

DISEASE DIAGNOSIS AND MANAGEMENT: AN INDUSTRY REPORT

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INTRODUCTION

Over the last 15 years the culture of shrimp has expanded to such a large commercial scale it is now a substantial commodity on the world market. The industry has grown extremely fast and for this reason far less is known about shrimp diseases than other commodities, such as cattle, pigs, and chickens that have been established for many years. Available information is based largely on clinical observation rather than information gathered from experimental studies designed to specifically understand an aspect of a particular disease. The industry is littered with references to diseases such as RDS, sea gull syndrome, bamboo back, OMMS, and others that are based on these field observations.

Disease diagnosis is the "art" of distinguishing one disease from another (Brock, 1986). It is important because effective control, through management decisions, requires that an accurate determination be made of the nature, and if possible, the cause of the specific disease. Careful study must be made of all aspects of the culture system, husbandry practices, feeds, and shrimp before an understanding of this cause and ultimately the control of the

disease can be confidently determined.

Disease control is accomplished through prevention and therapy practices in management. Effective disease control requires an understanding of the host, agent, and environmental factors that form the disease triad (Brock 1986) seen in Figure 1.



These factors can be manipulated to shift the balance of this interaction in favor of increasing the chances of survival of the shrimp and reduction or elimination of the potential for a disease outbreak. Prevention is obviously the most important part of disease control. Some preventative measures are maintenance of water quality including environmental factors such as oxygen, temperature fluctuation, and waste build-up. Isolation practices to prevent cross contamination and even the development of resistant, or pathogen free, stocks are also preventative management. In cases where disease prevention is not possible, or has not been practiced, specific treatment for a disease problem may

be necessary. Some examples are drug or chemical therapy, correcting nutritional deficiencies, removing toxic agents, or improving water quality.

This "art" is practiced throughout the world of shrimp culture. An attempt was made to survey people involved in shrimp aquaculture and document their observations and diagnoses in the field as well as their management strategies applied to combat these diseases.

METHODS

A questionnaire about shrimp disease diagnosis and management was sent to people representing different areas of the world where shrimp are cultured. I had some very good responses, primarily from the Latin countries dealing with diseases in *P. vannamei*. Asia was under-represented in the survey responses but some responses about *P. monodon* disease are included. Information about our experiences at Amoriant culturing *Penaeus vannamei* are included because they illustrate what seems to be a growing and widespread problem.

RESULTS

The responses were consolidated into groups according to subject such as hatchery or growout, geographic location (Asia or the Americas), and species cultivated (*Penaeus monodon* and *Penaeus vannamei*). The final listing of diseases was reduced because there was some difficulty in interpreting comments. When the only comment about a disease was that it killed shrimp, the comment was not included.

Table 1 lists the hatchery diseases by region. Interesting similarities between the two parts of the world were the incidence of Baculovirus and of luminescent vibrio bacteria. Both of these appeared in the responses of all respondents.

Table 2 shows the different PL quality control parameters practiced in Asia and the Americas. Microscopic examination and PL size were the standard measure of PL quality. There is some movement towards muscle to gut ratio being practiced with *P. monodon* and salinity stress tests with *P. vannamei*.

Table 3 lists the major farm diseases that were reported. Average stocking rates for the farms that responded are also listed. The most common, and serious, disease in Asia appears to be *Monodon Baculovirus* (MBV) with some occurrences of IHHN virus. In the Americas, the primary virus disease was IHHN but vibrio problems appeared to be the most problematic.

