Development of a Brood Stock of the Jumbo Tiger Prawn, *Penaeus monodon* Fabricius

1. Completion of the life cycle of *Penaeus monodon* in captivity

2. Ovarian rematuration of spent females of *Penaeus monodon* in captivity

Technical Report No. 1

May, 1976

AQUACULTURE DEPARTMENT
SOUTHEAST ASIAN FISHERIES DEVELOPMENT CENTER
Tigbauan, Iloilo, Philippines

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TABLE OF CONTENTS

INTRODUCTION	1
THE STUDY SITE	3
COMPLETION OF LIFE CYCLE	5
REMATURATION OF SPENT SPAWNERS	11
CONCLUSION	14
LITERATURE CITED	15
ACKNOWLEDGMENT	16

INTRODUCTION

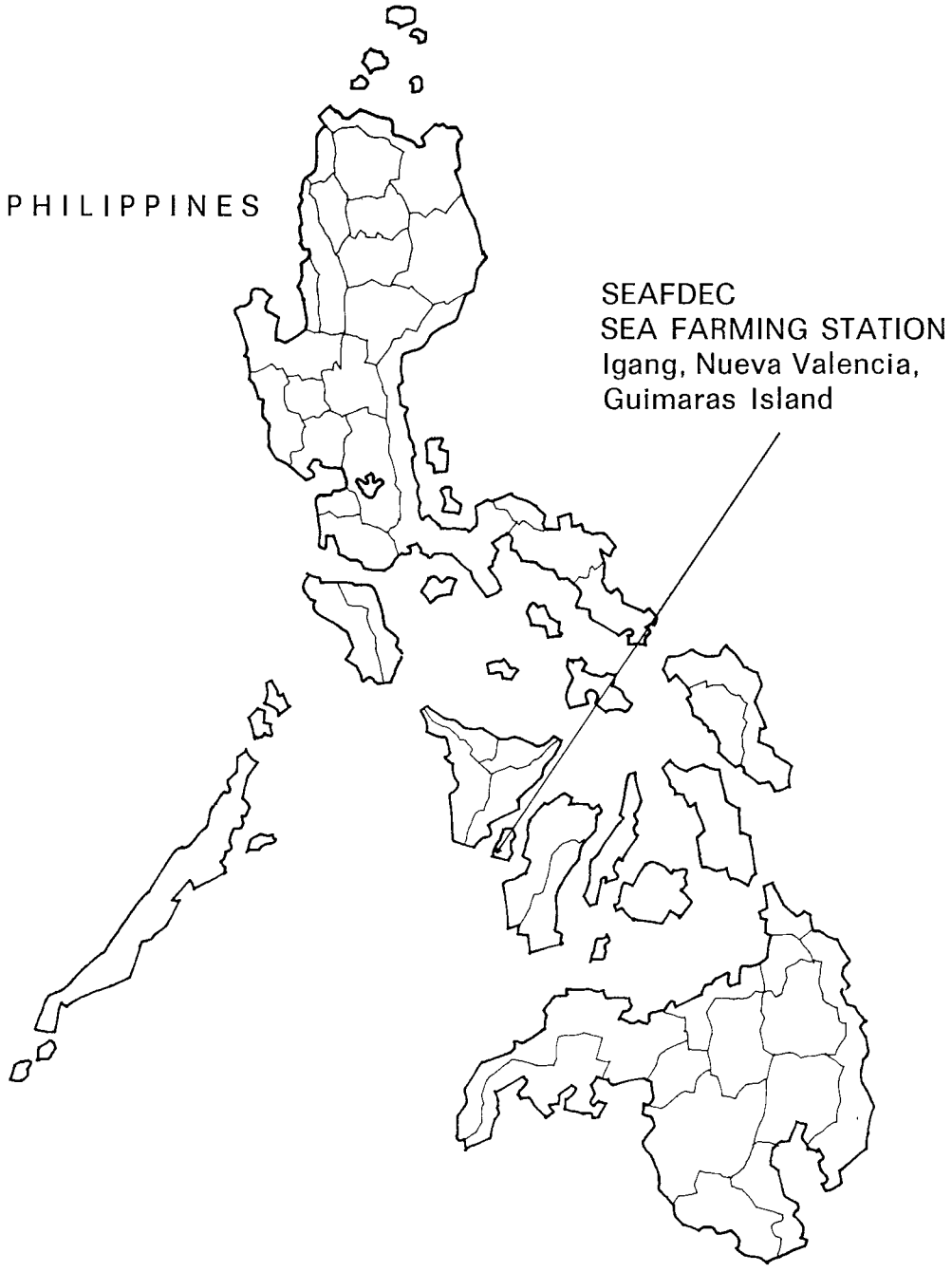
The full-scale cultivation of sugpo, *P. monodon* Fabricius, could only be realized if there is an assurance of continuous supply of fry. Obviously, a steady supply will depend largely on the availability of spawners. In the islands of Panay and Negros, they are normally gathered from fish traps and otter trawls and purchased at ₱5.00 each. Unfortunately, aside from being expensive, collection from their natural habitat is adversely affected by bad weather conditions and other uncontrolled variables. The main constraint at the SEAFDEC Tigbauan Hatchery as well as private hatcheries that may be established in the future, is how to secure an adequate supply of these spawners.

