

Disease Prevention Strategies for Penaeid Shrimp Culture

Shaun M. Moss

The Oceanic Institute
41-202 Kalanianaʻole Highway
Waimanalo, Hawaii 96795 USA
smoss@oceanicinstitute.org

Steve M. Arce

The Oceanic Institute
41-202 Kalanianaʻole Highway
Waimanalo, Hawaii 96795 USA
sarce@oceanicinstitute.org

Dustin R. Moss

The Oceanic Institute
41-202 Kalanianaʻole Highway
Waimanalo, Hawaii 96795 USA
dross@oceanicinstitute.org

Clete A. Otoshi

The Oceanic Institute
41-202 Kalanianaʻole Highway
Waimanalo, Hawaii 96795 USA
cotoshi@oceanicinstitute.org

Abstract

Penaeid shrimp aquaculture expanded significantly over the past two decades. However, shrimp farmers have suffered significant economic losses because of viral diseases. Researchers from the U.S. Marine Shrimp Farming Program (USMSFP) have developed novel approaches to mitigate the devastating impact of shrimp viruses, including the use of specific pathogen free (SPF) and specific pathogen resistant (SPR) shrimp, as well as the establishment of biosecure production systems that rely on pathogen exclusion. These approaches have evolved over the past decade in response to changing disease problems faced by U.S. shrimp farmers. In the late 1980's and early 1990's, U.S. farmers observed Runt Deformity Syndrome (RDS), an economically significant and frequent disease problem of cultured Pacific white shrimp, *Litopenaeus vannamei*. RDS is characterized by reduced growth rates and cuticular deformities and is caused by Infectious hypodermal and hematopoietic necrosis virus (IHHNV). The increasing incidence of RDS on commercial farms catalyzed USMSFP researchers to develop SPF stocks of *L. vannamei* that were free of IHHNV. High health offspring from these SPF stocks were made available to U.S. shrimp farmers, resulting in a significant increase in U.S. farmed shrimp production from 1992 - 1994. However, in mid-1995, Taura syndrome virus (TSV) was identified in south Texas, the major shrimp farming region in the U.S., and the resulting TSV epizootic contributed to a 164% decline in Texas shrimp production from 1994 to 1995. USMSFP researchers responded by initiating a selective breeding program to develop TSV-resistant *L. vannamei*. The use of these high-health SPR stocks, in conjunction with on-farm biosecurity practices, resulted in incremental increases in U.S. shrimp production from 1998 to the present. Although TSV-resistant shrimp improved production and profitability for those farmers who were experiencing crop losses from TSV, breeding shrimp for resistance to a single viral pathogen, using current selective breeding strategies, may not be the most effective course of action for the long-term viability of the shrimp farming industry. USMSFP researchers are now developing biosecure shrimp production systems which rely on pathogen exclusion, and research results indicate that it is possible to produce > 5 kg of market-sized shrimp (~ 20 g) per m² of raceway in about 12 weeks, using < 400 L of water per kg of shrimp. With advanced biosecure technologies available, the U.S. shrimp farming industry will be able to expand into areas away from the coast with greater control against the spread of disease and without adversely affecting the environment.

Introduction

Shrimp aquaculture expanded significantly during the 1980s and now represents a multi-billion dollar a year industry. In 2002, the global shrimp farming industry produced an estimated 1.6 million metric tons of shrimp, and production is projected to increase at a rate of 12-15% per year over the next several years (Rosenberry 2003). Although farmed shrimp now represent about 50% of the global penaeid shrimp supply,

farmers have suffered significant economic losses over the last decade, largely from viral diseases that have plagued the industry (**Table 1**). In Asia, mortalities of cultured shrimp due to White spot syndrome virus (WSSV) and Yellow head virus (YHV) have resulted in significant economic losses (Flegel and Alday-Sanz 1998), and Taura syndrome virus (TSV) is now spreading throughout this region. Similarly, in the Western Hemisphere, both WSSV and TSV have caused catastrophic losses on shrimp farms (Lightner 2003). In Ecuador alone, WSSV was responsible for an estimated 53% decline in shrimp production from 1998 to 2000, resulting in a loss of export revenue in excess of \$516 million (Rosenberry 2000).

Virus	Year of Emergence to 2001	Estimated loss
WSSV - Asia	1992	\$4 – 6 billion
WSSV - Americas	1999	> \$1 billion
TSV	1991 – 1992	\$1 – 2 billion
YHV	1991	\$0.1 – 0.5 billion
IHHNV ^a	1981	\$0.5 – 1.0 billion

^a Includes Gulf of California fishery losses for 1989 – 1994.

