Developing Specific Pathogen-Free (SPF) Animal Populations for Aquaculture: A Case Study for IHHN Virus of Penaeid Shrimp

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Abstract

To secure specific pathogen-free (SPF) broodstock for integration into nuclear breeding operations, the utmost care must be used to detect pathogens and to prevent the introduction of pathogens. Confidence in the SPF status of a founder population increases with the development of a lengthy history of negative diagnostic results and increases with the variety and sensitivity of the diagnostic methods employed. The relatively small number of animals needed and the finite time frame makes founder population acquisition easier than securing SPF seed. Wild stocks can be sources of SPF animals; however, aquaculture activities have spread pathogens to wild stocks, diminishing the supply of wild SPF animals. The procedures employed to secure SPF populations from wild stocks can, in principle, be extended to extract SPF individuals from contaminated populations.

Introduction

Since the discovery and subsequent description of infectious hypodermal and hematopoietic necrosis virus (IHHNV) (Lightner et al., 1983a), the virus has been a major force in determining the activities and directions of the U.S. shrimp farming industry. In fact, the widespread use of Penaeus vannamei rather than the faster growing P. stylirostris is largely because P. vannamei is more resistant to IHHNV-induced mortality.

IHHNV is a parovirus (Bonami et al., 1990) and its course of infection in P. stylirostris has been well documented (Bell and Lightner, 1984; 1987). Juveniles with acute IHHN disease exhibit reduced feeding, motting of the cuticle, unusual swimming behavior, and, ultimately, 80 - 95% mortality. The infection in P. vannamei, on the other hand, induces no clear signs of disease and no dramatic increase in mortality. Hence, P. vannamei was considered an asymptomatic carrier of the virus (Lightner et al., 1983b).
However, recently there has been evidence that IHHNV infections can cause disease syndromes in *P. vannamei* under typical aquaculture conditions. In 1989, an extensive epizootic of IHHNV infection in *P. vannamei* occurred throughout the U.S. shrimp farming industry as well as elsewhere in the Western Hemisphere. At the same time, there was an increase in reports of runting, deformities and decreased production on farms. In 1991, Kalagayan et al. linked IHHNV to runt-deformity syndrome (RDS) by demonstrating the presence of RDS in IHHNV-infected animals but not in IHHNV-free animals. Although the precise relationship between IHHNV and RDS has yet to be detailed, it is clear that IHHNV can have a severe negative impact on cultured *P. vannamei*. Concomitant with the demonstration that IHHNV can cause serious diseases in aquacultured penaeids, concern over the possible release of the exotic virus into U.S. coastal waters grew.

These two factors resulted in an effort within the United States to control the spread of IHHNV. The Gulf Coast Research Laboratory Consortium (GCRLC) is responsible for this effort. The GCRLC was formed in 1984 to accelerate the development of marine shrimp farming in the United States through research and technology transfer. It is comprised of six institutions working cooperatively (Fig. 1) and is funded by the United States Department of Agriculture.

In 1989, the six institutions comprising the GCRLC undertook to disinfect contaminated facilities at member institutions (four of the six had been contaminated with IHHNV), and re-stock them with IHHNV-free *P. vannamei*. A source of IHHNV-free *P. vannamei* had been located in Mexico by

![Figure 1. Institutional members of the Gulf Coast Research Laboratory Consortium.](image-url)
researchers at the University of Arizona. The strategy was to relocate IHHNV-free postlarvae to an isolation facility, grow them to broodstock, and produce postlarvae. The IHHNV-free postlarvae could then be provided to member institutions. In the summer of 1989, the sources of IHHNV-free animals in Mexico were infected with IHHNV. Lightner et al. (In press) have documented the introduction and spread of IHHNV throughout the aquaculture industry in the northern half of Mexico. In addition, Pantoja-Morales and Lightner (1991) have confirmed the introduction of IHHNV into the wild stocks of P. vannamei, P. stylirostris and P. californiensis of the Gulf of California. Lightner (pers. comm.) has also determined the existence of IHHNV in wild P. vannamei and P. occidentalis from the coast of Ecuador. Finally, Lotz et al. (1991) also found IHHNV in wild P. vannamei and P. stylirostris postlarvae in the Gulf of California and throughout the Pacific coastal waters of Central America (Figs. 2 and 3).

No animals whose status relative to IHHNV is unknown are allowed into clean facilities. Hence, a domestic supply of certified IHHNV-free animals was needed. The shrimp would need to be subjected to acute quality control measures, and a lengthy history of aggressive and repeated diagnostic testing for IHHNV would need to be developed. Eventually, a plan was developed for obtaining and maintaining a central supply of IHHNV-free animals that would be protected from IHHNV.

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**Figure 2.** Map of Mexico, Central America, and northern South America showing the distribution of Penaeus vannamei and Penaeus stylirostris. The two species have co-extensive distributions in the area shown.

**Figure 3.** The distribution of IHHNV and BP in penaeid shrimp. The viruses have co-extensive distributions. Shaded areas represent the occurrence of IHHNV and BP. Unshaded areas represent regions which have not been surveyed.
as well as other pathogens and from the dangers of genetic inbreeding. The plan has been initiated by the GCRLC, and the resulting program may be viewed as the initial phase in the domestication of _P. vannamei_.

**Founder Populations**

The nucleus of the protected stocks is kept in strict isolation and managed under a protocol to maintain sufficient genetic diversity and allow for the judicious selection of desirable production traits. The operation of the nuclear breeding facility is detailed in Wyban (this volume) and will not be dealt with further here. Instead, I will focus on the efforts of the GCRLC to secure SPF populations for the start or enhancement of stocks in the nuclear breeding facility. Populations destined to be incorporated into the nuclear breeding facility are referred to as founder populations.

The goal of any founder population acquisition is to provide specific pathogen-free shrimp that can increase the genetic diversity of the stock held at the nuclear breeding facility. Therefore, it would be desirable to secure several founder populations of shrimp from distinct geographic locations throughout the natural range of _P. vannamei_. The more animals that are obtained from more sites, the greater the increase in genetic diversity at the nuclear breeding facility. However, one must also consider what can actually be acquired and subsequently maintained. Attention to the resources available, the biology of the shrimp, and the experiences of animal breeding programs in poultry, salmon, and swine caused us to seek approximately 12 separate male-female crosses from each of three to ten sites. Therefore, the total number of animals needed (if collected from ten sites) is 240 (120 males and 120 females). Once these animals are in place, no more importations are necessary.

The small number of animals to be secured and the finite number of founder populations necessary to accomplish the goal sets this type of activity apart from the more extensive and continuous import of animals for general seed acquisition or production. Supplying animals to found a nuclear breeding facility entails low volume, but necessitates high confidence in assuring that pathogens of interest are absent from all 240 animals.

**Founder Population Acquisition**

The initial step in securing founder populations is to determine which pathogens and potential pathogens should be excluded. The next step is to locate potential sources of founder populations, and the third step is collecting the potential founder populations. The fourth step is to ensure the candidate founder populations are free of all pathogens that are to be excluded.

**Pathogens to be Excluded**

It is essential to itemize the specific pathogens to be excluded from the
founder populations. Goals such as "disease-free" are too general to be useful. No animal populations can be maintained free of disease; the term "disease" includes maladies of unknown and unforeseen causes. It is not possible to exclude agents that are unknown. Because new agents are being described from penaeid shrimp regularly, it is probably unavoidable that a new agent will eventually be detected in established nuclear breeding stocks.

