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PENAEID REARED BROOD STOCK: CLOSING THE CYCLE OF *P. monodon*, *P. stylirostris* AND *P. vannamei*

AQUACOP[1]
Centre Océanologique du Pacifique
CNEXO-COP
B.P. 7004
Taravao, Tahiti (French Polynesia)

ABSTRACT

Three shrimp species, *Penaeus monodon*, *P. stylirostris* (Panama and Mexican strains) and *P. vannamei* have matured and spawned in captivity at the Centre Océanologique du Pacifique (COP) laboratory. The shrimp, originally stocked as juveniles or postlarvae, were reared to adult size in tanks and ponds and have completed the cycle: F_3 generation for *P. monodon*, F_2 for *P. stylirostris* (Panama strain) and *P. vannamei* and F_1 for *P. stylirostris* (Mexican strain). Maturation occurs naturally under our rearing conditions or is induced by unilateral eyestalk ablation. Details are given on mating behavior, ovarian development, number of spawnings, egg viability and maintenance of captive brood stock. Partial results so far obtained for *P. monodon* and *P. stylirostris* clearly show a reared brood stock could sustain a commercial hatchery.

INTRODUCTION

In all countries, culture of penaeid shrimp is dependent on the capture of wild gravid females. Recent success in closing the cycle for different species has been obtained on an experimental scale; some mature and spawn spontaneously under certain culture conditions (Aquacop, 1975), others are induced to mature by thermal and light manipulations (Caubere et al., 1976, Laubier Bonichon, 1976-1978) or by eyestalk removal (Aquacop 1975, 1977a,b; SEAFDEC, 1976). Successive generations have been obtained (Aquacop, 1977b). In some cases immature wild males

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[1]Aquaculture team of the COP. Algae and mollusc cultures: J. L. Martin, O. Millous, Y. Normant, D. Gillet, O. Le Moine. Nutrition: A. Febvre, P. Vilmorin, J. J. Lainé, L. Mu, J. M. Guesne. Water quality control and treatment: J. Calvas, B. Couteaux, J. Bonfils, J. Y. Robert. Pathology: J. F. Le Bitoux, S. Robert. Crustacean and fish cultures: P. J. Hatt, M. Jarillo, J. P. Landret, J. Goguenheim, F. Fallourd, O. Avalle, J. Moriceau, D. Lacroix, S. Brouillet, R. Galzin, H. Pont, D. Amaru, V. Vanaa, A. Bennett, D. Sanford. Technology: J. F. Virmaux. Aquaculture program coordinator in tropical area: A. Michel.

and females of spawning age are collected and held in systems where maturation, mating and spawning occur (Environmental Research Laboratory 1978, Ralston Purina Company, St. Louis, Mo., personal communication).

To extend shrimp culture to areas of the world lacking native shrimp populations, to insure seed availability and to develop a genetic program, it is necessary to control maturation and reproduction in captivity. Since there is no indigenous species of penaeid shrimp for commercial culture in the waters of Tahiti, it has been particularly necessary for the Centre Oceanologique du Pacifique (COP) to perform such a program. Of seven different species under test, three (*Penaeus monodon*, *P. vannamei* and *P. stylirostris*) are the main candidates for intensive culture in a tropical environment. This paper deals with recent results obtained at the COP in rearing brood stocks and in closing the cycle for these species. Tentative answers are arrived at for the following basic questions: How does one rear the animals to spawning age? How should the brood stock be maintained? What are the maturation, mating and spawning processes in captivity? What is the rate of spawning, the number and viability of the eggs? What is the survival rate of larvae obtained by this process?

MATERIALS AND METHODS

The Vairao lagoon water is largely renewed by the swell action above the barrier reef; this gives a relatively stable environment which allows year-round culture of penaeid shrimp. The temperature of the water fluctuates from 25-29°C; the salinity is 35 ppt and pH remains constant at 8.2. A direct pumping in the lagoon provides continuously for all the COP facilities. In the ponds the temperature fluctuations are more important (23-34°C).

The first brood stock was reared from postlarvae for *P. vannamei* and from a few juveniles of *P. stylirostris* (Panama and Mexican strains). Both species have been obtained from Ralston Purina Company. For *P. monodon* the first brood stock comprised 14 juveniles and adults obtained from Fiji and New Caledonian Islands. The other brood stocks have been constituted through the new generations by the following procedure. Postlarvae are reared in 700 m^2 earthen ponds at a density of 30-60/m^2 until they reach 1-2 g size; they are then transferred into 2,500 m^2 earthen ponds at a density of 5-30/m^2 or to tanks of 800-1,200 m^2 at a density of 50/m^2 where they reach commercial size (15-22 g mean weight). At harvest, a few hundreds are stocked in earthen ponds at a density of 2-3/m^2 until they reach maturation size. Daily renewal of the water varies around 10% of the total volume. The brood stock is then transferred in a 400 m^2 tank with sand bottom where the water is injected through perforated plastic pipes which allow a self-cleaning action and avoid reduction of substrate. Density is 2-4 animals/m^2. Water depth is 2 m. In this tank animals are selected by divers and the largest and healthiest are stocked in twelve 12 m^2 circular maturation tanks with 80 cm of water.