Table 1. Estimated economic losses (in US\$) since the emergence of certain viral pathogens in penaeid shrimp aquaculture (modified from Lightner 2003).

In response to these viral pathogens, the global shrimp farming industry is changing the way shrimp aquaculture is practiced. In the United States (U.S.), researchers from the U.S. Marine Shrimp Farming Program (USMSFP) have developed novel approaches to mitigate the impact of shrimp viruses on domestic farm production. USMSFP member institutions involved in this effort include The Oceanic Institute (OI, Waimanalo, Hawaii), University of Arizona (UAZ, Tucson, Arizona), University of Southern Mississippi, Gulf Coast Research Laboratory (Ocean Springs, Mississippi), Waddell Mariculture Research Center (Bluffton, South Carolina), Texas A&M University (Port Aransas, Texas), and Tufts University (Boston, Massachusetts). Several of the approaches developed by the USMSFP have been used successfully in other meat-producing industries, and include the use of specific pathogen free (SPF) and specific pathogen resistant (SPR) stocks, as well as the establishment of biosecure production systems that rely on pathogen exclusion. Importantly, these approaches have evolved over the past decade in response to changing disease problems faced by U.S. shrimp farmers, and their evolution represents an interesting case study on the maturation of an important industry.

Pre-SPF Era for the U.S. Shrimp Farming Industry (1980's – 1991)

Although the first commercial shrimp farm in the U.S. was established in 1967, it wasn't until the late 1980's and early 1990s when the industry began to expand (Rosenberry 2003). During that time, the most commonly cultured species was the Pacific white shrimp, *Litopenaeus vannamei*, because it was considered to be highly resistant to Infectious hypodermal and hematopoietic necrosis virus (IHHNV), a member of the Parvoviridae family (Bonami et al. 1990). IHHNV was first recognized in 1981 when it was associated with catastrophic losses of cultured blue shrimp, *Litopenaeus stylirostris*, in Latin America (Lightner 1999). Despite the relative resistance of *L.*

vannamei to IHNV, shrimp farmers in the Western Hemisphere observed reduced growth rates and cuticular deformities in *L. vannamei* infected with IHNV. This condition is referred to as Runt Deformity Syndrome (RDS), and RDS represented an economically significant and frequent disease problem of cultured *L. vannamei* (Kalagayan et al. 1991). As much as 30% of cultured *L. vannamei* infected with IHNV exhibited RDS, and this reduced the market price of IHNV-infected shrimp by 10-50% relative to IHNV-free shrimp.

Establishment and Benefits of SPF Shrimp (1991 – 1994)

The increasing incidence of RDS on commercial farms in the U.S. catalyzed USMSFP researchers to develop SPF stocks of *L. vannamei* that were free of IHNV. Although the SPF concept was well established in other meat-producing industries (Zavala 2001), it had not yet been applied to shrimp. Guidelines for establishing SPF shrimp came from The International Council for the Exploration of the Sea’s (ICES) “Code of Practice to Reduce the Risks of Adverse Effects Arising from the Introduction of Non-indigenous Marine Species” (Sindermann 1990). Modifications of the ICES guidelines were used to develop the first SPF stock of penaeid shrimp for the U.S. shrimp farming industry from 1989-1991 (Wyban et al. 1993, Pruder 1994, Pruder et al. 1995). The guidelines stipulate that only disease-causing organisms that can be reliably diagnosed and physically excluded from a facility can be considered in an SPF program. The working list of specific pathogens for SPF penaeid shrimp in the U.S. has changed over time, as new pathogens have been identified and as more advanced disease diagnostic tools have become available. The current SPF list for the U.S. includes eight viruses, one prokaryote, and certain classes of parasitic protozoa (**Table 2**).