Further, no animal population can be maintained free of a pathogen that is undetectable or effectively undetectable. For example, the reo-like viruses presently are only detectable with the aid of a transmission electron microscope. To undertake the development of reo-free shrimp stocks would be premature. Nevertheless, it is of paramount importance that all pathogens in the stock be known, including those that may not actually be on the list of pathogens to be excluded. As the nuclear breeding stocks become more valuable to an industry, expensive measures may be justified to rid these stocks of a new agent.

Known obligate parasites of unknown effect should be excluded if possible. (In this paper, "parasite" is defined as any organism that uses another organism as its habitat. This includes bacteria and viruses). In general, it is not good practice to leave a parasite off the exclusion list simply because its effect is unknown; such an agent may become a problem in the future. A parasite that turns out to be a problem in a nuclear breeding stock is much more serious than the same agent in a farm pond. The investment in the nuclear breeding stock is too great to risk not excluding poorly understood parasites.

It is important that all pathogens on the exclusion list can be excluded from the populations. Attempting to exclude organisms such as Vibrio sp., facultative parasites of shrimp and humans that are ubiquitous in shrimp growout ponds and tanks, would be futile. However, it might be possible to exclude a serotype of a particular species of Vibrio, e.g., one of the human pathogenic Vibrio cholerae biovars.

On the other hand, some pathogens can be easily excluded from a population while in quarantine. For example, tetraphylidean cestode larvae are common in wild penaeids. The definitive hosts for these animals are elasmobranch fish; skates, for example. Since shrimp ponds do not have skates in them, the life cycle is not completed and the parasite is eliminated. Gregarines, which often use bivalves as intermediate hosts, are a potentially serious problem. While some intermediate hosts may be found in shrimp ponds, our experience with the gregarine Nematopsis penaeus from penaeid shrimp in the Gulf of Mexico suggests that the shrimp lose their infections several days after relocation to a quarantine tank.

The actual list of pathogens to be excluded also depends upon the species of focus and the geographic area under consideration for stock acquisition.
With the extensive movement of shrimp species around the world for aquaculture purposes, the geographic range of a particular pathogen may be less important than the species of shrimp under consideration.

**Pathogens of *Penaeus vannamei***

In this section, I will outline the pathogens considered for the exclusion list by the GCRLC. More detailed accounts of the pathogens may be found in Lightner (1983) or Sinder mann and Lightner (1988). Although viral pathogens are our primary concern, we considered many other disease agents. Despite our attention to numerous agents, however, prospective founder populations were invariably destroyed as a result of contamination with one of two viruses, IHHNV or Baculovirus *penaei* (BP).

**Viruses.** The main group of organisms targeted for exclusion from the founder populations are the viruses. Viruses are the most widespread of the serious pathogens of *P. vannamei*, and there are no proven therapeutics to eliminate viruses from infected animals. The initial focus was on IHHNV and BP because these are serious pathogens and they were likely to be encountered frequently. They were, in fact, the most common pathogens encountered. We also considered the other known baculoviruses (occluded and nonoccluded) and the parvo-like viruses to be unacceptable. However, with the possible exception of HPV (hepatopancreatic parvo-like virus), it was unlikely that we would find other known baculoviruses or parvo-like viruses in *P. vannamei*. The reo-like viruses (Krol et al., 1990) were not placed on the exclusion list because no reasonable diagnostic methods exist.

**Bacteria.** Most shrimp bacterial problems are caused by secondary invasion by free-living bacteria. Targeting these for exclusion would be fruitless. Furthermore, it is extremely difficult to distinguish between bacterial infections acquired in quarantine from those acquired in nature. Were bacteria to become a problem, it is likely that we could have eliminated them during quarantine by the judicious use of antibiotics.

The only bacterial agents we considered as potential primary parasites of shrimp are the rickettsia-like organisms. We therefore screened animals for hepatopancreatic granulomas that might have indicated an infection with rickettsia-like organisms (Krol et al., 1991). Founder populations containing rickettsia-like organisms would have been destroyed.

**Fungi.** Although fungi (e.g., *Fusarium solani*) are of concern in shrimp culture, they are not considered primary pathogens of shrimp (Lightner, 1988) and were, therefore, not to be excluded. However, the presence of a fungus in a sample may have precluded its incorporation into the nuclear breeding stock as it may have portended other stress-related problems.

**Protozoa.** The fouling protozoan ciliates (peritrichs such as *Zoothamnium*
sp., suctorian such as *Acinetia* sp., and apostomes such as *Hyalophyza* sp.) were not listed for exclusion. However, a heavy infestation could indicate stress in the quarantine facility and might have precluded the inclusion of a sample into the nuclear stocks for general health considerations.

Microsporans (e.g., *Amesia* sp., *Agmagoma* sp., *Pleistophora* sp. and *Theoharina* sp.) were placed on the exclusion list. However, they occur at low prevalence in wild populations, and infected individuals are easily detected and removed from a population during quarantine. In addition, there is some evidence that a piscine primer host may be necessary to allow transmission from one shrimp host to another (Iversen and Kelley, 1976). Microsporans, therefore, could have been eliminated from a contaminated population during the quarantine period because transmission would have been prevented by the absence of fish. The removal of affected individuals would have prevented pathogens from leaving the quarantine facility.

Gregarine protozoans may be of concern in some aquaculture settings. However, they are apparently eliminated spontaneously from shrimp during the quarantine phase. The exclusion of intermediate hosts from quarantine facilities prevents transmission. Therefore, gregarines were not placed on the exclusion list.

**Helminths.** We did not consider that helminths (cestodes, trematodes, and nematodes) needed to be excluded. The exclusion of intermediate hosts from the quarantine and nuclear breeding facilities would prevent transmission and eliminate most helminths.

**Crustaceans.** Crustaceans were not placed on the list. Crustaceans such as bopyrid isopods could have been eliminated by removal of infected individuals. These parasites also utilize an intermediate host (e.g., copepods), making transmission more difficult in quarantine.

**Locating Potential Sites for Acquisition**

Once a list of pathogens to be excluded was developed, the process of screening possible sources of founder populations began. The two options were culture facilities and wild populations. Culture facilities are often contaminated with IHNV as well as BP throughout the range of culture of *P. vannamei*. Therefore, we surveyed wild populations for the presence of the two agents of primary interest.

**Detection of Viruses**

All animals were screened for viruses by means of standard histological examination (Bell and Lightner, 1988). IHNV was detected by the presence of Cowdry Type A intranuclear inclusions in several tissues of ectodermal or mesodermal origin (Lightner et al., 1983a). BP was detected by the presence of intranuclear polyhedral occlusion bodies in cells of the hepatopancreas (Couch, 1974).
The sampling protocol prescribed that initially a site be screened by collecting a small grab sample of animals from the area, fixing the animals for histological examination while still in the field, and subsequently examining them for the presence of IHHNV and BP. If a sample was not positive after the examination of ten to 20 animals, then a large batch of live animals was to be obtained and placed into quarantine. In practice, however, the grab samples and the live samples were taken simultaneously wherever possible. Therefore, samples for examination were a combination of direct samples of wild postlarvae and adults, and a large number of wild postlarvae held in quarantine for 30 to 60 days.

We used postlarvae instead of juveniles or adults for several reasons. First, postlarvae are easier to handle and maintain in quarantine, allowing us to screen a larger number of animals. Second, because animals must be sacrificed for examination, we needed a large enough sample to insure that we would have animals left over. Third, postlarvae or young juveniles often show diagnostic signs of the two viruses better than older juveniles or adults (Bell and Lightner, 1987; LeBlanc and Overstreet, 1990).

If IHHNV was not detected after 60 days in quarantine, a bioassay diagnosis for IHHNV was performed. An IHHNV bioassay diagnosis consists of feeding a sample of *P. tannaei* that is suspected of carrying IHHNV to the more susceptible *P. stylirostris* (Lightner et al., 1983b). After nine to 30 days, any infected *P. stylirostris* will show mortality and the Cowdry Type A intranuclear inclusions characteristic of IHHNV infection.

