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SHRIMPS

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ABSTRACT

During 1988-1991, research at the Aquaculture Department of SEAFDEC on the shrimp *Penaeus monodon* has been directed towards a) the development of captive broodstock, b) the refinement of hatchery and grow-out techniques, c) the development of diets for the various stages of culture, and d) the prevention and control of diseases. Biochemical, morphological, and histological characterization of the male and female reproductive systems were conducted to provide basic information for the development of techniques for pond-reared broodstock. Studies on the refinement of hatchery techniques included determination of the environmental and feeding requirements of larvae and postlarvae to serve as basis for the improvement of management practices. Refinement of grow-out techniques included studies on the physiological response of this species to vital environmental factors and studies on the role of natural food organisms during culture. Nutrition studies have resulted in the formulation, testing, and improvement of diets for broodstock, larvae and postlarvae, juveniles, and subadult shrimps. Methods of prevention and control of the luminous bacterial disease, chronic soft shell syndrome, aflatoxicosis, *monodon baculovirus* (MBV) infection, and other relevant diseases have been investigated through the identification of causative agents and bioassay of possible chemo-therapeutants.

Studies to improve larval rearing of alternative shrimp species such as *P. indicus, P. merguiensis,* and *P. japonicus* have likewise been pursued. Nutritional requirements of the white shrimp species were evaluated to develop suitable formulated feeds for the different culture stages.

INTRODUCTION

Shrimp culture remains to be a fairly lucrative venture despite decline in prices. Like many growing industries, it is beset with diverse problems. Among the most pressing of these are the fluctuating and decreasing market price, occurrence of diseases in all phases of shrimp culture, and the dwindling supply of wild spawners or shrimp broodstock. Studies at the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC/AQD) during the last 4 years were focused on improvement of techniques towards more cost effective culture methods, disease control or prevention, and development of captive broodstock to address these problems and respond to the needs of the industry.

TIGER SHRIMP

Breeding

Induced maturation of *P. monodon* is usually achieved through ablation of one eyestalk. However, hatching rates of produced eggs are relatively low (Vogt et al. 1989). Hence, alternative methods which may elicit better reproductive performance were tested. Environmental manipulation through varying light quality was investigated as a possible factor influencing maturation. For unablated or ablated females, highest mean hatching rates of eggs and nauplii produced per female were obtained with green light and with the control (Primavera and Caballero, unpublished results). In addition, β-ecdysone and 17α-hydroxyprogesterone administration gave promising results in inducing maturation (Yashiro 1989).

Scarcity of spawners and declining number of adults from the wild, coupled with the inconsistent performance of larvae have spurred interest in developing shrimp broodstock in ponds. Studies on shrimp reproduction were conducted to develop techniques for growing captive broodstock. In one such study, Quinitio et al. (1990) isolated and described the ovarian protein vitellin and its related protein in the hemolymph, vitellogenin.

Based on histology and histochemistry, Tan-Fermin and Pudadera (1989) reclassified the existing stages of ovarian maturation into four: the previtellogenic, vitellogenic, cortical rod, and spent stages. For industry application, the ovarian width at the first abdominal segment must be measured to minimize arbitrariness; an ovarian width of at least 20 millimeter indicates readiness for spawning. There are no differences in the kind and appearance of oocytes in both ablated and unablated females. However, only the smallest (10-100 micrometer)or immature oocytes are left in the ovaries of unablated *P. monodon* after spawning. In contrast, larger (100-200 and 210-300 micrometer) oocytes in all stages of maturation are present in the ovaries of ablated females, indicating faster rematuration. Release of large but unripe oocytes during spawning may account for the low hatching rates obtained with ablated females (Tan-Fermin 1991, Vogt et al. 1989).

Lipid and protein levels (Millamena and Pascual 1990, Peñaflorida and Millamena 1990) in the ovaries were shown to significantly increase at early maturing to fully mature stages and decline at spent stage. Millamena and Pascual (1990) attribute the lipid increase to mobilization from the hepatopancreas to the ovary, while Peñaflorida and Millamena (1990) associated increases in ovarian protein concentration with synthesis of polypeptides, including yolk polypeptides, in the ovary. Amino acid and fatty acid (Peñaflorida and Millamena 1990, Millamena 1989, Millamena and Pascual 1990) profiles of the tissues were used to elucidate requirements of broodstock.

Studies on the early developmental stages of the reproductive system were done to determine the minimum age or size at which pond-grown *P. monodon* can be induced to mature or produce sperm. Results showed that primary oocytes can initially be observed among 2 to 3-month old female shrimps while early signs of spermatogenesis can be detected in 4- to 5-month old males (Toledo, personal communication).