Pathogen Type	Pathogen/Pathogen Group	Pathogen Category ²
Viruses	WSSV – nimavirus (new family)	C-1
	YHV, GAV, LOV – roniviruses (new family)	C-1
	TSV – picornavirus	C-1
	BP – occluded enteric baculovirus	C-2
	MBV –occluded enteric baculovirus	C-2
	BMN – nonoccluded enteric baculovirus	C-2
	IHNV –systemic parvovirus	C-2
	HPV – enteric parvovirus	C-2
Prokaryote	NHP – α -proteobacteria	C-2
Protozoa	Microsporidians	C-2
	Haplosporidians	C-2
	Gregarines	C-3

Table 2. A working list of “specific” and excludable pathogens for penaeid shrimp.¹

- ¹ Modified from D.V. Lightner, U.S. Marine Shrimp Farming Program FY03 Progress Report.
- ² Pathogen category (modified from Lotz et al. 1995) with C-1 pathogens defined as excludable pathogens that can potentially cause catastrophic losses in one or more penaeid species; C-2 pathogens are serious, potentially excludable; and C-3 pathogens have minimal effects, but may be excluded from NBCs, multiplication facilities, and some types of farms.

To develop an SPF stock, shrimp are collected from the wild and transferred to a primary quarantine facility where they are analyzed for specifically listed pathogens using appropriate disease diagnostic tools (**Fig. 1**). If shrimp test positive for any of the listed pathogens, they are destroyed in the primary quarantine facility. If shrimp test negative for specifically listed pathogens after several successive screenings, they are transferred to a secondary quarantine facility where they are matured and spawned to produce an F₁ generation of captive shrimp. Because some viruses can be transmitted from parent to offspring (vertical transmission), representative shrimp from the F₁ generation are analyzed for specifically listed pathogens. If shrimp from the F₁ generation test negative for specifically listed pathogens after several successive screenings, they are transferred out of the secondary quarantine facility and can be included as part of the germplasm in a nucleus breeding center (NBC). Shrimp that are maintained in a well-established NBC (i.e. where there is a history of negative disease status documented through a surveillance program) may be designated as SPF (Lotz 1997). However, once shrimp leave an SPF-NBC, they no longer are referred to as SPF even though they may be free of specifically listed pathogens. If shrimp are transferred from an SPF-NBC to a medium-biosecurity facility, their new designation is High Health (HH), indicating that these shrimp are at greater risk of pathogen exposure and infection. If shrimp are transferred to a low-biosecurity shrimp farm, they have entered the Commodity Production (CP) stream, which is most vulnerable to pathogen outbreaks, and the shrimp are neither SPF nor HH.

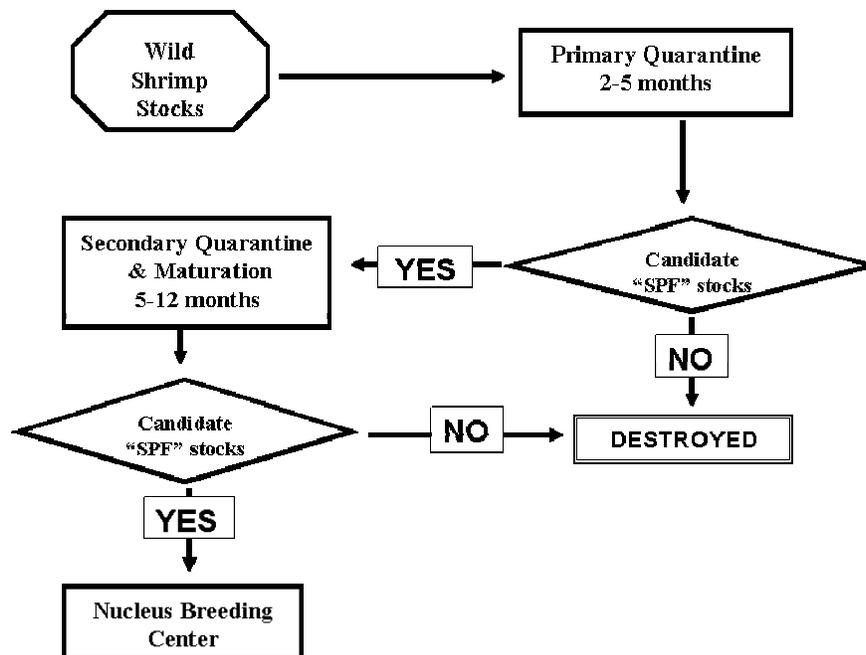


Figure 1. Procedures used to develop specific pathogen free (SPF) shrimp collected from the wild.

