BROODSTOCK MANAGEMENT AND SEED PRODUCTION OF *PENAEUS MONODON* (FABRICIUS)

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ABSTRACT

Research on the maturation of *Penaeus monodon* at AQD has focused on three broad areas, namely, reproductive biology and ecology, induced maturation and broodstock management. Studies on reproductive biology provided information on the life cycle, ovarian maturation stages, courtship and mating behavior, minimum size at sexual maturation (sperm occurrence, first spawning), and morphological egg types. Induced maturation has mainly been done through the eyestalk ablation method. Nutritional and environmental parameters were studied to enhance reproductive performance or as an alternative to ablation. Pond-reared and wild broodstock sources and marine pen and land-based tanks as maturation systems were also tested and compared. Size, shape, color, substrate material and other aspects of tank design and construction, sex ratio, stocking density, water management, and other parameters of the management system were also studied and refined.

Early techniques in larval and postlarval rearing of P. *monodon* at AQD were based on the community culture method of growing natural food in larval tanks. However, low and inconsistent survival led to a shift in rearing methods toward pure phytoplankton culture grown in separate tanks as food for the larvae. Henceforth, refinement of rearing methods have been conducted to improve larval survival through effective water management, nutrition, and disease control. Efforts are continuously being geared toward making the technology affordable to Filipino farmers.

INTRODUCTION

In 1973, when the Aquaculture Department was first established, there was no commercial prawn culture in the country to speak of - prawns and shrimps were harvested as incidental crops from milkfish

ponds. Recognition of penaeids as an important aquaculture commodity is evidenced by the start of prawn research that very same year. Since then, continuing research on prawn biology and culture at AQD has generated technology particularly in the areas of maturation and larval rearing which are highlighted in this paper.

BROODSTOCK DEVELOPMENT AND MANAGEMENT

Reproductive Biology and Ecology

Much of the available information on the life cycle of *Penaeus monodon,* can be traced to the five-year study of Motoh (1981) on the fisheries biology of the species. He described five ovarian maturation stages - undeveloped (1), developing (2), nearly ripe (3), ripe (4), and spent (5) - based mainly on progressively increasing mean egg diameter (35-235 μm) and external appearance. Other workers (Santiago 1977; Pudadera and Primavera 1981; Primavera 1982, 1983) also used external and dissected appearance of ovaries and histology to classify maturation stages. More recently, Tan and Pudadera (unpublished) gave a comprehensive classification into four stages using histological (egg diameter, gonadosomatic index) and histochemical criteria:

1. previtellogenic stage - oogonia and oocytes in chromatin nucleolus and/or perinucleolus eosinophilic,

2. vitellogenic stage - eosinophilic yolky oocytes,

3. cortical rod stage - oocytes with peripheral rod-like bodies, and

4. spent stage - remaining oocytes with yolk and/or cortical rods, thicker follicle layer, and few dark irregular perinucleolar oocytes.

Motoh (1981) found that over a three-year period, occurrence of wild *P. monodon* spawners peaked in March and October in Tigbauan, Iloilo (Southern Panay Is.) and in February, July, and November in Batan Bay, Aklan (Northern Panay Is.). Minimum spawner size was 48 mm carapace length (CL) and mean size was smaller (62 mm CL) in Batan Bay, a river mouth, compared to large sizes (64-72 mm CL) in the offshore waters of Tigbauan, Iloilo and Himamaylan, Negros Occidental (Motoh 1981).

Table 1 summarizes data on minimum size of *P. monodon* at sexual maturation: functional maturity or ability to mate of the male (petasma jointed) and female (presence of sperm in thelycum) and physiological maturity or production of eggs and sperm (Motoh et al 1976; Motoh 1981; Primavera 1978). Interestingly, pond stock reached maturity at sizes smaller than wild animals.