Assuming animals were negative for IHHNV after a “styrostris-bioassay,” the next step was to ship the remaining animals to a quarantine facility in Hawaii for further examination and clearing. From there, introduction into the nuclear breeding facility was to begin.

Direct integration of the founder population into the nuclear breeding facility is an option. However, in general, it is more prudent to grow the new population into broodstock and introduce tested offspring as families into the nuclear breeding facility.

The absence of a pathogen from a potential founder population can only be assured “to-the-best-of-our-abilities.” Thus, for certification, it is necessary to specify the methods of detection used, the number of times the diagnosis was applied, the number of animals to which the diagnosis was applied, and the length of quarantine. Confidence in the absence of a pathogen increases with increased sensitivity of the diagnostic techniques, greater number of tests performed, greater number of animals checked, and longer periods of quarantine.

**Quarantine Facilities and Procedures**

The primary means of assuring that a pathogen is not present in a founder
population is to develop a lengthy history of negative diagnostic test results. Quarantine is the crucial step in developing an appropriate history.

Quarantine serves three functions. An individual may have been infected with the pathogen of interest only recently, and, therefore, may not have developed the signs of infection. In this case, the quarantine period should provide the time necessary for the shrimp to develop signs of infection. Second, some infectious agents such as BP are more likely to be found if an infected host is stressed (Couch, 1974). Quarantine is usually stressful and can provide such a stimulus. Third, the quarantine procedure can amplify a disease agent within the quarantined sample. Infectious agents present in a small number of animals will eventually be transmitted to uninfected individuals. The parasite becomes more prevalent and can be detected by examining fewer individuals.

The danger in quarantine is, of course, that the quarantined populations are open to exposure and subsequent infection with important pathogens. The use of quarantine requires extremely tight quality control and precautions to prevent contamination of samples.

Quarantine Facilities

The quarantine facility and procedures used at the Gulf Coast Research Laboratory (GCRL) in Ocean Springs, Mississippi, U.S.A., will be examined. The two main goals of the quarantine facilities are preventing the contamination of samples with pathogens from surrounding areas and animals, and preventing the contamination of surrounding grounds and waters with exotic species and their pathogens.

The quarantine facilities at GCRL consist of a large greenhouse (15 m x 9 m) sited on a concrete slab with an 8-cm high lip completely surrounding the slab (Fig. 4). The greenhouse contains 15 2,000-L circular tanks. Each 2,000-L tank has an in-tank biofilter and can be drained individually into effluent PVC drain pipes. The effluent pipes empty into septic tanks equipped with chlorinators. From the chlorinating septic tank, the effluent can be pumped to the municipal sewer line. Contamination between tanks is prevented by operational procedures and the quality of the maintenance crew. The crew is well trained in isolation, sanitation, and disinfection procedures.

The other facility used for quarantine is a 3 m x 5 m isolation room that can accommodate four 650-L circular tanks. Each tank is separated from the others by shower curtains and each tank has its own in-tank biofilter. The tanks are not drained during the holding period and the water is chlorinated at the end of the holding period. Each tank has its own maintenance equipment: thermometer, nets, beakers, etc. The room has a center floor drain that empties into an outside septic tank equipped with a chlorinator. From the chlorinating septic tank, the effluent can be pumped to the municipal sewer line.
Cross contamination is prevented by operational procedures.

We employ sentinel tanks containing uninfected animals whenever possible. For example, we presently use sentinel tanks and aquaria containing IHHNV-free _P. stylirostris_ as a check against contamination. The sentinel tanks are treated as the other quarantine tanks and are subjected to the routine procedures employed for the other quarantine tanks.

**Quarantine Procedures**

To minimize the risk of releasing exotic organisms during quarantine activities, all effluent water is disinfected. Disinfection is accomplished with 100- to 200-ppm chlorine for 1 - 24 h prior to discharge into the municipal sewer lines that terminate at a landfill. All dead animals, molts, feces, etc. are disinfected with chlorine- or iodine-containing disinfectants or by autoclaving. Surfaces are cleaned with chlorine- or iodine-containing disinfectants.

To ensure that infectious diseases are not introduced into the quarantine facilities or transferred between tanks, we established routine sanitary work practices. These include restricted access, the use of foot baths at the entrances to all doors, regular cleaning and disinfecting of equipment and rooms, disinfection of shrimp waste and debris, and clean food preparation areas.

*Figure 4. Floor plan of the greenhouse quarantine facility at the Gulf Coast Research Laboratory.*
Most introductions and transfers of pathogens occur as a result of the transport of shrimp or shrimp parts. Therefore, special attention is paid to prevent the transfer of tank contents from one to another; similarly, equipment is segregated by tank with no overlap in use. Dry feed is kept away from shrimp and shrimp debris. Each tank has its own nets, and nets are disinfected after each use and allowed to dry completely between daily cleanings. Surgical gloves are used during routine tank cleaning, and gloves are disinfected and discarded after each tank is cleaned. Natural water is used in the facility; it is routinely settled, filtered and disinfected with chlorine.

The SPF Status of Wild Penaeus vannamei

The geographic range of *P. vannamei* extends from the northern Gulf of California to the northern portion of Peru (Fig. 2). Through the efforts of the GCRLC, the status of wild *P. vannamei* has been documented along most of its range (Lotz et al., 1990; Lightner et al., In press). The most widespread of the agents of interest is, unfortunately, the virus of most crucial interest: IHHNV.

Figure 3 shows the distribution of IHHNV in wild penaeids throughout the Pacific Coast of the Americas. The highest density of IHHN virus is in the Gulf of California, where all of the specimens in a sample of wild, adult *P. stylirostris* were found to be IHHNV-positive. It is significant that this area has one of the highest densities of *P. stylirostris* anywhere throughout its range. The range of *P. stylirostris* is coextensive with that of *P. vannamei*.

The Gulf of Panama also appears to have high levels of IHHNV, but lower than the Gulf of California. This area yielded fixed-in-the-field samples of *P. vannamei* postlarvae with IHHNV infections. The lowest density of IHHNV appeared to be in the northern portion of Central America. No positive fixed-in-the-field samples were found from Guatemala, Nicaragua, or northern Costa Rica; however, virus was detected in samples from Nicaragua and El Salvador after 30 to 60 days of quarantine. Southern Mexico has not been surveyed; its status is unknown. The waters surrounding Ecuador have yielded evidence that IHHNV is present in wild populations, but surveying has not been as extensive as that in Central America and Northern Mexico.

The survey of shrimp of the Pacific Ocean revealed the presence of numerous parasites in addition to the viruses. Peritrich and apostome ciliates were common on the gills. Gregarines were seen in the intestines. Finally, nematode, cestode, and trematode larvae were observed in the hepatopancreases, muscles and nerve cords of infected shrimp.

Acquiring SPF Stock From Contaminated Sources

Acquiring SPF animals from geographic areas free of the pathogens of interest is simply a matter of collecting
samples and subjecting them to the process of certification. However, if no pathogen-free sites are found, acquiring SPF animals is more complicated.

The data acquired from the survey throughout *P. vannamei*’s geographic distribution revealed that no area was unaffected by IHHNV. Since the widespread outbreak of IHHNV in 1989, no IHHNV-free potential founder populations have been found, despite extensive efforts and the quarantining of numerous samples of wild postlarvae. Therefore, our original objective of locating IHHNV-free geographic sites had to be reformulated. Instead of seeking IHHNV-free populations of shrimp, we now wish to secure a certain number of IHHNV-free individuals from populations of shrimp which are known to carry the virus.