Initial growout trials using HH *L. vannamei* indicated that these stocks outperformed non-HH stocks when evaluated at commercial shrimp farms in the U.S. (Wyban et al. 1993). In Texas, Jaenike et al. (1992) reported that HH shrimp obtained from the USMSFP produced a greater yield, higher survival, a more uniform size distribution, and a lower feed conversion ratio than non-HH shrimp. In Hawaii, Carpenter and Brock (1992) reported that HH shrimp produced a greater yield and higher survival than non-HH shrimp when cultured under semi-intensive and intensive culture conditions. Importantly, the HH crop yielded a 62.5% higher return than non-HH crops, and similar improvements were reported in South Carolina (Wyban et al. 1993). The overall effect of using HH shrimp in the U.S. was a significant increase in production from 1992-1994 (**Fig. 2**). This huge impact was most apparent in Texas where the majority of domestic shrimp farming occurs. During this time, production increased from 1.66 million pounds in 1991 to 3.8 million pounds in 1992 and 4.2 million pounds in 1993 (Rosenberry 2003), and this represents a 153% increase in production over two years.

U.S. Farmed Shrimp Production 1988 - 2002

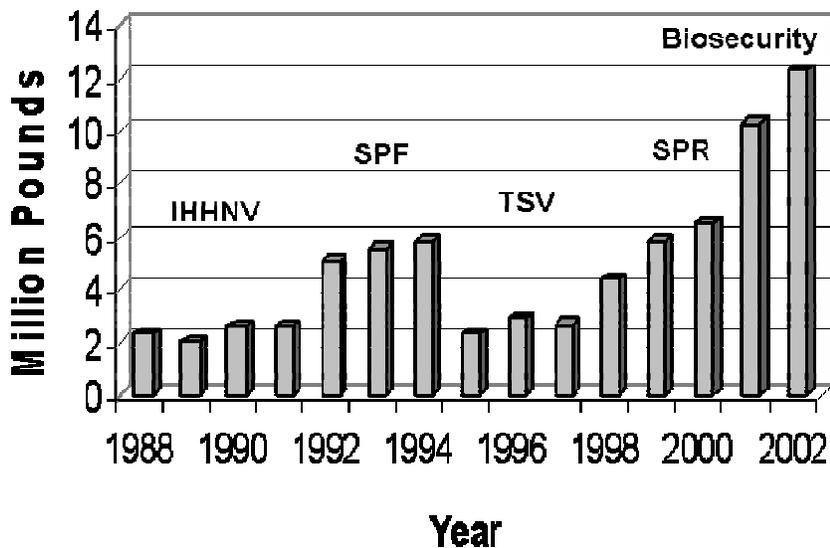


Figure 2. U.S. farmed shrimp production from 1988 – 2002.

Emergence and Impact of TSV in the U.S. (1995 – 1998)

Despite these encouraging results, HH shrimp were not a panacea for the disease problems plaguing the shrimp farming industry (Pruder 1994). In 1993, HH shrimp were cultured with wild-caught seed at a commercial shrimp farm near Rio Guayas in Ecuador. HH shrimp exhibited poor survival (7-43%) compared to wild seed (36-42%), and heavy mortalities were attributed to TSV infection. From this experience, it was demonstrated that HH shrimp cultured in environments with disease problems may not perform well. In mid-1995, TSV was identified in south Texas and the presence of this virus resulted in a significant decline in U.S. farmed shrimp production (Brock et al. 1997, **Fig. 2**). In Texas alone, shrimp production went from 3.69 million pounds in 1994 to 1.4 million pounds in 1995, a 164% decline in one year. Although shrimp production increased from

1995-1998, production levels never exceeded the pre-TSV years when SPF or HH shrimp were available.

Breeding of SPR Shrimp (1998 – present)

In response to the devastating effects of TSV on cultured shrimp in the U.S., USMSFP researchers initiated a selective breeding program to develop a TSV-resistant strain of *L. vannamei*. This approach seemed reasonable, especially in light of the tremendous improvements made through selective breeding of commercially important agriculture crops and animals (see Boyle 1999 for a review on the benefits of chicken breeding). Based on research conducted at OI since 1995, there appears to be additive genetic variation for resistance to TSV in *L. vannamei*, and significant improvements in TSV resistance have been made. In a recent research trial, shrimp selected for TSV resistance exhibited a mean family survival that was 18.4% higher than unselected control shrimp after a TSV-challenge test (Argue et al. 2002). Similar challenge tests conducted at UAZ from 1998-2000 revealed that mean survival of all TSV-challenged families increased from 24% to 39% during this period (White et al. 2002). In addition, mean survival of the best performing families increased from 65% in 1998 to 100% in 2000, and there are now commercial broodstock suppliers who claim to have families of *L. vannamei* that exhibit >90% survival to TSV (e.g. Wyban 2000). The use of TSV-resistant shrimp, in conjunction with on-farm biosecurity practices, contributed to a significant increase in production from 1998-2002 (**Fig. 2**). Again, this impact was most significant in Texas where production increased from 3.17 million pounds in 1998 to 8.27 million pounds in 2002 (Rosenberry 2003), representing a 161% increase in production over four years.