Table 1. Minimum size at first sexual maturation *of Penaeus monodon* (Motoh et al 1976, Primavera 1978, Motoh 1981)

Maturity	Male	Female
Functional	jointed petasma = 34 mm CL wild	+ sperm, thelycum = 47 mm CL wild = 39 mm CL pond
Physiological	+ sperm, testes = 37 mm CL wild = 31 mm CL pond = 40 g BW pond	maturation, spawning = 48 mm CL wild = 75 g BW wild

Copulation in closed thelycum penaeids, such as *P. monodon,* requires molting of the female. Primavera (1979) described three stages in the courtship and mating behavior of *P. monodon* starting with the parallel swimming of female and male or males up to the insertion of spermatophores inside the thelycum by one successful male. Such information has been valuable in designing maturation systems.

Based on his observations, Motoh (1981) concluded that first mating occurs at 4-5 mo in brackishwater areas where ovarian maturation also commences but full maturation is completed followed by spawning only after migration to offshore waters at around 10 mo.

A positive correlation was established between fecundity and female size in terms of CL (Motoh 1981) and body weight (Villegas et al 1986). Development of *P. monodon* eggs was described by Motoh

(1981); incubation period or hatching is 12-15 hr after spawning. Incubation period and hatch rate of eggs were observed under different temperature-salinity conditions (Reyes 1981). Time to hatching was inversely proportional to temperature whereas salinity did not affect it. Conversely, salinity affected hatch rate of eggs, but not temperature.

Based on morphology and hatch rates, Primavera and Posadas (1981) developed an egg classification system (Fig. 1) useful in predicting the number of nauplii from a given prawn. There is a highly significant linear relationship between the proportion of good (A_1) eggs and hatch rate.

Spawners are disinfected with 50-100 ppm formalin and spawning water with 10 ppm chlorine. Treatment of spawned eggs with 10 ppm detergent gave significantly higher hatch rates (97.2%) compared to those treated with other fungicides and untreated controls (Po and Sanvictores 1986).

Induced Maturation

Three basic approaches have been employed to induce ovarian maturation in penaeid shrimps: endocrine stimulation by ablation, nuttritional, and environmental. So far, maturation and successful spawning have been reported only for eyestalk-ablated *P. monodon* although diet and environmental parameters may also enhance reproductive performance.

Completion of the cycle of P. *monodon* was first achieved in 1975 (Santiago 1977). Maturation was observed in both unilaterally and bilaterally ablated females but the latter did not spawn and suffered total mortality. Unablated females as controls did not mature.

Although cutting, tying and cautery have been tried the preferred ablation method is by incision-pinching because of its convenience and lack of stress on the prawn (Primavera 1978, 1983).

Monitoring of tagged (Rodriguez 1976) ablated females shows an increase in rematuration rates in 1977-1979 from 10.4 to 23.2% for second spawnings and from 1.6 to 5.9% for third spawnings (Primavera 1982).

Nutrition plays an important role in maturation. In the wild, 85% of ingested food of adult *P. monodon* consisted of small crabs and shrimps and molluscs (Marte 1980). The more frequent occurrence