There were many similarities between diseases diagnosed and treatments practiced at hatcheries in the two regions surveyed. The use of antibiotics prophylactically or as direct treatments for bacteria and vibrio infections was a standard practice in most hatcheries. The higher incidence, or impact, of virus has

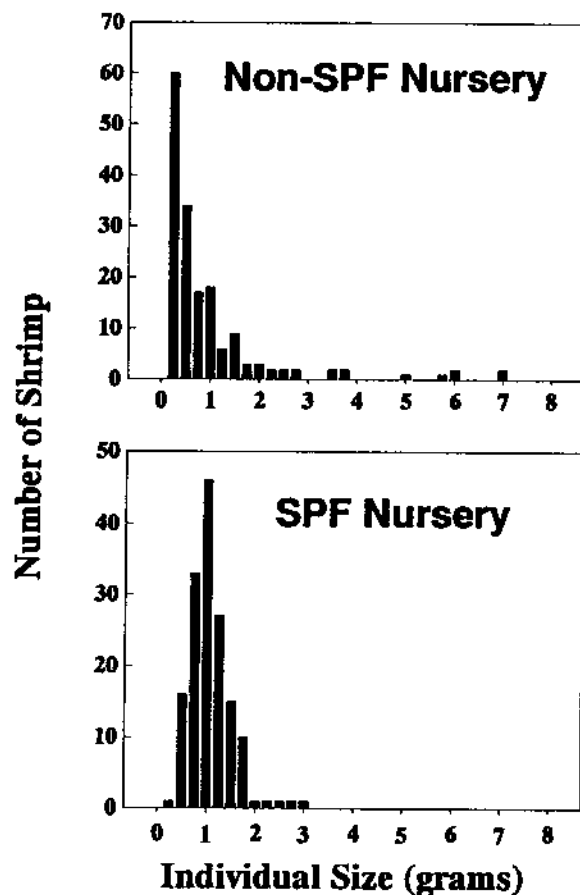


Figure 2. Harvest size distribution from successive earthen pond nurseries in same pond at Amoriant. A. Pond stocked with non-SPF (IHHN-positive) postlarvae. B. Pond stocked with SPF postlarvae.

prompted some changes in management. Whereas calcium hypochlorite has been used for many years as disinfectant, specifically for bacteria, it has recently been proven in laboratory tests to be effective against *Baculovirus penaei* at dosages of 200 ppm for 1 hour and 1600 ppm for as short as 20 seconds (LeBlanc and Overstreet, 1991a). Another common practice has been the periodic drying out of hatcheries. It is less labor intensive and cheaper than treating with calcium hypochlorite. Studies have shown 48 hours of desiccation to be long enough to inactivate *B. penaei* (LeBlanc and Overstreet, 1991b). Sodium hydroxide has also been reported to be effective against the spread of *B. penaei* in Ecuador (Akamine and Moores, 1989).

Quality of postlarvae produced in a hatchery has always been an issue. With feedback from the farms, standards are being developed so that hatcheries can judge the quality of their postlarvae produced. Whereas in the past the key parameters for determining viable postlarvae were basic microscopic examinations for bacterial infections and size/age relationships, muscle/gut ratios (Bauman, 1990) and stress tests are being adopted. One hatchery in Ecuador reported calculating the coefficient of variation (CV) within a population of postlarvae to estimate the incidence of IHHN virus as a measure of postlarval quality.

