Biotechnology-Aquaculture Interface:

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Biosecure Zero-Exchange Systems for Intensive Shrimp Culture:

Past Experiences, Biocomplexity Challenges, Future Opportunities

Shaun M. Moss

The Oceanic Institute

41-202 Kalanianaole Hwy.

Waimanalo, Hawaii 96795

smoss@oceanicinstitute.org

ABSTRACT

Shrimp farmers have suffered significant economic losses in recent years due to disease problems that have plagued the global shrimp farming industry. To mitigate crop loss, shrimp farmers and researchers are evaluating biosecure production systems designed to minimize the introduction and spread of pathogens and protect coastal resources. A hallmark of these systems is the elimination of water exchange as a management procedure because influent water can serve as a vector for disease and effluent discharge can pollute receiving waters. An important ramification associated with zero-exchange systems is the increased importance of *in situ* microorganisms both in regulating biogeochemical cycles within the culture environment and in directly affecting shrimp growth and survival. Our ability to manipulate this microbial "black box" to enhance shrimp performance is hampered by our lack of understanding about the complex interactions among the various microbial groups and how these microbes respond to system perturbations, including the input of exogenous shrimp feeds. Tremendous opportunities exist to exploit the synergistic relationships between the microbes, the shrimp, and exogenous inputs to maximize system productivity and profitability. Molecular-based tools can provide researchers with valuable insights into microbial community structure and function. Advanced technologies designed to monitor microbial metabolic activity can assist in system management, characterize system stability, and indicate impending system collapse. With these advances, biosecure zero-exchange systems will be able to produce shrimp at levels that are unattainable with existing technologies. This would represent a significant paradigm shift for aquatic animal production that goes beyond the culture of shrimp.

KEY WORDS: biocomplexity, biosecurity, microorganisms, shrimp

INTRODUCTION

The shrimp aquaculture industry expanded significantly throughout Asia and Latin America during the 1980's and this expansion was generated largely by abundant wild seed, static supplies of shrimp from capture fisheries, and high profits from cultured shrimp (Fast and Menasveta 2000). In 1999, farmers produced an estimated 814,250 metric tons of shrimp (Rosenberry 1999), and this represents about 25% of the total shrimp production worldwide. Despite high levels of production, shrimp farmers have suffered significant economic losses in recent years due to disease problems that have plagued the industry. In Asia, mortalities of cultured shrimp due to White Spot Syndrome Virus (WSSV) and Yellow Head Virus (YHV) have resulted in economic losses of about $1 billion per year since 1994 (Lightner et al. 1998). Similarly, in Ecuador alone, Taura Syndrome Virus (TSV) has been responsible for an estimated US$400 million loss in revenue per year, and this virus has had an equally devastating impact in other shrimp farming countries of the Western Hemisphere, including the U.S. (Brock et al.1997).

Although vaccines, medicated feeds, and immunostimulants are effective in combating some pathogens in other meat-producing industries, they are either unavailable to shrimp farmers or their efficacy is unproven. Various pond management strategies have been employed to mitigate the risk of disease outbreak, including the stocking of high-health seed, reducing water exchange rates, and screening influent water. Unfortunately, these are not effective disease prevention strategies in large, outdoor production ponds. For shrimp farmers to meet the growing demand for high-quality shrimp products, novel production systems must be designed to minimize the introduction and spread of pathogenic agents, as well as to protect coastal resources. Biosecure zero-exchange systems represent an emerging technology that provides a high degree of pathogen exclusion with minimal water exchange (see the contribution by Dr. Gary Pruder in these proceedings). An important ramification associated with reduced or zero water exchange is the increased importance of *in situ* microorganisms both in regulating biogeochemical cycles within the culture environment and in directly affecting shrimp growth and survival. In this paper, I review the importance of the *in situ* microbial community in affecting shrimp physiology, describe recent results from a prototype biosecure shrimp production system, highlight R&D opportunities to exploit the synergism between microbes, shrimp, and exogenous system inputs, and indicate significant economic opportunities that lie ahead for a domestic shrimp culture industry.

