

# Research on Crustaceans

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## Abstract

Crustacean research at the SEAFDEC Aquaculture Department in 1994-1999 focused on two commercially important species: the tiger shrimp *Penaeus monodon*, and the mud crab *Scylla serrata*. Research on tiger shrimp dealt with broodstock development, refinement of shrimp culture systems, and health management. Broodstock development aimed to develop a technology for a sustainable supply of good quality captive broodstock through selective breeding. Initial efforts identified polymorphic stocks with low disease prevalence as base population and development of screening protocol to assess their health status using non-lethal procedures. Improvement of reproductive performance through studies on nutritional requirements and sperm quality was also conducted. To refine shrimp culture systems, emphasis was placed on the physiological requirements of tiger shrimp, including salinity adaptation and osmoregulatory capabilities, improvement of formulated diets, and development of culture systems that are compatible with the environment. In shrimp health management, disease problems in various culture systems with emphasis on luminescent vibriosis and some viruses were defined. The quality of hatchery-reared post-larvae compared with those caught in the wild was assessed.

Research on the mud crab *Scylla serrata* started late in 1996. Studies were conducted on all culture phases: broodstock, hatchery, nursery, and grow-out. Broodstock development emphasized the development of an appropriate maturation system and a suitable maturation diet. The influence of eyestalk ablation and dietary history on reproductive performance was assessed. The completion of the mud crab life cycle in captivity was attained in 1997 when spawns from pond-reared females were further reared to produce second-generation broodstock. In the hatchery, larval rearing based on previous trials on feeding schemes, salinity tolerance, and water conditioning hastened progress in larviculture and formed the basis for large-scale production of mud crab juveniles. Research has shown the feasibility of direct stocking of crab megalopae in hapa nets in nursery ponds. In grow-out culture, studies have been done on the effects of stocking density, monosex culture, and practical diet development for the mud crab. Practical diets, formulated using local materials as ingredients, with or without vitamin and mineral supplementation, were found to be economically feasible for mud crab culture in ponds. Grow-out culture in mangrove pens appears to be an environment-friendly alternative to the usual open pond culture system.

## Introduction

Research on crustaceans at the SEAFDEC Aquaculture Department in 1994-1999 focused on two species of commercial importance: the tiger shrimp *Penaeus monodon*, and the mud crab *Scylla serrata*. Studies on the tiger shrimp were conducted on three major areas: broodstock development, refinement of culture systems, and health management. Studies on the mud crab were conducted on all phases of culture: broodstock, hatchery, nursery, and grow-out.

### Studies on the Tiger Shrimp *Penaeus monodon*

Research on the tiger shrimp aimed to develop a sustainable supply of good quality broodstock, ecologically sound pond grow-out management techniques, and environmentally sound methods of health maintenance.

#### *Broodstock development*

Preliminary studies leading to the development of a selective breeding program for *P. monodon* was initiated. L de la Peña (personal communication) compared the genetic diversity between wild and captive shrimp populations in the Philippines using a randomly amplified polymorphic DNA technique. Wild shrimps came from different regions: Palawan, Panay, Negros, and Quezon while cultured shrimps came from Antique and Negros provinces. Wild shrimps were found to be more polymorphic (66-71%) than cultured shrimps (54%), with Palawan samples showing the most genetically diverse population and Negros the least diverse. Palawan is a pristine area with extensive mangroves and no major aquaculture activity while Negros has severe mangrove loss and highly intensive shrimp culture systems. It appears that biological diversity in tiger shrimp populations is related to the status of mangroves and intensity of culture systems.

Further, JH Primavera *et al.* (personal communication) compared the prevalence of infectious hypodermal hematopoietic necrosis virus (IHHNV) in various wild populations of *P. monodon* and related this with shrimp culture intensification and mangrove status. Lower viral incidence in wild shrimp was found in sites with primary mangroves and no major shrimp farms, whereas higher levels were found in areas with intensive farms and severely degraded mangroves. Wild populations had a lower overall IHHNV incidence of 51% compared with 100% among F<sub>2</sub> and F<sub>3</sub> generations *P. monodon*. In another study, Primavera and Quintio (2000) observed some morphological abnormalities associated with the runt deformity syndrome (RDS) among an F<sub>3</sub> generation of *P. monodon* (>40 mm CL). RDS was characterized by a high carapace length-to-abdominal length ratio and a low carapace width-to-1st abdominal segment width ratio. Examination of external abnormalities and evaluation of morphometric ratios may complement existing protocols for disease and growth rates in shrimp breeding program.