When we conducted our survey using quarantine, it was desirable to collect as large a sample of shrimp as possible in order to be certain that the region was free of the virus. Our target for sampling postlarvae under the initial survey objective was to quarantine 10,000 to 20,000 animals for each sample. If one of those 10,000 to 20,000 animals was infected, then, in time, a large proportion would become infected and the virus would be easily detected after 60 days of quarantine. Presently, there is no way to select individual postlarvae from a contaminated sample. Consequently, if one animal in a sample is positive for the virus, the whole sample is destroyed.

Our inability to detect virus from some areas prior to 60 days in quarantine suggests that only a few animals were carrying the virus. If only a few animals from a given wild population carry the virus, a small sample containing 1,000 to 5,000 shrimp may be virus-free. If the prevalence of an agent in the wild is known, securing animals free of the agent is a statistical sampling problem. What is the optimum sample size to be certain no infected animals are present in a given sample?

The above problem is related to the problem fish inspectors face determining how many fish to examine from a particular batch to have a certain degree of confidence that they will find the pathogen. The American Fisheries Society “blue book” provides a table that recommends how many fish should be examined (Amos, 1985). For example, if a parasite is present in 10% of fish and the lot being evaluated contains 4,000 fish, 27 should be examined in order to be at least 95% confident that the pathogen will be detected, if present. The answer to the fish inspectors’ problem is determined from the hypergeometric statistical distribution and applies to sampling small target populations.

When the target population is very large (e.g., a wild population of shrimp) then the related binomial distribution applies. Table 1 provides the maximum sample sizes that ensure at least a 50% chance that no animals in the sample are infected with the pathogen of interest. Once the maximum
Table 1. Sample size that ensures a 50% chance of a parasite being absent from a sample.

<table>
<thead>
<tr>
<th>Prevalence in source population</th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>50.0% 1/2 infected</td>
<td>1</td>
</tr>
<tr>
<td>20.0% 1/5 infected</td>
<td>3</td>
</tr>
<tr>
<td>10.0% 1/10 infected</td>
<td>6</td>
</tr>
<tr>
<td>5.0% 1/20 infected</td>
<td>13</td>
</tr>
<tr>
<td>1.0% 1/100 infected</td>
<td>68</td>
</tr>
<tr>
<td>0.5% 1/200 infected</td>
<td>138</td>
</tr>
<tr>
<td>0.1% 1/1,000 infected</td>
<td>692</td>
</tr>
<tr>
<td>0.05% 1/2,000 infected</td>
<td>1,385</td>
</tr>
<tr>
<td>0.01% 1/10,000 infected</td>
<td>6,931</td>
</tr>
</tbody>
</table>

acceptable sample size is determined, how many separately packaged samples should be obtained to be 95% certain at least one sample is free of infection?

If there is a 50% chance (probability = 0.5) that an infected animal is present in a sample, the probability that it is present in both of two such samples is

\[ 0.5 \times 0.5 = 0.25 \ (25\%) \]

and, therefore,

\[ 100\% - 25\% = 75\%. \]

Hence, there is a 75% chance that the pathogen of interest is absent from either one or both samples. If three such samples are collected, the chances are that 87.5% of the time one of the three samples will have no infected individuals. Through this process, it is clear that if a parasite infects 50% of the packaged samples, one should collect five separately packaged samples to ensure that at least 95% (actually 96.8%) of the time an area is sampled, one of the five samples will be free of infected individuals. The likelihood of finding uncontaminated packages increases as more packages are collected.

Once the size of the sample and the number of samples to be collected is determined, then the collection of animals and the lengthy procedure of quarantine and subsequent development of the critical SPF history begins. Because shrimp will be selected from contaminated areas, the development of SPF histories is critical.

The ability to select animals from the wild rests on the assumption that not all animals from a contaminated wild population carry the pathogen. In principal, obtaining SPF individuals from contaminated culture facilities is the same as obtaining them from wild sources. However, the likelihood that there are specific pathogen-free individuals in a facility is reduced because the animals in culture are at higher densities than in nature. Hence, the
rate of transmission of pathogens is higher. In facilities with a history of IHNV, the prevalence of infection can be 100%, and is typically higher than that in most wild stocks carrying IHNV. Obtaining SPF animals from a contaminated facility would, therefore, probably necessitate screening individual shrimp.

Screening Individual Animals

If the technology to reliably screen individual animals was available, the necessity of clearing a whole sample of animals would be eliminated. In the batch quarantine method, an entire batch is rejected if one positive animal is found. However, screening individuals one at a time could yield a large number of negative animals, even if the prevalence of the virus is greater than 50%.

One problem associated with individual screening is the need to take a piece of the animal for histological evaluation. Postlarvae are too small and delicate to do this without sacrificing the animal; large juveniles and adults are more amenable to this procedure. Bell et al. (1990) compared diagnosis of IHNV using periopods with diagnosis from a more complete histological examination. However, the ability to nondestructively screen large animals is offset by two factors. As animals age (become larger) the likelihood of detecting infections of either IHNV or BP decreases. Additionally, removing a periopod on several occasions from a single individual increases the likelihood of mortality.

Recently, however, researchers at the University of Arizona have made substantial progress in the development of molecular diagnostic tools for IHNV and BP (see Lightner et al., this volume). The advances may eventually allow large-scale screening of individual broodstock. The molecular tools are already being tested for their ability to reliably screen individual broodstock.

The possibility of selecting uninfected animals from contaminated facilities and populations rests on the assumption that not all animals exposed to IHNV or other viruses develop an infection. This hypothesis has yet to be tested, but the prospects are very bright.

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Growth and Survival of Virus-infected and SPF *Penaeus vannamei* on a Shrimp Farm in Hawaii

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Abstract

In late 1982, Amorient Aquafarm initiated work with *Penaeus vannamei* at their maturation and hatchery site located in Kahuku on the island of Oahu, Hawaii. From 1983 to 1989, laboratory tests detected no known virus diseases or other obligate pathogens in shrimp cultured on the Amorient farm. In early 1989, however, infectious hypodermal and hematopoietic necrosis virus (IHHNV) was discovered in stocks of *P. vannamei* at the farm. The effect of IHHNV infection on shrimp production was dramatic and was expressed as a marked decrease in growth that is characteristic of runt-deformity syndrome (RDS) of *P. vannamei*. In the IHHNV-infected RDS groups, the coefficient of variation in size (CV) increased from 10 - 20% to 40%, and pond yields decreased accordingly. In mid-1990, *Baculovirus penaei* (BP) infections were also found at low prevalence and severity levels in samples of shrimp examined from Amorient ponds; however, no negative impact on production could be attributed to the BP infection. In January 1992, the farm was stocked with the progeny of SPF *P. vannamei* broodstock. This report considers the resulting disappearance of RDS and the production and yield improvements obtained when IHHNV-free shrimp were again cultured on the Amorient farm.

Introduction

Amorient Aquafarm, Inc. is a 175-acre (79.6-ha) shrimp and prawn farm located on the North Shore of Oahu, Hawaii. The farm was constructed in 1977 and was originally designed for the culture of the freshwater prawn, *Macrobrachium rosenbergii*. On the main farm site, there are 142 1-acre (0.46-ha) earthen ponds and one 0.5-acre (0.23-ha) concrete-sided round pond. There are also 10 0.25-acre (0.11-ha) broodstock ponds at an adjacent area 0.5
miles from the main farm site. The maturation/hatchery facility is located at a third site close to the broodstock pond area.

