like the enterprise budget, cash flow can be estimated, as is done in business plan development, or computed from actual sales/expenditures. Actual sales/spending can be compared to planned sales/spending to identify any substantial deviations; this provides management with time to make any corrections that keep the project on track. Actual cash flow budgeting provides a basis for planning the following year's cash flow budget, which then serves as a management guide.

TABLE 13.7 Investment Item Information Required for the Bio-Economic Model

Item	Total Cost per Greenhouse (\$)	Econ Life (yr)	Average Investment (\$)	Annual Depreciation (\$)	Annual Interest (\$)	Annual Repairs and Maintenance (%)	Repairs and Maintenance (\$)
A. CAPITAL COSTS							
Land for greenhouses	\$0		\$0		\$0		
Land for waste treatment, plant, and office	\$0						
GREENHOUSE COMPONENTS							
Structure	\$55,429	15	\$27,715	\$3695	\$2217	1.67	\$924
Covering	\$18,307	5	\$9153	\$3661	\$732	5.00	\$915
INTERIOR AUTOMATED ALUMINIZED SHADE SYSTEM							
Heating system	\$3743	7	\$1871	\$535	\$150	3.57	\$134
Cooling system	\$20,300	7	\$10,150	\$2900	\$812	3.57	\$725
Controls	\$2436	7	\$1218	\$348	\$97	3.57	\$87
Concrete for installation	\$14,747	20	\$7374	\$737	\$590	1.25	\$184
Prepaid freight	\$9153	20	\$4577	\$458	\$366	1.25	\$114
Installation cost	\$93,548	20	\$46,774	\$4677	\$3742	1.25	\$1169
GREENHOUSE ELECTRICAL SYSTEM							
Materials	\$5476	5	\$2738	\$1095	\$219	5.00	\$274
Labor	\$14,309	20	\$7154		\$572	1.25	\$179
RACEWAY CONSTRUCTION							
Materials	\$139,790	5	\$69,895	\$27,958	\$5592	5.00	\$6989
Labor	\$40,545	20	\$20,273	\$2027	\$1622	1.25	\$507
Equipment	\$4165	5	\$2083	\$833	\$167	5.00	\$208
Catwalk system	\$0	5	\$0			5.00	\$0
MECHANICAL AND LABORATORY BUILDING							
Materials	\$72,715	5	\$36,357	\$14,543	\$2909	5.00	\$3636
Labor	\$32,045	20	\$16,023	\$1602	\$1282	1.25	\$401
Equipment	\$6981	5	\$3491	\$1396	\$279	5.00	\$349

TABLE 13.7 Investment Item Information Required for the Bio-Economic Model—cont'd

Item	Total Cost per Greenhouse (\$)	Econ Life (yr)	Average Investment (\$)	Annual Depreciation (\$)	Annual Interest (\$)	Annual Repairs and Maintenance (%)	Repairs and Maintenance (\$)
RACEWAY HEATING SYSTEM							
Labor	\$12,205	20	\$6102	\$610	\$488	1.25	\$153
Equipment	\$72,312	5	\$36,156	\$14,462	\$2892	5.00	\$3616
MAJOR WATER TREATMENT AND CONTROL EQUIPMENT							
Labor	\$18,859	20	\$9430	\$943	\$754	1.25	\$236
Equipment	\$92,592	5	\$46,296	\$18,518	\$3704	5.00	\$4630
RACEWAY DRAINS AND HARVEST PIPES							
Materials	\$8348	5	\$4174	\$1670	\$334	5.00	\$417
Labor	\$4001	20	\$2001	\$200	\$160	1.25	\$50
WATER RETURN PIPING SYSTEM							
Materials	\$19,037	5	\$9519	\$3,807	\$761	5.00	\$952
Labor	\$7638	20	\$3819	\$382	\$306	1.25	\$95
Air supply piping system and raceway aeration							
Materials	\$10,723	5	\$5362	\$2145	\$429	5.00	\$536
Labor	\$3274	20	\$1637	\$164	\$131	1.25	\$41
FEED DELIVERY SYSTEM							
Materials	\$50,000						
Labor	\$10,000						
Hatchery evaluation laboratory and building	\$2050	5	\$1025	\$410	\$82	5.00	\$103
Effluent storage and evaporation ponds	\$0	5	\$0	\$0	\$0	5.00	\$0
Construction estimate, fencing, paving, stone, and asphalt		5	\$11,413	\$4565	\$913	5.00	\$1141
Concrete pads and installation for O ₂ tanks			\$1875	\$188	\$150	1.25	\$47

Continued

TABLE 13.7 Investment Item Information Required for the Bio-Economic Model—cont'd

Item	Total Cost per Greenhouse (\$)	Econ Life (yr)	Average Investment (\$)	Annual Depreciation (\$)	Annual Interest (\$)	Annual Repairs and Maintenance (%)	Repairs and Maintenance (\$)
Subtotal	\$921,603		\$405,653	\$114,531	\$32,452	1.25	\$28,812
B. EQUIPMENT/MACHINERY COSTS							
Hatchery equipment	\$3395	5	\$1697	\$679	\$136	5.00	\$170
Stand-by generator	\$17,000	5	\$8500	\$3400	\$680	5.00	\$850
Office equipment	\$10,000	5	\$5000	\$2000	\$400	5.00	\$500
All-terrain vehicle (golf cart w/bed)	\$5000	5	\$2500	\$1000	\$200	5.00	\$250
Large tractor	\$35,000	7	\$17,500	\$5000	\$1400	3.57	\$1250
Small tractor	\$0	7	\$0	\$0	\$0	3.57	\$0
Subtotal	\$70,395		\$35,197	\$12,079	\$2816		\$3020
Total	\$991,997		\$440,850	\$126,610	\$35,268		\$31,831

TABLE 13.8 Calculation of Initial Investment and Annual Replacement Costs

Item/year	0	1	2	3	4	5	6	7	8	9	10	SV ^a
A. CAPITAL COSTS												
Land for greenhouses	0											0
Land for waste treatment, plant, and office	0											0
GREENHOUSE COMPONENTS												
Structure	55,429	0	0	0	0	0	0	0	0	0	0	5543
Covering	18,307	0	0	0	0	0	18,307	0	0	0	0	1831
Interior automated aluminized shade system	50,297	0	0	0	0	0	0	0	0	0	50,297	5030
Thermal blanket	0	0	0	0	0	0	0	0	0	0	0	0
Heat system	3743	0	0	0	0	0	0	0	3743	0	0	374
Ventilation	0	0	0	0	0	0	0	0	0	0	0	0
Cooling systems	20,300	0	0	0	0	0	0	0	20,300	0	0	2030
Controls	2436	0	0	0	0	0	0	0	2436	0	0	244

TABLE 13.8 Calculation of Initial Investment and Annual Replacement Costs—cont'd

Item/year	0	1	2	3	4	5	6	7	8	9	10	SV ^a
Concrete for installation	14,747	0	0	0	0	0	0	0	0	0	0	1475
Prepaid freight	9153	0	0	0	0	0	0	0	0	0	0	915
Installation cost	93,548	0	0	0	0	0	0	0	0	0	0	9355
GREENHOUSE ELECTRICAL SYSTEM												
Materials	5476	0	0	0	0	0	5476	0	0	0	0	548
Labor	14,309	0	0	0	0	0	0	0	0	0	0	1431
RACEWAY CONSTRUCTION												
Materials	139,790	0	0	0	0	0	139,790	0	0	0	0	13,979
Labor	40,545	0	0	0	0	0	0	0	0	0	0	4055
Equipment	4165	0	0	0	0	0	4165	0	0	0	0	417
Catwalk system	0	0	0	0	0	0	0	0	0	0	0	0
MECHANICAL AND LAB BUILDING												
Materials	72,715	0	0	0	0	0	72,715	0	0	0	0	7271
Labor	32,045	0	0	0	0	0	0	0	0	0	0	3205
Equipment	6981	0	0	0	0	0	6981	0	0	0	0	698
RACEWAY HEATING SYSTEM												
Labor	12,205	0	0	0	0	0	0	0	0	0	0	1220
Equipment	72312	0	0	0	0	0	72,312	0	0	0	0	7231
MAJOR WATER TREATMENT AND CONTROL EQUIPMENT												
Labor	18,859	0	0	0	0	0	0	0	0	0	0	1886
Equipment	92,592	0	0	0	0	0	92,592	0	0	0	0	9259
RACEWAY DRAINS AND HARVEST PIPES												
Materials	8348	0	0	0	0	0	8348	0	0	0	0	835
Labor	4001	0	0	0	0	0	0	0	0	0	0	400
WATER RETURN PIPING SYSTEM												
Materials	19,037	0	0	0	0	0	19,037	0	0	0	0	1904
Labor	7638	0	0	0	0	0	0	0	0	0	0	764
AIR SUPPLY PIPING SYSTEM AND RACEWAY AERATION												
Materials	10,723	0	0	0	0	0	10,723	0	0	0	0	1072
Labor	3274	0	0	0	0	0	0	0	0	0	0	327

Continued

TABLE 13.8 Calculation of Initial Investment and Annual Replacement Costs—cont'd

Item/year	0	1	2	3	4	5	6	7	8	9	10	SV ^a
FEED DELIVERY SYSTEM												
Materials	50,000											
Labor	10,000											
Hatchery evaluation lab and building	2050	0	0	0	0	0	2050	0	0	0	0	205
Effluent storage and evaporation ponds	0	0	0	0	0	0	0	0	0	0	0	0
Construction Estimate, fencing, paving, stone, and asphalt	22,826	0	0	0	0	0	22,826	0	0	0	0	2283
Concrete pads and installation for O ₂ tanks	3750	0	0	0	0	0	0	0	0	0	0	375
Subtotal, capital investment	921,603	0	0	0	0	0	475,322	0	26,479	0	50,297	
B. EQUIPMENT/MACHINERY COSTS												
Feed Storage Bins (same thing as hoppers? Two 14 ton hoppers with fill pipe and auger-type dispenser per greenhouse)	0	0	0	0	0	0	0	0	0	0	0	0
Hatchery Equipment	3395	0	0	0	0	0	3395	0	0	0	0	339
Stand-by generator	17,000	0	0	0	0	0	17,000	0	0	0	0	1700
Office equipment	10,000	0	0	0	0	0	10,000	0	0	0	0	1000
All-terrain vehicle (golf cart w/ bed)	5000	0	0	0	0	0	5000	0	0	0	0	500
Large tractor	35,000	0	0	0	0	0	0	0	35,000	0	0	3500
Small tractor	0	0	0	0	0	0	0	0	0	0	0	0
Hopper for sodium bicarbonate	0	0	0	0	0	0	0	0	0	0	0	0
Miscellaneous	0	0	0	0	0	0	0	0	0	0	0	0
Subtotal, equip/machinery	70,395	0	0	0	0	0	35,395	0	35,000	0	0	
Total	991,997	0	0	0	0	0	510,717	0	61,479	0	50,297	93,200

^a SV = Salvage value; 10% used for all items.

A fourth output summarizes the ten annual cash flows. Table 13.12 and Fig. 13.1 show the initial investment as a negative in year 0 and varying positive and negative cash flows in subsequent years. Four pieces of information are required for investment analysis: (1) annual

net cash revenues, (2) initial investment, (3) salvage value of the investment, and (4) discount rate. Gross receipts and total costs come from the ten annual cash flow budgets, and the initial investment (\$991,997) comes from Table 13.7. The salvage value is derived from the

TABLE 13.9 Intermediate- and Long-Term Loan Payments of Annual Interest and Principal

Intermediate-Term Loan Terms and Annual Payment Amount							
Principal		Annual Interest Rate	Term (Years)		Periods per Year		Start Date
70,395		8.00%	7		1		1/1/2001
Periodic payment:			Number of payments:				
13,521			7				
Payment No	Month	Beginning Balance	Total Payment	Interest	Principal	Ending Balance	Cumulative Interest
1	Jan-01	70,395	13,521	5632	7889	62,505	5632
2	Jan-02	62,505	13,521	5000	8520	53,985	10,632
3	Jan-03	53,985	13,521	4319	9202	44,783	14,951
4	Jan-04	44,783	13,521	3583	9938	34,845	18,533
5	Jan-05	34,845	13,521	2788	10,733	24,111	21,321
6	Jan-06	24,111	13,521	1929	11,592	12,519	23,250
7	Jan-07	12,519	13,521	1002	12,519	0	24,251

Long-Term Loan Terms and Annual Payment Amount							
Principal		Annual Interest Rate	Term (Years)		Periods per Year		Start Date
921,603		8.00%	7		1		7/1/2001
Periodic Payment:			Number of payments:				
177,014			7				
Payment No	Month	Beginning Balance	Total Payment	Interest	Principal	Ending Balance	Cumulative Interest
1	Jul-01	921,603	177,014	73,728	103,286	818,317	73,728
2	Jul-02	818,317	177,014	65,465	111,549	706,767	139,194
3	Jul-03	706,767	177,014	56,541	120,473	586,294	195,735
4	Jul-04	586,294	177,014	46,904	130,111	456,183	242,638
5	Jul-05	456,183	177,014	36,495	140,520	315,664	279,133
6	Jul-06	315,664	177,014	25,253	151,761	163,902	304,386
7	Jul-07	163,902	177,014	13,112	163,902	0	317,498

TABLE 13.10 Enterprise Budget (Receipts, Variable Costs, Fixed Costs, Net Returns to Land) and Breakeven Prices for a Super-Intensive Shrimp Production System Consisting of Ten Greenhouses (Eight Grow-Out Raceways per Greenhouse and Two Nursery Raceways per Greenhouse) Based on Average of 10-yr Cash Flow

	Unit	Quantity	Price or Cost/Unit	Value or Cost	Percent of Costs	Value/Cost per lb.
1. GROSS RECEIPTS						
Farm-gate shrimp value, whole, heads-on	lb	338,044	\$3.27	\$1,104,215		\$3.27
(kg/m ³)		8.213				
2. VARIABLE COSTS						
FEED						
Grow-out	ton	222	\$1748	\$388,717	46.6%	\$1.15
Nursery	ton	23	\$1098	\$25,465	3.1%	\$0.08
LABOR, NURSERY, AND GROW-OUT						
Farm management	annual	1	\$140,875	\$140,875	16.9%	\$0.42
Hired labor, hourly	h	1	\$62,818	\$62,818	7.5%	\$0.19
Hatchery supplies	crop	9	\$962	\$8179	1.0%	\$0.02
PL ₁₂	\$/1000	3444	\$8.00	\$27,650	3.3%	\$0.08
UTILITIES						
Fuel, gasoline	\$/gal	1096	\$3.30	\$3,617	0.4%	\$0.01
Fuel, diesel	\$/gal	1460	\$3.95	\$5,767	0.7%	\$0.02
Electricity	\$/kwh	1359	\$0.08	\$39,677	4.8%	\$0.12
Water, fresh						
Initial raceway filling	\$/m ³ water	1489	\$0.14	\$208	0.0%	\$0.00
Evaporation replenishment	gal/all greenhouses/d	23,047	\$3.23	\$1178	0.1%	\$0.00
CHEMICALS						
Salt, Red Sea Salt	bag (2220 lb/bag)	90	\$650	\$5850	0.7%	\$0.02
Sodium bicarbonate	2450 lb (pallet)	54,000	\$0	\$8910	1.1%	\$0.03
Mineral additive to water	\$/yr			\$10,000	1.2%	\$0.03
Liquid oxygen						
Liquid oxygen tank rental	6000-gal tank/mo	1	\$1500	\$18,000	2.2%	\$0.05
Liquid oxygen supply	100 ft ³ /raceway per day	147.8	\$0.40	\$21,585	2.6%	\$0.06
Sludge removal	\$/gal	45	\$15.00	\$2017	0.2%	\$0.01

TABLE 13.10 Enterprise Budget (Receipts, Variable Costs, Fixed Costs, Net Returns to Land) and Breakeven Prices for a Super-Intensive Shrimp Production System Consisting of Ten Greenhouses (Eight Grow-Out Raceways per Greenhouse and Two Nursery Raceways per Greenhouse) Based on Average of 10-yr Cash Flow—cont'd

	Unit	Quantity	Price or Cost/Unit	Value or Cost	Percent of Costs	Value/Cost per lb.
Telephone expense	\$/month	12	\$200.00	\$2400	0.3%	\$0.01
Interest on operating capital	dollar	772,911	8.00%	\$61,833	7.4%	\$0.18
Total variable costs				\$834,744	100.0%	\$2.47
3. INCOME ABOVE VARIABLE COST				\$269,471		\$0.80
4. FIXED COST						
Land charge (not included)	dollar	0	8.00%	\$0	0.0%	\$0.00
Facility depreciation	dollar			\$114,531	58.5%	\$0.34
Machinery depreciation	dollar			\$12,079	6.2%	\$0.04
Repair and maintenance	annual			\$31,831	16.3%	\$0.09
Interest on raceway and greenhouse construction	dollar			\$32,452	16.6%	\$0.10
Interest on Equip./Mach. Purchases	dollar			\$2816	1.4%	\$0.01
Insurance on facilities and equipment	%/investment \$	991,997	0.21%	\$2067	1.1%	\$0.01
Property tax	\$/ac	6.60	\$9.48	\$63	0.0%	\$0.00
Total fixed costs				\$195,838	100.0%	\$0.58
5. TOTAL OF ALL SPECIFIED EXPENSES				\$1,030,583		\$3.05
6. NET RETURNS ABOVE ALL SPECIFIED EXPENSES^a				\$73,632		\$0.22
Net returns per greenhouse: Above specified variable costs				\$269,471		\$0.80
Above specified total costs				\$73,632		\$0.22
Breakeven price: To cover specified variable expenses				\$2.47		
To cover specified total expenses				\$3.05		

^a Labor and Management expenses have been included, but no expense has been included for land, therefore Net Returns to Land is represented by this budget.

calculation of depreciable assets, with a discount rate of 10% chosen for this analysis.

Table 13.12 can be used as a template and, in addition to the already-stated inputs, includes rows for entering investor dividends and

income taxes, if desired. (They are left blank here.) Information from the annual replacement cost schedule (Table 13.8) is entered into Table 13.12 as a necessary cost in the long-run upkeep of the infrastructure. Summed, these

Heating, natural gas	\$0.00	\$/therm	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Electricity	\$0.08	\$/kwh	1359	3370	3044	3370	3261	3370	3261	3370	3370	3261	3370	3261	3370	39,677
Water, fresh																
Initial 80 RW fill	\$0.14	\$/1000 gal	1489	208												208
Evaporation replacement	\$0.14	\$/1000 gal	8,412,155	100	90	100	97	100	97	100	100	97	100	97	100	1178
CHEMICALS																
Salt, Red Sea Salt	\$650	bag (2220lb/ bag)	90	58,500												58,500
Sodium bicarbonate	\$0.165	\$/lb	54,000	743	743	743	743	743	743	743	743	743	743	743	743	8910
Mineral additive to water	\$10,000	\$/yr per GH	1							10,000						10,000
Liquid oxygen																
Liquid oxygen tank rental	\$1500	11K-gal tank/mo	1	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	18,000
Liquid oxygen supply	\$0.40	100 ft. ³ vol/ RW per d	147.84	1833	1656	1833	1774	1833	1774	1833	1833	1774	1833	1774	1833	21,585
Sludge removal	\$15.00	\$/gal	45	168	168	168	168	168	168	168	168	168	168	168	168	2017
Telephone expense	\$200.00	\$/mo	12.00	200	200	200	200	200	200	200	200	200	200	200	200	2400
Insurance	0.21%	%/ investment \$	991,997	2067												2067
Property tax	\$9.48	\$/ac	7	63												63
SCHEDULED DEBT PAYMENTS:																
Long term																
Principal			921,603							103,286						103,286
Interest	8.00%	Percent	317,498							73,728						73,728

Continued

TABLE 13.11 Example of a One-Year Cash Flow Generated as an Output From Cash Flow, Year 1, for a Recirculating Biosecure Shrimp Production Facility—cont'd

Month	Price, \$/lb	Unit	Annual Quantity	Jan-01	Feb-01	Mar-01	Apr-01	May- 01	Jun-01	Jul-01	Aug-01	Sep-01	Oct-01	Nov- 01	Dec-01	Total
<i>INTERMEDIATE TERM</i>																
Principal			70,395	7889												7889
Interest	8.00%	Percent	24,251	5632												5632
Total cash outflow				138,467	62,650	63,163	62,992	63,163	62,992	250,177	63,163	62,992	63,163	62,992	63,163	1,019,077
Cash available				−138,467	−62,150	180,775	117,783	54,620	230,556	−19,621	−62,526	−62,492	−62,663	161,637	98,474	
New borrowing				138,967	62,650	0	0	0	0	20,121	63,026	62,992	63,163	0	0	410,919
Payment on																
Principal										221,738					0	221,738
Interest	8.00%	Percent								9125						9125
Ending cash balance				500	500	180,775	117,783	54,620	230,556	−230,364	500	500	500	161,637	98,474	98,474

TABLE 13.12 Bio-Economic Model Output

Item/Yr	0	1	2	3	4	5	6	7	8	9	10
Gross receipts	0	937,496	1,176,127	1,172,674	1,172,603	945,138	1,176,127	1,404,291	938,837	1,171,356	1,040,700
Total costs	0	1,249,941	1,242,665	959,423	959,423	959,423	959,423	959,423	768,888	768,888	768,888
Investor dividend	0	0	0	0	0	0	0	0	0	0	0
Taxable income	0	−312,445	−66,537	213,251	213,180	−14,285	216,704	444,868	169,949	402,469	271,812
Income taxes	0	0	0	0	0	0	0	0	0	0	0
Net income	0	−312,445	−66,537	213,251	213,180	−14,285	216,704	444,868	169,949	402,469	271,812
Depreciation	0	126,610	126,610	126,610	126,610	126,610	126,610	126,610	126,610	126,610	126,610
Net income + depreciation	0	−185,835	60,072	339,860	339,789	112,325	343,314	571,478	296,559	529,078	398,422
Initial investment and replacement costs	−991,997	0	0	0	0	0	510,717	0	61,479	0	50,297
Net cash flow	−991,997	−185,835	60,072	339,860	339,789	112,325	−167,403	571,478	235,080	529,078	348,125
Average selling price used \$/lb	3.27										
Pay-back period	yr	4.55									
Discount rate	%	10.00%									
Net present value	\$	102,641									
Internal rate of return	%	11.72%									

Ten-yr cash flow for calculating payback period, net present value, and internal rate of return for a super-intensive recirculating shrimp production system using hyper-intensive 35% crude protein feed, stocking at 324 juveniles/m³, juveniles weighing 4.7 g and grown to 27 g, having a 1.59 FCR, grown for 77 days.

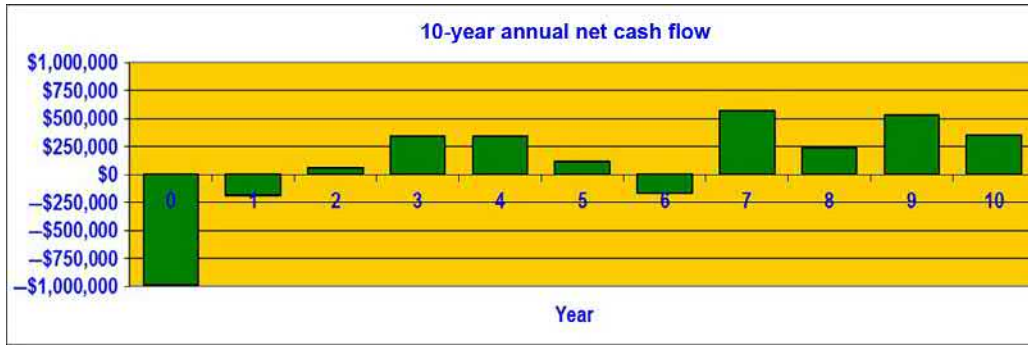


FIG. 13.1 Ten-year annual net cash flow.

items provide an annual outcome—positive or negative—used in investment analysis. This information is used to calculate financial measures of profitability: net present value (NPV), internal rate of return (IRR), and investment payback period.

The NPV accounts for the time value of money in an investment based on the stream of future cash flows over the life of the project and a discount rate. It is the sum of the present values for each year's net cash flow less the initial cost of the investment. The formula is as follows:

$$\text{Net present value} = -C + \left(P_1 / (1+i)^1 \right) + \left(P_2 / (1+i)^2 \right) + \dots + \left(P_n / (1+i)^n \right) \quad (13.20)$$

where C is the initial investment, P_n is the net cash flow in year n , and i is the discount rate.

Excel has a built-in function for calculating NPV:

$$= \text{NPV}(\text{rate}, \text{value1}, \text{value2} \dots \text{value11}) \quad (13.21)$$

where "rate" is the discount rate, "value1" is the initial investment (sometimes referred to as Year 0), and "value2" through "value11" are annual net cash flows for Years 1 through 10. These "values" must be equally spaced in time and represent the end of each period. NPV interprets the order of "value1," "value2" through

"value11" as the order of cash flows. The Excel NPV function can be set up in a few ways, with documentation available in Excel's spreadsheet help site.

The IRR is closely related to NPV and also incorporates the time value of money concept. The IRR is the discount rate that makes the NPV equal to zero. Its formula is as follows:

$$\text{Net present value} = -C + \left(P_1 / (1+i)^1 \right) + \left(P_2 / (1+i)^2 \right) + \dots + \left(P_n / (1+i)^n \right) = 0 \quad (13.22)$$

where NPV is set equal to zero and the equation is solved for i , the discount rate.

Because NPV is set to zero, the formula can be rearranged with the investment C on the left side of the equation, making the NPV of net revenue flows equal to the investment cost:

$$C = \left(P_1 / (1+i)^1 \right) + \left(P_2 / (1+i)^2 \right) + \dots + \left(P_n / (1+i)^n \right) \quad (13.23)$$

Excel has a built-in IRR function (see Excel's help site for documentation):

$$= \text{IRR}(\text{values}, [\text{guess}]), \quad (13.24)$$

where the "values" parameter references the cells that contain the year-zero investment and the net cash flows for years 1 through 10. The "guess" parameter is an estimate of the discount

rate that “seeds” Excel’s iterative technique for calculating IRR. The result is accurate within 0.00001 percent.

If Excel cannot find a result after 20 iterations, an error message is returned and a new “guess” can be entered. The “guess” parameter may, however, be omitted; in this case, the IRR function starts with a trial discount rate of 0.10 (10 percent).

The payback period is the number of years it takes for an investment to return its original cost through the annual net cash revenues that it generates. Its formula is as follows:

$$\text{Payback period} = \frac{\text{investment}}{\text{average annual net cash flow}} \quad (13.25)$$

The payback period is one way to rank investments. The project with the fastest payback period is favored. It does not, however, take into account the timing of cash flows or flows that occur after payback has been reached. Nonetheless, it is easy to calculate and quickly identifies investments with the fastest cash returns.

The bio-economic model also allows for quick sensitivity analysis to be conducted for production, facility, and financing items in the model. This is done by changing the desired parameter, rerunning the model, and then comparing the new results with those of the base model. This identifies the variables that have the greatest effect on project profitability.

Economic analyses of commercial facilities have been based mainly on the results of research trials that have been extrapolated to larger scale operations. But commercial operations capture efficiencies owing to economies of scale that are not available in a research setting. Such extrapolations thus must be interpreted with care.

A full-scale commercial operation thus is the real test of the profitability of this super-intensive recirculating shrimp production technology. Much depends on an operation’s location, expertise, technology, biosecurity, and markets. Good decisions in these areas

will produce viable operations based on this technology.

Regarding commercial facilities, Florida Organic Aquaculture, LLC in Fellsmere, FL used a modified nursery and grow-out technology developed by Dr. Samocha and described in this manual.

All models use assumptions and this bio-economic model is no exception. When extrapolating research data to a larger scale, the following assumptions are made:

- production cycles run smoothly and continuously year-round
- a sufficient number of healthy PL10 is available year-round
- shrimp selling price is known
- changes made in sensitivity analyses are justified by the researcher’s core knowledge

Regarding the third assumption, the future price of shrimp cannot be predicted with certainty, so historical price trends using 10-yr average prices and knowledge of current trends are used. Regarding the last assumption, the knowledge accumulated by the research team is essential in defining operationally reasonable parameter changes and in identifying any “ripple effects” that accompany these changes. For example, changing stocking density may change mortality in a predictable way that must be addressed in interpreting the sensitivity analysis.

13.3 CAPITAL INVESTMENT EXAMPLES

Information on the cost of raceway construction using alternative materials, raceway dimensions, and capital items for large and small systems fills in the gaps regarding what is needed to build these systems. Capital costs vary by locale and over time; those itemized here are estimates. Anyone delving deeper into construction of such systems must research these costs or hire a competent professional to perform this work.

13.3.1 Greenhouse/Raceway Design, Materials, Construction, and Economies of Scale

Investment costs include land purchase (including land preparation cost) for an area at least large enough for greenhouses, waste treatment pond, and office/lab space. The greenhouse, raceways, and their components are included in the initial investment. Fig. 13.2 diagrams a typical greenhouse with units to enclose eight raceways.

Building construction estimates differ according to the structure and materials (Ogershok

and Pray, 2004). Costs for a preengineered steel building, a wood-frame barn, and an as-built greenhouse to cover 4350 ft² (404 m²) are presented in Table 13.13. The as-built greenhouse and wood-frame barn have similar costs and are less expensive than the steel building. Cost alone, however, should not be the sole determining factor when selecting a structure because the production technology may require exposure (or no exposure) to sunlight, and the climate maybe temperate or subtropical.

Raceway width must be harmonized with the width of the enclosing structure and raceway

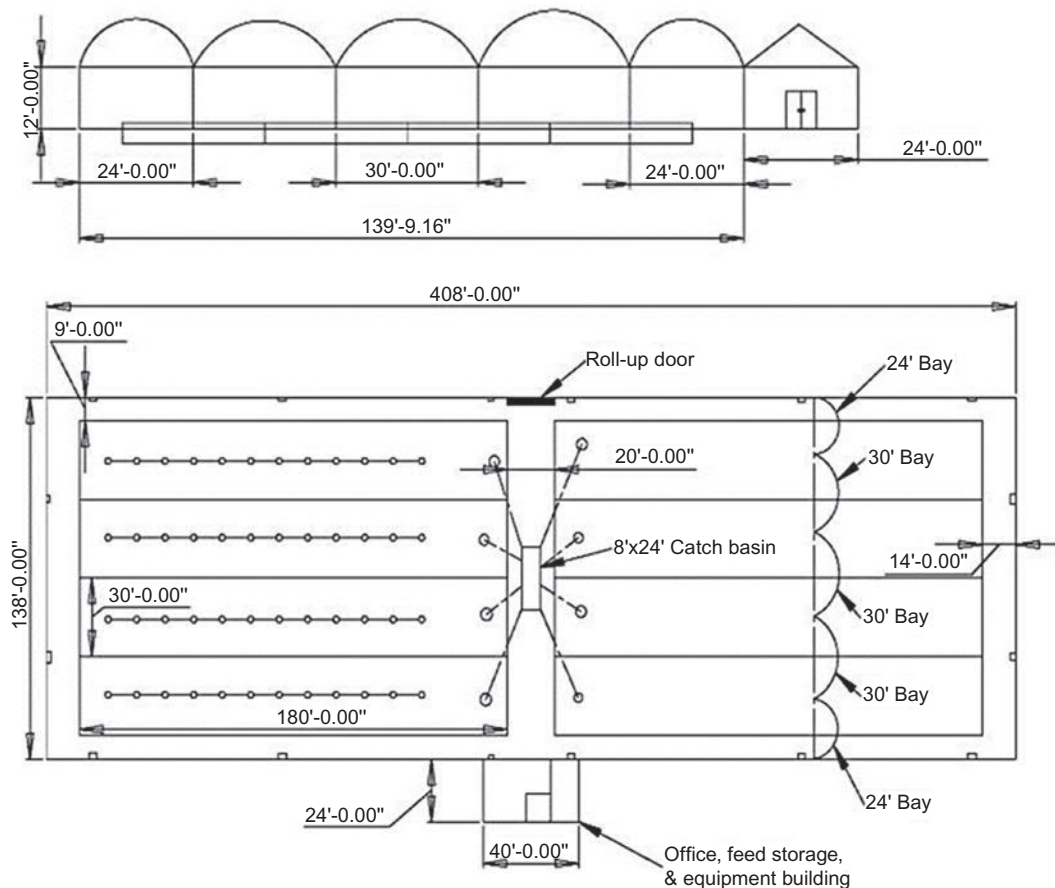


FIG. 13.2 Greenhouse structure to cover eight 500-m² (four per side) raceway units sharing a central harvest area.

TABLE 13.13 Three Building Structure Options to Enclose Raceway Units

Building Options	Material	Quantity	Unit	Material (\$)	Labor (\$)	Cost/Unit (\$)	Total Cost (\$)
Preengineered steel building	Steel structure	\$4350	ft ²	3.41	4.05	7.46	32,451
	Foundation/Footings	\$13.00	yd ³	84.70	67.90	152.60	1984
	Total						34,435
Wood-frame barn	2 × 8 × 16' Rafter	3600	LF	0.66	1.26	1.92	6912
	2 × 4 × 8' Stud	3200	LF	0.46	0.65	1.11	3552
	1/2" Ext. paneling 4x8	90	pc.	16.55	9.83	26.38	2374
	Roof fiberglass Corrugated	4785	ft ²	0.81	0.33	1.14	5455
	Foundation/footings	13.00	yd ³	84.70	67.90	152.60	1984
	Purlins 2 × 4	1450	LF	0.32	0.30	0.62	899
	Total						21,176
As built greenhouse	2 × 12 × 16' Treat. (23)	368	LV	1.33	0.97	2.30	846
	1/2" Plywood 4 × 8 Trt. CDX	16	P.	3.97	9.97	33.94	543
	Jaderloon package	1		14,125	4500	18,625	18,625
	Total						20,014

length will determine slope and minimum depth requirements. Construction can be done with cinder blocks, poured cement, or wood-frame walls; raceway bottoms can be constructed using slab concrete or sand; and all use high density polyethylene (HDPE) or Ethylene Propylene Diene Monomers (EPDM) liners.

Table 13.14 provides example costs for these raceway construction methods and shows the potential range of costs that may be expected (Ogershok and Pray, 2004). The most cost-effective option for raceways is the wood frame, followed by block walls with a sand bottom.

Raceways have large drains and a shared central harvest basin. Adjacent raceways share walls, and each has a center divider plus shared catwalks for access. There is debate about the optimum number and size of raceways per

greenhouse. Structures with either eight or ten raceways have been designed along with detailed costs. These have been analyzed in several publications (Hanson and Posadas, 2005; Hanson et al., 2007; McAbee et al., 2006; Posadas and Hanson, 2003; Posadas and Hanson, 2006; Samocha et al., 2008).

Table 13.15 presents data for the economy of scale as a function of raceway size based on wood-post and liner construction estimates. Factors other than size, such as ease of management, quantity at harvest, and production control, may override this cost factor.

13.3.2 Construction Cost for a Large Greenhouse With Ten 500 m³ Raceways

The design in Section 13.2 called for one large greenhouse with 10 raceways, two for nursery

TABLE 13.14 Estimated Raceway Construction Costs for Two Wall Types and Slab or Sand Bottoms, and As-Built Raceway Cost

Type	Material	Quantity	Unit	Material (\$)	Labor (\$)	Cost/Unit (\$)	Total Cost (\$)
Block walls slab bottom	Slab 6" 3000 PSI Concrete	2930	ft ²	2.46	1.20	3.66	10,724
	Block	1136	ft ²	1.87	4.52	6.39	7259
	Excavation w/ backhoe	452	yd ³	NA	4.60	4.60	2079
	Liner	4760	ft ²	0.30	0.70	1.00	4760
	Total						24,822
Block walls w/sand bottom	Total						14,098
Pored walls slab bottom	Slab 6" 3000 PSI Concrete	2930	ft ²	2.46	1.20	3.66	10,724
		43	yd ³	70.90	16.20	87.10	3745
	Forms	2272	SFCA	1.86	4.63	6.49	14,745
	Excavation w/ backhoe	452	yd ³	NA	4.60	4.60	2079
	Liner	4760	ft ²	0.30	0.70	1.00	4760
	Total						36,054
Pored walls w/sand bottom	Total						25,330
As-built raceway	6 × 6 × 10' post Trt	71	Pc.	19.40	17.20	36.60	2599
	2 × 8 × 16' Trt (19)	304	LF	1.00	0.91	1.91	581
	Liner	4760	ft ²	0.30	0.70	1.00	4760
	2 × 12 × 16' Trt (72)	1152	LF	1.33	0.97	2.30	2650
	Excavation w/ backhoe	452	yd ³	NA	4.60	4.60	2079
	Total						12,668

and eight for grow-out. Each had a 500-m² surface area and 1-m deep. The total raceway area thus is 5000m² and the total volume is 5000m³.

The greenhouse is equipped with electrical, catwalk, raceway heating, water treatment and control, drains and harvest, water-return piping, air supply piping, aeration, and feed delivery

systems (Table 13.7). It also includes an automated shade system, heating, and cooling. Freight and installation are included in the estimate. Mechanical and lab buildings house blowers and equipment for filtration, oxygenation, and water quality analysis. Other required facilities are a nursery evaluation lab and

TABLE 13.15 Raceway Economies of Scale With Post and Liner Construction

Type	Material	Quantity	Unit	Material (\$)	Labor (\$)	Cost/Unit (\$)	Total Cost (\$)
As-built raceway	6 × 6 × 10' post Trt (71)	71	pc.	19.40	17.20	36.60	2599
268 m ²	2 × 8 × 16' Trt (19)	304	LF	1.00	0.91	1.91	581
	Liner	4760	ft ²	0.30	0.70	1.00	4760
	2 × 12 × 16' Trt (72)	1152	LF	1.33	0.97	2.30	2650
	Excavation w/ backhoe	452	yd ³	NA	4.60	4.60	2079
	Subtotal						12,668
	Cost per m ²						47.27
As-built raceway	6 × 6 × 10' post Trt (71)	97	pc.	19.40	17.20	36.60	3550
500m ²	2 × 8 × 16' Trt (19)	384	LF	1.00	0.91	1.91	733
	Liner	7826	ft ²	0.30	0.70	1.00	7826
	2 × 12 × 16' Trt (72)	1536	LF	1.33	0.97	2.30	3533
	Excavation w/ backhoe	627	yd ³	NA	4.60	4.60	2884
	Subtotal						18,527
	Cost per m ²						37.05
As-built raceway	6 × 6 × 10' post Trt (71)	117	pc.	19.40	17.20	36.60	
750m ²	2 × 8 × 16' Trt (19)	473	LF	1.00	0.91	1.91	903
	Liner	11,100	ft ²	0.30	0.70	1.00	11,100
	2 × 12 × 16' Trt (72)	1892	LF	1.33	0.97	2.30	4352
	Excavation w/ backhoe	947	yd ³	NA	4.60	4.60	4356
	Subtotal						24,993
	Cost per m ²						33.32
As-built raceway	6 × 6 × 10' post Trt (71)	128	pc.	19.40	17.20	36.60	4684
1000 m ²	2 × 8 × 16' Trt (19)	520	LF	1.00	0.91	1.91	993
	Liner	14,520	ft ²	0.30	0.70	1.00	14,520
	2 × 12 × 16' Trt (72)	2080	LF	1.33	0.97	2.30	4784
	Excavation w/ backhoe	1232	yd ³	NA	4.60	4.60	5667
	Subtotal						30,649
	Cost per m ²						30.65

building, an effluent storage and evaporation pond(s), fencing, and stone paving for roads. Concrete pads for liquid oxygen tanks also are required. Equipment for this scale of greenhouse-raceway configuration includes hatchery equipment, generator, office items, ATVs, and tractors.

The approximate cost for this fully equipped greenhouse enclosing ten raceways is \$991,997. Depending on location, this could vary from \$750,000 to \$1.25 million. This system is the basis for the analysis in [Section 13.4](#) of how changing key criteria affects financial viability.

13.3.3 Construction Cost for a Small Greenhouse With Six 40 m³ Grow-Out Raceways

There is increasing interest in smaller intensive facilities, such as a greenhouse with six 40 m³ raceways. Economic analysis of 2014 production trials with this smaller system is based on cost itemizations in [Table 13.16](#). The capital and equipment investment was \$252,382.

13.3.4 Construction Cost for a Small Greenhouse With Two 100m³ Raceways

Compared to the 5000m³ facility, a greenhouse with two 100m³ raceways is less expensive but also has a much smaller grow-out volume. [Table 13.17](#) lists greenhouse and raceway components and other items. The overall capital and equipment investment was \$197,138.

13.4 FACTORS AFFECTING COST OF PRODUCTION AND FINANCIAL VIABILITY

Super-intensive, biosecure, recirculating shrimp systems incorporate advanced engineering and management to achieve high output per unit area. Production modules can be replicated to achieve economies of scale. To the extent that

these systems are economical, they will have a bright future in the United States and beyond. Economic analyses presented here will assist investors in evaluating the system's commercial viability for a specific site ([Hanson et al., 2007, 2009](#)).

Sensitivity of the base model's cost of production (COP) and financial viability to changes in critical biological, investment, and price factors was evaluated by increasing (or decreasing) these factors by 20% and then rerunning the model ([Hanson et al., 2009](#)). Differences in COP, NPV, and IRR between the base case and each recalculated model were ranked, with larger differences signifying factors with a greater impact on financial measures.

Assumptions used in the base model included specifying inputs for grow-out and nursery areas, number of greenhouses, capital construction costs, financing terms, initial operating costs, land area, raceway carrying capacity, stocking density, beginning and ending shrimp size, selling price, growth rate, FCR, and survival ([Table 13.18](#)).

The base scenario includes ten greenhouses, each with two nursery raceways and eight grow-out raceways for 40,000m³ of grow-out area and 10,000m³ of nursery area. Crop length was 86 days (including two days between cycles), resulting in 4.25 crops of 20-g shrimp per year, or 2.6 million pounds (~ 1179 metric tons) annually. The system featured continuous water circulation, oxygen injection, wood-frame raceways at \$1.70 ft² (\$18.29/m²), heating during winter, availability of high-saline water, and sedimentation ponds. Cost data are presented in [Table 13.18](#).

Baseline results indicate that the variable cost of producing shrimp was \$2.05/lb (\$4.52/kg); when fixed costs are included, the total cost of production was \$2.43/lb (\$5.36/kg). Based on a selling price of \$3.27/lb (\$7.21/kg) for whole 20-g shrimp, the payback period was 3.2 years.

TABLE 13.16 Fixed Costs for Constructions and Equipment/Machinery for the Texas A&M-ARML Indoor Recirculating Shrimp Production Facility, Six 40 m³ Raceways, 2014

Item	Unit	Cost/ Unit (A) (\$)	Number (B)	Cost (A × B) (\$)	Salvage Value per Item (C) (\$)	Useful Life Years (D)	Annual Deprec. (\$)	Interest on Investment/3 (A × B) × IR (\$)	Repairs Maintenance Cost/Year (\$)
<i>A. Capital cost</i>									
Land purchase	ac	50,000	0.5	25,000				875	
Greenhouse structural components	various	8897	1.0	38,897	3131	10	3577	1471	389
Greenhouse electrical system	various	25,000	1.0	25,000	2500	10	2250	963	250
Raceways	various	3982	6.0	23,892	2389	10	2150	1338	239
Water quality laboratory	various	50,422	0.5	25,211	–	10	2521	882	252
Major water treatment and control equipment	various	24,635	1.0	24,635	–	10	2464	862	246
Raceway drains and harvest pipes	various	4556	1.0	4556	456	10	410	175	46
Water return piping system	various	5847	1.0	5847	585	10	526	225	58
Air supply piping system and raceway aeration	various	10,829	1.0	10,829	1083	10	975	417	108
Feed delivery system	various	5080	1.0	5080	508	10	457	196	51
Office building	various	15,000	0.5	7500	750	10	675	276	75
Effluent storage and evaporation ponds	various	10,750	0.5	5375	538	10	484	188	54
Harvest basin and equipment	various	1320	0.5	660	66	10	59	23	7
Construction (fencing, paving, stone, and asphalt)	various	10,000	0.5	5000	500	10	450	175	50
Concrete pads and installation for O ₂ tanks	various	650	1.0	650	65	10	59	23	7

Continued

TABLE 13.16 Fixed Costs for Constructions and Equipment/Machinery for the Texas A&M-ARML Indoor Recirculating Shrimp Production Facility, Six 40 m³ Raceways, 2014—cont'd

Item	Unit	Cost/ Unit (A) (\$)	Number (B)	Cost (A × B) (\$)	Salvage Value per Item (C) (\$)	Useful Life Years (D)	Annual Deprec. (\$)	Interest on Investment/3 (A × B) × IR (\$)	Repairs Maintenance Cost/Year (\$)
Subtotal				208,132	12,571		17,056	8089	1831
<i>B. Equipment/machinery</i>									
Feed storage bins	ea	9000	0.5	4500	450	10	405	189	45
Stand-by generator	ea	15,500	0.5	7750	775	10	698	326	78
Office equipment	ea	2000	0.5	1000	100	10	90	42	10
General storage container	ea	\$8000	0.5	4000	400	10	360	168	40
All-terrain vehicle (golf cart w/bed)	ea	3000	0.5	1500	150	10	135	63	15
Fork lift	ea	10,000	0.5	5000	500	10	450	210	50
Vehicle	ea	15,000	0.5	7500	750	10	675	315	75
Wheel barrows	ea	50	1.0	50	5	10	5	2	1
Miscellaneous tools	per pond	500	0.5	250	25	10	23	11	3
Miscellaneous power tools	ea	\$1000	0.5	500	50	10	45	21	5
Water supply	various	7200	1.0	7200	720	10	648	302	72
Miscellaneous	ea	10,000	0.5	5000	500	10	450	210	50
Subtotal				44,250	4425		3983	1859	443
Total				252,382	16,996		\$21,039	9947	2274

Note: These costs do not include any raceway heating system. For six 40 m³ raceways it is estimated that a heating system would cost approximately \$60,160 installed.

The 40 m³ raceways were not built to accommodate our current use. If we are to build a new system it will not be of 40 m³ but at least 100 m³ working volume.

The biological improvement that reduced production cost the most and increased NPV and IRR was a 20% increase in grow-out survival (from 70% to 84%). This resulted in a \$0.36/lb (\$0.79/kg) decrease in the cost of production—

from \$2.43 to \$2.10/lb (\$5.36 to \$4.63/kg)—and a near doubling of NPV, from \$10.79 to \$21.27 million (Table 13.19).

Increasing grow-out stocking density by 20%, from the baseline 500 PL/m³ to 600 PL/m³,

TABLE 13.17 Fixed Costs for Constructions and Equipment/Machinery for the Texas A&M-ARML Indoor Recirculating Shrimp Production Facility, Two 100 m³ Raceways, 2014

Item	Unit	Cost/ Unit (A) (\$)	Number (B)	Cost (A × B) (C) (\$)	Salvage Value per Item (D) (\$)	Useful Life years (E)	Annual Deprec. (F) (\$)	Interest on Investment/3 (A * B) * IR (\$)	Repairs Maintenance Cost/Year (\$)
<i>A. Capital cost</i>									
Land purchase	ac	50,000	0.5	25,000				875	
Greenhouse structural components	various	7389	1.0	27,389	2115	10	2527	1033	274
Greenhouse electrical system	various	2500	1.0	12,500	1250	10	1125	481	125
Raceways	various	7200	2.0	14,400	1440	10	1296	605	144
Water quality laboratory	various	50,422	0.5	25,211	–	10	2521	882	252
Major water treatment and control equipment	various	12,765	1.0	12,765	–	10	1277	447	128
Raceway drains and harvest pipes	various	4794	1.0	4794	479	10	432	185	48
Water return piping system	various	3309	1.0	3309	331	10	298	127	33
Air supply piping system and raceway aeration	various	3320	1.0	3320	332	10	299	128	33
Feed delivery system	various	2540	1.0	2540	254	10	229	98	25
Office building	various	15,000	0.5	7500	750	10	675	276	75
Effluent storage and evaporation ponds	various	10,750	0.5	5375	538	10	484	198	54
Harvest basin and equipment	various	1320	0.5	660	66	10	59	24	7
Construction (fencing, paving, stone, and asphalt)	various	10,000	0.5	5000	500	10	450	184	50
Concrete pads and installation for O ₂ tanks	various	–	–	–	–	10	–	–	–
Subtotal				149,763	8055		11,671	5543	1248

Continued

TABLE 13.17 Fixed Costs for Constructions and Equipment/Machinery for the Texas A&M-ARML Indoor Recirculating Shrimp Production Facility, Two 100 m³ Raceways, 2014—cont'd

Item	Unit	Cost/ Unit (A) (\$)	Number (B)	Cost (A × B) (C) (\$)	Salvage Value per Item (D) (\$)	Useful Life years (E)	Annual Deprec. (F) (\$)	Interest on Investment/3 (A*B)*IR (\$)	Repairs Maintenance Cost/Year (\$)
<i>B. Equipment/Machinery</i>									
Feed storage bins	ea	9000	0.5	4500	450	10	405	189	45
Stand-by generator	ea	15,500	0.5	7750	775	10	698	326	78
Office equipment	ea	2000	0.5	1000	100	10	90	42	10
General storage container	ea	8000	0.5	4000	400	10	360	168	40
All-terrain vehicle (golf cart w/bed)	ea	3000	0.5	1500	150	10	135	63	15
Fork lift	ea	10,000	0.5	5000	500	10	450	210	50
Vehicle	ea	15,000	0.5	7500	750	10	675	315	75
Wheel barrows	ea	50	1.0	50	5	10	5	2	1
Miscellaneous tools	per pond	500	0.5	250	25	10	23	11	3
Miscellaneous power tools	ea	1000	0.5	500	50	10	45	21	5
Water supply	various	10,325	1.0	10,325	1033	10	929	434	103
Miscellaneous	ea	10,000	0.5	5000	500	10	450	210	50
Subtotal				47,375	4738		4264	1990	474
Total				197,138	12,793		15,935	7533	1721

reduced the cost of production by \$0.19/lb (\$0.42/kg) from \$2.43 to \$2.24/lb (\$5.36 to \$4.94/kg)—and increased the NPV by \$6.16 million, from \$10.79 to \$16.95 million.

Other biological improvements, such as grow-out growth rate, FCR, and nursery survival improved the financial outlook by lesser amounts. Increasing the shrimp selling price by 20% increased the NPV by \$9.57 million (+12.5% IRR) and had no effect on the cost of production.

Feed price and PL price also were analyzed, but because these are controlled by parties outside of the production environment, it is not as informative to consider 20% drops in these factors. Reducing the initial investment and acquiring a greater share from investors (80 to 100%) rather than from bank loans (20 to 0%) were important, in improving financial viability.

Continued improvements in super-intensive production technologies and management are occurring. These include increasing growth rate,

TABLE 13.18 Base Scenario Conditions Used in Bio-Economic Model Run

Southern location		Raceway carrying capacity, kg/m ³	7.0
Coastal, Mid-Atlantic state			
Rearing area per greenhouse		Initial stocking density, PL/m ³	500
Grow-out, m ²	4000	Stocking size of PL,	1.0
Nursery, m ²	1000	Crops/yr	4.25
Greenhouse modules	10	Shrimp selling price, \$/lb	3.27
Greenhouse cost, \$/ft ²	10.59	Growth rate, g/wk	1.5
Raceway size, ft ²	5382	FCR	2.0
Raceway cost, \$/ft ²	11.42	Survival, percent	70
Other construction cost, \$/ft ²	4.91	Harvest size, g	20
<i>Capital financing</i>		<i>Interest rate, %</i>	
From the bank	20%	Short term	10
From equity investors	80%	Intermediate term	7
		Long term	7
Initial operating cost, \$	1,000,000	Annual production, million lb	2.6
Land needs, acres	20	Land cost, \$/ac	20,000

stocking and survival rates, and reducing the variable and fixed costs of shrimp production. Genetic improvement specific to intensive recirculating systems can be expected to favor higher yields and reduce costs. Critical-factor analysis, such as outlined before, helps focus on areas that can sharpen the competitiveness of these systems, making them commercially attractive in the United States.