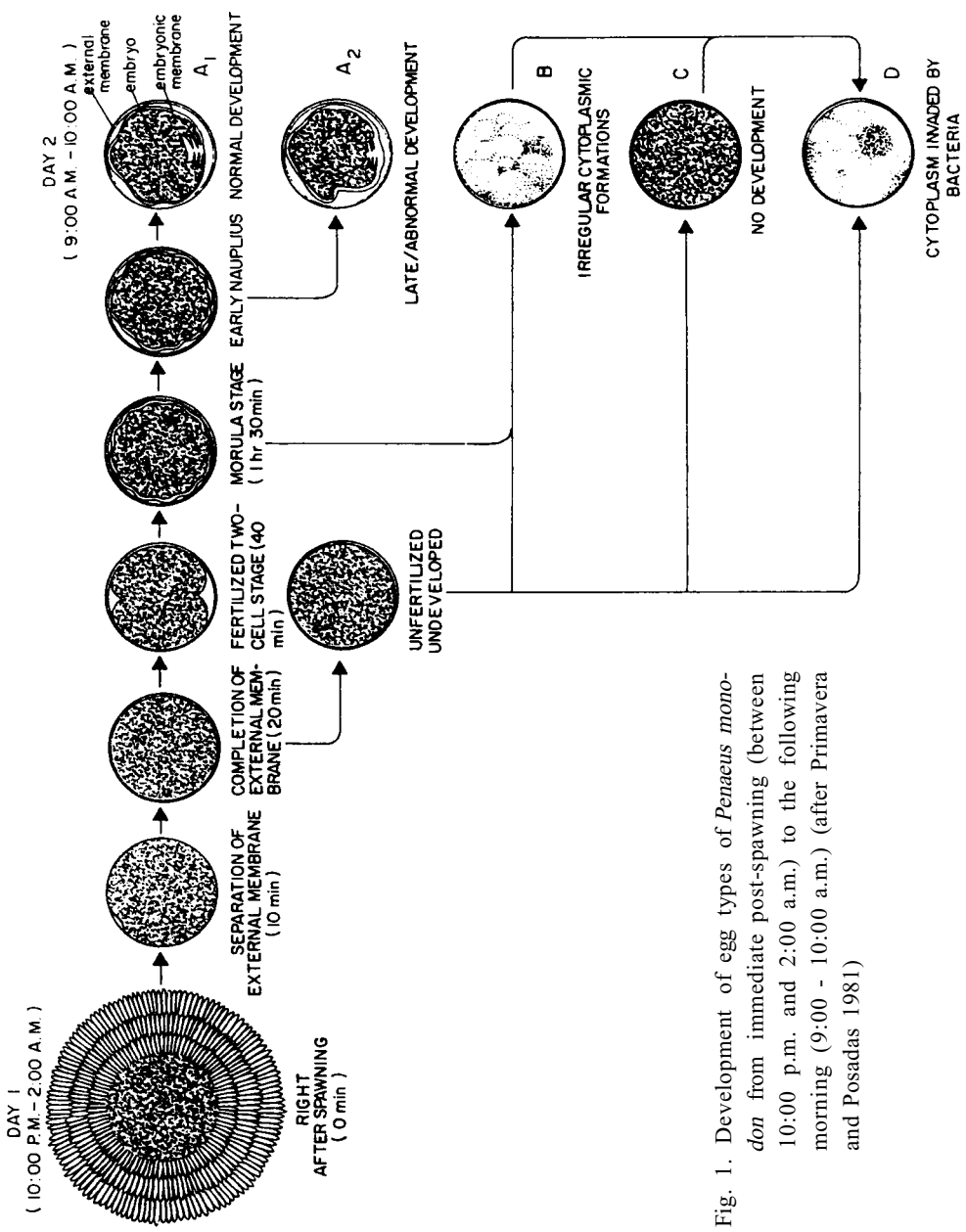

Fig. 1. Development of egg types of *Penaeus monodon* from immediate post-spawning (between 10:00 p.m. and 2:00 a.m.) to the following morning (9:00 - 10:00 a.m.) (after Primavera and Posadas 1981)

of molluscs and other non-crustaceans during months of higher feeding index may reflect changes in dietary requirements related to gonad development during the spawning season (Marte 1982).

Wild immature *P. monodon* females showed an increase in ovarian lipid levels upon reaching full maturity from 5.8 to 17.0% and from 7.5 to 21.9% in unablated and ablated females, respectively (Millamena et al unpublished). The fatty acid profile showed 12.1-24.9% and 11.8-21.5% total fatty acids in unablated and ablated females, respectively, consisting of 20:4ω6, 50:5ω3 and 22:6ω3 fatty acids. The same polyunsaturated fatty acids (PUFA) were reflected in the spawned eggs, indicating their importance in the reproductive process.

A study of different feeding regimes showed highest larval production and hatch rates when ablated *P. monodon* were fed with molluscs (squid, mussel) in combination with a pellet (Primavera et al 1979). More recently, Millamena et al (1986) obtained the best reproductive performance from ablated females given pellets with cod liver oil (longer chain C_{20} and C_{22} PUFA and higher ω3:ω6 ratio) compared to those with soybean lecithin (C_{18} PUFA and lower ω3:ω6 ratio).

A few environmental studies at AQD have focused on light. In an early study, unablated *P. monodon* attained only partial maturation under blue and natural light but not under red light (Pudadera and Primavera 1981). Only ablated females reached full maturation and spawned. An ongoing study incorporating recent improvements in broodstock management and nutrition will verify these results.