In late 1982, 78 adult, wild-caught *Penaeus vannamei* were shipped from Ecuador and introduced in quarantine at the Amorient Aquafarm maturation/hatchery site in Kahuku, Oahu, Hawaii. The intent of the introduction was for the Amorient staff in Hawaii to develop appropriate technology and gain experience in shrimp maturation and larval rearing, and to eventually transfer this knowledge to the company’s 1,000-acre (455-ha) commercial shrimp farm in Ecuador.

In mid-1986, a subpopulation of adult *P. monodon* obtained from the Aquaculture Development Program, State of Hawaii, were stocked into the Amorient Aquafarm maturation facility. This group of shrimp originated as offspring from a wild-caught spawner collected in waters off Sabah, Malaysia. After introduction to Hawaii and repetitive direct histopathology and shrimp bioassay evaluation during the 18 months of quarantine following introduction, a small group of adult shrimp was transferred to the Amorient Company.

On the basis of initial success in reproduction, spawning, production of postlarvae and favorable results in growout with *P. vannamei*, the decision was made to engage 80% of the Kahuku farm in shrimp culture. Over the next several years, shrimp production was rather consistent, ranging between 2,400 and 3,000 lbs/acre/yr (2,400 - 3,000 kg/ha/yr). Starting in late 1986, *P. monodon* were also stocked for growout, but by early 1988 culture of this species was limited due to poor pond performance relative to *P. vannamei* under the environmental conditions and husbandry practices in use at that time on the Amorient farm.

In mid-1987, an outbreak of infectious hypodermal and hematopoietic necrosis virus (IHNNV) disease occurred on a shrimp farm near Amorient Aquafarm. The origin of the IHNNV in this outbreak is not determined. However, by late 1988, IHNNV was detected in samples of F6 generation shrimp collected from the Amorient maturation/hatchery area, and by mid-1990, the virus was widespread in growout ponds on the farm. Also, in August 1990, *Baculovirus penaei* (BP) infection was found in shrimp sampled from the Amorient farm.

In December 1990, The Oceanic Institute provided P1 generation, Mexican-derived, specific pathogen-free (SPF) *P. vannamei* broodstock to Amorient Aquafarm from which SPF postlarvae were produced and stocked into growout ponds.

**Materials and Methods**

Comparison between non-SPF-derived and SPF shrimp pond production and

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1 The term “high health” animals is preferred by Wyban (this volume) when referring to animals that have been removed from an SPF quarantine facility.
Table 1. A summary of the IHNV and BP histopathology results for *Penaeus vannamei* samples from Amoriont AquaFarm ponds for the period of 1987 through 1991.

<table>
<thead>
<tr>
<th>Year</th>
<th>Stock</th>
<th># Ponds sampled</th>
<th># Shrimp examined</th>
<th># BP+</th>
<th>#IHNV+</th>
</tr>
</thead>
<tbody>
<tr>
<td>1987</td>
<td>Ecuador</td>
<td>25</td>
<td>155</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1988</td>
<td>Ecuador</td>
<td>4</td>
<td>40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1989</td>
<td>Ecuador</td>
<td>8</td>
<td>150</td>
<td>0</td>
<td>70 (61%)</td>
</tr>
<tr>
<td>1990</td>
<td>Ecuador</td>
<td>6</td>
<td>60</td>
<td>12 (20%)</td>
<td>52 (87%)</td>
</tr>
<tr>
<td>1991</td>
<td>Mexico</td>
<td>24</td>
<td>270</td>
<td>11 (4%)</td>
<td>0</td>
</tr>
</tbody>
</table>

Growth performance was done by determination of the total weight of shrimp harvested from ponds, percent survival, weekly growth rate, feed conversion ratio, and size distribution (mean, standard deviation and coefficient of variation) for shrimp weight from random samples (minimum N = 100) of different populations.

From 1983 onwards, diagnostic examination for the detection of known penaeid viruses and other obligate pathogens were periodically conducted on the offspring of *P. vannamei* and *P. monodon* cultured at the Amoriont site, and on stocks produced from the Amoriont maturation/hatchery facility that were distributed to other locations in Hawaii.

For disease monitoring, shrimp were sampled at various sizes/ages, including postlarvae (PL6-12), 0.5- to 1.5-g nursed juveniles, 4- to 10-g subadults from growout ponds, and >35-g broodstock. Specimens were either frozen for the *P. stylirostris* bioassay test (Lightner et al., 1985) or killed by injection and immersion in Davidson's fixative (Humason, 1979) for histopathology evaluation. Histological processing and slide preparation followed standard procedures. Tissue sections were stained with hematoxylin and eosin (Luna, 1968).

Prior to restocking the ponds in 1991 with the progeny of SPF broodstock, the ponds were dried for two weeks, and 800 lbs of agricultural limestone was spread over the pond bottoms. The bottom gravel of the round pond was dried and then partially filled to cover the substrate, which was treated with 10 mg/L of chlorine overnight before the pond was refilled.

In the growout trials, a 25% protein, locally produced pellet was fed to shrimp stocked into earthen ponds, and a 45% protein, imported pelleted ration was provided to the shrimp in the round pond.

**Results and Discussion**

The histopathology examination results for *P. vannamei* sampled from the Amoriont farm and maturation/hatchery areas for the period from 1987 through 1991 are listed in Table 1. In addition, *P. stylirostris* bioassay trials were conducted on shrimp that originated from
the Amorient population up to mid-1987. For example, between January and May 1987, indicator shrimp bioassay tests were carried out on tissue samples from three groups (N = 50) of Amorient subadult to adult *P. vannamei*. Prior to 1989, infections by either BP or IHHN viruses were not detected in bioassays or by direct histopathology evaluation of shrimp from the Amorient farm. Direct histopathology tests conducted on juvenile through adult (N = 30) *P. monodon* sampled from several ponds in August 1987 were also negative for known obligate shrimp pathogens.

However, IHHN virus infection was detected histologically in samples of postlarvae collected from the Amorient hatchery in early 1989 (Fig. 1). As the year progressed, the frequency of IHHN virus-positive groups increased, and by May of that year, IHHNV was detected in 100% of the postlarval groups sampled. Within IHHNV-positive groups, the average number of individuals with histologically diagnosable IHHNV infection increased through 1989 and 1990. Average prevalence of infection in early 1990 was 26.2%, but during the summer of 1990, prevalence of infection increased to an average of 43.1%. Coincidentally, broodstock were replaced every four to six months, and the increased prevalence of infection may have reflected higher levels of IHHNV infection in the older broodstock.

In late 1990, The Oceanic Institute provided several groups of SPF nauplii (Mexico stock) to Amorient Aquafarm. The postlarvae produced with these nauplii tested negative by histological

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![Graph showing percentage of IHHNV-positive individuals within groups of postlarvae tested for IHHNV.](image-url)
criteria for IHNV infection, whereas postlarvae produced in sister tanks using nauplii from IHNV-infected broodstock (Amorent’s Ecuador stock) continued to test positive for IHNV (Fig. 1). At no point during this period did any of the postlarval populations \(N = 71\) harvested from the hatchery test positive for BP.

Unfortunately, histogram assessments were not carried out for shrimp cultured on the Amorent farm before IHNV was detected, from 1982 through 1988. RDS was not apparent in early 1989; this is demonstrated by random histograms of populations from 1-acre earthen ponds in which the size coefficient of variation (CV) was only 17% (Fig. 2a). However, a nursery harvest at that time contained an unusually high number of “small” juveniles — a large percentage weighed 0.3 g or less (Fig 3a). As time passed, on average, the CV slowly increased, peaking at 46% in late 1990 (Fig. 2b). The increasing CV was also apparent in successive crops harvested from the intensive, 0.5-acre round pond (Figs. 4a, b). As a result of RDS, the average harvest size decreased from 11.9 g to 8.5 g in the 2 growout trials conducted during this period.

