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Bacterial diseases in tiger shrimp culture in the Philippines

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Intensive monoculture of giant tiger shrimp, *Penaeus monodon*, was introduced in the Philippines in the 1980s. Its acceptance was spurred by the availability of hatchery-reared postlarvae and artificial diets. Intensive systems, however, soon became plagued with infectious diseases, of which the most economically disastrous had bacterial etiologies. Many shrimp farmers have now reduced their culture runs.

Groups of bacteria causing diseases in shrimp

Two groups of bacteria cause serious diseases in various phases of shrimp culture: (1) *Leucothrix* sp. and (2) several species of *Vibrio* (Lavilla-Pitogo 1995). These bacteria cause fouling of exoskeletal and respiratory surfaces, create cuticular or subcuticular localized lesions on the shell and appendages, or cause internal or systemic bacterial infection.

Leucothrix is a filamentous bacterium that normally lives as an epiphyte on macroscopic marine algae and arthropods. Infestations on respiratory surfaces become serious problems if coupled with critically low dissolved oxygen levels in the rearing water. Bacterial filaments may also trap other organisms and debris that complicate the stressful larval condition (Baticados *et al.*, 1990a). The condition is corrected upon complete molting and improvement of the rearing environment although industry practitioners occasionally use drugs as remedial measures.

Shell disease or necrosis in tiger shrimp larvae is quite common. Although the disease seldom causes serious mortality, the burn spots on the shell or missing appendages on marketable postlarvae is enough cause for rejection in the highly competitive market for postlarvae.

Bacterial exosketal lesions are also common in tank- and pond-reared tiger shrimp juveniles with incidence rates of 36% and 20%, respectively (Lio-Po & Lavilla-Pitogo1990). Disease incidence is related to prawn age or duration of culture. Bacterial isolation yields mostly *Vibrio* spp.

Although minor to moderate bacterial fouling and shell disease seldom cause mortality, their presence on larval surfaces affects marketability. The grow-out farmers choose their postlarvae based on several criteria that are indicators of good hatchery husbandry (Baumann & Jamandre 1990). Postlarvae with necrosis or carrying fouling organisms are usually rejected.

Various species of vibrios have been reported in the Philippines and other shrimp growing areas in Asia (Lavilla-Pitogo, 1995). Many vibrios associated with serious losses in shrimp culture systems are non-sucrose fermenters such as *Vibrio harveyi* (Lavilla-Pitogo et al. 1990, Harris et al. 1996), *V. damsela* (Song et al. 1993), *V. vulnificus* (West & Colwell 1984), *V. parahaemolyticus* (Alsina & Blanch 1994), and *V. penaeicida* (Ishimaru et al. 1995). These non-sucrose fermenting bacteria usually form green colonies, and are considered the 'bad' vibrios. In contrast, the 'good' vibrios are sucrose-fermenting bacteria that form yellow colonies. Some shrimp farmers monitor the balance between the greens and the yellows.

Luminescent vibriosis in the hatchery

The first shrimp hatchery system in the Philippines was patterned after the Japanese community culture method (Shigeno 1975) using 100 - 200 ton concrete tanks. Gravid shrimp females are placed in tanks after an array of larval food organisms have been satisfactorily established by inoculation and fertilization. But this system allowed the proliferation of microorganisms, many of which were not eaten by larvae and instead became pests during culture, causing molting difficulties or mortality. The primary problem was fungal infection (Baticados et al. 1990).

The second shrimp hatchery system was the Galveston method (Mock & Murphy 1971) which employed smaller rearing tanks. Natural food were grown separately in other tanks. This method underwent several modifications, eventually evolving into a simpler and easily adaptable system (Platon 1978, Parado-Estepa et al. 1996). This method is still being used by shrimp hatcheries.

Whereas the Japanese culture system harbored a complex community of organisms, the Galveston method and its modifications were largely based on clean rearing water in which nauplii, unicellular algae and diatoms, zooplankton and other substances were added. This husbandry method created a niche for opportunistic pathogens, specifically, bacteria. Thus, in this context, luminescent vibriosis is a result of the shift of the hatchery system from one that is ecologically balanced to one that accommodates opportunists.

Excerpted from the paper first presented at the Symposium on Diseases in Marine Aquaculture; Hiroshima, Japan; October 1997 and published in ***Fish Pathology*** *Vol. 33, No. 4, 1998*

Bacterial epizootics due to luminescent bacteria were first recognized in mid 1980s. The outbreaks were notable because shrimp hatchery operations by then had reached industry scale. There was a high incidence of luminescent vibriosis due to *V. harveyi* (Lavilla-Pitogo et al. 1990). Of the 97 colonies picked at random from larval and water samples, 93 were identified as *V. harveyi* and 4 as *V. splendidus*.

