EFFECT OF UNILATERAL EYESTALK ABLATION AND SEROTONININJECTION ON THE REPRODUCTIVE PERFORMANCE OFMARINE SHRIMP *PENAEUS (MARSUPENAEUS) JAPONICUS*

By

El-Telbany M. M.[1] , W. N. El-Hawarry[1] , F. A. Abdel- Razek[2]
[1]Dept of. Animal Husbandry- Fac. Vet. Med . Alex. Univ.
[2]Invertebrate Aquac. Dep. National Inst. of Oceanography and Fisheries (NIOF)

SUMMARY

The effect of unilateral eyestalk ablation and serotonin injection on the ovarian maturation and spawning of wild spent Penaeus japonicus was investigated through two experiments. The first one (June-July); adult spent P. japonicus females with mean 29.24g b.w.., 153 mm TL , and males with 16.15g b.w.., and 131mm TL were compared against a non ablated control group for 36 days. The weekly analysis shows a high rate of maturation through day 7, 14, 21 and 35. Meanwhile, the first spawn following ablation was observed on the 6th day, P. japonicus ablated females generated an average of 114306 ±4233 eggs / spawn, hatching eggs (39184 ±8210 nauplii) with an approximately 48% hatching rate, and zoea stage (31380 ± 11753) with an approximately 75.91 % metamorphosis rate.
The second experiment (30th September- October); Adult spent P. japonicus females with mean (35 g b.w., 164mm TL), and males with mean (23.84g b.w., 148 mm TL) were used in this experiment. In this experiment both unilateral eyestalk ablation and injection of 5-HT creatinine sulfate at 60 µg g^{-1} b.w., at days 1, 14 and 28 were used as a major variables under study compared to a control group injected the three injections using a sterile NaCl solution 0.85% conc. The study was undertaken for 42 days following unilateral eyestalk ablation, animals started to develop ovaries. The weekly analysis showed a high rate of maturation through day 7, 14, 21, 35 and 42 in case of ablated females. The results of 5-HT injection showed ovarian maturation starting on the 7th day and generating a low rate of induction during the experimental period. Serotonin injection program does not seems to be a practical alternative to eyestalk ablation; results obtained from this study suggest abnormal ova development where the histological features of stage III ovaries of eyestalk ablated females never differentiated cortical rods, whereas, stage IV derived from wild matured females showed typical stage IV histological features with

clearly differentiated cortical rods which, may partially explain the inability of eggs obtained from ablated shrimp to hatch or the quality reduction of their offspring.

INTRODUCTION

The effect of eyestalk ablation on the gonadal development was first discovered by Panouse (1943) in *Plaemon seratus* shrimp. However, the importance of this finding to shrimp hatchery was dormant for thirty years until applied to *Penaeus duorarum* by Caillouet (1972) to improve gonadal development then subsequently confirmed in other shrimp species (Aquacop, 1977). A lot of research concerning unilateral eyestalk ablation to various species has been proposed under various conditions (Wyban et al., 1987; Yano and Wyban, 1993). However, many associated problems have been reported, as deterioration in spawn quality and quantity overtime (Emmerson, 1980; Primavera, 1985), and conflicting results on spawn size, hatch success and other variables (Browdy, 1992). Eyestalks are the endocrine center for regulating many physiological mechanisms such as molting, metabolism, sugar balance, heart rate, pigmentation and gonad maturation. Therefore, unilateral eyestalk ablation affects all aspects of shrimp physiology (Quackenbush, 1991).

Inhibitory hormones in the eyestalk (produced in the X organ sinus gland) prevent further ovarian growth, especially if nutritional environmental conditions such as those while females are held captive in tanks, are seemed unfavorable to females. This block on ovarian development can be removed by eyestalk ablation induce further ovarian development and spawning, (Michael et al., 2000). Females, which mature in captivity, are thought to produce low numbers of eggs and superior nauplii, regardless of whether eyestalk ablation is used when compared to wild matured females. In controlled comparisons made under controlled laboratory conditions a trend toward reduced fecundity was well documented (Beard and Wickins, 1980; Emmerson, 1980; Browdy et al., 1987).