Broodstock Management and Maturation System

Broodstock from the wild or pond source may be stocked in maturation tanks or pens, and retrieved as gravid females, eggs or nauplii (Primavera 1985).

Broodstock source. *P. monodon* broodstock may be obtained from the wild or from ponds. Wild broodstock are caught from coastal waters by trawler or motorized pumpboat such as a baby trawler and from estuaries by tide-dependent stationary gear such as fish corrals, lift nets, and lever nets (Motoh 1980, Primavera 1983). Age at ablation from spawning varies from 5 mo (Primavera 1978) to15 mo (Santiago 1977). Satisfactory maturation, spawning, and metamorphosis of larvae to postlarvae have been obtained from females with a minimum age of 8 mo (Primavera 1982, unpublished; Millamena et al 1986). In the wild, *P.*

monodon attains full maturity and spawning at 10 mo (Motoh 1981).

Early studies showed poor reproductive performance of pond stock compared to wild stock with the proportion of good eggs and corresponding hatch rates lowest from pond-ablated followed by wild ablated and best hatching from wild spawners (Primavera and Posadas 1981). Hence for the last three years, studies at AQD have concentrated exclusively on pond stock.

Even with production of good quality larvae and postlarvae from pond-reared *P. monodon,* the state-of-the-art is to use wild broodstock for three reasons. First, the non-availability of pond broodstock-holding the prawns beyond the 3-4 mo cropping period means a loss to the farmers. Second, wild broodstock give a faster turnover producing in 6 weeks the same number of nauplii pond broodstock can yield in 12 weeks. Lastly, pond stock require a maturation pellet often not commercially available (Millamena et al 1986) whereas wild broodstock can be maintained on natural food alone.

Maturation systems. Primavera (1983, 1985) traced the evolution of a suitable maturation system for *P. monodon* dating back to the Igang pens in 1975 through varying sizes of land-based tanks to the present 12 m^3 tank. The tank system offers advantages of security and convenience in maintenance and broodstock sampling over the pen.

Tanks may be made of cement, ferrocement, fiberglass, and plastic or canvas-lined aluminum or wooden tanks. Tank size is a compromise between the biological requirements of the broodstock and the convenience of the hatchery personnel as can be seen in the popularity of the 10-12 m^3 circular tank. In a recent study, Primavera et al (unpublished) observed consistently higher total egg and nauplii production and hatch rates from females in a black-walled tank compared to those in unpainted tanks. A white sand substrate gave significantly higher nauplii production and hatch rates from ablated *P. monodon* compared to black sand (Pudadera et al 1980a).

Ideally, maturation tanks should have a flow-through water system with 100-400% daily exchange rate. However, water should be recirculated when flow-through is not feasible as when sea water is polluted or turbid during typhoons (Primavera 1983).

Depending on water quality and exchange rate and prawn size, stocking density of P. *monodon* is 2-7 / m^2 (Primavera 1985). Sex ratios

of 1-2 females to 1 male are more economical because they maximize egg and larval production per tank (Pudadera et al 1980b).

Although ablated *P. monodon* can mature and spawn at a salinity range of 15-32 ppt, full salinity is required for incubation and hatching of eggs (Posadas 1986).

Monitoring and Retrieval

Nauplii collectors have been tested for *P. monodon* (A.T. Young pers. comm,), but spawner retrieval gives the advantages of individual records of body measurements, egg numbers and hatch rates, and easier processing of eggs (Primavera 1983). Examination of broodstock and retrieval of spawners is more manageable in 10-12 m^3 tanks than in tanks larger than 20 m^3 ,and more efficient with frequent monitoring of broodstock. Nightly checking of a 12 m^3 tank yielded 48 spawnings producing 6.8 × 10^6 nauplii compared to only 3.0 × 10^6 nauplii from 29 spawnings with thrice weekly monitoring (Pudadera et al 1980a).

The maturation requirements for *P. monodon* including size and age, feeding and tank parameters are summarized in Table 2.