In late December 1990, Amorent Aqua-farm received SPF \(P.\) vannamei broodstock. These shrimp were foundergeneration stock collected by the U.S. Shrimp Consortium as postlarvae in Mexico and grown to broodstock at the quarantine facility of The Oceanic Institute in Hawaii.

Figure 2. Size variation in \(P.\) vannamei during growout.
Figure 3. Size variation in Penaeus vannamei in the nursery phase.

Figure 4. Size variation of Penaeus vannamei in round pond growout trials.
Improved production was noted immediately with the progeny of the SPF broodstock. Note, for example, the size distributions from two consecutive nursery harvests depicted in Figures 3b and 3c. Both ponds were stocked at a similar density and reared for the same number of days. Figure 3b represents non-SPF postlarvae; many of the harvested shrimp were 0.25 g or less. Figure 3c gives the data for the SPF animals; their average size at harvest was approximately 1 g.

We also observed a dramatic reduction of RDS in the earthen semi-intensive ponds and in the intensive round pond. Figure 2c is a size histogram for an earthen pond stocked with SPF *P. vannamei*. The CV for this pond was 19%. Figure 4c contains similar data for a population harvested from the round pond — the CV was 9%.

The production data for the growout ponds discussed above are in Tables 2 and 3. For the IHHNV-infected shrimp, there was a general decrease in growth rate, mean harvest size and lbs/acre/crop. However, once the SPF shrimp were stocked, there was less size variability and production improved.

As indicated by the data, one benefit of the SPF broodstock was a reduction in the level of RDS in growout and nursery. This was extremely important from a marketing point of view. Using three successive round pond harvests as an example (Table 3), the SPF crop yielded a 62.5% higher return than the non-SPF harvests. In the non-SPF harvest on Nov. 16, 1990, 8% of the shrimp were below marketable size and 53% were under 8.5 g; these animals commanded a low price. In comparison, 100% of the SPF crop was sellable, and only 3% weighed less than 9.5 g. Similar results were obtained for the earthen ponds.

IHHN virus was detected histologically in samples collected from ponds on the Amorient farm in 1989 (Table 1). In 1989, the average prevalence of IHHNV-infected *P. vannamei* was 61%, but increased to 89% in 1990. Since SPF shrimp have been stocked at Amorient, IHHN virus infection has not been detected histologically in *P. vannamei* sampled (N = 270) from ponds on the site. However, more study is required before the status of IHHN virus in the earthen ponds of the Amorient farm will be understood.

Furthermore, the prevalence of BP infection declined from 20% to 4% in pond-reared shrimp between 1990 and 1991. In laboratory experiments, LeBlanc and Overstreet (1991) demonstrated that BP is inactivated by desiccation. Perhaps the two-week drying period between crops partially inactivated infectious BP in the pond sediments. Further study is required to clarify this issue.

In summary, stocking the progeny of SPF broodstock on an IHHNV-contaminated farm where RDS was a serious problem resulted in the virtual elimination of RDS and improved production and profitability.
Table 2. Production data from non-SPF and SPF growout crop at Amorlent in 1989-91.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Semi-intensive growout</th>
<th>Intensive growout</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Non-SPF</td>
<td>SPF</td>
</tr>
<tr>
<td>Density (No./m²)</td>
<td>15.0</td>
<td>14</td>
</tr>
<tr>
<td>Duration (days)</td>
<td>83</td>
<td>77</td>
</tr>
<tr>
<td>Growth rate (g/wk)</td>
<td>0.78</td>
<td>0.54</td>
</tr>
<tr>
<td>FCR</td>
<td>2.2</td>
<td>2.6</td>
</tr>
<tr>
<td>Harvest CV (%)</td>
<td>17</td>
<td>46</td>
</tr>
<tr>
<td>Harvest size (g)</td>
<td>9.2</td>
<td>6.9</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>66</td>
<td>73</td>
</tr>
<tr>
<td>Total crop (lbs.)</td>
<td>826</td>
<td>623</td>
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</table>

Table 3. Marketing impact of using non-SPF versus SPF shrimp.

<table>
<thead>
<tr>
<th>Count</th>
<th>Size (g)</th>
<th># Shrimp</th>
<th>Percent</th>
<th>Pounds</th>
<th>Price</th>
<th>Value ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsellable</td>
<td>&lt; 4</td>
<td>6</td>
<td>2</td>
<td>75</td>
<td>0.00</td>
<td>0</td>
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<tr>
<td>71-110</td>
<td>4 - 6</td>
<td>23</td>
<td>6</td>
<td>227</td>
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<tr>
<td>51-70</td>
<td>6.5 - 8.5</td>
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<td>13</td>
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<td>5</td>
<td>189</td>
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<td>803</td>
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<td>10.0 - 11.0</td>
<td>45</td>
<td>12</td>
<td>458</td>
<td>4.50</td>
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<tr>
<td>36-40</td>
<td>11.5 - 12.5</td>
<td>63</td>
<td>17</td>
<td>649</td>
<td>4.75</td>
<td>3,083</td>
</tr>
<tr>
<td>31-35</td>
<td>13.0 - 14.5</td>
<td>83</td>
<td>22</td>
<td>841</td>
<td>5.25</td>
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<tr>
<td>26-30</td>
<td>15.0 - 17.5</td>
<td>55</td>
<td>15</td>
<td>561</td>
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<tr>
<td>21-25</td>
<td>18.0 - 21.5+</td>
<td>32</td>
<td>9</td>
<td>342</td>
<td>6.25</td>
<td>2,137</td>
</tr>
<tr>
<td>Total</td>
<td>374</td>
<td></td>
<td></td>
<td>3,838</td>
<td></td>
<td>18,580</td>
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<table>
<thead>
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<th>Count</th>
<th>Size (g)</th>
<th># Shrimp</th>
<th>Percent</th>
<th>Pounds</th>
<th>Price</th>
<th>Value ($)</th>
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<tr>
<td>Unsellable</td>
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<td>32</td>
<td>8</td>
<td>248</td>
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<td>67</td>
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<td>531</td>
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<td>108</td>
<td>28</td>
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<tr>
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<td>18</td>
<td>562</td>
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<td>374</td>
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<td>6</td>
<td>184</td>
<td>5.25</td>
<td>966</td>
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<td>26-30</td>
<td>15.0 - 17.5</td>
<td>11</td>
<td>3</td>
<td>90</td>
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<td>517</td>
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<td>21-25</td>
<td>18.0 - 21.5+</td>
<td>1</td>
<td>1</td>
<td>27</td>
<td>6.25</td>
<td>169</td>
</tr>
<tr>
<td>Total</td>
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<th>Percent</th>
<th>Pounds</th>
<th>Price</th>
<th>Value ($)</th>
</tr>
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<tr>
<td>51-70</td>
<td>6.5 - 8.5</td>
<td>3</td>
<td>2</td>
<td>77</td>
<td>4.00</td>
<td>308</td>
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<td>9.0 - 9.5</td>
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<td>1</td>
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<td>4.25</td>
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<td>1,145</td>
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<td>11.5 - 12.5</td>
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<td>19</td>
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<tr>
<td>Total</td>
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<td></td>
<td>4,271</td>
<td></td>
<td>20,326</td>
</tr>
</tbody>
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References


Shrimp Production in Texas Using Specific Pathogen-Free Stocks

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Los Fresnos, Texas 78566, U.S.A.