Another study determined the effects of tags and tank color. Neither variable affects reproductive performance, but tagging causes a significant decrease in survival (Primavera and Caballero 1989).

Hatchery

The increase in number of hatcheries in the Southeast Asian region indicates that adoption of existing techniques for larval rearing is already economically feasible. Thus, studies concentrated on refinement of techniques to obtain consistent or higher survival or growth rates, lower production costs, or simplify rearing methods. Modification of the major activities affecting postlarval production were focused on water management and feeding practices.

Past methods using untreated seawater for rearing *P. monodon* larvae (SEAFDEC/AQD Working Committee 1984) have been revised to include pretreatment with chlorine and sodium EDTA (Parado-Estepa et al. 1991). EDTA improves survival by chelating heavy metals in the medium (Licop 1988) and chlorine significantly reduces bacterial populations in the water by 90%. Bacterial load builds up after 6 hours following neutralization, thus, use of treated water is highly recommended (Baticados and Pitogo 1991).

Larval rearing involves feeding of both artificial diets and phytoplankton. Several inexpensive protein sources were screened as possible substitutes for animal protein to lower cost of larval diets. These feed components were evaluated using an index based on the amino acid profile of whole tissues (Peñaflorida 1989). In addition, kappa-carrageenan microbound diet (C-MBD) was formulated (Bautista et al. 1989) and tested as larval food for *P. monodon.* In 40-liter culture tanks, natural food (NF) or C-MBD fed alone or in combination (NF+C-MBD) gives similar survival rates. However, verification tests in 10-ton tanks showed significantly higher survival with NF+C-MBD than natural food alone (Bautista et al. 1991).

Natural food is important for shrimp larvae; thus, several algal species were evaluated for nutritional quality. *Tetraselmis chuii* has higher protein and crude fat levels comparable to *Chaetoceros calcitrans* or *Skeletonema costatum* (Millamena et al. 1990b).

The optimal feeding levels using *Tetraselmis* was defined by determining the incipient limiting level (ILL) or the lowest food density to provide maximum ingestion rates at each substage. ILL increases with age of larvae until M_{III} and declines at postlarva 1. However, growth or percentage molting is significantly affected by food density only at the protozoeal substages (Loya-Javellana 1989). Therefore, *Tetraselmis* must be maintained at a density not lower than 10,000 cells/milliliter (ILL) at the protozoeal stage. For the mysis and postlarval 1 stages, 20,000 and 30,000 cells/milliliter (lowest densities tested), respectively, can be used and still attain comparable growth rates.

To overcome the problem of synchronization of natural food production with hatchery activities, Millamena et al. (1990a) developed a method for harvesting and preserving algae. Sun dried *Chaetoceros* or *Tetraselmis* proved to be acceptable to shrimp larvae.

Artemia is given at the mysis and postlarval stages. Tests showed that survival of postlarvae fed *Artemia* maintained on algae is higher compared with those fed *Artemia* maintained on ricebran (Millamena et al. 1988).

The pollution of culture water by excess feeds was demonstrated by Millamena (1990). Excess feed increases the biochemical oxygen demand (BOD), depresses dissolved oxygen (DO), and elevates NH_4-N and NO_2-N to levels detrimental to shrimp postlarvae.

Grow-out

Pond culture studies have focused on the refinement of techniques involved in extensive and semi-intensive methods. Growth of natural food in the pond can be enhanced through fertilization, which may also cause deterioration of soil or water conditions when applied indiscriminately. Comparison of fertilization schemes showed that biweekly application of 15 kilograms of nitrogen and 30 kilograms phosphorus per hectare with or without chicken manure increases shrimp yields (Subosa and Bautista l991a,b). Further increases in the amount of fertilizers do not improve yields.

Triño and Bolivar (1990) correlated shrimp production with the presence of lablab (a microbenthic complex of blue-green algae, diatoms, and microscopic plants and animals), lumut (composed mostly of filamentous green algae like *Chaetomorpha* and *Enteromorpha),* or digman *(Najas graminea)* in extensive ponds. Mean body weight and total production (kilograms) are highest in digman ponds. Primavera and Gacutan (1989) similarly found that juveniles fed live *N. graminea* attain highest survival and a high mean length compared with those fed either live or decaying *Ruppia maritima* or decaying *N. graminea.*

Studies related to semi-intensive culture concentrated on the improvement of supplementary diets for *P. monodon.*

Catacutan (1990) evaluated diets containing various carbohydrate levels based on survival, growth, and apparent digestibility. In another study, inositol, choline, and ascorbic acid were identified as the most essential vitamins for normal development of juvenile shrimps (Catacutan and de la Cruz 1989). Semi-intensive culture trials, however, resulted in similar survival or growth of shrimps fed diets with or without vitamins, suggesting that requirements may have been satisfied by the natural food (Triño et al. 1992).