13.5 ECONOMIC ANALYSIS OF 2013 AND 2014 RESEARCH TRIALS

Economic analyses of the production of Pacific White Shrimp in zero-exchange, biofloc-dominated nursery and grow-out systems have been conducted at the Texas A&M-ARML at Flour Bluff, Corpus Christi, Texas over the last decade. These systems produce large quantities of high-quality shrimp but also have a high initial investment and high operating costs.

13.5.1 2013 Trials—Economic Analysis of Two Feeds

This study compared commercially available feed to an experimental feed. Both were formulated for super-intensive, biofloc-dominated shrimp systems. The Hyper-Intensive (HI-35) 35%-protein diet cost \$0.874/lb (\$1.93/kg) and the Experimental (EXP) 40%-protein diet cost \$0.884/lb (\$1.95/kg). Each was applied in three 40 m³ raceways filled with a mixture of biofloc-rich and natural seawater. Salinity was 30 ppt. The 4.7-g juveniles stocked in each at 324/m³ were from a cross between Taura Resistant and Fast-Growth genetic lines developed by Shrimp Improvement, Islamorada, FL. The study ran over 77 days with no water exchange.

Survival and FCR were better with the HI-35 diet, but growth was better with the EXP diet; larger shrimp thus were harvested with the latter treatment (Table 13.20). Production for HI-35 was 8.21 kg/m³, compared to 7.79 kg/m³ for EXP.

TABLE 13.19 Change in Net Present Value (NPV), Internal Rate of Return (IRR), and Cost of Production (COP) With 20% Improvement in Critical Production Factors

Grow-Out Components	Change	Change From Base ^a		
		NPV \$mil.	IRR %	Cost of Production \$/lb ^b
1. Survival	+20%	+10.48	+13.7	−0.36
2. Shrimp price	+20%	+9.57	+12.5	0.00
3. Stocking density	+20%	+6.16	+8.1	−0.19
4. Initial investment	−20%	+2.24	+6.8	−0.04
5. Growth rate	+20%	+2.23	+6.4	−0.19
6. Nursery and grow-out feed price	−20%	+2.37	+3.1	−0.18
7. Feed conversion ratio	−20%	+2.12	+3.0	−0.17
8. Source of financing	20/80–0/100	+1.79	+2.4	−0.02
9. Nursery survival	+20%	+1.12	+1.5	−0.15
10. PL price	−20%	+1.01	+1.2	−0.08

^a Compared to the base scenario total cost of production of \$2.43 per pound (\$2.05 per pound variable cost and \$2.43 per pound for variable plus fixed costs), net present value of \$10.79 million and internal rate of return of 25.3%.

^b The change in cost of production is the difference between full cost of production, including variable and fixed costs, for the critical factor change and the base scenario.

(Source: Hanson, T.R., Posadas, B.C., Samocha, T.M., Stokes, A.D., Losordo, T.M., Browdy, C.L., 2009. Economic factors critical to the profitability of super-intensive biofloc recirculating shrimp production systems for marine shrimp. In: L. vannamei. In: Browdy, C.L., Jory, D.E. (Eds.), *The Rising Tide, Proceedings of the Special Session on Sustainable Shrimp Farming, Aquaculture 2009, The World Aquaculture Society, Baton Rouge, Louisiana, USA*, pp. 243–259.)

TABLE 13.20 2013 Study Results Comparing Hyper-Intensive 35% Protein Feed (HI-35) to a 40% Protein Experimental Feed (EXP-40)

		HI-35	EXP-40
Stocking	Juveniles/m ³	324	324
Survival	%	93.1	83.4
Growth	g/wk	2.05	2.16
Stocking size	g	4.7	4.7
Final weight	g	27.2	28.8
FCR		1.59	1.72
Length of crop	d	77	77
Production	kg/m ³	8.21	7.79

Production results were extrapolated over 10 years to project cash flow for eight 500m³ grow-out raceways and two 500m³ nursery raceways (Hanson et al., 2014). Initial investment was \$991,997 and an 8% interest rate was assumed for loans. Cost of production, net returns to land, NPV, IRR, and payback period were calculated.

The sensitivity of total annual sales (Table 13.21) and net returns, payback period, NPV, and IRR (Table 13.22) at two selling prices—\$7.20/kg (\$3.27/lb.) and \$8.82/kg (\$4.00/lb.)—was analyzed. The higher sales price obviously produced greater revenue from each treatment, with HI-35 being higher owing to its positive effect on shrimp yield.

TABLE 13.21 Summary of 2013 Production Results Extrapolated to a Greenhouse With Eight 500-m³ Grow-Out Raceways and Two 500-m³ Nursery Raceways and Two Shrimp Selling Prices

	HI-35%	HI-35%	EXP (HI-40%)	EXP (HI-40%)
Selling price, \$/lb	3.27	4.00	3.27	4.00
Production, lb/crop	71,924	71,924	68,077	68,077
Crops/yr, no.	4.7	4.7	4.7	4.7
Production, lb/yr	338,044	338,044	319,960	319,960
Production, ton/yr	169	169	160	160
Total sales/yr, \$ million	1.1	1.4	1.0	1.3

The cost of production was less for the HI-35 diet (\$3.05/lb or \$6.73/kg) than for the EXP diet (\$3.28/lb or \$7.23/kg). Similarly, the net return above all costs was greater for the HI-35 diet. Comparing the \$3.27/lb (\$7.21/kg) shrimp selling price for each diet, EXP had a negative net return (Table 13.22). At the higher shrimp price (\$4.00/lb or \$8.82/kg), the HI-35 and EXP diets both had positive net returns, with HI-35 returns greater.

The NPV and IRR followed this pattern as well: The greatest IRR (38%) was for the HI-35 diet, followed by 29% for EXP at the higher selling price. At the lower price, the IRR was 12% for HI-35 and -1% for EXP. At the higher price, payback was 2.0–2.5 years for the two diets.

The overall economic conclusion is that the lower priced HI-35 feed resulted in better production and, when combined with either selling price, was profitable. An important caveat must be emphasized: these results were extrapolated from small-scale research trials. Additionally, the model assumed 4.7 crops/yr, which requires year-round PL supply. Thus far, however, the research facility has been limited to only one crop per year. This must be considered seriously

TABLE 13.22 Summary of Economic Analysis for the 2013 Trials Extrapolated to a Greenhouse With Eight 500-m³ Grow-Out Raceways and Two 500-m³ Nursery Raceways at Two Shrimp Selling Prices

	HI-35%	HI-35%	EXP (HI-40%)	EXP (HI-40%)
Gross receipts, \$/lb	3.27	4.00	3.27	4.00
Variable cost, \$/lb	2.47	2.47	2.67	2.67
Income above variable cost, \$/lb	0.80	1.53	0.60	1.33
Fixed cost, \$/lb	0.58	0.58	0.61	0.61
Total of all specified expenses, \$/lb	3.05	3.05	3.28	3.28
Net return above all costs, \$/lb	0.22	0.95	(0.01)	0.72
Payback period, y	4.5	2.0	11.0	2.5
Net Present Value (\$ million)	0.1	1.7	-0.7	1.1
Internal Rate of Return (%)	12	38	-1	29

when evaluating commercial-scale operations based on this technology and strongly argues for a pilot project that is properly equipped for year-round production trials.

13.5.2 2014 Trials—Analysis of Nursery and Grow-Out in 100m³ and 40m³ Raceways

Trials were run in six 40m³ and two 100m³ raceways. Economic analysis was performed

without extrapolation to a larger facility (Hanson et al., 2015) because of interest in smaller scale production units.

The four trials analyzed were as follows:

- a) Nursery performance of Pacific White Shrimp, two dietary regimes, PL5–10 stocked at 675 PL/m³ in six 40 m³ raceways reared for 62 days to approximately 5.6 g/ind.
- b) Nursery production of Pacific White Shrimp with a³ injectors, PL5–10 stocked at 540 PL/m³ in two 100 m³ raceways reared for 62 days to approximately 6.5 g/ind.
- c) High-density Pacific White Shrimp production with the effect of *Vibrio* outbreak, 6.5-g juveniles at 458/m³ in two 100 m³ raceways and grown for 38 days to 18 to 19 g/ind.
- d) High-density Pacific White Shrimp production, two feeds of different protein content, about 5.6-g juveniles at 457/m³ in six 40 m³ raceways for 48 days to 21 g/ind.

The 100 and 40 m³ systems had no temperature control in the 2014 nursery study, and cool weather during the 3 weeks after stocking negatively affected performance. There was less temperature variation in the 100 m³ raceways, as is expected for larger volume of water. The lower temperature meant a longer production period to reach 6.5 g/ind. This led to higher electrical and manpower expenses (Table 13.23).

The 100 m³ nursery raceway had the lower cost per 1000 juveniles (Table 13.24). There were higher power expenses in the smaller raceways because of the six blowers, six pumps, and higher manpower requirements to run 6 raceways compared to the two larger ones. The former had a higher stocking density that would be more typical of a commercial operation, and the increase in production reduced costs on a per-thousand-juvenile basis.

The 2014 grow-out study had lower survival because of *Vibrio* infections. Raceways thus were harvested earlier than planned (Table 13.25). Lower survival led to higher FCRs, even though

TABLE 13.23 Summary of 2014 Nursery Study Comparing Production of Shrimp Grown in Two Different Greenhouse/Raceway Configurations

	Two 100 m ³ Raceways	Six 40 m ³ Raceways
Stocking (PL5-10/m ³)	540	675
Survival (%)	96	85
Growth (g/wk)	0.73	0.60
Yield (kg/m ³)	3.36	3.16
Final weight (g)	6.5	6.4
FCR	0.81	0.89
Length of crop (d)	62	62

TABLE 13.24 Summary of 2014 Nursery Study Cost of Shrimp Production Raised in Two Different Greenhouse/Raceway Configurations

	Two 100 m ³		Six 40 m ³	
	Total \$	\$/1000 Juveniles	Total \$	\$/1000 Juveniles
Variable costs	6006	58	10,122	73
Fixed costs	1422	14	1897	14
Total expenses	7428	72	12,019	87

weekly growth was above 2 g/ind. Total expenses were lower for the six smaller tanks than for the two 100 m³ tanks, but when viewed in terms of the biomass produced, the larger raceways had the lower breakeven point: \$8.99/kg, or \$4.08/lb (Table 13.26).

The 100 m³ raceways were more cost efficient. This is attributed to greater efficiency in labor and energy usage.

Increased survival is key to improving performance. This is especially challenging when confronted with a *Vibrio* outbreak. In the 9 grow-out

TABLE 13.25 Summary of 2014 Grow-Out Study Comparing Production of Shrimp Grown in Two Different Greenhouse/Raceway Configurations and Fed Two Diets in the Greenhouse With Six Raceways

	Six 40m ³ Raceways		Two 100m ³ Raceway
	HI-35 Diet	EXP14 Diet	EXP14 Diet
Stocking (PL5-10)	457	457	458
Survival (%)	80	76	76
Growth (g/wk)	2.1	2.3	2.3
Yield (kg/m ³)	7.2	7.4	6.5
Final weight (g)	19.8	21.5	18.7
FCR	1.68	1.62	1.84
Length of crop (days)	48	48	38 (<i>Vibrio</i>)

TABLE 13.26 Summary of 2014 Grow-Out Study Cost of Shrimp Production Grown in Two Different Greenhouse/Raceway Configurations and Fed Two Diets in the Greenhouse Having Six Raceways

Total (\$)	Six 40m ³ Raceways		Two 100m ³ Raceways
	HI-35	Exp14	Exp14
Variable costs	8976	8911	10,077
Fixed costs	1761	1761	1549
Total expenses	10,737	10,672	11,627
Breakeven price, \$/kg, to cover			
Variable costs	10.33	10.09	7.79
Total expenses	12.36	12.08	8.99

trials in the 40m³ system, only one had very low survival. Although *Vibrio* outbreaks occurred in another two trials, survival was above 75% in each.

Overall, there was an 11% complete loss due to *Vibrio*, 22% partial mortality, and 33% if one

considers all trials in which *Vibrio* affected the system.

Out of the five grow-out trials in the 100m³ raceways, two suffered *Vibrio* outbreaks that resulted in survival as low as 70%. Thus, although complete crop losses were avoided 40% of the time, *Vibrio* still negatively affected production. (Poor FCRs also are thought to have been caused by *Vibrio* interfering with feed digestibility.) A sure solution for controlling *Vibrio* certainly would advance production management.

Another factor that would have improved the financial indexes is production of larger shrimp, at least to the 21- to 26-count (per lb) market size, that is, about 17 to 22 g/ind.

13.6 MARKETING

13.6.1 General Marketing Principles

New producers often do not address marketing until harvest is near, but understanding markets and marketing is essential to obtaining the best price for a crop.

A market unites sellers, buyers, and distributors in an arena for organizing and facilitating their business transactions. Market activities inform business decisions that can be framed in terms of several basic economic questions:

- What should be produced?
- How much should be produced?
- Who are the customers?
- How is the product distributed?
- What is the best sales price?

In a broad sense, market decisions hinge on the quantity of product supplied by producers and the quantity demanded by consumers. Factors that affect supply include the price of inputs, technology, expectations, taxes, and subsidies; those that affect demand include income level, prices of competing goods, personal tastes and expectations, taxes, and subsidies provided

to consumers. The intersection of supply and demand curves defines the equilibrium price and quantity for a product (Colander, 2006).

Marketing activities are conducted by the firm selling product. Many aspects must be considered, some of which relate to answering the following questions:

- Is there a market for the product?
- What is the product's full market potential?
- What factors affect demand for, and prices of, the product?
- What market segments can be penetrated?
- Can the product be distributed and sold efficiently?
- What are the institutional constraints?

Marketing shrimp involves the flow of products and services from the point of production to the plate of seafood consumers. Management is responsible for identifying customers' needs and supplying them efficiently and profitably. Marketing thus begins on the farm and ends with satisfied customers.

Part of marketing's utility is getting the product to the desired place: moving shrimp from the farm-gate to the supermarket. This involves timing (getting the product to market directly or storing the processed product), product form (transformation of live shrimp into fresh or frozen shrimp, heads-on or -off, shelled or not), and possession (consignment of ownership during each stage of the product's route through the marketing channel).

Marketing functions include the transfer of title through buying and selling. Buying involves finding sources of supply and assembling the correct product quantities. Selling involves merchandising, advertising, and packaging.

The physical aspects of marketing solve problems related to when a product must be delivered to a location and in a specific form. This involves storage, transportation, handling, and processing.

Marketing ensures the smooth performance of exchange and physical functions, including standardization, financing, risk bearing, and

market intelligence. Standardization establishes uniform product grades; financing involves the use of money to carry on marketing activities; risk bearing is acceptance of possible loss in the market chain; and intelligence is the collection, organization, interpretation, and dissemination of market data.

The flow of information through a market channel transmits data on product quantity, quality, price, time availability, origin, and so on, from the end-consumer through intermediaries (retailers, wholesalers, processors) back to producers (Fig. 13.3).

The producer provides information about the amount of shrimp available, grade, and quality to the processor. The processor adds their costs, determines a price for the processed product, and provides this information to other middlemen along the chain (wholesalers, retailers). The middlemen add their costs (transport, storage, etc.) and provide this information to their customers at restaurants, grocery stores, or other purchasers. Finally, the customer determines if the purchase price is agreeable for the product being sold.

This information—the quantity, quality, price, time, and place of product shipment—is sent back through the middlemen to the producers. If sales conditions are acceptable, then there is a flow of physical product from the producer through intermediaries to the final consumer. When product is received, there is a transactional flow that concludes the sale, that is, the flow of money, check, or other payment medium that fulfills the contract.

Distribution channels for shrimp can be direct—from producer to consumer—or more complicated, going through many levels before being consumed (Fig. 13.4). Each additional level generally adds costs, but also adds value; the selling price thus increases at each level. Lower prices usually found are in direct sales.

A marketing axiom is that large-volume producers typically sell to processors equipped to

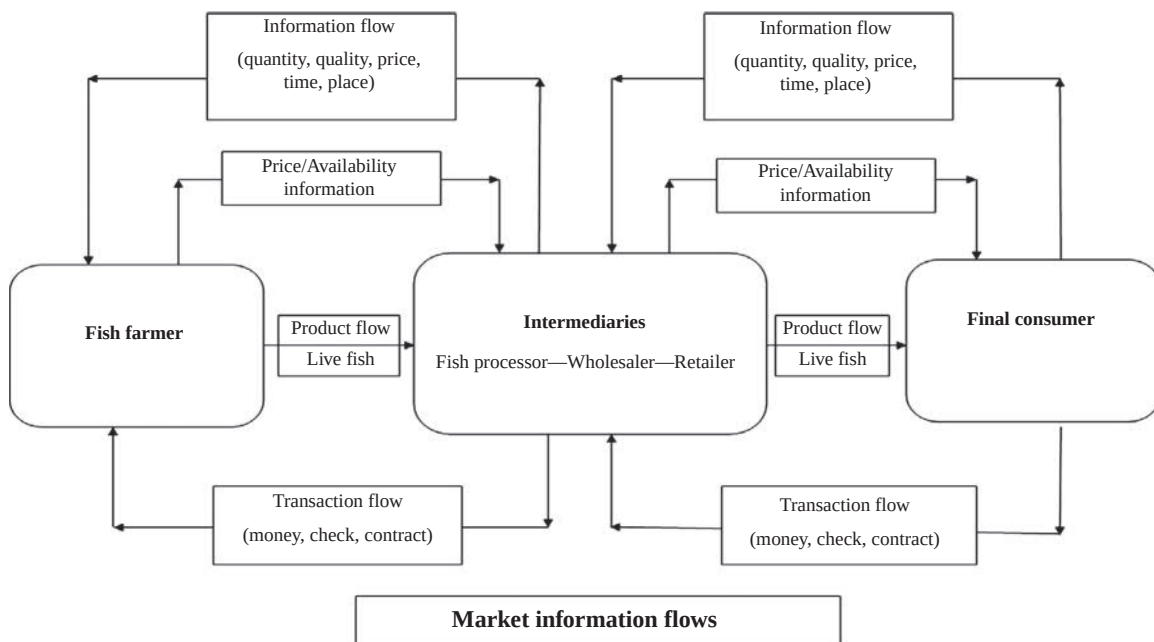


FIG. 13.3 Marketing network with flows of information on product demand, price/availability, product supply, and transactions.

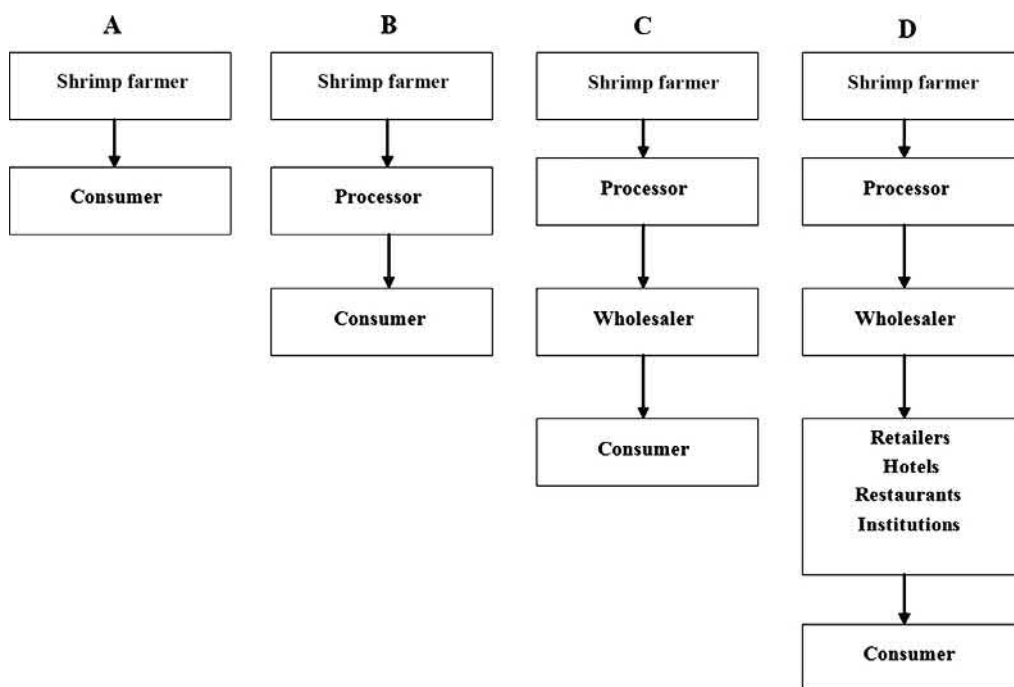


FIG. 13.4 Example distribution channels for shrimp.

handle large volumes of shrimp. In these cases, the producer typically is a “price taker,” meaning the producer accepts the price offered by the processor or there is no sale.

Small-volume producers can sell directly to certain market segments for which they assume the role of “price maker,” meaning that they set the sales price, as long as they are not placed in competition with large-volume producers.

13.6.2 Historical Shrimp Prices, Shrimp Size Categories, and Their Effect on Profitability

Selling price is crucial to the viability of any enterprise. The U.S. Department of Commerce provides value and quantity information for imported shrimp products that are a basis for prices used in feasibility studies. These may be found on the National Marine Fisheries Service site: <http://www.st.nmfs.noaa.gov/commercial-fisheries/foreign-trade/applications/monthly-product-by-countryassociation> (accessed 22 October 2018). A private company, Urner Barry, provides a subscription service for shrimp prices (<http://www.urnerbarry.com/> accessed 22 October 2018).

Prices change over time, by place of origin, product size, product form, and also according to the prices of competing sources of shrimp. Urner Barry provides historical data for two product forms over many size categories: shell-on headless and peeled headless. Shell-on headless shrimp originate from the Gulf of Mexico, Central and South America, Asia, India, and Bangladesh. Peeled headless product originates from Asia and the Gulf of Mexico. There are 5 to 13 categories of count-per-weight (pieces per lb) for each form. There are many competing sources of shrimp, and the cost of production, including a price mark-up, must be below the price of alternative products.

Two example pricing trends are offered here. Fig. 13.5 shows historical prices for all size categories of Gulf of Mexico Brown Shrimp (shell-on

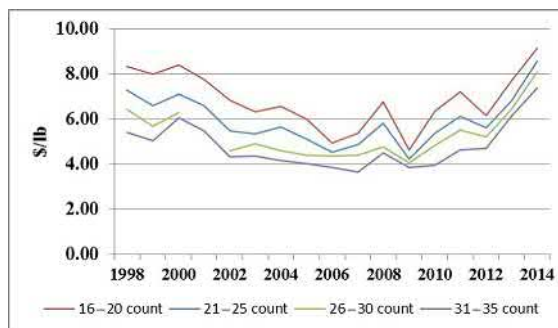


FIG. 13.5 Historical Gulf of Mexico Brown Shrimp (shell-on headless) prices at first point of sale, 1998–2014. (Courtesy of Urner Barry.)

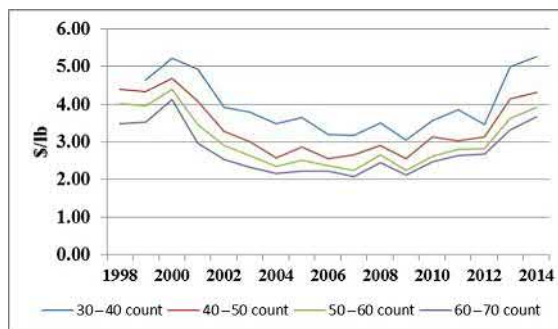


FIG. 13.6 Farm-raised Pacific White Shrimp prices, Central and South America (head-on) at first point of sale, 1998–2014. (Courtesy of Urner Barry.)

headless). Prices declined from the early 2000s to 2006 and have been increasing from 2012 to the present (2016). A similar trend is seen for farm-raised Pacific White Shrimp from Central and South America (Fig. 13.6).

The enterprise budgets generated earlier must choose a selling price to determine gross receipts. Prices in both figures are for sales to the first receiver—the US importer—and so are not strictly appropriate in a business plan for a US producer. The information on the range of prices by size category, the source of shrimp with which an enterprise will compete, and general pricing trends nevertheless is informative and will assist in understanding the market.

The analysis in [Section 13.4](#) indicated that increasing selling price by 20% was the second most important factor in improving NPV and IRR. The analysis in [Section 13.5.2](#) considered two price levels to provide insight into the price that turns an enterprise with a negative net return into one with a positive net return.

Shrimp harvest size also determines the length of the crop cycle and, therefore, the number of crops/yr. The number of crops/yr for the model in this chapter is computed by dividing 365 d/year by the sum of grow-out duration (d/crop) plus inter-crop downtime (d/crop). Whether or not this number of crops can be realized is critical to the validity of model projections. If a supply of healthy PL can be delivered as needed, then the probability of completing several crops/year is enhanced.

From a profitability standpoint, this leads to the question: Is it better to grow fewer large shrimp or more smaller shrimp in a year? The answer lies partly in the selling price for different sizes ([Table 13.27](#)). Larger shrimp command a higher market price, but the highest shrimp price may not produce the greatest net return when the number of production cycles per year is considered. We know that we can produce 30-g shrimp from 1- to 2-g juveniles stocked at high density that grow at more than 2 g/wk. The important economic question relates to whether or not the price for the larger shrimp—for

example, 30 g vs 25 g—justifies the cost of extending the crop.

The gap between the price for larger shrimp and grow-out cost is presented in [Table 13.28](#), in which the effect of shrimp size on crops/year, production quantity, COP, net returns, and other financial measures is compared for four product sizes: 15, 20, 25, and 30 g/ind. These data are from model projections based on costs and biological parameters presented earlier.

Ten crops/year are possible when 15-g shrimp are produced, but only 4.2 with 30-g shrimp ([Table 13.28](#)). The additional crops, despite producing lower priced smaller shrimp, increase annual production and receipts. The increased production offsets the lower price. Interestingly, variable costs do not change much between the size grades and fixed costs do not vary at all. Net returns above all costs are highest for the smallest size at \$286,943. The cost of production follows this same trend, with \$2.05/lb (\$4.52/kg) for 15-g shrimp increasing to \$3.05/lb (\$6.73/kg) for 30-g shrimp. The NPV and IRR are positive and highest for the smallest shrimp.

A big advantage of the indoor recirculating system analyzed before is that it can be sited near large urban markets. Product thus may be marketed as “fresh, never-frozen” in local markets. The production process also may be more easily adapted to serve niche markets that might not attract competition from large-volume producers of commodity shrimp. Market research efforts thus will benefit by determining local preferences in shrimp size and product form.

Finally, the flexibility to serve a mix of seafood buyers—from niche to commodity to retail—can reduce the risk of an outlet changing suppliers or no longer dealing with one’s product form. Niche markets may provide higher selling prices but may not be able to handle millions of pounds of shrimp. Wholesalers, on the other hand, may pay a lower price but can handle much greater quantities of product. One can make the same level of profit selling greater quantities at a lower marginal price or selling less product at a higher

TABLE 13.27 Historical Ex-Vessel Price (\$/lb) for Heads-on Shrimp From the Northern Gulf of Mexico

Shrimp Size, Count (#/lb)	Shrimp Weight (g)	10-yr Average Price (\$/lb)
Under 15	>30	5.02
15–20	22–30	4.28
21–25	18–22	3.27
26–30	15–18	3.13
31–35	13–15	2.77

TABLE 13.28 The Effect of Shrimp Size on Production and Economic Measures^a

Item	15g	20g	25g	30g
Crop duration, days	35	52	69	86
Number of crops/yr	10.1	6.8	5.2	4.2
Production, lb	401,710	363,864	344,396	332,535
Shrimp price, \$/lb	3.13	3.27	4.28	5.02
Receipts, \$	1,312,182	1,188,558	1,124,966	1,086,222
Variable costs, \$	829,400	832,932	834,748	835,855
Fixed costs, \$	195,838	195,838	195,838	195,838
Net returns above all costs, \$	286,943	159,788	94,380	54,529
Cost of production covering all costs, \$/lb	2.55	2.83	2.99	3.10
NPV, \$	1,477,959	708,674	261,381	−59,038
IRR, %	34.62	22.07	14.42	9.02

^a Based on greenhouse, grow-out and nursery raceway, investment and other specifications detailed in the bio-economic model of this chapter.

price. A mix of outlets may result in a higher average price than if shrimp are sold exclusively to only one type of outlet.

The message of this section is that comprehensive market research is absolutely essential before beginning production. It is the best way to project selling price at harvest and the quantity one might expect to sell (Hanson et al., 2006; Wirth and Davis, 2001). The aquaculturist thus must become a marketer/sales person or hire someone with the skills to fill this critical function.

13.7 CONCLUSIONS

Biofloc systems are becoming less expensive with better building material and economies of scale. Construction costs can be reduced with different materials, techniques, and scale. For example, substituting greenhouse coverings for preengineered steel buildings results in substantial savings. Substituting lined-bottomed raceways for concrete slab bottoms, and wood frames for block or poured concrete walls, also reduce the initial investment.

The economies of scale is evident in the lower cost per unit area of larger raceways. For raceways alone (no greenhouse covering), construction decreased from \$47/m² for a 268m³ raceway to \$31/m² for a 1000m³ raceway. Construction decreased from \$1052/m² for six 40m² raceway/greenhouse units to \$986/m² for two 100m³ units to \$198/m² for ten 500m³ units.

Years of research have resulted in technically feasible biofloc systems. Financial analyses demonstrate that their viability depends on production scale and losses from disease (*Vibrio*). The 2013 research trials had production costs of \$3.05 and \$3.28/lb. The 2014 trials assessed a possible new approach that involved raising PL to 6.5 g and then restocking those for final grow-out to 20-g. *Vibrio* outbreaks reduced survival in those trials to 76%, resulting in a very high production cost of \$4.08/lb.

Mortality was the most important factor affecting the cost of production, net returns, net present value, and the internal rate of return. Sensitivity analysis indicated that, for the 5000m² raceway/greenhouse complex, a 20% improvement in survival reduced the cost of

production by \$0.36/lb, increased NPV by \$10.48 million, and increased IRR by 13.7%. *Vibrio* seems to be the most important disease affecting shrimp production in super-intensive systems and its control needs to be the priority in commercial production.

While high production costs affect financial viability, selling price plays a key role in the final determination of economic viability. Shrimp prices can be volatile. From 2004 to 2011, prices were low but rose quickly in 2012 to 2014 owing to diseases in the shrimp farming sector. The higher prices make these recirculating systems much more viable and attractive investments.

Shrimp selling price varies with size. In super-intensive greenhouse systems, producing more crops per year of smaller shrimp is more profitable than producing fewer crops (and quantity) of larger shrimp. Marketing is a deciding factor in selecting the best size because niche markets may pay a very high premium for larger shrimp, especially if these are not readily available.

Those considering biofloc shrimp production must develop a business plan that integrates the biological, technical, physical, and financial aspects required for a viable business.

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Research and Results

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The following is a summary of nursery and grow-out trials conducted at the Texas A&M-AgriLife Research Mariculture Lab (ARML) over 16 year period with *Litopenaeus vannamei*. In most cases, nursery and grow-out trials were conducted in diluted natural seawater (NSW) with salinity of about 30 ppt. The main objectives were to improve management and economic viability of these systems when operated at high densities with no water exchange under biofloc-dominated conditions.

14.1 NURSERY TRIALS

14.1.1 Nursery Trials in the 40m³ Raceway System

14.1.1.1 1998–1999

Table 14.1 summarizes nursery studies from 1998 and 1999 under different stocking densities. Postlarvae (PL) were fed 50% and 45% crude protein feeds 6 times per day and supplemented with live *Artemia* nauplii the first week after stocking. These trials were conducted in water temperatures between 26.9 and 29.9°C, DO between 6.9 and 7.3 mg/L, pH between 7.8 and 8.3, TAN between 0.1 and 10.4 mg/L, and salinity between 16 and 21 ppt.

Each raceway had a pressurized sand filter to control particulate matter. Water-use efficiency varied between 1.2 and 1.8 m³/kg shrimp. The calculated water use included water to fill the raceway plus water to replace losses from evaporation, leakage, and filter backwashing. FCRs were below 1.0.

14.1.1.2 2000

Table 14.2 summarizes a follow-up 50-d nursery trial (Cohen et al., 2005) in two raceways stocked at 3700 PL8–10/m³ and supplemented with pure oxygen. Feed type and management were similar to those in 1998 and 1999. Average water temperature was slightly above 28°C (range: 24.5 to 31.5°C).

TAKE-HOME MESSAGES FROM THE 2000 NURSERY TRIAL—40 M³ RACEWAY SYSTEM:

- ✓ The nursery was capable of supporting biomass >4.6 kg/m³ of juvenile shrimp (av. wt. 1.1 to 1.23 g) with high survival (>97%), FCR below 1, and maximum water use of 352 L/kg shrimp,
- ✓ A swimming pool pressurized sand filter was capable of maintaining TSS below 200 mg/L,
- ✓ It was possible to maintain low ammonia (≤2 mg/L) throughout the trial,

TABLE 14.1 Summary of 40 m³ Nursery Trials (1998 and 1999) With Pacific White Shrimp Postlarvae at Different Stocking Densities

Density (PL10/m ³)	Duration (d)	Final Wt. (g)	Yield (kg/m ³)	Survival (%)	FCR	Water Use	
						(%/d)	(L/kg Shrimp)
1500	35	0.70	0.93	86.8	0.61	0.34	1197
1500	35	0.58	0.89	99.7	0.65	0.45	1302
1500	35	0.42	0.72	111.1	0.68	0.79	1772
2500	42	0.54	1.10	82.1	0.68	1.24	1378
2500	42	0.60	0.89	59.2	0.97	1.46	1816
3500	48	0.81	2.51	89.9	0.92	5.29	1410

TABLE 14.2 Summary of 50-d Nursery Trial in 2000 With PL8–10 (0.8 mg) Pacific White Shrimp at 3700 PL/m³ in 40 m³ Raceways With Sand Filter and Supplemented Pure Oxygen

Raceway ID	Final Wt. (g)	Yield (kg/m ³)	Survival (%)	FCR	Water Use	
					(%/d)	(L/kg Shrimp)
1	1.23	4.6	97	0.86	1.24	352
2	1.10	4.7	106	0.98	1.24	344

- ✓ Nitrite–N increased steadily from <4 mg/L in Week 5–26.4 mg/L in the last week,
- ✓ High survival and growth suggest no negative impact from this high nitrite exposure for about a week under trial conditions,
- ✓ Moderate increase (from 0.2 mg/L to 17 mg/L in 50-d) in nitrate–N concentration during the nursery, and
- ✓ Further information related to the nursery trials conducted in 1999 and 2000 can be found in: [Cohen et al., 2005](#); [Samocha et al., 2002](#).

a different method for removing excess particulate matter: a common swimming pool pressurized sand filter with manual backwash, an automated bead filter, and a large foam fractionator ([Fig. 14.1](#)).

Feed type and management were similar to those in 1998 and 1999. Temperatures ranged from 27.0 to 28.5°C, DO from 6.0 to 6.3 mg/L, pH from 7.5 to 7.6, and salinity was 25 ppt.

Weekly changes in nitrogen species and TSS are presented in [Fig. 14.2](#). Nursery water characteristics and production results are presented in [Fig. 14.3](#) and [Table 14.3](#).

14.1.1.3 2003

Water exchange and pressurized sand filters were used to control particulate matter in earlier trials. The transition into low- or no-water exchange required a more efficient method. The first step was to compare the particle removal capacity of other devices ([Handy et al., 2004](#)). A 74-d nursery trial was conducted in three raceways, each with

TAKE-HOME MESSAGES FROM THE 2003 NURSERY TRIAL—40 M³ RACEWAY SYSTEM:

- ✓ Shrimp tolerated TAN of 23 mg/L with no adverse effect on survival,
- ✓ High survival (96%) was achieved even with high nitrite concentration (30 mg/L NO₂–N) for almost a week,

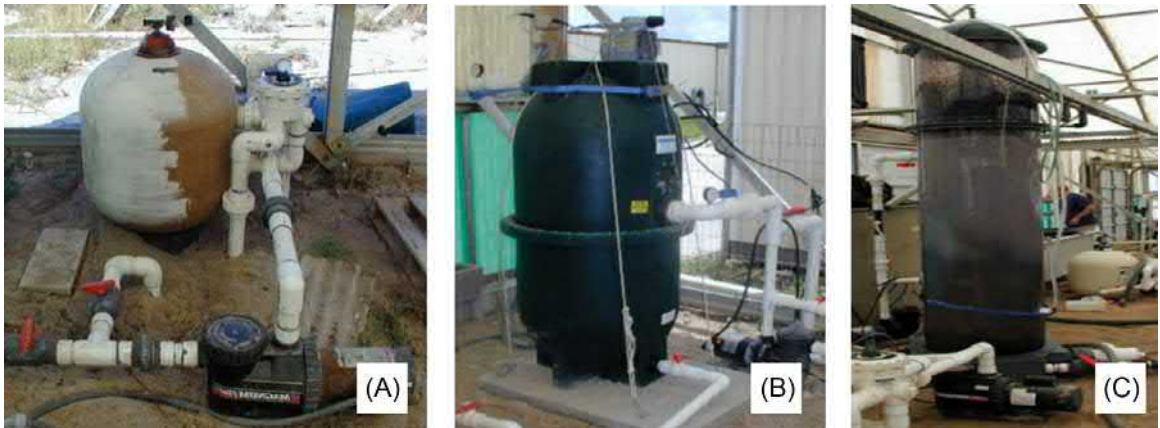


FIG. 14.1 (A) A common swimming pool pressurized sand filter with manual backwash, (B) an automated bead filter, and (C) a large foam fractionator used to control particulate matter in three separate raceways in the 2003 nursery trial.

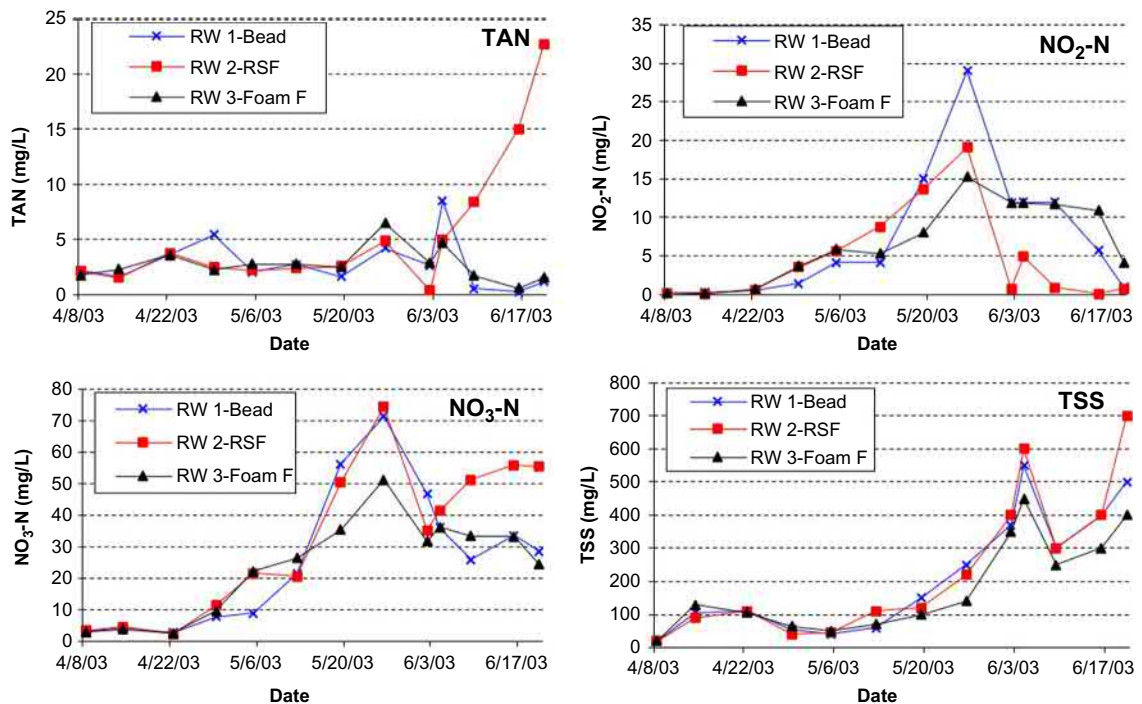


FIG. 14.2 Weekly changes in TAN, NO₂-N, NO₃-N, and TSS in trials with three different particle control methods.



FIG. 14.3 (A) Heavy foam developed in the raceway with the pressurized sand filter, (B) a persistent algal bloom developed in the raceway with a foam fractionator during the 2003 nursery trial, (C) Imhoff cones, showing (left to right) water coloration in the raceways operated with bead filter, sand filter, and foam fractionator.

TABLE 14.3 Summary of a 74-d Nursery Trial (2003) With 40 m³ Raceways With 0.6-mg PL5–6 Pacific White Shrimp at 4300, 7300, and 5600 PL/m³ With a Bead Filter (BF), Pressurized Sand Filter (PSF), and Foam Fractionator (FF)

Treatment	Final Wt. (g)	Yield (kg/m ³)	Survival (%)	FCR	Water Use	
					(%/d)	(L/kg Shrimp)
BF	0.65	2.7	96	1.70	1.5	780
PSF	0.85	5.9	100	1.09	0.5	235
FF	0.69	3.7	98	1.50	2.3	727

- ✓ Without adding nitrifying bacteria, it took 8 weeks for NOB to reduce nitrite,
- ✓ Oversized foam fractionators are not recommended for biofloc control because it strips large portion of the heterotrophic and nitrifying bacteria, allowing development of algal blooms ($4\text{--}5 \times 10,000,000$ cell/mL see Fig. 14.3b),
- ✓ Low water exchange reduces shrimp stress and mortality,
- ✓ Partial water exchange was required to reduce TSS in the raceway with the bead filter,
- ✓ The pressurized sand filter was not capable of controlling TSS and required manual removal of TSS from the surface, but with no need for water exchange,
- ✓ The raceway with the sand filter and manual biofloc removal could support 5.9 kg/m³ of

0.85 g juvenile shrimp with excellent survival (100%), low FCR (1.1), and low water use (235 L/kg shrimp) when stocked at 7300 PL/m³ in 74 days,

- ✓ An improved method is needed to crop biofloc,
- ✓ The highest yield required pure oxygen at 40 L/min during the last 2 weeks before harvest, and
- ✓ Further information related to the nursery trial conducted in 2003 can be found in: [Handy et al., 2004](#).

14.1.1.4 2004

Based on the good results from the previous trial with the sand filter and the need to improve particulate matter control, a 71-d nursery study

was conducted to compare raceways with a sand filter and homemade foam fractionator (Fig. 14.4) under reduced exchange (3.35%/d) to raceways with only a sand filter and increased exchange (9.37%/d) (Mishra et al., 2008).

The trial was conducted in four raceways with two replicates at 4000 PL4-5/m³. Feeds and feed management were as described for 1998 and 1999. Mean water temperatures varied between 26.2 and 27.4°C, DO between 5.9 and 6.3 mg/L, pH between 7.2 and 7.3, salinity about 27 ppt with average TSS <300 mg/L in both treatments.

TAKE-HOME MESSAGES FROM THE 2004 NURSERY TRIAL—40 M³ RACEWAY SYSTEM:

- ✓ Water exchange of 9.37%/d was effective in keeping low TAN (1–2 mg/L),
- ✓ TAN in the two raceways with daily exchange of 3.35% resulted in levels as high as 27 mg/L,
- ✓ Different daily exchange rates did not prevent nitrite-N from increasing to about 20 mg/L during Week-7 in one raceway of each treatment,
- ✓ Although shrimp in one low-exchange raceways reached high nitrite level, survival was very high (92%),

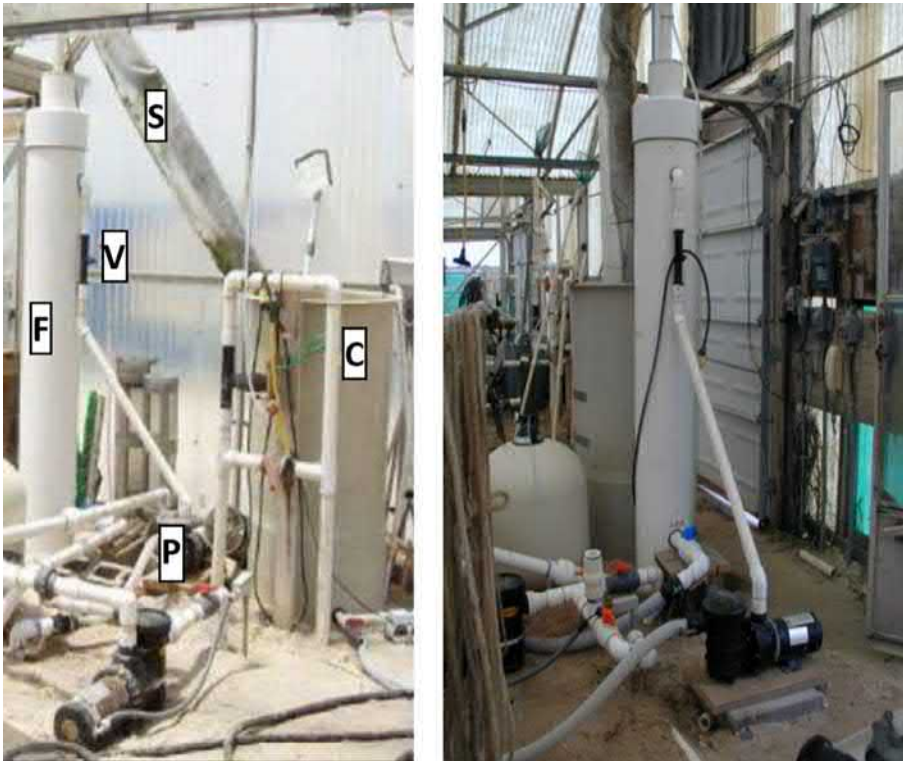


FIG. 14.4 Homemade foam fractionators (F) with a designated pump (P), Venturi injector (V), polyethylene foam-diverting sleeve (S), and foam collection tank (C).

TABLE 14.4 Results From a 71-d Nursery (2004) in 40m³ Raceways With 0.6mg Pacific White Shrimp PL at 4000/m³ and Particulate Matter Controlled by Water Exchange (WE) of 9.37%/d or a Combination of Pressurized sand Filters and Homemade Foam Fractionators (FF) with 3.35%/d Exchange in Two Replicates

Treatment	Size at Harvest (g)	Yield (kg/m ³)	Survival (%)	FCR
FF	1.9 ^a	7.6 ^a	100 ^a	0.97 ^a
FF	2.0 ^a	6.9 ^a	92 ^a	1.08 ^a
WE	1.7 ^b	3.9 ^b	56 [*]	1.64 ^a
WE	1.4 ^b	4.7 ^b	82 ^a	1.36 ^a

* Mortality due to mechanical failure.

Values within a column with similar superscripts are not significantly different ($P > .05$).

- ✓ 3.35% daily water exchange improved performance compared to 9.37% daily exchange with survival of: 92% and 100% vs. 82%, size: 1.9 and 2.0 g vs. 1.4 g, FCR: 0.97 and 1.08 vs. 1.36, yield: 6.9 and 7.6 kg/m³ vs. 4.7 kg/m³, health: intestinal histology showed lower bacteria load in shrimp from low-exchange treatment,
- ✓ Although performance was excellent with reduced exchange, the large homemade foam fractionators, and pressurized sand filters (Table 14.4), required frequent filter backwashes and intermittent operation of foam fractionators suggest the need for more suitable biofloc control, and
- ✓ Further information related to the nursery trial conducted in 2004 can be found in Mishra et al., 2008.

14.1.1.5 2009

A 62-d nursery study was designed to evaluate the effect of high- and low-protein feeds on growth, survival, and certain water-quality indicators under limited exchange (Correia et al., 2014). The trial was conducted in four

raceways with 5000 PL10–12/m³. The homemade foam fractionator used in the previous study was hard to regulate because the size was too large for 40m³ raceways and required a separate pump. Each raceway had a small commercial foam fractionator (Model VL65, Aquatic Eco-systems, Inc., Apopka, FL, US - see Video # 3) operated by the same 2-hp pump for aeration and circulation. Furthermore, because of the sand filters' limited biofloc cropping capacity (e.g., a very short run-time before backwash was required), biofloc control was solely by the foam fractionators.

Raceways had an online DO monitoring system (5200A YSI Inc., Yellow Springs, OH, US) that contributed to refining feed management and use of organic carbon supplementation to control inorganic nitrogen and promote biofloc development.

Water was inoculated with the diatom *Chaetoceros muelleri* to facilitate transition of PL from the hatchery to the nursery environment. It was fertilized (2.62 mg N/L, 0.25 mg P/L, and 1.66 mg Si/L) and inoculated with the diatom (70,000 cells/mL) one day before stocking.

Until Day 43, shrimp were fed four equal daily rations. From Day 44 on, 70% of the ration was offered during the day and the rest at night via three belt feeders per raceway. Beginning on Day 27, shrimp in two raceways were fed 30% protein feed; those in the other two were fed a 40% protein feed. Rations were adjusted based on observed consumption and distributed by hand four times per day.

From Day 10 to 18, each raceway received 0.5 L of molasses every other day. From Day 19 to 29, molasses was added when TAN rose above 3 mg/L. Molasses supplementation was calculated based on a nitrogen–carbon ratio of 1:6. It was not added after Day 30 because TAN was consistently below 0.5 mg/L.

Foam fractionators were operated only during the final two weeks, during which SS was >15 mL/L and/or TSS was >400 mg/L. Raceways were exposed to similar water

temperatures (26.6–28.7°C), DO (5.6–5.7 mg/L), pH (7.3–7.5), and salinity (29–31.5 ppt).

DO was always very high in the morning during the first 43 days. A drop in DO was noticed soon after feeding, with recovery just before the next feeding. DO recoveries always were to a level slightly lower than before the previous feeding, with a downward trend from morning to afternoon. It started few hours after the last feeding and reached the highest concentration just before the first feeding.

As mentioned, from Day 44, only 70% of the daily ration was fed in 4 equal portions during the day, while the rest was fed throughout the night by three belt feeders per raceway. DO monitoring showed that this feed delivery prevented the drop-and-recovery pattern observed before. Monitoring also helped schedule molasses additions that avoided significant DO drops and enabled more accurate pure oxygen use, saving money.

TAKE-HOME MESSAGES FROM THE 2009 NURSERY TRIAL—40M³ RACEWAY SYSTEM:

- ✓ Weight, survival, FCR, yield, and water usage were slightly better with the high-protein feed (Table 14.5),

- ✓ Inoculation with diatoms plus organic carbon supplementation (molasses) prevented high TAN (Fig. 14.5A),
 - ✓ Diatom inoculations and molasses did not prevent nitrite from reaching high levels (up to 25 and 20 mg/L NO₂-N for the high and low protein treatment, respectively—see Fig. 14.5B),
 - ✓ Diatom inoculations and applications of molasses did not accelerate establishment of nitrite-oxidizing bacteria (NOB) since it took 46 to 54 days for nitrite to start going down (Figs. 14.5B and D), which may suggest a need for a method to accelerate NOB development,
 - ✓ Nitrite and nitrate were significantly higher in the high-protein feed trials (Figs. 14.5B and C),
 - ✓ Except for the last week, when less attention was paid to TSS, the foam fractionators were capable of maintaining the TSS at ≤500 mg/L (Fig. 14.5E),
 - ✓ The online DO monitoring was valuable in optimizing DO levels, and
 - ✓ Further information related to the nursery trial conducted in 2009 can be found in: [Correia and Samocha, 2010](#); [Correia et al., 2014](#); [Samocha, 2009](#); [Samocha et al., 2010a, 2011a, b, 2012b](#).
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TABLE 14.5 Summary of 62-d Nursery Trial (2009) With 1-mg Pacific White Shrimp PL10–12 in 40m³ Raceways at 5000 PL/m³ Offered 30% and 40% Crude Protein (CP) Feeds

Variables	30% CP	40% CP
Final weight (g)	0.94 ± 0.00	1.03 ± 0.02
SGR ^a (%/d)	11.03 ± 0.01	11.19 ± 0.05
Survival (%)	82 ± 11	84 ± 6
FCR	0.91 ± 0.05	0.82 ± 0.05
Yield (kg/m ³)	3.7 ± 0.5	4.2 ± 0.2
Water use (L/kg)	303 ± 12	279 ± 2

^a Specific growth rate.