The SEAFDEC Aquaculture Department, right after its establishment in mid-1973, has considered the enormous significance of the problem and has launched several studies to minimize, if not to entirely solve this. In December 1975, roughly after 2-1/2 years of intensive study, for the first time in the world, SEAFDEC Aquaculture Department has succeeded in inducing *P. monodon* to mature and produce normally the first generation of postlarval fry, thereby successfully effecting the completion of *P. monodon's* life cycle while in captivity.

Another significant study the Department has initially carried out which could help augment and stabilize the supply of spawners and eventually stimulate the establishment of more prawn hatcheries and the development of ponds for prawn culture as a major export-oriented, dollar-earning industry, is the possible development of ovarian rematuration of spent spawners. This recycling process, aside from being relatively inexpensive, would have the additional advantage of affecting fuel savings and reducing manpower in catching spawners in the natural fishing grounds.

These two consecutive successful studies to induce gonadal maturation will eventually lead to the solution of two major problems in prawn aquaculture. These are the high cost of obtaining gravid females in natural fishing grounds, and dependence on seasonal periodicity of gonadal maturation of wild female stock.

Fig. 1. Location of SEAFDEC Sea Farming Station.



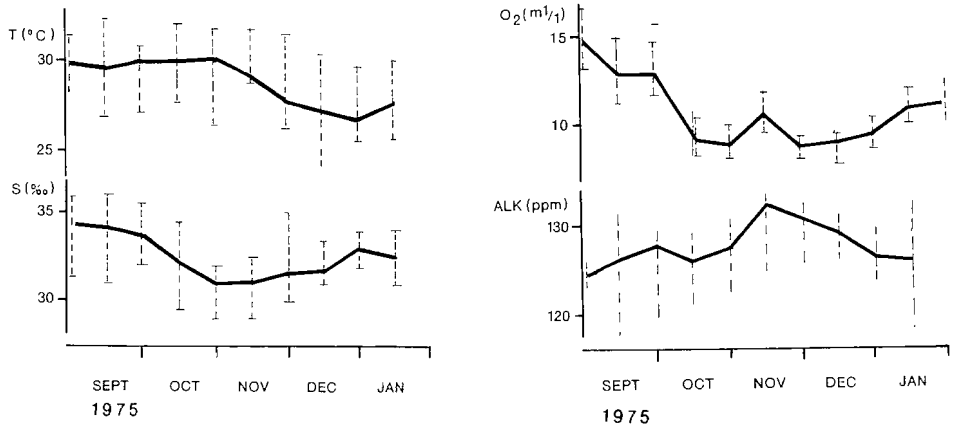
THE STUDY SITE

The studies were conducted inside Humaraon, a tidal cove of about two hectares located in Igang, Nueva Valencia, Guimaras Island (Fig. 1). The cove is naturally protected on all sides by extensively weathered coralline rock hills and opens to the outlying waters through two channels measuring 84 m and 82 m wide respectively. Depth of water in the mud-bottomed pen area is four meters at chart datum. The dominant hillside vegetaton is ipil-ipil *Leucaena glauca* and *Cassia sp.)*. There are considerable stands of the mangrove *Rhizophora mangele* along the shorelines.

Hydrological data collected from within the enclosure included air and water temperature, total alkalinity, salinity and dissolved oxygen (Fig. 2). Water samples were taken at 1 m and 4 m depth and analyzed by standard titration methods. Standard Secchi disc readings taken at 1000 hours once each week during the month of October, 1974, ranged from 4.0 m to 2.6 m depth, with an average of 3.4 m. Parallel hydrological data were collected at a second station situated 100 m beyond the mouth of the cove at 12 depth (chart datum) in order to compare conditions within and outside the cove.

Water temperature recordings from within the pens at 1 m depth ranged between a minimum of 22.5°C and a maximum of 33.2°C. Mean water temperature over the 9-month experimental period was 28.9°4C ($\pm$1.81°C). Highest temperatures were recorded during May through July of 1975, lowest temperatures during December, February and March of 1975 and January of 1976. Temperatures within the enclosures and outside the cove were very similar at all times. Bottom (4 m) temperatures within the cove followed a similar monthly pattern with bimonthly means averaging 0.37°C lower than at 1 m.

Fig. 2. Hydrology of Igang Bay, Guimaras Island, site of prawn maturation experiments.