BENEFITS OF A MICROBIALLY DOMINATED SHRIMP PRODUCTION SYSTEM

It has been assumed that shrimp reared in intensive production systems receive little or no nutrition from natural pond biota and are almost entirely dependent on high-quality, formulated feeds to satisfy their nutritional requirements. However, studies have shown 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 (Leber and Pruder 1988, Moss et al. 1992, Moss 1995). This growth enhancing effect of pond water is significant because there are no feed additives or ingredients that can be incorporated into formulated diets to improve shrimp performance to this extent. In addition to juvenile shrimp, pond water can enhance the growth of postlarvae 416% over growth rates attained in clear well water and growth rates of broodstock shrimp can be improved by 33%. Ontogenetic differences in the ability of shrimp to exploit the growth enhancing effect of pond water may be related to the size and structure of the shrimp's feeding appendages because growth enhancement likely results from the assimilation of organic particles produced autochthonously in the culture environment. Smaller shrimp may be able to manipulate and ingest these particles more effectively than larger shrimp.

In an attempt to characterize these growth-enhancing particles, a size fractionation experiment was conducted to compare the effects of pond water, with selected particles removed, on the growth of juvenile Pacific white shrimp, *Litopenaeus vannamei* (Moss et al. 1992). Particles were removed by passing the water through a series of mechanical filters and an activated carbon filter. In the presence of suspended particles between 0.5 and 5.0 µm, shrimp growth rates increased 53% over growth rates attained in clear well water, and particles greater than 5.0 µm improved growth by an additional 36%. Particles less than 0.5 µm, including dissolved organic matter, did not contribute to shrimp growth. In this study, almost half of the particulate organic carbon in the pond water was living carbon in the form of pennate and centric diatoms (Moss and Pruder 1995). Diatoms are readily digestible by shrimp because of their low fiber content and they generally contain high levels of nutritious polyunsaturated fatty acids. Penaeid shrimp are known to consume diatoms in the wild and in aquaculture ponds, and diatoms were probably an important dietary item for shrimp in this study. Fecal strands excreted by shrimp contained numerous empty diatom frustules, but it is unclear if diatoms were ingested live or as a component of microbial-detrital aggregates. In a laboratory feeding study, it has been shown that juvenile shrimp can survive and grow on a live monoculture of the diatom, *Chaetoceros* sp., as their sole source of food (Moss 1994). In this study, shrimp fed *Chaetoceros* grew 0.32 grams per week, which is similar to growth rates reported for unfed juvenile shrimp reared in tanks receiving flow-through pond water from an intensive shrimp pond. In addition, shrimp fed *Chaetoceros* exhibited nucleic acid concentrations and RNA/DNA ratios that were almost identical to values reported for unfed shrimp reared in the microbially rich pond water. It appears that the live diatom component of pond water contributed significantly to the growth enhancing effect reported in these pond water studies. However, the relative nutritional importance of different microbial groups is unclear, and in some systems heterotrophic bacteria and associated detritus are considered the dominant sources of microbial nutrition for shrimp (McIntosh 1999).

In addition to a direct nutritional benefit, pond water affects both the abundance and species composition of microflora in shrimp mid- and hindguts (Moss et al. 2000). Guts of shrimp reared in pond water had significantly lower numbers of aerobic bacteria and a higher aerobic gut floral diversity compared to guts of well water-reared shrimp. Although the role of gut flora in penaeid shrimp nutrition is unknown, they may contribute exogenous enzymes, supply essential compounds lacking in the shrimp's diet, or out-compete pathogenic bacteria. Pond water also stimulates digestive enzyme activity in shrimp, including serine protease, collagenase, amylase, cellulase, laminarinase, and lipase (Moss et al. 2001a). Finally, pond water affects the innate immune response in shrimp by stimulating serum agglutination in response to pathogen exposure (Primavera et al. 2000). Shrimp reared in pond water exhibited an enhanced serum agglutination response to formalin-killed *Vibrio harveyi* compared to shrimp reared in clear well water.

In the studies summarized above, pond water originated from a 337-m^2 round pond used for intensive shrimp culture. Management protocols for this pond include daily water exchanges that average about 30-50% of the total pond volume per day throughout the growout period. However, in light of the devastating disease problems currently plaguing the global shrimp farming industry, water exchange has become a risky management option for maintaining acceptable water quality. This situation has resulted in the emergence of novel shrimp production systems that rely on pathogen exclusion, including reduced or zero water exchange. With these new production systems, shrimp farmers and researchers have a unique opportunity to further exploit the *in situ* microbial community to enhance shrimp growth and survival.