Table 2. Summary of maturation requirements for *Penaeus monodon,* SEAFDEC Aquaculture Department, 1975-1987

Size	70-150 g female, 45-120 g male
Source	Pond or wild
Age, pond	Minimum 10 mo (from spawning)
Ablation	Required
Feeding	
Regime	Pellet + natural food (mussel, squid, annelids)
Lipid source (pellet)	6% cod liver oil
Light intensity	Reduced (~ 100 lux)
Light quality	Ongoing study
Tank size	12 m^3 tank, circular
Tank color (inner walls)	Black
Substrate	White sand
Water	100-400% flow-through
Density	3-5/m^2 (300-400 g/m^2)
Sex ratio	1-2 females to 1 male

LARVAL AND POST-LARVAL REARING

Rearing Facilities

Seed production of *Penaeus monodon* in SEAFDEC AQD started in 1975 with rearing techniques similar to those used in the MSU-IFRD. Larvae were reared in rectangular 50, 120, and 200-t concrete tanks installed under a structure with translucent plastic roofing (Platon 1979). With the barangay hatchery concept, a more compact hatchery model was introduced in 1977. The basic unit consisted of 2-t larval rearing tanks, 2 units 1-t algal tanks, 2 units 1-t *Brachionus* culture tanks, and 1 unit 1-t *Chlorella* culture tank, a small compressor or blower for aeration, and 2 sets of water pump. Larval tanks were made of marine plywood shaped into a cylinder with an octagonal cross section and a conical bottom fitted with a drain valve to facilitate draining and harvesting (Platon 1978).

In 1981, the use of shallow (not more than 1 m deep) bathtub-shaped larval tanks made of concrete, wood or fiberglass was recommended. Instead of gate valves, 2 inch PVC drain pipes were installed for draining and harvesting. *Brachionus* and *Chlorella* culture tanks were no longer necessary as the feeding scheme was modified. Aeration was provided by portable electric aerators (Gabasa 1981).

The continuous search for low-cost materials for tanks led to the use of locally-available materials such as bamboo and wooden slats with polyethylene plastic sheet linings (Anon. 1984). There was more flexibility in the materials and shapes of larval tanks to be used as shown in Table 3.

Rearing Methods

Seed production of *P. monodon* at SEAFDEC AQD started with techniques adopted from the community culture method where phytoplankton was allowed to bloom in the larval rearing water as food for the animals. A modification in the system was the hatching of eggs in the spawning tank and not in the larval tank (Anon. 1975). Water was introduced to the larval tanks to a depth of 2 m and was fertilized so that there will be a diatom bloom by the time animals molt to the zoea stage. Nauplii were stocked at a density of 6 000/t as mass mortality occurred at densities beyond this. Baker's yeast was introduced at the zoea and *Artemia* nauplii or rotifers *(Brachionus)* at the mysis stage

Table 3. Comparison of larval tanks used in the seed production of *Penaeus monodon*.

Category	Capacity	Material	Description
Large-scale hatchery[a]	50-200 t	concrete	rectangular
Barangay hatchery[b]	2 t	marine plywood	cylindrical with an octagonal cross-section and a conical bottom; with gate valve for draining
Small-scale[c]	preferably 1.5-12 t	concrete, wood, or fiberglass	bath-tub type; about 0.8 m deep with 2" PVC drain pipe
Small-scale[d]	preferably 3-5 t	concrete, fiberglass, marine plywood, or locally-available materials such as bamboo or wood slats with plastic sheet lining	circular, rectangular or square; sloping bottom; with drain pipe

[a]Platon (1979)

[b]Platon (1978)

[c]Gabasa (1981)

[d]Anon. (1984)

(Table 4). About 20% of the total water volume was changed daily starting at mysis stage. Survival rate at harvest (P_9) was very low and inconsistent, attributed to the non-selective production of natural food and uncontrolled blooms that fouled the water.

An important innovation in the feeding method was initiated in 1976. Diatoms such as *Chaetoceros* or *Skeletonema* were grown in separate algal tanks and concentrated with the use of a sand filter before feeding (Yap 1979). This lessened the possibility of unwanted algal blooms and allowed the culturist to choose the species of phytoplankton to be fed.