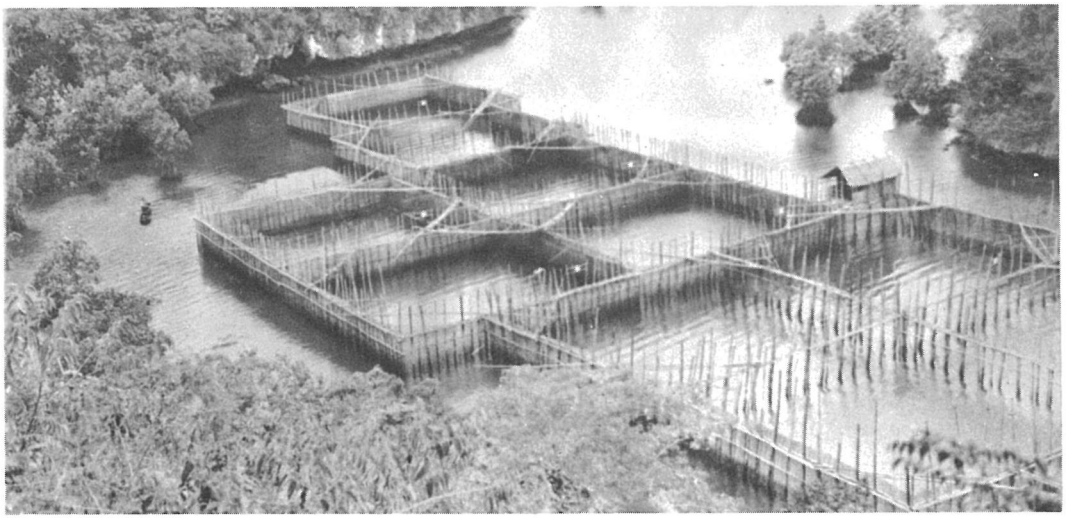
Salinities within the enclosures at 1 m depth ranged between 27.0 ppt and 36.4 ppt. Mean salinity over the 9-month period was 33.02 ppt ($\pm$ 1.56 ppt). Highest salinities were recorded during the dry months of April through September and lowest salinities occurred in October through January. Salinities were lowered by heavy rainfall but observed turbidities did not change appreciably. Salinities generally showed the greatest bimonthly range when the mean was high and did not differ significantly from those outside the cove ($t_{.05} = 2.03$).

Dissolved oxygen values ranged from 3.3 ppm to 19.5 ppm within the pens, and 4.9 ppm to 22.8 outside the cove. Low values were observed to occur usually during the morning readings, especially under calm conditions. Mean dissolved oxygen during the experiment was 9.29 ($\pm$ 3.14) ppm inside the pens. Mean bimonthly dissolved oxygen levels decreased from July (when these readings began) to mid-December in 1975. Thereafter the trend reversed through late December and January of 1976. There was no significant difference between dissolved oxygen levels recorded inside or outside the cove ($t_{.05} = 2.03$).

Mean bimonthly ranges in total alkalinity (expressed as carbonate) were between 94 and 155 ppm inside the cove; and between 93 and 145 ppm in the adjacent area of the sea. Mean alkalinity over the study period was 129.8 and 126.1 ppm at the two stations respectively. From visual inspection of the data, there is no suggestion of a correlation of alkalinity with trends in other physical-chemical parameters recorded, nor is there a significant difference between bimonthly means inside and beyond the cove ($t_{.05} = 2.06$). Short-term trends appear similar in both localities.

Resident planktonic biota within the pens, as determined by bi-weekly tows included an abundance of *Nitzschia sigma, Melosira nummuloides, Navicula sp.* and *Cocconeis splendica.* The dominant algal genera found were *Oscillatoria* and *Cladophora.* Zooplankton consisted of copepods, ciliates, and dipteran larvae.

The maturation pens.



COMPLETION OF LIFE CYCLE

Review of Literature

The present study was stimulated by the success of shrimp culturists in various parts of the world in completing the life cycles of other penaeid species of commercial importance using eyestalk ablation techniques to induce gonadal development in females where it does not occur spontaneously in captivity. However, most successful applications of this technique, employed chiefly by commercial shrimp farming companies overseas, are of recent origin and not yet recorded in the scientific literature.

Crustacean eyestalks are known to contain the storage and distribution centers of the gonad and moult inhibiting hormones. Published work to date, mainly on crabs (Adiyodi, 1970) has shown that eyestalk removal is likely to produce either precocious molting or precocious gonad development depending upon the relative interactions of other ambient environmental factors, and the age of the animal when operated upon. In more recent commercial applications of the technique, it has been shown that the removal of only one eyestalk, rather than both, is sufficient to initiate gonadal development while reducing risks of mortality, impairment of normal behavioral responses for feeding and mating, and/or suppression of gonadal responses to light and photoperiod.