BIOSECURE SHRIMP PRODUCTION

A number of commercial shrimp farms and research organizations are evaluating zero-exchange shrimp production systems. With this approach, organic particles and inorganic nutrients remain in the culture environment and inorganic nitrogen control depends on *in situ* biofiltration. From the commercial sector, Belize Aquaculture, Ltd. has taken the lead in developing a zero-exchange system using low-protein feeds and heavy aeration. As described above, this system takes advantage of microorganisms and associated detritus as a source of supplemental food for the shrimp. However, unlike the previously described experiments, there is a predictable shift in microbial community structure over the course of the growout, where mixed photoautotrophic/heterotrophic microbial communities are replaced by heterotrophic bacterial "floc". These heterotrophs assist in maintaining system stability and enable the pond to assimilate large amounts of organic inputs. The success of Belize Aquaculture represents a significant milestone for the global shrimp aquaculture industry and their approach to shrimp culture reflects an important paradigm shift from traditional shrimp farming methods. However, the production systems at Belize Aquaculture are not biosecure and the potential for a significant disease outbreak exists. In fact, it was recently reported that Belize Aquaculture has experienced a shrimp virus outbreak that will adversely affect shrimp production and profitability. In an effort to develop biosecure technologies for the U.S. shrimp farming industry, the Oceanic Institute (OI) is evaluating a prototype biosecure system for the intensive production of *L. vannamei*. The system consists of a covered, 58-m^2 raceway that allows for the continuous flow of water in a circular pattern around a central baffle. The flow of water produces a scouring velocity to keep solids in suspension. An aspirator-type aerator is used to provide both aeration and water movement. For water filtration, the system relies on a 25-ft^3 propeller-washed bead filter (PBF) for solids removal and biofiltration. Importantly, the PBF allows a sufficient amount of microalgae and other microbes to pass through it so that a "green water" environment can be maintained. In this type of environment, shrimp can take advantage of the growth enhancing effect of pond water described earlier.

Similar to outdoor pond systems, the availability of live algal cells is not constant in the OI raceway due to periodic blooms and crashes. After an algal crash, sedimented cells rapidly decompose by physical, chemical, and biological agents. However, unlike detritus derived from vascular plants, microalgal detritus is not refractory and shrimp can obtain substantial amounts of nutrients, including nitrogen, from this detrital resource. In an effort to manage algal blooms in the raceway, and as part of the operational protocol for the PBF, the PBF is periodically cleaned or backwashed to remove accumulated solids. Because of this activity, replacement water is added to the raceway at a rate of <0.5% of the total raceway volume per day throughout the growout period. Water is also added to replace evaporative loss and it is important to note that all replacement water is fresh water, so the system requires no exchange of seawater. Backwash from the PBF contains uneaten feed, feces, molts, detritus, and microorganisms that are nutritionally valuable to the shrimp. In a recent experiment, it was determined that backwash from the raceway PBF can increase shrimp growth 172% over growth rates of shrimp without access to PBF backwash, despite shrimp in both treatments receiving a commercial shrimp feed *ad libitum*. This study indicates that bead filter backwash can more than double shrimp growth and calls into question the need to remove these particles from the culture environment.

In a recent evaluation of OI's prototype biosecure system, juvenile shrimp were stocked at a density of 200/m^2 and were grown to a harvest weight of 23.3 g in 16 weeks. During this trial, shrimp grew 21.1 g, growth rate was 1.32 g/wk, survival was 85%, and production was 4.0 kg/m^2 (40,000 kg/ha/crop). This level of production is an order of magnitude greater than the mean production reported at Bowers Shrimp Farm in south Texas, and this farm is considered one of the most successful shrimp farms in the U.S. (Table 1). In addition, production in OI's biosecure system is double the production reported at Belize Aquaculture under commercial, zero-exchange conditions.

Table 1. Comparison of shrimp performance among three shrimp production systems.

System	Density (# /m^2)	Growth Rate (g/wk)	Survival (%)	Size at Harvest (g)	Production (kg/m^2)	Crops Per Year
Bowers Farm, Texas[a]	36-50	1.05	70-74	18.0	0.4 – 0.6	1
Belize Aquac. Ltd[b]	120	1.00	83	18.8	1.9	2 (?)
OI raceway[c]	200	1.32	85	23.4	4.0	2.5 –

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[a]information from *Fish Farming News*, Jan/Feb 2000, Vol. 8

[b]information from *Global Aquaculture Advocate*, Dec 1999, Vol. 2

[c]data from OI's recent trial in a 58-m^2 raceway

Importantly, in the recent trial at OI, the amount of water used to produce one kg of whole shrimp was about 370 liters, and this is two to three orders of magnitude less than what is commonly used by the existing shrimp farming industry. Typically, the amount of water used by shrimp farmers to produce one kg of whole shrimp ranges from 39,000 to 199,000 liters, with a mean value of 86,000 liters (Hopkins and Villalón 1992). Information used to determine these values came largely from semi-intensive shrimp farms and these values include exchange water plus one pond volume for drain harvest. In more intensive systems with minimal water exchange, water use per kg of shrimp produced can be reduced significantly (Table 2).