14.1.1.6 2010

Growth is a major factor affecting the economic viability of intensive shrimp systems. It thus is important to use genetic lines with high growth potential. A 52-d no-water-exchange nursery trial was conducted to (1) monitor shrimp performance and changes in water quality throughout a nursery trial with no water exchange; (2) determine the impact of inoculating diatoms (40,000 cells/ml), adding nitrifying bacteria (3m³ of nitrifying-rich water/raceway), and supplementing molasses on ammonia and nitrite levels; (3) determine if the small foam fractionators are adequate for biofloc control; and (4) evaluate performance of an online DO

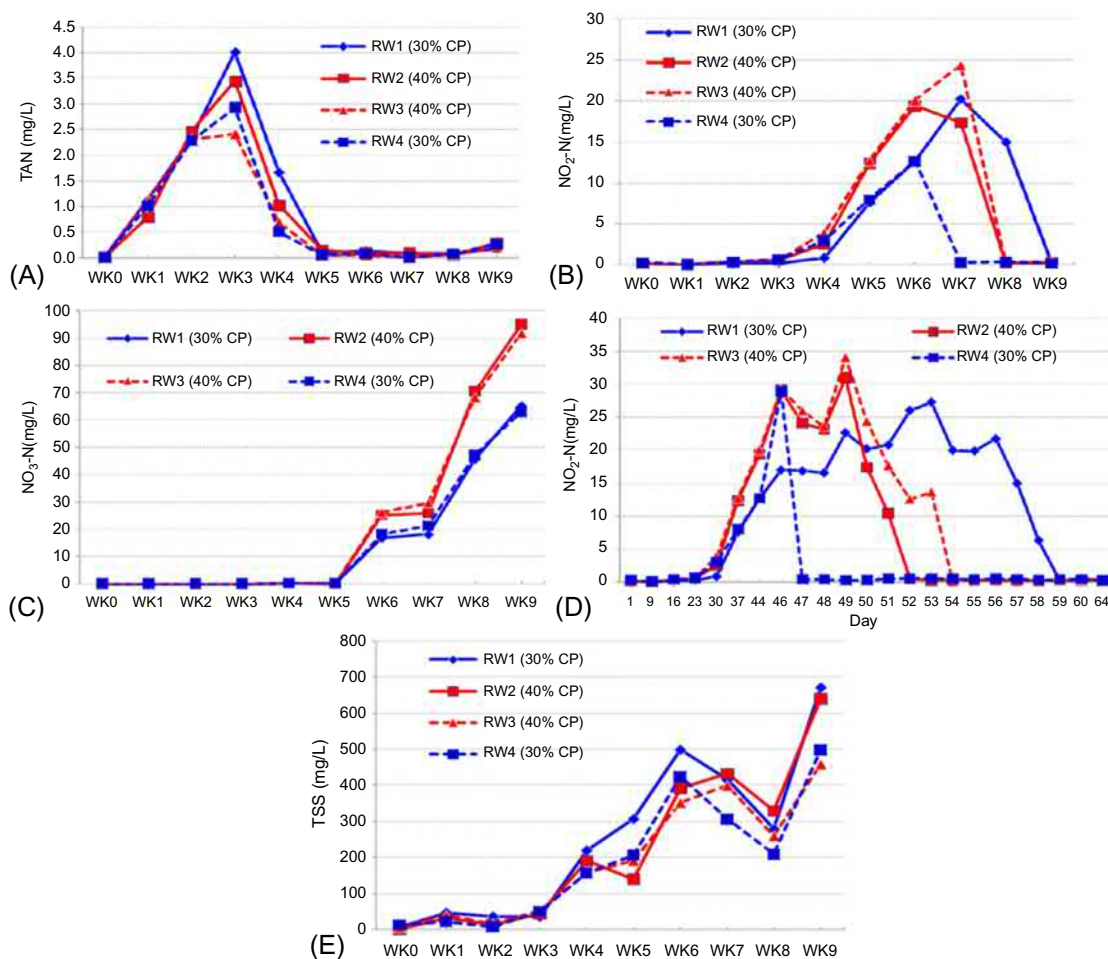


FIG. 14.5 Weekly changes in ammonia (A), nitrite (B), nitrate (C), daily changes in nitrite (D), and weekly changes in TSS (E). All data from a 62-d nursery trial in 2009 with Pacific White Shrimp PL10–12 in four 40 m³ raceways at 5000 PL/m³ fed 30% and 40% crude protein (CP) feeds.

monitoring system (YSI 5200A, Yellow Spring, OH, US) with polarographic sensors and external wiper.

Four raceways were stocked with 11-day-old PL at 3500/m³. Postlarvae were from two genetic lines: the Fast-Growth line and the slower-growth Taura-Resistant line.

Molasses supplementation was more aggressive than in earlier trials: 0.5 L/d on days 1–4, 8–11, 14–17, 21–22, 24–25, 27, and 1 L/d/raceway

on days 28–30. It varied on Day 18 between 2.85 and 3.5 L, depending on ammonia concentration in each raceway (e.g., adding 6 g of carbon for each 1 g of ammonia). From Day 35 until harvest, no molasses was added because ammonia was consistently below 0.5 mg/L. Molasses supplementation prevented ammonia accumulation but not nitrite. Nitrite-N increased up to 34.9 mg/L in one RW (Fig. 14.6) before dropping to low levels during Weeks 5 and 6.

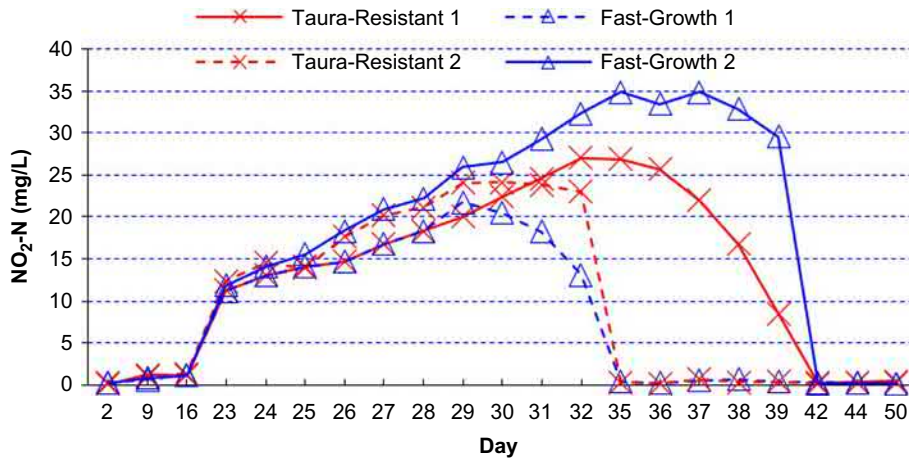


FIG. 14.6 Daily NO₂-N in a 52-d nursery trial (2010) with Pacific White Shrimp at 3500 PL11/m³ in four 40 m³ raceways and no water exchange.

TAKE-HOME MESSAGES FROM THE 2010 NURSERY TRIAL—40M³ RACEWAY SYSTEM:

- ✓ Algal inoculation, along with nitrifying-rich water and the organic carbon supplementation, helped maintain low ammonia (<5 mg/L) throughout the trial,
- ✓ These additions did not prevent nitrite from reaching high concentrations, but they shortened the time for NOB to be established by more than 10 days (Fig. 14.6),
- ✓ Shrimp tolerated up to 17-d of exposure to NO₂-N between 11.9 and 34.9 mg/L with no adverse effect on survival (>97%),
- ✓ Nitrate-N increased throughout the trial, reaching almost 160 mg/L,
- ✓ Foam fractionators maintained TSS below 500 mg/L,
- ✓ Once again, the online DO monitoring system helped regulate feed and molasses applications and prevented DO drops below required levels,
- ✓ Molts prevented smooth operation of the DO probe's wipers, suggesting the need for a more reliable method of cleaning the membrane,
- ✓ Survival in both treatments was high, but Taura-Resistant shrimp had higher final weights and better FCRs than Fast-Growth shrimp (Table 14.6), and
- ✓ Further information related to the nursery trial conducted in 2010 can be found in: [Samocha et al., 2011a](#).

TABLE 14.6 Performance of Fast-Growth and Taura-Resistant Pacific White Shrimp PL in a 52-d Nursery (2010) in Four 40 m³ Raceways at 3500 PL11/m³ and No Water Exchange in a Two-Replicate Trial

Treatment	Wt. (g)	Yield (kg/m ³)	Survival (%)	FCR	Water Use (L/kg Shrimp)
Taura-Resistant	0.97 ^a	3.7 ^a	97 ^a	1.01 ^a	350 ^a
Taura-Resistant	0.82 ^a	3.1 ^a	100 ^a	1.05 ^a	394 ^a
Fast-Growth	0.71 ^b	2.9 ^a	100 ^a	1.12 ^a	396 ^a
Fast-Growth	0.76 ^b	3.1 ^a	100 ^a	1.21 ^a	375 ^a

Values in columns with the same superscripts indicate no significant differences ($P > .05$).

14.1.1.7 2012

Many nurseries rely heavily on *Artemia* as feed for postlarvae during the first few days after stocking. *Artemia* nauplii also ease the transition of PL from the hatchery to the nursery. *Artemia* cysts are collected from natural sources, so their availability (and price) fluctuates from year to year. Further, as a wild-harvest product, *Artemia* have the potential for introducing pathogens. This risk is minimized by decapsulation.

These concerns have motivated evaluation of alternative larval and postlarval feeds. Partial replacement has been successful for many species, but complete substitution remains difficult. Attractability, palatability, digestibility, and potential negative impacts on water quality are only a few of the impediments to successful replacement of live or frozen *Artemia* (Zmora et al., 2013).

EZ *Artemia* (ZBI, Gardners, PA, US) mimics the color, taste, texture, and nutritional value of *Artemia* nauplii while eliminating the expense of hatching and processing *Artemia* cysts. EZ *Artemia* has ingredients selected for their quality, attractability, and digestibility; it also contains probiotics to enhance the health and survival of the target organism.

EZ *Artemia* was evaluated as a supplement for young postlarvae in a 49-d nursery study in six 40 m³ raceways with no water exchange. The trial also was designed to determine if inoculation with biofloc-rich water prevents high nitrite.

Additionally, the galvanic probe of the YSI 5200A DO monitoring system was replaced with a new system (YSI 5500D) operated with optical probe. Unlike the galvanic probe that requires a water current of 7–30 cm/s and membrane cleaning for reliable measurements, the optical probe does not require water flow or frequent maintenance.

Each raceway was filled with a mixture of seawater (20 m³), municipal freshwater (10 m³), and biofloc-rich water (10 m³) from a previous

grow-out study. Raceways were stocked at 1000/m³ with PL9 (2.5 ± 0.9 mg) from a hybrid of Fast-Growth and Taura-Resistant lines.

For the first 11 days, postlarvae in three control raceways were fed 50% protein dry feed (PL Raceway Plus, ZBI, Gardners, PA, US). Those in three other raceways were fed 52% protein EZ *Artemia* (25% by weight) and dry feed (75% by weight). All postlarvae were fed EZ *Artemia* in the hatchery. Shrimp in both treatments received 50% protein dry feed (PL Raceway Plus, ZBI) and 40% protein dry feed (Shrimp PL 40-9, ZBI) for the remainder of the trial.

Molasses was added at 500 mL/raceway on days 3, 13, 14, 15, and 1 L/raceway on days 4–5, 7–12, and 16–22. No molasses was added from Day 23 until the end of the trial.

A foam fractionator was used to control biofloc. Salinity was kept at 30 ppt with chlorinated tap water. Mean temperature, DO, and pH were 28.1°C, 5.92 mg/L, and 7.58, respectively. There were no significant differences in water quality between treatments (Table 14.8).

TAKE-HOME MESSAGES FROM THE 2012 NURSERY TRIAL—40 M³ RACEWAY SYSTEM:

- ✓ EZ *Artemia* resulted in slight, but not statistically significant, improvement in performance compared to shrimp fed dry feed throughout the trial (Table 14.7),

TABLE 14.7 Performance of Fast-Growth and Taura-Resistant Pacific White Shrimp PL9 (2.5 mg) in a 49-d Nursery Trial (2012) in 40 m³ Raceways at 1000 PL/m³ and No Exchange

	Dry Feed (Control)	EZ <i>Artemia</i> + Dry Feed
Final weight (g)	3.6 ± 0.1	3.6 ± 0.2
Yield (kg/m ³)	2.7 ± 0.1	2.8 ± 0.2
FCR	0.84 ± 0.04	0.81 ± 0.04

TABLE 14.7 Performance of Fast-Growth and Taura-Resistant Pacific White Shrimp PL9 (2.5 mg) in a 49-d Nursery Trial (2012) in 40 m³ Raceways at 1000 PL/m³ and No Exchange—cont'd

	Dry Feed (Control)	EZ <i>Artemia</i> + Dry Feed
Survival (%)	76 ± 1	77 ± 2
Water use (L/kg)	412 ± 19	414 ± 8

TABLE 14.8 Water Quality in a 49-d Nursery Trial (2012) in 40 m³ Raceways With Pacific White Shrimp at 1000 PL9/m³ and No Exchange

Parameter	Mean	Range
Alkalinity (mg/L as CaCO ₃)	170	96–235
Dissolved oxygen (mg/L)	5.9	4.0–8.8
NO ₂ -N (mg/L)	0.94	0.01–9.80
NO ₃ -N (mg/L)	54	0.1–68.0
pH	7.6	7.3–8.2
PO ₄ (mg/L)	5.3	0.1–10.7
Salinity (ppt)	30.4	25.9–32.5
SS (mL/L)	7	0–20
TAN (mg/L)	0.56	0.01–6.20
Temperature (°C)	28.1	24.2–31.9
TSS (mg/L)	146	5–685

- ✓ Increase in the volume of nitrifier-rich water (10 m³/raceway, or 25% of total volume), together with molasses supplementation, helped maintain average TAN below 2.5 mg/L (Fig. 14.7),
- ✓ Inoculation and carbon supplementation reduced the time to establish stable NOB to less than four weeks (Fig. 14.7),
- ✓ Average nitrite-N was below 7 mg/L (Fig. 14.7),
- ✓ Maximum nitrate-N was between 100 and 168 mg/L,

- ✓ Foam fractionators maintained average TSS below 330 mg/L and SS below 14 mL/L (Fig. 14.7),
- ✓ The 5500D online DO system with the optical probes performed very well and delivered accurate readings with minimal maintenance, and
- ✓ Further information related to the nursery trial conducted in 2012 can be found in: [Samocha et al., 2013a,b,c](#).

14.1.1.8 2014

Two 62-d nursery trials were run in 2014, one in the 40 m³ raceways and the other in the 100 m³ raceways. To avoid exposing shrimp to high nitrite while nitrite-oxidizing bacteria developed, trials evaluated acceleration of nitrification with either water rich in nitrifying bacteria or a commercial nitrification product.

Because of sporadic *Vibrio* outbreaks previously observed in our grow-out systems, yellow and green *Vibrio* colonies were measured on TCBS agar (see Section II.B—Appendix II). Green colonies were considered pathogenic. Sampling was twice weekly throughout the two trials with water enriched with a commercial nitrifying bacterial supplement and a probiotic. The trial in 40 m³ raceways also compared postlarvae performance when fed according to different feeding regimes.

Six raceways were stocked at 675 PL/m³ with PL5–10 (0.9 ± 0.6 mg) produced by hybridization of Fast-Growth and Taura-Resistant specific-pathogen-free (SPF) genetic lines.

Raceways were filled with 30 ppt natural seawater and then run without water exchange. Two days before stocking, each received 4 m³ of nitrifying-bacteria-rich water produced over three weeks in 6-m³ outdoor tanks with KI Nitrifier (Keeton Industries, Wellington, CO, US).

KI Nitrifier and white sugar were added as needed for the first five weeks after stocking to accelerate development of nitrifying bacteria. White sugar also was used as the organic carbon

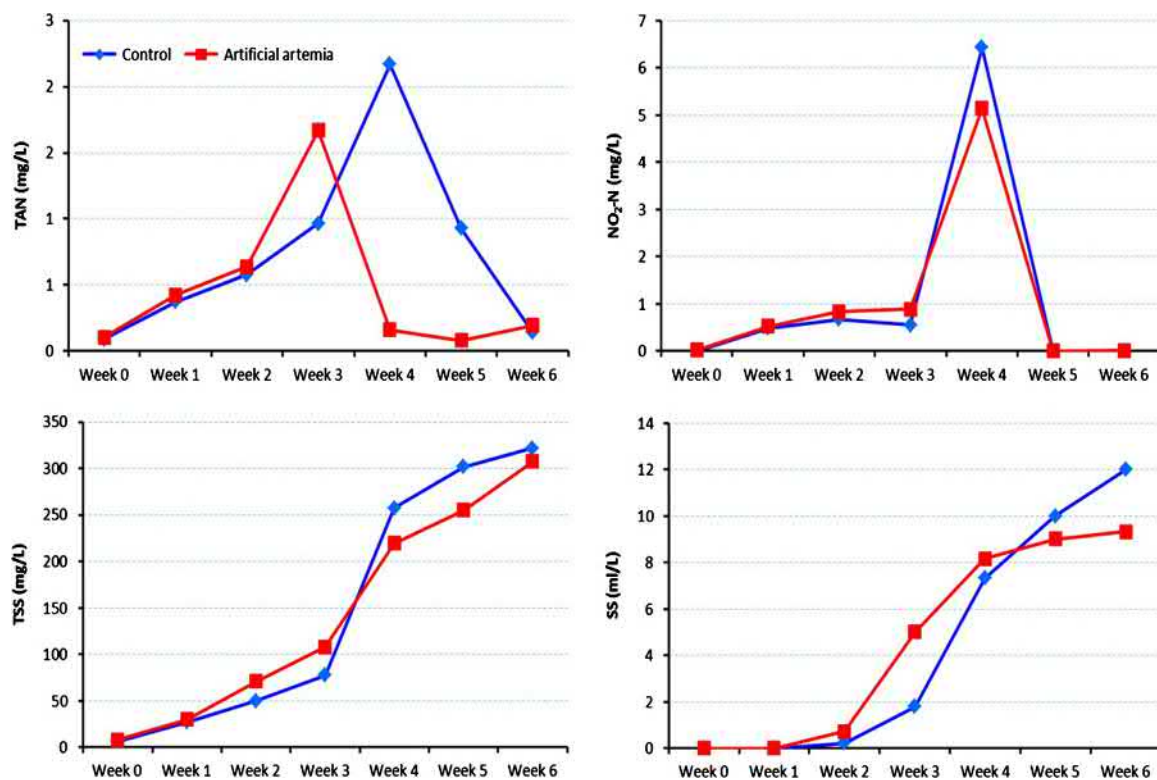


FIG. 14.7 Weekly changes in TAN, NO₂-N, TSS, and SS in a 49-d nursery trial (2012) in six 40m³ raceways with Pacific White Shrimp at 1000PL9/m³ and no exchange.

source instead of molasses. Each raceway received a bacterial supplement (Ecopro, EcoMicrobials, LLC., Miami, FL, US) every 1–3 days.

Pump-driven mixing was minimal during the first three weeks, during which raceways were manually mixed every second day to prevent development of anoxic zones. Mixing and aeration were increased gradually with the equipment in each raceway. The YSI 5500 DO monitoring system with optical probes was used.

Unlike previous trials, solids concentration was controlled with three tools: foam fractionators, settling tanks, and multicyclone filters.

To improve DO and reduce feed leaching, the old practice—30% of daily ration distributed at night by belt feeders—was changed to continuous feeding with six belt feeders per raceway.

Postlarvae in three raceways were fed a combination of dry feed (55% crude protein) and EZ *Artemia* for the first 10 days. Those in the other three raceways were fed only the 55% crude protein dry feed. Extremely high size variation at stocking necessitated abandoning the dry-feed-only treatment two days after stocking because many postlarvae had empty guts. After the second day, feed was distributed continuously by belt feeders. Feed size and feeding rates were adjusted according to growth, shrimp size variation (once every 2 weeks), expected growth, FCR, and survival.

After adjusting the feed program, there were no significant differences in final survival, weight, growth rate, yield, or FCR between the two treatments (Table 14.9). A significantly

TABLE 14.9 Summary of 62-d Nursery Trial (2014) With Pacific White Shrimp PL5–10 (0.9 ± 0.6 mg) at 675 PL/m^3 in 40 m^3 Raceways Fed EZ *Artemia* and Dry Feed in Biofloc-Dominated Water With No Exchange

Indicator	Mean $\pm$ SD
Survival (%)	85 ± 11
Final weight (g)	5.6 ± 0.6
Yield (kg/m^3)	3.2 ± 0.2
FCR	0.88 ± 0.06
Water use (L/kg)	464 ± 26
Sugar added (kg/m^3)	0.77 ± 0.07
Bicarbonate added (kg/m^3)	0.17 ± 0.04

low FCR (0.9) was obtained raising juveniles to 5.6 g. Despite good results, extra effort was required to accommodate postlarvae of different sizes. The coefficient of variation in shrimp size decreased from about 60% to 44% at harvest. A controlled study is needed to determine whether or not careful adjustment of feed particle size played any role in this reduction. Results underline the need for low size variation to streamline the nursery process.

The problem with the small postlarvae fed only dry feed emphasizes the importance of being alert to unexpected events, such as small or variable sizes. Under these conditions, EZ *Artemia* was key in providing proper nutrition during the earliest phases and so contributed to harvest success. Proactive management also was essential in controlling FCR and water quality.

There were no differences in water quality among raceways. Mean temperature, salinity, DO, and pH were 26.6°C ($20.8\text{--}30.2^\circ\text{C}$), 30.4 ppt ($29.4\text{--}31.5$ ppt), 6.47 mg/L ($4.43\text{--}8.52 \text{ mg/L}$), and 8.20 ($7.63\text{--}8.54$), respectively. Inoculation with nitrifier-rich water, controlled organic carbon additions, and use of commercial nitrifying bacteria concentrate were effective in preventing ammonia and nitrite from increasing to levels observed in previous trials. Mean TAN

and $\text{NO}_2\text{-N}$ were $0.79\text{--}1.17 \text{ mg/L}$ (max: 4.95 mg/L) and $1.4\text{--}3.2 \text{ mg/L}$ (max: 10.9 mg/L), respectively, and had no observed negative impact on postlarvae. Green *Vibrio* colony concentration remained below 100 CFU/mL , less than 28% of the yellow colony concentration.

TAKE-HOME MESSAGES FROM THE 2014 NURSERY TRIAL— 40 m^3 RACEWAY SYSTEM:

- ✓ It is extremely important to determine the size variation of each new batch of PL, and if the CV is $>10\%$, then feed particle size must be adjusted to accommodate all PL,
- ✓ Close monitoring of feed consumption and particle size is vital to prevent starvation and optimize nursery performance,
- ✓ Inoculation with nitrifying bacteria and careful use of organic carbon can prevent the increase in ammonia and nitrite to high levels,
- ✓ Commercial nitrifying bacteria concentrate can expedite development of nitrifying bacteria,
- ✓ In addition to avoiding high ammonia and nitrite, inoculation shortens the time to establish nitrification,
- ✓ TCBS agar plates are a good tool for quantifying pathogenic *Vibrio*,
- ✓ Probiotics may have contributed to the low FCR in this trial, and
- ✓ Further information related to the nursery trial conducted in 2014 can be found in: [Samocha et al., 2015a,b,c](#).

14.1.2 Nursery Trials in the 100 m^3 Raceways

14.1.2.1 2014

The only nursery trial conducted in the two 100 m^3 raceways was in 2014. Postlarvae source and size were the same as for the small raceways, but stocking density was lower (540 PL5-10/m^3). An additional objective to those mentioned for the trial in the 40 m^3

raceway system was to determine if a^3 injectors had an impact on postlarvae performance.

Two days before stocking, raceways were filled with 90m^3 of 30ppt natural seawater and 10m^3 of water with nitrifying bacteria. Municipal water was added periodically to compensate for losses from foam fractionators and settling tanks, but there was no water exchange during the trial. The same DO monitoring system was used, but each raceway had two optical DO probes.

White sugar additions kept ammonia below 3mg/L and KI Nitrifier (added on days 1, 4, 7, 10, and 32 at 26.42g/raceway) accelerated nitrification. The bacterial supplement Ecopro was added every 3 days at 20g/raceway , with 40g/raceway on Day 39 and 30g/raceway on Day 42. Solids were controlled by the foam fractionator and settling tank described in Sections 5.9.1.3 and 5.9.2.3.

Shrimp were fed EZ *Artemia* and dry feed. Feed size and rate were based on shrimp growth and size variation. Feed was delivered continuously via six belt feeders per raceway. Yellow- and green-colony *Vibrio* were monitored twice weekly (two replicates) using TCBS agar plates.

A 2-hp pump provided mixing and maintained DO above 4.5mg/L throughout the trial. The a^3 injectors were operated from the first day. The mesh size of pump intake filter screens was increased from 0.5 to 0.8 to 1.0mm as shrimp

grew. Because of high size variation, each screen change was delayed to avoid drawing small postlarvae into the pump.

Manual adjustment of water flow to each a^3 injector was made by ball valve. These were key to maintaining adequate DO and preventing damage to young postlarvae from strong mixing for the first days after stocking. Video #23 shows the fine mesh screens on the pump intakes.

Water temperature was low for the first few weeks. Other parameters were suitable for Pacific White Shrimp: mean temperature, salinity, DO, and pH were 26.6°C ($22.2\text{--}30.2^\circ\text{C}$), 30.4ppt ($29.7\text{--}31.1\text{ppt}$), 6.67mg/L ($4.41\text{--}8.46\text{mg/L}$), and 8.1 ($7.63\text{--}8.48$), respectively.

Mean TAN was $0.76\text{--}0.80\text{mg/L}$ (max: 2.72mg/L) and mean $\text{NO}_2\text{-N}$ was 1.60 to 2.27mg/L (max: 5.5mg/L). Nitrifier-rich water, white sugar, and the commercial nitrifying bacteria product were more effective in preventing the high TAN and nitrite of the other system. Maximum TAN and nitrite were about one-half of those in the small raceways (Fig. 14.8). As water temperature, mixing, and the amount of feed were different in the systems, more studies are needed to determine the main reason for the faster development of the nitrifying bacteria in these raceways. Green *Vibrio* colonies were below 50CFU/mL and less than 2% of yellow colonies throughout the trial.

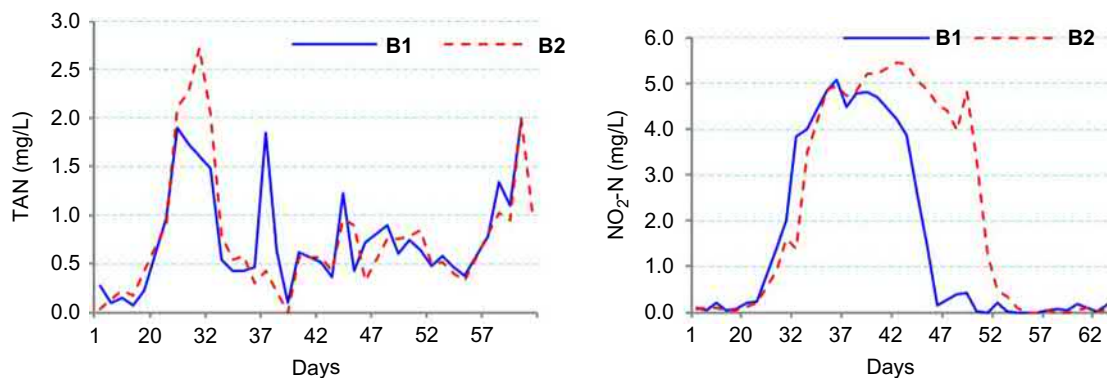


FIG. 14.8 Changes in TAN and $\text{NO}_2\text{-N}$ in a 62-d nursery trial (2014) with the Pacific White Shrimp PL5–10 ($0.9 \pm 0.6\text{mg}$) at $540/\text{m}^3$ in two 100m^3 raceways with no exchange.

TABLE 14.10 Summary of a 62-d Nursery Trial (2014) With Pacific White Shrimp PL5–10 (0.9 ± 0.6 mg) at 540 PL/m^3 in 100 m^3 Raceways fed EZ *Artemia* and Dry Feed in Biofloc-Dominated Water With No Exchange

	Raceway B1	Raceway B2
Survival (%)	98	95
Final weight (g)	6.5	6.4
Yield (kg/m^3)	3.4	3.3
FCR	0.81	0.81
Water use (L/kg)	420	447
Sugar added (kg/m^3)	0.33	0.33
Bicarbonate added (kg/m^3)	0.26	0.25

Average harvest weight (6.5 g) after 62 days was greater than that of shrimp from the 40 m^3 raceways (5.6 g). Low temperatures ($20.8\text{--}26.7^\circ\text{C}$) during the first four weeks caused a longer-than-normal nursery duration in both 40-m^3 and 100-m^3 systems.

Postlarvae size variation prompted frequent monitoring to adjust feed particle size properly. One 2-hp pump supported 3.4 kg/m^3 of shrimp biomass with no need for oxygen supplementation. Survival was very high and FCR was low (Table 14.10).

The 100 m^3 raceway was more uniformly mixed than the 40 m^3 raceway. Biofloc developed sooner, alkalinity declined faster, and nitrifying bacteria were established earlier.

Mean morning and afternoon DO throughout the trial was slightly higher ($6.55\text{--}6.79 \text{ mg/L}$) than in the 40 m^3 raceways ($6.36\text{--}6.57 \text{ mg/L}$) despite higher biomass. This suggests that the design of the 100 m^3 raceways with a^3 injectors provided a superior environment for nitrifying bacteria by enhanced mixing and higher DO, as demonstrated by the greater amount of bicarbonate required ($0.25\text{--}0.26$ vs. 0.17 kg/m^3) to maintain alkalinity.

TAKE-HOME MESSAGES FROM THE 2014 NURSERY TRIAL— 100 m^3 RACEWAY SYSTEM:

- ✓ Survival, growth, and yield were higher in the larger raceways,
- ✓ The very low (0.8) FCR for the 6.5 g shrimp suggested that similarly low FCRs are possible for market-size shrimp,
- ✓ Good shrimp performance, low pathogenic *Vibrio*, and lower ammonia and nitrite might be partly attributed to probiotics and nitrifying bacteria during the nursery phase,
- ✓ Establishment of nitrifying bacteria was faster than in the smaller raceways,
- ✓ Ammonia and nitrite maxima were lower than in other trials,
- ✓ Manually adjusting a^3 flow during the first few weeks was time consuming: A better option might be programmable variable-speed pumps to control water flow and mixing when raising young postlarvae, and
- ✓ Further information related to the nursery trial conducted in 2014 can be found in: [Samocha et al., 2015c](#).

Table 14.11 provides a summary of the nursery trials at the Texas A&M AgriLife Research Mariculture Laboratory (1998–2014).

14.2 GROW-OUT TRIALS

14.2.1 Grow-Out Trials in 40 m^3 Raceways

Grow-out trials in the 40 m^3 raceways started in 2006; those in the 100 m^3 raceways in 2010. Structural and management modifications were made over time to streamline production and make the systems more economically viable.

To calculate water-use efficiency when raceways were filled with water from a prior nursery trial, the added volume was subtracted from the total volume used for grow-out (e.g., taking into account the volume of new sea- and freshwater

TABLE 14.11 Nursery Trials in Raceways at the Texas A&M AgriLife Research Mariculture Laboratory (1998–2014)

Trial	Days	Stock (g/ind)	Harvest (g/ind)	Yield (kg/m ³)	Survival (%)	FCR	Water (L/kg)	References
1998–1999 40m ³ pp. 287	35– 48	PL10 (0.001)	0.42–0.81	0.72–2.51	59–111	0.61to 0.97	1197 to 1816	Samocha et al. (2002)
2000 40m ³ Page 287–288	50	PL8–10 (0.0008)	1.10 1.23	4.6 4.7	97 106	0.86 0.98	344 352	Cohen et al. (2005)
2003 40m ³ pp. 288–290	74	PL5–6 (0.0006)	0.65 0.69 0.85	2.7 3.7 5.9	96 98 100	1.1 1.5 1.7	235 727 780	Handy et al. (2004)
2004 40m ³ pp. 290–292	71	PL4–6 (0.0006)	1.9 2.0 1.7 1.4	7.6 6.9 3.9 1.4	100 92 82	1.0 1.1 1.4 1.6	438 485 1952 1614	Mishra et al. (2008)
2009 40m ³ pp. 292–293	62	PL10–12 (0.001)	0.94 1.03	3.7 4.2	82 84	0.82 0.91	279 303	Correia et al. (2014); Correia and Samocha (2010); Samocha (2009); Samocha et al. (2010a); Samocha et al. (2011a,b); Samocha et al. (2012b)
2010 40m ³ pp. 293–295	52	PL11–12 (0.001)	0.71 0.76 0.82 0.97	2.9 3.1 3.1 3.7	97 100 100 100	1.01 1.05 1.12 1.21	350 375 394 396	Samocha et al. (2011c)
2012 40m ³ pp. 296–297	49	PL9 (0.0025)	3.56 3.65	2.7 2.8	76 77	0.81 0.84		Samocha et al. (2013a,b,c)
2014 40m ³ pp. 297–299	62	PL5–10 (0.0009)	5.57	3.2	85	0.88	464	Samocha et al. (2015a,b,c)
2014 100m ³ pp. 299–301	62	PL5–10 (0.0009)	6.43 6.49	3.3 3.4	95 98	0.81 0.81	420 447	Samocha et al. (2015a,b,c)

added in the initial filling and for makeup). For example, if 25 m^3 of aged water from the nursery was used to partially fill a raceway for the grow-out trial, then only 15 m^3 of new water was needed to fill the raceway to capacity. If another 20 m^3 of replacement water (fresh and saline) was added during the grow-out trail, the net water use was $15 + 20 = 35\text{ m}^3$.

Studies were conducted in the same raceways used for nursery trials. To avoid bias in stocking, shrimp were harvested from nursery raceways (to determine survival, yield, etc.) and transferred to a single tank before restocking. This handling imposed additional stress that does not exist in a commercial setting. To take advantage of the benefits of preconditioned nursery water and to ensure equal experimental conditions, this water was collected, mixed, and returned to raceways. Because of storage limitations, this prolonged the start of grow-out trials and may have increased stress that does not exist in commercial settings. In a few cases, in fact, when juvenile harvest and stocking were done under high TSS, high water temperature, and low DO, we documented the direct link between stress and pathogenic *Vibrio* outbreaks in grow-out.

14.2.1.1 2006

A 94-d grow-out trial was set with four objectives: (1) determine if the shallow raceways used for the nursery trials could produce marketable shrimp at high stocking density and no water exchange; (2) monitor growth, survival, and FCR with limited water exchange; (3) compare the impact of foam fractionators and water exchange on water quality and shrimp performance; (4) determine if molasses supplementation is required to avoid ammonia and nitrite accumulation.

Six raceways with water from a previous 60-d nursery trial plus new seawater (75%:25%) were stocked with juveniles ($0.76 \pm 0.08\text{ g}$) at $279/\text{m}^3$. Shrimp were fed a 35% crude protein commercial feed (HI-35, ZBI, Gardners, PA, US) distributed by hand in four equal portions per day. Rations were calculated weekly, assuming FCR of 1.4, growth of 1.2 g/wk , and mortality of $1\%/wk$.

Two raceways had homemade foam fractionators (Fig. 14.4) and were run with limited water exchange. Another two were operated with low water exchange but without foam fractionators. For these four, molasses was added whenever TAN was above 1 mg/L . The last two raceways were operated with a little higher water exchange, no foam fractionators, and no molasses supplementation. All raceways had a short (45-cm) HDPE extruded net around the perimeter to prevent jumping losses (Fig. 14.9).

There were no significant differences in water quality among raceways: water temperature ($28.1\text{--}30.1^\circ\text{C}$), DO ($5.4\text{--}5.8\text{ mg/L}$), pH (6.7), and salinity (34–36 ppt).

TAN never exceeded 1 mg/L in the raceways designated to receive molasses, so none was added. In fact, TAN remained below 1 mg/L in all six raceways, with no significant differences among treatments. Except for higher reactive phosphorus ($13\text{ vs. }11\text{ mg/L PO}_4$) in the four raceways with reduced exchange, there were no significant differences in any of the other indicators. Nitrite-N in all raceways was low ($<2.5\text{ mg/L}$) and maximum Nitrate-N averaged 74 mg/L .

Owing to heavy losses from jumping (1%–5% of the population per night), the trial was terminated when shrimp reached $15.9\text{--}17.4\text{ g}$.



FIG. 14.9 A photo of the black HDPE-extruded netting around the perimeter of a 40 m^3 raceway used in 2006 in a 94-d grow-out trial with Pacific White Shrimp juveniles ($0.76 \pm 0.08\text{ g}$) at $279/\text{m}^3$.

TABLE 14.12 Performance of Pacific White Shrimp Juveniles (0.76 ± 0.08 g) Stocked at $279/\text{m}^3$ in a 94-d Grow-Out Trial (2006) in Six 40m^3 Raceways Operated in Duplicates With Three Treatments: No Foam Fractionator and Limited Water Exchange (No-FF), Foam Fractionator With Limited Water Exchange (FF), and No Foam Fractionator With Increased Water Exchange (WE) When Fed 35% Protein Feed

Treatment	Av. Wt. (g)	Growth (g/wk)	Yield (kg/m ³)	Survival (%)	FCR	Water Use(L/kg Shrimp)
No FF	17.2 ^a	1.3 ^a	4.1 ^{ab}	86 ^b	1.28 ^{ab}	170 ^a
No FF	17.2 ^a	1.3 ^a	3.9 ^{ab}	82 ^b	1.34 ^{ab}	112 ^a
FF	16.1 ^b	1.2 ^b	4.2 ^a	94 ^a	1.25 ^a	131 ^a
FF	15.9 ^b	1.2 ^b	4.3 ^a	96 ^a	1.24 ^a	113 ^a
WE	17.0 ^a	1.3 ^a	3.8 ^b	81 ^b	1.37 ^b	202 ^b
WE	17.4 ^a	1.3 ^a	3.8 ^b	77 ^b	1.41 ^b	203 ^b

Columns with the same superscript letters suggest no statistically significant differences ($P > .05$).

This underscored the need to add a short fence around each raceway (Fig. 14.9).

Average weight and weekly growth with the foam fractionators were significantly lower than in the other two treatments. Yields and water exchange in these raceways, however, were much higher and FCR was significantly less than in raceways with increased water exchange. Survival was greater with the foam fractionators (Table 14.12), and those shrimp showed no signs of viral or bacterial infections.

TAKE-HOME MESSAGES FROM THE 2006 GROW-OUT TRIAL— 40M^3 RACEWAY SYSTEM:

- ✓ Shallow raceways produced subadults (15.9–17.4 g) with good survival (77.2%–96.1%), low FCR (1.24–1.41), and moderate yield (3.75–4.26 kg/m³),
- ✓ Raceways required higher netting to prevent jumping losses,
- ✓ Venturi injectors on atmospheric air (i.e., without pure oxygen) met the DO demand of biomass at least as high as 4.2 kg/m³,
- ✓ Shrimp survival was higher with foam fractionators,

- ✓ Aged water helped maintain low NO₂-N (0.3 mg/L) and TAN (<1 mg/L), alleviating the need to add molasses, and
- ✓ Further information related to the grow-out trial conducted in 2006 can be found in: [Austin et al., 2007](#); [Samocha et al., 2013d](#).

14.2.1.2 2007

The 2007 trial explored use of settling tanks for solids control. This 92-d trial took place in four raceways, two with foam fractionators and two with settling tanks. The trial's objectives were: (1) determine if shallow nursery raceways could produce marketable shrimp at high density with no water exchange; (2) monitor growth, survival, and FCR with no or limited exchange; (3) compare the impact of foam fractionators and settling tanks on selected water-quality indicators with no exchange; (4) evaluate the benefit(s) of continuous DO monitoring.

Foam fractionators were the same as in the previous trial. Settling tanks had conical bottoms, a total volume of 8.6 m³, and a working volume of 4.9 m³. Four raceways were filled with water

from an earlier nursery trial (aged for 78 days). Juveniles (1.3 ± 0.2 g) were stocked at $531/\text{m}^3$.

Shrimp were fed the same feed with the same frequency and ration sizes as in the previous trial. Daily ration was reduced gradually from 5.0 to about 4.8 kg/d in the last week of the trial.

TSS control began on Day 29. Foam fractionators were operated intermittently, targeting a TSS of about 400 mg/L. Settling tanks received a constant flow (4 L/min) until Day 79 when water supply was stopped through the end of the trial because TSS was below 175 mg/L. There was no water exchange. Municipal freshwater or seawater was used to adjust salinity and compensate for operational losses.

An online DO monitoring system (5200A, YSI Inc.) with a polarographic DO and temperature sensors in each raceway was installed on Day 29. On reaching a biomass of $5\text{--}6\text{ kg}/\text{m}^3$, DO dropped from about 4 mg/L to 2.5 mg/L shortly after each feeding. A gradual recovery followed. From Day 53 forward, these fluctuations were minimized by feeding 2/3 of the daily ration in four equal portions during the day and the remainder through night from three belt feeders. Until Day 73, with estimated biomass of about $6\text{ kg}/\text{m}^3$, oxygen demand was met solely by the pump-driven Venturi injectors on atmospheric air. Beginning on Day 74, air was enriched with pure oxygen at 3.5 L/min.

No shrimp were lost to jumping. Shrimp submitted for disease diagnosis showed no signs of viral or bacterial infections.

There was no significant difference in water quality among treatments: mean water temperature was 29.4°C , salinity 33 ppt, pH 7.3, and DO 4.8 mg/L. TAN was low (0.1 mg/L) in all raceways. Raceways with foam fractionators had higher $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ than those with settling tanks. Higher nitrite may have stemmed from intermittent use of the foam fractionator, which removed large amounts of NOB and prevented continuous nitrification. Lower nitrate in the settling tank treatment suggested removal of nitrate by denitrification in settling tanks. Nitrite in raceways with foam fractionators peaked at about 10 mg/L $\text{NO}_2\text{-N}$ and was below 1 mg/L from Day 63. The nitrate-N drop to 20 mg/L in all raceways during the harvest week suggested active denitrification.

Table 14.13 summarizes performance over the 92-d study. Shrimp in raceways with settling tanks had higher final weights and yields; one yielded $9.3\text{ kg}/\text{m}^3$. Differences in yields, FCR, growth, and survival between the treatments were not statistically significant.

Homemade foam fractionators used in previous trials were operated with a 1-hp pump at flow rates of 260–300 L/min. To avoid reducing biofloc to suboptimal levels, foam fractionators were activated and deactivated every few days.

TABLE 14.13 Summary of a 92-d Grow-Out Trial (2007) in four 40 m^3 Raceways With Pacific White Shrimp Juveniles (1.3 ± 0.2 g) at $531/\text{m}^3$ Fed a 35% Crude Protein Feed and No Water Exchange

Treatment	Av. wt. (g)	Growth (g/wk)	Yield (kg/m^3)	Survival (%)	FCR	Water Use (L/kg Shrimp)
ST	18.4 ^a	1.3 ^a	9.3 ^a	88 ^a	1.21 ^a	62
ST	18.5 ^a	1.2 ^a	8.6 ^a	81 ^a	1.36 ^a	49
FF	17.4 ^b	1.2 ^a	8.6 ^a	81 ^a	1.40 ^a	53
FF	17.3 ^b	1.3 ^a	7.9 ^a	80 ^a	1.30 ^a	63

Foam fractionators (FF) and settling tanks (ST) for solids control with two replicates per treatment. Values with the same superscript in a column indicate no significant difference.

The resulting wide fluctuation in biofloc may have created unfavorable growing conditions (e.g., suboptimal concentrations of ammonia- and nitrite-oxidizing bacteria, fluctuations in DO, pH, etc.).

TAKE-HOME MESSAGES FROM THE 2007 GROW-OUT TRIAL—40M³ RACEWAY SYSTEM:

- ✓ Shallow raceways produced as much as 9.3 kg/m³ with good survival and low water use,
 - ✓ Surrounding raceways with tall netting prevented jumping losses,
 - ✓ Intermittent operation of oversized foam fractionators can create quick changes in biofloc concentration,
 - ✓ For the first 8 weeks, denitrification in settling tanks reduced nitrate,
 - ✓ Online DO monitoring helped formulate a feeding schedule that reduced feed-related DO drops,
 - ✓ Aged water contributed to lowering nitrite levels, and
 - ✓ Further information related to this grow-out trial can be found in: [Samocha, 2010](#); [Samocha et al., 2011b, 2012a, 2013a,b,c](#).
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14.2.1.3 2009

To address the unacceptably high biofloc fluctuations in the previous year's work that was attributed to the homemade foam fractionators, smaller commercial units (VL65, Aquatic Eco System, Apopka, FL, US) requiring much lower water flow (6–10 L/min) were tested in 2009. Unlike the foam fractionators that required a separate pump, these operated via a side loop on the discharge pipe of the 2-hp pump that circulated and aerated each raceway (see [Video # 3](#)).

A 108-d grow-out trial was run with juveniles (1.0 ± 0.2 g) stocked at 450/m³. Two raceways each had one of the smaller foam fractionators; two others had a settling tank.

The objectives were to (1) confirm that shallow raceways produce marketable Pacific White Shrimp at high density and no water exchange, (2) compare the effect of small commercial foam fractionators and settling tanks on water quality and shrimp performance, and (3) further evaluate continuous DO monitoring.

Raceways were filled with water from a recently completed 62-d nursery trial. For the first week, shrimp were fed a combination of nursery feed (30% protein, #4, Rangen Inc., Buhl, ID, US) and a 35% protein grow-out feed (HI-35, ZBI, Gardners, PA, US) formulated for intensive systems with limited discharge. The daily ration was divided into four equal portions until Day 18. From Day 19, 2/3 of the ration was fed in four equal portions during the day and 1/3 was provided continuously through the night with four belt feeders. Daily rations were adjusted based on an assumed FCR of 1.4, growth of 1.4 g/wk, and mortality of 0.5%/wk.

Use of settling tanks and foam fractionators was not required until Day 23. Water supply to these devices was adjusted to maintain TSS between 400 and 500 mg/L and settleable solids between 10 and 14 mL/L. Flow to the settling tanks varied between 2 and 6 L/min. Collected solids were drained every 6–8 weeks. No water was exchanged throughout the trial. Municipal chlorinated freshwater was added to compensate for water losses.

Water temperature, salinity, DO, and pH were monitored twice daily (YSI 600, YSI Inc., Yellow Springs, OH, US). Alkalinity and settleable solids were monitored every 2–3 days. Dissolved inorganic nitrogen and phosphate were monitored weekly. Alkalinity and pH were controlled by adding sodium bicarbonate, targeting an alkalinity of 160 mg/L and pH above 7.3.

Each raceway had the YSI 5200A DO system with a polarographic probe and external wiper. Data were uploaded to a lab computer with remote access. Oxygen supplementation did not begin until Day 68. For 40 days (Days 68–102), oxygen was used intermittently at 1 L/min for 30–60 min following daytime

feeding and whenever DO dropped below 3mg/L. Oxygen was provided continuously at 0.3–0.5L/min during the final week (Days 102–108).

There were no significant differences in water quality among treatments: mean water temperature was 29.3°C, salinity 30.6ppt, pH 6.8, and DO 5.0mg/L. Mean alkalinity with foam fractionators was less than with settling tanks (124 vs. 129mg/L), but this difference is small as a practical matter.

Mean nitrate was higher with foam fractionators (232 vs. 193mg/L NO₃-N), with higher nitrate on the last day (459 vs. 359mg/L NO₃-N). Alkalinity and nitrate differences again pointed to denitrification in settling tanks.

Inoculating raceways with nitrifier-rich water helped maintain very low ammonia (<1mg/L TAN) and nitrite (<1.5mg/L NO₂-N) in all raceways despite high shrimp biomass (>9.3kg/m³) and no organic carbon additions. Settleable solids reached 33mL/L in one raceway on Day 43, but concentrations mostly were between 10 and 30mL/L. TSS rose as high as 790mg/L on one occasion, but concentrations generally were 400–500mg/L. There were no signs of bacterial infection during this trial.

The YSI 5200A DO monitor proved to be a valuable management tool. Its data contributed to a significant reduction in pure oxygen use over previous trials. Approximately 37L of pure

oxygen and 15.4kW of electricity were used to produce 1kg of shrimp.

Except for higher survival in raceways with foam fractionators, there were no other significant differences between treatments, although slightly greater final weights were obtained with foam fractionators (Table 14.14). As juveniles were of the Taura-Resistant line, slower growth was not a surprise. High survival and yields in both treatments offset the extended growth period and relatively high FCR.

TAKE-HOME MESSAGES FROM THE 2009 GROW-OUT TRIAL—40M³ RACEWAY SYSTEM:

- ✓ High yield and good performance can be obtained in shallow raceways,
- ✓ Except for improved survival, there was no difference in shrimp performance between the small foam fractionators and settling tanks,
- ✓ Maintaining TSS between 400 and 500mg/L, with occasional increases up to 790mg/L, did not have a negative impact on shrimp performance,
- ✓ Yields were higher with aged water and no exchange or organic carbon supplementations was needed to keep TAN and NO₂-N below 1.0 and 1.5mg/L, respectively,

TABLE 14.14 Pacific White Shrimp Performance in a 108-d Grow-Out Trial (2009) in Four 40m³ Raceways with 1.0g Juveniles at 450/m³ Each Operated With a Foam Fractionator (FF) or Settling Tank (ST) for TSS Control With Two Replicate per Treatment

Treatment	Final Weight (g)	Growth (g/wk)	Yield (kg/m ³)	Survival (%)	FCR	Water use (%/d) (L/kg Shrimp)	O ₂ (L/min-Last 7 Days)
ST	22.0	1.4	9.3	95 ^a	1.60	0.28	32 0.19
ST	21.8	1.4	9.5	95 ^a	1.57	0.27	27 0.16
FF	22.5	1.4	9.5	97 ^b	1.53	0.24	27 0.36
FF	22.4	1.4	9.8	96 ^b	1.57	0.22	24 0.19

Values with the same superscript letters within a column indicate no significant difference ($P > .05$).

- ✓ Good survival was obtained in both treatments, where the cutoff point for DO supplementation was below 3mg/L,
 - ✓ No adverse effect on survival was noticed even though NO₃-N at harvest was 462mg/L,
 - ✓ Removing solids accumulated in the settling tanks every 6–8 weeks helped reduce nitrate,
 - ✓ Online DO monitoring system helped modify feeding practices to prevent undesirable DO fluctuations,
 - ✓ The external wiper for the polarographic sensor is not recommended for biofloc conditions owing to high maintenance,
 - ✓ Extrapolation to a commercial system of eight grow-out and two nursery tanks with 3.7 crops/yr projects a payback period, Net Present Value, and Internal Rate of Return of 2.8 years, \$1,081,000, and 32.8%, respectively (10-year horizon, 10% discount rate).
- Chapter 13 has a more complete discussion, and
- ✓ Further information related to this grow-out trial can be found in: [Correia and Samocha, 2010](#); [Haslun et al., 2012](#); [Samocha, 2010](#); [Samocha et al., 2010a,b, 2011a,b, 2012a, 2013a,c](#).