In these tanks, previously described (Aquacop, 1975), the substrate is coral sand and the water renewal is 2-3 times the total volume daily through perforated plastic pipes imbedded in the sand. The water temperature fluctuates between 24 and 29°C but the pH remains stable at 8.2. Natural light is reduced 60-90% by shading. The sex ratio is 1:1 and

different densities have been tested (40-160/tank). In some experiments males and females of *P. stylirostris* and *P. vannamei* are maintained in separate tanks and mature females are sorted only a few hours before spawning and placed with the males. Compound pellets with a supplement of squid flesh are distributed. Unilateral eyestalk ablation is practiced on *P. monodon* and in some experiments on *P. stylirostris* and *P. vannamei*, by simple pinching of the eyestalk. Females are examined each night for ovarian development. For *P. monodon*, which has a dark carapace, this is done with a waterproof handlight which permits viewing the ovaries without handling, thus avoiding stress. When the ovarian color, shape and texture indicate readiness to spawn the animal is removed from the maturation tank. The *P. monodon* females are placed in a 2 m^3 tank where they achieve maturation or directly in a 500-liter spawning tank depending on the degree of ovarian development. A supplement of fresh troca flesh (*Trochus niloticus*) is added to the 2 m^3 tank. In the case of *P. stylirostris* and *P. vannamei*, before transferring the female to the 500-liter spawning tanks, it is necessary to check for spermatophore deposition on the thelycum. The spawning quality is determined by the percentage of normal, abnormal and unfertilized eggs. Larvae from each spawn are subsequently reared in 500-liter or 2 m^3 tanks following the procedure already described (Aquacop, 1977b).

RESULTS

The three species acclimatize well to rearing conditions. The shrimps molt regularly and no cannibalism is observed. However, behavior and disease resistance vary among species. *P. monodon* lies more often on the substrate and rarely burrows; the swimming activity is low day and night. *P. vannamei* burrows in the sand or earth substrate during the day and becomes active at the end of the afternoon. The two strains of *P. stylirostris* are active day and night and burrow rarely. No mass mortality problems have been encountered with *P. monodon* and *P. vannamei*, but *P. stylirostris* seems sensitive to a *Vibrio* giving the "white pleura disease" especially when temperatures are above 30°C. The molting periodicity in the maturation tanks is four to six weeks. An extension of this period is an indication of too old or weak animals and that the stock must be renewed.

In all species, sexual dimorphism appears early, and males and females can be easily distinguished after they reach 2 g size. The males present spermatophores around 10 g but successful deposition is seen only for males of 25 to 35 g.

It takes five to twelve months to reach the first maturation size depending on species and conditions in the grow-out period. In the maturation tanks, for biomass above 300 g/m^2, maturations are rare and spawning does not occur. The optimum stocking density seems to be 20 females between 50 and 130 g and 20 males from 35-60 g for *P. monodon*. It can be doubled for the smaller species such as *P. vannamei*. Under these conditions maturations and spawnings are frequent.

No mortality occurs during unilateral eyestalk ablation except for freshly molting females. Maturation after ablation can be very rapid; some females are able to develop full ovaries and spawn in three or four days; some others molt and it takes two or three weeks before development occurs. All the ablated females develop ovaries and spawn at least one time if they stay healthy. For *P. monodon* this operation is a

necessity and only a few females have matured with both eyes. For *P. vannamei* and *P. stylirostris* (Panama strain), the ablation gives earlier and more numerous maturations, but also a shorter life. For *P. stylirostris* (Mexican strain) the females have matured rapidly without eyestalk ablation. For the three species the maturation process once begun can be achieved in four days or can last two weeks whether the females are ablated or not. Each cohort of breeders can give spawnings for two to five months according to the species and it is necessary to have a regular renewal of the animals. The losses during a three-month period are around 10% and mainly involve the females which have to endure stress due to the handling (observations and and spawning process). The following procedure is also applied: once a month a maturation tank is emptied and the animals are used to bring the other tanks up to optimum density. Animals which show necrosis or damaged appendages are discarded. The empty tank is cleaned, dried and restocked with new animals selected from the 400 m^2 tank. Every six months the stock of the 400 m^2 tank is renewed.