Tiger shrimp larvae and postlarvae exposed to 10^2 *V. harveyi* cells per ml can die within 48 h. When seen through scanning electron microscopy, infected larvae had a plaque of bacteria on the mouth and on its feeding apparatus, implying an oral route of entry for the pathogen.

While the ecology, natural abundance and 'free-living' status of *V. harveyi* is relatively well-known, reports of the diseases they cause in aquaculture started to emerge only in this decade.

The course of infection in the hatchery was studied at AQD (Table 1). Spawners, whose midgut bacterial flora contain 16 to 17% luminescent vibrios of its total *Vibrio* population, are significant sources of luminescent vibrios. Interestingly, spawners have been observed to release large amounts of fecal material during spawning, thus facilitating bacterial colonization of newly spawned eggs (Lavilla-Pitogo 1995).

The most common approach to prevent or control luminescent vibriosis in the hatcheries was through the use of chemicals. This approach, however, should be considered as a last alternative because most drugs were found ineffective and induced deformities in treated larvae (Baticados et al. 1990).

Table 2 lists the measures that could be adapted, modified, or improved to prevent luminescent vibriosis in tiger shrimp hatcheries. Seawater harbors luminous bacteria in numbers ranging from 1 to 63 cells per ml composed of 60 to 70% *Vibrio (Lucibacterium) harveyi* (Orndorf & Colwell 1980). The other significant luminous bacterial species in warm waters is *V. fischeri*.

Chlorination is a practical way of reducing the number of pathogens in the water, but repopulation of dechlorinated seawater occurs rapidly if bacteriostatic levels of chlorine are used (Baticados & Pitogo 1990). An alternative is the use of microbially matured seawater that has been tested to select non-opportunistic bacterial flora (Skjermo et al. 1997). In some shrimp hatcheries, the use of commercially available 'probiotics' or biological products has been tried with varying results. The use of benign bacteria to compete with pathogens is a promising approach in aquaculture and studies towards their application have been done (Dopazo et al. 1988, Lemos et al. 1991). These techniques are geared to restoring ecological balance in the system, but modifications to suit various systems should be developed.

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TABLE 1 **Sources of *Vibrio harveyi** in tiger shrimp hatcheries** (Lavilla-Pitogo et al. 1992)

Sample	Presumptive *Vibrio* (PV) count	Luminescent *Vibrio*
Nearshore seawater	average of 10^2 cfu per ml	< 10 cfu per ml
Artemia salina		
Nauplii	33-89% of individual flora	0-0.005% of PV
Hatching water	41-61% of total bacteria/ml	0.003-0.17% of PV
Spawners		
Midgut contents	66-77% of midgut bacteria	16 - 17% of PV
Exoskeleton	6 - 23% of exoskeletal flora	3 - 8% of PV
Phytoplankton		
Chaetoceros calcitrans	0.85 - 3.5% of total bacteria	0

*Differentiation of *V. harveyi* from other luminescent vibrios follows West & Colwell (1984) and Alsina & Blanch (1994).

TABLE 2 Measures that could be adapted, modified, or improved to prevent luminescent vibriosis in tiger shrimp hatcheries

Remedial measure	References
Water conditioning or treatment	
Chlorination	Baticados & Pitogo 1990, Lio-Po et al. 1989
Use of microbially matured seawater	Skjermo et al. 1997
Spawning	
Removal of spawners immediately after spawning	Lavilla-Pitogo et al. 1992
Egg washing	Lio-Po et al. 1989
Larval rearing	
Reduction of stocking density	FD Estepa (pers. comm.)
Feed sanitation	
Disinfection of zooplankton resting stages prior to hatching	Lio-Po et al. 1992
Rinsing of *Artemia* nauplii and other zooplankton	Lavilla-Pitogo et al. 1992
Rearing water	
Use of diatoms with inhibitory effects against vibrios	Lavilla-Pitogo et al. 1992
Use of benign bacteria as competitors or inhibitors of pathogen growth	Dopazo et al. 1988 Lemos et al. 1991

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the mangrove area.

To reach his conclusions, Dr. Wiedemeyer had to study the tropic structure and the nutritional interactions of representative animal species and food sources by analyzing stomach contents, conducting monodietary experiments, starvation experiments, and using the stable isotopes of carbon, nitrogen, and sulfur for his analyses. - MTC

Gavino on research trends of PUFA

Dr. Victor Gavino, Associate Professor from the University of Montreal (Canada), discussed the research trends of omega-3 polyunsaturated fatty acids on August 19 at AQD's Tigbauan Main Station.