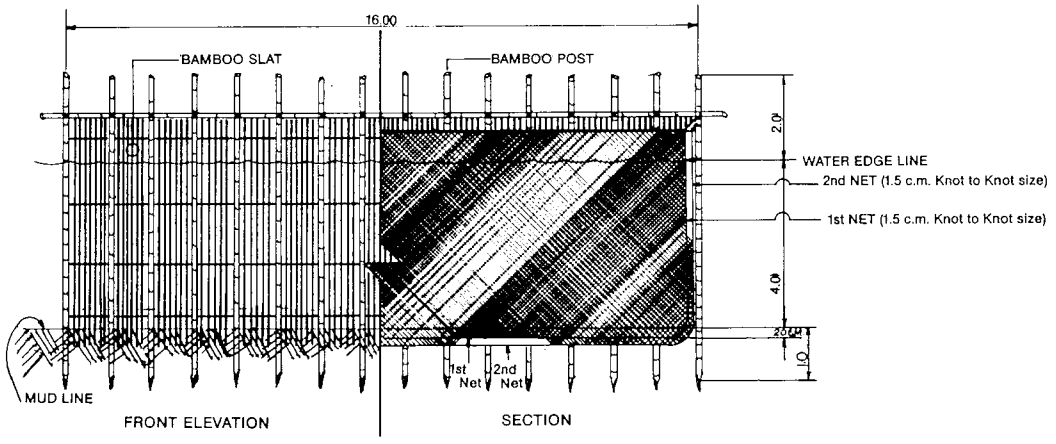
Although ovulation, maturation and the subsequent spawning of unfertilized or non-viable eggs by captive *Penaeus monodon* have been achieved (Liao, 1973; Arnstein & Beared, 1974; Alikunhi *et al, 1975)*, all previous attempts to complete the life cycle of the species, as far as is known, have failed. Because of the importance of dependable supplies of *P. monodon* fry to meet the needs of the growing shrimp pond culturists within Southeast Asia, studies have been recently initiated by SEAFDEC in attempts to achieve sexual maturity in captive stock and the subsequent mass production of viable young.

Methods

Penaeus monodon fry hatched from females captured from the sea and reared to 25-day old post-larvae (P_{25}) in the hatchery of the Mindanao State University Institute of Prawn Research and Development, were transferred to the SEAFDEC ponds at Leganes and reared for the next four months. Immediately thereafter, 1500 specimens with average weight of 16.43 grams were transported to experimental prawn maturation pens for further observation under fully marine conditions.

The pen system at Igang is of 12 compartments, each with a surface area of 250 sq m and tightly fenced with bamboo slats (Fig. 2). A double netting with 1.5 cm knot-to-knot minimum mesh size was suspended within each pen and buried 20 cm below the muddy substratum. Dried twigs of *Rhizophora* and *Avicennia* were set at the center of each compartment for additional sanctuary and shade.

Fig. 3. Structural detail of prawn maturation pens.



Three groups of 5-month old prawns were each stocked in 3 of 12 compartments at the rate of 500 animals per compartment. For the next 10 months, the groups were rotated monthly between adjacent pens, except in May and June when two monthly transfers were required because of extensive algal fouling.

At the age of 15 months, 600 shrimps were selected for the experiment commencing on April 25, 1975 and treated in three groups each consisting of 100 females and 100 males. Females in two of the groups were subjected to bilateral ablation and unilateral ablation respectively. The third group of normal animals served as the control. Males were left unablated as were the control. Males were left unablated as spermatogenesis had been observed previously to occur spontaneously in captivity. Each of the three groups was separately stocked as earlier trials resulted in total mortality of bilaterally ablated animals, thought to be due to cannibalism by sighted prawns. Rotation between pens continued as before.

The animals were fed twice daily with chopped mussel meat, trash shrimps, and tuna fish at a minimum daily rate of 10% of the estimated total shrimp biomass, using circular trays 30 cm in diameter distributed around the perimeter of the pens.

During the period of study, ending on January 27, 1976, 30 animals were sampled monthly from each compartment and examined for determination of body weight, total length (measured from base of rostrum to posterior margin of telson), carapace length (measured from the base of rostrum to middorsal carapace margin), and the stage of ovarian development defined according to the five maturation stages used by Rao (1969) and previously confirmed to be applicable to *P. monodon* (Villaluz *et al.*, 1972). All monthly samples were returned to the pens except for three bilaterally ablated females, seven unilaterally ablated females and five controls which were sacrificed for histological studies during the course of the work. Survival figures in Table 1 have not been adjusted to account for these sacrifices. Ovaries were fixed in Bouin's solution section and stained for histological examination using Heidenhein's Azan.

Spawning and hatching were carried out in seven fiberglass tanks (one m^3) filled with sea water which had been previously filtered through a plankton net (64 micron mesh). One gravid female was placed in each of the tanks which contained two airstones to ensure adequate agitation and aeration for newly-spawned eggs. On the morning following spawning, spent females together with detritus were removed from the tanks while the released eggs were examined and counted.

Newly-hatched nauplii were not fed until all residual yolk had disappeared after two days. Some 40,000 zoea larvae were retained in each of the tanks and fed with cultured *Skeletonema costatum* twice daily at a constant concentration of approximately 20,000 cells per ml. Mysis stages were given a supplementary diet of bread yeast at the rate of 2 gm per day and rotifers at 30 cells per ml. A further food supplement of minced tuna meat was fed daily to the postlarvae between the first and 15th day at a daily rate equal to their estimated total biomass. During the rearing of postlarvae, the tank bottom was occasionally stirred and 20% of the water was replaced each day. All larvae, except the progeny of spawner No. 7 were reared outdoors, as indoor night temperatures were thought to be too low (20-25°C) for satisfactory feeding and larval growth, usually best at 27°C.