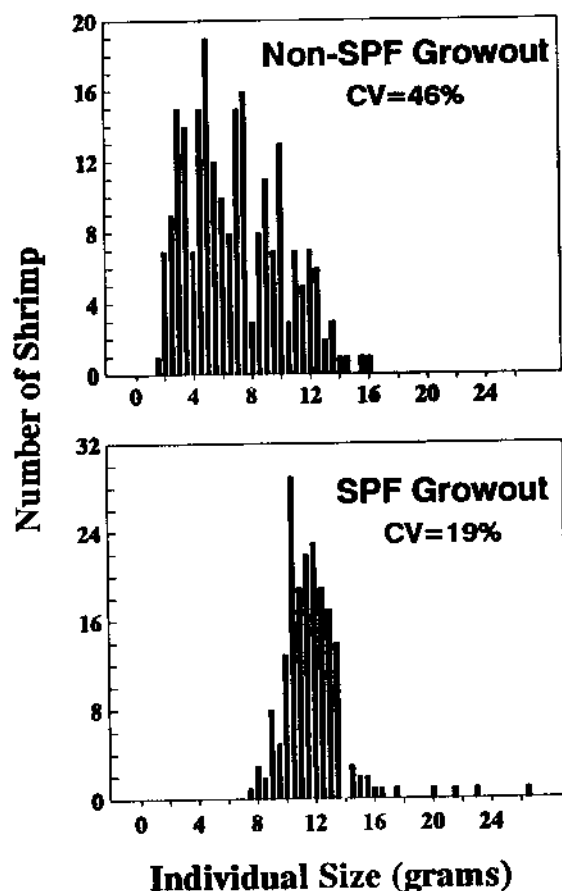


Figure 3. Harvest size distribution from semi-intensive growout crops in 1-acre earth ponds at Amorient. A. Pond was direct-stocked with non-SPF (IHHN-positive) postlarvae. B. Pond stocked with SPF postlarvae.

The impact of shrimp diseases on farm production range from minor to complete loss of product, depending on severity of infection. Farm management has also had to adapt to the increasing problems with diseases. Stress-causing agents within the culture system are being monitored more closely due to opportunistic bacteria and virus such as MBV and BP that, even though present only impact weakened shrimp. Generally, poor water quality and inadequate nutrition are the basic determinants of disease outbreaks (Sindermann and Lightner, 1988). However parasites like *Gregarinas nematopsis* are finding shrimp to be good hosts and are having a negative impact on production (Miller, 1991).

DISCUSSION

Disease diagnosis, prevention, and therapy is possible in hatcheries, most semi-intensive and all intensive culture systems. In some semi-intensive and all of the extensive shrimp culture systems it is usually impractical to treat disease outbreaks even if they are diagnosed. This makes prevention and animal quality even more important factors.

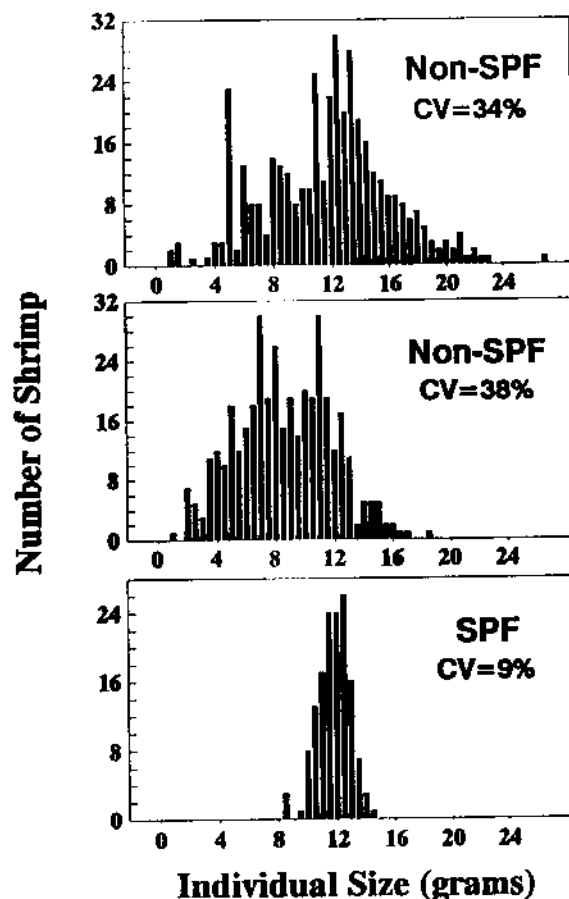


Figure 4. Harvest size distribution from successive crops in intensive pond at Amorient. A & B pond was stocked with non-SPF (IHHN positive) juveniles. C. Pond stocked with juveniles from SPF broodstock.

Many diseases thought to be site or geography specific are now found all over the world. Examples of acquired diseases and the management practices developed to reduce impacts on production include IHHN and BP virus now present in the State of Hawaii. Until mid-1989, the *P. vannamei* at Amorient Aquafarm were BP and IHHN-free. These were progeny from an original 78 broodstock brought from Ecuador in 1982. A closed cycle system had been developed and successfully managed for many years eliminating the need to import any more shrimp. Over those years another company built an intensive farm next to Amorient. They imported their own shrimp which ended up being IHHN positive. The ensuing massive mortalities forced the eventual closure of that facility. Within a year the disease had migrated over to Amorient Aquafarm and soon after that BP was also discovered in the farm-raised shrimp.