14.2.1.4 2010

Because of slow growth in the previous trial (1.4g/wk), objectives of the 2010 grow-out work were to compare performance of juveniles of the Fast-Growth line with those of the Taura-Resistant line. Grow-out was conducted in four 40m³ raceways filled with water from the previous 52-d nursery trial and stocked at 550/m³. Initial weight was 0.74g for Fast-Growth juveniles and 0.90g for Taura-Resistant juveniles. Each raceway had only the small commercial foam fractionator for solids control. Feed, feed management, DO monitoring, and water exchange practices were similar to those of the previous trial.

Although results were poor, the following summary describes the events that occurred and the steps taken to find workable solutions for the poor performance.

The first mortality was observed on Day 44 in a raceway with Fast-Growth juveniles when mean weight was 8g and biomass was about 4.4kg/m³. Mortality subsequently continued in this raceway, with daily losses in the tens to as many as 2000. Despite exchanging more than 100% of the volume, mortality continued and reached a maximum of 5400/day. Production was terminated on Day 72 when shrimp averaged 15g and survival was 16.3%. Mortality in the other three raceways was slightly less, so the trial was continued for another 69 days to evaluate ways of halting this unusually high mortality.

No mortality was noticed in the two nearby 40m³ raceways, of which one was only 0.5m from the raceway where *Vibrio*-related mortality was first noticed. These had been stocked with Taura-Resistant postlarvae harvested at 8.5g after 105 days and used in the first 2010 grow-out trial in the 100m³ raceways. Survival in this 87-d trial was >89.5%, suggesting that the health of these shrimp was not compromised during the 61 days they spent near the *Vibrio*-infected raceway.

Although shrimp in the affected raceways showed morphological signs resembling Noda virus infection (Fig. 14.10A), testing indicated that this virus was not present. Many shrimp in each raceway showed tail deformities (Fig. 14.10B). With initial results from disease diagnostic laboratories suggesting *Vibrio* infection, salinity was reduced from 30 to 15ppt on Day 91, but without any positive response.

On Day 95, shrimp feed was coated with 1.1% Activate (Novus International Inc., Saint Charles, MO, US), although the manufacturer recommends that the product be applied with extruded feed. Activate contains a blend of organic acids and methionine hydroxy analog, a highly bio-available source of methionine. The organic acids in Activate are designed to reduce the pH of the gastrointestinal tract and promote desirable and more balanced intestinal flora, thus aiding digestion, providing more nutrients from feed, and improving performance.

This treatment did not reduce mortality, so on Day 105 feed was also coated with 0.0275% EZ

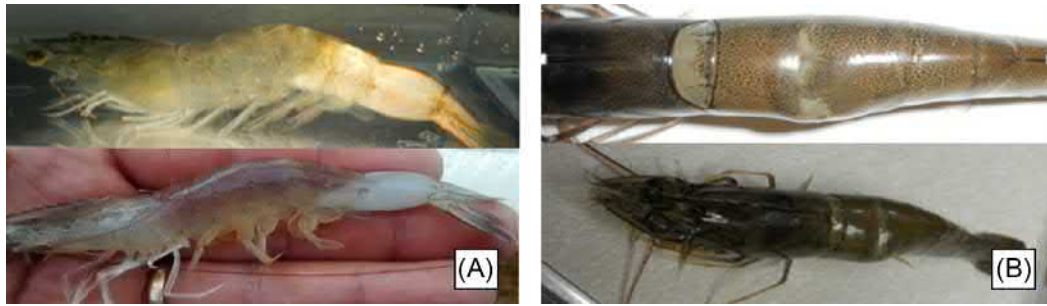


FIG. 14.10 Pacific White Shrimp showing tail necrosis (A) and tail deformities (B).

Bio (ZBI), a multifunctional biological aquaculture feed additive of nonpathogenic bacteria recommended to be added during feed preparation. It is specifically formulated for use in shrimp and fish hatcheries to combat pathogenic bacteria such as *Vibrio*. No significant improvement in mortality was noticed.

Vibrio parahaemolyticus was isolated from shrimp hemolymph and determined to be sensitive to oxytetracycline (OTC). A special INAD permit (Investigational New Animal Drug) was obtained and shrimp in two raceways were provided a medicated feed (4.4 g of OTC/kg feed) for 14 days. A clear reduction in daily mortality subsequently was observed in both raceways. Average weight at harvest after 141 days was 34 to 37 g, with survival of 5.6%–7.9%.

Ammonia and nitrite were very low before the disease was discovered, although there were several short intervals of high water temperature ($>34^{\circ}\text{C}$), TSS ($>1083\text{ mg/L}$), SS (150 mL/L), and low DO ($\leq 3.5\text{ mg/L}$). These may have contributed, separately or together, to triggering the outbreak, but this is speculation, not a confident explanation of the origin of the problem.

TAKE-HOME MESSAGES FROM THE 2010
GROW-OUT TRIAL—40M³ RACEWAY SYSTEM:

- ✓ Extensive effort should be made not to expose shrimp to stressors that compromise their

immune system and open the door for pathogenic *Vibrio* outbreaks,

- ✓ Massive water exchanges did not stop *Vibrio*-related mortalities,
 - ✓ Activate (a blend of organic acids and methionine hydroxy analog, Novus International Inc.) and EZ Bio (a multifunctional aquaculture feed additive of nonpathogenic bacteria, Zeigler Bros. Inc.) did not halt mortality,
 - ✓ Oxytetracycline (OTC) was effective in stopping the mortality, and
 - ✓ Further information related to this grow-out trial can be found in: [Samocha et al., 2011d](#).
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14.2.1.5 2011

Members of the United States Marine Shrimp Farming Program (Oceanic Institute in Hawaii, Gulf Coast Research Lab in Mississippi, Wadell Mariculture Center in South Carolina, and Texas A&M AgriLife Research) initiated a comparative study using economic modeling and other metrics to evaluate the intensive biofloc systems and management practices of each member. Participating facilities attempted to standardize salinity, stocking density, feed, and postlarvae sources to allow meaningful comparisons. The objective was to study changes in water quality and performance of Fast-Growth juveniles stocked at high density with no water exchange.

TABLE 14.15 Summary of the 2011 Grow-Out Trial With Pacific White Shrimp Juveniles in Five 40 m³ Raceways at 500/m³ With No Water Exchange and Fed a 35% Protein Feed

Raceway	Av. Weight (g)		Days	Growth (g/wk)	Survival (%)	Yield (kg/m ³)	FCR	Water Use (L/kg Shrimp)	Salinity (ppt)
	Stocking	Harvest							
1	1.9	22.2	81	1.8	88	9.7	1.39	147	18
2	1.9	23.6	82	1.9	82	9.6	1.44	139	18
3	1.9	23.4	82	1.8	82	9.4	1.45	126	18
4	1.9	23.8	83	1.9	79	9.4	1.45	138	18
5	1.4	25.1	85	2.0	79	9.9	1.44	127	30
Av.		23.6		1.9	82	9.6	1.43	135	
SD		0.9		0.1	0.3	0.2	0.02	9	

Four 40m³ raceways were filled with a mixture of 12m³ seawater, 8.5m³ biofloc-rich water from an earlier 42-d nursery trial, and 19.5m³ of municipal freshwater to adjust salinity to 18ppt. Juveniles (1.9g) produced on-site from nauplii received from the Oceanic Institute were stocked at 500/m³ and harvested 81–83 days later (Table 14.15). A fifth raceway with a salinity of 30ppt was stocked at the same density with Fast-Growth juveniles (1.4g). These were harvested 85 days after stocking.

Each raceway had a small commercial foam fractionator. Solids targets were 200–300mg/L TSS and 10–14mL/L SS. The TSS target was increased on Day 30 to 400–500mg/L to minimize algal blooms. A homemade 550-L settling tank (Fig. 5.30) was added to each raceway on Day 43 because of the inability of foam fractionators to maintain TSS at the desired level. Alkalinity was adjusted to 150–200mg/L with sodium bicarbonate. All raceways had the DO monitoring system (YSI 5200A) described earlier.

Shrimp were fed a 35% protein feed (HI-35, ZBI). Daily rations were calculated assuming an FCR of 1.2, growth of 2.0g/wk, and mortality of 0.25%/wk. Rations were based on observed consumption and growth monitored twice per

week. Two-thirds of the daily ration was fed in four equal portions during the day and one-third through the night with three belt feeders per raceway.

Seawater and freshwater were used to maintain salinity and offset evaporative and operational losses. There was no water exchange. Oxygen supplementation began on Day 44 when estimated biomass was 6.5kg/m³. Molasses was applied only when TSS was below 200mg/L to accelerate heterotrophic bacteria development and prevent algal blooms.

There were no statistically significant differences in water quality among raceways: mean water temperature was 29.4°C (28.2–30.7°C), DO was 5.7mg/L (4.0–7.1mg/L), and pH was 7.3 (6.9–7.9). Calculated carbon dioxide in the four raceways averaged 18.6±2.4mg/L (6.7–35.5mg/L). It was 22.5±11mg/L (7.6–63.1mg/L) in the raceway with salinity of 30ppt. TAN remained below 0.7mg/L and nitrite below 1mg/L NO₂-N in all raceways. Nitrate increased from about 10mg/L to a maximum of 350mg/L NO₃-N at the end of the trial.

Growth, survival, FCR, and yields were high (Table 14.15). Except for greater survival in one of the 18ppt raceways, survival at 30ppt was

comparable. Slightly better harvest weight, growth, and yield were observed at 30 ppt.

Poor performance in trials at other institutions made it impossible to compare results. Waddell Mariculture Center achieved 6.6 kg/m³, but with mediocre production parameters caused by a late start of grow-out, poor-quality postlarvae, and blue-green algae growth during the seasonal transition. The other two institutions lost crops entirely.

TAKE-HOME MESSAGES FROM THE 2011
GROW-OUT TRIAL—40M³ RACEWAY
SYSTEM:

- ✓ Aged water helped maintain healthy nitrifying bacteria in the culture water that prevented increase in TAN and nitrite in all five raceways even at high shrimp yields,
 - ✓ When TSS levels are reduced, unintentionally, to below 150 mg/L (e.g., a drastic reduction in the nitrifying bacterial population in the system), temporary organic carbon supplementation at a rate which will allow the heterotrophic bacteria to convert all excess TAN to bacterial biomass, can prevent increase in TAN and nitrite and provide the slower growing nitrifying bacteria the time for them to recover,
 - ✓ When concentration of TSS is low, organic carbon supplementation can be used to increase heterotrophic bacteria concentration to reduce light penetration and prevent algal blooms,
 - ✓ The commercial foam fractionators alone could not keep TSS within the required range,
 - ✓ Shrimp raised in 18 ppt salinity grew better than previously (1.8–1.9 g/wk), yielding 9.4 and 9.7 kg/m³, and those in 30 ppt salinity grew at 2 g/wk and yielded 9.9 kg/m³, and
 - ✓ Further information related to this grow-out trial can be found in: [Hanson et al., 2013a,b](#), [Samocha et al., 2011a,b, 2012b](#).
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14.2.1.6 2012

Based on the encouraging 2011 results, the 2012 study in the 40 m³ raceways evaluated the impact of two commercial 35% crude-protein feeds of different quality and price on shrimp performance and water quality under high stocking density and no water exchange. One feed (HI-35, ZBI) was formulated for super-intensive production systems and the other (SI-35, ZBI) for outdoor semi-intensive production ponds.

The 67-d trial was run in six raceways filled with 18 m³ of water used in a preceding 49-d nursery study plus 22 m³ of natural seawater and municipal freshwater to reach 30 ppt. Raceways had small commercial foam fractionators and the small homemade settling tanks described earlier. The YSI 5200A DO monitor was replaced with the YSI 5500D, which uses optical probes.

This study stocked a cross of Fast-Growth and Taura-Resistant lines developed by Shrimp Improvement Systems (Islamorada, FL, US). Postlarvae mortality in the first shipment provided an unplanned opportunity to study the performance of juveniles of two distinct size classes when cultured together at high density. The two groups were produced from two batches received eight days apart and reared at 1000 and 3000/m³ to average weights of 3.7 and 0.9 g/ind, respectively. Of the 20,000 stocked in each raceway (500/m³), 12,000 (300/m³) came from the higher weight group to form a population average of 2.7 g/ind.

Three raceways were fed HI-35 feed (\$1.75/kg) and three the SI-35 (\$0.99/kg). Feed was distributed manually for the first three days. From Day 4–11, both manual feeding and automatic belt feeders were used. From Day 12–47, feed was delivered by four belt feeders over 12 h. Beginning on Day 48, shrimp were fed with 24-h belt feeders.

For the first month, daily rations were based on an assumed growth of 1.5 g/wk, an FCR of 1.4, and mortality of 0.5%/wk. Rations later

were adjusted based on consumption and results of twice-weekly sampling. Growth eventually was adjusted to 2.6 g/wk.

Use of foam fractionators began on Day 7 and settling tanks on Day 44. These biofloc control tools were operated intermittently, targeting TSS of 200–400 mg/L and SS of 10–12 mL/L. Flow rates varied from 8.5 to 12 L/min for the settling tanks and 6 to 10 L/min for the foam fractionators. There was no water exchange; fresh and seawater were added as in previous trials.

Water temperature, salinity, dissolved oxygen, and pH were monitored twice daily with a YSI 650 handheld multiprobe. Settleable solids were monitored daily and alkalinity twice per week, adjusted to 150–200 mg/L with sodium bicarbonate as needed. TSS was monitored three times per week and kept within 200–400 mg/L. Nitrogen and phosphate were monitored weekly.

From Day 17 through Day 38, oxygen supplementation was intermittent and related to daily events (feeding, molasses addition). From Day 39 when estimated biomass was 6 kg/m³, oxygen was provided continuously (3.4–8.2 L/min) owing to chronic low DO. The YSI 5500D monitor was a reliable tool in combating low DO; the optical probes reduced calibration and maintenance time. There were no differences in water quality between treatments (Table 14.16).

This study confirmed that partial use (<50%) of biofloc-conditioned water from the nursery was effective in establishing nitrifying bacteria in grow-out raceways. As a result, ammonia and nitrite remained low throughout the study, with no significant differences between treatments.

Nitrate increased steadily from 40 to 359 mg/L NO₃-N with HI-35 and from 46 to 286 mg/L with SI-35, with no significant difference between treatments.

Average TSS with SI-35, 278 mg/L (155–460 mg/L), was significantly greater than 223 mg/L (115–552 mg/L) with HI-35. These differences could be related to the higher fiber (2.69% vs. 1.61%) and ash (11.11% vs. 9.55%) in SI-35 feed. Higher TSS in the SI-35 treatment

TABLE 14.16 Water Quality in the 2012 Grow-Out Trial With Pacific White Shrimp Juveniles in 40m³ Raceways at 500/m³ With No Water Exchange and 35% Protein Feed

Parameter	Mean	Range
Dissolved oxygen (mg/L)	5.7	4.6–7.6
NO ₂ -N (mg/L)	0.44	0.06–2.34
NO ₃ -N (mg/L)	138	40–359
pH	7.1	6.2–7.5
PO ₄ (mg/L)	9.5	0.3–21.1
Salinity (ppt)	28.3	24.4–36.7
SS (mL/L)	10	2–27
TAN (mg/L)	0.24	0.08–0.51
Temperature (°C)	30.1	27.5–31.5

may explain the 11% increase in oxygen consumption, greater use of settling tanks and foam fractionators, and slightly lower water-use efficiency (Table 14.17).

TAKE-HOME MESSAGES FROM THE 2012 GROW-OUT TRIAL—40M³ RACEWAY SYSTEM:

- ✓ The YSI 5500D monitoring system was a reliable tool in preventing low DO and the optical probes reduced calibration and maintenance time,
- ✓ Continuous feeding prevented feeding-related reduction in DO observed when feed was distributed manually 4 times per day,
- ✓ Aged water helped maintain low TAN and nitrite,
- ✓ Stocking juveniles of two distinct size groups—a 2.8 g difference in mean weight—did not affect production of marketable shrimp,
- ✓ The coefficient of variation of 100 individuals collected randomly from each raceway at harvest was 4.5% lower in the HI-35 treatment (21.8% vs. 26.3%),

TABLE 14.17 Pacific White Shrimp Performance in a 67-d Grow-Out Trial (2012) With 2.7 g Juveniles in Six 40 m³ Raceways at 500/m³ Fed Two Commercial Feeds, No Water Exchange, With Foam Fractionators (FF) and Settling Tanks (ST) to Control Biofloc

Feed	Av. Wt. (g)	Growth (g/wk)	Yield (kg/m ³)	Survival (%)	FCR	Water Use (L/kg Shrimp)	Operation (h) FF	ST
HI-35 ^a	22.1	2.0	9.7	87	1.25	125	812	87
SI-35 ^b	19.7	1.8	8.7	88	1.43	138	1253	391
Diff	2.4	0.2	1.0	(1.0)	0.18	14	441	304

^a HI-35, ZBI, Gardners, PA, US.

^b SI-35, ZBI, Gardners, PA, US.

- ✓ Although raceways were stocked with juveniles from a cross of Fast-Growth and Taura-Resistant lines, growth rates were high—between 1.8 and 2.0 g/wk,
- ✓ The HI-35 feed significantly improved mean harvest weight, yield, weekly growth, and FCR compared to the SI-35 feed,
- ✓ HI-35 feed resulted in lower FCR than SI-35 (1.25 vs. 1.43),
- ✓ Controlling TSS in SI-35 tanks took more effort (hours of operation of the foam fractionators and the settling tanks),
- ✓ Preliminary economic analysis indicates that, despite the cost difference (HI-35: \$1.75/kg vs. SI-35: \$0.99/kg), both are commercially viable ([Chapter 13](#)), and
- ✓ Further information related to this grow-out trial can be found in: [Braga et al., 2016](#); [Hanson et al., 2013a,b](#), [Samocha et al., 2012c, 2013a,b,c](#).

14.2.1.7 2013

Based on the improved performance with the HI-35 feed, a 77-d grow-out trial was designed to determine if a high-quality feed with 40% protein would further improve performance. Objectives were to (1) compare the 35% protein HI-35 feed of the previous years with an experimental 40% protein feed (EXP-40), (2) study the effect of the two feeds on water quality with no water exchange, and (3) further evaluate continuous DO monitoring.

The trial was conducted in six 40 m³ raceways with three replicates per treatment. Each raceway was filled with 35 m³ of biofloc-rich water from an earlier nursery trial and 5 m³ natural seawater with salinity adjusted to 30 ppt. Juveniles (4g) produced from PL provided by KAVA Farms (Los Fresnos, TX, US) from a cross of Fast-Growth and Taura-Resistant lines were stocked at 324/m³. For the first week, daily rations were based on an assumed growth of 1.5 g/wk, an FCR of 1.4, and mortality of 0.5%/wk. Feed was delivered continuously by six belt feeders per raceway. Rations were adjusted based on consumption and results of twice-weekly sampling.

Foam fractionators and settling tanks were operated intermittently, targeting TSS between 200 and 300 mg/L and SS between 10 and 14 mL/L. Seawater and freshwater were added to compensate for evaporative and operational losses.

Water temperature, salinity, DO, and pH were monitored twice daily; nitrogen species and phosphorus, weekly. Settleable solids and TSS were measured every two days. Alkalinity was monitored twice weekly and adjusted to 180 mg/L with sodium bicarbonate and soda ash. There was no difference in water quality between the treatments ([Table 14.18](#)).

The YSI 5500D system monitored DO and their optical probes again proved valuable by allowing quick adjustments that minimized stress. Setting upper and lower DO limits helped optimize oxygen use.

TABLE 14.18 Water Quality in a 77-d Grow-Out Trial (2013) With Pacific White Shrimp Juveniles in Six 40m³ Raceways at 324/m³ Fed Commercial (HI-35) and Experimental (EXP-40) Feed With No Water Exchange

Parameter	Mean	Range
Dissolved oxygen (mg/L)	5.0	3.7–6.5
NO ₃ -N (mg/L)	194	60–401
pH	7.4	7.0–7.9
PO ₄ (mg/L)	62	10–218
Salinity	29.6	25.3–33.6
SS (mL/L)	13	0–40
TAN (mg/L)	0.61	0.05–3.35
Temperature (°C)	29.1	25.2–30.9

Oxygen supplementation began on Day 8. Until Day 57, oxygen use depended on daily events (e.g., molasses addition). Beginning Day 58 when estimated biomass was 7.2 kg/m³, oxygen was used continuously because air was insufficient to maintain DO above 4 mg/L.

Mean TAN and NO₂-N were low (1.8 and 2.4 mg/L, respectively) even with mortality that started on Day 22. The higher TAN from the higher protein EXP-40 feed may account for the elevated TSS (428 ± 124 mg/L, range: 250 to 692 mg/L) compared to TSS with the HI-35 feed (381 ± 114 mg/L, range: 142 to 617 mg/L). This was not, however, statistically significant. Nitrate-N increased from 61 mg/L to a maximum of 401 mg/L at the end of the trial.

There was no difference in mean weight, yield, weekly growth, or FCR (Table 14.19). For the first 31 days, improved growth was noticed in shrimp fed EXP-40 (3.4–4.4 g/wk vs. 3.0–4.0 g/wk). Over the same period, FCRs were similar for both treatments, roughly 0.45–1.20. Harvested shrimp displayed little sexual maturity or sex-related size variability.

Survival with HI-35 was significantly higher. Mortality was observed on Day 22 in one of the EXP-40 raceways. This spread into the other

TABLE 14.19 Pacific White Shrimp Performance in a 77-d Grow-Out Trial (2013) in Six 40m³ Raceways at 324/m³ Fed Commercial (HI-35) and Experimental (EXP-40) Feed With No Water Exchange

	HI-35	EXP-40
Final Weight (g)	27.2 ± 0.9	28.8 ± 1.8
Growth (g/wk)	2.2 ± 0.1	2.2 ± 0.3
Total Biomass (kg)	328 ± 12	312 ± 45
Yield (kg/m ³)	8.2 ± 0.3	7.8 ± 1.1
FCR	1.59 ± 0.01	1.72 ± 0.08
Survival (%)	93 ± 3 ^a	83 ± 3 ^b

Values with different superscript letters within a row suggest statistically significant differences between treatments ($P > .05$).

raceways and ended on Day 52, with highest mortality in the EXP-40 raceways. No mortality was observed after Day 52, but growth was substantially reduced. This resulted in poor FCRs for both treatments.

Preserved and live shrimp were submitted for disease diagnosis. Histology identified enteric and systemic bacterial infections, suggesting *Vibriosis* as the likely cause. 16S rRNA sequencing on three isolates from live shrimp suggested presence of several *Vibrio* species: *V. parahaemolyticus*, *V. owensii*, *V. communis*, and *V. alginolyticus*. RT-PCR (Reverse Transcription-Polymerase Chain Reaction, a diagnostic microbiological technique) indicated no signs of infection by TSV, YHV, IMNV, or PvNV.

TAKE-HOME MESSAGES FROM THE 2013 GROW-OUT TRIAL—40M³ RACEWAY SYSTEM:

- ✓ Growth and FCRs during the first 3 1/2 weeks were excellent in both treatments (when no signs of pathogenic *Vibrio* infection were noticed), with slightly better performance with the higher protein feed,
- ✓ Significant decline in performance is expected in the presence of pathogenic *Vibrio*, but good

- yields and survival of market-size shrimp (27.2 and 28.8 g) were achieved nevertheless,
- ✓ Performance was better with the HI-35 feed, including higher yield (8.2 vs. 7.8 kg/m³), survival (93% vs. 83%), and improved FCR (1.59 vs. 1.72),
 - ✓ Improved survival of the HI-35 shrimp might be owed in part to VPak, an all-natural, highly purified additive reported by the manufacturer to increase disease resistance, survival, and yields (more testing is needed to examine this hypothesis),
 - ✓ Harvested shrimp showed little sexual maturity or sex-related size variation,
 - ✓ The *Vibrio* infection significantly increased FCR,
 - ✓ Aged water maintained TAN and nitrite low even with *Vibrio*-related shrimp mortality,
 - ✓ The YSI 5500D DO system and optical sensors again proved their value,
 - ✓ Preliminary analysis of profitability (Chapter 13) indicated that both feeds are commercially viable under the conditions of this trial when shrimp are sold at \$13.2/kg (\$6.00/lb), and
 - ✓ Further information related to this grow-out trial can be found in: [Castro et al., 2014](#); [Hanson et al., 2014](#).

14.2.1.8 2014

The 2014 work focused on identifying any benefits of the improved 40% protein feed. A 49-d trial was conducted in four raceways, each configured as in the previous study. Raceways were filled with 35 m³ of mature culture water from the previous 62-d nursery run plus 5 m³ of natural seawater. Salinity was 30 ppt and there was no water exchange. Freshwater was added twice weekly to maintain salinity and to compensate for losses from evaporation and solids control.

Juveniles (5.3 g) raised from hybrid postlarvae (Fast-Growth and Taura-Resistant lines, Shrimp Improvement Systems, Islamorada, FL,

US) were stocked at 457/m³. Both feeds were produced by ZBI. Two raceways were fed HI-35 (2.4 mm, 35% protein) and two others EXP-40 (2.4-mm, 40% protein). Feed was delivered continuously by six evenly spaced automatic belt feeders. Raceways were inspected for uneaten feed daily with a dip net. Daily rations were adjusted between growth samplings based on consumption, measured growth, expected growth, FCR, and survival.

A commercial probiotic, Ecopro (EcoMicrobials LLC., Miami, FL, US), was added every 1–3 days as a *Vibrio*-control measure. Pure oxygen was added as needed from Day 14 to maintain DO above 4 mg/L. Alkalinity was increased to 160 mg/L with sodium bicarbonate every second day. NaOH was used to increase pH above 7 on Days 33–40. No supplemental organic carbon was added. TSS and SS ranges were 200–300 mg/L and 10–14 mL/L, respectively.

Temperature, salinity, DO, and pH were monitored twice daily; SS, daily; TSS and alkalinity, every second day; nitrogen and PO₄, weekly. There was no difference in water quality between the two treatments (Table 14.20).

TABLE 14.20 Water Quality in a 49-d Grow-Out Trial (2014) With Pacific White Shrimp Juveniles in Four 40 m³ Raceways Fed Two Commercial Feeds With No Water Exchange

Parameter	Mean	Range
Dissolved oxygen (mg/L)	5.4	3.5–6.9
NO ₂ -N (mg/L)	0.24	0.01–2.25
NO ₃ -N (mg/L)	125	46–232
pH	7.5	6.8–8.0
Salinity	30.3	29.6–31.2
SS (mL/L)	19	4–90
TAN (mg/L)	1.4	0.2–6.0
Temperature (°C)	29.9	27.8–31.8
TSS (mg/L)	356	150–550

Vibrio concentrations were monitored twice weekly, in duplicate, in all raceways by spreading water samples on TCBS agar and, at the end of the trial, on CHROMagar *Vibrio*. Water samples were individually blended for 20 s to release *Vibrio* cells from particulate solids. Agar plates were inoculated with a 10- μ L sample and incubated for 24 h at 32°C, after which the number of yellow and green colonies were counted on TCBS. Blue colonies (*V. vulnificus*), mauve colonies (*V. parahaemolyticus*), and white/colorless colonies (*V. alginolyticus*) were counted on CHROMagar.

Mean alkalinity was significantly lower with EXP-40 (143 mg/L CaCO₃ vs. 158 mg/L). This required more bicarbonate (40.8 kg vs. 27.5 kg) to maintain alkalinity and suggests more nitrification from higher TAN produced by the higher protein feed.

Nitrate and phosphate accumulated over time. As expected, nitrification was higher with EXP-40: NO₃-N was 232 mg/L, compared to 189 mg/L for HI-35. There was, however, no significant difference in mean final NO₃-N between treatments. Phosphate increased to 57 mg/L for EXP-40 and 39 mg/L for HI-35. Mean phosphate was significantly lower for HI-35 than EXP-40 (26 vs. 32 mg/L).

There were no significant differences in *Vibrio* counts between treatments (Table 14.21). Total

Vibrio counts increased over time, particularly in the final week (up to 35,500 CFU/mL). Higher mortality near the end of the trial corresponded with an increase in yellow colonies. CHROMagar plating and API suggested the presence of *V. parahaemolyticus*, *V. vulnificus*, and *V. alginolyticus* in moribund shrimp hemolymph and hepatopancreas tissue. 16S rRNA sequencing confirmed the presence of *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus*, *V. harveyi*, and *V. mytili* in moribund shrimp hemolymph. Biochemical profiling with Biolog and PCR (culture water, hemolymph, and hepatopancreas) identified *V. parahaemolyticus* as the likely pathogen associated with mortalities.

Feed type did not affect *Vibrio* counts, although the number and proportion of green colonies was greater in raceways fed EXP-40. Dietary protein has been shown to affect biofloc composition and also may have affected *Vibrio* populations between treatments, either directly or through differences in NO₃-N and PO₄ concentrations.

The likely etiological agent identified in moribund shrimp, *V. parahaemolyticus*, is a common disease agent in shrimp farming responsible for substantial economic losses. Biofloc is thought to have a probiotic effect, but *Vibrio* outbreaks nevertheless are common. Outbreaks usually are associated with one or more stressors, for example, high temperature, low DO, high TSS.

TABLE 14.21 Mean *Vibrio* Colony Counts on TCBS over a 49-d Grow-Out Trial (2014) in Four 40 m³ Raceways Fed 35% and 40% Protein Feeds (HI-35 and EXP-40)

<i>Vibrio</i> Colonies (CFU/mL)	HI-35		EXP-40	
	Mean $\pm$ SD	Min–Max	Mean $\pm$ SD	Min–Max
Total	11,200 $\pm$ 1200	2700–30,150	13,650 $\pm$ 3600	3600–35,550
YCFU ^a	7400 $\pm$ 3000	1600–25,050	7000 $\pm$ 2700	700–20,900
GCFU ^b	3900 $\pm$ 1800	600–10,600	6700 $\pm$ 900	1850–15,900
% GCFU	39 $\pm$ 8	3–70	55 $\pm$ 18	8–87

^a YCFU: Yellow colony forming units.

^b GCFU: Green colony forming units.

There were no significant differences in any variables at $P = .05$.

Non-sucrose-fermenting (GCFU) *Vibrio*, which includes *V. parahaemolyticus*, were much more abundant in the grow-out study (600–15,900 CFU/mL) than in the prior nursery phase (<100 CFU/mL) (see results from 2014 trial in [Section 14.1.1.8](#)). This might be related to transfer stress. In addition, although water quality was acceptable, ranges included high TAN, nitrite, and nitrate, and low DO, and pH. Each is potentially stressful, particularly if the immune response was compromised by *Vibrio*.

Probiotics have been effective in preventing *Vibrio* infections in juvenile Pacific White Shrimp in biofloc systems ([Balcázar et al., 2007](#); [Krummenauer et al., 2014](#)), so a commercial probiotic was added to raceways. It may have delayed *Vibrio*-related mortalities but did not prevent them. *Vibrio* development corresponded with clinical indications of vibriosis and higher mortality, thus reinforcing the need to monitor *Vibrio* in super-intensive biofloc systems.

There were no significant differences in mean survival, harvest weight, growth, yield, PER, or FCR between the treatments ([Table 14.22](#)). Shrimp fed EXP-40 grew faster and weighed more at harvest; those fed HI-35 had better

survival. The result was a similar yield for the two feeds.

TAKE-HOME MESSAGES FROM THE 2014 GROW-OUT TRIAL—40M³ RACEWAY SYSTEM:

- ✓ There was no significant improvement in performance from the inclusion of Vpak,
- ✓ Increasing dietary protein from 35% to 40% in the presence of pathogenic *Vibrio* did not improve growth, survival, FCR, or PER,
- ✓ Water usage per kg of shrimp fed the high-protein feed was much lower (29 vs. 50 L/kg),
- ✓ Growth rate was high (average: 2.33 g/wk) even when infected with *Vibrio*,
- ✓ Shrimp fed the 40% crude protein feed had a higher growth rate (2.33 vs. 2.1 g/wk),
- ✓ Monitoring *Vibrio* is useful for anticipating disease outbreaks and any effects of probiotics on pathogenic *Vibrio* counts,
- ✓ CHROMagar helps in identification of pathogenic *Vibrio*,
- ✓ Feeding 40% crude protein resulted in greater nitrification, which required higher bicarbonate supplementation, and
- ✓ Further information related to this grow-out trial can be found in: [Prangnell et al., 2016](#); [Samocha et al., 2015b,c](#).

TABLE 14.22 Pacific White Shrimp Performance in a 49-d Grow-Out Trial (2014) in four 40 m³ Raceways fed 35% and 40% Crude Protein Feeds With No Water Exchange

	HI-35	EXP-40
Survival (%)	80±5	77±13
Final Weight (g)	19.8±0.4	21.5±1.7
Growth (g/wk)	2.10±0.02	2.33±0.21
Yield (kg/m ³)	7.2±0.6	7.4±0.7
PER ^a	1.72±0.23	1.55±0.21
FCR ^b	1.68±0.22	1.63±0.22
Water use (L/kg)	50	29

^a PER (protein efficiency ratio) = Biomass gain (g)/protein intake (g).

^b FCR (feed conversion ratio) = Total feed intake (g)/Total biomass gain (g).

[Table 14.23](#) summarizes the results from the grow-out trials in 40 m³ raceways at the Texas A&M-ARML (2006–2014).

14.2.2 Grow-Out Trials in the 100m³ Raceways

14.2.2.1 2010

Grow-out trials in 40 m³ raceways demonstrated the need to supplement culture water with pure oxygen to produce high yields. Preliminary estimates suggested that the cost of oxygen to produce 1 kg of shrimp was \$0.81. The first trial of 2010 focused on incorporating

TABLE 14.23 Grow-Out Trials in 40 m³ Raceways at the Texas A&M-ARML (2006–2014)

Trial	Days	Stock (g/ind)	Harvest (g/ind)	Yield (kg/m ³)	Survival (%)	FCR	Growth (g/wk)	Water (L/kg)	References
2006 40 m ³ <i>pp. 303–304</i>	94	0.76	15.9	3.8	82	1.24	1.2	1.17	Austin et al. (2007); Samocha et al. (2013d)
			16.1	3.8	86	1.25	1.2	113	
			17.2	3.9	94	1.28	1.3	131	
			17.2	4.1	96	1.34	1.3	170	
				4.2		1.37	1.3	202	
				4.3		1.41	1.3	203	
2007 40 m ³ <i>pp. 304–306</i>	92	1.25	17.3	7.9	80	1.21	1.2	49	Samocha (2010); Samocha et al. (2011b); Samocha et al. (2012a); Samocha et al. (2013a,b,c)
			17.4	8.6	81	1.30	1.2	53	
			18.4	8.6	81	1.36	1.3	62	
			18.5	9.3	88	1.40	1.3	53	
2009 40 m ³ <i>pp. 306–308</i>	108	0.99	21.8	9.3	95	1.53	1.4	24	Correia and Samocha (2010); Haslun et al. (2012); Samocha (2010); Samocha et al. (2010a,b); Samocha et al. (2011a,b); Samocha et al. (2012a); Samocha et al. (2013a,c)
			22.0	9.5	95	1.57	1.4	27	
			22.4	9.5	96	1.57	1.4	30	
			22.5	9.8	97	1.60	1.4	32	
2010 40 m ³ <i>pp. 308–309</i>	72	0.90	15.0	1.0	6	na	1.4	na	Samocha et al. (2011d)
	141	0.74	34.4	1.2	6				
			37.4	1.5	8				
					16				
2011 40 m ³ <i>pp. 309–311</i>	81	1.9	22.2	9.7	88	1.39	1.8	147	Hanson et al. (2013a,b); Samocha et al. (2011a,b); Samocha et al. (2012b)
	82		23.6	9.6	82	1.44	1.9	139	
	82		23.4	9.4	81	1.45	1.8	126	
	83		23.8	9.4	79	1.45	1.9	138	
	85		25.1	9.9	79	1.44	2.0	127	
2012 40 m ³ <i>pp. 311–313</i>	67	2.66	22.1	9.7	87	1.25	2.0	125	Hanson et al. (2013a,b); Samocha et al. (2012c); Samocha et al. (2013a,b,c)
			19.7	8.7	88	1.43	1.8	138	
2013 40 m ³ <i>pp. 313–315</i>	77	4.7	27.2	8.2	93	1.59	2.1	na	Castro et al. (2014); Hanson et al. (2014)
			28.8	7.8	83	1.72	2.2		
2014 40 m ³ <i>pp. 315–317</i>	49	5.3	29.8	7.2	80	1.68	2.1	50	Prangnell et al. (2016); Samocha et al. (2015a,b)
			21.5	7.4	76	1.63	2.3	29	

For further details and results, refer to the pages listed under the TRIAL column.

the a^3 injectors into the newly constructed 100m^3 raceways. Objectives were to (1) evaluate the ability of injectors to mixing the larger tanks and maintain high DO without pure oxygen, (2) evaluate their effect on water quality and shrimp performance with no water exchange, (3) determine the benefit from using the YSI 5200 online DO monitoring system with polarographic sensor, (4) determine if a homemade foam fractionator and one a^3 injector could control biofloc concentrations, and (5) to test the feasibility of harvesting the shrimp using the concrete harvest basin and a fish pump.

Each 100m^3 raceway had two high pressure pumps, one 3hp and one 2hp, and one homemade foam fractionator (see [Section 5.9.2.3](#) and [Fig. 5.46](#)). Only one of the pumps was operated during the initial grow-out phase, when biomass was relatively low. Although raceways have a capacity of 100m^3 , only 80m^3 was used. Each was filled with 50m^3 seawater and 30m^3 of biofloc water from a previous 52-d nursery trial. There was no exchange and 0.7m^3 of municipal freshwater was added weekly to maintain salinity and offset operational losses.

Taura-Resistant juveniles (8.5g) were stocked at $270/\text{m}^3$ and fed the Zeigler Bros. Inc. (ZBI) 35% protein HI-35 feed 4 times per day in equal rations calculated by assuming growth of $2\text{g}/\text{wk}$, FCR of 1.4, and mortality of $0.5\%/\text{wk}$. Rations were adjusted based on twice-weekly sampling. Each raceway had the YSI 5200A DO monitor with polarographic sensor and external wiper.

Mean water temperature was 30°C , salinity 30.8ppt, pH 7.0, and DO $5.8\text{mg}/\text{L}$. TAN declined from $0.8\text{mg}/\text{L}$ in the first week to

$0.2\text{mg}/\text{L}$ for most of the trial. Nitrite-N declined from less than 2 to $0.5\text{mg}/\text{L}$. Nitrate-N increased from 61 to $400\text{mg}/\text{L}$ at harvest.

Foam fractionators operated about half the time and kept TSS between 200 and $400\text{mg}/\text{L}$. Using only air, the 14 a^3 injectors maintained DO from 4.7 to $5.5\text{mg}/\text{L}$ and kept biofloc in suspension. At maximum flow, the two pumps and injectors generated a surface current of $30\text{cm}/\text{s}$.

As water temperature declined in the fall, shrimp were harvested with a six-in (stands for 6") fish pump (Magic Valley Heli-Arc and Mfg., Inc., Twin Falls, ID, US) before the system reached carrying capacity. Harvest biomass was $6.4\text{kg}/\text{m}^3$. More than 12 weeks were required to reach average weights of 25.7g and 26.6g, with biomass of 6.3 and $6.6\text{kg}/\text{m}^3$ in the two raceways. The relatively slow growth (1.38 and $1.45\text{g}/\text{wk}$) were not unexpected because slow growth has been observed in Taura-Resistant shrimp.

Of much greater concern was the extremely high FCR: 2.36 and 2.56 ([Table 14.24](#)). Harvested shrimp had a slightly "beaten-up" appearance that might have resulted from physical damage inflicted by the fast current. This also may have forced shrimp to expend energy on swimming that otherwise might have been used for growth.

Based on these results, the 3-hp pump was replaced with a 2-hp pump to reduce the total horsepower from 5 to 4hp. Water depth was increased by 20cm to provide a total working volume of 100m^3 . The greater volume and higher stocking density meant a significant increase in the number of shrimp in each raceway, requiring at least a 30% increase in daily feed.

TABLE 14.24 Summary of 87-d Grow-Out Trial (2010) in Two 100m^3 Raceways With Pacific White Shrimp Juveniles (8.5g) at $270/\text{m}^3$ With No Water Exchange

Raceway	Av. Wt. (g)	Growth (g/wk)	Survival (%)	FCR	Yield (kg/m^3)	Freshwater Use (%/day)	Water Use (L/kg Shrimp)
1	25.7	1.4	90	2.56	6.3	0.125	228
2	26.6	1.5	91	2.36	6.6	0.125	210

TAKE-HOME MESSAGES FROM THE 2010 GROW-OUT TRIAL—100M³ RACEWAY SYSTEM:

- ✓ Stocking the raceways with shrimp of 8.5 g in size presented no operational problem,
- ✓ Operating the a³ injectors with a total of 5 hp per raceway provided good water mixing and adequate DO to support 6.6 kg/m³ of marketable shrimp under no water exchange using solely atmospheric air,
- ✓ In addition to improved feed management (e.g., prevent DO decrease because of high feed input), the online DO monitoring system showed that the 14 injectors were suitable for maintaining high DO throughout the trial,
- ✓ The two pumps created a strong current (up to 30 cm/s) which may have resulted in a slightly “beaten-up” appearance of the shrimp,
- ✓ Use of 30 m³ aged water, out of the 80-m³ working volume was adequate to maintain TAN and nitrite-N < 1 mg/L throughout the trial,
- ✓ Nitrate-N increased from the initial concentration of 61 to 400 mg/L at harvest,
- ✓ The homemade foam fractionator operated by one a³ injector was capable of controlling TSS levels in the raceways,
- ✓ Shrimp growth was low (1.38 and 1.45 g/wk), FCR was high (2.36 and 2.56), and so was the survival (90 and 91%),
- ✓ The concrete harvest basin and the fish pump worked without any problems and help completing the harvest in less than 1.5 h, and
- ✓ Further information related to this grow-out trial can be found in: [Samocha et al., 2011a,b, 2012a, 2013c](#).

14.2.2.2 2011

The objectives of the 2011 trial were similar to those of the previous year: (1) evaluate the a³ injectors’ ability to maintain DO and mixing

without supplemental oxygen at increased volume and density, (2) evaluate the ability of the homemade foam fractionator to control biofloc but with higher feed input, (3) evaluate the impact of manually feeding 50% of the ration during the day and belt-feeding 50% at night, and (4) evaluate the effect of the injectors on performance when stocking smaller shrimp.

Each raceway was filled to 100 m³ with 55 m³ of seawater, 10 m³ of chlorinated freshwater, and 35 m³ of biofloc-rich water from a previous nursery trial. No water was exchanged. Freshwater (0.3 m³/d) was added weekly to compensate for evaporative and other losses.

Raceways were stocked at 390/m³ with 3.1-g Taura-Resistant juveniles produced from PL received from Shrimp Improvement System (Islamorada, FL, US). Shrimp were fed HI-35 feed as in the previous trial. Half of the daily ration was offered in four equal portions during the day and the remainder was fed through the night by four belt feeders. Initial rations were based on an assumed FCR of 1.4, growth of 1.2 g/wk, and mortality of 0.5%/wk. Rations were adjusted based on twice-weekly growth sampling and observations of feed consumption.

Biofloc was controlled by the homemade foam fractionator set at a flow rate of 28 L/min. Alkalinity was adjusted to 150 to 200 mg/L using sodium bicarbonate and calcium hydroxide.

Water temperature, salinity, DO, and pH were monitored twice per day. Nitrogen species, alkalinity, SS, and TSS were monitored at least weekly. Raceways had the 5200A DO monitoring system. Mean water-quality indicators for the 100 m³ raceways are in [Table 14.25](#).

Initial TSS and SS targets were 200–300 mg/L and 10–14 mL/L, respectively. Targeted TSS was increased on Day 30 to 400–500 mg/L to see if this might reduce daily ration and thus improve FCR. About 8 weeks into the study, it was determined that the foam fractionators were not removing the required amount of solids. On Day 62 with biomass estimated at 6.5 kg/m³,

TABLE 14.25 Water Quality in a 106-d Grow-Out Trial (2011) in 100m³ Raceways Stocked With 3.1 g Juvenile Pacific White Shrimp at 390/m³, a³ Injectors, HI-35 Feed, and No Exchange

Parameter	Mean	Range
Dissolved oxygen (mg/L)	5.8	4.4–7.3
NO ₂ -N (mg/L)	0.25	0.1–2.2
NO ₃ -N (mg/L)	10 (at stocking)–562.7 (at harvest)	
pH	7.1	6.3–7.9
Salinity	28.5	24.3–32.4
TAN (mg/L)	0.45	0.1–2.9
Temperature (°C)	29.8	27.6–32.2

DO dropped below 4.5 mg/L, so the second 2-hp pump was engaged. Some mortality was observed during this period of high solids, high temperature, and moderate DO. Most mortality likely occurred because of gill fouling during the two weeks when TSS and SS exceeded 1000 mg/L and 39 mL/L, respectively. Supplemental oxygen reduced mortality. On Day 74, a newly constructed 2m³ settling tank was added to each raceway and operated at 7.5 to 12 L/min. This helped reduce TSS to 200 mg/L within 4–5 days. Oxygen was discontinued after solids returned to normal and mortality had tapered off.

Shrimp were harvested by fish pump on Day 106. Survival was good (mean: 83%) with average growth of 1.5 g/wk and mean harvest weight 25.2 g (Table 14.26).

14.2.2.3 2012

Objectives were to evaluate (1) performance of Fast-Growth × Taura-Resistant hybrids (as compared to the Taura-Resistant juveniles used in the previous trials) at higher density, no water exchange, and fed a commercial feed for intensive biofloc systems; (2) a³ injectors in zero-exchange super-intensive raceways; and (3) continuous feeding (e.g., no manual feeding) and avoiding feeding near pump intakes on FCR.

This 63-d grow-out trial was run in the two 100m³ raceways with the same foam fractionators and settling tanks as in the previous trial. Raceways initially were filled to 72m³ with seawater (23m³), municipal chlorinated freshwater (24m³), and biofloc-rich water (25m³) from a previous nursery trial. On Day 7, both raceways

TABLE 14.26 Summary of a 106-d Grow-Out Trial (2011) in Two 100m³ Raceways Stocked With 3.1 g Juvenile Pacific White Shrimp at 390/m³, a³ Injectors, HI-35 Feed, and No Exchange

Raceway	Stocking		Harvest (g)	Growth (g/wk)	Survival (%)	Yield (kg/m ³)	FCR	Water Use (L/kg Shrimp)
	(Shrimp/m ³)	Av. Wt.(g)						
1	390	3.1	25.1	1.5	80	8.0	1.83	123
2	390	3.1	25.4	1.5	86	8.7	1.70	109
Av.			25.3	1.5	83	8.4	1.77	116
SD			0.2	0.0	3	0.3	0.06	10

TABLE 14.27 Summary of a 63-d Trial (2012) in two 100m³ Raceways With 3.6-g Pacific White Shrimp Juveniles at 500/m³, a³ Injectors, HI-35 Feed, and No Exchange

Raceway	Stocking (Juveniles/m ³)	Av. Wt. (g)	Harvest Av. Wt. (g)	Growth (g/wk)	Survival (%)	Yield (kg/m ³)	FCR	Water Use (L/kg)
1	500	3.6	22.8	2.1	81	9.2	1.43	112
2	500	3.6	22.7	2.1	78	8.9	1.53	121
Average			22.7	2.1	80	9.0	1.48	117

were filled to capacity (100m³) with 14m³ of freshwater and 14m³ of seawater. Freshwater was added weekly (equivalent to about 0.475m³/d) to compensate for water losses.

Raceways were stocked at 500/m³ with 3.6g juveniles from the Fast-Growth × Taura-Resistant cross (Shrimp Improvement Systems). Shrimp were fed the same ZBI HI-35 feed used in earlier trials. It was distributed continuously on 4 24-h belt feeders per raceway. Initial daily rations were based on an FCR of 1.4, growth of 1.5g/wk, and mortality of 0.5%/wk. Rations were adjusted based on results of twice-weekly growth sampling and feed consumption.

Raceways had the same YSI 5200A DO system as previous trials. Water temperature, salinity, DO, and pH were monitored twice daily. Alkalinity was measured twice weekly and adjusted to 160mg/L with sodium bicarbonate. SS was measured daily and TSS at least twice weekly. Nitrogen species and PO₄ were monitored weekly. Mean water temperature, salinity, DO, and pH were 29.6°C, 29.3 ppt, 5.5mg/L, and 7.1, respectively. TAN and NO₂-N remained low (<0.6 and <1.5mg/L, respectively), and NO₃-N increased from 67 to 309mg/L at harvest.

Foam fractionators were started on Day 8 and use of settling tanks began on Day 23 when SS reached 23mL/L in one of the raceways. Flow was 28L/min for the foam fractionators and 8.5–20L/min for the settling tanks. With both solids-removal devices used intermittently, mean TSS and SS were 292mg/L and 12mL/L, respectively.

Minor mortality was observed from the third week. Supplemental oxygen was provided on Day 22 to alleviate potential stress and hopefully stem mortality. It had no perceptible effect, so the second 2-hp pump was started on Day 44 when biomass was about 8.2kg/m³. Supplemental oxygen was discontinued 3 days later (Day 47).

Shrimp were harvested on Day 64 with a fish pump. Mean final weight was 22.7g/ind. Shrimp grew an average of 2.1g/wk, yielding about 9.0kg/m³ (Table 14.27), compared with 8.4kg/m³ in the previous study. FCR was lower (1.48 vs. 1.77) and survival was moderate (80%).

Operating foam fractionators and settling tanks at flow rates up to 28L/min and 20L/min, respectively, maintained TSS within the targeted range when daily feed was as high as 22kg/raceway (220g/m³). The a³ injectors prevented biofloc settling and maintained adequate DO (>5mg/L) to support the high yield.

TAKE-HOME MESSAGES FROM THE 2012 GROW-OUT TRIAL—100M³ RACEWAY SYSTEM:

- ✓ Continuous feeding eliminated the DO drops observed when hand-feeding,
- ✓ a³ injectors driven by 4-hp pumping capacity supported the DO needs of 9.2kg/m³ in 100m³,
- ✓ Very importantly: sustained growth > 2g/wk reduced the production cycle from 106 to 63 days,

- ✓ Fast-Growth × Taura-Resistant juveniles grew well even stocked at 500/m³,
- ✓ Average FCR for the two raceways, 1.48, was the lowest observed for this system. The reason for this is not clear, but it might relate to the higher and more uniform DO that resulted from continuous feeding and feed delivery away from pump intake screens (thus preventing feed loss),
- ✓ NO₃-N concentration at harvest was below 309 mg/L,
- ✓ Foam fractionators and settling tanks adequately controlled solids at daily loads up to 22 kg,
- ✓ Preliminary economic analysis suggests far better economic viability compared to the 40 m³ system (see [Chapter 13](#)), and
- ✓ Further information related to this grow-out trial can be found in: [Hanson et al., 2013a,b](#), [Samocha et al., 2011a,b, 2012a,b,c, 2013a,b,c](#).

14.2.2.4 2014

This trial was designed to further improve management and production practices. The original objective was to determine the impact of probiotics on performance and water quality. When *Vibrio*-related mortality started 10 days into the trial, emphasis shifted to monitoring the interaction between probiotics and *Vibrio*.

This 38-d trial was conducted with juveniles (6.5 g) derived from a Fast-Growth × Taura-Resistant cross by Shrimp Improvement Systems (see details for the 2014 nursery trial [Section 14.1.2](#)).

Prior to stocking, juveniles were harvested with dip nets (no suitable fish pump was available). Because of limited holding space, high biomass from one raceway (>316 kg or 7.9 kg/m³) was kept for 24 h in a 40 m³ raceway. With high water temperature (>30.9°C), high TSS (>500 mg/L), and sporadic exposure to low DO (2.5 mg/L), mortality reached more than 13% before transfer to the 100 m³ raceway.