Results

Bilaterally ablated females had suffered total mortality by 196 days, but interim samplings showed that the ovaries had developed to Stage III in one specimen by 40 days, in three more specimens by 60 days, and two specimens by 142 days (Table 1). Those with one eyestalk removed showed a similar

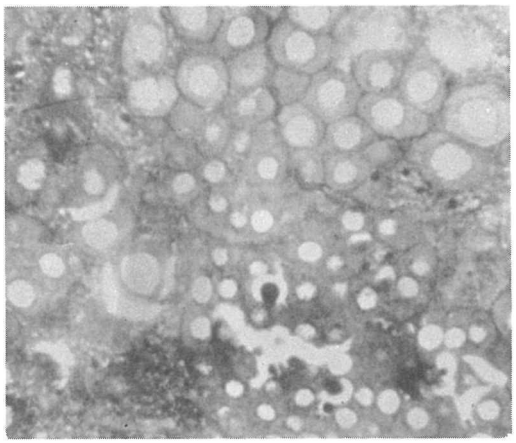
Table 1. Survival and maturation of 15-month old hatchery produced female Penaeus monodon reared in captivity.

	Unablated		One Eye Ablated		Two Eyes Ablated	
Date	Stock	Stage-No.	Stock	Stage-No.	Stock	Stage-No.
4-25-75	100	None	100	None	100	None
6-5-75	83	II-1	67	II-3 III-1	46	III-1
7-2-75	-	-	61	III-2	40	III-3
8-14-75	68	II-1	55	None	27	II-3
9-17-75	64	II-1 III-1	47	II-2	20	III-2
10-16-75	56	II-1 IV-1	38	II-1	4	None
11-8-75	49	II-1	38	None	0	-
12-21-75	46	II-3 IV-1	36	IV-3	-	-
12-29-75	40	II-3	32	III-3	-	-
1-16-76	39	II-1	27	II-8 III-4	-	-

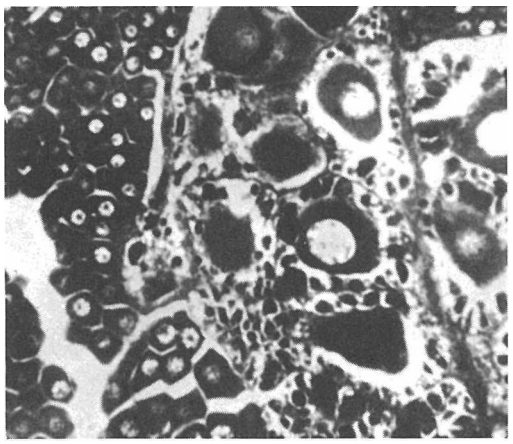
rate of development, but 38% of these survived beyond 196 days. Of the 30 females sampled after 142 days only a single unablated female had developed to Stage II. Two specimens had reached Stage IV at 171 and 239 days. By 265 days, no other specimens had developed beyond Stage II. Survival of the controls (39%) over the experimental period was 12% higher than in the group with one eyestalk removed.

Histological sections (Fig. 4 a, b, c, d) of developing ovaries of unilaterally ablated females showed that vitellogenesis occurred from Stage II, and that the sequence of oogenesis and maturation was similar to that described for other penaeid shrimps (Cummings, 1961; Fujinaga, 1942; King, 1948; Rao, 1969). Subsequent hatching of viable F_1 larvae suggests normal embryological development among fertilized eggs released by unilaterally ablated animals.

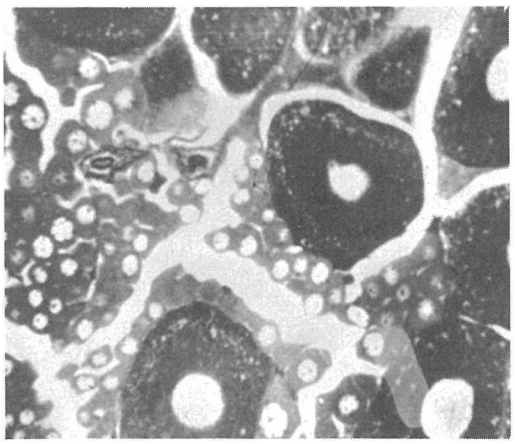
Fig. 4. Cross sections of Penaeus monodon ovary.



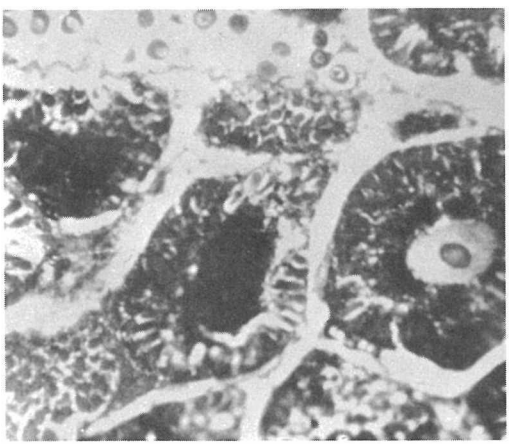
A. Stage I, immature;



B. Stage II, early developing;



C. Stage III, late developing;



D. Stage IV, mature.

Table 2. Spawning record of unilaterally ablated Penaeus monodon.