The standard management practice for dealing with IHHN virus has been to stock a farm with wild postlarvae or with hatchery postlarvae reared using nauplii from wild gravid females. Experience has shown a higher incidence of the virus in shrimp from maturation nauplii passed on from the IHHN-

positive broodstock. In Hawaii, there are no wild broodstock populations. With each successive batch of pond reared broodstock used in our closed system the incidence of IHHN in their offspring got worse.

Figure 4a & b contains histograms of successive crops in Amorient's half acre intensive round pond during this period. You can see a slight increase in the coefficient of variation and a decrease in the growth rate. Most of the ponds at Amorient are 1 acre earthen ponds and Figure 3a shows a histogram from one of these production ponds at the height of the infection with a coefficient of variation of 46%.

In late 1990 Amorient received some Specific Pathogen Free (SPF) *P. vannamei* broodstock that were P1 generation of shrimp collected by the U.S. Shrimp Consortium as postlarvae and grown to broodstock at the quarantine facility of The Oceanic Institute in Hawaii (Wyban et al. 1992). Postlarvae were grown at Amorient using nauplii from these broodstock. They tested negative for IHHN virus using histopathology and were stocked onto the farm. The ponds themselves were prepared by drying for two weeks and then spreading 800 pounds of calcium carbonate on the bottom before filling. The difference was quite extraordinary. Figure 2a & b compares histograms of successive crops in the same nursery pond when stocked with IHHN-positive followed by SPF postlarvae. Figure 4c shows the production from the round intensive pond after being stocked with SPF juveniles. At harvest, it had a coefficient of variation of only 9%. The financial and marketing aspect of these 3 successive round pond harvests is shown in Table 4. There was a 62.5% higher return from the SPF crop compared to the previous harvest (non-SPF) and marketing was easier because there were no runts to be sold. The earthen ponds also had significant improvements as seen in Figure 3b. The incidence of IHHN and BP virus in shrimp sampled has dropped from a high in 1990 of 87% and 20% respectively to only 2% in 1991. Thus, stocking postlarvae from SPF broodstock onto the previously IHHN-contaminated farm where Runt Deformity Syndrome was a serious problem, eliminated the RDS and improved profitability.

I would like to comment on a few recommendations people submitted regarding research priorities directed to help shrimp aquaculture. Nearly all were interested in the development of fast, non-lethal methods for virus diagnosis. Many showed an interest in SPF shrimp and the work being done to improve strains of commercially grown shrimp. There also appeared to be a need to work with the FDA and get some antibiotics cleared for aquaculture use, much like the work done with TNHP by Bell et al. in Texas (submitted). The importance of this is mounting with the increasing occurrences and high mortality being attributed to bacteria and vibrio blooms in ponds. There was also a recommendation to study the life history of Gregarinas nematopsis to see if there is an intermediate host that could be eliminated to solve this problem.

ACKNOWLEDGEMENT

The disease list and comments made in this paper are by no means complete, there are a multitude of shrimp diseases, it is just what the people surveyed felt was most important. I would like to thank the people on the panel who provided their honest comments. I received some anonymous replies from people who had somehow heard about this project and wanted their observations noted. It is that kind of interaction that will benefit us all in finding cures for disease outbreaks.

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Table 1. Hatchery diseases by region

Asia: *Penaeus monodon**Penaeus monodon* Baculovirus (MBV)

- Symptoms - Round, spherical occlusion bodies can be observed with a microscope in the hepatopancreas and feces of the larvae.
 - Animals become lethargic, disoriented, and feeding slows down. The resultant slower growth puts them 1 to 2 days behind the normal postlarval growth curve. PL age to total length relationship is abnormal. - Some postlarvae with MBV were reported to be reddish in color.
- Treatment - None is conclusively known. Many people reported egg and nauplius washing with iodine, assuming that the virus was introduced from broodstock feces.