To scale down the hatchery technology to a level which can be adopted by local farmers with a minimum of financial and technical input, the barangay hatchery was conceptualized in 1977. Smaller tanks and a more compact hatchery model was used (Platon 1978). Stocking density of larvae was increased to 30-50/1 and harvest was at P_5. The 1977 barangay hatchery feeding scheme in Table 4 was used. However, the basic concern was the culture of natural food which needs specific technical skill and which involved use of more tanks and synchronization of mass production of food with larval rearing. Studies on optimization and simplification of production techniques were thus conducted.

Optimization studies included testing of media for phytoplankton production (Anon. 1977) and screening of phytoplankton species for larval food (Aujero et al 1983). Simplification of production techniques involved studies on the use of preserved natural food so these may be cultured during off-culture periods of larvae.

The use of 100 ppm alum or lime or NaOH to raise the pH of the phytoplankton medium and cause floc formation was tried. The harvested phytoplankton were successfully preserved by freezing at -20 to -22°C with a cryoprotectant but had to be neutralized before use (Aujero and Millamena 1981). This technique was not adopted by private operators as this entailed use of a pH meter which is quite costly. Frozen rotifers and sun-dried phytoplankton were also tested as food but did not perform as well as live phytoplankton (Anon. 1979).

Other attempts in lessening phytoplankton requirement or dependence on this were made by testing possible phytoplankton supplements such as baker's yeast (Villegas and Kanazawa 1980), marine yeast (Aujero et al 1984), whole egg, brown mussel meat, trash fish, soybean cake (Quinitio et al 1983) and egg yolk (Quinitio and Reyes 1983).The

Table 4. Feeding schemes used in the larval rearing of *P. monodon* at SEAFDEC AQD

	ZI	ZII	ZIII	MI	MII	MIII	P1	P2	P3	P4	P5
1. Modified community culture method[a]											
Phytoplankton	----	----	----	----	----	----	----	----	----	----	----
+Baker's yeast	----	----	----	----	----	----	----	----	----	----	----
+*Artemia* or *Brachionus*				----	----	----	----	----	----	----	----
2. Barangay hatchery scheme[b]											
Skeletonema or *Chaetoceros* (x 10^3 cells/ml)	30	50		80							
+*Tetraselmis* (x 10^3 cells/ml)		5	10		20			5	10		
+*Brachionus* (ind/ml)				5	8	10					
+*Artemia* (ind/ml)									5		
3. Small-scale hatchery scheme[c]											
Tetraselmis (x 10^3 cells/ml)	----	----	5	----	----	----					
+Egg yolk (particles/ml)			15	25							

	+Artemia (ind/ml)			10 – 15	15 – 20
	+Baker's yeast (if not *Tetraselmis*)	0.1-0.3/t/day			
4.	Modified egg yolk feeding method[2]				
	Skeletonema or *Chaetoceros*	5 – 10			
	or *Tetraselmis*	2.5 – 5			
	or mixed diatoms (x 10^5 cells/ml)	5 – 10			
	or mixed diatoms (x 10^5 cells/ml)	5 – 10			
	+Egg yolk (particles/ml)		15-25	2 – 5	
	+Artemia (ind/ml)				2-5
5.	Present feeding scheme[3]				
	Skeletonema or *Chaetoceros* (x 10^5 cells/ml)		20 – 50		
	+Microparticulate diet (g/t/day)		1 – 3		
	+Artemia (in/ml)			0.5 – 5	

most popular among those tested was the eggyolk introduced by Gabasa (1981). Using the feeding scheme in Table 4, an average survival rate of 52.9% (P_5) was obtained while only 2.5% resulted from the previous scheme (Gabasa 1981). The stocking density of nauplii was increased to 50-100/1, so more larvae could be reared in a given volume of the tank. Furthermore, tanks for *Chlorella* and *Brachionus* cultures were no longer necessary. The ratio of algal tank to larval tank volume, therefore, decreased (Table 5). With this technology, rearing of P_5 for 10-15 more days in nursery tanks before transferring to ponds was recommended. A further modification of this culture method (Anon. 1984a) is presented in Table 4. This allowed more freedom in selecting the phytoplankton species to be given as food to the larvae.