Spawner No.	Stage of Maturity	Carapace Length (mm)	Body Weight (g)	Count (10^3)				
				Eggs	Nauplii (N)	Zoea (Z)	Mysis (M)	Post-Larva (P)
1	IV	65	113	355.0	263.0	248.0	0	0
2	IV	58	90	95.0	77.0	30.0	0	0
3	III	54	110	80.0	32.0	18.5	17.0	6.5
4	III	54	108	130.0	76.0	20.0	5.5	0.9
5	III	69	160	110.0	48.0	15.5	5.0	0.8
6	III	54	120	375.0	0	0	0	0
7	III	50	100	270.0	170.0	108.0	36.0	(Still being reared)

Numerals suffixing N, Z, M, P indicate days surviving in each stage.

Two unilaterally ablated females with Stage IV ovaries were spawned on the night of December 21, 1975. Eggs from this spawning hatched after 18 hours at 27°C. One spawner produced 188,700 nauplii from 255,000 eggs being equivalent to a hatch rate of 74%, the other spawner yielded 95,000 eggs resulting in 77,000 nauplii, a hatch rate of 81% (Table 2). Five more females with Stage III ovaries were subsequently induced to spawn, resulting in an average hatch rate of 42.1% exclusive of the eggs of one female (#6) which were not fertilized.

Of the seven unilaterally ablated females that released eggs, six produced viable larvae. Survival over 26 days from one-day old nauplii (N_1) to 15-day old postlarvae (P_{15}) allowed P_{15} to be reared from each of three females. The first female produced 1,510 P_{15} (0.47% survival from N_1) and the second and third females 48 (0.06%) and 31 (0.06%) respectively. Larvae from two further females did not survive past the second and third day as zoeae, respectively after 5 and 6 days from hatching. This was thought due to a very poor bloom of *Skeletonema* in the outdoor tanks. As of January 27, 1976, 8,000 mysis larvae from another female were still surviving after 9 days from hatching.

Discussion

Although the results of the work can only be regarded as preliminary, they provide valuable data and information for future work aimed at propagating *Penaeus monodon* under controlled conditions. Viable F_1 larvae can be obtained using unilateral ablation techniques to activate ovary maturation. Bilateral ablation causes high mortality among adult stock and cannot therefore be considered successful. It is possible that viable larvae can be produced from unablated females, but failure to induce spawning in these, and the diminishing numbers with developing ovaries in samples taken during the latter part of the experiment, suggest that the ovaries may regress before spawning occurs. Until this hypothesis is tested it will not be possible to determine whether unilateral ablation is solely responsible for the success of the experiment, or such success is due in part to a fortuitous choice of experimental site.

Hydrological data obtained during this study indicate that a mean water temperature of around 29°C and almost fully marine salinities (33 ppt) are suitable for ovary maturation, breeding and successful spawning. The animals are, however, tolerant of quite wide variations about the respective means. Dissolved oxygen values exceeding saturation levels appear desirable, although some of the higher readings recorded in this work may be due to experimental error occurring in the relatively primitive field facilities presently available for analytical work. The physico-chemical data also confirm that among parameters measured, conditions within the cove are almost precisely similar to those outside where wild spawners occur. The fact that spawners do not occur naturally within the cove may well be attributed to lack of suitable food, and this emphasizes a further need for detailed studies on dietary requirements during maturation under controlled, rather than captive conditions. The period of time required for ovary maturation in *Penaeus monodon* may be as little as two months after unilateral ablation. Failure to obtain successful spawning for a further six months can be attributed to infrequent sampling and the fact that the ripe females detected earlier may have died and been eaten after spawning leaving no indication of their spent condition.

Hatching rates of 74% and 87% obtained from Stage IV spawners, and an average of 42% from Stage III animals compares favorably with data obtained from females captured from the sea. The SEAFDEC hatchery records also indicate that egg numbers are similar to those occurring in wild stock. Larval survival was relatively low in most cases, but these mortalities can be almost entirely attributed to specific problems occurring in the hatchery, and the unfortunate failure of larval food supply for two of the spawnings. The larvae themselves showed no abnormalities under microscopic examination, and such observations suggest complete viability among progeny here produced by way of unilateral ablation techniques.