Although there is no doubt that TSV-resistant shrimp can improve production and profitability for those farmers who experience a TSV outbreak, there are compelling reasons why breeding shrimp for resistance to a single viral pathogen, using current selective breeding strategies, may not be the most prudent course of action for the long-term viability of the shrimp farming industry (Moss et al. in press). Similar to other organisms, there appears to be a trade-off between disease resistance and shrimp growth (Chevassus and Dorson 1990, Henryon et al. 2002, CENIACUA and AKVAFORSK 2002). Also, there are concerns that results from laboratory disease-challenge tests may not be predictive of survival in commercial ponds. Importantly, there are growing concerns about viral mutations, whereby previously resistant shrimp strains may become susceptible to evolving viruses. In fact, this situation occurred recently with TSV.

In 2001, significant mortalities of *L. vannamei* occurred at shrimp farms in Belize resulting from TSV epizootics (Rosenberry 2001), and there were concerns that a new TSV strain had emerged. Researchers from UAZ compared a TSV isolate from Belize with the reference isolate from Hawaii to identify possible differences, using selected OIE (Office of International de Epizooties) diagnostic methods and sequence analysis of nucleotides and amino acids in the viral genome. These researchers concluded that the two isolates exhibited different characteristics and thus represented different strains of the virus (Erickson et al. 2004). Importantly, broodstock suppliers to Belize reported that shrimp bred for resistance to the “old” Taura strain (Hawaii isolate) succumbed to the “new” Belize strain. In response to these concerns, researchers from OI and UAZ explored the possibility of developing selectively bred families of *L. vannamei* that exhibited resistance both to the Hawaii and Belize TSV strains. In a recent trial, several shrimp families from OI’s germplasm were identified as having high survival to the Belize strain, and offspring from these families were produced and distributed to U.S.

broodstock suppliers and subsequently challenged with both TSV strains (Moss et al. 2003). Selectively bred shrimp exhibited 95% survival after exposure to the Hawaii TSV. This was 75% higher than unselected control shrimp, which exhibited 20% survival after exposure to the same virus. Importantly, selected shrimp exhibited 63% survival after exposure to the Belize TSV, whereas all of the control shrimp died by day 4 of the challenge test. These results indicate that the Belize strain of TSV was more lethal than the Hawaii TSV, although it is possible to develop lines of shrimp that exhibit some resistance to both virus strains.

The Need for Biosecure Production Systems

In light of the limitations in breeding for disease resistance, selective breeding should not be perceived as a panacea for the health problems plaguing the shrimp farming industry. Rather, the industry needs to adopt strict biosecurity protocols to ensure its future. On-farm biosecurity strategies were rapidly adopted by U.S. shrimp farmers in the aftermath of the TSV epizootic in Texas in 1995, and included reduced water exchange rates, filtering of pond influent, drying out of ponds over the winter, and screening of postlarvae for diseases. Although these biosecurity measures, along with the use of HH-SPR shrimp, contributed significantly to the increase in U.S. shrimp production from 1998-2002, diseases continue to impact the domestic shrimp farming industry. Recently, WSSV was reported in Hawaii and TSV has emerged again in south Texas. In addition, necrotizing hepatopancreatitis (NHP) continues to be problematic in Texas, for shrimp farmers, as its seasonal appearances have been ongoing since the mid-1980s (Pantoja et al. 2003). Although no shrimp viruses were detected in Texas in 2002, necrotizing hepatopancreatitis (NHP) continues to be problematic, as its seasonal appearances have been ongoing since the mid-1980s (Pantoja et al. 2003). For shrimp farmers to meet the growing demand for high-quality shrimp products, novel production systems and management protocols must be designed to minimize the introduction and spread of pathogenic agents, as well as to protect coastal resources. Biosecure shrimp production systems represent an emerging alternative to traditional shrimp culture, and provide a high degree of pathogen exclusion with minimal water exchange. In an effort to develop biosecure technologies for the U.S. shrimp farming industry, several researchers from the USMSFP are evaluating prototype systems that may have commercial application (Browdy and Bratvold 1998, Moss et al. 1998, Ogle and Lotz 1998, Samocha and Lawrence 1998).