Later studies, however, revealed that egg yolk was deficient in polyunsaturated fatty acids which was shown to be an important component of larval diet (Millamena and Quinitio 1984.) This was one of the causes of the shift toward the use of commercially available micro¬particulate diets (Table 4 present feeding scheme). Use of locally available feed such as powdered *Acetes* also gave promising results (Kungvan¬kij et al 1983). However, feeding of these artificial diets still involved use of natural food (Table 4). With the carageenan-microbound diet formulation, preliminary results showed that this could be used as a total phytoplankton substitute (Bautista and Millamena unpublished).

The Aquaculture Department has also probed into the possibility of using substitutes for *Artemia.* But *Brachionus, Moina* sp. and *Tisbintra* as food for mysis and postlarvae did not perform as well and were not as convenient to use as *Artemia* (Anon. 1982). To make this expensive food item available locally, *Artemia* culture in tanks (Sorgeloos et al 1980) and in salt ponds (De los Santos et al 1980; Primavera et al 1980; Jumalon et al 1983) has been successfully undertaken.

Studies on biology and larval stage identification by Motoh (1979) were valuable as feeding and water management in a hatchery system vary with larval stage. Investigation of the larval tolerance to various environmental factors such as salinity (Reyes 1981) and nitrite and ammonia (Catedral 1977) also contributed to the determination of optimal rearing conditions.

Diseases and Parasites

Research on the prevention and control of diseases and parasites includes (1) isolation and identification of possible pathogens, (2) screening

Table 5. Feeding schemes at SEAFDEC AQD

Feeding Scheme	Food Items Used Until P_5 Rearing	Ratio of Tanks for Natural Food Culture to Larval Tanks
Modified Community Method (Anon. 1975)	Phytoplankton Baker's yeast *Artemia* or *Brachionus* or Copepods	No separate tanks for natural food culture
Barangay Hatchery Method (Platon 1977)	Diatoms *Brachionus* (to be fed with *Chlorella)* *Tetraselmis* *Artemia*	1-1.5:1
Small-scale Hatchery Method (Gabasa 1981)	*Tetraselmis* sp. Egg yolk *Artemia*	0.5:1
Small-scale Hatchery Method (Anon. 1984)	Diatoms or *Tetraselmis* sp. Egg yolk *Artemia*	0.17-0.2:1
Present Method	Diatoms Microparticulates *Artemia*	0.17-0.2:1

for a suitable prophylactic (prevention or chemotherapeutic (control), and (3) bioassay experiments to test larval tolerance to the chemotherapeutic.

Several microorganisms have been identified to cause mortalities in the larval stages. These include the suctorean parasite *Ephelota gemmi¬para* (Gacutan et al 1977), the ciliates *Zoothamnium, Vorticella,* and *Epistylis* (Gacutan 1979), fungi of the *Lagenidium* (Baticados et al 1977)

and *Haliphthoros philippinensis* (Hatai *et al* 1980) species, and bacteria such as *Leucothrix* and *Vibrio* (Anon. 1985). Infestation by ciliates were, in many instances, cast off by successful molt. These pathogens were not very lethal to *P. monodon* (Gacutan 1979).

Lagenidium and *Haliphthoros* were the most prevalent causes of larval mortalities in prawn hatcheries in the late seventies (Gacutan 1979). Several chemicals were tested for the prevention and control of these mycotic agents. At its effective dose, malachite green was tolerated only by mysis and post-larvae (Lio-Po et al 1978) while furanace not only caused reduction in phytoplankton population (Baticados and Gacutan 1977) but also caused deformities in the larvae (Gacutan et al 1978). Mycostatic and mycocidal chemicals were identified by Lio-Po et al (1982 and 1985) as potential control agents. Tolerance experiments indicated that Treflan-R at a dose of 0.2 ppm was effective against both pathogens and did not cause significant reduction of larval population (Lio-Po and Sanvictores 1986).

SUMMARY

Based on techniques used in the community culture and the Galveston (separate culture of natural food) methods, SEAFDEC AQD has adopted the technology to suit local conditions. It has also scaled down the hatchery technology to a level local farmers can afford.

Despite the achievements of the Department, several production constraints could be identified:

1. lack of spawner or nauplii supply
2. lack of technicians, and
3. occurrence of disease.

Research must, therefore, be directed toward improvement of the reproductive performance of pond-reared or wild broodstock and the optimization of rearing conditions for increased survival rate.

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