Table 2. Water use per kilogram of shrimp produced in intensive, low water exchange systems.

Shrimp Species	**Water Exchange Per Day (%)**	**Stocking Density (# /m^2)**	**Water Per Kg Shrimp (liters/kg)**	**Reference**
L. setiferus	25.0	40	64,000	Hopkins et al. 1993
L. setiferus	2.5	40	9,000	Hopkins et al. 1993
L. setiferus	0	20	6,000	Hopkins et al. 1993
L. vannamei	0	63-121	2,000	Rosenberry 1999
L. vannamei	<10.0	35	1,500	Hamper 2000
L. vannamei	< 0.5	100	483	Moss et al. 2001b
L. vannamei	< 0.5	200	370	Moss et al.

				2001b

The importance of reduced or zero water exchange can not be understated. In addition to reducing the opportunity for pathogen introduction, this approach minimizes nutrient and biological pollution of the natural environment and self-pollution of the shrimp culture environment. Clearly, results from research designed to investigate zero-exchange systems indicate that high shrimp biomass can be supported under these conditions. The intensive shrimp production systems described above all depend on a healthy and diverse microbial community to enhance shrimp performance and maintain acceptable water quality. However, critical research is needed to further exploit all of the benefits derived from the *in situ* microbial community, to identify beneficial microbes that contribute to system production and profitability, and to develop best management strategies to ensure their persistence in the system.

MICROBIAL BIOCOMPLEXITY CHALLENGES

Interrelationships between various functional groups within the microbial community are poorly understood and relationships between microbes, system inputs, water quality, and shrimp health are complex. Shrimp culture systems can exhibit rapid changes in ambient nutrient concentrations that result in unpredictable blooms and crashes of eukaryotic microalgae. Nutrient fluxes result from the periodic additions of exogenous inputs and poorly understood changes in microbial biogeochemical cycling. In addition, shifts in heterotrophic bacterial community structure can favor opportunistic pathogens, thereby creating animal health problems. A potential obstacle to increasing production and profitability of biosecure zero-exchange systems is the inability to maintain the persistence of a beneficial microbial community that enhances shrimp performance and promotes acceptable water quality. However, if such a microbial community can be identified and maintained in the culture environment throughout the growout period, shrimp production can increase to levels that are unattainable with existing technologies.

With biosecure zero-exchange systems, shrimp farmers and researchers have an opportunity to introduce a defined microbial community into the culture environment to work synergistically with the shrimp and the exogenous feeds to maximize system productivity and stability. In light of this goal, significant R&D opportunities exist to: 1) characterize microbial community structure and function in a biosecure zero-exchange shrimp production system; 2) identify a defined inoculum of "effective microorganisms" (EM) and maintain their persistence over the growout period; and 3) identify critical control variables that impact microbial community structure and function.

Most microbial populations cannot be cultured and isolated from the environment using standard microbiological techniques, and the application of these techniques to characterize microbial community structure is inappropriate. Significant opportunities exist to apply recent advances in molecular biology to characterize microbial community structure using such approaches as multiplexed, bead-based methods and DNA-chip technology for microbe identification. Information generated from these approaches will enable researchers to make inferences about microbial community function in biosecure zero-exchange systems over the course of a growout period. Successional changes in microbial community function can be made by tracking the presence or absence of key microbial groups, including photoautotrophic eukaryotes, nitrifying bacteria, denitrifiers, sulfate reducers, and pathogenic vibrios. This information will allow researchers to identify patterns of system stability, as well as evidence of impending system collapse. Importantly, information generated from this research can be used to identify candidate "effective microorganisms" for use as constituents in a defined microbial inoculum.

The concept of "effective microorganisms" (EM) was developed for terrestrial agriculture and refers to the use of mixed cultures of beneficial and naturally occurring microorganisms that can be applied as an inoculum to increase microbial diversity of soils for increased crop production (Higa and Wididana 1991). However, no attempt has been made to use this approach in aquatic animal husbandry despite the huge potential for increasing production of the target species. Candidate EM's can be selected from the *in situ* microbial community for particular characteristics, including ectoenzymatic activity, high nutritional value for shrimp, or enhanced ability to oxidize ammonia. Alternatively, an inoculum can be "created" using genetically modified microorganisms that exhibit desirable traits.