Abstract

Harlingen Shrimp Farms has obtained yields averaging 2.5 - 3 MT/ha in recent years, but some ponds have produced more than 4.5 MT/ha/crop. To achieve more consistent yields, a cooperative research agreement was entered into with the Gulf Coast Research Laboratory Consortium (GCRLC) in September 1990. The GCRLC supplied Harlingen Shrimp Farms with specific pathogen-free (SPF) broodstock to produce postlarvae for commercial-scale comparisons with selected farm stocks, termed Texas broodstock source (TBS), which were IHHNV positive. The SPF broodstock were maintained in isolation from the farm stocks housed in the same facility. Regular inspection of the postlarvae indicated that the offspring were also SPF. The ponds stocked with postlarvae produced from the SPF broodstock outperformed the TBS postlarvae in terms of survival, overall yield and decreased size variation.

Introduction

Harlingen Shrimp Farms, located in South Texas and originally founded in 1980 as Laguna Madre Shrimp Farms, was formed in October 1990. The farm produces P. vannamei in 180 ha of semi-intensively managed ponds. Postlarvae are supplied by a hatchery on site. Ponds range in size from 2 - 15 ha, and are direct-stocked at densities up to 50 postlarvae/m².

The climate in South Texas is considered subtropical, and the growing season for a tropical shrimp such as P. vannamei is restricted to 245 days at the farm site. The need for higher returns per ha, per unit time has pushed most farms toward intensification. To maximize yields, most U.S. farms use high-quality feeds and aerate at 5 - 9 hp/ha. With high aeration and efficient application of high-quality feeds, stocking densities have been increasing. Some farms stock more than 70 postlarvae/m² and achieve yields over 6 MT/ha in a single crop. A consistent supply of high-quality postlarvae is required to make such intensive systems successful.

At Harlingen Shrimp Farms, yields have averaged 2.5 - 3 MT/ha in recent years, but some ponds have produced
more than 4.5 MT/ha/crop. These inconsistent yields have resulted from low survival and growth rates. The low growth rates have been impacted by runt-deformity syndrome (RDS), which has been causally linked with IHNV (Kalogayan et al., 1991), but the low survival rates remain unexplained. In an attempt to achieve more consistent yields, a cooperative research agreement to utilize high-health stocks of shrimp was entered into with the Gulf Coast Research Laboratory Consortium (GCRLC) in September 1990.

The GCRLC supplied Harlingen Shrimp Farms with enough specific pathogen-free (SPF) broodstock to produce postlarvae for commercial-scale comparisons with selected farm stocks ("Texas broodstock source," or "TBS"), that were IHNV positive. SPF broodstock, by definition, are free of IHNV, hepatopancreatic parvo-like virus, Baculovirus penaei, microsporidians, gregarines, nematodes and cestodes. Ponds stocked with postlarvae produced from the SPF broodstock (hereafter referred to as "high-health" animals) outperformed the offspring of the TBS in terms of survival, overall yield and decreased size variation.

**Methods and Materials**

The hatchery at Harlingen Shrimp Farm is housed within a 3,600-m² concrete building and includes a water treatment facility, two maturation rooms, broodstock holding and acclimation areas and over 500 tons of larval rearing capacity. The maturation area consists of two light- and temperature-controlled rooms, each containing 12 8-ton circular fiberglass tanks. The tanks are plumbed in groups of four; each group has a biofilter. In preparation for receiving SPF broodstock, one of these four tank systems was physically isolated from the rest of the tanks with a plastic curtain. A new biofilter was installed, and the whole area was carefully sterilized. A protocol for receiving and isolating the SPF broodstock and their progeny was developed in conjunction with Dr. Paul Frelier and the Gulf Coast Research Laboratory Consortium (Frelier, pers. comm). The protocol was strictly followed. Standard maturation methodologies utilizing unilateral eyestalk ablation were used with both groups of broodstock. Nauplii produced from the SPF broodstock were isolated from the TBS nauplii, and initially were reared in an isolated part of the larval rearing area.

The larval rearing area consisted of rows of fiberglass tanks that drain into trenches for harvesting. One of these rows of tanks was physically isolated from the others with a plastic curtain. Identical, standard hatchery methods were used for both groups, except that the high-health postlarvae were initially stocked at lower densities because fewer broodstock were sourced for nauplii. The high-health nauplii were initially isolated from the TBS nauplii. After several million postlarvae were produced in isolation, the larval rearing area was no longer physically segregated. The nauplii produced from the two broodstock groups were segre-
Table 1. Comparison of the average growth rate, feed conversion ratio (FCR), survival and percentage of population with rostral and/or tail deformities at harvest.

<table>
<thead>
<tr>
<th>Pond</th>
<th>Animal type</th>
<th>Stocking density (No/m²)</th>
<th>Ave. growth per week after 1g (g/wk)</th>
<th>FCR</th>
<th>Survival (%)</th>
<th>% deformed at harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-1</td>
<td>TBS</td>
<td>50</td>
<td>0.80</td>
<td>3.1</td>
<td>43</td>
<td>30</td>
</tr>
<tr>
<td>G-4B</td>
<td>TBS</td>
<td>50</td>
<td>0.84</td>
<td>2.6</td>
<td>46</td>
<td>11</td>
</tr>
<tr>
<td>A-4</td>
<td>TBS</td>
<td>75</td>
<td>0.85</td>
<td>3.6</td>
<td>40</td>
<td>16</td>
</tr>
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<td>G-2</td>
<td>TBS</td>
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<td>0.87</td>
<td>3.2</td>
<td>30</td>
<td>29</td>
</tr>
<tr>
<td>N-2</td>
<td>SPF</td>
<td>37</td>
<td>0.74</td>
<td>2.7</td>
<td>43</td>
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</tr>
<tr>
<td>G-3</td>
<td>SPF</td>
<td>37</td>
<td>0.77</td>
<td>2.5</td>
<td>72</td>
<td>2</td>
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<tr>
<td>N-3</td>
<td>SPF</td>
<td>50</td>
<td>1.06</td>
<td>2.3</td>
<td>25</td>
<td>*</td>
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<tr>
<td>N-4</td>
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<td>50</td>
<td>0.89</td>
<td>2.3</td>
<td>32</td>
<td>3</td>
</tr>
<tr>
<td>N-6</td>
<td>SPF</td>
<td>50</td>
<td>0.90</td>
<td>2.3</td>
<td>60</td>
<td>5</td>
</tr>
<tr>
<td>N-7</td>
<td>SPF</td>
<td>37</td>
<td>1.03</td>
<td>2.3</td>
<td>64</td>
<td>*</td>
</tr>
<tr>
<td>A-2</td>
<td>SPF</td>
<td>75</td>
<td>0.67</td>
<td>2.9</td>
<td>60</td>
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</tr>
</tbody>
</table>

*Data not available.

Eleven ponds of varying sizes, totaling 50 ha, were stocked with either high-health postlarvae or TBS postlarvae (Table 1). Although the hauling tank and transfer baskets were sanitized with chlorine prior to stocking with high-health postlarvae, no attempt was made to use segregated equipment or supplies in the management of the growout ponds. Management strategies were applied according to stocking density. For example, aeration rates ranged from no aeration at the stocking density of 18 postlarvae/m² to nearly 15 hp/ha for the smaller ponds stocked at 75 postlarvae/m². The average daily water exchange rates ranged from ten to 45% per pond, depending on the biomass estimate. All ponds were fed two to three times per day using 45% protein crumbles until shrimp reached 1 g; thereafter, 30% protein prawn pellets were used. Feeding rates were calculated using a standard feed curve based on percent biomass, and were adjusted according to observed consumption rates monitored with feed trays. Ponds were evaluated on a weekly basis. Each week, 100 to 200 shrimp from each pond were sampled using cast nets. The average weight was determined, and shrimp were inspected for state of health and vigor, signs of stress, feeding activity, deformities, size variation and shell lesions. Samples of stocked postlarvae, 30 day-old juveniles and adults nearing harvest were collected and fixed in Davidson’s solution for disease testing. All samples were examined at Texas A&M University, where diagnoses for
Table 2. Average number of nauplii produced per sourced female and percentage of total females mated and sourced per day.