Dr. Gavino said that omega-3 polyunsaturated fatty acids (w3PUFA) have received increasing attention in recent years. These fatty acids are essential to most vertebrates and are ultimately derived from both terrestrial and aquatic plants in the food chain. It has been generally accepted that the essentiality of w3PUFA is related to its participation in eicosanoid metabolism. Recently, these fatty acids become known for other important roles in biochemistry and physiology.

In particular, the w3PUFA 22:6n-3 docosahexanoic acid (or DHA) is important in neural tissue development. It is now generally agreed that DHA deprivation results in lower visual acuity in all species tested to date, including man. Data on brain function is controversial in that DHA appears to be related to mental capacity. DHA accretion in neural tissue occurs in early development which means that improper nutrition among infants, particularly those born pre-term may place this population at risk.

Lastly, Dr. Gavino reminded his AQD audience that there is a lack of data on DHA content of different types of food consumed in the Philippines. - MBS

Hygienic procedures such as egg washing have been recommended for the hatchery to reduce bacterial and viral contamination at the start of culture (Lio-Po et al. 1989, Sano & Momoyama 1992). But many hatchery operators skip this procedure altogether in favor of mass spawning in larval rearing tanks due to the additional labor required. Mass spawning favors the proliferation of opportunistic pathogens (Lavilla-Pitogo *et al.,* 1992), thus making chemoprophylaxis or chemotherapy inevitable options for successful production.

To prevent bacterial diseases in the hatchery, technicians already have better alternatives -- the use of microbially mature or aged seawater, application of "probiotics," and washing eggs -- to use in place of chemicals. But these techniques need to be refined to make the approaches user-friendly. These measures do not discount the positive effects of immunostimulation or vaccination although it is generally recognized that these approaches can also benefit from more research (Plumb, 1995).

Diseases in the grow-out systems

In shrimp grow-out culture, early reports of bacterial problems were limited to shell disease, filamentous bacterial infestation, and tail rot. In the last quarter of 1993, however, mass mortality associated with massive bacterial infection in the digestive organ of shrimp started occurring and contributed largely to the collapse of shrimp grow-out activities.

A monitoring program was carried out in 1994 and 1995 to understand the course of the disease in shrimp grow-out culture. Intensive monitoring of the bacterial population of the water, both from the source and within the ponds, as well as monitoring of the bacterial flora of newly stocked postlarvae and their hepatopancreata were done. The following discussion is based on the results of Leano et al. (1998) and Lavilla Pitogo et al. (1998).

Like larval luminescent vibriosis, samples of affected shrimp from grow-out ponds harbored significantly high numbers of vibrios. The target organ of infection is the hepatopancreas. Hispathology showed severe inflammation in and around hepatopancreatic tubules of the entire organ, and intense pathological disruption of the hepatopancreas was observed in smaller animals within the sample group. In larger animals, however, melanized lesions were found in the proximal region of the hepatopancreas. These lesions affect the digestive function of the organ as the necrotic parts become non-functional. This observation explains the two consequences of the infection -- mortality and slow growth. Total necrosis and dysfunction, as seen in acutely inflamed digestive organs, lead to death, while partial dysfunction causes slow growth as not all tubules function in digestion, absorption and storage. Growth impairment is a direct function of dysfunctional area. The potential for recovery of shrimp with partially impaired digestive organs is high, provided that the growth points of this organ located distally remain unaffected. The poor production performance of stocks affected by vibriosis is a consequence of damage incurred in the hepatopancreas by *Vibrio*.

To understand the course of infection in the grow-out system, the bacterial flora of pond-cultured tiger shrimp and the rearing water were quantified using various microbiological culture media during the first 60 days of culture. The purpose of these studies was to determine the level of luminescent *Vibrio* that tiger shrimp harbors during disease epizootics and during non-disease situations.

Results showed that during disease outbreaks, luminescent vibrios dominate the population of bacteria in the hepatopancreas, up to 90%, compared to 50% in healthy animals. At 18 to 31 days of culture, a mean bacterial load of 9.0 x 10^4 cfu per hepatopancreatic tissue was obtained from diseased animals, compared to

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7.0 x 10^1 in healthy animals of the same stage. This shows that a threshold level of 104 cfu per hepatopancreas could be maintained in the first 30 days of culture. The use of antimicrobials may not be necessary for animals harboring luminescent bacteria below that level. The study of Liu et al. (1996) showed that shrimp-associated *V. harveyi* isolates are pathogenic at levels ranging from 4.87 x 10^4 to 8.65 x 10^4 cfu per g live prawn in the challenge test.