Substitution of feed components to lower production cost of shrimps has been tested. Pascual et al. (1990) demonstrated that defatted soybean meal can replace up to 35% of animal protein. *Leucaena leucocephala* leaf meal as a protein source, however, is not suitable for shrimps (Pascual and Catacutan 1990).

Hemolymph calcium levels of P. *monodon* at different molt stages and salinities were investigated. Total hemolymph calcium is largely influenced by molt stage and to a lesser degree by salinity (Parado-Estepa et al. 1989).

Diseases

One of the major problems experienced by the shrimp industry during the past few years is the occurrence of diseases. In the hatchery phase, extensive mortalities occurred due to the luminous bacteria, identified as *Vibrio harveyi* and *V. splendidus* (Lavilla-Pitogo et al. 1990b). Chemical control against this disease was tested but appears to be limited because of the ineffectiveness and prohibitive cost of readily available drugs, and the morphological deformities produced in treated larvae (Baticados et al. 1990b). Thus, potential sources and routes of entry into the larval rearing system were identified to establish a set of preventive measures. Results showed that *V. harveyi* can enter the hatchery system mainly through the fecal matter from spawners, the seawater, or unwashed *Artemia* cysts (Lavilla-Pitogo et al. 1990a). These further suggest that breeders must be removed and the eggs washed after spawning to prevent infection of stock. Treatment of seawater and rinsing *Artemia* nauplii prior to use in the hatchery are also recommended.

Another disease of shrimps is caused by the *P. monodon-type* baculovirus identified by Baticados et al. (1991). Affected shrimps exhibit pale bluish-gray to dark-blue coloration, loss of appetite, and retarded growth (Baticados et al. 1990a).

The chronic soft-shell syndrome, a disease caused by several factors such as nutritional deficiency, presence of toxic elements, and poor water and soil conditions, affects juvenile and adult shrimps (Baticados et al. 1990a). This disease can also be induced by exposure to 1.5 to 150 parts per billion of the commonly used pesticide Gusathion A (Baticados and Tendencia 1991). Levels of calcium do not significantly vary between soft-shelled and healthy animals (Baticados et al. 1986). It was suggested that the Calcium-to-Phosphorus (Ca:P) ratio, and not calcium alone, may exert a greater influence on shell hardening. Subsequently, Bautista and Baticados (1990) showed that soft-shelling is reversed after feeding a diet containing a 1:1 Ca:P ratio.

Other diseases of shrimps are the shell disease (Lio-Po and Lavilla-Pitogo 1990) and red disease. The latter may be caused by aflatoxin or rancid feeds (Baticados et al. 1990a). Infection can be prevented by storing feeds in well-ventilated areas at a temperature of 10-20°C to retard oxidation (De la Cruz et al. 1989).

ALTERNATIVE SPECIES

Studies similar to those conducted for *P. monodon* were done for *P. merguiensis* and *P. indicus.* Changes in hemolymph vitellogenin levels during maturation (Quinitio, unpublished results), salinity tolerance of larvae (Yashiro, unpublished results), and pond culture using different densities (Mesa and Alava, unpublished results) are some of the studies being conducted for these species. Diets for all stages of *P. indicus* and *P. merguiensis* have been formulated and tested (Bautista, unpublished results; Peñaflorida, personal communication). For *Penaeus japonicus,* its life cycle in captivity was completed under Philippine conditions (Quinitio et al., in press).

CONCLUSION/RECOMMENDATIONS

One of the most urgent problem now facing the industry is the lack of *P. monodon* spawners or adults. Basic information from initial work must be considered and applied to the development of captive broodstock. For both *P. monodon* and alternative species, continued efforts must be exerted to refine hatchery and pond rearing techniques, with emphasis on maximizing cost effectivity.

Disease occurrence may continue to adversely affect the shrimp industry. Thus, research must also be directed towards finding immediate but practical means of disease prevention and control.

REFERENCES

Baticados MCL, Coloso RM, Duremdez RC. 1986. Studies on the chronic soft shell syndrome in the tiger prawn, *Penaeus monodon* Fabricius, from brackishwater ponds. Aquaculture 56: 271-285.