OI's system consists of a concrete 58-m² raceway that is filled with seawater (34 ppt) from an underground aquifer to a depth of about 60 cm. A 2-HP, aspirator-type aerator is used to provide aeration and to move water in a circular pattern around a central baffle. Water flow produces a scouring velocity to keep solids in suspension. For filtration, a 25-ft³ propeller-washed bead filter is used for solids removal and biological filtration (Malone et al. 1998). The bead filter allows a sufficient amount of microalgae and other suspended particles to pass through so a "green water" environment was maintained. This is important because shrimp reared in water with high concentrations of microalgae and microbial-detrital aggregates grow better than shrimp reared in clean, filtered seawater (Moss 2002). Clear, plastic sheeting (6 mil) is used to cover the raceway as a biosecurity feature to reduce pathogen introduction by airborne vectors. The cover also serves as an effective thermal insulator to maintain desirable water temperatures.

Shrimp production data from this system are encouraging. In a recent trial, juvenile shrimp were stocked at a density of 300/m² and were grown to a harvest weight

of 19.9 g in 12 weeks (Moss et al. 2002). During this trial, shrimp growth rate was 1.47 g/wk, survival was 86.3%, and production was 5.2 kg/m² (52,000 kg/ha/crop equivalent). Importantly, the amount of water used to produce one kg of whole shrimp was about 352 L, and this is two to three orders of magnitude less than what is commonly used by the existing shrimp farming industry. In 1992, Hopkins and Villalón reported the volume of water used by farmers to produce one kg of whole shrimp ranged from 39,000 to 199,000 L. Information used to determine these values came largely from semi-intensive shrimp farms where liberal water exchange was a common management protocol. Over the past decade, the global shrimp farming industry has made a concerted effort to reduce the amount of water used during shrimp growout. The primary impetus for this change came from efforts to mitigate the introduction of pathogens into the shrimp culture environment, although the collateral benefits of reduced effluent discharge also were recognized. Over the past several years, research institutions and commercial shrimp farms have evaluated intensive shrimp production systems that rely on reduced or zero-water exchange and results indicate that it is possible to reduce significantly the amount of water used to culture shrimp (**Table 3**).

Table 3. Amount of water used to produce one kilogram of whole shrimp. Data are from research institutions and commercial shrimp farms that culture shrimp under intensive conditions and rely on reduced or zero-water exchange.

Species	Water Exchange (%/Day)	Stocking Density (Shrimp/m ²)	Water Use (L/kg shrimp)	Reference
<i>L. setiferus</i>	25.0	40	64,000	Hopkins et al. (1993)
<i>L. setiferus</i>	2.5	40	9,000	Hopkins et al. (1993)
<i>L. setiferus</i>	0	20	6,000	Hopkins et al. (1993)
<i>L. vannamei</i>	0	63-121	2,000	Fast & Menasveta (2000)
<i>L. vannamei</i>	< 10.0	35	1,500	Hamper (2000)
<i>L. vannamei</i>	< 0.5	100	483	Moss et al. (2002)
<i>L. vannamei</i>	< 0.5	200	370	Moss et al. (2002)
<i>L. vannamei</i>	< 0.5	300	352	Moss et al. (2002)

Conclusions

Results from USMSFP research indicate that it is possible to produce > 5 kg/m² of market-sized shrimp (~ 20 g) in a biosecure production system in about 12 weeks, using < 400 L of water per kg of shrimp. Although these results are encouraging, the application of this capital-intensive technology only makes sense if shrimp can be produced at a competitive price. Unfortunately, from June 2000 to June 2003, the Urner Barry shell-on white shrimp index dropped from US\$6.50 per pound to less than US\$3.50 per pound (Rosenberry 2003), thus making it very difficult for U.S. shrimp farmers to make a profit. The good news is that per capita shrimp consumption in the U.S. reached a record level in 2002 to 3.7 pounds. It is hoped that, with advanced

biosecure technologies available, the U.S. shrimp farming industry will be able to meet these growing market needs by providing consumers with a high-quality product at a competitive price. Such technologies will allow shrimp farmers to expand shrimp production into areas away from the coast with greater control against the spread of disease and without adversely affecting the environment.

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