<table>
<thead>
<tr>
<th></th>
<th>March TBS</th>
<th>March SPF</th>
<th>April TBS</th>
<th>April SPF</th>
<th>May TBS</th>
<th>May SPF</th>
<th>June TBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nauplii x 10^3</td>
<td>88.5</td>
<td>144.9</td>
<td>87.4</td>
<td>139</td>
<td>87.1</td>
<td>148.7</td>
<td>70.1</td>
</tr>
<tr>
<td>Percent females mated and sourced per day</td>
<td>6.1</td>
<td>5.2</td>
<td>5.5</td>
<td>5.6</td>
<td>5.0</td>
<td>6.4</td>
<td>4.6</td>
</tr>
</tbody>
</table>

IHHNV and other disease agents were made by direct histology. The size distribution in each pond was determined by individually weighing samples of shrimp and by examining final processing packout reports.

**Results**

Over 85 million nauplii were produced by 140 female, SPF broodstock from March through June, 1991. The percentage of females mated per day and the average number of nauplii per spawn for both broodstock groups are listed in Table 2. The average number of nauplii per spawn takes into account all females that were mated and placed into spawning tanks (sourced).

The SPF and TBS broodstock performed similarly in terms of percent females mated per day; however, the SPF broodstock averaged more nauplii per spawn.

Not all of the nauplii produced from the SPF females were segregated in larval rearing. Overall, 36 million postlarvae were produced from segregated SPF nauplii. Survival from nauplii to postlarvae was better in the high-health postlarvae (Table 3). The survival rates listed in Table 2 represent only the results from 9,000-L tanks. The size, vigor and appearance of the high-health and TBS postlarvae were similar.

Approximately 11.5 million high-health postlarvae were segregated in growout ponds. The results from eleven ponds stocked with either high-health or TBS postlarvae will be discussed here (Table 1). Growth rates did not differ greatly between the high-health and TBS ponds. Average time from PL5 to 1 g average weight was 32 d for high-health animals and 38 d in the TBS ponds. Furthermore, growth from 1 g to harvest weight averaged .87 g/wk and .84 g/wk in high-health and TBS ponds, respectively (Table 1).

A dramatic difference, however, was observed in the degree of size variation observed in the high-health and TBS groups. A typical TBS pond population averaging 1 g in size contained some shrimp that were less than 0.1 g and others that weighed more than 3 g. A typical high-health population, by contrast, had a size distribution ranging only from 0.5 g to 1.5 g. This difference became more pronounced as the growout period progressed — the size distribution in TBS ponds increased...
weekly, whereas the high-health populations maintained a tight size distribution throughout the culture period. As a result, a much more uniform product was harvested from the high-health ponds (Fig. 1a, b).

The level of rostral or tail deformities detected during weekly samples and at harvest typically ranged from 15 to 25% in TBS ponds, but was less than 3% in the high-health animals (Table 1).

Survival rates were higher in high-health ponds as compared to the TBS ponds. Average survival from PL5 to harvest for high-health animals was 51%; the best pond had 72% survival at harvest. Average survival for the TBS animals was only 40%; the best pond had a 46% survival rate. Feed conversion rates (FCRs) were also much better in SPF ponds (Table 1).

**Discussion**

The SPF broodstock and their high-health offspring outperformed the TBS broodstock and their progeny in the hatchery; however, the differences between the two groups were even more pronounced during growout.

The SPF broodstock produced more nauplii per spawning female than the TBS broodstock; however, the SPF broodstock were much larger, and there is a positive correlation between broodstock size and spawn size. The percentage of females mating and spawning per day was good for both groups and did not appear to be different. The sourcing pressure on the SPF broodstock was slightly more intensive due to fewer females producing.

Overall survival of the high-health nauplii was higher than that of the TBS nauplii. The greatest difference was in April, when survival of the high-health and TBS nauplii was 72% and 59%, respectively (Table 3). At that time, however, the high-health tanks were stocked at lower densities, and in our hatchery, lower densities in larval rearing often translate into increased survival. In May, the two groups were similar in terms of survival from nauplii to postlarva, but in June and July, when densities were similar, the high-health tanks yielded better survivals.
than TBS tanks. Finally, the appearance of postlarvae was similar between the two groups.

Additional comparisons between the high-health and TBS nauplii and postlarvae were conducted at two universities. Individual families of nauplii were sent to the University of Houston Clearlake for comparison of resistance to Baculovirus penaei (BP). The experiment, which was designed to compare differences between broodstock groups and between families within broodstock groups, entailed feeding BP-laden Artemia to mysis larvae and monitoring mortality. No differences in resistance to BP were detected (Lester, pers. comm.).

Four groups of postlarvae from each broodstock source were sent to Texas A&M University for a replicated experiment conducted in a controlled envi-

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Figure 1a. Comparison of size distribution at harvest for four ponds.
ronment tank system (Castille et al., 1992). This comparison evaluated postlarval performance for 30 and 60 days, beginning with PL6. Survivals were similar; however, high-health postlarvae posted significantly better growth rates and also had a significantly smaller size variation than the TBS postlarvae.

The differences between high-health and TBS animals were more evident in growout. Initial sampling revealed a uniform size distribution in high-health ponds and a wide size variation in the TBS ponds. These differences increased as growout progressed (Fig. 1a, b). The uniform size distribution of the high-health animals allowed for more accurate average weight and total biomass estimates and resulted in more efficient feed management, as revealed by the FCRs (Table 1). Feed is the largest operational cost in produc-

![Size Distribution at Harvest](image)

*Figure 1b. Comparison of size distribution at harvest for four ponds.*
ing shrimp — the lower the FCR, the better the profit margin.

The more uniform size distribution was also advantageous at harvest. Shrimp buyers prefer a uniform product; runts are regarded as liabilities. The uniform distribution of the high-health animals benefited fresh or heads-on marketing where grading is more labor intensive. Batches that could be sold either as whole shrimp at pondside, or as "bulk-ungraded" at market, had lower handling and processing costs.

The average survival rate of high-health animals was better than that of the TBS shrimp (Table 1). Historically, our farm site has experienced wide ranges in pond survival rates, which are still largely unexplained. The high-health shrimp ponds also exhibited a wide range of survival rates (25% to 72%). There is increasing evidence that the low survival rates at our farm are due to unknown factors related to the growout ponds and are not the direct result of postlarval quality. The higher average survivals of the high-health animals simply indicates that that they have a greater capacity to tolerate these unknown factors. In addition, the growth rates of high-health shrimp were slightly higher than the TBS animals.

The average growth rate in the SPF ponds, .87 g/wk after 1 g, was lower than anticipated. This may have been due to underfeeding resulting from higher-than-expected survivals.

In summary, with better survival, more uniform size, easier management resulting in lower FCRs and more marketing options, the high-health animals performed better than the TBS progeny.

Acknowledgments

Harlingen Shrimp Farms is grateful to The Oceanic Institute and the Gulf Coast Research Laboratory Consortium (GCRLC) for providing the SPF shrimp stocks. It is important for research groups such as the GCRLC to be at the forefront of production-oriented projects. Dr. Paul Frelier and his staff at Texas A&M University spent hours analyzing the samples and helping to develop the protocol for maintaining the SPF stock as disease-free. Finally, the entire staff at Harlingen Shrimp Farms was instrumental in producing the shrimp and being conscientious about the SPF project.

Literature Cited