Meanwhile, to improve their reproductive performance, studies on nutritional requirements and sperm quality of captive broodstock were conducted. Pangantihon-Kuhlmann *et al.* (1998) evaluated the effect of dietary supplementation with various levels (0, 50, 100, 150, 200 ppm) of astaxanthin, a major pigment in shrimps, on reproductive performance. A 50 ppm astaxanthin supplementation improved the fecundity; however, egg hatch rates and larval metamorphosis did not vary among levels tested.

Quintio and Parado-Esteva (1995) found that sperm quality improved in captive males of at least 65g body weight and recommended this to be the minimum size requirement for broodstock. Regardless of size, tank-held males showed a decline in sperm quality after 35 days. Eyestalk ablation of males had a negative effect on sperm quality and produced more abnormal (deformed

body, spikeless, bent spike or a combination of these characters) sperm, although the sperm counts did not differ.

### **Refinement of shrimp culture systems**

Studies to refine existing shrimp culture systems were focused on salinity adaptation and osmoregulatory capability of juvenile tiger shrimp, improvement of shrimp grow-out diets, and development of ecologically sound pond management practices.

The ability of *P. monodon* to adapt to changes in salinity was studied by Parado-Esteba (1998). *P. monodon* postlarvae and juveniles were transferred from ambient salinity to salinities of 4-50 ppt at temperatures of 22 C, 28 C and 33 C. The ability to tolerate a wide range of salinities improved with age and survival, but was generally lower at 33°C. Another study showed that osmolality, sodium and chloride concentrations in the hemolymph were efficiently regulated at 8-32 ppt at all temperatures tested. However, loss of ability to osmoregulate was observed when *P. monodon* juveniles were exposed to extremes of salinity and temperature (i.e., 4 ppt and 22 C, 40 ppt or higher and 33 C).

Several studies were conducted to improve formulated shrimp diets for grow-out. Millamena *et al.* (1996, 1997a, 1998, 1999b) established the quantitative requirements of *P. monodon* for ten essential amino acids, which is crucial in optimizing growth and feed efficiency and in developing cost-effective shrimp diets. Peñaflorida (1999) determined the phosphorus requirement of *P. monodon* by dietary supplementation with various levels of phosphorus, P (0, 0.5, 1.0, 1.5, 2.0 ppm). Highest weight gain was obtained at 0.5 ppm P, which represents the optimum supplementation level. At this level, P pollution from shrimp culture effluents may be minimized.

Golez and Millamena (1998) studied the utilization of processed soybean meal (trade name: HP 300) as an alternative source of protein for shrimp feeds. Processed soybean meal can replace 6-9% of fishmeal, 5% of shrimp head meal, and 4-6% unprocessed soybean meal in the diet of *P. monodon* juveniles. Millamena and Triño (1997c) developed a low-cost diet using mostly locally available materials. Local fishmeal (*Thunnus sp*) and cowpea meal (*Vigna unguiculata*) were the main sources of dietary protein in a diet without vitamins or mineral supplements. The low-cost diet can effectively support pond production of *P. monodon* at 1.9-2.6 tons per ha at stocking densities of 5-10 per m<sup>2</sup> and alleviates the high cost of shrimp grow-out feeds.

Bautista-Teruel and Subosa (1999) evaluated the effects of processing conditions, heating time and temperature, on urease activity (UA) levels and on nutrient quality of soybean meal (SBM). Protein quality of SBM in terms of amino acid content was not significantly affected by processing condition. Heat treatment for SBM was adequate at 120 C for 20 min. Diets processed under this condition had the highest growth and survival among shrimps fed this diet. UA was minimal at 0.32 ppm compared with 20-25 ppm in diets with unprocessed SBM.

### **Shrimp health management**

Studies on health management in culture systems focused on common disease problems in cultured shrimps: luminescent vibriosis and viruses. Lavilla-Pitogo *et al.* (1998a) reported the following observations on luminous bacterial (LB) disease: 1) the underlying cause of mortality are the vibrios, mainly *Vibrio harveyi*; 2) the mode of infection is oral with the hepatopancreas, a major storage organ for nutrients, as the target organ; 3) vibrios are attracted to chitin, a component of crustacean exoskeleton; 4) vibrios exhibit a wide tolerance to environmental parameters compared with its host *P. monodon*, making control of vibrio infection by environmental manipulation difficult; and 5) exposure of post-larvae to luminous *Vibrio* levels equal to or greater than 10<sup>3</sup> colony forming units (cfu) per ml

in the rearing water can cause mass mortalities. Quantification of the bacterial flora of affected shrimps showed a high luminous bacterial population of  $9.0 \times 10^4$  cfu per hepatopancreas (hp) compared with 70 cfu per hp of healthy shrimps (Leaño *et al.*, 1998). They suggested some preventive measures against *Vibrio* infection as follows: 1) since the growth of vibrios is suppressed in a dry pond bottom, thorough pond drying should be adopted as part of pond management protocol; 2) the use of reservoirs to reduce luminous bacterial population in a water supply source by sedimentation followed by disinfection with chemicals such as hypochlorite.