Selection of ready-to-spawn females is simple for *P. vannamei* and *P. stylirostris* as their carapaces are translucent, permitting the color of the ovaries to be seen without hand-netting the animals. The gonad, which is first whitish, then yellow and reddish, turns golden brown or greenish brown on the day of spawning and presents a constriction in the top of the first abdominal segment. The males deposit the spermatophores only on hard-shell females which will spawn a few hours later. The courtship and mating behavior already described (Aquacop, 1977b) begins in the afternoon in relation to light intensity: it can be observed as early as 2:00 P.M. on cloudy days or just at sunset on bright days and last some hours. In some periods the chasing behavior is very intense and in others it is very low; this phenomenon seems independent of the presence of ripe females. The mating success on ripe females is around 50% for *P. stylirostris* (Mexican strain), 30% for *P. stylirostris* (Panama strain), and only 10% for *P. vannamei*. Sometimes the spermatophores are glued at a wrong place due to interference of another male or sudden interruption of the swimming against the tank wall. The percentage of sperm deposited can be increased by separation of males and females, and transferring only the ripe females into the male tank.

For *P. monodon* the observed courtship and mating behavior agrees with the detailed report of Primavera (1978). It takes place just after the molting of a female when the shell is soft. The spermatophores are injected in the thelycum where the sperm stay until the next molting. The percentage of successful impregnation is high, around 90%. The color of the gonad is whitish at the beginning, turning greenish or dark green on spawning day. There is no constriction in the first abdominal segment but on the contrary a swelling appears.

Each female can give several spawnings. Fourteen *P. stylirostris* (Mexican strain) gave 46 spawnings in two months. Six *P. monodon* gave 18 spawnings in three months. Sometimes spawning occurs in a short period of time between two molts; some *P. stylirostris* and *P. vannamei* have spawned twice at five-day intervals; one *P. monodon* gave three good spawnings within two weeks without molting; after each spawning the gonad appeared completely empty. For *P. monodon*, which has a closed thelycum, the same stock of sperm is used for the different spawnings between two molts. For the other species, which present an open thelycum, a new spermatophore must be attached prior to each spawning.

Regression of developing ovaries is quite frequent in *P. monodon* and the reason is unknown. Stress during handling has been suspected but it occurs also with undisturbed females observed only under waterproof flash light. In *P. stylirostris* and *P. vannamei* regression is rare and development of the ovaries leads almost every time to spawning.

The spawning process, which is always observed between 8:00 P.M. and 1:00 A.M. in the tanks, begins by sudden jumps and active swimming of the female. Then the animal spawns remaining stationary in the middle of the water, generally with the head on the tank wall and the pleopods actively dispersing the newly extruded eggs. It can occasionally happen that the female does not move and in that case the eggs are extruded in one jelly mass. The whole process lasts about one minute. Sometimes the ova which are in the head part of the gonad are spawned; the next morning the ova of abdominal lobes are always clearly visible but regression occurs within two or three days.

The cortical reaction is very rapid and the first segmentation occurs in a few minutes. The eggs under the microscope are of three types (Aquacop, 1977b): unfertilized eggs with two or three big cells and many small ones; normal fertilized eggs which are clearly recognizable by the fecundation membrane and the presence of well developed nauplii; and abnormal fertilized eggs in which development began but has stopped at various stages; these latter eggs generally show a broken fertilization membrane and some nauplii from such eggs are deformed with isolated cells and they may hatch to give larvae with two heads, missing appendages and other anomalies.

For *P. monodon* the percentage of normal eggs is high and can reach 95%, but some spawnings are apparently unfertilized despite the presence of sperm in the thelycum. Most of the females which are transferred directly from the maturation tanks to the spawning tanks give non-viable eggs or a low percentage of normal eggs. If the females finish their ovarian development in separate smaller tanks where the only difference is the supplement of fresh troca flesh, the spawning is much better. Among the different compounded pellets tested the best results have been obtained with those containing high protein (60%) including squid meal. For *P. vannamei* and *P. stylirostris* (Panama strain) the percentage of good eggs is very low and most of the impregnated females give abnormal or unfertilized eggs. The reason is not known. Under the exact conditions, in the same tank and on the same day, one female can give a good spawn and the others poor spawns. For *P. stylirostris* (Mexican strain) 50% of the eggs gave viable nauplii. The number of spawned eggs varies according to species size and individuals: 60,000 to 600,000 for *P. monodon* of 45 to 130 g size; 60,000 to 200,000 for *P. vannamei* of 30 to 45 g size; 100,000 to 250,000 for *P. stylirostris* (Panama strain) of 60 to 80 g size; and 70,000 to 100,000 for *P. stylirostris* (Mexican strain) of 30 to 40 g size. This number does not decrease in successive generations already observed.