Juveniles from the second raceway were not subjected to the same stress. Grow-out raceways were stocked at 458/m³.

Raceways were filled with nitrifier-rich water (88%) from a previous nursery trial and natural seawater (12%). There was no exchange and freshwater additions compensated for losses. A probiotic, Ecopro (EcoMicrobials, Miami, FL, US), was added every other day at 2 g/m³.

Unlike the previous trial, raceways had the YSI 5500D DO system with two optical probes in each raceway. Temperature, salinity, DO, and pH were monitored twice daily; SS was monitored daily; alkalinity every second day; TSS and nitrogen species twice weekly; and PO₄ weekly. Alkalinity was adjusted to 160 mg/L and pH to >7 with additions of NaHCO₃ and Ca(OH)₂.

Foam fractionators and settling tanks were operated at the same rate and frequency as in the previous trial. The TSS target was 200–300 mg/L and the SS target 10–14 mL/L.

Shrimp were fed EXP-40 (40% protein, 9% lipid). Daily rations were determined assuming an FCR of 1.2 to 1.3, growth of 1.5 g/wk, and mortality of 0.5%/wk. Feed was adjusted based on twice-weekly growth sampling and feed consumption. Feed was distributed continuously by six belt feeders per raceway.

Vibrio concentrations were monitored twice weekly in duplicate in both raceways (see details for the 2014 nursery trial in [Section 14.1.2](#)). Mean water-quality indicators for the 100 m³ raceways are presented in [Table 14.28](#).

There was a 7-d delay in the increase of green *Vibrio* colonies in the raceway (B2) with non-stressed juveniles. From Day 15, however, green colony *Vibrio* counts in that raceway were mostly higher than in the other ([Fig. 14.11](#), [Table 14.29](#)). Green colony counts in both were much higher than in the nursery. Monitoring yellow- and green-forming colonies was useful in anticipating outbreaks.

One week after stocking, a wave of mortality started in the raceway with the stressed

TABLE 14.28 Water Quality in a 38-d Grow-Out Trial (2014) in Two 100 m³ Raceways With 6.4-g Hybrid (Fast-Growth × Taura-Resistant) Pacific White Shrimp Juveniles at 458/m³

Parameter	Mean	Range
Alkalinity (mg/L as CaCO ₃)	138	117–159
Dissolved oxygen (mg/L)	6.1	4.6–7.2
NO ₂ -N (mg/L)	0.18	0.10–0.58
NO ₃ -N (mg/L)	112	62–187
pH	7.6	6.7–7.9
PO ₄ (mg/L)	32	22–57
Salinity	30.4	29.3–31.0
SS (mL/L)	20	4–41
TAN (mg/L)	1.20	0.27–2.85
Temperature (°C)	30.3	28.8–31.6
TSS (mg/L)	353	163–600

juveniles. It spread to the other raceway a few days later. Vibriosis-related mortality was confirmed by identification of different pathogenic *Vibrio* species in culture water and moribund shrimp. Because mortality increased over time and reached several thousand per day, the trial

was terminated. Unexpectedly, the raceway with stressed juveniles (B1) had greater survival; it also had slightly smaller shrimp, lower growth and yield, higher FCR, and lower protein efficiency (PER) (Table 14.30 and 14.31).

TAKE-HOME MESSAGES FROM THE 2014 GROW-OUT TRIAL—100M³ RACEWAY SYSTEM:

- ✓ The YSI 5500D monitoring system with optical sensors required less maintenance and calibration than the YSI 5200,
- ✓ Growth in both raceways was high (2.2 and 2.3 g/wk) despite the *Vibrio* outbreak,
- ✓ One raceway had 80% survival, but the FCR was very high (2.07),
- ✓ The high mortality from the outbreak forced early termination of the trial,
- ✓ Exposure to stress—low DO, high temperature, high TSS, crowding—during the nursery harvest might trigger pathogenic *Vibrio* during grow-out,
- ✓ Monitoring yellow- and green-forming colonies was useful in anticipating *Vibrio* outbreaks,
- ✓ The Ecopro probiotic was not effective in controlling this *Vibrio* outbreak. This was

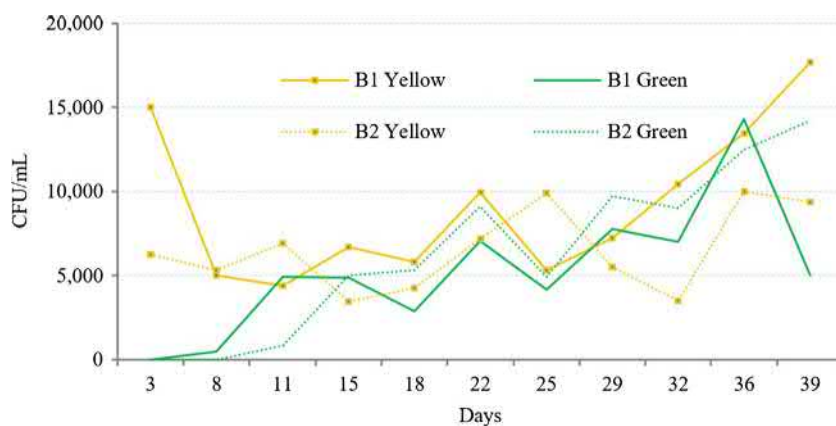


FIG. 14.11 Yellow & green *Vibrio* counts in a 38-d grow-out trial (2014) in 100 m³ raceways with hybrid (Fast-Growth × Taura-Resistant) juveniles (6.4 g) at 458/m³.

TABLE 14.29 *Vibrio* Counts in a 38-d Trial (2014) in two 100 m³ Raceways With Hybrid (Fast-Growth × Taura-Resistant) Juveniles (6.4 g) at 458/m³

<i>Vibrio</i> Colonies (CFU/mL)	Mean	Range
Total (×1000)	18.0	5.3–31.7
Yellow (×1000)	12.2	3.5–28.1
Green (×1000)	5.9	0.0–14.3
% Green	39.0	0.0–72.0

confirmed by a study with shrimp from the high-survival raceway (B1) stocked into an empty 100 m³ raceway with the same water, and

- ✓ Further information related to this grow-out trial can be found in: [Samocha et al., 2015a,b,c](#).

14.3 CURRENT AND FUTURE RESEARCH DIRECTIONS

Extensive work at the Texas A&M-ARML at Flour Bluff has helped identify further research needs to make the super-intensive, no-exchange systems more competitive and economically viable. Following is a list of areas requiring development of additional tools and practices to overcome some of the present limitations of this technology:

- *Disease prevention and minimization*
 - Develop dependable probiotics and probiotics designed to control specific bacterial and fungal diseases.
 - Isolate bacteriophages that target specific virulent bacteria, with emphasis on *Vibrio*.
 - Develop fast-growth breeding lines that perform well under crowded conditions and are resistant to pathogenic *Vibrio* and other bacteria.

TABLE 14.30 Summary of a 38-d Grow-Out Trial (2014) in Two 100 m³ Raceways With Pacific White Shrimp (6.4 g) at 458/m³, a³ Injectors, EXP-40 Feed, and No Exchange

	Raceway B1	Raceway B2
Survival (%)	80	72
Final Weight (g)	18.4	19.0
Growth Rate (g/wk)	2.2	2.3
Yield (kg/m ³)	6.0	6.9
PER ^a	1.25	1.59
FCR ^b	2.07	1.61
Water use (L/kg)	34	35

^a PER (protein efficiency ratio) = Biomass gain (g)/protein intake (g).

^b FCR (feed conversion ratio) = Total feed intake (g)/Total biomass gain (g).

- *Changes in water and shrimp tissue with water reuse*
 - Characterize accumulation and depletion of selected ions and determine optimal range for nitrate concentration in culture water.
 - Characterize accumulation and impact of dissolved organics and nitrate.
- *Maintaining optimal water quality and shrimp tissue*
 - Identify natural ion exchange to balance specific anions and cations.
 - Test specially formulated feeds with and without specific minerals.
 - Use of denitrification side loops for nitrate removal and alkalinity restoration.
- *Waste disposal and/or reuse*
 - Develop profitable uses of shrimp molts.
 - Identify effects of increasing biofloc protein content on shrimp performance.
 - Study collection and reuse of dried biofloc.
 - Test use of wet/dry biofloc as a soil amendment.
- *General shrimp performance*
 - Develop high-growth lines for high density and low temperature.

TABLE 14.31 Summarizes the Grow-Out Trials in Two 100m³ Raceways at the Texas A&M-ARML (2010–2014)

Trial	Days	Stock (g/ind)	Harvest (g/ind)	Yield (kg/m ³)	Survival (%)	FCR	Growth (g/wk)	Water (L/kg)	References
2010 80m ³ <i>pp.</i> 317–320	87	8.5	25.7 26.6	6.3 6.6	90 91	2.56 2.36	1.4 1.5	228 210	Samocha et al. (2011a,b); Samocha et al. (2012a); Samocha et al. (2013c)
2011 100m ³ <i>pp.</i> 320–321	106	3.14	25.1 25.4	8.0 8.7	80 86	1.83 1.70	1.5 1.5	123 109	Samocha et al. (2011a,b); Samocha et al. (2012b); Samocha et al. (2013c)
2012 100m ³ <i>pp.</i> 321–323	63	3.6	22.8 22.7	9.2 8.9	81 78	1.43 1.53	2.1 2.1	112 121	Hanson et al. (2013a,b) Samocha et al. (2011a,b) Samocha et al. (2012a,b,c) Samocha et al. (2013a,b,c)
2014 100m ³ <i>pp.</i> 323–325	38	6.4	18.4 19.0	6.0 6.9	80 72	2.07 1.61	2.2 2.3	34 35	Samocha et al. (2015a,b,c)

For further details and results, refer to the pages listed under the TRIAL column.

- Develop genetic lines with low size variation.
- Determine whether natural light improves shrimp performance.
- Develop specially formulated feeds and production practices to support growth above 5 g/wk with FCR below 1.
- Establish feed and feeding strategies to optimize performance, including alternate use of feeds of different qualities.
- Establish transfer and harvest protocols to minimize shrimp stress and losses.
- Develop reliable and cost-effective methods to estimate the shrimp population in culture tanks.

- Compare the economics of shrimp production in two-, three-, and four-phase systems.

Of these research needs, the priority areas are *Vibrio* control, changes in water ionic composition over successive production cycles, and waste disposal. *Vibrio* infections affect production worldwide and closed biofloc systems are especially vulnerable because of their extremely high densities. Developing reliable *Vibrio* control measures—such as nutritional improvements, probiotics and prebiotics, bacteriophages, biosecurity protocols, genetic improvements, and advanced system design for stress reduction—will increase production and harvest consistency.

Some evidence suggests that specific ions and heavy metals may accumulate or become depleted over successive production cycles in closed biofloc systems. This may diminish shrimp and biofloc performance, as well as restrict marketability. Measures must be developed to maintain and restore optimal ionic composition. Nitrate and phosphate also accumulate, while alkalinity is depleted. Developing in-cycle denitrification systems that remove nitrate, restore alkalinity, and control phosphate will improve water quality.

Solids must be removed from closed biofloc systems to maintain optimum TSS and culture water eventually must be disposed. Waste disposal represents a cost and potential environmental issue. Techniques for treating and safely reusing waste, such as digesters, must be refined to improve system sustainability and biosecurity. Alternative uses for solid waste, such as soil amendments and feed additives, should be explored. More efficient feeds and feeding strategies that optimize growth and reduce solids production will limit waste disposal needs.

14.4 PERSPECTIVES

The information presented in this manual summarizes progress made over 16 years by the Texas A&M-ARML at Flour Bluff, Corpus Christi, Texas, toward development of sustainable, super-intensive, biofloc-dominated production of marketable shrimp. System design and operation began with simple shallow tanks operated with water exchange, crude aeration systems, and limited carrying capacity. This simple system evolved into the super-intensive production technology described in detail in this manual.

This work underscores the importance of monitoring and controlling key water-quality indicators. The online DO monitoring system has been invaluable in refining nursery and

grow-out practices. When properly used—and with experience—inexpensive foam fractionators and settling tanks control biofloc. Incorporating the a³ injectors allowed yields of marketable shrimp at more than 9 kg/m³, high survival, and low FCRs with only atmospheric air. Our experience suggests that yields higher than 9 kg/m³ can be achieved in these systems, but we strongly recommend that those who start with this technology target lower yields (up to 7 kg/m³) until production procedures are refined.

The work also highlighted the impact of feed quality and feeding practices on shrimp performance, as well as the need for efficient temperature control to operate these systems year-round in seasonally cold locales.

Developments described in this manual could not have been achieved without the hard work and diligence of a cast of very dedicated employees, students, and researchers who spent many long hours carrying out these studies. A significant enhancement of our research capacity was achieved by strong ties with local, national, and international institutions; shrimp producers; feed mills; manufacturers; and aquaculture equipment suppliers.

The information and technology generated at the facility has been transferred to users and researchers through numerous presentations in national and international meetings and in publications. This manual responds to a demand for a comprehensive summary of the design, management, and economics of our super-intensive system and is intended for a wider audience of stakeholders.

Super-intensive, biofloc-dominated, no-water-exchange technology continues to expand but, largely owing to high operating costs, is not at the point at which it can compete with mass production of “commodity” shrimp in outdoor ponds—although its application to the nursery phase for commercial operations in outdoor ponds can make that sector more sustainable and more efficient.

For this reason, the biofloc systems that are the subject of this manual focus on providing fresh, never-frozen, high-quality shrimp to niche markets that serve consumers who value domestic production and will support higher prices. As the market for sustainably produced seafood expands—driven partly by more strict regulations on aquacultural discharge—so will the need for the type of systems described in this manual.

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Troubleshooting

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Observations and Potential Remediation Steps for Super-Intensive, Indoor, Biofloc-Dominated Shrimp Production Systems

Problem	Possible Cause(s)	Solution(s)	Additional Information
WATER QUALITY			
Low alkalinity (<140 mg/L CaCO ₃)	1. High nitrification activity	• Increase alkalinity by adding bicarbonate or carbonate liming agent • Denitrification	pp. 41–42, 43–46, 49–50, 135–138 pp. 211–215, 305–307
	2. Strong algal bloom, when NH ₃ is the main metabolite	• See “Dense algae bloom”	pp. 53, 140, 147–148, 326–327
High ammonia (>3 mg/L TAN)	1. Nitrifying bacteria not fully established	• Add organic carbon to allow heterotrophic bacteria to consume more ammonia • Add a commercial nitrifying bacteria product • Reduce feeding rate	pp. 45–47, 128, 130, 138–141, 173, 299–300, 300–301 188–191
	2. Toxins preventing bacterial growth, (e.g., from nonaquaculture grade tank liner)	• Identify toxin and source (e.g., tank material, water supply, water disinfection residues, etc.); treat appropriately (e.g., water exchange, install new tank liners) • Flush and allow toxins to leach from liner before use	pp. 72, 124

Continued

Observations and Potential Remediation Steps for Super-Intensive, Indoor, Biofloc-Dominated Shrimp Production Systems—cont'd

Problem	Possible Cause(s)	Solution(s)	Additional Information
Low DO (<4 mg/L)		Only use inert and nontoxic culture tank materials, such as aquaculture-grade EPDM	
	3. Low solids concentration	- Cease or reduce flow through solids filtration equipment - Add organic carbon	pp. 130, 138–141, 141–142
	4. Low pH (<6.8) and/or low alkalinity (<75 mg/L CaCO ₃) restricting nitrification (inorganic carbon limitation)	- Increase pH with NaOH or Ca(OH)₂ - Increase alkalinity by adding a bicarbonate or carbonate - Denitrification - Water exchange	pp. 49, 135, 136 pp. 137, 212–214 pp. 43–44
	1. High shrimp biomass	Partial or complete harvest	pp. 133–134, 201
		Provide supplemental O₂	pp. 82–84, 133–134
	2. Overfeeding	- Remove uneaten feed - Reduce feeding rate	p. 171 pp. 172–173, 189–192
	3. High TSS or SS	Remove excess solids (solids filtration equipment)	pp. 84–87, 103–106, 141–142
	4. Inadequate mixing	- Clean/redirect mixing equipment - Manual mixing	pp. 149, 150
	5. High temperature	- Reduce temperature if practical - Provide supplemental O₂	pp. 64–68, 135 pp. 82–84, 133–134
	6. Carbon addition too rapid	- Provide supplemental O₂ - Stagger or reduce future carbon addition	pp. 82–84, 133–134, 141, 174
	7. Algal bloom crash	- Provide supplemental O₂ - Remove settled algal biomass if possible	pp. 82–84, 133–134
	8. Air blower or pump failure	- Clean, repair, or replace equipment - Provide supplemental O₂ until problem is rectified	pp. 77–78, 79–80 pp. 82–84, 133–134
	9. O ₂ bottle empty	Change bottle	pp. 83–84

Observations and Potential Remediation Steps for Super-Intensive, Indoor, Biofloc-Dominated Shrimp Production Systems—cont'd

Problem	Possible Cause(s)	Solution(s)	Additional Information
	10. Faulty DO probe	Clean and calibrate DO probe	p. 84
Presence of hydrogen sulfide (>0.005 mg/L)	1. Solids accumulation on base of culture tanks causing anoxic conditions	- Clean/redirect mixing equipment and perform manual mixing to remove areas of solids accumulation on the base of culture tanks - Increase air or water flow - Remove accumulated uneaten feed - Increase pH to reduce H₂S toxicity	pp. 86, 149, 361 pp. 114, 135, 149, 173
High nitrate (>400 mg/L NO ₃ -N) (30 ppt salinity)	1. End product of nitrification	- Denitrification - Water exchange	pp. 52–53, 115, 137, 212–213 p. 44
High nitrite (>10 mg/L NO ₂ -N)	1. Nitrifying bacteria not fully established	- Add organic carbon to reduce the amount of NH₃ available for conversion to NO₂ - Add a commercial nitrifying bacteria product - Reduce feeding rate	pp. 45–47, 138–141 pp. 128, 299–300, 300–301 pp. 173, 188–191
	2. Low solids concentration (<100 mg/L TSS)	- Turn off or reduce flow through solids filtration equipment - Add organic carbon	pp. 84–88, 103–106, 141–142 pp. 130, 138–141
	3. Low pH (<6.8) and/or low alkalinity (<75 mg/L CaCO ₃) restricting nitrification	- Increase pH with NaOH or Ca(OH)₂ - Increase alkalinity by adding a bicarbonate or carbonate - Denitrification - Water exchange	p. 135 p. 136 pp. 52–53, 137, 212–214 p. 44
Low pH (<7.0)	1. Nitrification (acid-forming reaction)	- Increase pH with NaOH or Ca(OH)₂ - Increase aeration to drive off more CO₂ - Water exchange - Denitrification treatment	p. 135 pp. 49, 135 p. 43 pp. 137, 212–214
	2. High biomass (high CO ₂ production)	- Increase pH with NaOH or Ca(OH)₂ - Increase aeration to drive off more CO₂	p. 135 pp. 49, 135 p. 44

Continued

Observations and Potential Remediation Steps for Super-Intensive, Indoor, Biofloc-Dominated Shrimp Production Systems—cont'd

Problem	Possible Cause(s)	Solution(s)	Additional Information
		• Water exchange • Remove biomass through partial harvest or solids reduction (See “High solids concentration”)	pp. 84–88, pp. 103–106, 141–142, 201
	3. Low alkalinity	• Increase alkalinity by adding a bicarbonate or carbonate • Denitrification • Water exchange	p. 136 pp. 137, 212–214 p. 44
	4. Natural state of groundwater (associated with high CO ₂)	• Degassing pretreatment	p. 135
High pH (>8.5)	1. High phytoplankton concentration (algal bloom)	• Injection of bottled CO₂ via air diffusers or Venturi • Reduce phytoplankton concentration (See “Dense algae bloom”)	p.135 pp. 130, 138–141, 147–148
High phosphate	1. Accumulation in system from feed	• Biological (denitrification/digestion) treatment • Chemical (flocculent) treatment	pp. 115, 137, 143, 211, 212, 215 pp. 137, 143
High salinity	1. Evaporation 2. Mixing error	• Add freshwater • Water Exchange	pp. 142–143 p. 44
Low solids concentration (TSS <250mg/L, SS <10mL/L, Turbidity <75 NTU)	1. New system 2. Excessive solids removal	• Decrease operation of solids filtration equipment • Add organic carbon	pp. 84–88, 103–106, 141–142 pp. 130, 138–141
High solids concentration (TSS >350mg/L, SS >15mL/L, Turbidity >200 NTU)	1. Insufficient solids removal 2. Overfeeding 3. Algal bloom	• Increase operation of solids filtration equipment • Water exchange • Remove uneaten feed • Reduce feeding rate • See “Dense algae bloom”	pp. 84–88, 103–106, 141–142 p. 44 pp. 171, 173 pp. 173, 188–191 pp. 130, 138–141, 147–148
Temperature outside optimum range (28–31°C)	1. Seasonal or diurnal variation 2. Failure of heating equipment/system	• Adjust or redesign temperature control system • Repair or adjust heating equipment/system	pp. 64–68, 135 pp. 64–68, 135

Observations and Potential Remediation Steps for Super-Intensive, Indoor, Biofloc-Dominated Shrimp Production Systems—cont'd

Problem	Possible Cause(s)	Solution(s)	Additional Information
Deficient elements in culture water, or ionic ratios out of optimum range	1. Depletion in system over time	• Restore with a trace element product or specific ion source (e.g., muriate of potash for potassium) • Water exchange	pp. 55, 126–127, 143–147 p. 44
	2. Already deficient in source water	• Add relevant trace element product/s or specific ion source as part of the pretreatment protocol • Change water source	pp. 38–39, 40–41, 127 pp. 37–41
Excessive heavy metals in culture water	1. Accumulation in system over time	• Removal via external settling/digestion tanks, filtration, adding chelators or dosing with ozone (externally) • Water exchange • Solids removal	pp. 55, 127, 144–146 pp. 141–142, 214
	2. Present in source water	• Remove via filtration, settling, chelation, or ozone treatment as part of the pretreatment protocol • Change water source	p. 127 pp. 37–41

CULTURE SYSTEM

Biofloc not developing	1. Excessive solids removal	• Turn off or reduce flow through solids filtration equipment • Add organic carbon	pp. 84–88, 103–106, 141–143 pp. 130, 138–141
	2. Toxins preventing bacterial growth, (e.g., from nonaquaculture grade tank liner)	• Identify toxin and source (e.g., tank material, water supply, water disinfection residues); treat appropriately (e.g., water exchange, install new tank liners) • Flush and leach toxins from liner before use • Only use inert and nontoxic culture tank materials, such as aquaculture-grade EPDM	pp. 72, 126–127
	3. Low alkalinity (<75 mg/L CaCO ₃)	• Increase alkalinity by adding a bicarbonate or carbonate	p. 136

Continued

Observations and Potential Remediation Steps for Super-Intensive, Indoor, Biofloc-Dominated Shrimp Production Systems—cont'd

Problem	Possible Cause(s)	Solution(s)	Additional Information
“Dead patches” in culture tank	1. Inadequate mixing	• Clean or redirect mixing equipment • Increase air/water flow, depending on postlarvae age • Manual mixing	p. 150 pp. 110, 150
	2. Overfeeding	• Remove uneaten feed • Reduce feeding rate	pp. 171–172 pp. 173, 189–190
Excessive foam on water surface	1. Biofloc not yet fully established	• Mix foam back into water column with jets of water off the recirculation line • Manual mixing	pp. 149–150
	2. Algal bloom crash	• Remove settled algal biomass if possible	
	3. Excess dissolved carbon	• Reduce carbon addition if ammonia and nitrite concentrations are low	pp. 45–47, 138–141
Dense algae bloom	1. High nutrient load (NH ₃ , NO ₃ , or PO ₄) combined with low TSS/turbidity	• Increase organic carbon addition to enhance heterotrophic bacterial populations and limit ammonia availability to microalgae • Maintain TSS above 250 mg/L (turn off or reduce flow through solids filtration equipment, and add organic carbon) • Reduce exposure to sunlight	pp. 104, 130, 138–141, 148 pp. 84–88, 106–109, 141–142 pp. 130, 138–142 pp. 46, 147
SHRIMP			
High FCR	1. Overfeeding	• Reduce feeding rate • Check protocol for estimating growth and survival, and determining feeding rate • Regularly inspect culture tanks for uneaten feed and adjust feeding rate accordingly	pp. 173, 189 pp. 166–167, 170, 184, 187–189, 191–193 p. 189
	2. Poor water quality	• Rectify specific water quality problem • Increase water quality monitoring frequency	pp. 133–150 pp. 91, 133–150

Observations and Potential Remediation Steps for Super-Intensive, Indoor, Biofloc-Dominated Shrimp Production Systems—cont'd

Problem	Possible Cause(s)	Solution(s)	Additional Information
Loss of appetite	3. Poor feed quality	• Check condition and age of stored feed • Improve feed storage conditions • Use a higher quality brand of feed	pp. 186–187, 237 pp. 186–189 pp. 21–24, 185
	4. Poor shrimp quality	• Source postlarvae from a different hatchery with a superior genetic line	pp. 24–25, 153–154
	5. Disease	• See “Disease outbreak”	
	6. Gut flora poorly developed	• Add probiotics/prebiotics to feed	pp. 88, 128–129, 238
	1. Poor water quality	• Rectify specific water quality problem	pp. 133–150
	2. Disease	• See “Disease outbreak”	
Empty or partially empty guts	1. Underfeeding	• Increase feeding rate • Check protocol for estimating growth and survival, and determining feeding rate	pp. 165, 172, 187–189 pp. 169–171, 174, 184, 185, 186, 187–189, 192–194
	2. Inappropriate feed particle size	• Match feed to shrimp size according to growth sampling	pp. 168, 174
	3. Inadequate feed distribution	• Adjust location or increase number of auto-feeders • Distribute some feed by hand • Check that culture tank water is evenly mixed	pp. 88–90, 169–170, 172–173, 191–192 p. 192 pp. 150, 173
	4. Poor feed quality	• Check condition and age of stored feed • Improve feed storage conditions • Use a higher quality brand of feed	pp. 165–171, 186–187, 237–239 pp. 185–187 pp. 21–24, 185
	5. Poor water quality	• Rectify specific water quality problem	pp. 91, 133–150
	6. Disease	• See “Disease outbreak”	

Continued

Observations and Potential Remediation Steps for Super-Intensive, Indoor, Biofloc-Dominated Shrimp Production Systems—cont'd

Problem	Possible Cause(s)	Solution(s)	Additional Information
Slow growth	1. See “Empty or partially empty guts”		pp. 166–170, 173, 299
	2. Poor shrimp quality	Source postlarvae from a different hatchery with a superior genetic line	pp. 24–25, 153–154, 190
	3. Gut flora poorly developed	Add probiotics/prebiotics to feed	pp. 129, 237–238
Large size variation	1. Variation at stocking	- Contact source hatchery to suggest postlarvae grading improvements - Grade at nursery harvest	pp. 153–155
	2. Underfeeding	- Increase feeding rate - Check protocol for estimating growth and survival, and determining feeding rate	pp. 172, 166–169, 187–191 pp. 169–170, 184–185, 187–189
	3. Inadequate feed distribution	- Adjust location or increase number of auto-feeders - Distribute some feed by hand - Check that culture tank water is evenly mixed	pp. 88–90, 149–150, 172, 192 p. 192 pp. 150, 173
	4. Inappropriate feed particle sizes	Match feed to shrimp size according to growth sampling	pp. 169–170, 174
	5. Genetic growth differences	- Source postlarvae from a different hatchery with a superior genetic line - Contact source hatchery to suggest postlarvae and grading improvements	pp. 24–25, 153–154 p. 154
	6. Gut diseases such as hemocytic enteritis	See “Disease outbreak”	pp. 220–223
High incidence of cannibalism	1. See “Empty or partially empty guts”		pp. 165, 171, 299
	2. Large size variation	See “Large size variation”	
Unexplained mortality	1. Poor water quality	- Check all water quality parameters - Rectify specific water quality problem	pp. 133–150 pp. 133–150

Observations and Potential Remediation Steps for Super-Intensive, Indoor, Biofloc-Dominated Shrimp Production Systems—cont'd

Problem	Possible Cause(s)	Solution(s)	Additional Information
	2. Toxins in water (e.g., disinfection residue, some tank/liner materials, impurities in organic/inorganic carbon source)	- Identify toxin and source; treat appropriately (e.g., water exchange, install new tank liners) - Flush and leach toxins from liner before use - Only use inert and nontoxic culture tank materials, such as aquaculture-grade EPDM	pp. 70, 123 pp. 70–72
	3. Disease	See “Disease outbreak”	
	4. Damage from excessive air or water flow	Adjust aeration and mixing equipment	pp. 110, 149
	5. Handling (e.g., broken rostrums)	Improve sampling and nursery harvest techniques	pp. 171–172, 174
Increased rate of molting (sustained significant increase in exuviae in system)	1. Poor water quality	Rectify specific water quality problem	pp. 133–150
	2. Disease	See “Disease outbreak”	pp. 165, 221
Shrimp unable to molt or die while molting (soft shell, exuviae still partially attached to mortality)	1. Poor water quality	Rectify specific water quality problem	pp. 133–150
	2. Disease	See “Disease outbreak”	pp. 165, 221
	3. Shell fouling	- Check for stressors (poor water quality, disease) - Assess disinfection protocol of new water	pp. 133–150, 165, 222 pp. 119–126
	4. Nutritional deficiency	- Check condition and age of stored feed - Improve feed storage conditions - Use a higher quality brand of feed	pp. 186–189, 237–238 pp. 186–189 pp. 21–24, 185
	5. Ionic deficiency in culture water (e.g., low Ca^{2+} or K^{+})	- Supplement deficient ion in feed or culture water - Assess pretreatment protocol and water source	pp. 40–41, 55, 127, 143, pp. 37–41, 54, 127, 143
Poor appearance (missing appendages, black marks, short antennae, etc.)	1. Cannibalism	See “High incidence of cannibalism”	pp. 165–166, 171
	2. Predation	Improve predator exclusion methods	pp. 90, 221, 235–236

Continued

Observations and Potential Remediation Steps for Super-Intensive, Indoor, Biofloc-Dominated Shrimp Production Systems—cont'd

Problem	Possible Cause(s)	Solution(s)	Additional Information
	3. Excessive water or air flow, particularly in the nursery phase	For the first week following stocking, operate air and water flow at the minimum level required to maintain DO, then gradually increase flow as shrimp grow and biomass increases	pp. 111, 149
	4. Poor water quality	Rectify specific water quality problem	pp. 133–150
	5. Disease	See “Disease outbreak”	
	6. Short antennae are common in high-density culture		p. 221
Fouling such as algae or protozoans on body	Inadequate grooming due to lethargy caused by a stressor such as disease or poor water quality.	- See “Disease outbreak” - Rectify specific water quality problem	p. 221 pp. 133–150
Gill fouling	1. High TSS	See “High solids concentration”	pp. 222–223
	2. Disease	See “Disease outbreak”	
	3. Poor water quality	Rectify specific water quality problem	pp. 133–150
Abnormal coloration or marks	1. Poor water quality	Rectify specific water quality problem	pp. 133–150
	2. Disease or parasites	See “Disease outbreak”	pp. 195, 221
	3. Handling	Improve nursery harvest and sampling protocols to limit stress to shrimp	pp. 171–172, 178
Unusual behavior (e.g., corkscrewing, extended surface swimming, excessive jumping, lethargy, shrimp gathered around aeration devices)	1. Poor water quality (usually low DO or high ammonia/nitrite)	Rectify specific water quality problem	pp. 133–150, 160, 171–172, 220
	2. Disease	See “Disease outbreak”	
	3. Gill fouling	See “Gill fouling”	pp. 222–223
Tail cramping	1. Stressors such as handling during periods of high temperature	Minimize handling and air exposure	pp. 172, 222, 223
	2. Mineral imbalance such as high manganese or low potassium	Supplement deficient ion in feed or culture water	pp. 40–41, 55, 127, 143

Observations and Potential Remediation Steps for Super-Intensive, Indoor, Biofloc-Dominated Shrimp Production Systems—cont'd

Problem	Possible Cause(s)	Solution(s)	Additional Information
Many shrimp gathered under belt feeders or rapidly surfacing when feed is added	1. Inadequate feed distribution	- Adjust location or increase number of auto-feeders - Distribute some feed by hand - Check that culture tank water is evenly mixed	pp. 88–90, 169–173, 191 p. 191 pp. 149, 171
	2. Underfeeding	- Increase feeding rate - Check protocol for estimating growth and survival, and determining feeding rate	pp. 169–170, 189–192 pp. 169–171, 174, 184, 189–191, 192–194
Disease outbreak ^a	1. Stressor such as poor water quality or sudden change in a parameter such as temperature	- Rectify specific water quality problem - Stabilize system - Adjust probiotic/prebiotic inoculation regime	pp. 133–150 pp. 49, 135 pp. 88, 128–130, 237–238
	2. Stress from nursery harvest and restocking	- Improve harvest protocols to limit stress to shrimp - Increase probiotic/prebiotic inoculation pre- and postharvest	p. 178 pp. 88, 128–130, 237–238
	3. Poor feed quality (nutritional deficiency)	- Check condition and age of stored feed - Improve feed storage conditions - Use a higher quality brand of feed - Optimize feeding rate	pp. 186–189, 237 pp. 186–189 pp. 21–24, 185 pp. 21–24, 172, 189 pp. 166–172
	4. Poor shrimp quality	Use postlarvae from a different hatchery with a superior genetic line	pp. 25, 153–154
	5. Lapse in biosecurity	Audit and improve biosecurity protocols, particularly disinfection	pp. 119–127, 234–238
	6. High biomass	- Reduce solids concentration - Partial harvest - Review stocking protocols	pp. 84–88, 107–108, 141–142 pp. 134, 196–197 pp. 183–184

^a For disease treatment options, see pp. 296–305, 311–314.

Glossary

- Acclimation** Process of adjusting shrimp to a different set of physical and chemical parameters, usually in preparation for transport or stocking.
- Ammonia-Oxidizing Bacteria (AOB)** Aerobic bacteria that transform ammonia to nitrite.
- Aquaculture** Rearing aquatic organisms for food, pharmaceuticals, nutraceuticals, stock enhancement, or as ornamentals for home aquaria.
- Autotrophy** Mechanism of “self-feeding,” whereby an organism synthesizes organic compounds from inorganic components. Can be chemoautotrophic or photoautotrophic (e.g., algae).
- Biofloc** Suspended aggregates of aquatic detritus and microorganisms.
- Biofloc Technology (BFT)** Aquaculture technology that uses biofloc to process dissolved metabolites and serve as a source of nutrition for cultured species.
- Biomass** The total mass of a population or community of organisms.
- Biosecurity** Protocols that exclude pathogens and predators from a culture facility.
- Catabolize** The act of breaking down a substance (protein or carbohydrate) to derive energy.
- Chemical Oxygen Demand (COD)** Measure of the amount of oxygen required to oxidize all organic substances in a volume of water.
- Chemoautotrophic** Metabolic mechanism that derives nutrition and energy from inorganic sources.
- Compensatory Growth** Accelerated growth that follows a period of poor growth under suboptimal conditions. Also called “catch-up” growth.
- Constructed wetland** An area designed to settle solids and absorb nutrients via photosynthesis, usually for the purpose of environmental mitigation.
- Culture tank** Container in which the target species is grown.
- Denitrification** Anaerobic microbial process that chemically reduces nitrate to nitrogen gas.
- Digestibility** Quantification of how well a substance is absorbed by an organism.
- Disinfection** Cleaning that reduces many harmful microorganisms, not to be confused with sterilization, which kills *all* organisms present.
- Exuviae** Cast-off exoskeleton resulting from ecdysis (molting) by crustaceans or insects.
- Five-day Carbonaceous Biochemical/Biological Oxygen Demand (cBOD₅)** Measurement of oxygen depletion by living organisms in a sample of water over a five-day period.
- Fouling** Clogging or covering by something undesirable and/or harmful. Fouling agents can be nonliving (e.g., carbonate deposits) or living (e.g., biofouling organisms like barnacles or algae).
- Fixation** Preservation of a sample for processing at a later time.
- Foam Fractionator (“Protein Skimmer”)** Device that produces fine bubbles of air,

- oxygen, or ozone that capture dissolved organics and small particles.
- Greenwater culture** A form of aquaculture that uses algae to process metabolic wastes.
- Grow-out** Stage of the culture cycle that produces marketable product.
- Halophyte** Plant with an affinity or tolerance for salt.
- Hazard Analysis and Critical Control Points (HACCP)** Analysis used in food handling to identify the most likely points of contamination and spoilage.
- Hemolymph** Circulatory fluid in crustaceans and insects.
- Hepatopancreas** Shrimp internal organ responsible for production of digestive enzymes.
- Heterotrophic** Strategy of deriving nutrition and energy from organic sources.
- Inoculation** Seeding a small, established culture of microorganism(s) to stimulate a larger culture.
- Internal Rate of Return (IRR)** A way to evaluate an investment that accounts for the time value of money. The IRR is the discount rate at which the Net Present Value (see below) equals zero.
- Investment Payback Period** The time needed to recover an investment through net cash revenues.
- Mariculture** Aquaculture of brackish water or marine organisms.
- Melanization** Concentration of dark pigments (melanin), often an indication of stress, injury, or infection in shrimp.
- Mixotrophic** Biofloc culture with auto- and heterotrophic microorganisms in floc aggregates.
- Mole (abbr. mol)** Just like a dozen is a collection of 12 of anything (e.g., a dozen eggs, a dozen years), a mole is also a collection, albeit a *much* larger one: Instead of 12, it is roughly 6 followed by 23 zeros. The mole—or parts thereof, such as the micromole, which is one-thousandth of a mole—is particularly useful when expressing quantities of the very small entities that participate in chemical reactions.
- Moribund** At the point of death.
- Necrosis** Dead tissue.
- Net Present Value (NPV)** A measure that accounts for the time value of money in an investment based on the stream of future cash flows over the life of the project and a discount rate.
- Nitrification** Two-step microbial process of oxidizing ammonia to nitrite and then to nitrate.
- Nitrite-Oxidizing Bacteria (NOB)** Bacteria responsible for converting nitrite to nitrate.
- Nursery** A stage in the culture cycle in which juveniles are raised. Nurseries use space more efficiently and promote health, thereby increasing survival and performance during grow-out.
- Organic Shrimp** Marketing strategy that advertises use of “organic” practices, though there are various definitions of “organic.” The belief is that the “organic” label justifies a premium price.
- Oxidize** Chemical addition of oxygen. More generally, the loss of electrons by a reactant.
- Pathogen** A disease-causing agent such as certain bacteria, viruses, and fungi.
- Photoautotrophic** Metabolic strategy in which organic compounds are synthesized from inorganic substances utilizing light as the energy source.
- Protein Skimmer** See Foam Fractionator (above).
- Raceway (RW)** Elongated culture tank in which water flows in one end and out the other or around a central partition. RWs described in this manual are of the second type.
- Recirculating Aquaculture System (RAS)** Culture system designed to minimize the amount of water added or exchanged. Typically indoors for better environmental control.
- Salinity** The total concentration of dissolved salts in a solution. The current oceanographic standard is Absolute Salinity, expressed as g/

kg. Salinities reported in this manual were made with a conductivity meter, for which the previous standard, Practical Salinity Units (psu), is appropriate. Parts-per-thousand (ppt) still is more common in aquaculture and is used herein.

Sterilization Process that kills all living organisms. Not to be confused with disinfection (see above).

Stocking Introduction of culture organisms into a culture container.

Super-intensive Level of culture generally considered to be above the industry norm in terms of both inputs and yields.

Sustainability Ability to continue an activity or practice indefinitely without detrimental effects to the environment or society.

Traceability Ability to verify through documentation all inputs (feed ingredients, chemicals, water), handling, and storage at every stage of the production chain.

Venturi A flow device consisting of a constricted tube that increases fluid speed, by creating a region of low pressure through which another fluid or gas can be drawn in and efficiently mixed. Used in aquaculture for aeration and the injection of chemicals.

List of Abbreviations

cBOD₅	five-day carbonaceous biochemical/ biological oxygen demand	NPV	net present value
AOB	ammonia-oxidizing bacteria	ORP	oxidation redox potential
BFD	biofloc dominated	PL	postlarvae
BFT	biofloc technology	ppm	parts per million
cfm	cubic feet per minute	ppt	parts per thousand
cfu	colony forming units	PSF	pressurized sand filter
cmm	cubic meters per minute	RAS	recirculating aquaculture system
COD	chemical oxygen demand	RW	raceway
CP	crude protein	SD	standard deviation
CV	coefficient of variation	SPF	specific pathogen-free
DO	dissolved oxygen	SPR	specific pathogen-resistant
FCR	feed conversion ratio	SPT	specific pathogen-tolerant
FF	foam fractionator	SS	settleable solids or suspended solids
GCFU	green colony forming (<i>vibrio</i>) units	ST	settling tank
GH	greenhouse	Texas A&M-ARML	Texas A&M AgriLife Research Mariculture Lab
HACCP	hazard analysis and critical control points	TAN	total ammonia nitrogen, sometimes called ammonia, a summation of the un-ionized ammonia (NH ₃) and ionized ammonium (NH ₄ ⁺)
ind	individual	TCBS	thiosulfate citrate bile salts
IMTA	integrated multi-trophic aquaculture	TDS	sucrose agar
IRR	internal rate of return	TSS	total dissolved solids
IWG	inches of water gauge or pressure	UV	total suspended solids
MCF	multicyclone filter	VSS	ultraviolet (light)
mWG	meters of water gauge or pressure	w/w	volatile suspended solids
LC₅₀	lethal concentration of toxicant which kills 50% of a population at specified exposure time	YCFU	wet weight
NOB	nitrite-oxidizing bacteria		yellow colony forming (<i>vibrio</i>) units

I

Water Quality Testing Procedures and Alternatives

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I.A DISSOLVED OXYGEN

There are test kits to measure DO, but the most common and easiest method is with an oxygen electrode that measures the rate at which oxygen diffuses across a membrane. The electrode is connected to a meter for continuous monitoring or spot-checks of DO. Meters can be oxygen specific or multiparameter. Most measure temperature because this affects oxygen saturation. Meters must be regularly calibrated and membrane heads cleaned or changed as required. Calibrate at the salinity and temperature (usually 25°C) required by the particular model. Special attention must be paid to membrane integrity and avoiding air bubbles under the membrane.

Optical probes have no membrane and do not consume oxygen during measurement. This eliminates the need to move water past the probe. They are 50%–100% more expensive. Optical probes connected to an online monitoring and alarm system (YSI 5500D Multiparameter Monitoring System, Yellow Springs Instruments, Yellow Springs, OH, US) were used successfully at the Texas A&M-AgriLife Research Mariculture Lab (ARML).

I.B TEMPERATURE

Electronic thermometers can continuously monitor temperature in culture systems or be used for spot-checks. Both are recommended for aquaculture. Electronic thermometers can be temperature specific or part of multiparameter units. Mercury or alcohol-filled glass thermometers with a scale of 0.1°C are a backup and also are used for calibration.

I.C pH

The simplest and most accurate method to measure pH is with an electrode. Probes should be cleaned and calibrated weekly against pH standards (e.g., pH 4, 7, and 10). Pen-type pH meters are available from as little as \$10 but are less accurate. Pen-type meters that require calibration generally perform better than those that do not. The lifespan of regular pH probes is usually longer than pen-type meters. pH electrodes typically last up to 2–3 years if well maintained. There are also many brands of pH test kits in the form of reagent tests

or test strips. Test kits are useful to cross-check pH probes or as a backup if the probe malfunctions.

I.D ALKALINITY

Alkalinity is measured by titration following the method in [Eaton et al. \(1995\)](#). Measurement with a photometer or spectrophotometer is faster and easier than traditional titration. These are less accurate and consistent, but they indicate alkalinity sufficiency or deficiency. There are field titration and digital titration kits with improved accuracy and cheaper than photometer and spectrophotometer tests.

I.E AMMONIA

Ammonia should be measured within a few hours of taking water samples because oxidation of ammonia can occur within the sample bottle. If this is not possible, then samples should be refrigerated. Samples do not usually require filtering, but this should be confirmed in the manual for the particular test. “Stained” water interferes with the test color of some kits and this distorts results.

Ammonia test kits vary in price depending on their accuracy and ease of use. They can be in the form of reagent tests or test strips. Ammonia-specific photometers and tests using general photometers and spectrophotometers are more accurate than simple test kits. If ammonia is higher than the test range, then the sample must be diluted. For example, for a twofold dilution, mix 5 mL of deionized water with 5 mL of sample and, after analysis, multiply the result by 2. Some tests require a conditioning reagent for salt water to prevent precipitation. Wear safety gloves whenever handling reagents involved in these tests.

Ammonia in seawater also can be measured accurately by probe (ion-selective electrode). Probes for laboratory use (ion-selective electrodes) typically range in price from \$600 to

\$800, plus the cost of calibration standards and membrane kits.

[Tables AI.1–AI.3](#) display the percentage of un-ionized ammonia at three salinities: 23–27 ppt, 18–22 ppt, and freshwater.

I.F NITRITE

Measure nitrite within a few hours of taking water samples because oxidation of ammonia and nitrite can occur within the sample bottle. Nitrite tests include various types of reagents or test strips for both low range and high range. They are much simpler than ammonia and nitrate tests. Nitrite-specific photometers (and tests that rely on general photometers and spectrophotometers) are more accurate than test kits. If nitrite is higher than the test range, the sample must be diluted. Most tests measure nitrite-nitrogen ($\text{NO}_2\text{-N}$), the nitrogen in nitrite. Multiplying $\text{NO}_2\text{-N}$ by 3.284 converts to NO_2 . Wear safety gloves whenever handling the reagents for these tests.

I.G NITRATE

Nitrate can be difficult to measure accurately because of the more complex methods involved and the presence of compounds that interfere with the reading. As nitrate accumulates, samples may require dilution to fall within the test kit range. This also dilutes interfering compounds and improves test reliability. Many kits convert nitrate to nitrite, and then measure the nitrite. For this reason, the nitrite in the sample is subtracted from the measured nitrate to obtain the actual concentration. Measure nitrate within a few hours of taking samples because of oxidation within the bottle increases its concentration.

Nitrate tests can be in the form of various types of reagent tests or test strips. Nitrate-specific photometers are more accurate than test kits. If nitrate is higher than the test range, then the sample must be diluted. Most tests measure

TABLE AI.1 Percentage of Toxic (Unionized) Ammonia in the 23–27ppt Salinity Range at Different Temperatures and pH

pH	Temp (°C)													
	20	21	22	23	24	25	26	27	28	29	30	31	32	33
7.0	0.33	0.36	0.38	0.41	0.44	0.48	0.52	0.55	0.59	0.63	0.67	0.72	0.77	0.83
7.1	0.42	0.45	0.48	0.52	0.56	0.60	0.65	0.69	0.74	0.79	0.84	0.90	0.97	1.04
7.2	0.52	0.56	0.61	0.65	0.70	0.75	0.81	0.87	0.93	1.00	1.07	1.14	1.22	1.30
7.3	0.66	0.71	0.76	0.82	0.88	0.94	1.02	1.09	1.16	1.25	1.34	1.43	1.53	1.63
7.4	0.82	0.89	0.95	1.02	1.10	1.19	1.28	1.36	1.46	1.56	1.67	1.79	1.91	2.05
7.5	1.03	1.11	1.20	1.29	1.38	1.47	1.61	1.71	1.83	1.96	2.10	2.24	2.39	2.57
7.6	1.30	1.40	1.51	1.62	1.74	1.87	2.01	2.14	2.29	2.45	2.62	2.81	2.99	3.20
7.7	1.63	1.76	1.88	2.02	2.17	2.34	2.52	2.68	2.86	3.06	3.28	3.50	3.74	3.99
7.8	2.04	2.20	2.36	2.53	2.72	2.93	3.12	3.33	3.57	3.82	4.08	4.35	4.65	4.95
7.9	2.55	2.75	2.95	3.16	3.39	3.66	3.89	4.17	4.44	4.76	5.09	5.43	5.78	6.17
8.0	3.19	3.44	3.69	3.95	4.24	4.57	4.85	5.18	5.56	5.92	6.30	6.71	7.19	7.63
8.1	3.98	4.29	4.61	4.93	5.26	5.68	6.02	6.45	6.90	7.35	7.83	8.33	8.85	9.43
8.2	4.97	5.35	5.71	6.13	6.54	7.05	7.46	8.00	8.47	9.09	9.71	10.31	10.87	11.63
8.3	6.17	6.62	7.09	7.58	8.13	8.72	9.26	9.80	10.53	11.11	11.89	12.66	13.33	14.08
8.4	7.65	8.16	8.70	9.31	9.95	10.72	11.30	12.02	12.84	13.59	14.34	15.33	16.17	17.08
8.5	9.44	10.02	10.73	11.42	12.25	13.10	13.76	14.66	15.58	16.41	17.34	18.43	19.44	20.43

(Based on EIFAC, 1986. Report of the working group on terminology, format, and units of measurement as related to flow-through and recirculation systems. European Inland Fisheries Advisory Commission (EIFAC) Tech. Pap. No. 49. Rome, Italy. FDEP, 2001. Calculation of Un-ionized Ammonia in Fresh Water. Florida Department of Environmental Protection Chemistry Laboratory Methods Manual, Tallahassee, Florida. Available from: https://floridadep.gov/sites/default/files/5-Unionized-Ammonia-SOP_1.pdf (Accessed 9 March 2018).)

TABLE AI.2 Percentage of Toxic (Unionized) Ammonia in the 18–22ppt Salinity Range at Different Temperatures and pH

pH	Temp (°C)													
	20	21	22	23	24	25	26	27	28	29	30	31	32	33
7.0	0.35	0.37	0.40	0.43	0.46	0.51	0.53	0.57	0.61	0.65	0.69	0.75	0.80	0.86
7.1	0.44	0.46	0.50	0.54	0.58	0.64	0.67	0.71	0.76	0.82	0.88	0.94	1.01	1.08
7.2	0.56	0.58	0.63	0.67	0.72	0.81	0.84	0.90	0.96	1.03	1.10	1.18	1.26	1.35
7.3	0.70	0.73	0.79	0.85	0.91	1.01	1.06	1.12	1.20	1.29	1.38	1.48	1.59	1.69
7.4	0.88	0.92	0.99	1.06	1.14	1.28	1.33	1.41	1.51	1.62	1.73	1.85	1.98	2.12

Continued

TABLE AI.2 Percentage of Toxic (Unionized) Ammonia in the 18–22ppt Salinity Range at Different Temperatures and pH—cont'd

pH	Temp (°C)													
	20	21	22	23	24	25	26	27	28	29	30	31	32	33
7.5	1.11	1.15	1.24	1.34	1.43	1.59	1.66	1.77	1.89	2.03	2.17	2.32	2.48	2.65
7.6	1.39	1.45	1.56	1.68	1.80	2.01	2.09	2.21	2.37	2.54	2.72	2.90	3.10	3.31
7.7	1.74	1.82	1.95	2.09	2.25	2.51	2.61	2.77	2.96	3.17	3.39	3.62	3.87	4.13
7.8	2.18	2.28	2.44	2.62	2.81	3.14	3.23	3.45	3.69	3.95	4.21	4.50	4.81	5.13
7.9	2.73	2.85	3.06	3.28	3.51	3.91	4.03	4.31	4.61	4.93	5.25	5.62	5.99	6.37
8.0	3.41	3.56	3.82	4.10	4.39	4.88	5.03	5.38	5.71	6.13	6.51	6.94	7.41	7.87
8.1	4.26	4.44	4.76	5.10	5.46	6.07	6.25	6.67	7.09	7.58	8.09	8.62	9.17	9.71
8.2	5.30	5.52	5.92	6.33	6.76	7.62	7.75	8.26	8.77	9.35	9.97	10.64	11.24	11.90
8.3	6.58	6.85	7.35	7.87	8.40	9.28	9.52	10.20	10.87	11.49	12.18	12.99	13.70	14.49
8.4	8.15	8.44	9.02	9.66	10.28	11.40	11.62	12.51	13.26	14.05	14.89	15.73	16.62	17.58
8.5	10.00	10.37	11.13	11.86	12.65	13.84	14.15	15.26	16.08	16.97	17.91	18.91	19.98	21.03

(Based on EIFAC, 1986. Report of the working group on terminology, format, and units of measurement as related to flow-through and recirculation systems. European Inland Fisheries Advisory Commission (EIFAC) Tech. Pap. No. 49. Rome, Italy. FDEP, 2001. Calculation of Un-ionized Ammonia in Fresh Water. Florida Department of Environmental Protection Chemistry Laboratory Methods Manual, Tallahassee, Florida. Available from: https://floridadep.gov/sites/default/files/5-Unionized-Ammonia-SOP_1.pdf (Accessed 9 March 2018).)