Studies were conducted to understand microbial interactions in the shrimp hatchery (Lavilla-Pitogo *et al.*, 1998b). These studies showed that the natural microflora associated with diatoms, *Chaetoceros* and *Skeletonema*, are potential sources of bio-control agents. In another study, the bacteria, *Pseudomonas* spp, was shown to possess vibriolytic activity (Dalisay, 1999). To develop environmentally sound methods for disease control, Lavilla-Pitogo and Paner (1999) attempted to use probiotics or bioaugmentation implements that are available commercially. Probiotic treatment involves the addition of benign bacteria through the feed to alter shrimp gut microflora or as water and soil conditioner to create a healthy environment. Variable results were obtained with commercial probiotics due to factors like the lack of information regarding probiotics' biological components, mode of action, and conditions under which they may be effective, and loss of viability during transport and storage.

E Cruz-Lacierda (personal communication) examined viral diseases in *P. monodon* postlarvae and broodstock. Major viruses present in shrimp postlarvae were monodon baculovirus (MBV) and hepatoparvovirus (HPV) with infection rates of 32% and 30%, respectively. These viruses were also detected among *P. monodon* broodstock, in addition to IHHNV and lymphoid organ baculovirus (LOW). Further studies to compare the infection rates of MBV and LB in hatchery-reared and in wild-caught shrimp postlarvae were conducted. Infection rates for both MBV (23%) and LB (79%) were significantly higher in cultured postlarvae (4% and 40%, respectively).

Another disease detected by Lavilla-Pitogo *et al.* (1999) in tiger shrimp postlarvae was the swollen hind gut syndrome manifested by enlargement and distention of the hind gut folds and its junction with the mid gut. Disease prevalence ranged from 8 to 12% of yearly batches examined. Postlarvae affected by the disease have high mortality rates and exhibited wide size variation within batches, making these postlarvae unsuitable for culture in grow-out ponds.

### **Studies on the Mud Crab *Scylla serrata***

Research studies on mud crab aimed at developing sound and reliable technologies on all phases of culture to promote consistent spawning and hatching of good quality larvae, increase survival and production of crablets, and improve pond yield.

#### ***Broodstock development***

Good quality eggs and larvae are produced by broodstock that are housed in optimal living facilities and given a suitable diet. In mud crab broodstock development, emphasis was placed on developing an appropriate maturation system and a suitable maturation diet. The maturation system consisted of 10-m<sup>3</sup> circular concrete tanks provided with flow-through seawater supplied in an upwelling flow, sand substrate, and a shelter per female. Females were stocked at a low density of 1 per m<sup>3</sup>. Multiple re-maturation and spawning, usually two to three times per female, are possible under this scheme. Using this maturation system, studies to develop a suitable maturation diet for *S. serrata* were conducted by Millamena and Qunitio (1999a). A combination diet of natural food (mussel meat and fish by-catch) and a SEAFDEC-formulated diet gave more consistent maturation

and spawning of good quality eggs and larvae. The influence of eyestalk ablation on reproductive performance was further studied. Eyestalk ablation shortened the latency period but did not improve reproductive performance. The technique may be used when there is an immediate need for seeds in mud crab hatcheries. Studies to compare the reproductive performance of pond-reared females with those caught in the wild showed that rearing broodstock on a defined diet in ponds improved their reproductive performance (Millamena and Quintio, 1997b). Completion of the mud crab life cycle in captivity was attained in 1997 when spawns from pond-reared females grew and were reared in ponds to produce the second-generation broodstock.

### **Larval rearing**

The major bottleneck in crab culture is low and inconsistent survival of larvae. In the hatchery, larval rearing based on previous trials on feeding schemes, salinity tolerance, water conditioning schemes, and health management hastened progress in larvi-culture and formed the basis for large-scale production of mud crab juveniles.

The major food items and feeding density used in larval rearing of *S. serrata* are: *Brachionus plicatilis* at 10-15 individuals per ml and *Artemia sp* at 1-5 individual per ml. The suitability of alternative feeds was tested, which aimed at reducing feed costs. A micro-particulate larval diet developed by SEAFDEC can be used as partial substitute, thereby reducing dependence on natural food. Larval survival (9.3-10.5%) in this diet was improved compared with natural food alone (3.3%). Toledo *et al.* (1998) has shown that mixed zooplankton, *Acartia* and *Pseudodiaptomus* nauplii that are normally found in brackishwater ponds, can be used as supplement for rotifers.