Baticados MCL, Cruz-Lacierda ER, de la Cruz MC, Duremdez-Fernandez RC, Gacutan RQ, Lavilla-Pitogo CR, Lio-Po GD. 1990a. Diseases of penaeid shrimps in the Philippines. Aquaculture Extension Manual No. 16. SEAFDEC/AQD, Tigbauan, Iloilo. 46 p.

Baticados MCL and Pitogo CL. 1991. Chlorination of seawater used for shrimp culture. Israeli J. Aquacult. -Bamidgeh 42: 128-130.

Baticados MCL, Lavilla-Pitogo, CR, Cruz-Lacierda ER, dela Peña L, Suñaz NA. 1990b. Studies on the chemical control of luminous bacteria *Vibrio harveyi* and *V. splendidus* isolated from diseased *Penaeus monodon* larvae and rearing water. Dis. of Aquat. Org. 9: 133-139.

Baticados MCL, Tendencia EA. 1991. Effects of Gusathion A on the survival and shell quality of juvenile *Penaeus monodon.* Aquaculture 93: 9-19.

Baticados MCL, Pitogo CL, Paner MG, de la Peña LD, Tendencia EA. 1991. Occurrence and histopathology of *Penaeus monodon* baculovirus infection in hatcheries and ponds in the Philippines. Israeli J. Aquacult. - Bamidgeh 43: 35-41.

Bautista MN and Baticados MCL. 1990. Dietary manipulation to control the chronic soft-shell syndrome in tiger prawn, *Penaeus monodon* Fabricius. p. 341-344. In: The Second Asian Fisheries Forum. Hirano R and Hanyu I (Eds.). Asian Fisheries Society, Manila, Philippines. 991 p.

Bautista MN, Millamena OM, Kanazawa A. 1989. Use of kappa-carrageenan microbound diet (C-MBD) as feed for *Penaeus monodon* larvae. Mar. Biol. 103: 169-173.

Bautista MN, Parado-Estepa FD, Millamena OM, Borlongan E. 1991. Large scale production of *Penaeus monodon* using natural food and artificial diets. Israeli J. Aquacult. - Bamidgeh 43: 137-144.

Catacutan MR. 1990. Apparent digestibility of diets with various carbohydrate levels and the growth response of *Penaeus monodon.* Aquaculture 95: 89-96.

Catacutan MR and dela Cruz M. 1989. Growth and midgut cells profile of *Penaeus monodon* juveniles fed water-soluble-vitamin deficient diet. Aquaculture 81: 137-149.

De la Cruz M, Erazo G, Bautista MN. 1989. Effect of storage temperature on the quality of diets for the prawn *Penaeus monodon.* Aquaculture 80: 87-95.

Lavilla-Pitogo CR, Albright LJ, Paner MG, Suñaz NA. 1990a. Studies on the sources of luminescent *Vibrio harveyi* in *Penaeus monodon* hatcheries. A paper presented at the Symposium on Diseases. 26-29 November 1990. Bali, Indonesia.

Lavilla-Pitogo CR, Baticados MCL, Cruz-Lacierda ER, de la Peña L. 1990b. Occurrence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. Aquaculture 91: 1-13.

Licop MSR. 1988. Sodium EDTA effects on survival and metamorphosis of *Penaeus monodon* larvae. Aquaculture 74: 239-247.

Lio-Po GD, and Lavilla-Pitogo C. 1990. Bacterial and exoskeletal lesions of **the tiger** prawn *Penaeus monodon.* p.701-704. In: The Second Asian Fisheries Forum. Hirano R, Hanyu I (Eds). Asian Fisheries Society, Manila, Philippines. 991 p.

Loya-Javellana G. 1989. Ingestion saturation and growth responses of *Penaeus* larvae to food density. Aquaculture 81: 329-336.

Millamena OM. 1989. Effect of fatty acid composition of broodstock diet on tissue fatty acid patterns and egg fertilization and hatching in pond-reared *P. monodon.* Asian Fish. Sci. 2: 127-134.

Millamena OM 1990. Organic pollution resulting from excess feed and metabolite build-up: effect on *Penaeus monodon* postlarvae. Aquacult. Eng. 9: 143-150.

Millamena OM, Aujero E, Borlongan IG. 1990a. Techniques in algae harvesting and preservation for use in culture as larval food. Aquacult. Eng. 9: 295-304.

Millamena OM, Bombeo RF, Jumalon NA, Simpson KL. 1988. Effects of various diets on the nutritional value of *Artemia* sp. as food for the prawn *Penaeus monodon* Fabricius. Mar. Biol. 98: 217-222.

Millamena OM and Pascual FP. 1990. Tissue lipid content and fatty acid composition of *Penaeus monodon* Fabricius broodstock from the wild. J. World Aquacult. Soc. 21: 116-121.