Critical control variables need to be identified to monitor the health of the *in situ* microbial community, characterize evidence of system collapse, and develop best management practices to promote maximum system productivity and stability. In addition to standard water quality parameters (i.e. pH, alkalinity, dissolved oxygen concentrations, nutrient concentrations, organic pool sizes, suspended solids concentrations, and light intensity), microbial metabolic activity can be monitored and this information can be used to develop appropriate management protocols to allow for the detection of system instability. Technologies that can monitor metabolic activity of *in situ* microbes in real time would offer significant advantages over alternative approaches that require a lag time between sample collection and the generation of useful data.

FUTURE ECONOMIC OPPORTUNITIES

Shrimp is the number one preferred seafood in the U.S., and consumer demand continues to grow each year. In 1998, per capita consumption reached a record 1.3 kg, and this represents about 20% of the total per capita U.S. seafood consumption (Rosenberry 1999). At the same time that demand for shrimp products is increasing in the U.S., domestic aquaculture production of shrimp remains stagnant and is insignificant compared to the major shrimp-producing countries in Asia and Latin America. In 1999, the global shrimp farming industry produced an estimated 814,250 metric tons of whole shrimp, whereas U.S. farmers produced only 1,500 metric tons, or 0.18% of total world production. In terms of domestic shrimp supplies, wild harvest from U.S. trawlers was only 126,100 metric tons in 1998, the lowest since 1983. As a consequence, shrimp imports in 1999 reached a record 332,000 metric tons valued close to $3.1 billion. The disparity between demand and production accounted for a $2.9 billion trade deficit in 1999. For 2000, shrimp imports were estimated to reach 318,000 metric tons with a value of about $3 billion. In light of the significant demand for shrimp products in the U.S. and a limited domestic supply, a tremendous opportunity exists for the expansion of a domestic shrimp culture industry. However, in order for such an industry to emerge, research is needed to develop and apply advanced technologies to support biosecure, zero-exchange production systems as a viable alternative to traditional shrimp farming.

The biosecure zero-exchange system described above relies on a holistic approach to shrimp production that combines the benefits of major advances used in successful meat-producing industries with novel approaches to aquatic animal husbandry. We believe that this approach will provide U.S. shrimp farmers with an economically viable approach to shrimp culture that will make them competitive with foreign producers. In a recent economic analysis (Leung and Moss 2000), it was shown that a hypothetical biosecure, zero-exchange production system would be profitable if high stocking densities (200 shrimp/m^2), survival (80%), and growth rates (1.5 grams/week) were attained. With these production parameters, a profit of $2.39/kg could be realized (assuming a farm-gate price of $12.10/kg), with a 17.7% return on investment. However, if biosecure zero-exchange systems are to compete with conventional shrimp farms, higher levels of production and higher profit margins need to be realized. Through applied research and the application of biotechnology, we can expect significant improvements in shrimp performance with a concomitant increase in profitability. For example, if stocking density, survival, and growth rate increase to 483 shrimp/m^2, 90%, and 3.0 grams/week, respectively, a profit of $5.56/kg could be realized, with a 107.5% return on investment. With these profit margins, U.S. shrimp farmers would be poised to significantly reduce the federal trade deficit in shrimp products while simultaneously creating a new industry for U.S. workers.

RECOMMENDATIONS

Short-term (1-3 years):

1. Using molecular-based approaches, characterize microbial community structure in biosecure production systems and quantify spatial and temporal variability of these communities.
2. Identify candidate "effective microorganisms" (EM) to use in a defined inoculum to increase system productivity and profitability.

Mid-term (4-7 years):

1. Using biosensor technology and fluorescence sensing instrumentation, identify critical control variables that impact microbial community structure and function.
2. Develop best management strategies to maintain the persistence of beneficial microbes contained in the EM inoculum.
3. Operate a successful, large-scale biosecure, zero-exchange shrimp production system (with a defined microbial inoculum, specialty feeds, and genetically improved shrimp) that can produce 10 kg/m^2.

Long-term (8-10 years):

1. Using transgenic technology, create a defined microbial inoculum with desirable characteristics that can out-perform an EM inoculum derived from *in situ* microorganisms.
2. Develop mathematical models that integrate interactions of inoculum microbes with exogenous system inputs so that system outputs can be predicted and maximized.

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