Luminescent Bacteria (Lumbac)

- Symptoms - Many rod-shaped vibrios in the gut, hemolymph, and sinuses. - This bacteria shows luminescence in the dark. Animals are weak with severe mortality if infected before the postlarval stages.
- Treatment - Monitor bacterial levels and treat the larvae with antibiotics based on sensitivity. Examples are: oxytetracycline, furazolidone, sulfa-trimethoprim.

Necrosis

- Symptoms - Gut necrosis is a breakdown of the cells lining the hepatopancreas causing reduced feeding. - Regular necrosis is external. In the earlier stages the terminal ends of the appendages and uropods appear eaten away.
- Treatment - This is treatable with antibiotics, based on sensitivity.

North, Central, and South America: *Penaeus vannamei**Baculovirus Penaei* "BP"

- Symptoms - Polyhedral occlusion bodies observed microscopically in hepatopancreas and intestine. - Inactivity and reduced feedings. - Depending on the degree of infection and the stage of the larvae/postlarvae, slow dwindling or complete mortality in larval rearing tanks can be observed.
- Treatment - None is known. If the virus occurs, the recommendation is to shut down and completely disinfect the facility. All tanks and pipes should be soaked with a 200 ppm solution of hypochlorite for a minimum of 1 hour, but usually overnight. - Walls, floors, and equipment should be sprayed with a 1200 ppm solution of calcium hypochlorite and allowed to remain damp for 30 seconds before rinsing. - The most effective is complete dry out of facility for at least 4 days.

"Bolitos"

- Symptoms - Sloughing of cells lining the hepatopancreas producing round vacuoles in the hepatopancreas and foregut. - Reduced activity and feeding thus lengthening the larval cycle.
- Treatment - Antibiotics

Luminescent Bacteria

- Symptoms - Green to greenish yellow vibrios colonizing on larvae that glow in the dark.
- Pseudomonas - Usually presents itself in late mysis/early post larval stages, it can cause severe mortalities. - Increased frequency in the warmer seasons.
- Treatment - Iodine dip the nauplii before stocking to avoid introduction. - Treat water source with ozone or ultra-violet radiation. Increase water exchange to 200-250% per day. - Based on sensitivity, treat with antibiotics.

TABLE 2. QUALITY CONTROL OF POSTLARVAE

Asia: *Penaeus monodon*

Key parameters - Size (length/age relationship) - Muscle to gut ratio in the sixth tail segment in 12 to 13 millimeter postlarvae of 4:1 or better. - Microscopic examination to check feeding, bacterial, protozoal infestations, as well as to examine the hepatopancreas for specific diseases. - Salinity or formalin stress tests.

Salinity: instantaneous 32 to 17 ppt for 2 hours, 100% survival. Formalin: instantaneous 100 ppm for 1 hour 100% survival

North, Central, South America: *Penaeus vannamei*

Key parameters - Size (6.5 mm for a PL10 from tip of telson to base of eyestock). - Gill development, most posterior gills should have at least 4 branches. - For a PL10 a salinity stress test dropping immediately from 35 ppt to 0 ppt for 30 minutes. Then putting them back into 35 ppt for another 30 minutes and finally counting the survivors. Greater than 75% survival is acceptable.

TABLE 3. FARM DISEASES BY REGION

Asia: *Penaeus monodon*

Stocking rates - Semi-intensive 15/m² juvenile stocked and intensive 30/m² direct stocked with postlarvae.

Penaeus Monodon Baculovirus (MBV)

Source - Vertically transmitted.

Symptoms - Round, spherical occlusion bodies can be observed in the hepatopancreas. - Animals become lethargic, feeding slows down and some darker than normal coloration has been reported.

Treatment - Dry ponds completely one month between cycles. - Control seed quality and source.

Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHN)

Source - Believed to be vertically and horizontally transmitted.

Symptoms - Hunchback deformities - Animals are weak, lethargic, and mortalities seen.

Treatment - Dry ponds between cycles. - Control seed quality and source.