Larval rearing results differ according to species. *P. monodon* is certainly the most difficult to culture in our conditions as it seems very sensitive to a bacterial disease causing rapid necrosis of the appendages (Aquacop, 1977a) between the zoea II and P_{10}. *P. vannamei* and *P. stylirostris* are much more resistant. For all species the first losses during larval rearing can occur at the first zoeal stage; some of the newly molted larvae do not ingest algae and die in a few hours. The

percentage of non-feeding animals seems related to a high percentage of abnormal development in the spawned eggs. The larvae that feed immediately at zoea stage 1 continue to develop normally. In the absence of bacterial attack, survival is around 80% at a density between 25 and 140 P_1/liter.

By December 1978 we had obtained the third generation of *P. monodon*, the second of *P. vannamei* and *P. stylirostris* Panama strain, and the first reared brood stock of *P. stylirostris* (Mexican strain) is beginning to spawn. For *P. monodon* and *P. vannamei* maturation and reproduction in captivity occur throughout the year with temperature above 24°C. For *P. stylirostris* (Panama strain), the maturation appears to decrease during the hot months (February to June) but this could be an artifact due to the age of the stocks under experiment. For the Mexican strain we need to work with more animals to reach a conclusion; so far we have had maturations from December to April. Main results are summarized in Table 1.

TABLE 1. Summary of Maturation Experiments with Different Species

Species	Unilateral ablation	% Maturation	Age of first maturation (month)	Wt of gravid females (g)	% Sperm	No. spawning /♀/cycle	No. ♂/♀ x 1000	Spawning (month)	% Regression
P. monodon	yes	90	07-12	45-130	90	1-4	60-600	2-3	40
P. vannamei	yes-no	90	12 15	30-45	10	1-3	60-200	2-3	10
P. stylirostris (Panama strain)	yes-no	90	07-09	45-80	30	1-3	10-250	2-3	10
P. stylirostris (Mexican strain)	no	90	05-07	30-40	50	1-3	70-100	2-3	10

DISCUSSION

In the past decade a vast amount of research has been directed toward the understanding and manipulation of the reproductive cycle in penaeid prawns. Unilateral eyestalk pinching is very successful under our culturing conditions. It is a prerequisite for *P. monodon* and enhances greatly the results for *P. stylirostris* and *P. vannamei*; however, these two species also spawn naturally.

A number of relevant problems must be solved to attain good reliability. *P. monodon* so far gives the best promise in view of the number of good spawnings, but the proportion of regression among females which have started their ovarian development must be decreased. For *P. vannamei* and *P. stylirostris* Panama strain, maturations and spawnings occur frequently but the problem is the low percentage of successful mating and normal eggs. For *P. stylirostris* Mexican strain, if the data obtained during a two-month period can be confirmed during the whole year, the percentage of normal eggs is already sufficient.

From the preceding results and if the remaining technical problems are solved, the following conclusions can be drawn:

1. It is possible to rear enough large, healthy animals in captivity to constitute brood stocks in five to ten months depending upon the species, and rearing conditions. New stock must be available every

six months to work on a year-round basis. This stock will be used to fill and replenish the maturation tank ponds.

2. Tanks of 12 m^3 with sand substrate are of suitable size to give maturation and mating of the three species.

3. In each maturation tank, the same animals give a high number of spawnings and good quality eggs in a two or three month period.

A mean result of one spawning/month/female could probably be achieved on a routine basis giving a minimum of four million eggs per tank. If we can obtain the percentage of good eggs for *P. vannamei* and *P. stylirostris* (Panama strain) that is already obtained for *P. monodon* and *P. stylirostris* (Mexican strain), one tank could easily supply two million nauplii per month.

If we assume a 50% survival during the larval period and a 50% survival from P_5 to commercial size, two tanks could sustain a production of 20 tons/month.

This solution seems more convenient than operating a boat the whole year in search of gravid females. It will also enable development of genetic selection.

CONCLUSION

The use of reared penaeid brood stocks through successive generations, as has been done in *Macrobrachium* culture, must be achieved in the near future. However, it seems necessary to have a strict brood stock rotation plan to optimize the period when the maximum number of spawnings of viable eggs may be obtained.

The results presented in this paper show that it will be possible under these conditions to obtain spawning the entire year at a sufficient rate for the three species. The problem of the low viability of the spawned eggs of *P. vannamei* and *P. stylirostris* (Panama strain) remains to be solved. Two research topics seem of prime importance: determination of the exact nutritional requirements during the maturation process and developing methods for hormonal control of the cycle.

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