Monitoring of the bacterial population in the rearing water showed that the flora obtained is diverse during the first few weeks of flooding the ponds with water. However, the diversity is lost after three weeks. Non-sucrose fermenting vibrios (the 'greens') begin to dominate in the water at the beginning of the fourth week (Figure 1). In ponds that were monitored, the percentage of yellow vibrios decreased relative to the greens, suggesting a change in the population of the culturable bacteria three to four weeks into the culture. The rearing process evidently caused substantial deterioration of bacterial diversity and favored the potential pathogens. Since it has been determined that continuous exposure of postlarvae to non-sucrose-fermenting luminescent bacterial counts of 10^2 or higher results in significant mortality, this diversity should be maintained. The change in the bacterial populations highlights the importance of bacterial monitoring in shrimp and rearing water to determine management approaches for control.

Monitoring of the intake water from brackishwater rivers or nearshore showed that up to 10^2 cfu per ml of luminescent vibrios is present in the water. Farmers were advised to stock shrimp only if the microbial flora is not dominated by potential pathogens. It was, therefore, necessary for them to allocate part of their watered areas for a reservoir, wherein water could be conditioned and be rid of particulate-associated bacteria. The strategy was to maintain an environment that is conducive to the growth of postlarvae while they are still small.

During a two-year monitoring of farms with luminescent vibriosis, several risk factors were identified as being closely associated with the disease problem including, the duration of exposure to high luminescent *Vibrio* population (above 10^2), the age and size of postlarvae upon stocking, nutritional stress, and the presence of gut-infecting viruses in newly-stocked postlarvae.

Antibiotics have been used to control luminescent bacterial diseases in ponds with little sucess. Aside from an unreliable method of delivery through topdressing on feeds, sick animals become anorexic thus making chemotherapeutants unavailable to the shrimp that need them most. The principal drawback of antibiotic application is the development of antibiotic resistant organisms and their spread in the environment (Baticados et al. 1990).

Research needs

There is a need for basic research to determine whether more than one species of luminescent *Vibrio* exists and the pathogenicity of these bacteria. These studies may help explain the difference in

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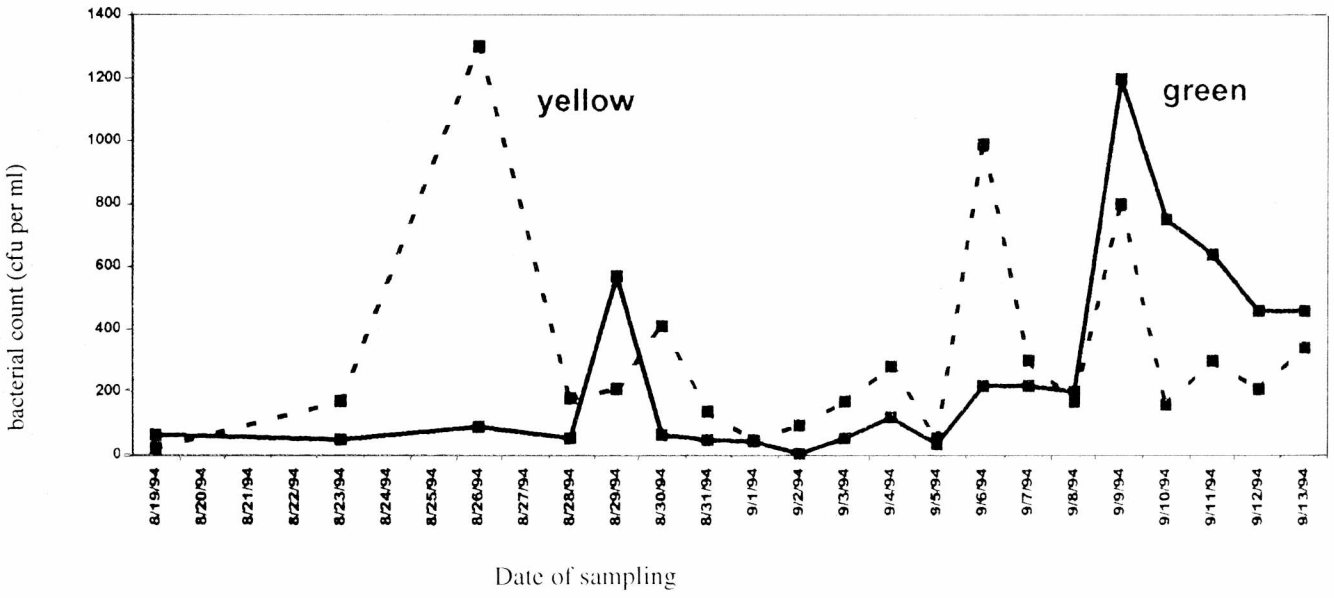
Figure 1 The diversity of vibrios in shrimp rearing ponds shift from the predominance of sucrose-fermenting types (yellow colonies) during the first few weeks of culture to the predominance of non-sucrose fermenters (green colonies) three weeks after flooding.