Bamboo Back

Source - Unknown for sure, possibly nutritional deficiency.

Symptoms - Succeeding abdominal shell segments do not overlap properly, allowing muscle to protrude and be cut off.

Treatment - None proven conclusively at this time.

One Month Mortality Syndrome (OMMS)

Source - Believe to be vibrio sp. related.

Symptoms - Shrimp stop feeding, followed by mass mortalities. Usually occurs between 25 and 45 days in direct stocked ponds.

Treatment - None conclusively known.

Black Spot

Source - Caused by chitinous bacteria, mostly vibrio.

Symptoms - Black necrotic spots on the outer shell or appendages.

Treatment - Increased water exchange and the use of tea seed cake was reported.

Red Disease

Source - Unknown for sure but one suggestion was that it came from feeding rancid feed.

Symptoms - Progressive discoloration of the shrimp, beginning with the gills and pleopods and eventually turning the entire body red. The hepatopancreas can also be effected with necrosis and atrophy. Depending on severity, high mortality can occur.

Treatment - Nothing conclusive, except of course avoiding feeding any rancid feed.

Cotton Shrimp Disease (Whiteback or Microsporidiosis)

- Source - Microsporida infection of shrimp
- Symptoms - Whiteness and striation of the tail muscle, it basically looks cooked or opaqueness of gonads. Spores can be found in the muscle.
- Treatment - Shrimp microsporidia require prior ingestion by an intermediate host, usually a shrimp predator such as fish, before the spores become infective for shrimp. Thus elimination of this intermediate host is advised.

Black (Brown) Gill Disease

- Source - Exposure to toxic levels of cadmium, copper, potassium permanganate, ozone, crude oil, low pH, ammonia, or nitrite. It is also seen from other infections by disease such as IHHN, bacteria or vibrios, and even vitamin C deficiency.
- Symptoms - Black discoloration of the gill lamellae. In severe cases necrosis and atrophy of the gill may be seen.
- Treatment - Depends on determination of the probable cause and if these can be controlled.

Vibrio Disease

- Source - Gram negative bacteria belonging to the genus *Vibrio*. Examples are *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum*, and *V. harveyi*.
- Symptoms - Erratic or disoriented swimming behavior. Loss of appetite and lethargy. Infections may cause slow dwindling or rapid, complete mortality.
- Treatment - On a commercial farm the treatment of a vibrio outbreak with antibiotics, such as in the feed, can be costly and not always effective. Prevention is the best practice by maintaining good water quality, density, and adequate feed for the system used.

North, Central, and South America: *Penaeus vannamei*

- Stocking rates - Semi-intensive 10-17/m² juvenile stocked and 28/m² direct stocked with postlarvae.

Baculovirus penaei "BP"

- Source - Vertical transmission
- Symptoms - Polyhedral occlusion bodies observed in hepatopancreas and intestines.
- Inactivity, reduced feedings, and eventual mortality.
- Treatment - This disease occurs periodically and is best managed by avoiding stress.
- Dry ponds completely between cycles.

Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHN)

- Source - Believed to be vertically and horizontally transmitted.
- Symptoms - Runt deformity syndrome (RDS) is observed.
- Treatment - Control seed quality and source. A higher incidence of RDS has been found in shrimp produced from IHHN positive broodstock in a maturation facility. A lower incidence has been found in shrimp produced using nauplii from wild, gravid, females and no incidence at all in shrimp produced from SPF, IHHN free, adults in a maturation facility.

Black Spot

- Source - Caused by chitinous bacteria.
- Symptoms - Black necrotic spots on the outer shell. Lengthy molts have also been reported.
- Treatment - Increased water exchange and the use of antibiotics lessens the incidence.

Body Cramp

- Source - Unknown but nutritional and/or environmental factors could be the cause. It is suspected that handling of shrimp in warm, humid air that is considerably warmer than the culture system could cause cramping.
- Symptoms - Rigid flexure of the tail in live shrimp that cannot be straightened out.
- Treatment - None is conclusively known but avoidance of handling of the shrimp during warm periods, when the symptom occurs and minimizing stress would be advised.