Millamena OM, Peñaflorida VD, Subosa PF. 1990b. The macronutrient composition of natural food organisms mass cultured as larval feed for fish and prawns. Israeli J. Aquacult.-Bamidgeh 42: 77-83.

Parado-Estepa FD, Ladja JM, de Jesus EG, Ferraris RP. 1989. Effect of salinity on hemolymph calcium concentration during the molt cycle of *Penaeus monodon.* Mar. Biol. 102: 189-193.

Parado-Estepa FD, Quinitio ET, Borlongan E. 1991. Prawn hatchery operations. Aquaculture manual no. 19. SEAFDEC/ AQD, Tigbauan, Iloilo. 44 p.

Pascual FP and Catacutan MR. 1990. Defatted soybean meal and leucaena leaf meal as protein sources in diets for *Penaeus monodon* juveniles, p. 345-348. In: The Second Asian Fisheries Forum. Hirano R, Hanyu I (Eds). Asian Fisheries Society, Manila, Philippines. 991 p.

Pascual FP, Cruz E, Sumalangcay A Jr. 1990. Supplementary feeding of *Penaeus monodon* juveniles with diets containing various levels of defatted soybean meal. Aquaculture 89: 183-191.

Peñaflorida V. 1989. An evaluation of indigenous protein sources as potential component in the diet formulation for tiger prawn, *Penaeus monodon,* using essential amino acid index (EAAI). Aquaculture 83: 319-330.

Peñaflorida V, Millamena OM. 1990. Variation in the biochemical composition of *Penaeus monodon* tissues during the reproductive cycle. Israeli J. Aquacult-Bamidgeh 42: 84-90.

Primavera JH, Caballero R. 1989. Effect of tagging on maturation and survival of ablated *Penaeus monodon* in painted and unpainted tanks. Philipp. Sci. 26: 5-20.

Primavera JH, Gacutan RQ. 1989. Preliminary results of feeding aquatic macrophytes to *Penaeus monodon* juveniles. Aquaculture 80: 189-193.

Quinitio ET, Hara A, Yamauchi K, Fuji A. 1990. Isolation and characterization of vitellin from the ovary of *Penaeus monodon.* Inv. Reprod. Dev. 17: 221-227.

Quinitio ET, Parado-Estepa FD, Coniza E. Notes on the completion of the life cycle of *Penaeus japonicus* in captivity in the Philippines. Phil. J. Science 120. (In press).

SEAFDEC AQD Working Committee. 1984. Prawn hatchery design and operation. 2nd Ed. Aquaculture Ext. Manual no. 9. Tigbauan, Iloilo, Philippines. 50 p.

Subosa PF, Bautista M. 1991a. Influence of stocking density and fertilization regime on growth, survival and gross production of *Penaeus monodon* Fabricius in fertilized brackishwater ponds. Israeli J. Aquacult.- Bamidgeh 43: 69-76.

Subosa P, Bautista M. 1991b. Yield of *Penaeus monodon* Fabricius in brackishwater ponds given different fertilizer combinations. Aquaculture 94: 39-48.

Tan-Fermin JD. 1991. Effects of unilateral eyestalk ablation on ovarian histology and oocyte size frequency of wild and pond-reared *Penaeus monodon* (Fabricius) broodstock. Aquaculture 93: 77-86.

Tan-Fermin JD, Pudadera R. 1989. Ovarian maturation stages of the wild tiger prawn, *Penaeus monodon* Fabricius. Aquaculture *77:* 229-242.

Triño AT, Peñaflorida VD, Bolivar EC. 1992. Growth and survival of *Penaeus monodon* juveniles fed a diet lacking vitamin supplements in a modified extensive culture system. Aquaculture 101: 25-32.

Triño A, Bolivar E. 1990. Growth performance of *Penaeus monodon* in lablab, lumut, and digman ponds under various farm practices. J. Aquacult. Trop. 5: 123-129.

Vogt G, Quinitio ET, Pascual FP. 1989. Interaction of the midgut gland and the ovary in vitellogenesis and consequences for the breeding success: a comparison of unablated and ablated spawners of *Penaeus monodon.* p. 581-592. In: Aquaculture - a biotechnology in progress. De Pauw N, Jaspers E, Ackeford H, Wilkings N (Eds.). European Aquacult. Soc. Bredene, Belgium.

Yashiro R. 1989. Biochemical, immunological, and histological studies on ovarian maturation of *Penaeus monodon* Fabricius. A doctoral dissertation submitted to the University of the Philippines Dilliman Quezon City, Philippines. 64 p.