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host susceptibility. Serological, hybridization, or molecular biological methods may be necessary for a more detailed taxonomic classification of the organisms.

Research on environmental risk factors may help define the conditions responsible for epidemic diseases. There is also a need to examine the rearing protocols in the hatchery that may influence the health and survival of postlarvae in ponds, especially during the first two months of culture.

The rearing process in the farms evidently caused substantial deterioration of bacterial diversity. Since its dramatic appearance in late 1993, luminescent vibriosis has remained prevalent and caused significant reduction of rearing activities. Data that link the disease to water quality and environmental conditions must be obtained and examined for a holistic prevention and control program to be implemented.

LIST OF REFERENCES WILL BE PROVIDED UPON REQUEST

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Pangasinan were demolished by the Task Force created by the national government. After the demolition, it was estimated that only 30% of fishpens and fishcages are still in operation. The offshore cages in Bolinao, Sual and Alaminos, Pangsinan were not affected by the demolition.

The proliferation of pens and cages in marine waters since 1996 coincided with the intensification of culture in ponds particularly in Visayas and Mindanao. These ponds were formerly intensive prawn ponds. So, throughout the country, milkfish is being cultured in high densities.

The price of milkfish continued to increase from 1989 to the first quarter of 1997 (Table 2). But the industry's expansion resulted in oversupply. This became apparent when there was a big drop in prices in the third quarter of 1997. The price increased towards the end of 1997 but it is still low compared to 1996 to early 1997 prices. The decrease in price leads to more losses of most cage, pen and pond operators.

Table 2 Wholesale prices of milkfish (1989-1998)

Year	2-3 pcs per kg	3-4 pcs per kg	4-5 pcs per kg
1989	35	30	25
1990	40	35	30
1991	50	45	40
1992	50	45	42
1993	50	45	42
1994	55	50	45
1995	60	55	50
1996	70	60	50
1997 1st quarter	90	70	60
2nd	80	60	55
3rd	60	50	45
4th	70	60	55
1998 1st quarter	65	60	55
2nd	55	45	38
3rd	55	45	38

At present, the fishpen and fishcage operators who are still in the business are the original operators who were able to make a lot of profit during their first year of operation and those who have efficient systems and low production cost. The latest Typhoon Gading flooded most areas in Pangsinan which resulted to more losses. Even the offshore cages were not spared by the typhoon. The stocks from at least twenty (20) units of Polar Cirkel cages were lost due to damage in nets. Some fishpens in Bolinao were also damaged. Those fishpens which were reinforced with new wooden and bamboo poles prior to the typhoon suffered minimal losses.

In the Visayas and Mindanao areas, most fishpond operators have stopped stocking milkfish in their farms because of low prices. With these developments, most fishpen and fishcage operators are hopeful that the prices will increase in the next few months.

Netcage in SEA ... from p 25

Singapore

The Primary Production Department (PPD) of Singapore in 1986, identified suitable species for intensive cage culture as: grouper (*Epinephelus tauvina,* sea bass (*Lates calcarifer*) and golden snapper (*Lutjanus johni*). Floating net cage was found to be a more advantageous aquaculture system because it can be adjusted to adverse conditions. The Straits of Johore are suitable sites for floating net cage culture where waves are normally less than 0.5 m in height. PPD recommends the following parameters for net cage culture:

Fingerlings of 7.5-10.0 cm (3-4") can be stocked in hapa nets at 100-150 fish per m^2. Cages of 2 x 2 x 2 m can hold 400-600 fingerlings until they reach a size of 12.5-15.0cm (5-6") which then should be size graded into 44 fish per m^2. A nursery cage of 5 x 5 x 3 m can hold 1,100 fish. After 2-3 months, fish are transferred to grow out cages where they are cultured for 6-8 months. Trash fish are used as feeds, the size of which would depend on the size of the fish. For fingerlings, feeds are chopped finely at 1 cm (0.4") and for grow out, around 2.5 cm (1"). Feeding is done once or twice daily at 10% BW.

Regular net changing would ensure good water exchange in the net and obtain optimal environmental conditions for the fish.

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