Gregarinas nematopsis "Gregarines"

- Source - Life cycle is unknown.
 Symptoms - Particularly a problem during the nursery phase they grow in the gut causing blockage. As they grow they suck nutrients from the gut wall leaving lesions for infection to set in. The resultant effect is weak shrimp with slow growth.
 Treatment - None is conclusively known.

Sea Gull Syndrome

- Source - Higher than usual bacterial counts in pond water. Thought to be associated with *V. parahemolyticus* and *V. alginolyticus*.
 Symptoms - Stressed shrimp swim at the surface where birds attack them. - Sudden mortality in nursery ponds greater than 30 days old.
 Treatment - This disease is caused by vibrio infestation yet treating with antibiotics has not proven successful.
 - The stress could be related to a density/biomass relation in the nurseries. Many people are adjusting their schedules to harvest nurseries after 21 days and before 30 days. This strategy has raised the nursery survivals from 30% to 80- 90%. Another management tool is to avoid nurseries altogether and direct-stock growout ponds.

Texas Necrotizing Hepatopancreatitis (TNHP)

- Source - *Vibrio* or vibrio-like bacteria
 Symptoms - Loss of appetite, lethargy, soft shells, incresed mortality.
 Treatment - The addition of antibiotics into the feed, particularly oxytetracycline (Bell et. al. 1991).

Table 4. IHHN-POSITIVE ROUND POND CROP - JULY 27, 1990

COUNT	GR. SIZE	NUMBER SHRIMP	PERCENT	POUNDS	PRICE	VALUE
UNSELLABLE	<4	6	2	75	0.00	0.00
51-70	6.5-8.5	47	13	496	4.00	1984.00
46-50	9.0-9.5	20	5	189	4.25	803.25
41-45	10.0-11.0	45	12	458	4.50	2061.00
36-40	11.5-12.5	63	17	649	4.75	3082.75
31-35	13.0-14.5	83	22	841	5.25	4415.25
26-30	15.0-17.5	55	15	561	5.75	3225.75
21-25	18.0-21.5+	32	9	342	6.25	2137.50
TOTAL		374		3838		\$18,560.75
IHHN-POSITIVE ROUND POND CROP - NOV. 16, 1990						
UNSELLABLE	<4	32	8	248	0.00	0.00
71-110	4-6	67	17	531	3.75	1991.25
51-70	6.5-8.5	108	28	876	4.00	3504.00
46-50	9.0-9.5	33	8	248	4.25	1054.00
41-45	10.0-11.0	69	18	562	4.50	2529.00
36-40	11.5-12.5	48	12	374	4.75	1776.50
31-35	13.0-14.5	23	6	184	5.25	966.00
26-30	15.0-17.5	11	3	90	5.75	517.50
21-25	18.0-21.5+	1	1	27	6.25	168.75
TOTAL		392		3140		\$12,507.00
SPF ROUND POND CROP - MAR. 15, 1991						
51-70	6.5-8.5	3	2	77	4.00	308.00
46-50	9.0-9.5	1	1	35	4.25	148.75
41-45	10.0-11.0	38	27	1145	4.50	5152.50
36-40	11.5-12.5	74	52	2212	4.75	10507.00
31-35	13.0-14.5	27	19	802	5.25	4210.50
TOTAL		143		4271		\$20,326.75

Table 5. Production data from SPF and non-SPF growout crops at Amorient in 1990-91.

Parameter	Semi-intensive growout		Intensive Growout		
	Non-SPF	SPF	Non-SPF	Non-SPF	SPF
Density (#/m ²)	14	13.7	110	98.5	91.5
Duration (days)	77	106	91	101	104
Growth rate (g/wk)	0.54	0.76	0.87	0.54	0.74
FCR	2.6	1.7	1.85	3.37	2.1
Harvest CV (%)	46	19	34	38	9
Harvest size (g)	6.9	11.8	11.9	8.5	11.8
Survival (%)	73	74	66.4	86.1	91
Total crop (lbs.)	623	1055	3838	3140	4271