TABLE AI.3 Percentage of Toxic (Unionized) Ammonia in Freshwater (TDS=0mg/L) at Different Temperatures and pH

pH	Temp (°C)													
	20	21	22	23	24	25	26	27	28	29	30	31	32	33
7.0	0.395	0.425	0.457	0.491	0.527	0.564	0.607	0.651	0.697	0.747	0.797	0.855	0.914	0.977
7.1	0.498	0.535	0.575	0.617	0.663	0.711	0.763	0.818	0.876	0.938	1.00	1.07	1.15	1.23
7.2	0.625	0.673	0.723	0.776	0.833	0.894	0.958	1.03	1.10	1.18	1.25	1.35	1.44	1.54
7.3	0.786	0.845	0.908	0.975	1.05	1.12	1.20	1.29	1.38	1.48	1.58	1.69	1.81	1.93
7.4	0.988	1.06	1.14	1.22	1.31	1.41	1.51	1.62	1.73	1.85	1.98	2.12	2.26	2.42
7.5	1.24	1.33	1.43	1.54	1.65	1.76	1.89	2.03	2.17	2.32	2.48	2.65	2.83	3.03
7.6	1.56	1.67	1.80	1.93	2.07	2.22	2.37	2.54	2.72	2.91	3.11	3.32	3.54	3.78
7.7	1.95	2.10	2.25	2.41	2.59	2.77	2.97	3.18	3.40	3.63	3.88	4.14	4.42	4.71
7.8	2.44	2.63	2.82	3.02	3.24	3.47	3.71	3.97	4.24	4.53	4.84	5.16	5.50	5.86
7.9	3.06	3.28	3.52	3.77	4.04	4.33	4.53	4.94	5.28	5.64	6.01	6.41	6.83	7.27

TABLE AI.3 Percentage of Toxic (Unionized) Ammonia in Freshwater (TDS=0mg/L) at Different Temperatures and pH—cont'd

pH	Temp (°C)													
	20	21	22	23	24	25	26	27	28	29	30	31	32	33
8.0	3.81	4.10	4.39	4.70	5.03	5.37	5.75	6.15	6.56	7.00	7.44	7.94	8.44	8.98
8.1	4.76	5.10	5.47	5.85	6.25	6.69	7.14	7.62	8.12	8.65	9.21	9.79	10.40	11.05
8.2	5.92	6.34	6.79	7.25	7.75	8.27	8.82	9.40	10.00	10.70	11.30	12.02	12.80	13.50
8.3	7.34	7.86	8.39	8.96	9.56	10.20	10.90	11.50	12.30	13.00	13.80	14.70	15.50	16.40
8.4	9.07	9.69	10.30	11.00	11.70	12.50	13.30	14.10	15.00	15.90	16.80	17.80	18.80	19.90
8.5	11.13	11.90	12.70	13.50	14.40	15.21	16.20	17.20	18.20	19.20	20.26	21.40	22.60	23.80

(Based on Thurston, R.V., Russo, R.C., Emerson, K., 1979. *Aqueous ammonia equilibrium-tabulation of percent un-ionized ammonia*. EPA-600/3-79-091, Environmental Research Laboratory, Duluth, Minnesota, USA. Available from NTIS (PB80-103518). EIFAC, 1986. *Report of the working group on terminology, format, and units of measurement as related to flow-through and recirculation systems*. European Inland Fisheries Advisory Commission (EIFAC) Tech. Pap. No. 49. Rome, Italy. USEPA, 1987. *Nonpoint Source Guidance*. U.S. Environmental Protection Agency, Office of Water and Office of Water Regulations and Standards, Washington, DC, USA.)

NO₃-N (nitrate-nitrogen). This is converted to NO₃ when multiplied by 4.427. Wear safety gloves whenever handling the reagents for these tests.

When microalgae are present in large numbers, place the Imhoff cones in a dark place to minimize flotation of settleable solids. A shorter settling period of 10–20 min can be used as long as a consistent time is followed. [Fig. AI.1](#) shows Imhoff cones filled with biofloc water.

I.H SETTLEABLE SOLIDS (SS)

Volumetric test for settleable solids (SS) ([Eaton et al. 1995](#)):

- *Equipment*
 1. Imhoff cone
- *Method*
 1. Add 1 L of a well-mixed water sample to the Imhoff cone.
 2. Let the sample settle for 45 min, then gently stir the sides of the cone with a rod or spin by hand.
 3. Let the sample settle for another 15 min.
 4. Record the volume of settleable solids at the base of the cone as mL/L. If there are any open areas of liquid between the settled solids, then estimate the volume of these and subtract from the total volume of settleable solids.

I.I TOTAL SUSPENDED SOLIDS (TSS)

Example protocol for TSS following Standard Methods 2540D and 2540E in [Eaton et al. \(1995\)](#) (gravimetric method):

- *Equipment*
 1. GF/A Glass-fiber filter disks (sized to fully cover base of evaporation dishes). The size, grade, and price of these filter paper disks can vary significantly. 7.6-cm glass fiber filter circles of adequate grade will typically cost \$126 per 100.
 2. Drying oven, set to 103–105°C.
 3. Vacuum pump.
 4. Vacuum flask with rubber crucible holder.



FIG. AI.1 Imhoff cones with bacterial floc.

5. Desiccator, containing desiccant with a color indicator showing moisture concentration.
 6. Electronic balance (0.1-mg readability).
 7. 7.7-cm Buchner funnel.
 8. Magnetic stirrer with TFE stirring bar.
 9. Metal forceps/tongs.
 10. Plastic forceps.
 11. Timer.
 12. Wash bottle filled with DI water.
- *Method*
 - *Part I—Crucible Preparation*
 1. Ensure that the oven is between 103 and 105°C.
 2. Hook up a 1-L vacuum flask to the vacuum pump and insert the black rubber Buchner funnel holder into the top of the flask.
 3. Place the funnel on the suction and use a deionized rinse bottle to seat the preweighed filter with three successive squirts of water, starting from the center of the filter and squirting in a circular motion out to the walls of the crucible.
 4. Record initial weight of the preweighed filter paper (number written in pencil on the bottom of the aluminum tray) on a data sheet.
 - *Part II—Filtration and oven drying (TSS)*
 1. Make sure samples are very well mixed using a magnetic stirrer with a TFE stirring bar.
 2. Using forceps, vacuum the Buchner funnel. Prerinse with three squirts of deionized water to reseal the filter.
 3. Use a graduated cylinder or syringe to transfer an accurate sample into the Buchner funnel. The sample size can be up to 50mL or more. Add enough sample so that the filter is completely covered in matter, but not so loaded that water flow is severely restricted. Record mL of sample added.
 4. Rinse the sample with three consecutive rinses of 10mL deionized water, making sure not to add the 10mL of water until the previous addition of water has completely filtered through. Allow the vacuum to run for a few moments after the last amount of deionized water has been filtered.

5. Return the filter paper to the aluminum-lined baking tray.
6. Place the entire baking tray in the preheated oven (103–105°C).
7. Leave samples in the oven for at least an hour to remove all remaining water in the filter.
8. Remove the entire tray from the oven after one hour and transfer to the desiccator.
9. Cool with the lid cracked for 2 min, then close the lid and let the samples cool for another 18 min.
10. After 18 min, weigh all samples in the same order they were weighed during Part I and record on a data sheet.

○ *Part III—Calculation*

After entering the weights and mL of the samples into the TSS [Excel Sheet #20](#)—Appendix VII, the final values will be calculated automatically in the last two columns. Here is the calculation:

$$\text{TSS (mg total suspended solids/L)} = \frac{(A - B) \times 1000}{V}$$

where A = Weight 2 (Filter plus residue in mg after oven), B = Weight 1 (empty filter after furnace), V = Sample volume (mL).

Page [#416](#) in Appendix VII is an example form for calculating and recording TSS. [Excel Sheet # 19](#) named *Vibrio* TSS VSS Alkalinity Calc—Appendix VII provides the template for data entry and the calculation.

Tips

1. Keep the laboratory at a constant temperature—22°C (about 71°F) throughout the entire process.
2. If the crucibles are not allowed to cool with the lid cracked, the desiccator will be very difficult to open once they are completely cooled.
3. When taking weights, replace the lid immediately after removing each crucible.

4. Prewedged filter paper on aluminum trays for gravimetric analysis can be purchased from several companies. These come with the weight of each filter pan printed on an attached heat-resistant label so that Part I (Crucible Preparation) can be skipped, saving considerable time and labor. Aluminum dishes also cool faster than porcelain crucibles so that less cooling time is required. Furthermore, the producers who use this method were able to do away with a desiccator without sacrificing accuracy.

TSS probes can monitor TSS continuously, often in conjunction with other parameters, including SS and turbidity. Probes provide much faster results, but their accuracy currently is limited. They must be calibrated regularly against known TSS values. Readings should be compared with traditional gravimetric results to check accuracy. Costs vary considerably, ranging from \$1500 to \$5500 for a multiparameter unit. Some spectrophotometers measure TSS, for example, the Hach TSS Method 8006. This is much faster and gives consistent results that generally are 20%–30% lower than the gravimetric TSS Standard Method procedure. Spectrophotometry can be used to monitor TSS if results are periodically compared with the gravimetric Standard Method. This establishes a relationship between these two methods for the system being tested. Accredited laboratories and some wastewater treatment plants may be willing to measure TSS for comparison with on-site measurements.

I.J TURBIDITY

Turbidity is measured with an electronic portable turbidimeter, turbidity probes, most spectrophotometers, and photometers. These are easy to use and give fast, reproducible results. Begin analysis as soon as possible after sampling, preferably at a similar pH and

temperature. Gently agitate samples immediately prior to analysis to ensure a representative sample and avoid dilution if possible (Eaton et al., 1995). Calibrate meters with known standards close to the expected results before each use.

I.K SALINITY

Salinity usually is measured by one of the following methods:

1. A refractometer (Fig. AI.2) measures the refractive index of a water sample as light passes through it. To use: Open the plate and add one or two drops of sample water to the lens. Gently close the plate so that the sample spreads out over the entire lens evenly without air bubbles. Look through the eyepiece, focus if necessary, and read the salinity off the scale (the point at which the boundary line between the white and blue sections meets the scale) (Fig. AI.2). Most refractometers have ppt and specific gravity scales and temperature compensation. Check the manual for the calibration procedure.

2. Conductivity measures the ability of a solution to carry an electric current (Eaton et al. 1995). It gives a more precise measure than a refractometer or hydrometer. Conductivity increases with temperature, so it is measured at a reference temperature of 25°C or converted via a coefficient. Some automatically compensate for temperature and convert the reading to salinity in ppt; others read out micro- or millisiemens per centimeter ($\mu\text{S}/\text{cm}$ or mS/cm). To convert from mS/cm to ppt, use an online conversion calculator. For example: <http://www.fivecreeks.org/monitor/sal.shtml> (accessed 10 June 2018). Page # 417 — Appendix VII shows conversion table from conductivity to salinity in different water temperatures.

I.L PHOSPHATE

Phosphorus/phosphate tests are available for photometers and spectrophotometers. If the concentration is higher than the test range, the sample will need to be diluted. Wear safety gloves whenever handling the reagents involved in this tests.

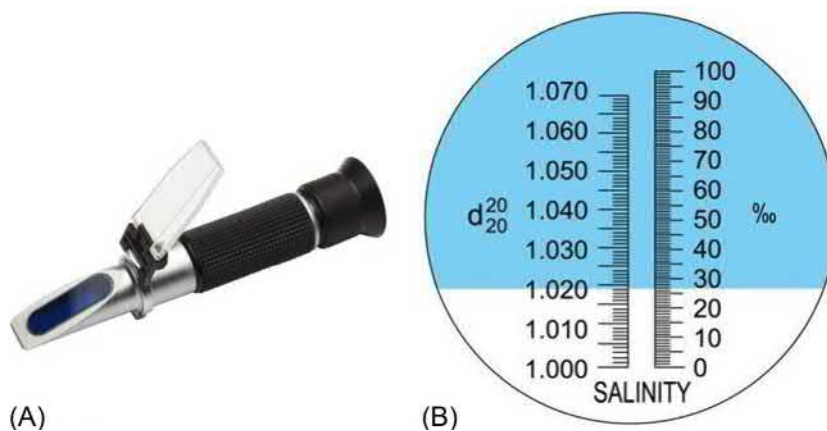


FIG. AI.2 Refractometer (A) and scale visible when looking through the refractometer eye piece (B), with specific gravity on the left and salinity (ppt) on the right.

I.M CHLORINE

Chlorine tests are available for photometers and spectrophotometers, along with a variety of chlorine-specific colorimeters and titration-based test kits. If the concentration is higher than the test range, the sample will need to be diluted. Chlorine can be neutralized after treatment with vigorous aeration for 24h or more quickly with chemicals—usually sodium thiosulfate, although Vitamin C and hydrogen peroxide can be used (see [Section 6.2.1](#)).

References

Eaton, D.E., Clesceri, L.S., Greenberg, A.E., 1995. *Standard Methods for the Examination of Water and Wastewater*, nineteenth ed. Publication Office, American Public Health Association, Washington, DC.

Further Reading

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II

Microbiological Tests

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II.A VIBRIO MONITORING

Vibrio spp. are gram-negative, facultative anaerobic, chemoautotrophic bacteria. They can grow under aerobic or anaerobic conditions in the presence of inorganic ions serving as electron acceptors, such as oxygen, nitrate, and sulfate. Regular monitoring of *Vibrio* spp. allows time to prevent a disease outbreak and test the effectiveness of probiotic treatments.

Vibrio concentration is monitored with thiosulphate-citrate-bile salt sucrose (TCBS) agar (see Appendix IIb for a detailed method). TCBS has a high pH (8.5–9.5) that suppresses growth of most non-*Vibrio* bacteria, so it is highly selective for *Vibrio*. Sucrose-fermenting *Vibrio* produce yellow colonies and non-sucrose-fermenting *Vibrio* produce green colonies (Table AII.1 and Fig. AII.1—Appendix VII). Yellow colonies generally are nonpathogenic; green colonies are considered pathogenic to shrimp.

Vibriosis often is observed in intensive closed systems when green-colony *Vibrio* species, particularly *V. parahaemolyticus*, increase relative to yellow-colony species (Fig. AII.1). Some pathogenic species, however, such as *V. harveyi*, *V. alginolyticus*, and *V. campbelli*, also may express

yellow colonies (Doug Ernst, Natural Shrimp, personal communication). *V. harveyi* and *V. splendidus* also can exhibit variations in color on TCBS, that is, yellow or green (Jeffrey Turner, TAMU-CC, personal communication).

Some probiotic species also can form as yellow colonies on TCBS, and *Pseudomonas* spp. and *Aeromonas* spp. occasionally form blue-green colonies. The method described in Appendix IIb can be used for CHROMagar *Vibrio* plates (also known as RambaCHROM *Vibrio*), with colonies appearing as mauve (*V. parahaemolyticus*), green-blue to turquoise-blue (*V. vulnificus* and *V. cholerae*), or white (colorless) (*V. alginolyticus*) (Fig. AII.2).

CHROMagar can be more specific for *Vibrio* than TCBS (Di Pinto et al., 2011), although it covers fewer species. A detailed biochemical key for *Vibrio* identification is found in Nogueroles and Blanch (2008). Ray et al. (2010) describe three other methods for monitoring microbial communities in biofloc systems: visual microscopy abundance quantification, epifluorescence microscopy with image analysis quantification, and bacterial fatty acid assessment by gas chromatography.

Black colonies growing on TCBS indicate sulfate-reducing bacteria. This indicates that

TABLE AII.1 Colony Color Formed by Different Pathogenic *Vibrio* spp. on TCBS Agar Plates According to Sucrose (Yellow) or Nonsucrose Fermenting (Green) (Noguera and Blanch, 2008; Doug Ernst, personal communication; Jeffrey Turner, TAMU-CC, personal communication)

<i>Vibrio</i> sp.	Colony Color	% Colonies Forming Color
<i>V. alginolyticus</i>	Yellow	75–89
<i>V. anguillarum</i>	Yellow	≥90
<i>V. campbelli</i>	Green	≥90
<i>V. cholerae</i>	Yellow	≥90
<i>V. damsela</i>	Green	–
<i>V. fluvialis</i>	Yellow	≥90
<i>V. furnissii</i>	Yellow	≥90
<i>V. harveyi</i>	Green (often with a lighter halo); luminescence	≥90
<i>V. hepatarius</i>	Yellow	≥90
<i>V. metschnikovii</i>	Yellow	≥90
<i>V. mimicus</i>	Green	≥90
<i>V. mytili</i>	Yellow	≥90
<i>V. nereis</i>	Yellow	≥90
<i>V. nigrapulchritudo</i>	Green	≥90
<i>V. pacinii</i>	Yellow	≥90
<i>V. parahaemolyticus</i>	Bluish-green	≥90
<i>V. penaeicida</i>	Green	≥90 (Poor growth on TCBS)
<i>V. ponticus</i>	Yellow	75–89
<i>V. splendidus</i> (I)	Yellow	75–89 (Weak)
<i>V. splendidus</i> (II)	Green	≥90
<i>V. tubiashi</i>	Yellow	≥90
<i>V. vulnificus</i>	Green (can be yellowish)	75–89/≥90 depending on strain



FIG. AII.1 TCBS agar plates with *Vibrio* colonies. (A) Yellow (light gray in print version) dominant [only one green (dark gray in print version)], (B) Higher proportion of green colonies.



FIG. AII.2 A CHROMagar *Vibrio* agar (CHROMagar-France) with mauve (*V. parahaemolyticus*), green-blue (light gray in print version) to turquoise-blue (dark gray in print version) (*V. vulnificus*/*V. cholerae*), and white (colorless) (*V. alginolyticus*) colonies. (Alberto Lerner, CHROMagar, <http://www.chromagar.com/>. Used with permission.)

highly toxic hydrogen sulfide is being generated, usually from an area of accumulated sludge. If this occurs, immediately raise DO, reduce feed ration, and—if it does not create another problem by raising un-ionized ammonia to unsafe levels—increase pH (Panakorn, 2016; Bob Rosenberry, personal communication). Maintain adequate mixing to avoid areas of sludge accumulation where anoxic conditions promote H_2S production.

II.B TCBS PLATE TESTING METHOD FOR VIBRIO

(Based on Doug Ernst, personal communication)

Equipment

- Sample bottles
- Spray bottle containing a liquid surface disinfectant (e.g., 90% ethanol)
- Hand disinfectant
- Deionized water
- Blender (hand-held or bench-top)
- Micropipette and tips
- Inoculating loop

- Bunsen burner or alcohol lamp
- TCBS agar plates (Thiosulfate citrate bile-salts sucrose agar) (or more specific equivalent such as CHROMagar)
- Incubation oven
- Black fine tip marker

Method

Disinfect all work surfaces and equipment in the fume hood or sterile cabinet. Apply a liquid surface disinfectant, such as 90% ethanol, with a spray bottle. Wash hands thoroughly with freshwater and an alcohol-based hand disinfectant.

1. Collect water samples (200 mL) in labeled sterilized bottles. Sterilize the bottles with liquid disinfectant, rinse with deionized water and allow to air dry (preferably in an oven at $>105^\circ\text{C}$) prior to collecting samples. Once samples are collected, rinse the outside of the sample bottles with freshwater and spray with surface disinfectant prior to placing on the workbench.
2. Label Petri dish covers with the sample ID and volume inoculated using a black fine tip marker.

3. Blend the sample for 20 s to release the *Vibrio* cells from solids, either in the sample bottle or a disinfected container. Disinfect the blender and container with liquid disinfectant and rinse thoroughly with deionized water between samples.
4. Apply water samples to the plates using a micropipette with disposable tips. Target 30–300 colonies per plate for ease of counting. Apply a volume of 100 μ L if the *Vibrio* count is expected to be <3000 cfu/mL. Apply 10 μ L if the *Vibrio* count is expected to be >3000 cfu/mL. Inoculate replicate plates for a few samples to check results.
5. Place the sample drop on the plate while keeping the lid mostly covering the plate. Only open the lid for as short a time as needed to complete inoculation. Let the drop flow down one direction on one side of the plate, keeping it at least 1 cm away from the inside edges. Heat the inoculating loop in the flame (until red hot) and let it cool in air or in the sterile agar before using. Gently distribute the sample on the agar surface with the inoculating loop by using multiple parallel strokes in a perpendicular direction, working first in one direction and then perpendicular to the original direction, and finally in a third direction, covering the entire plate surface. Keep the sample away from the edges of the Petri dish. Just touch the surface of the agar without digging in. Resterilize the inoculating loop after each use. Bacterial plating methods and diagrams are available on the internet.
6. Incubate the plates for 18–24 h at 30–32°C (Matching culture tank temperature).
7. Perform plate counts immediately after removal from the incubator as some colonies may change color at room temperature. Hold the plate upside down over or up to a light source. Count the colonies, using a black fine tip marker to mark the colonies on the underside of the plate as they are counted.
8. Report the number of yellow colonies, green colonies, and total colonies as cfu/mL and multiply the number by the dilution factor (10- μ L sample $\times$ 100, 100- μ L sample $\times$ 10). If the count is too high to reasonably count, divide the plate into equal segments (e.g., four quadrants), count the colonies on a portion of the segments (e.g., two quadrants), and multiply the result accordingly (e.g., by 2) for the total count. An example form and an [Excel Sheet # 19](#) for recording *Vibrio* colony numbers are available in Page # 415 named *Vibrio* & Alkalinity Form_Examples & Calc Sheet—Appendix VII. The whole *Vibrio* monitoring process is shown in [Video # 29](#)—Appendix VIII.

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- Di Pinto, A., Terio, V., Novello, L., Tantillo, G., 2011. Comparison between thiosulphate-citrate-bile salt sucrose (TCBS) agar and CHROMagar *Vibrio* for isolating *Vibrio parahaemolyticus*. *Food Control* 22 (1), 124–127.
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- Ray, A.J., Seaborn, G., Leffler, J.W., Wilde, S.B., Lawson, A., Browdy, C.L., 2010. Characterization of microbial communities in minimal-exchange, intensive aquaculture systems and the effects of suspended solids management. *Aquaculture* 310, 130–138.

III

Sample Fixation With Davidson's AFA Fixative, Storage, Labeling, and Transport

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The procedure for preparing Davidson's AFA Fixative Solution is as follows (based on [Lightner, 1996](#)):

To prepare 1 L of solution, mix the following together:

1. 330 mL of 95% ethyl alcohol
2. 220 mL of 100% formalin (Approx. 38% formaldehyde)
3. 115 mL of glacial acetic acid
4. 335 mL of distilled or tap water

If less solution is needed, divide the proportions of each component equally. Prepare the solution in a well-ventilated area located away from any source of heat or open flame. Avoid inhaling or touching the solution. Wear safety equipment, including safety glasses, gloves, a filter mask, and a laboratory coat when preparing and handling the solution. Store Davidson's solution at room temperature in a flammable liquids cabinet in a closed container to avoid evaporation. Store used solution in a chemical waste container and dispose at an approved waste disposal facility for incineration. Check local regulations for specific disposal requirements.

The procedure for fixation, storage, sample labeling, and transport is as follows (from [Lightner, 1996](#)):

Larvae and Early Postlarvae:

1. Immerse selected shrimp directly in the fixative.
2. Fix for 12–24 h and then transfer to 75%–90% ethyl alcohol for storage.

Larger Postlarvae, Juveniles, and Adults:

1. Inject fixative (0.1–10 mL depending on size) into the living shrimp with needle and syringe (needle gauge depends on shrimp size, i.e., 27 gauge needle for small juveniles).
2. The site of injection should be laterally in the hepatopancreas proper, in the region anterior to the hepatopancreas, in the anterior abdominal region, and in the posterior abdominal region ([Fig. AIII.1](#)).
3. The fixative should be divided between the different regions, with the cephalothoracic region, specifically the hepatopancreas, receiving a larger share than the abdominal region.

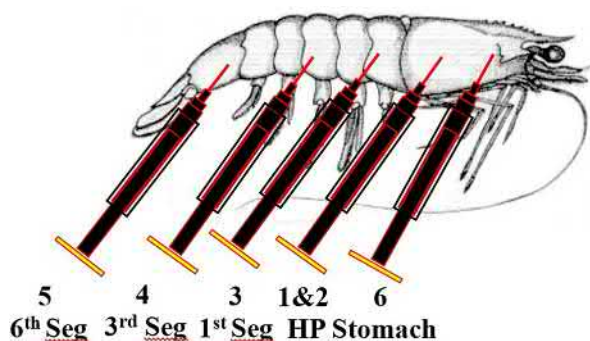


FIG. AIII.1 Injection points for fixation of whole shrimp.

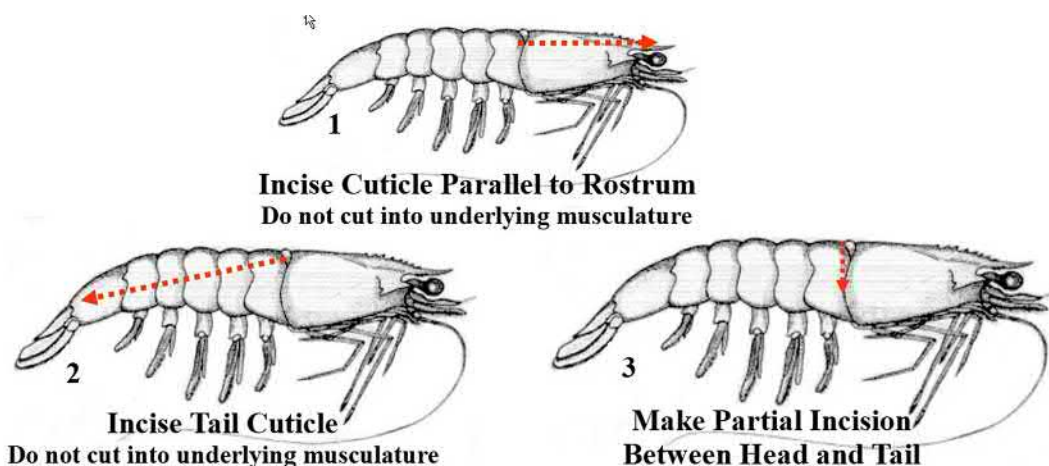


FIG. AIII.2 Incision locations for fixation of whole shrimp.

4. A good rule of thumb: Inject an equivalent of 5%–10% of the shrimp's body weight; all signs of life should cease and visible color change should occur in injected areas.
5. Immediately following injection, slit the cuticle with dissecting scissors from the sixth abdominal segment to the base of the rostrum, paying particular attention not to cut deeply into the underlying tissue. The incision in the cephalothoracic region should be just lateral to the dorsal midline, while that in the abdominal region should be approximately mid-lateral (Fig. AIII.2).
6. Shrimp larger than ~12g then should be transversely bisected at least once just posterior to the abdomen/cephalothorax junction, and (optional) again mid-abdominally.
7. Following injection, incisions, and bisection/trisection, immerse the specimen in the remainder of the fixative (one part tissue to ten parts fixative (by volume), for example, a shrimp of 10-mL volume would require 100 mL of fixative).
8. Allow shrimp to remain in the fixative at room temperature for 24–48h, depending on size (larger shrimp stay in longer).

9. Following proper fixation, transfer the specimens to 70% ethanol, where they can be stored for weeks.
10. Record a complete history of the specimen at the time of collection: gross observations on the condition of the shrimp, species, age, weight, source (pond, tank, culture tank, and identifying number), source of parent stock, fixation method, and any other pertinent information that may at a later time provide clues to the source and cause of the problem. Use a soft-lead pencil on paper (plastic paper such as tracing paper is recommended as it will not fall apart in the fixative).
2. Wrap with paper towels to completely cover.
3. Place towel-wrapped specimen in a sealable plastic bag and saturate with 70% ethyl alcohol. Sufficient ethyl alcohol should be used to keep the shrimp moist (~25–30 mL).
4. Label: Include the history, as recorded before, with the shipment. Use soft-lead pencil on paper (plastic paper such as tracing paper is recommended).
5. Place bag within a second sealable bag.
6. Multiple small sealable bags again can be placed within a large sealable bag.
7. Seal bags using duct tape.

Transportation or Shipment for Processing:

1. Remove the specimens from the 70% ethyl alcohol.

Reference

Lightner, D.V. (Ed.), 1996. *A Handbook of Pathology and Diagnostic Procedures for Diseases of Penaeid Shrimp*. World Aquaculture Society, Baton Rouge, LO.

IV

Water Quality Laboratory and Safety Procedures

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This appendix lists recommended equipment for a water quality laboratory at a large-scale intensive shrimp farm or research facility. Not all of this equipment will be necessary for smaller facilities and more affordable substitutes can be used. For example, a benchtop spectrophotometer may eliminate the need for TSS and alkalinity measuring equipment.

IV.A SUGGESTED WATER QUALITY LABORATORY EQUIPMENT

See [Table AIV.1](#).

IV.B BASIC LABORATORY SAFETY

Potential hazards to worker health in the water quality laboratory must be minimized. Worker safety can be maintained through:

1. Safe storage and labeling of chemicals

- a. The laboratory must have cabinets to safely store chemicals, that is, a cabinet for storing

flammable chemicals such as ethanol, acetone, and formalin and a cabinet for storing corrosive chemicals (such as acids).

- b. Some chemicals, such as bleach and muriatic acid, should not be stored near each other owing to potential reaction.
 - c. Store used hazardous chemicals (including chemical waste from water quality test kits) in clearly labeled, sealed inert containers away from high temperatures, and dispose in accordance with local laws and regulations.
 - d. Store used and unused chemicals in tubs or trays within their storage areas to limit any spread of minor spills.
 - e. Clearly label all chemicals and stored samples with the contents, date of arrival, and date of opening. Keep the original label with all relevant hazard information intact on the container.
- ### 2. Availability of protective equipment
- a. All workers must use protective equipment relevant to each potential hazard—laboratory coats, respirators, safety glasses,

TABLE AIV.1 Recommended Water Quality Laboratory Analyses, Equipment, and Supplies

	Equipment and Supplies	Purpose/s	Potential Substitutes
Infrastructure	Water analysis and evaluation room	General	
	Air conditioner	General	
	Cabinets	Storage	
	Fume hood ^a	Chemical handling safety	
	Sink with running freshwater	General	
	Work bench	General	
	Eyewash station	Chemical safety	
	Corrosive liquids cabinet	Chemical storage	
	Flammable liquids cabinet		
Equipment	Oven (150°C) ^a	TSS analysis	TSS probe, spectrophotometer, or photometer
	Muffle furnace ^a		
	Vacuum pump ^a		
	Filtration manifold ^a		
	Filtration funnels and fittings ^a		
	2-L Suction Erlenmeyer's ^a		
	Crucibles (ceramic or aluminum) ^a		
	Aluminum trays ^a		
	Metal tongs (large) ^a		
	Desiccators ^a		
	Desiccant ^a		
	Bunsen burner (alcohol or gas)	Bacterial monitoring	Send samples to external laboratory
	Inoculation (wire) loops		
	Blender		
	Incubation oven (20–45°C)		
	Spray bottle		
	250-mL glass sample bottles		
	125-mL Erlenmeyer flasks ^a	Alkalinity titration	Test kit, digital titrator, spectrophotometer, or
	25-mL burette ^a		
	Adjustable burette stand and clamp ^a		
	Stirring plate ^a		
	Magnetic stirring bars ^a		

TABLE AIV.1 Recommended Water Quality Laboratory Analyses, Equipment, and Supplies—cont'd

Equipment and Supplies	Purpose/s	Potential Substitutes
Imhoff cones	SS Determination	
Reverse osmosis unit ^a	Deionized water production	Bottled deionized water
Hemocytometer ^a	Cell counts	
Refrigerator	General	
Freezer	General	
Computer	General	
Micropipettes (electronic or manual) (100–1000 µL; 0.5–10 mL)	General	
Electronic balance, 1-mg readability	General	
Electronic balance, 0.1-g readability	General	
Dissecting microscope	General	
Compound microscope ^a	General	
Calculator	General	
Tally (handheld counter)	General	
Timer	General	
Table lamp	General	
Alcohol thermometers (–20 to 110°C)	General	
Metal forceps	General	
Plastic forceps	General	
Dissecting scissors	General	
Drying rack	General	
Paper towel dispenser	General	
Deionized water bottles (500 mL)	General	
Beakers (50, 100, 500, 1000 mL)	General	
Funnels	General	
Graduated cylinders (100 mL, 1 L)	General	
Test tubes (10 mL)	General	
Miscellaneous glass and labware	General	
Broken glassware and sharps disposal container	Lab waste	
Chemical waste disposal containers	Lab waste	
Rubbish bins	Lab waste	

Continued

TABLE AIV.1 Recommended Water Quality Laboratory Analyses, Equipment, and Supplies—cont'd

	Equipment and Supplies	Purpose/s	Potential Substitutes
Consumables	GF/A Glass-fiber filter disks ^a	TSS analysis	
	TCBS/RambaCHROM agar plates	<i>Vibrio</i> monitoring	
	27G Needles	Hemolymph sampling	
	Micropipette tips (1000 µL)	General	
	Micropipette tips (5 mL)	General	
	Micropipette tips (10 mL)	General	
	1-mL syringes	General	
	5-mL syringes	General	
	50-mL syringes	General	
	Filter paper	General	
	250-mL plastic sample bottles	General	
	Detergent	General	
	Hand sanitizer	General	
	Delicate task wipes	General	
	Paper towels	General	
	Brushes	General	
	Fine tip black markers	General	
	Pencils	General	
Chemicals and reagents	Bromocresol Green or Bromocresol green—Methyl red indicator powder pillows ^a	Alkalinity titration	Test kit, digital titrator, spectrophotometer, or photometer
	Methyl Red ^a		
	Phenolphthalein or phenolphthalein indicator powder pillows ^a		
	Sulfuric acid (concentrated) ^a		
	Tris (Hydroxymethyl) Aminomethane (THAM) ^a		
	Reagents for measuring nitrogenous and other compounds (using a flow-injection analyzer, spectrophotometer, or photometer)	Measuring TAN, NO ₂ , NO ₃ , PO ₄ etc.	Compound-specific test kits
	Ethyl alcohol (Ethanol)	Disinfection and sample preservation	
	Formalin solution	Sample preservation	
	Glacial acetic acid	Sample preservation	

TABLE AIV.1 Recommended Water Quality Laboratory Analyses, Equipment, and Supplies—cont'd

	Equipment and Supplies	Purpose/s	Potential Substitutes
Water quality testers	Sodium hypochlorite	Disinfection	
	Sodium thiosulfate	Neutralizing chlorine	
	Multiparameter probes (DO, pH, salinity, temperature)	Measuring DO, pH, salinity, and temperature	
	Refractometers	Measuring salinity	
	Spectrophotometer	Measuring dissolved compounds	Photometer, compound-specific test kits
	Turbidimeter ^a	Measuring turbidity	Spectrophotometer or photometer
Safety equipment	TSS Probe ^a	Measuring TSS	Gravimetric method, spectrophotometer, or photometer
	Laboratory coats	Personal safety equipment	
	Safety glasses		
	Respirators and filters		
	Dust masks		
	Autoclave gloves ^a		
	Chemical gloves		
	Disposable nitrile examination gloves		
	MSDS File	Chemical information	
	First Aid Kit	First Aid	
Other	Program or application for water quality calculations ^a	Water quality calculations	Manual calculations

^aOptional, depending on the scale of the facility.

chemical-resistant boots and gloves. Keep this equipment clean and in good order. Each worker should have their own set and be aware of what protective equipment is required for each hazard.

- b.** A functioning eyewash station and (preferably) shower should also be available.

- 3.** Access to and understanding chemical information

- a.** Maintain an up-to-date MSDS (Material Safety Data Sheet) file in an accessible location within the laboratory. This file should contain MSDS for each chemical in the lab in alphabetical order.

- b.** All workers should know how to interpret an MSDS and the DOT labels (or equivalent) on all chemicals in use.
 - c.** Display warning signs and charts concerning hazards in the laboratory in prominent locations near the hazards in question.
- 4.** An up-to-date first-aid kit
 - a.** An up-to-date first aid kit must be accessible in the laboratory.
- 5.** A clean and tidy laboratory (good housekeeping practices)
 - a.** Maintain proper storage for all equipment and chemicals. Return every item to its storage location after use.
 - b.** Implement an equipment cleaning procedure. For example, clean used glassware with a laboratory detergent (such as Alconox), rinse several times in tap water, then rinse several times in deionized water and dry on a dedicated drying rack.
 - c.** Clean up spills immediately, following relevant safety procedures and notifying other workers of the spill hazard.
- 6.** Regular training in safe laboratory practices
 - a.** Train all workers in laboratory safety procedures, chemical use, interpreting MSDS and labels, and evacuation procedures when they begin employment and regularly, with updates, thereafter.
 - b.** One staff member should be responsible for laboratory health and safety, ensuring that all procedures are followed, updating procedures as required, and maintaining safety equipment, MSDS file, and the first aid kit.
 - c.** All staff is responsible for reporting potential hazards to the health and safety officer or manager.

Staff should be trained in basic first aid by a certified instructor.

V

The Water-Quality Map

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V.A WATER QUALITY IS WATER CHEMISTRY

Successful aquaculture depends on successful water-quality management, especially at very high biomass (at which dramatic changes can happen very quickly) and in closed systems (in which the manager does not have the “luxury” of flushing water-quality problems to the environment).

Aquaculturists have a long list of water-quality concerns: temperature, salinity, pH, Total Alkalinity (TA), dissolved oxygen (DO), un-ionized ammonia (UIA or NH_3), carbon dioxide (CO_2), nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), total suspended solids (TSS), the saturation states of calcite (Ω_{ca}) and aragonite (Ω_{ar}), dissolved nitrogen gas (N_2), various heavy metals, and disease vectors.

The management challenge is particularly daunting because many of these variables are interrelated in complex ways. As a result, adjusting one to a safe level unintentionally may change others in a harmful way. Wurts and Durborow (1992) accurately summarized the situation facing water-quality managers:

Many of the principles of chemistry are abstract (e.g., carbonate-bicarbonate buffering) and difficult to grasp. However, a fundamental understanding of the concepts and chemistry

underlying the interactions of pH, CO_2 , alkalinity...is necessary for effective and profitable pond management. There is no way to avoid it: Water quality is water chemistry.

And water chemistry is a very technical subject.

The WQ Map (Water-quality Map) is an application that helps managers quickly and accurately perform routine and exceptional water-quality tasks by means of a visually informative interface that hides all chemical and mathematical details.

The WQ Map evolved from the *pH & Alkalinity* module of aquaCalc (Staresinic, 1998), a set of software tools that solves a variety of aquaculture design and operations problems. The WQ Map is a thorough upgrade that uses the latest formulae from marine chemistry with a greatly enhanced graphical interface. Currently proprietary, it has been used successfully in the Samocha biofloc system described in this manual. This appendix introduces it in nontechnical terms to

- demonstrate how to solve a water-quality problem that arises regularly in RAS
- illustrate two widely held misconceptions about water quality that are more easily grasped graphically than through tables of numbers or equations

V.B THE WQ MAP: LIKE GOOGLE MAPS FOR WATER QUALITY

Travelers who know where they are and where they want to go, but who do not know how to get from one place to the other, rely on *Google Maps* for quick and accurate directions. The WQ Map provides a similar service for the water-quality “traveler”—the aquaculture manager. Similar to a conventional traveler, the manager knows the system’s current and target water quality, but perhaps not how to change from one to the other.

Briefly, the manager enters water temperature and salinity to define the system’s water quality as a map. Then, as in *Google Maps*, the starting and destination points are set. These are not geographical locations, of course, but are entered as pH and alkalinity. The WQ Map then uses this information to calculate the amount of chemical reagents that must be added

to reach the desired water-quality conditions. It also illustrates the path to the target, along with regions of dangerously high un-ionized ammonia and CO_2 .

V.C THE WQ MAP: A QUICK TOUR

The WQ Map is based on a graphical approach to analyzing carbonate equilibria introduced by [Deffeyes \(1965\)](#). [Fig. AV.1](#) shows the WQ Map set for 28°C , 34.5 ppt, and typical atmospheric pressure at sea level. Total Alkalinity is on the y-axis. Dissolved Inorganic Carbon (DIC), the sum of dissolved carbon dioxide (CO_2), bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}), is on the x-axis. pH is projected onto the WQ Map as a family of straight lines, each representing a single pH value. The pH lines (also called pH isopleths) have the following features:

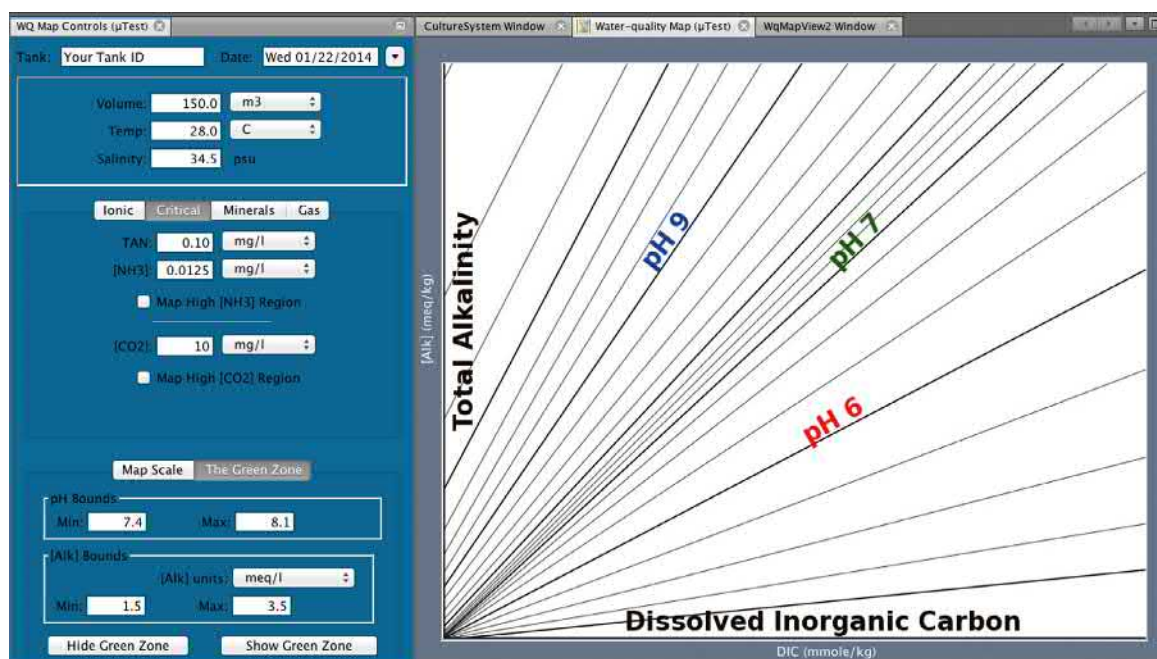


FIG. AV.1 Layout of the Basic WQ Map.

- they radiate outward from near the origin
- they are more widely spaced farther from the origin
- lower pH is in the lower right of the map
- higher pH is in the upper left of the map
- lines are not equally spaced, for example, pH 7–8 lines are closer than pH 6–7 lines
- line position varies with temperature, salinity, and pressure

Lines of equal pH may be interpreted in the way that lines of equal elevation are read on a topographic map: Where they are closer together, pH changes more rapidly; where they are farther apart, pH changes more slowly. The farther apart they are, the better the pH buffering.

V.D ASSUMPTIONS

Several assumptions are required to use the WQ Map to solve water-quality problems. The most important is that the culture water is effectively closed to atmospheric exchange of CO₂. This does not mean that it is completely isolated from the atmosphere; instead, it means that dissolved CO₂ is not in equilibrium with the atmosphere.

This assumption holds very well for many systems, including high-density biofloc systems, because the dissociation reactions that determine pH and alkalinity proceed much faster than the exchange of CO₂ across the air-water interface. One indication of this is that pH in such systems typically is much lower than it would be if the culture water were equilibrated with the atmosphere. In that case, pH would be about 8.1.

Other noteworthy assumptions are that the system is homogeneous (it contains only the liquid phase) and is at chemical equilibrium. Aquaculture systems are neither: They are

heterogeneous (in addition to the liquid phase, they contain solids and—of course—organisms) and are dynamic (owing mainly to changing biological processes, such as respiration and nitrification).

For measurements made between short time intervals—generally on the order of hours for high-density biofloc systems—these two assumptions are sufficiently satisfied that the WQ Map provides very useful guidance in managing aquaculture water quality.

An additional consideration arises in systems with very high concentrations of organic bases, such as may be expected in long-running closed systems. If these bases account for a large fraction of Total Alkalinity, then CO₂ calculations will be compromised. This is a topic of current research in natural water chemistry ([Hunt et al., 2011](#); [Abril et al., 2015](#); [Ulfsbo et al., 2015](#)) but, as far as the author is aware, is yet to be studied thoroughly in aquaculture. The WQ Map nevertheless has proven useful in managing water quality in the Samocha biofloc system, so any modifications to this approach must await data on the contribution of humic and fulvic acids to Total Alkalinity in long-running reuse systems.

Readers interested in greater technical details are referred to several recognized works on water chemistry that were consulted in developing the WQ Map ([Butler, 1982](#); [Morel and Hering, 1993](#); [Stumm and Morgan, 1996](#); [Zeebe and Wolf-Gladrow, 2005](#)). Highly informative, very well-written, and accessible articles by [Weaver \(2016a, b\)](#) and [Holmes-Farley \(2002a,b\)](#) are also well worth studying.

V.E AN EXAMPLE

Working through a problem that arises regularly in closed systems—adjusting pH and alkalinity—is a good way to become familiar with the WQ Map.

PROBLEM

A 40 m³ grow-out tank has a temperature of 28°C and a salinity of 30 ppt. pH is 6.8 and Total Alkalinity is 1.5 meq/kg (75 ppm CaCO₃). Both pH and alkalinity are lower than desired.

Sodium bicarbonate (NaHCO₃, baking soda) and sodium hydroxide (NaOH, caustic soda) are available adjustment reagents.

How much of each must be added to raise pH to 7.3 and alkalinity to 2.0 meq/kg (100 ppm)?

To solve this common problem, the manager first enters temperature (28°C), salinity (30 ppt), and tank volume (40 m³) in the Input Data panel (Fig. AV.2, left). This sets the family of pH lines that represents the water-quality “topography” of the culture system.

Starting and ending water-quality points are plotted next. This usually would mean entering values for DIC (*x*-axis) and Total Alkalinity (*y*-axis). Total Alkalinity is measured by titration in the normal course of a production run, but it is impractical to measure DIC in aquaculture because the analytical equipment is very expensive and sample preparation is complicated. Instead, Total Alkalinity is paired with an easily measured pH value, and these are entered in the *Starting WQ* and *Ending WQ* panels of the user interface (Fig. AV.2, center). Tapping the *Get WQ Map Directions* button (Fig. AV.2, lower right) plots these points on the map.

Fig. AV.3 shows the initial waypoint (Black & Gold) at pH 6.8 and TA 1.5 meq/kg and the target waypoint (blue “A”) at pH 7.3 and TA 2.0 meq/kg.

The broken blue line that extends from the initial point toward the upper right (Fig. AV.3) traces water-quality points that can be reached by adding sodium bicarbonate. It is displayed when the NaHCO₃ box is checked in the Adjustment Options menu (Fig. AV.4).

Because this bicarbonate vector (the blue line) does not pass through the target point, bicarbonate additions alone cannot produce the desired adjustment. This reflects the general situation: Two reagents usually are required for proper water-quality adjustment.

Selecting NaOH in the Adjustment Options menu adds the sodium hydroxide vector to the map and fills the adjustment zone (Fig. AV.5, light yellow region) between it and the bicarbonate vector. The adjustment zone contains all water-quality points that can be reached by adding some combination of sodium bicarbonate and sodium hydroxide under the specified temperature and salinity. The desired water quality—here, pH 7.3 & TA 2.0 meq/kg—is within that zone, so some combination of these two reagents will change the initial conditions to those of the target.

The WQ Map calculates the needed amounts of these reagents when *Get WQ Map Directions* again is clicked. Results are displayed in the lower right panel of the user interface when the *Results* tab is open (Fig AV.6)

The screenshot shows the 'Water-quality Trek Data' interface with three main sections:

- Input Data:**
 - Volume: 40.0 m³
 - Temp: 28.0 °C
 - Salinity: 30.0 psu
 - Altitude: 0.0 m
 - Buttons: Basic, Critical, Minerals, Gas
- Water-quality Trek Data:**
 - Tank Code: RW-IV
 - Date: Wed 07/15/2015
 - Starting WQ:** pH: 6.8 (NBS scale), [Alk]: 1.5 meq/l
 - Ending WQ:** pH: 7.3 (NBS scale), [Alk]: 2.0 meq/l
 - O₂ sat: 3.0
- Adjustment Options:**
 - Output Units: kg
 - Checked: ☒ NaHCO₃
 - Unchecked: ☐ NaOH, ☐ Na₂CO₃, ☐ CaCO₃, ☐ Ca(OH)₂, ☐ CaO
 - Buttons: Remove CO₂, Add CO₂, Add HCl
 - Radio buttons: ☐ L&D, ☐ Calc. DIC, ☐ Calc. DIC & TA
 - Button: Get WQ Map Directions

FIG. AV.2 The WQ Map’s data input panels for the example problem in the text.

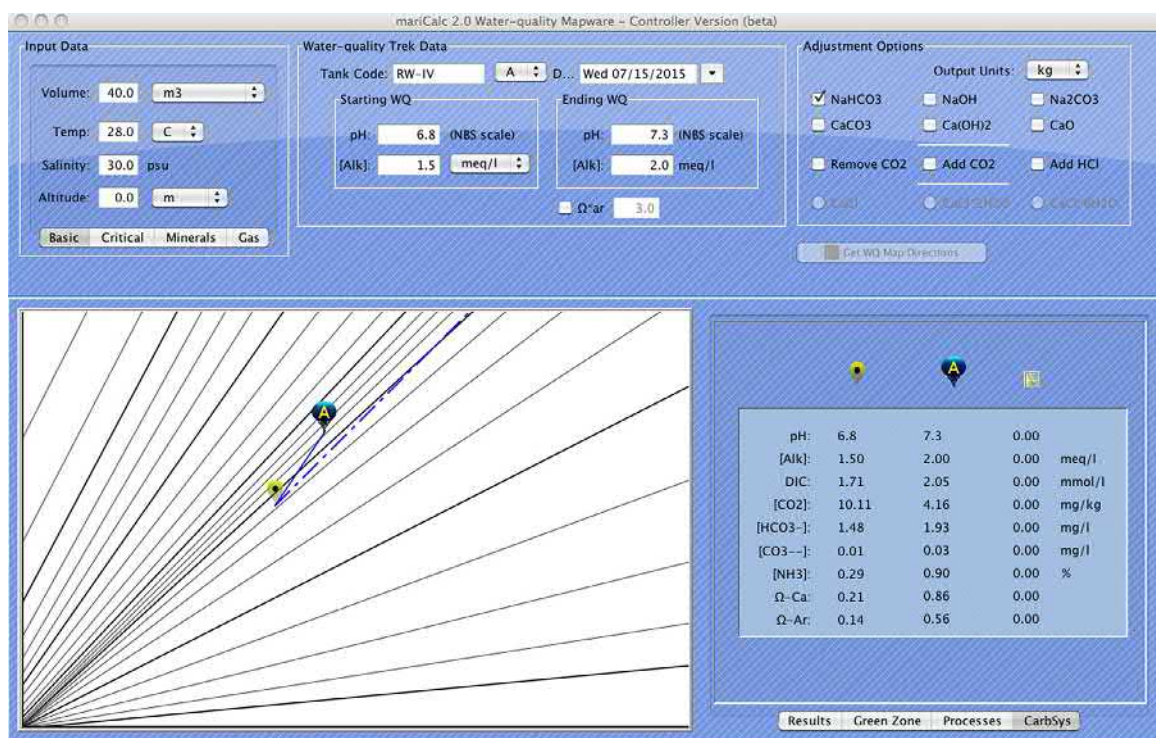


FIG. AV.3 The WQ Map for the example problem with initial and target points plus the bicarbonate vector.