Major morphological changes during larval development were characterized through light and electron microscopy. The major changes observed by G Loya-Javellana (personal communication) were setation, sclerotization, appearance and development of the gland filter, gastric mill and cardiac chamber. The implications on larval feeding ability and size-specific feeding regimes are: feeding may not be critical at the early zoea-1 stage and complicated feed types are suitable only at zoea-3. Parado-Esteva and Quintio (1997) did studies to determine the optimum salinity levels for the zoeal stages and found that *Scylla sp.* larvae can tolerate a wide salinity range of 20-32 ppt. Gradual lowering of seawater salinity from ambient to 25 ppt starting at late zoeal stages, zoea-4 or zoea-5, until the megalopae stage improved larval survival. Further studies were conducted on water conditioning schemes. Aging, chlorination, and dechlorination with prophylaxis treatment were found to be suitable methods of water conditioning and were used as components of the larval rearing protocol.

Studies on health management in the hatchery conducted by Lavilla-Pitogo *et al.* (1998c) have shown that larval mortalities are mainly due to systemic bacterial infection. The major causative agent is the vibrios and all inputs to hatcheries such as spawned eggs, natural food, and untreated seawater supply are possible sources of infection.

Quintio and Parado-Esteva (2000) conducted studies on simulated transport of mud crab zoeae and megalopae. They found that the optimal loading densities were:  $10 \times 10^3$  individuals per liter for zoeae and 50 individual per liter for megalopae. These densities should be used during transport for subsequent stocking in nursery ponds.

### **Nursery rearing**

Studies on nursery rearing, from megalopae to crablet, focused on nutrition and feeds, effects of

stocking density, shelter and substrate requirements. Tank studies under controlled conditions and nursery pond studies in hapa nets were conducted.

E Quintio *et al.* (personal communication) assessed the effect of various natural diets; *Acetes*, *Artemia*, mussel and snail, on survival and growth of crab instar-1. Specific growth rate of crablets fed *Acetes* was significantly higher compared with other diets. Survival after 30 days did not significantly differ among treatments. Large-scale production of crab megalopae using the best feeding and management schemes defined in small-scale experiments resulted in survival of 0.4 to 50% (zoea-1 to megalopae) and 19 to 47.3% (megalopae to instar-2). Subsequently, hatchery-produced megalopae have been cultured in ponds of private hatchery operators (Quintio *et al.*, 1997)

G Loya-Javellana (personal communication) examined the shelter and substrate preference of *S. serrata* juveniles. Larger juveniles, 10-14 g body weight, preferred net shelters. Gravel may replace mud as substrate for rearing smaller juveniles while sand may replace mud for rearing larger juveniles. Rodriguez *et al.* (1998) have shown the feasibility of direct stocking of crab megalopae in hapa nets in nursery ponds. Megalopae were stocked at 10, 20, and 30 individuals per m<sup>3</sup> in 20 m<sup>2</sup> hapa nets provided with dry coconut fronds as shelter and cultured for 30 days. Survival rates of 35.6-53.3% and mean final body weight of 2.9-3.4 g did not significantly differ among treatment groups. Growth rates in ponds were found to be 6-10 times higher than in tanks, demonstrating that direct stocking of megalopae in ponds can circumvent problems of inadequate nutrition and cannibalism.

### **Grow-out culture**

Grow-out culture studies examined the effects of stocking density and monosex culture. The development of practical diets for the grow-out was also attempted. Triño *et al.* (1999) evaluated the effects of stocking density and monosex (male or female) culture of *S. serrata* stocked at 0.5, 1.5 and 3.0 per m<sup>2</sup> in ponds. All-male culture gave significantly higher mean body weight (410-475 g) than all-female culture (325-332 g). Both male and female monosex cultures had high net revenue and return on investment of more than 100%. Stocking crabs at a density of 0.5-1.5 individuals per m<sup>2</sup> was economically viable, environmentally sound, and profitable.

Pond production of mud crab fed a formulated diet made of local materials as ingredients, with or without vitamin and mineral supplementation, was compared with trash fish diet as control. Growth of crabs fed diet with vitamin supplementation was similar to crabs fed trash fish but significantly better than those fed without vitamin supplements. The economics of feeding a formulated diet may outweigh the greater growth obtained with trash fish and can save on costs of storage equipment and electricity (Triño *et al.*, 1998).

Mud crab farming in net pens or enclosures in mangroves was evaluated as an alternative to pond culture. Triño and Rodriguez (1998) showed that a stocking density of 0.5-1.5 individuals per m<sup>2</sup> gave survival of 30-52% and final body weight of 296-350 g. This culture system demonstrates the viability of an environment-friendly farming system.

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