REMATURATION OF SPENT SPAWNERS

Review of Literature

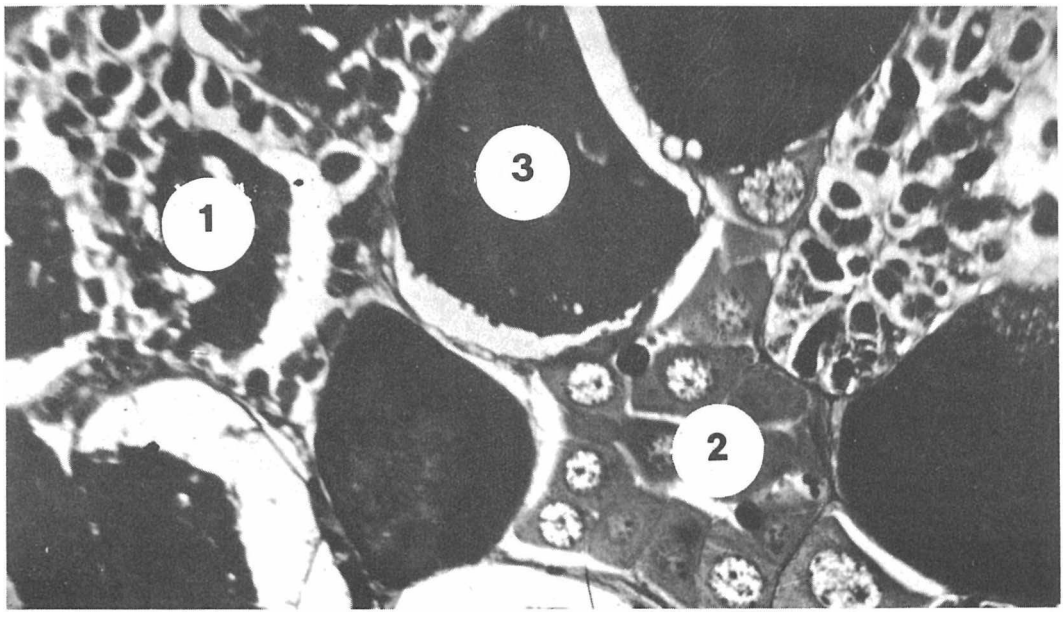
The number of times an individual prawn could breed during its lifetime is hard to document in nature. However, it is generally accepted that although a few crustacean species breed only once, in most species breeding follows a definite pattern (Carlisle and Knowles, 1959).

In some cases the number of times an individual penaeid could spawn during its lifetime can be inferred from the size range of spawners caught in nature as in *Parapenaeopsis stylifera* (Menon, 1953; Rao, 1968). Some penaeids are multiple spawners during a single season such as *Penaeus setiferus*, with only a few probably surviving to spawn during a second season (Lindner & Anderson, 1955).

Rao, 1968, noted that *Penaeus indicus* spawned 5 times during a lifetime. Ovarian rematuration of *Penaeus indicus* in captivity has been accomplished at the Naawan hatchery, Naawan, Misamis Oriental (MSU-IFRD Tech. Report 1975).

So far, there has been no record available on the number of times *Penaeus monodon* could spawn during its lifetime. However, some preliminary histological examination of Stages III and IV ovaries of wild spawners indicated the occurrence of previous spawning. The simultaneous presence in the maturing ovary of atretic follicles, and a large number of developing oogonia and oocytes respectively, are clear indications of previous and forthcoming spawning. (Fig. 5)

Fig. 5. Photomicrograph of a 10 micron section of a Stage III ovary taken from a wild spawner showing 1) atretic follicle, 2) oogonia, 3) oocyte.