Adjustment Options

Output Units: kg

☒ NaHCO₃ ☐ NaOH ☐ Na₂CO₃
☐ CaCO₃ ☐ Ca(OH)₂ ☐ CaO
☐ Remove CO₂ ☐ Add CO₂ ☐ Add HCl
☐ CaO ☐ CaCl₂·2H₂O ☐ CaCl₂·6H₂O

Get WQ Map Directions

FIG. AV.4 Adjustment Options menu with sodium bicarbonate selected.

There are other ways to make this adjustment. For example, if sodium carbonate (Na₂CO₃, soda ash) is substituted for sodium hydroxide, then the map and adjustment results will be as displayed in Fig. AV.7.

The steeper slope of the sodium carbonate vector—it is twice that of the sodium bicarbonate vector—is owed to that compound's different chemical properties, the details of which are not discussed here.

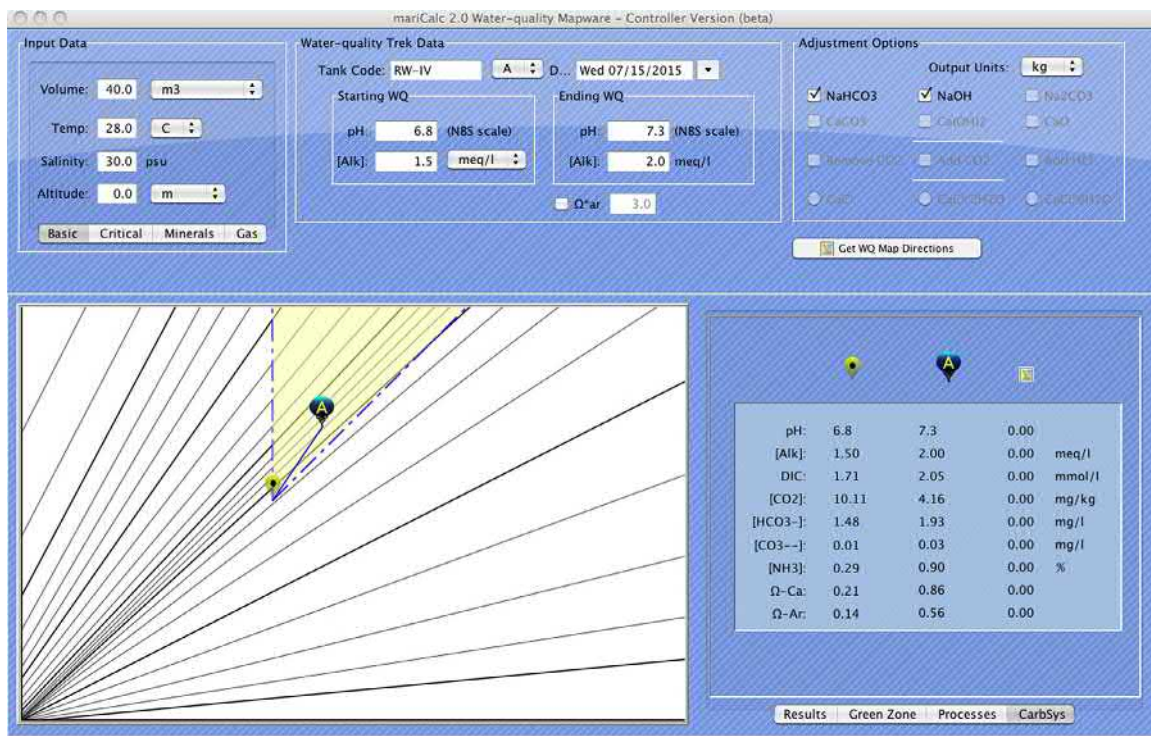


FIG. AV.5 Water-quality points in the yellow adjustment zone can be reached by adding Na-bicarbonate and Na-hydroxide.

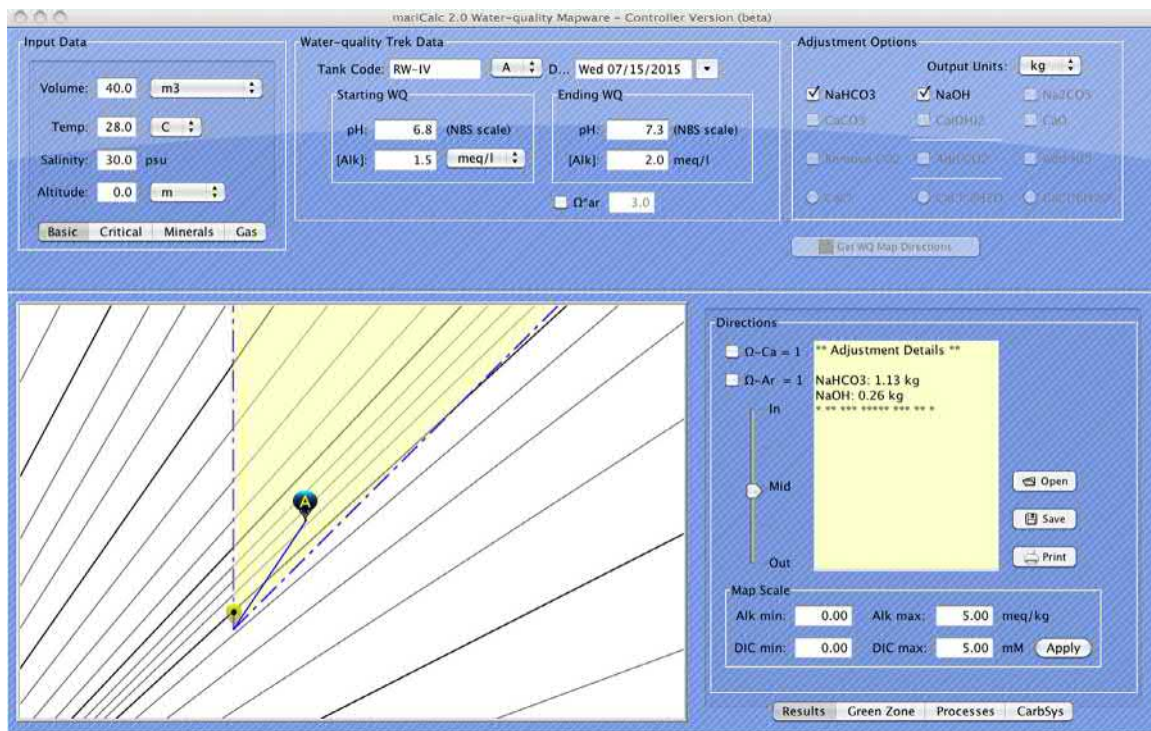


FIG. AV.6 Adding 1.13kg of Na-bicarbonate and 0.26kg of Na-hydroxide solves the example problem.

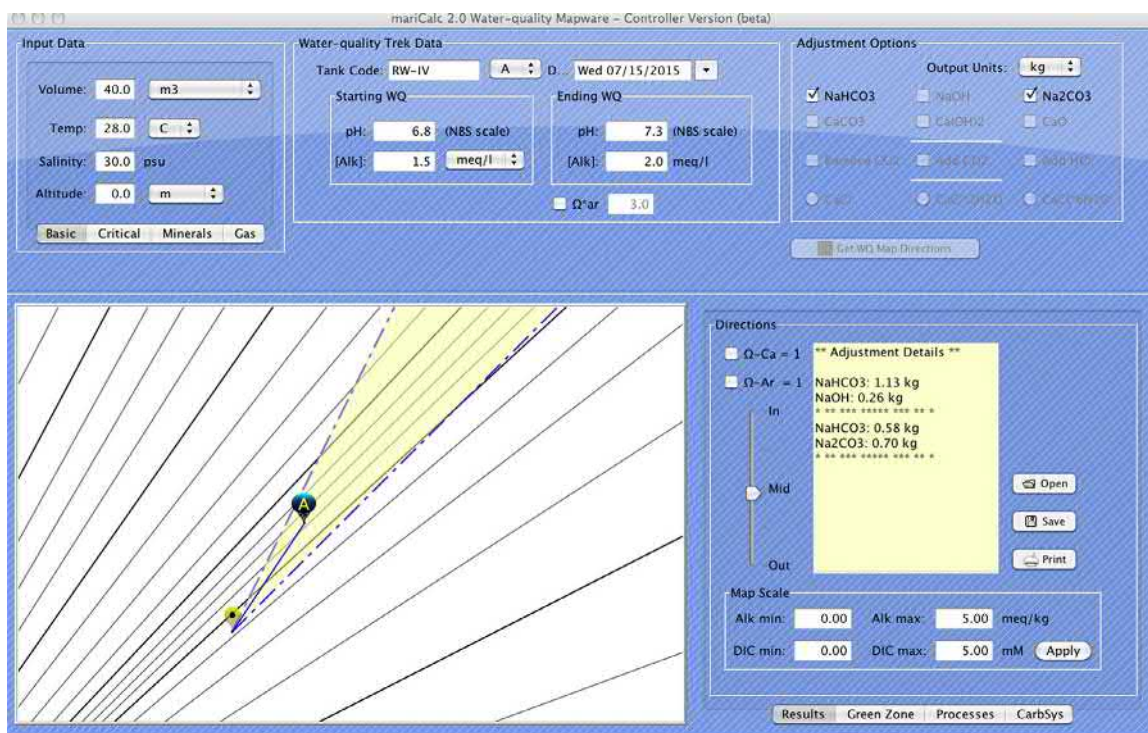


FIG. AV.7 Adding 0.58kg of Na-bicarbonate and 0.70kg of Na-carbonate also solves the example problem.

Not every set of adjustment reagents can reach the target. Fig. AV.8 shows that the adjustment zone for sodium carbonate and sodium hydroxide does not include the target point of the example problem. No amount of these reagents will solve the example problem.

V.F DECORATING THE WQ MAP

Other features may be displayed on the map to enhance its value to aquaculture water-quality management. These include the region that contains a species' safe water quality—its Green Zone—and danger zones of undesirably high concentrations of carbon dioxide and un-ionized ammonia. These three areas are illustrated in Fig. AV.9 for the example problem.

The Green Zone is set with controls in the lower right panel. The default Green Zone is defined by pH and alkalinity. This can be refined to exclude undesirable levels of CO_2 , un-ionized ammonia, and mineral saturation. The Green Zone in Fig. AV.9 is bounded by pH 7.0, pH 8.0, TA 0.85 meq/kg (42.5 ppm CaCO_3), and TA 3.0 meq/kg (150 ppm CaCO_3).

CO_2 and un-ionized ammonia danger zones are defined in the Input Data panel (upper left of the user interface) under the Critical tab (Fig. AV.10).

In this case, critical CO_2 —the maximum concentration tolerated by the cultured species—is set at 20 mg/L. Once entered, the map displays the reddish-orange region in which CO_2 is unacceptably high (Fig. AV.9).

The critical level of un-ionized ammonia-nitrogen is set at 0.0125 mg/L (12.5 $\mu\text{g/L}$).

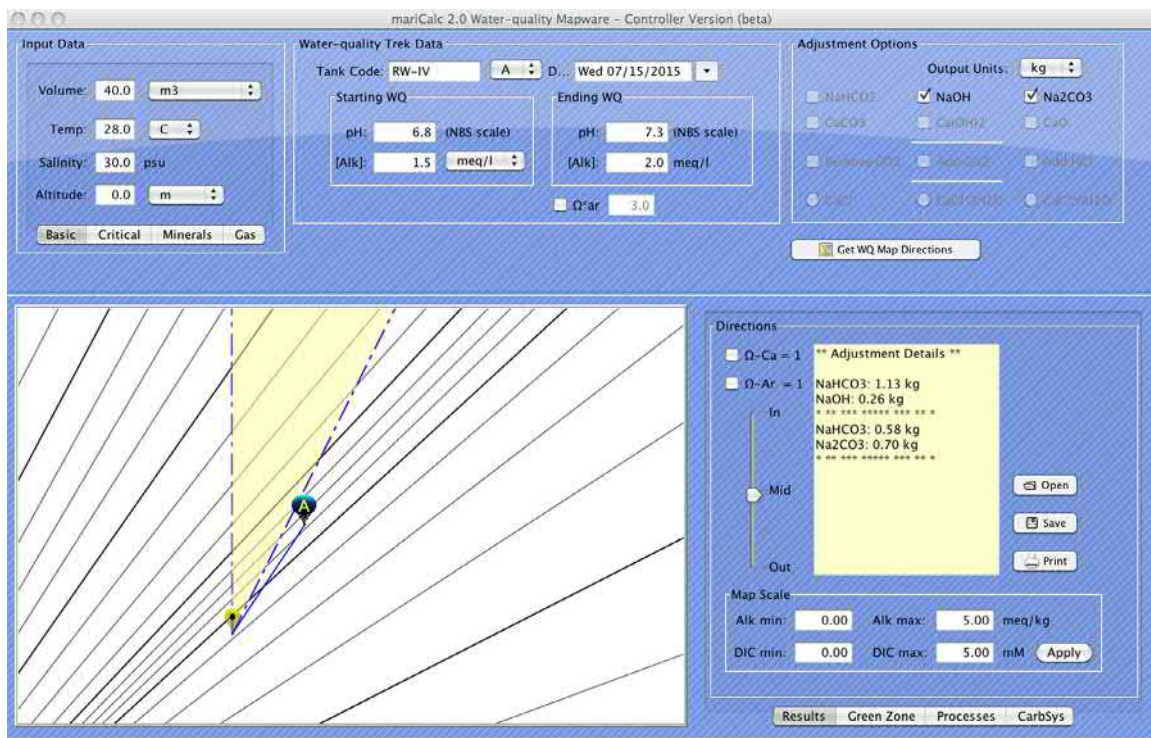


FIG. AV.8 No amount of Na-carbonate and Na-hydroxide can reach the target of the example.

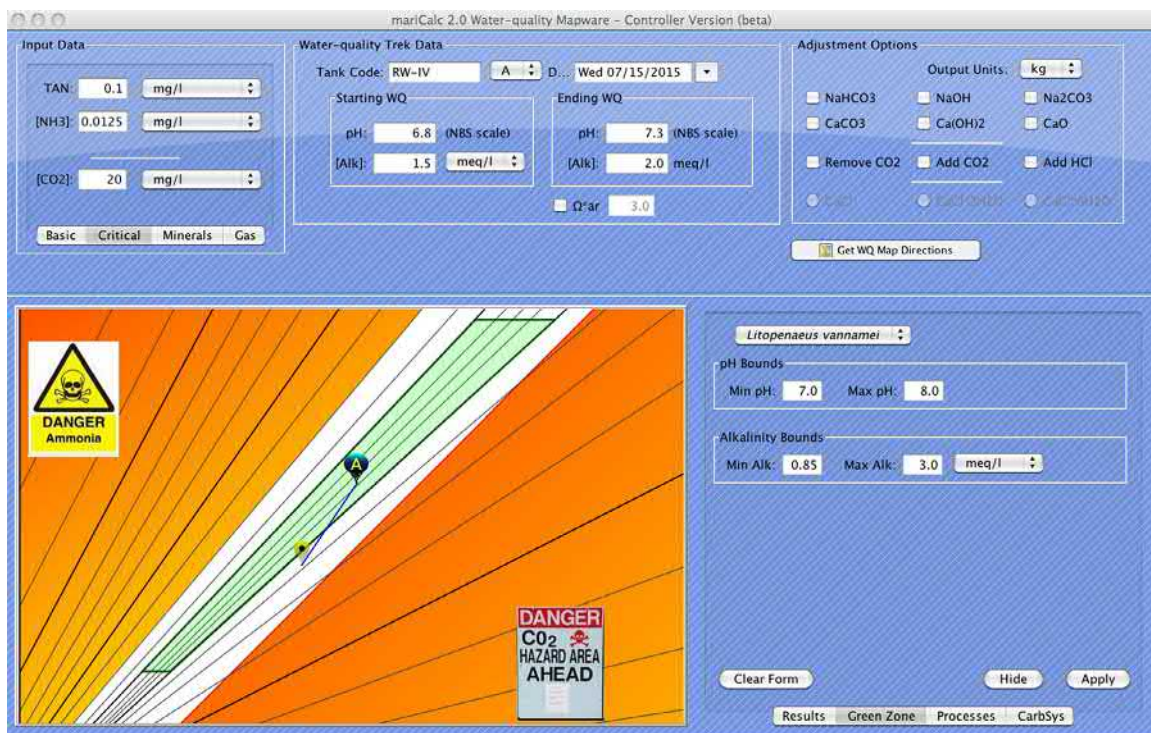


FIG. AV.9 WQ Map decorated with the Green Zone (safe area) plus UIA & CO₂ danger zones.

FIG. AV.10 Setting critical values of un-ionized ammonia and dissolved carbon dioxide.

Calculating the UIA danger zone also requires entering TAN (Total Ammonia Nitrogen), which is 0.1 mg/L in this example. The ammonia danger zone then is displayed as the reddish-orange area in the upper left (Fig. AV.9).

With these important features mapped, the manager's 'game' can be described as making water-quality adjustments that keep pH and alkalinity within the Green Zone and out of the danger zones. The WQ Map helps the aquaculturist win this game.

Among other available map decorations, the Ω ("omega") zones where calcite and aragonite are super-saturated may be plotted. These forms of calcium carbonate are important components of the shells of many marine species, including shrimp. An example is not developed here, but the controls for this feature are found under the Minerals tab of the Input Data panel (Fig. AV.8).

V.G PREDICTING WATER QUALITY

The WQ Map can predict future water quality by computing the net effect of biological, chemical, and other processes that take place over the

production cycle. Some of these processes are as follows:

- respiration, which adds CO_2
- excretion, which adds ammonium
- nitrification, which removes ammonium and produces nitrate
- denitrification, which removes nitrate
- water exchange, which changes pH and un-ionized ammonia

This feature is illustrated by predicting the water quality 6 1/2h after feeding 120kg of shrimp 1.5% of their body weight/d. Data are entered under the Respiration tab of the Processes dialog. The result is plotted on the WQ Map as a black circle and displayed in the dialog (Fig. AV.11).

In this case, the prediction is that pH will drop from 7.3 to about 7.0 and CO_2 will more than double from 4.2 to 8.7 mg/L. Overall, the system's water quality is predicted to end near the border of the Green Zone (Fig. AV.11). This is valuable information for managers who can use it to anticipate future water-quality conditions under different operational scenarios.

CO_2 added from respiration increases DIC and does not change alkalinity, so it maps as

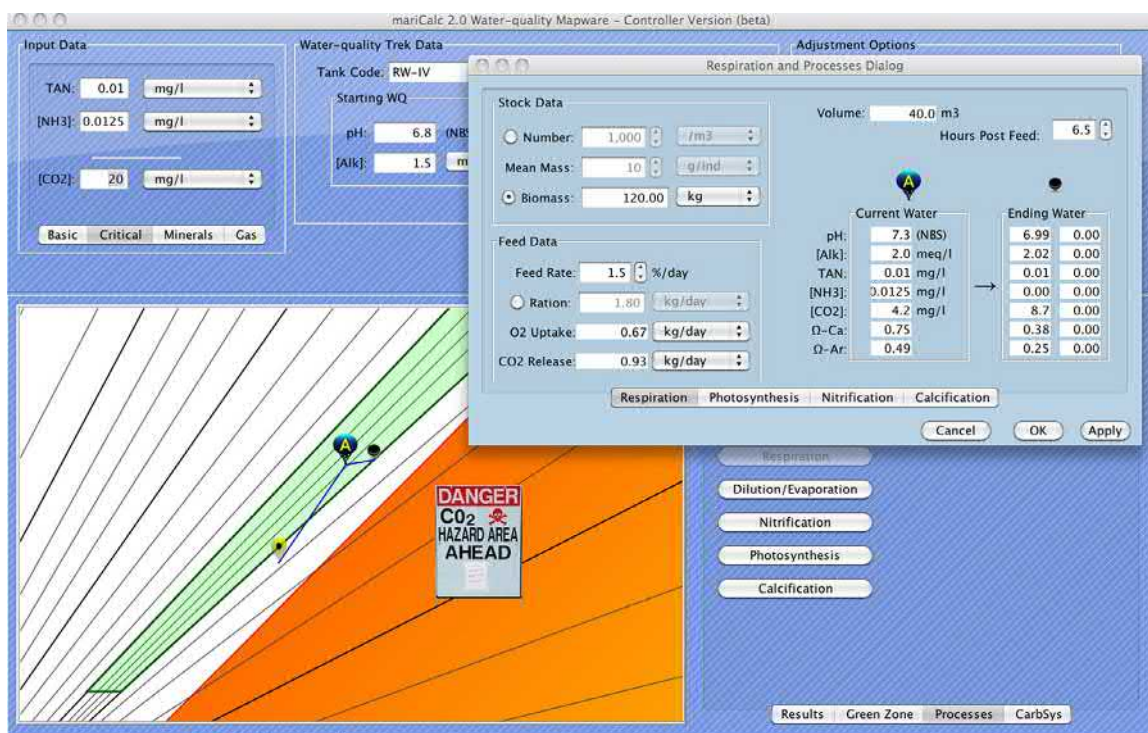


FIG. AV.11 Predicted water quality 6 1/2h after feeding 120kg of shrimp at 1.5%/day (black circle).

a horizontal vector directed to the right toward higher DIC and lower pH. The observant reader might point out that the line connecting point “A” and the predicted water quality (black circle) is not, however, horizontal; instead, it is directed slightly upwards. This is because ammonium, the main form of nitrogen excreted by shrimp, increases Total Alkalinity. It thus adds a component directed vertically upward toward higher y-axis values. The net result is the prediction vector mapped in Fig. AV.11.

The WQ Map can combine any of the processes listed above to predict their net effect on water quality. The bottom line is that this feature allows managers to run very useful *what-if* scenarios that help plan for near-term changes in aquaculture water quality.

V.H COMMON MISCONCEPTIONS ABOUT BICARBONATE AND CO₂

The Effect of Bicarbonate on pH

Adding bicarbonate *always* increases alkalinity. The trajectories of the bicarbonate vectors mapped in Figs. AV.3–AV.7 make this clear: The bicarbonate vector always rises to higher values of alkalinity than the alkalinity at which it started.

Many managers are convinced that bicarbonate always increases pH, too; but it does not. Adding bicarbonate can increase, decrease, or even have essentially no effect on pH.

Bicarbonate’s effect on pH depends on the starting pH and alkalinity (as well as on temperature, salinity, and pressure). This fact is complicated to demonstrate using the highly

interrelated chemical reactions that compose the carbonate system, but it is very easily visualized with the WQ Map. Figs. AV.12–AV.14 illustrate the effects of bicarbonate additions from three different starting points.

Fig. AV.12 illustrates a case in which adding bicarbonate *increases* pH. With initial pH 6.0 and initial alkalinity 1.0 meq/kg, the bicarbonate vector crosses lines of ever-higher pH. For example, adding 7.38 kg of bicarbonate raises pH 0.5 units from 6.0 to 6.5. Alkalinity increases from 1.0 to about 3.2 meq/kg (160 ppm CaCO_3).

Fig. AV.13 illustrates a case in which adding bicarbonate *decreases* pH. With initial pH 8.75 and initial alkalinity again 1.0 meq/kg, the bicarbonate vector crosses lines of ever-lower pH. Adding 6.72 kg of bicarbonate lowers pH 0.5 units from 8.75 to 8.25. This is accompanied

by an increase in alkalinity from 1.0 to about 3.0 meq/kg (150 ppm CaCO_3).

Finally, Fig. AV.14 illustrates a case in which adding bicarbonate has essentially no effect on pH. With initial pH 7.50 and initial alkalinity yet again 1.0 meq/kg, the bicarbonate vector is roughly parallel to the pH 7.50 line. As such, unlike the two previous cases, adding bicarbonate has almost no effect on pH. Adding as much as 12 kg of bicarbonate raises pH from 7.50 to about 7.53, a change of only 0.03 pH units that will not be measured confidently in most aquaculture labs. This very small pH change is accompanied by an increase in alkalinity to almost 4.5 meq/kg (225 ppm CaCO_3).

This is not just a theoretically interesting artifact of carbonate-system chemistry applied to an aquaculture problem. Aquaculture often is conducted between pH 7.0 and 7.5, a range within

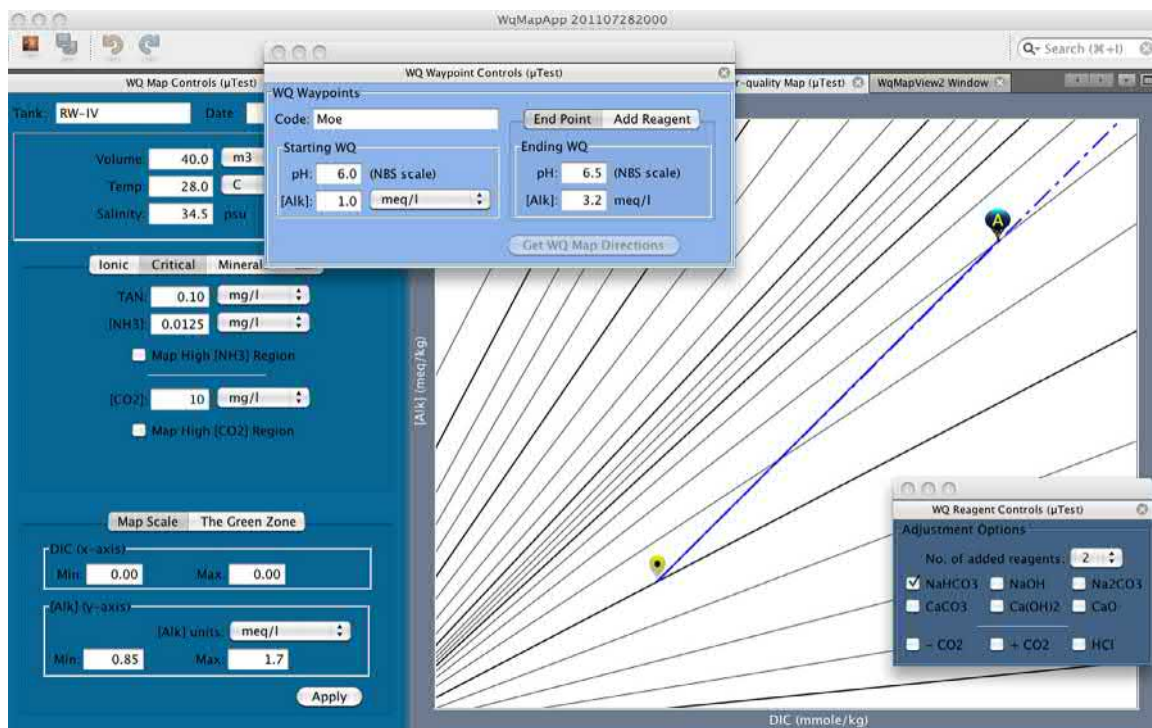


FIG. AV.12 A case in which adding NaHCO_3 increases pH.

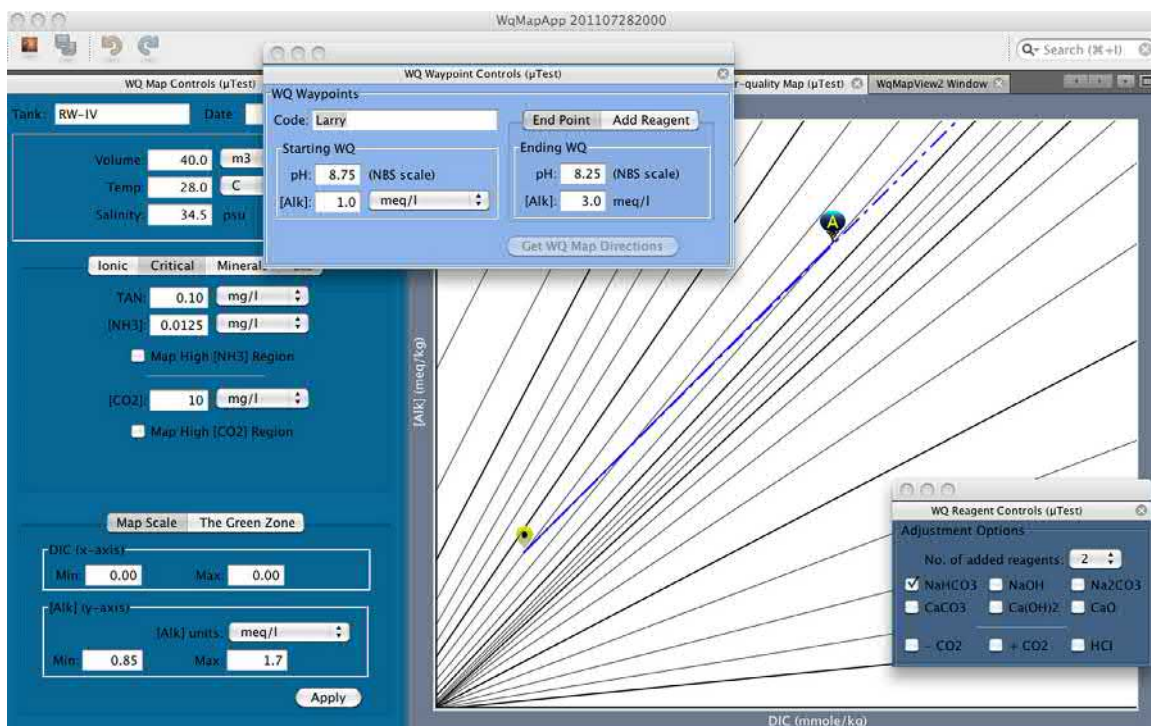


FIG. AV.13 A case in which adding NaHCO₃ decreases pH.

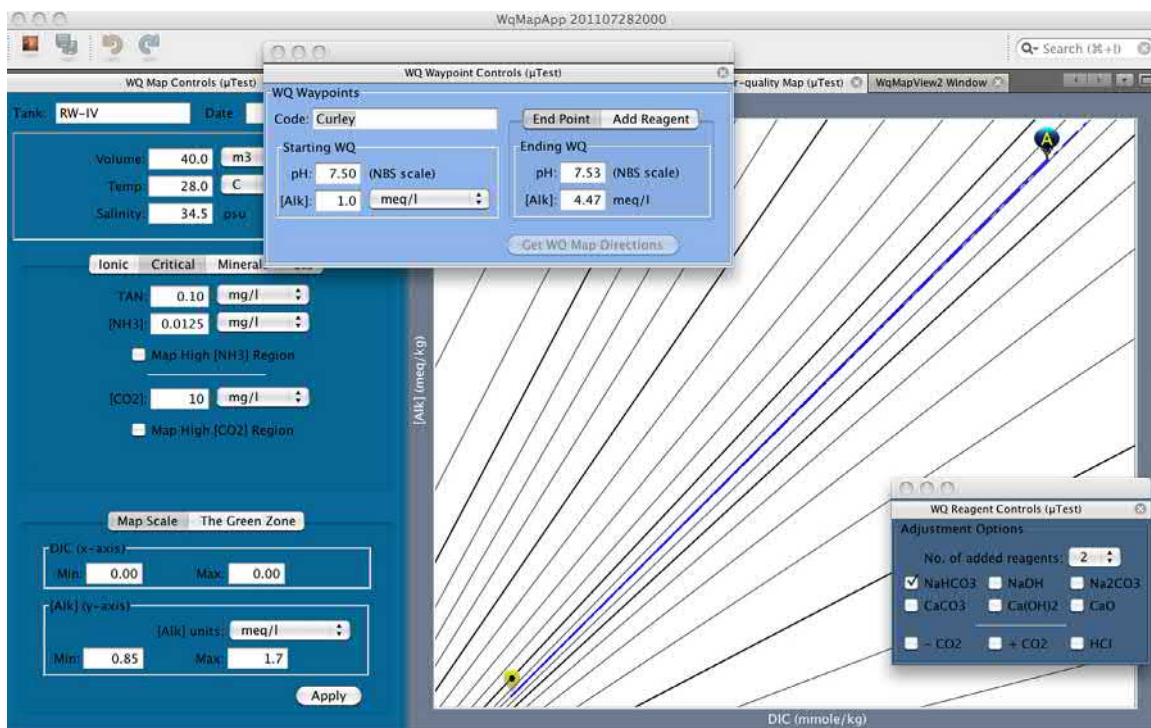


FIG. AV.14 A case in which adding NaHCO₃ does not change pH.

which bicarbonate affords very limited pH control. The author is familiar with situations in which managers have added truly massive amounts of bicarbonate with the intention of raising pH, but found that pH did not change. [Figs. AV.12–AV.14](#) provide a visual explanation of why this is so.

The main takeaway: Bicarbonate always increases alkalinity but, depending upon initial water quality, it will increase pH, decrease pH, or not significantly change pH at all.

The Effect of CO₂ on Alkalinity

Another common misconception is that a change in carbon dioxide causes a change in alkalinity. Adding or removing CO₂, however, has no effect on alkalinity. This is because CO₂ carries no charge. Adding or removing it does not upset the charge balance and so does not affect alkalinity.

This generally is an unsatisfying explanation for those who point out that CO₂ reacts with water to form carbonic acid and then quickly dissociates to negatively charged HCO₃⁻ and CO₃⁻². That certainly is true but, without offering a technical explanation, the carbonate system is constructed in a way that this does not result in a net change in charge.

Thorough explanations are available for those with a background in chemistry (e.g., [Butler, 1982](#); [Zeebe and Wolf-Gladrow, 2005](#); [Wolf-Gladrow et al., 2007](#)). [Wolf-Gladrow et al. \(2007\)](#) provide an excellent discussion of their explicitly conservative formulation of Total Alkalinity. This very clever approach simplifies quantifying the effect on alkalinity of various water-quality components, including carbon dioxide.

The WQ Map graphically depicts the effect of changes in carbon dioxide in a way that is easy to grasp. Because carbon dioxide is a component of DIC but not of alkalinity, adding CO₂ (e.g., by shrimp and biofloc respiration) plots as a horizontal vector directed toward higher DIC values

([Fig. AV.15](#)). This vector runs parallel to the x-axis, so alkalinity (on the y-axis) does not change.

Similarly, removing CO₂ (e.g., by degassing to the atmosphere) also maps as a vector parallel to the x-axis, but now directed toward lower DIC values ([Fig. AV.16](#)). Removing CO₂ thus does not change alkalinity.

A related point is that there is a limit to how much CO₂ can be degassed from a system because this is driven by the difference in partial pressures between the air and the culture water. When CO₂ in the air of a poorly ventilated building exceeds that in the open atmosphere, a more severe limit is placed on the amount of CO₂ that can be driven off from culture water (and, therefore, the degree to which pH can be raised by degassing).

The main takeaway: Changing CO₂ does *not* change alkalinity.

V.I SUMMARY

The WQ Map quickly and accurately solves aquaculture water-quality problems that arise routinely, especially in closed systems stocked at very high biomass, such as the Samocha bio-floc system, in which it has been tested.

It also generates accurate predictions of future water quality. This helps managers anticipate problems that otherwise might threaten a crop. The accuracy of this predictive feature will improve when coupled with modern machine-learning algorithms fed a system's historical water-quality data.

Like other software, the WQ Map will be most effective in the hands of an experienced manager who, very often, will encapsulate important practical information about a given culture system that cannot be entered into current applications. In this way, it should contribute to improving RAS yields and expanding environmentally sustainable aquaculture.

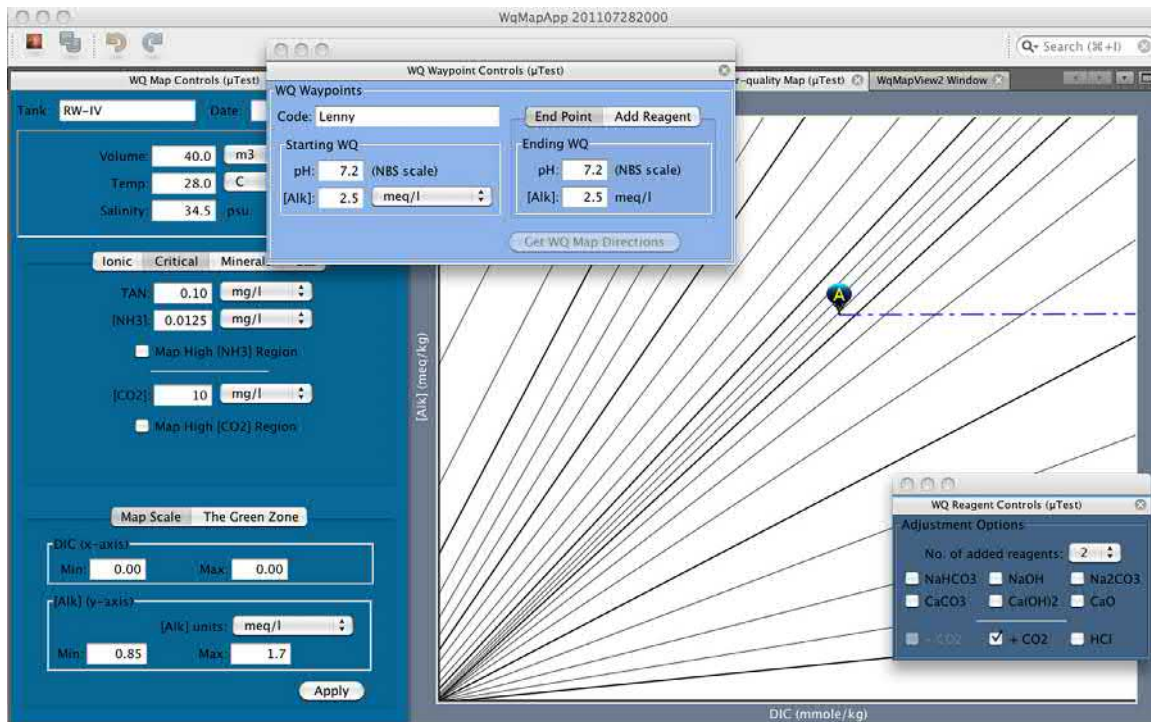


FIG. AV.15 Adding CO_2 lowers pH without changing Total Alkalinity.

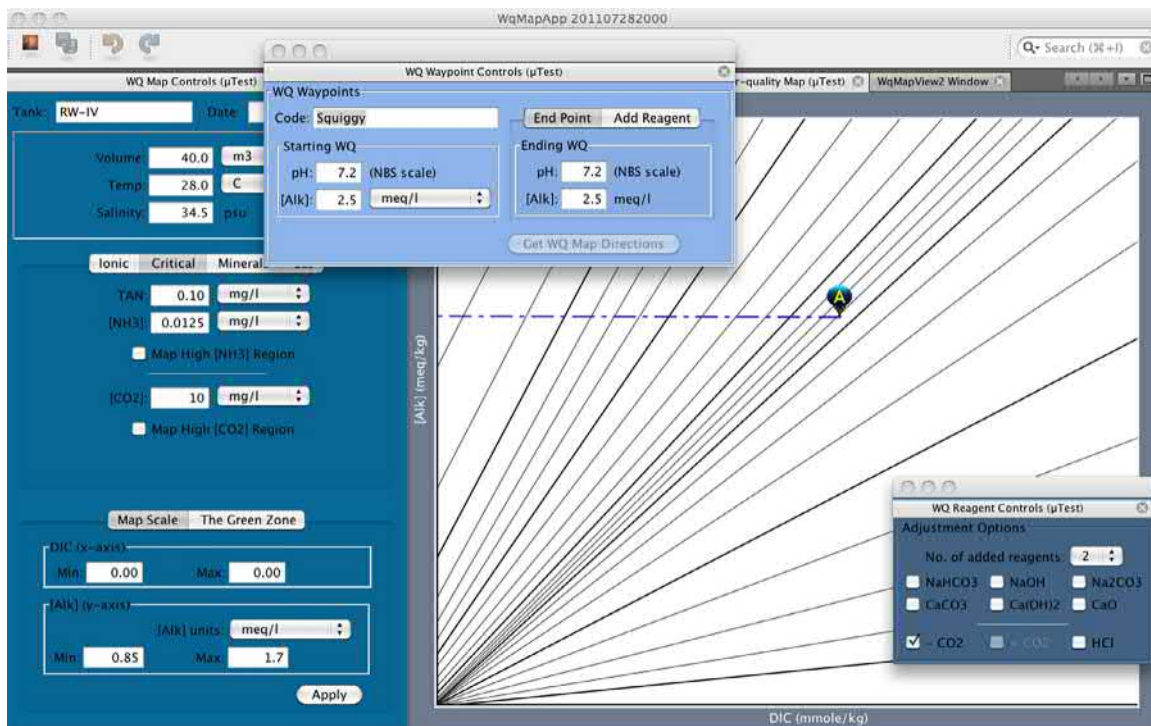


FIG. AV.16 Removing CO_2 raises pH without changing Total Alkalinity.

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VI

Technical Sheets

VI.A UNIT CONVERSION

See [Table AVI.1](#).

TABLE AVI.1 Unit Conversion Table

Unit	Multiplied by	Equals/Unit	Multiplied by	Equals
<i>Length</i>				
Millimeters	0.03937	Inches	25.4001	Millimeters
Centimeters	0.3937	Inches	2.5400	Centimeters
Centimeters	0.03281	Feet	30.48	Centimeters
Centimeters	0.01094	Yards	91.44	Centimeters
Meters	39.37	Inches	0.0254	Meters
Meters	3.2808	Feet	0.3048	Meters
Meters	1.0937	Yards	0.9144	Meters
Kilometers	0.62137	Miles	1.609	Kilometers
<i>Area</i>				
Millimeters ²	0.00155	Inches ²	645.16	Millimeters ²
Centimeters ²	0.155	Inches ²	6.4516	Centimeters ²
Centimeters ²	0.001076	Feet ²	929	Centimeters ²
Centimeters ²	0.000116	Yards ²	8,631	Centimeters ²
Meters ²	1550	Inches ²	0.000645	Meters ²
Meters ²	10.764	Feet ²	0.0929	Meters ²

Continued

TABLE AVI.1 Unit Conversion Table—cont'd

Unit	Multiplied by	Equals/Unit	Multiplied by	Equals
Meters ²	1.196	Yards ²	0.836	Meters ²
Kilometers ²	0.3861	Miles ²	2.59	Kilometers ²
Hectares	2.471	Acres	0.4047	Hectares
<i>Mass</i>				
Grams	0.035274	Ounces	28.3495	Grams
Grams	0.002205	Pounds	453.5924	Grams
Kilograms	2.2046	Pounds	0.4536	Kilograms
Tons (metric)	2204.62	Pounds	0.0004536	Tons (metric)
<i>Volume</i>				
Liters	0.9463	Quarts (US)	1.057	Liters
Liters	0.2642	Gallons (US)	3.7854	Liters
Liters	0.035315	Feet ³	28.3168	Liters
Centimeters ³	0.06102	Inches ³	16.39	Centimeters ³
Meters ³	35.3144	Feet ³	0.0283	Meters ³
Meters ³	1.308	Yards ³	0.7646	Meters ³
Meters ³	264.172	Gallons (US)	0.003785	Meters ³
<i>Pressure</i>				
kiloPascals (kPa)	0.14504	Pounds/sq. inch	6.89476	kiloPascals
Atmospheres	14.696	Pounds/sq. inch	0.068	Atmospheres
Atmospheres	33.957	Ft. of water	0.295	Atmospheres
Bars	0.9869	Atmospheres	1.01325	Bars
Bars	14.5036	Pounds/sq. inch	0.06895	Bars
<i>Power</i>				
Kilowatt (kW)	1.3405	hp (electric)	0.746	Kilowatt

VI.B TEMPERATURE CONVERSION (CELSIUS—FAHRENHEIT)

See [Table AVI.2](#).

VI.C FRICTION LOSS TABLES

(A) PVC Pipe Frictional Head Loss/100 ft for Schedule 40 Pipe—English Units ([Timmons and Ebeling, 2013](#). Used with Permission)

TABLE AVI.2 Temperature Conversion ($T (^{\circ}\text{F}) = T (^{\circ}\text{C}) \times 1.8 + 32$)

$^{\circ}\text{C}$	$^{\circ}\text{F}$	$^{\circ}\text{C}$	$^{\circ}\text{F}$
−40	−40.0	24	75.2
−20	−4.0	25	77.0
−10	14.0	26	78.8
−5	23.0	27	80.6
0	32.0	28	82.4
5	41.0	29	84.2
10	50.0	30	86.0
15	59.0	31	87.8
16	60.8	32	89.6
17	62.6	33	91.4
18	64.4	34	93.2
19	66.2	35	95.0
20	68.0	40	104.0
21	69.8	50	122.0
22	71.6	60	140.0
23	73.4	100	212.0

	Head Loss			Head Loss			Head Loss			Head Loss			Head Loss			Head Loss			Head Loss		
	Velocity GPM (fps)	(ft/ 100 ft)	(psi)	Velocity (fps)	(ft/ 100 ft)	(psi)	Velocity (fps)	(ft/ 100 ft)	(psi)	Velocity (fps)	(ft/ 100 ft)	(psi)	Velocity (fps)	(ft/ 100 ft)	(psi)	Velocity (fps)	(ft/ 100 ft)	(psi)	Velocity (fps)	(ft/ 100 ft)	(psi)
	1/2 inch			3/4 inch			1 inch			1-1/4 inch			1-1/2 inch			2 inch			3 inch		
1	1.07	0.99	0.43	0.61	0.25	0.11															
2	2.14	3.57	1.55	1.22	0.91	0.39	0.75	0.28	0.12	0.43	0.07	0.03									
5	5.35	19.4	8.43	3.05	4.95	2.15	1.88	1.53	0.66	1.09	0.40	0.17	0.80	0.19	0.08						
7	7.49	36.3	15.7	4.27	9.23	4.00	2.63	2.85	1.23	1.52	0.75	0.32	1.12	0.35	0.15						
10				6.10	17.9	7.74	3.76	5.51	2.39	2.17	1.45	0.63	1.60	0.68	0.30	0.97	0.20	0.09			
15							5.64	11.67	5.06	3.26	3.07	1.33	2.40	1.45	0.63	1.45	0.43	0.19			
20							7.52	19.87	8.61	4.35	5.23	2.26	3.19	2.47	1.07	1.94	0.73	0.32	0.88	0.11	0.05
25										5.43	7.90	3.42	3.99	3.73	1.62	2.42	1.10	0.48	1.10	0.16	0.07
30	4 inch									6.52	11.1	4.80	4.79	5.22	2.26	2.91	1.55	0.67	1.32	0.23	0.10
35													5.59	6.95	3.01	3.39	2.06	0.89	1.54	0.30	0.13
40	1.02	0.10	0.04										6.39	8.89	3.85	3.88	2.63	1.14	1.76	0.38	0.17
45	1.15	0.13	0.06													4.36	3.27	1.42	1.98	0.48	0.21
50	1.28	0.15	0.07													4.84	3.98	1.72	2.20	0.58	0.25
60	1.53	0.22	0.09													5.81	5.58	2.42	2.64	0.81	0.35
70	1.79	0.29	0.13	6 inch												6.78	7.42	3.21	3.08	1.08	8.47
75	1.92	0.33	0.14																3.30	1.23	0.53
80	2.04	0.37	0.16	0.90	0.05	0.02													3.52	1.39	0.60
90	2.30	0.46	0.20	1.01	0.06	0.03													3.96	1.72	0.75
100	2.55	0.56	0.24	1.13	0.08	0.03	8 inch												4.40	2.10	0.91
125	3.19	0.84	0.37	1.41	0.11	0.05													5.50	3.17	1.37
150	3.83	1.18	0.51	1.69	0.16	0.07	0.97	0.04	0.02										6.60	4.44	1.92
175	4.47	1.57	0.68	1.97	0.21	0.09	1.14	0.06	0.02	10 inch											
200	5.11	2.01	0.87	2.25	0.27	0.12	1.30	0.07	0.03												

250	6.39	3.04	1.32	2.81	0.41	0.18	1.62	0.11	0.05	1.03	0.04	0.02	12 inch		
300				3.38	0.58	0.25	1.95	0.15	0.07	1.24	0.05	0.02			
350				3.94	0.77	0.33	2.27	0.20	0.09	1.44	0.07	0.03	1.02	0.03	0.01
400				4.50	0.99	0.43	2.60	0.26	0.11	1.65	0.09	0.04	1.16	0.04	0.02
450				5.06	1.23	0.53	2.92	0.32	0.14	1.36	0.11	0.05	1.31	0.05	0.02
500				5.63	1.49	0.65	3.25	0.39	0.17	2.06	0.13	0.06	1.45	0.06	0.02
750				8.44	3.15	1.37	4.87	0.83	0.36	3.09	0.27	0.12	2.18	0.12	0.05
1000							6.50	1.41	0.61	4.12	0.47	0.20	2.90	0.20	0.09
1250										5.15	0.70	0.31	3.63	0.30	0.13
1500										6.19	0.99	0.43	4.36	0.42	0.18
2000													5.81	0.72	0.31
2500															
3000															

Light gray area recommended flowrates to minimize settlement of solids (<1 to 2 fps) and dark gray to avoid scouring of walls and junctions (<5 fps).