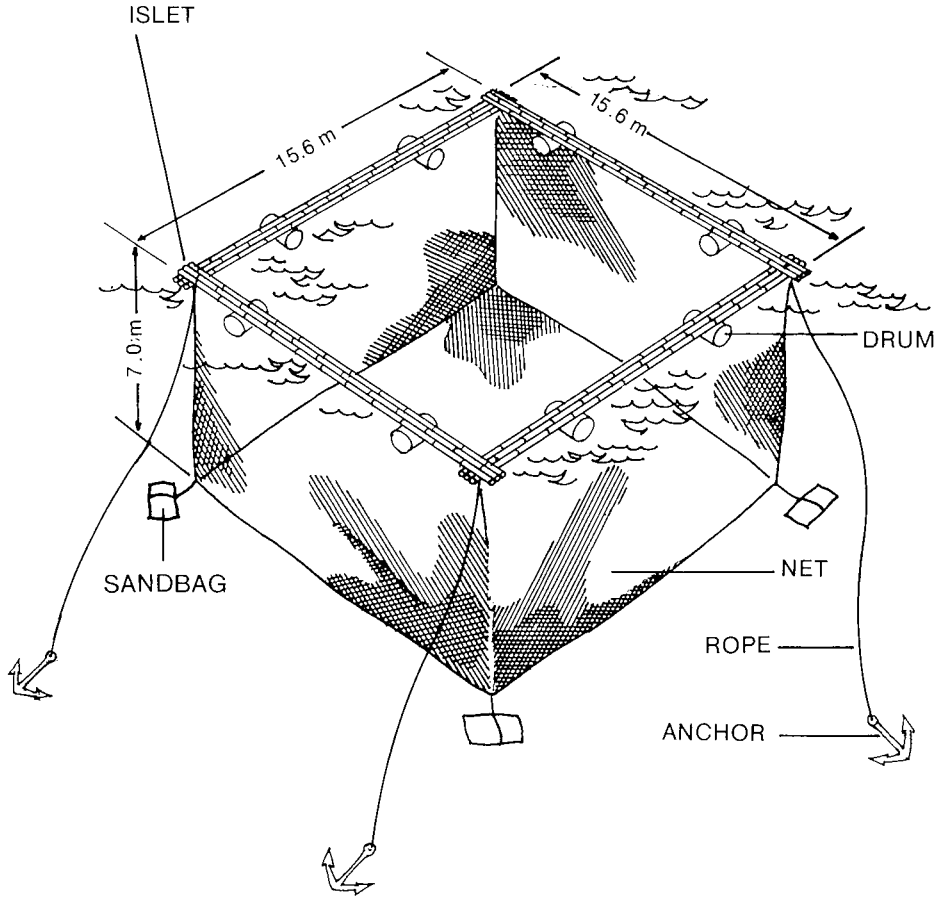


Methods

Stages III & IV spawners, sorted out from nearby fish traps in Batan, Aklan and Himamaylan, Negros and also from commercial trawlers plying the area were transported to the hatchery at Tigbauan and allowed to spawn. A total of 189 of these spent spawners, gradually accumulated at the SEAFDEC Tigbauan Hatchery from August 30, 1975 to November 4, 1975 were transported in six batches to the culture pen at SEAFDEC's Igang Sea Farming Station. Similarly, a total of 194 fifteen to twenty-four month-old males taken from SEAFDEC's Leganes Experimental Prawn Ponds was transported in six batches and kept in the same culture pen.

The culture pen used in this study consists of a rectangular nylon net measuring 15.6 m^2 and 7 m deep with 1.5 cm mesh, stretched. This is attached to a 15.6 m^2 bamboo skeletal framework suspended by 10 air-filled 210-liter oil drums (Fig. 2). The net is held firmly by 3 pieces of No. 12 metal anchor, aside from a 15 kilo sandbag attached to each of the four bottom corners.

Fig. 6. Floating pen used in the rematuration of spent spawners.



The whole stock was examined three times in a four-month period (October to February) by totally lifting the net to observe primarily any progress in ovarian rematuration and secondarily to determine the effects of the ablation treatment on survival rates.

Three treatments on eyestalk ablation were made on a total of 189 spent spawners. The treatments consist of (a) control, without ablation; (b) unilateral ablation; and (c) bilateral ablation. Of the 189 spawners used, 40 each were ablated unilaterally and bilaterally and the remaining stock of 109 served as control.

Results and discussion

Table 3 outlines the result of the experiments. No ovarian development was observed on October 26, 1975. In the second thorough examination on February 18, 1976, out of a total of 91 surviving females, 12 showed Stage II ovaries. Further observation revealed that all these 12 were unilaterally ablated. None of the controls showed any signs of ovarian development, and all the bilaterally ablated animals perished. Out of 63 surviving control animals, 53 were further ablated unilaterally because of encouraging results observed on the use of this treatment.

Table 3. Survival and rematuration of spent Penaeus monodon spawners after ablation of one eyestalk. Rematuration occurred inside a floating pen at Igang, Guimaras Island. Unablated females showed no sign of rematuration. All stage III and IV individuals were sent to the hatchery for spawning.

Date	Total No.	Maturity Stage				Unablated (control)
		II	III	IV	V	
10/26/75	114[a]	0	0	0	40	34
2/18/76	91	12	0	0	16	63[b]
2/29/76	54	10	3	1	36	4
3/9/76	81[c]	19	0	0	62	0
3/16/76	71	6	1	1	63	0
3/23/76	66	19	2	0	45	0
3/29/76	60	0	5	1	54	0

a. 40 animals were bilaterally ablated, but none survived.

b. Reduced to 10 animals, 53 ablated due to encouraging development of experimental animals.

c. Additional stock of 38 spawners added March 8, 1976.

The third spawner inventory was done on February 29, 1976. Fifty-four females were recovered. As usual, no ovarian development was observed in the four remaining females under the control. Fourteen unilaterally ablated females exhibited ovarian development from Stages II to IV (Table 2). Out of this number, four females developed to Stages III and IV. They were kept alive in a 60-liter plastic pail and transported by boat to the Tigbauan Hatchery. Unfortunately, two of the females spawned while in transit. The other two spawned completely at the SEAFDEC Hatchery in Tigbauan and produced on March 1, 1976 a total of 600,000 nauplii. They have undergone normal development thru zoea and are now at the mysis stage.

Periodic examination of the stock of spent spawners should be carried out more often because it is possible that rematuration and respawning of other prawns could have taken place unnoticed considering that it took only eleven days to transform some of the Stage II females to Stage IV.

The foregoing experiment indicates the significance of unilateral ablation in inducing rematuration of *P. monodon* in captivity. It also shows the rapid, phenomenal ovarian development from Stage II to Stage IV. In a matter of only eleven days, some females of Stage II have developed into Stages III and IV. The experiment further indicates that unilaterally ablated animals compare well with normal ones with regard to survival rate.

CONCLUSION

This first successful completion of the life-cycle of *Penaeus monodon* in captivity and the first ovarian rematuration of spent spawners in captivity are likely to have considerable economic implications in the Southeast Asian region, especially within the Philippines. The Aquaculture Department of SEAFDEC plans to continue these experiments, and to broaden its scope to include mass propagation of spawners under more closely controlled conditions to ensure an adequate and continuing supply of fry for the industry. Improved ablation techniques known to reduce mortality are under review. Studies to determine the most suitable age at which to ablate pond stock are under way and work on the nutritional requirements during ovarian maturation has been intensified. Male shrimps are also being closely studied so as to ensure successful fertilization of ablated females. Future success in this project and reliable mass propagation of fry at all times of the year becoming closer to reality will provide the necessary impetus for increased production to bring more of the 12.2 million potential hectares within Southeast Asia under pond cultivation.

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