(B) PVC Pipe Frictional Head Loss/100 ft for Schedule 40 Pipe—Metric Units (Timmons and Ebeling, 2013. Used with Permission)

	Head Loss			Head Loss			Head Loss			Head Loss			Head Loss			Head Loss			Head Loss		
	Velocity Lps (mps)	(m/100 m)	Velocity (psi) (mps)	(m/100 m)	Velocity (psi) (mps)	(m/100 m)	Velocity (psi) (mps)	(m/100 m)	Velocity (psi) (mps)	(m/100 m)	Velocity (psi) (mps)	(m/100 m)	Velocity (psi) (mps)	(m/100 m)	Velocity (psi) (mps)	(m/100 m)	Velocity (psi) (mps)	(m/100 m)	Velocity (psi) (mps)	(m/100 m)	Velocity (psi) (mps)
	16 mm			20 mm			25 mm			32 mm			40 mm			50 mm			63 mm		
0.05	0.34	1.29	1.8	0.22	0.4	0.6															
0.1	0.68	4.65	6.6	0.44	1.6	2.2	0.28	0.5	0.8												
0.2	1.36	16.77	23.8	0.87	5.7	8.1	0.56	2.0	2.8	0.34	0.6	0.8									
0.3	2.04	35.50	50.4	1.31	12.1	17.2	0.84	4.1	5.9	0.51	1.2	1.8	0.33	0.4	0.6						
0.5				2.18	31.1	44.1	1.40	10.7	15.2	0.85	3.2	4.5	0.55	1.1	1.5	0.35	0.4	0.5			
0.75							2.11	22.6	32.1	1.28	6.8	9.6	0.82	2.3	3.3	0.52	0.8	1.1			
1.0										1.71	11.5	16.3	1.10	3.9	5.5	0.70	1.3	1.9	0.44	0.4	0.6
1.25	90 mm									2.14	17.4	24.7	1.37	5.9	8.4	0.87	2.0	2.8	0.55	0.6	0.9
1.5				110 mm									1.64	8.2	11.7	1.05	2.8	3.9	0.66	0.9	1.3
1.75	0.38	0.21	0.3										1.92	11.0	15.6	1.22	3.7	5.2	0.77	1.2	1.7
2.0	0.43	0.27	0.4	0.29	0.10	0.1	125mm									1.40	4.7	6.7	0.88	1.5	2.2
2.5	0.54	0.41	0.6	0.36	0.15	0.2										1.75	7.1	10.1	1.10	2.3	3.3
3.0	0.65	0.57	0.8	0.43	0.22	0.3	0.34	0.12	0.2							2.10	10.0	14.1	1.33	3.3	4.6
3.5	0.76	0.76	1.1	0.51	0.29	0.4	0.39	0.15	0.2	160 mm									1.55	4.3	6.2
4.0	0.87	0.98	1.4	0.58	0.37	0.5	0.45	0.20	0.3										1.77	5.6	7.9
4.5	0.97	1.22	1.7	0.65	0.46	0.7	0.50	0.25	0.4	0.31	0.07	0.1							1.99	6.9	9.8
5.0	1.08	1.48	2.1	0.72	0.56	0.8	0.56	0.30	0.4	0.34	0.09	0.1	200 mm								
6.0	1.30	2.07	2.9	0.87	0.78	1.1	0.67	0.42	0.6	0.41	0.13	0.2				225 mm					
7.0	1.51	2.75	3.9	1.01	1.04	1.5	0.79	0.56	0.8	0.48	0.17	0.2	0.31	0.06	0.1				250 mm		
8.0	1.73	3.52	5.0	1.16	1.33	1.9	0.90	0.71	1.0	0.55	0.21	0.3	0.35	0.07	0.1	0.28	0.04	0.1			
10				1.45	2.01	2.9	1.12	1.08	1.5	0.68	0.32	0.5	0.44	0.11	0.2	0.35	0.06	0.1	0.28	0.04	0.1
12				1.74	2.82	4.0	1.35	1.51	2.1	0.82	0.45	0.6	0.53	0.15	0.2	0.42	0.09	0.1	0.34	0.05	0.1

14	2.03	3.75	5.3	1.57	2.01	2.9	0.96	0.60	0.9	0.61	0.20	0.3	0.48	0.12	0.2	0.39	0.07	0.1
16				1.80	2.58	3.7	1.10	0.77	1.1	0.70	0.26	0.4	0.55	0.15	0.2	0.45	0.09	0.1
18				2.02	3.20	4.5	1.23	0.96	1.4	0.79	0.33	0.5	0.62	0.18	0.3	0.51	0.11	0.2
22							1.51	1.40	2.0	0.97	0.47	0.7	0.76	0.27	0.4	0.62	0.16	0.2
26							1.78	1.90	2.7	1.14	0.64	0.9	0.90	0.36	0.5	0.73	0.22	0.3
30							2.05	2.48	3.5	1.32	0.84	1.2	1.04	0.47	0.7	0.84	0.28	0.4
40										1.76	1.43	2.0	1.38	0.80	1.1	1.12	0.48	0.7
50													1.73	1.21	1.7	1.40	0.73	1.0
60													2.08	1.70	2.4	1.68	1.02	1.4
70																1.96	1.36	1.9

Light gray area recommended flowrates to minimize settlement of solids (<0.3 to 0.6 m/s) and dark gray to avoid scouring of walls and junctions (<1.5 m/s).

V.I.D PERIODIC TABLE

Source: Wikimedia Commons, Free Software Foundation Inc. <https://commons.wikimedia.org/wiki/File:Periodic-table.jpg> Author: LeVanHan (2008).

PERIODIC TABLE

Atomic Properties of the Elements

NIST
National Institute of
Standards and Technology
U.S. Department of Commerce

Frequently used fundamental physical constants

For the most accurate values of these and other constants, visit physics.nist.gov/constants

1 second = 9 192 631 770 periods of radiation corresponding to the transition between the two hyperfine levels of the ground state of ¹³³Cs

speed of light in vacuum	<i>c</i>	299 792 458 m s ⁻¹ (exact)
Planck constant	<i>h</i>	6.626 07 × 10 ⁻³⁴ J s (<i>h</i> = <i>h</i> /2π)
elementary charge	<i>e</i>	1.602 177 × 10 ⁻¹⁹ C
electron mass	<i>m_e</i>	9.109 38 × 10 ⁻³¹ kg
proton mass	<i>m_p</i>	1.672 622 × 10 ⁻²⁷ kg
fine-structure constant	<i>α</i>	1/137.035 999
Rydberg constant	<i>R_∞</i>	10 973 731.569 m ⁻¹
	<i>R_∞c</i>	3.289 841 960 × 10 ¹⁵ Hz
	<i>R_∞hc</i>	13.605 69 eV
Boltzmann constant	<i>k</i>	1.380 6 × 10 ⁻²³ J K ⁻¹

□ Solids
□ Liquids
□ Gases
□ Artificially Prepared

Physical Measurement Laboratory
www.nist.gov/pml

Standard Reference Data
www.nist.gov/srds

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
IA	IIA	IIIB	IVB	VB	VIB	VII	VIII	VIII	VIII	IB	IIB	IIIA	IVA	VA	VIA	VIIA	VIIIA	
1	H Hydrogen 1.008 1s																	He Helium 4.002602 1s
2	Li Lithium 6.94 2s	Be Beryllium 9.0121831 1s ² 2s											B Boron 10.81 1s ² 2s ² 2p	C Carbon 12.011 1s ² 2s ² 2p ²	N Nitrogen 14.007 1s ² 2s ² 2p ³	O Oxygen 15.999 1s ² 2s ² 2p ⁴	F Fluorine 18.99840316 1s ² 2s ² 2p ⁵	Ne Neon 20.1797 1s ² 2s ² 2p ⁶
3	Na Sodium 22.98976928 (Ne)3s	Mg Magnesium 24.305 (Ne)3s ²											Al Aluminum 26.9815385 (Ne)3s ² 3p	Si Silicon 28.0855 (Ne)3s ² 3p ²	P Phosphorus 30.97376200 (Ne)3s ² 3p ³	S Sulfur 32.06 (Ne)3s ² 3p ⁴	Cl Chlorine 35.45 (Ne)3s ² 3p ⁵	Ar Argon 39.948 (Ne)3s ² 3p ⁶
4	K Potassium 39.0983 (Ar)4s	Ca Calcium 40.078 (Ar)4s ²	Sc Scandium 44.955908 (Ar)3d ¹ 4s ²	Ti Titanium 47.867 (Ar)3d ² 4s ²	V Vanadium 50.9415 (Ar)3d ³ 4s ²	Cr Chromium 51.9961 (Ar)3d ⁵ 4s ¹	Mn Manganese 54.938044 (Ar)3d ⁵ 4s ²	Fe Iron 55.845 (Ar)3d ⁶ 4s ²	Co Cobalt 58.933194 (Ar)3d ⁷ 4s ²	Ni Nickel 58.6934 (Ar)3d ⁸ 4s ²	Cu Copper 63.546 (Ar)3d ¹⁰ 4s ¹	Zn Zinc 65.38 (Ar)3d ¹⁰ 4s ²	Ga Gallium 69.723 (Ar)3d ¹⁰ 4s ² 4p ¹	Ge Germanium 72.630 (Ar)3d ¹⁰ 4s ² 4p ²	As Arsenic 74.921595 (Ar)3d ¹⁰ 4s ² 4p ³	Se Selenium 78.971 (Ar)3d ¹⁰ 4s ² 4p ⁴	Br Bromine 79.904 (Ar)3d ¹⁰ 4s ² 4p ⁵	Kr Krypton 83.798 (Ar)3d ¹⁰ 4s ² 4p ⁶
5	Rb Rubidium 85.4678 (Kr)5s	Sr Strontium 87.62 (Kr)5s ²	Y Yttrium 88.90584 (Kr)4d ¹ 5s ²	Zr Zirconium 91.224 (Kr)4d ² 5s ²	Nb Niobium 92.90637 (Kr)4d ⁴ 5s ¹	Mo Molybdenum 95.95 (Kr)4d ⁵ 5s ¹	Tc Technetium (98) (Kr)4d ⁵ 5s ²	Ru Ruthenium 101.07 (Kr)4d ⁷ 5s ¹	Rh Rhodium 102.90550 (Kr)4d ⁸ 5s ¹	Pd Palladium 106.42 (Kr)4d ¹⁰	Ag Silver 107.8682 (Kr)4d ¹⁰ 5s ¹	Cd Cadmium 112.414 (Kr)4d ¹⁰ 5s ²	In Indium 114.818 (Kr)4d ¹⁰ 5s ² 5p ¹	Sn Tin 118.710 (Kr)4d ¹⁰ 5s ² 5p ²	Sb Antimony 121.760 (Kr)4d ¹⁰ 5s ² 5p ³	Te Tellurium 127.60 (Kr)4d ¹⁰ 5s ² 5p ⁴	I Iodine 126.90447 (Kr)4d ¹⁰ 5s ² 5p ⁵	Xe Xenon 131.293 (Kr)4d ¹⁰ 5s ² 5p ⁶
6	Cs Cesium 132.9054520 (Xe)6s	Ba Barium 137.327 (Xe)6s ²		Hf Hafnium 178.49 (Xe)4f ¹⁴ 5d ² 6s ²	Ta Tantalum 180.94788 (Xe)4f ¹⁴ 5d ³ 6s ²	W Tungsten 183.84 (Xe)4f ¹⁴ 5d ⁴ 6s ²	Re Rhenium 186.207 (Xe)4f ¹⁴ 5d ⁵ 6s ²	Os Osmium 190.23 (Xe)4f ¹⁴ 5d ⁶ 6s ²	Ir Iridium 192.217 (Xe)4f ¹⁴ 5d ⁷ 6s ²	Pt Platinum 195.084 (Xe)4f ¹⁴ 5d ⁹ 6s ¹	Au Gold 196.966569 (Xe)4f ¹⁴ 5d ¹⁰ 6s ¹	Hg Mercury 200.592 (Xe)4f ¹⁴ 5d ¹⁰ 6s ²	Tl Thallium 204.38 (Xe)4f ¹⁴ 5d ¹⁰ 6s ²	Pb Lead 207.2 (Xe)4f ¹⁴ 5d ¹⁰ 6s ²	Bi Bismuth 208.98040 (Xe)4f ¹⁴ 5d ¹⁰ 6s ²	Po Polonium (209) (Xe)4f ¹⁴ 5d ¹⁰ 6s ²	At Astatine (210) (Xe)4f ¹⁴ 5d ¹⁰ 6s ²	Rn Radon (222) (Xe)4f ¹⁴ 5d ¹⁰ 6s ²
7	Fr Francium (223) (Rn)7s	Ra Radium (226) (Rn)7s ²		Rf Rutherfordium (261) (Rn)5f ¹⁴ 6d ² 7s ²	Db Dubnium (268) (Rn)5f ¹⁴ 6d ³ 7s ²	Sg Seaborgium (271) (Rn)5f ¹⁴ 6d ⁴ 7s ²	Bh Bohrium (272) (Rn)5f ¹⁴ 6d ⁵ 7s ²	Hs Hassium (277) (Rn)5f ¹⁴ 6d ⁶ 7s ²	Mt Meitnerium (276) (Rn)5f ¹⁴ 6d ⁷ 7s ²	Ds Darmstadtium (281) (Rn)5f ¹⁴ 6d ⁸ 7s ²	Rg Roentgenium (280) (Rn)5f ¹⁴ 6d ⁹ 7s ²	Cn Copernicium (285) (Rn)5f ¹⁴ 6d ¹⁰ 7s ²	Uut Ununtrium (284) (Rn)5f ¹⁴ 6d ¹⁰ 7s ²	Fl Flerovium (289) (Rn)5f ¹⁴ 6d ¹⁰ 7s ²	Uup Ununpentium (293) (Rn)5f ¹⁴ 6d ¹⁰ 7s ²	Lv Livermorium (293) (Rn)5f ¹⁴ 6d ¹⁰ 7s ²	Uus Ununseptium (294) (Rn)5f ¹⁴ 6d ¹⁰ 7s ²	Uuo Ununoctium (294) (Rn)5f ¹⁴ 6d ¹⁰ 7s ²
			La Lanthanum 138.90547 (Xe)5d ¹ 6s ²	Ce Cerium 140.116 (Xe)4f ¹ 5d ¹ 6s ²	Pr Praseodymium 140.907 (Xe)4f ² 6s ²	Nd Neodymium 144.242 (Xe)4f ³ 6s ²	Pm Promethium (145) (Xe)4f ⁴ 6s ²	Sm Samarium 150.36 (Xe)4f ⁵ 6s ²	Eu Europium 151.964 (Xe)4f ⁶ 6s ²	Gd Gadolinium 157.25 (Xe)4f ⁷ 6s ²	Tb Terbium 158.92535 (Xe)4f ⁸ 6s ²	Dy Dysprosium 162.500 (Xe)4f ⁹ 6s ²	Ho Holmium 164.93033 (Xe)4f ¹⁰ 6s ²	Er Erbium 167.259 (Xe)4f ¹¹ 6s ²	Tm Thulium 168.93422 (Xe)4f ¹² 6s ²	Yb Ytterbium 173.054 (Xe)4f ¹³ 6s ²	Lu Lutetium 174.9668 (Xe)4f ¹⁴ 6s ²	
			Ac Actinium (227) (Rn)5f ¹ 6d ¹ 7s ²	Th Thorium 232.0377 (Rn)5f ² 6d ² 7s ²	Pa Protactinium 231.03688 (Rn)5f ² 6d ¹ 7s ²	U Uranium 238.02891 (Rn)5f ³ 6d ¹ 7s ²	Np Neptunium (237) (Rn)5f ⁴ 6d ¹ 7s ²	Pu Plutonium (244) (Rn)5f ⁶ 6d ¹ 7s ²	Am Americium (243) (Rn)5f ⁷ 6d ¹ 7s ²	Cm Curium (247) (Rn)5f ⁸ 6d ¹ 7s ²	Bk Berkelium (247) (Rn)5f ⁹ 6d ¹ 7s ²	Cf Californium (251) (Rn)5f ¹⁰ 6d ¹ 7s ²	Es Einsteinium (252) (Rn)5f ¹¹ 6d ¹ 7s ²	Fm Fermium (257) (Rn)5f ¹² 6d ¹ 7s ²	Md Mendelevium (258) (Rn)5f ¹³ 6d ¹ 7s ²	No Nobelium (259) (Rn)5f ¹⁴ 6d ¹ 7s ²	Lr Lawrencium (262) (Rn)5f ¹⁴ 6d ¹ 7s ²	

[†]Based upon ¹²C. () indicates the mass number of the longest-lived isotope.

[†]IUPAC conventional atomic weights; standard atomic weights for these elements are expressed in intervals; see iupac.org for an explanation and values.

For a discussion of the data, visit physics.nist.gov/data

NIST SP 966 (September 2014)

VI.E VOLUME CALCULATIONS

To determine the volume of culture tanks or vessels, measure the internal dimensions and calculate as follows:

Rectangle

$$\text{Volume} = l \times w \times h$$

where l = length, w = width, and h = height.

Example: What is the volume of a 1.0-m $\times$ 0.75-m rectangular tank with water depth of 0.45 m (45 cm)?

$$\begin{aligned}\text{Volume} &= 1.0 \times 0.75 \times 0.45 \\ &= 0.3375\text{m}^3 \\ &= 337.5\text{L}\end{aligned}$$

For a raceway with a bottom sloping to a drain at one end, measure the depth at each end and in the middle of the raceway, and calculate the average depth.

Example: What is the volume of a 30-m $\times$ 3-m raceway with depth of 1.0 m (shallow end), 1.15 m (middle), and 1.3 m (deep end)?

$$\begin{aligned}\text{Volume} &= 30 \times 3 \times \left(\frac{1 + 1.15 + 1.3}{3} \right) \\ &= 103.5\text{m}^3 \\ &= 103,500\text{L}\end{aligned}$$

Cylinder

$$\text{Volume} = \pi r^2 h$$

$$r = \frac{\phi}{2}$$

where $\pi = 3.141$, r = radius, h = height, ϕ = diameter.

Example: What is the maximum volume of a circular reservoir tank with an internal diameter of 4 m and height of 6 m?

$$\begin{aligned}\text{Volume} &= 3.141 \times (2)^2 \times 6 \\ &= 75.384\text{m}^3 \\ &= 75,384\text{L}\end{aligned}$$

Cone

$$\text{Volume} = \frac{1}{3}(\pi r^2 h)$$

where $\pi = 3.141$, r = radius, h = height.

Example: What is the water volume of a conical bottom settling tank, with a total water depth of 1.5 m, maximum diameter of 0.7 m, and cone height of 0.35 m?

Calculate the volume of the bottom cone and upper cylinder separately.

$$\begin{aligned}\text{Cone Volume} &= \frac{1}{3} \left(3.141 \times (0.35)^2 \times 0.35 \right) \\ &= 0.04489\text{m}^3 \\ &= 44.89\text{L}\end{aligned}$$

$$\text{Total Volume} = 44.89 + 442.49 = 487.38\text{L}$$

$$\begin{aligned}\text{Cylinder Volume} &= \left(3.141 \times (0.35)^2 \times (1.5 - 0.35) \right) \\ &= 0.44249\text{m}^3 \\ &= 442.49\text{L}\end{aligned}$$

Reference

Timmons, M.B., Ebeling, J.M. (Eds.), 2013. *Recirculating Aquaculture*, third ed. Ithaca Publishing Company, Ithaca, NY.

VII

Excel Sheets and Forms—Summary

No.	File Name	Description
Folder Name: Nursery		
1	PL Acclimation Data Recording Form	For data recording during acclimation to nursery tanks: PL samples, mortalities, water volume, and water quality. Page # 401
2	PL Evaluation Data Recording Form	For PL microscopic physical evaluation following receipt from the hatchery and during early nursery stages. Page # 402
3	Nursery WQ, Feed, & More_Form	For recording daily water quality, equipment operation, feed rations, and other inputs. Page # 403
4	Nursery Group Sampling_Form & Calc	For weekly shrimp growth sampling, with calculations for average weight and growth (g/day and g/week) given in a separate spreadsheet. Page # 404
5	Nursery Ration Growth FCR Survival	For input of Excel Sheet #4 data and calculating average weight, growth, biomass, FCR, and rations. A separate spreadsheet with example data from 2014 is included.
6	Nursery WQ Feed Growth FCR Electronic Data Recording Form Example & Cal	For collating data for individual nursery tanks over a full nursery cycle (from Sheet # 3 and # 4)—water quality, equipment operation, feed rations, shrimp weight, and other inputs. Includes columns to calculate FCR and growth. A separate spreadsheet with example data from 2012 is included. Page # 405
7	Nursery Individual wt. Frequency distribution & Feed Calc_Examples	Example spreadsheets (2014 nursery trials) for calculating shrimp size distribution and allocating feed type and size to individual nursery tanks.
8	Shrimp PL Age and Length	For estimating <i>L. vannamei</i> PL weight at given total lengths and PL size at given ages. Covers PL5–30. Page # 406
9	Nursery Sampling Before Transfer_Form	For recording weight and size distribution prior to nursery tank harvest. The collected data can be added to a separate spreadsheet which includes formulae for calculating average weight and size variation. Page # 407

Continued

No.	File Name	Description
10	Juvenile Transfer Form & Calc	For recording weight of harvested shrimp during transfer to grow—out tanks. The collected data can be added to a separate spreadsheet which includes formulae for calculating total harvest weight, yield, shrimp number, survival, average weight, and size variation. Page # 408
Folder Name: <i>Grow—out</i>		
11	Grow—out WQ Operation Feed <i>Vibrio</i> Inputs Data Recording Form	For recording daily water quality, equipment operation, feed rations, and other inputs. Page # 409
12	Grow—out Group Sampling_Form & Calc	For weekly shrimp growth sampling, with calculations for average weight and growth (g/d and g/wk) given in a separate spreadsheet. Page # 410
13	Grow—out 40 & 100 m ³ RWs Growth. FCR. Ration Calc_Examples	Example spreadsheets (2012 & 2013 Grow—out trials) for calculating shrimp average weight, growth, biomass, FCR, and rations.
14	Grow—out Ration Growth FCR Survival	For input of Sheet # 12 data and calculating average weight, growth, biomass, FCR, and rations. A separate spreadsheet with example data from 2014 is included.
Folder Name: <i>General</i>		
15	Calc & Example FCRs 100 m ³ RW	For recording feed offered and shrimp growth, and calculating FCR, biomass, average weight, and growth. A separate spreadsheet with example data from 2012 is included. Page # 411
16	Group Weight Sampling_Form & Calc	For recording group weight of harvested shrimp from nursery or grow—out tanks. The collected data can be added to a separate spreadsheet which includes formulae for calculating total harvest weight, shrimp number, average weight, and size variation. Page # 412
17	Individual Weight Sampling_Form & Calc	For recording individual weight of 100 sampled shrimp from nursery or grow—out harvest. Data can be added to a separate spreadsheet that includes formulae for calculating average weight and size variation. Page # 413
18	Organic Carbon Supplementation_Examples & Calc	Examples on how to calculate organic carbon requirements for feed with different crude protein and different organic C sources and user input to calculate molasses/white sugar requirements. Page # 414
Folder Name: <i>Water Quality</i>		
19	<i>Vibrio</i> & Alkalinity Form_Examples & Calc	For recording and calculating <i>Vibrio</i> plate counts and alkalinity (standard titration method). Examples are given for each. Page # 415
20	TSS Form_Example & Calc	For recording and calculating TSS (standard titration method). Examples are given. Page # 416
21	pH Calc	Spreadsheet showing the calculation of [H ⁺] from known pH and how to calculate an average pH from multiple pH values
22	Changes in WQ during Grow—out_2012 40 m ³ RW System_Example	Example spreadsheets for weekly water quality data from the 2012 grow—out trial. Includes data and graphs of TAN, NO ₂ —N NO ₃ —N, PO ₄ , TSS, VSS, alkalinity, turbidity, cBOD ₅ , and COD changes over time.
	Salinity TDS Conductivity_Conversions Table	Conversion table Conductivity to salinity. Page # 417

Postlarvae Acclimation Data Recording Form

PL Source: _____ Bag #: _____ Sample Vol. (mL): _____ Live PL/ Sample: _____ Total PL/Bag: _____	Date: _____ Bag Vol. (L): _____ PL/Sample: _____ Dead PL/Sample: _____ TAN (mg/L): _____

Time	Temp.	DO	pH	Salinity	Vol. Added (L)

Postlarvae Microscopic Evaluation, after Davis et al. (2004) and Villalon (1991).

Date: _____ Hatchery: _____
 PL Age (d): _____ Genetic Line: _____
 Sample Size: _____ Population CV: _____
 Av. Wt. (mg): _____

Indicator	Comments	Score
Mucus & debris on setae:		
Fouling: (sessile ciliates, filamentous bacteria, benthic algae, fungi etc.), especially on gills:		
Broken walking legs (periopods) or antennae:		
Lesions on walking legs/swimming legs (pleopods) and antennae along with/without chitinoclastic bacterial infection:		
Evidence of brown spots on the body such as chitinoclastic bacteria:		
Deformities in eye stalks, rostrum, first and second antennae, tail segments, and walking leg:		
Opacity of tail segments and swimming legs:		
Body pigmentation and hepatopancreas color:		
Gut fullness, hepatopancreas lipid content and deformities:		
Gill Development:		
Other:		

Date: ____/____/____ DOC: ____
 Day: M, T, W, T, F, S, S

Nursery Production - *L. vannamei*
 PL Source _____

PL/m³: ____

Tank	FW	SW	FF	MCF	ST	TSS	SS	NH ₄ -N	NO ₂ -N	NO ₃ -N	Turb	Alka	Vibrio (CFU/mL)		DO		pH		Sal	Dead
ID	(m ³)	(m ³)	(h)	(h)	(h)	(mg/L)	(mL/L)	(mg/L)			(NTU)	(mg/L)	Yellow	Green	am	pm	am	pm	(ppt)	(#)
1																				
2																				
3																				
4																				
5																				
6																				
7																				
8																				
9																				
Tank	Belt Feeders (kg)							Total Fed	Sugar	Bicarbo	Carbo	Probio	O ₂		REMARKS					
ID	EZ Art	<400µm	400-600µm	600-860µm	1 mm	1.5 mm	2 mm	(kg)	(g)	(kg)	(kg)	(g)	(L/min)	(h)						
1																				
2																				
3																				
4																				
5																				
6																				
7																				
8																				
9																				

Excel Sheet # 3_Nursery WQ, Feed, & More_Form

Nursery Production - *Litopenaeus vannamei* PL Source: _____

Stocking Date: _____

DOC: _____

Sampling Date :				Days from last Sampling :			7
TK	Current						Av. Wt. 7 Days
ID	Group Wt. (g)	# Shrimp	Tare (g)	Av. Wt. (g)	(g/day)	(g/wk)	Earlier (g)
1							
2							
3							
4							
5							
6							
7							
8							

Excel Sheet # 4_Nursery Group Sampling_Form

Example for electronic nursery data entry templet used by Texas A&M-ARML for 40 m³ raceway

Stocking Date: Raceway Volume (m³):40 # of PLs stocked: Stocking Density (PL/m²): PL Size (g):
 Tank ID: RW 1 PL Genetic Info: Cross: Fast-Growth/Taura-F # of shrimp: 40,294

Comments	Alka (mg/L)	TSS (mg/L)	TAN (mg/L)	NO ₃ -N (mg/L)	NO ₂ -N (mg/L)	O ₂ (lpm)	Venturi (h)	New SW (m³)	New FW (m³)	Volume (m³)	ST (h)	FF (h)	NaHCO ₃ (g)	CaCO ₃ (g)	Current Bio (g)	Av. Weight (g)	Bio Incr from Last Feed from Last (g)	FCR From T ₁	FCR from Last	Feed Types	Total Feed (g)	Feed Type 2 (g)	Feed Type 1 (g)	pH	DO (mg/L)	DO (%)	Temperature (°C)	Time (PM)	SS (mL/L)	Turbidity (NTU)	pH	DO (mg/L)	DO (% Saturation)	Salinity (ppt)	Temperature (°C)	Time (AM)	PL Age (days)	Day of culture	Date (M/D/Y)	
	1	9																																						
	2	10																																						
	3	11																																						
	4	12																																						
	5	13																																						
	6	14																																						
	7	15																																						
	8	16																																						
	9	17																																						
	10	18																																						
	11	19																																						
	12	20																																						
	13	21																																						
	14	22																																						
	15	23																																						
	16	24																																						
	17	25																																						
	18	26																																						
	19	27																																						
	20	28																																						

Excel Sheet # 6_Typical Nur WQ Feed_records

Relationship of PL age and size

(Doug Ernst, NSC, 4/25/14)

Data compiled from various sources, internal and external.

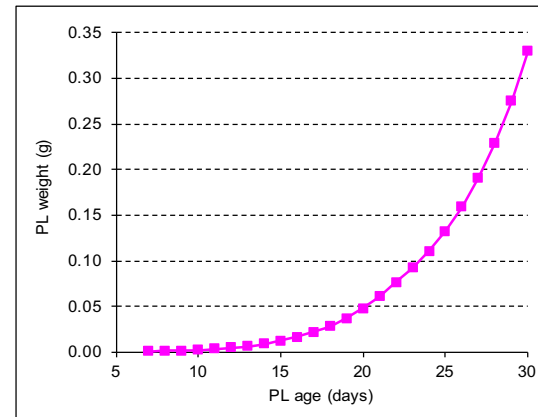
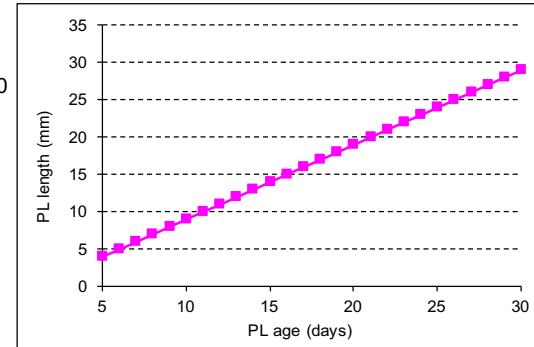
Age and size relation shown below is an approximation and varies with PL culture conditions and growth rate.

Given that it's easier to measure length than weight for PL stocking (PL8 - 12), this chart is used to estimate mean weight based on mean length.

PL age	Min Ln (mm)	Max Ln (mm)	Mean Wt (g)
5	3	4	
6	4	5	
7	5	6	0.0010
8	6	7	0.0015
9	7	8	0.0020
10	8	9	0.0025
11	9	10	0.0035
12	10	11	0.0049
13	11	12	0.0069
14	12	13	0.0094
15	13	14	0.0127
16	14	15	0.0169
17	15	16	0.0219
18	16	17	0.0285
19	17	18	0.0371
20	18	19	0.0482
21	19	20	0.0614
22	20	21	0.0768
23	21	22	0.0922
24	22	23	0.1106
25	23	24	0.1327
26	24	25	0.1593
27	25	26	0.1911
28	26	27	0.2293
29	27	28	0.2752
30	28	29	0.3302

Insufficient branchial development and osmo-regulatory capacity for salinity less than about 30 ppt.

Acceptable range in PL development for osmo-regulatory capacity (salinity reduction) and shipping in sealed bags (metabolic loading).



Excel Sheet # 8_Shrimp PL Age and Length

Nursery Tank Sampling Before Transfer

Tank ID: _____ Date: _____

Sample	Total Wt. (g)	# Shrimp	Tare (g)	Av. Wt. (g)
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				

Excel Sheet # 9_Nursery Sampling Before Transfer_Form

Juvenile Transfer - Data Recording Sheet

PL Source: _____		Moved to GO Tank: _____	
Tank Vol. (m³): _____			
Nursery ID: _____		Trans. Date: _____	
Weight (kg)	Cumulative Weight (kg)	Weight (kg)	Cumulative Weight (kg)
1 _____	_____	26 _____	_____
2 _____	_____	27 _____	_____
3 _____	_____	28 _____	_____
4 _____	_____	29 _____	_____
5 _____	_____	30 _____	_____
6 _____	_____	31 _____	_____
7 _____	_____	32 _____	_____
8 _____	_____	33 _____	_____
9 _____	_____	34 _____	_____
10 _____	_____	35 _____	_____
11 _____	_____	36 _____	_____
12 _____	_____	37 _____	_____
13 _____	_____	38 _____	_____
14 _____	_____	39 _____	_____
15 _____	_____	40 _____	_____
16 _____	_____	41 _____	_____
17 _____	_____	42 _____	_____
18 _____	_____	43 _____	_____
19 _____	_____	44 _____	_____
20 _____	_____	45 _____	_____
21 _____	_____	46 _____	_____
22 _____	_____	47 _____	_____
23 _____	_____	48 _____	_____
24 _____	_____	49 _____	_____
25 _____	_____	50 _____	_____

Date: ____/____/____DOC: _____

Grow-out - *L. vannamei*

Stocking Juveniles/m³: _____

Day: M, T, W, T, F, S, S

Tank	FW	SW	FF	MCF	ST	TSS	SS	NH ₄ -N	NO ₂ -N	NO ₃ -N	Turb	Alka	Vibrio (CFU/mL)		DO		pH		Sal	Dead
ID	(m ³)	(m ³)	(h)	(h)	(h)	(mg/L)	(mL/L)		(mg/L)		(NTU)	(mg/L)	Yellow	Green	am	pm	am	pm	(ppt)	(#)
1																				
2																				
3																				
4																				
5																				
6																				
7																				
8																				
Tank	Belt Feeders (kg)				Total Fed	Sugar	Bicarbo	Carbo	Probio	O ₂		REMARKS								
ID	1 mm	1.5 mm	2 mm	2.4 mm	(kg)	(g)	(kg)	(kg)	(g)	(L/min)	(h)									
1																				
2																				
3																				
4																				
5																				
6																				
7																				
8																				

Grow-out Production - *Litopenaeus vannamei* PL Source: _____

Stocking Date: _____ DOC: _____

Sampling Date:				Days from Last Sampling:			7
TK	Current						Av. Wt. 7 Days Earlier (g)
ID							
1							
2							
3							
4							
5							
6							
7							
8							

Excel Sheet # 12_Grow-out Group Sampling_Form & Calc

Computing FCR in 100 m³ RW

Total shrimp stocked in RW:		50,000		Stocking density (shrimp/m³):		500			
Date	Doc	Total Feed (g/day)	Total Feed (g)	Inter FCR	Overall FCR	Feed Last wt.	Biomass Increase (g)	Av. wt. (g)	Biomass (g)
	1								
	2								
	3								
	4								
	5								
	6								
	7								
	8								
	9								
	10								
	11								
	12								
	13								
	14								
	15								
	16								
	17								
	18								
	19								
	20								
	21								
	22								
	23								
	24								
	25								

Group Weight - Nursery / Grow-out Harvest - Data Recording Sheet								Tank ID:
PL Source:		Transfer / Final Harvest				Date:		
Tot. Wt. (g)	# shrimp	Tare	Av. Wt.	Tot. Wt. (g)	# shrimp	Tare	Av. Wt.	
1				21				
2				22				
3				23				
4				24				
5				25				
6				26				
7				27				
8				28				
9				29				
10				30				
11				31				
12				32				
13				33				
14				34				
15				35				
16				36				
17				37				
18				38				
19				39				
20				40				

Excel Sheet # 16—Group weight sampling form & calc

Individual Weight - Data Recording Sheet

Tank ID:	Nur.	GO	Trans. Harvest Date:
1 _____	26 _____	51 _____	76 _____
2 _____	27 _____	52 _____	77 _____
3 _____	28 _____	53 _____	78 _____
4 _____	29 _____	54 _____	79 _____
5 _____	30 _____	55 _____	80 _____
6 _____	31 _____	56 _____	81 _____
7 _____	32 _____	57 _____	82 _____
8 _____	33 _____	58 _____	83 _____
9 _____	34 _____	59 _____	84 _____
10 _____	35 _____	60 _____	85 _____
11 _____	36 _____	61 _____	86 _____
12 _____	37 _____	62 _____	87 _____
13 _____	38 _____	63 _____	88 _____
14 _____	39 _____	64 _____	89 _____
15 _____	40 _____	65 _____	90 _____
16 _____	41 _____	66 _____	91 _____
17 _____	42 _____	67 _____	92 _____
18 _____	43 _____	68 _____	93 _____
19 _____	44 _____	69 _____	94 _____
20 _____	45 _____	70 _____	95 _____
21 _____	46 _____	71 _____	96 _____
22 _____	47 _____	72 _____	97 _____
23 _____	48 _____	73 _____	98 _____
24 _____	49 _____	74 _____	99 _____
25 _____	50 _____	75 _____	100 _____

Excel Sheet # 17—Individual weight sampling_form & calc

Amount of different organic carbon sources required to convert all TAN generated from feed into biomass of heterotrophic bacteria

Specific Example

1. Assuming tank volume of: 100,000 L
 2. Level of TAN in tank: 3 mg/L
 3. Providing the heterotrophic bacteria all organic C requires to convert the TAN into bacteria biomass

Example 2:

F	1 kg		
PC	40%	=	0.40
C	0.144		
TAN/day	= 0.0576 kg		

Example 3:

F	1 kg		
PC	45%	=	0.45
C	0.144		
TAN/day	= 0.0648 kg		

Calculation for molasses: $100,000 \text{ L (TK vol.)} \times 3 \text{ (TAN conc.)} \times 6 \text{ (required C)} \times 1.3 \text{ (molasses spec. wt.)} \times 24\% \text{ (C in molasses)} / 1,000 \text{ (conv. To g from mg)}$

Carbon Source	Available C	Required Organic Carbon
Molasses*	24.0%	5,769.23 mL
White sugar	42.1%	4,275.53 g
Lactose	42.1%	4,275.53 g
Dextrose	40.0%	4,500.00 g
Glucose	40.0%	4,500.00 g
Acetate	40.0%	4,500.00 g
Glycerol	39.1%	4,603.58 L
Cellulose	44.4%	4,054.05 g
Starch	44.4%	4,054.05 g
Cassava meal	43.4%	4,147.47 g
Corn flour	43.4%	4,147.47 g
Rice brane	43.4%	4,147.47 g
Sorghum meal	43.4%	4,147.47 g
Tapioca	43.4%	4,147.47 g
Wheat flour	43.4%	4,147.47 g
Wheat brane	43.4%	4,147.47 g

* Assuming 24% W/W carbon concentration and specific weight of 1.3 g/ml

Calculation of daily TAN production in no exchange production system

Assumptions:

F = Daily Ration:	1 kg		
PC = Protein Concentration:	35%	=	0.35
Constant for TAN generation in no exchan	0.144		
Nitrogen in Protein:	16%		

$$\text{TAN (kg)} = \text{F (kg)} \times \text{PC (decimal value)} \times 0.144$$

$$0.0504 \text{ kg} = 1 \times 35\% \times 0.144$$

Example 1:

F	1 kg		
PC	30%	=	0.30
C	0.144		
TAN/day	= 0.0432 kg		

Example 4:

F	1 kg		
PC	50%	=	0.50
C	0.144		
TAN/day	= 0.0720 kg		

Example 5:

F	1 kg		
PC	55%	=	0.55
C	0.144		
TAN/day	= 0.0792 kg		

Steps to enhance development of nitrifying bacteriaAssumptions: Tank volume: 40,000 L

- 1/3 of the TAN generated from feed per day is taken by the heterotrophic bacteria
- 2/3 of the TAN generated from feed per day is left for the nitrifying bacteria to process
- Tank was inoculated with at least 10% of its volume with nitrifying-rich water
- Alkalinity, TAN, nitrite, nitrate and pH are monitored daily
- Daily ration: 1 kg
- Feed protein concentration: 55%
- Amount of TAN generated: 0.0792 kg
- TAN concentration in 1.98 mg/L $(0.0792 \times 1,000 \times 1,000 / 40,000)$
- Amount of TAN left for the nitr 0.0523 kg (0.0792×0.66)

Day 1

TAN	1.98 mg/L
Alkalinity	140 mg/L (as CaCO ₃)
pH	7.8
NO ₂	0.01 mg/L
NO ₃	0.001 mg/L
Organic C supplementat	0.2981 kg $[(0.0523 \times 6 - (0.0523 \times 6 \times 0.05))$

5% reduction in organic C below the amount needed to convert all TAN into heterotrophic bacterial biomass to free-up TAN for the nitrifying bacteria

Day 2

TAN	0.01 mg/L
Alkalinity	128 mg/L (as CaCO ₃)
pH	7.8
NO ₂	0.02 mg/L
NO ₃	0.001 mg/L
Organic C supplementat	0.2824 kg $(0.3138 - 0.0314)$

Alkalinity reduction along with slight increase in nitrite suggest nitrification activity 10% reduction in organic C supplementation along with increase in alkalinity to 140 mg/L are suggested to free-up TAN for the nitrifying bacteria

Excel Sheet # 18_Organic Carbon Requirement Examples & Cal

Vibrio Counts

Date: _____ Time Inoculated: _____
 Time Counted: _____
 Sample size (μL): _____

RACEWAY	Yellow		Green		Total	
	CFU/Plate	CFU/mL	CFU/Plate	CFU/mL	CFU/Plate	CFU/mL
RW 1						
RW 2						
RW 3						
RW 4						
RW 5						
RW 6						
RW 7						
RW 8						
RW 9						
RW 10						

Alkalinity

	SULFURIC ACID USED (mL)			Date:
	Initial Reading	End Point Reading	Difference (mL)	Alkalinity (mg/L CaCO ₃)
RW1			0.00	
RW2			0.00	
RW3			0.00	
RW4			0.00	
RW5			0.00	
RW6			0.00	
B1			0.00	
B2			0.00	

Normality of H ₂ SO ₄ Solution	Jun-14	0.019023462

*Calculated normality of H₂SO₄ solution- should be close to 0.02

TSS Monitoring Form*

DATE:

RW	Sample	Empty (g)	Dry (g)	Empty (mg)	Dry (mg)	TSS (mg/L)	Av. TSS (mg/L)	Dry 2 (g)	Dry 2 (mg)
Control									
RW1									
RW2									
RW3									
RW4									
RW5									
RW6									
RW7									
RW8									
RW9									
RW10									

* This form is based on Standard Method procedure (Eaton et al. 1995); To save time and simplify monitoring we recommend the use of Pre Weighed 7.7. cm filter papers



Conversion Table for Changing Conductivity into Salinity

Conductivity*							Salinity
0°C	5°C	10°C	15°C	20°C	25°C	30°C	ppt
1.200	1.400	1.500	1.700	2.000	2.200	2.400	1
2.220	2.500	2.900	3.300	3.700	4.100	4.500	2
3.200	3.700	4.200	4.700	5.300	5.900	6.500	3
4.100	4.700	5.400	6.100	6.900	7.600	8.400	4
5.000	5.800	6.600	7.500	8.400	9.300	10.300	5
5.900	6.800	7.900	8.800	9.900	11.000	12.100	6
6.700	7.800	8.900	10.100	11.300	12.600	13.900	7
7.600	8.800	10.100	11.400	12.800	14.200	15.700	8
8.500	9.800	11.200	12.700	14.200	15.800	17.400	9
9.300	10.800	12.300	13.900	15.600	17.300	19.100	10
10.200	11.800	13.400	15.200	17.000	18.900	20.800	11
11.000	12.800	14.500	16.500	18.900	20.400	22.500	12
11.900	13.700	15.600	17.600	19.700	21.900	24.100	13
12.600	14.600	16.700	18.700	21.100	23.400	25.800	14
13.400	15.600	17.800	20.100	22.400	24.900	27.400	15
14.200	16.400	18.800	21.200	23.800	26.400	29.100	16
15.000	17.400	19.800	22.400	25.100	27.800	30.700	17
15.800	18.300	20.900	23.600	26.400	29.300	32.300	18
16.600	19.200	21.900	24.800	27.700	30.700	33.900	19
17.400	20.100	23.000	25.900	29.000	32.200	35.500	20
18.200	21.100	24.000	27.100	30.300	33.600	37.000	21
19.000	22.000	25.100	28.300	31.600	35.000	38.600	22
19.800	22.900	26.100	29.400	32.900	36.500	40.100	23
20.600	23.800	27.100	30.600	34.200	37.900	41.700	24
21.400	24.700	28.100	31.700	35.400	39.300	43.200	25
22.100	25.500	29.100	32.800	36.700	40.700	44.800	26
22.800	26.400	30.100	33.900	37.900	42.100	46.300	27
23.600	27.300	31.100	35.100	39.200	43.500	47.800	28
24.400	28.100	32.100	36.200	40.400	44.800	49.400	29
25.200	29.000	33.100	37.300	41.700	46.200	50.900	30
26.000	30.000	34.100	38.500	43.000	47.600	52.400	31
26.800	30.900	35.100	39.600	44.200	49.000	53.900	32
27.500	31.700	36.100	40.700	45.400	50.300	55.400	33
28.300	32.600	37.100	41.800	46.700	51.700	56.800	34
29.100	33.500	38.100	42.900	47.900	53.000	58.300	35
29.700	34.200	39.000	44.000	49.100	54.400	59.800	36
30.500	35.100	40.000	45.100	50.300	55.700	61.300	37
31.200	36.000	41.000	46.200	51.500	57.100	62.800	38
32.000	36.800	41.900	47.200	52.700	58.400	64.200	39
32.700	37.700	42.900	48.300	53.900	59.700	65.700	40

* Conductivity values are given in millisiemens/cm

Data derived from the equation of P.K. Weyl, Limnology and Oceanography; 9,75 (1964).

VIII

Videos

VIII. A FOLDER NAME: 40 M³ RWS

Sub Folder Name: <i>Equipment 40 m³ RW</i>			
No.	File Name	Length	Description
1	Aeration ring	00:00:13	Operating aeration ring around the pump intake standpipe in a 40 m ³ RW to keep the screen clear of debris and fouling material.
2	Aeration-mixing components and FF return_40 m ³ RWs	00:00:28	View of aeration/mixing components (air diffusers, bottom manifold (aeration from nozzles), and air lift pumps) and water return from foam fractionators.
3	Operation of a small foam fractionator_40 m ³ RWs	00:05:54	Operation of small foam fractionator and solids collection; adjusting flows and some components explained.
4	Underwater view of 40 m ³ RWs & shrimp PLs—early nursery phase	00:01:06	Underwater view of shrimp postlarvae on the center partition and an air-lift pump in operation.
Sub Folder Name: <i>Manual harvest</i>			
5	Manual harvest of marketable shrimp—40 m ³ RW	00:01:27	Extended clip of dip net harvests, showing shrimp capture, into baskets, lid on baskets and weighing.
6	Manual harvest—40 m ³ RW_Capture only	00:00:18	Harvesting shrimp with dip nets into harvest baskets.
7	Weighing harvested juvenile shrimp_nursery	00:00:44	Explanation of weighing juvenile shrimp in a basket and waiting for water to drain.
Sub Folder Name: <i>Shrimp sampling 40 m³ RW</i>			
8	Juvenile full guts	00:00:19	Sampled juveniles showing full guts and intact antennae.

Continued

Sub Folder Name: <i>Equipment 40 m³ RW</i>			
No.	File Name	Length	Description
9	Sampling & shrimp jumping_40 m ³ RWs	00:01:46	Sampling shrimp with a cast net and dip net. Shrimp jumping following sampling.
10	Sampling with a cast net_40 m ³ RWs	00:01:27	Explanation of shrimp sampling with a cast net; alternative method of using cast net.
11	Shrimp sampling process explained_40 m ³ RWs	00:03:13	Explanation of whole shrimp sampling and weighing process—cast net, weighing, and counting.
12	Weighing a shrimp sample-alternative method_40 m ³ RWs	00:01:10	Weighing a shrimp sample—adding shrimp directly from a dip net to a tared bucket on a balance.
Sub Folder Name: <i>Transfer of juveniles</i>			
13	Juvenile shrimp in a hauling tank	00:00:14	Healthy juveniles in an aerated hauling tank—ready for grow-out.

VIII. B FOLDER NAME: 100 M³ RWS

Sub Folder Name: <i>Equipment 100 m³ RW</i>			
No.	File Name	Length	Description
14	a ³ injector operation & temperature manipulation_100 m ³ RWs	00:02:30	Description of a ³ injector operation and temperature control through snorkel length.
15	Cleaning the a ³ injector assembly	00:01:29	Disassembling and cleaning the a ³ injector assembly.
16	Large foam fractionator_100 m ³ RWs	00:00:36	Description of a large foam fractionator operating on a 100 m ³ raceway.
17	Large foam fractionator_100 m ³ RWs.2	00:00:36	Annotated view of a large foam fractionator operating on a 100 m ³ raceway.
18	Standpipes in 100 m ³ Raceways	00:00:22	View of screened pump intake standpipe, harvest standpipe, and discharge from FF.
19	Underwater view of 100 m ³ RWs & a ³ injector operation—early nursery phase	00:01:23	Underwater view of shrimp postlarvae and a ³ injectors in operation.
Sub Folder Name: <i>Harvest with a fish pump</i>			
20	Harvest with a fish pump_100 m ³ RWs	00:01:28	Harvest with a fish pump, displaying different components both pre- and during-operation.
Sub Folder Name: <i>Nursery 100 m³ RW</i>			
21	Clean raceway condition following nursery harvest	00:00:17	View of clean 100 m ³ raceway bottom and walls following nursery harvest with no accumulation of feed.

Sub Folder Name: <i>Equipment 100 m³ RW</i>			
No.	File Name	Length	Description
22	Gentle aeration and mixing following postlarvae stocking	00:00:26	View of gentle aeration and mixing from a ³ injectors in the 100 m ³ RWs one day after stocking postlarvae.
23	Pump intake pipes and screens during the early nursery phase—100 m ³ RWs	00:01:02	Description of pump intake pipes and different sized screens to prevent sucking postlarvae into the pump.
	Sub Folder Name: <i>Shrimp sampling 100 m³ RW</i>		
24	Shrimp sampling in the 100 m ³ Raceway 1	00:01:14	Shrimp sampling procedure 1: Sampling shrimp with a cast net, concentrating shrimp samples in a dip net; weighing in a tared bucket on a balance, counting back into the RW.
25	Sampling shrimp in the 100 m ³ Raceway 2	00:01:37	Shrimp sampling procedure 2: Sampling shrimp with a cast net, adding to a preweighed bucket, weighing on a balance, counting back into the RW, reweighing the bucket.
	Sub Folder Name: <i>Shrimp behavior</i>		
26	Shrimp feeding on surface biofloc mat	00:00:57	Adult and juvenile shrimp feeding on surface biofloc mat.
27	Shrimp jumping	00:00:20	Shrimp jumping in RW, demonstrating the need for jump-netting.
	Sub Folder Name: <i>Venturi setup</i>		
28	Dosing chlorine into a reservoir tank using a Venturi	00:01:13	Safely dosing chlorine (bleach solution) into a reservoir tank using a Venturi injector.
	Sub Folder Name: <i>Vibrio monitoring</i>		
29	<i>Vibrio</i> monitoring procedures using TCBS agar plates	00:09:51	Description of procedures for <i>Vibrio</i> monitoring using TCBS agar plates—preparation, equipment, plate inoculation, incubation, counting colony forming units, and data recording.
	Sub Folder Name: <i>Water quality monitoring</i>		
30	DO display & probe	00:00:25	View of YSI 5200 DO monitor screen and optical DO probe in a raceway.
31	Measuring settleable solids with an Imhoff cone	00:00:21	Measuring settleable solids with an Imhoff cone.

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SUSTAINABLE BIOFLOC SYSTEMS FOR MARINE SHRIMP

TZACHI MATZLIACH SAMOCHA



A how-to-manual of everything you want to know about the design and operation of high-density indoor biofloc shrimp farming

Sustainable Biofloc Systems for Marine Shrimp is a comprehensive guide that describes the biofloc-dominated aquaculture systems developed over 20 years of research at Texas A&M AgriLife Research Mariculture Laboratory for the nursery and grow-out production of the Pacific White Shrimp, *Litopenaeus vannamei*.

Sustainable Biofloc Systems for Marine Shrimp is written in a generally nonacademic style useful for all stakeholders, with special attention on entrepreneurs interested in building a pilot biofloc-dominated system. In addition to the content of its 15 chapters that cover topics on design, operation, and economic analysis, the book includes appendices that expand on relevant topics, links to Excel sheets that assist in certain calculations, and video links that illustrate important operations tasks.

Key Features

- Emphasis on the most recent trials on nursery & grow-out of *L. vannamei*
- Discussion of site selection, equipment options, and water sources
- Step-by-step guides from tank preparation through water quality monitoring and control through feeding management and harvest
- A troubleshooting table with references to relevant sections of the book
- Fifteen Chapters with appendices, and links to Excel spreadsheets & videos

Dr. Tzachi Matzliach Samocha, Consultant, a former Regents Fellow & Professor at Texas A&M AgriLife Research Mariculture Laboratory at Flour Bluff, Corpus Christi, Texas, where he conducted shrimp culture trials in raceways and ponds to develop new concepts in intensive nursery and grow-out techniques with no water exchange to include: use of halophyte for aquaculture effluent water remediation and source for oil and plant protein for human and animal consumption, and the use of microalgae for biofuel and production of by-products. He succeeded with transfer of aquaculture technology to commercial producers and industry-related businesses, which is the basis of this work.

Dr. Samocha has received 140 research grants, published more than 120 papers in peer-reviewed and popular magazines, and has won over eight awards, including the Texas Agricultural Experiment Station Vice Chancellor's Award in Excellence, the Texas A&M University System, Regents Fellow Service Award, and three times the "Researcher of the Year" award from the Texas Aquaculture Association. In addition, he presented his basic and applied research findings in more than 400 national and international conferences and workshops.



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