The first report to evaluate the effect of serotonin injection on the ovarian maturation of marine shrimp was carried out in wild P. vannamei females (Vaca and Alfaro, 2000). The serotonin (5-hydroxytryptamine 5-HT) injection approach relies on the role that neurotransmitters play in regulating gonad maturation. Its secretion stimulates the release of neurohormones (Sarojini et al., 1994), induces maturation in vivo and in vitro, (*Procambarus clarkia*) by simulating the release of a gonad-stimulating hormone (GSH) from the brain and thoracic ganglia (Sarojini et al., 1995).

Many undesirable characteristics have been observed in captive reproduction after eyestalk ablation. There is some evidence that eyestalk ablation causes abnormal ova development, which could be expected to reduce the quality of the offspring (Bray and Lawrence, 1992). Moreover several studies of paenaid shrimp have found no histological distinctions between ovaries produced with or without eyestalk ablation (Duronslet et al, 1975; Teshima et al, 1988; Michael et al., 2000).

The performance of wild spent brood stock (wild and captive), induced to remature and spawn by eyestalk ablation or gonad stimulating release hormone injection, and the extreme cost of gravid penaeid collection, calls for a great need to rely on the captive matured brood stock as an alternative of wild ones for hatchery operations. Therefore, the aim of this experiment was to evaluate the reproductive behavior of marine shrimp, through studying the reproductive biology and performance (ovarian maturation and spawning) at different seasons and ages (sizes), after unilateral eyestalk ablation and serotonin injection.

MATERIAL AND METHODS

Broodstock collection:

Adult *P. japonicus*, mature females and males were obtained by trawling off Golf Abu-Kir - Alexandria. This fishing method was operated by two fishermen in small boats, using a kind of trammel net locally known as Kanar was used. Alive shrimp arrived to the shore in a very good condition at early morning. Selection was done according to the degree of ovarian maturation, size and healthy condition of the brood stock. In the meantime only impregnated females where the sperm mass can be seen as a whitish substance under the transparent cuticle of the sperm storage organ (thelycum) on the ventral side of the head (cephalothorax) between the 4th and 5th walking legs were selected. Selected prawns were transported in 60 L plastic pen containing freshly well-oxygenated seawater (using air pump functioned by battery) to the National Institute of Oceanography and Fisheries (NIOF) shrimp hatchery (invertebrate laboratory).

Spawning assessment:

Gravid females, upon arriving at the hatchery, were gradually acclimated to the tanks water temperature and kept well aerated, to reduce the transportation stress. Thereafter, wet weight was determined to the 0.01 g on a top loading balance after being blotted with tissue paper. Morphometric measurements, total length (TL), carapace length (CL), body length (BL), were taken to the nearest o.1cm using a 30 cm ruler. Thereafter, gravid females with size ranges 13.4 to 20.8 cm in TL were placed in 80L, 280L and 500L disinfected spawning tanks containing filtered, sediment free well- aerated seawater.

All the spawning trials were carried out at 25 - 30° C ambient temperature and 30 - 34 ppt salinity, during the period from May up to October, while those made at April, electrical heaters were used and adjusted to maintain the water temperature at 28° C. Successful spawners were either completely or partially spawn the ripe eggs. Holding the female against a bright light where some of the anterior or posterior portions of the ovaries remain in partial spawners, while complete spawners have no traces of the ovaries recognized partial spawning. Successful spawners were removed to storage tanks for subsequent induced spawning.

Quality of spawns was evaluated in terms of number of eggs per spawn, hatching rate, and number of nauplii per spawn for all females, however, metamorphosis rate from nauplius to zoea stage, and number of nuplii per spawn were also considered for some females (n=8). The techniques employed for brood

stock collection, spawning and larval rearing were essentially similar to that developed by Abdel Rahman, (1993), and Taha (2000) for *P. japonicus* seed production.

Induced maturation and spawning & Eyestalk ablation technique :

Unilateral eyestalk ablation was made using the pinching crushing technique, by slitting one eye with a razor blade to allow free flow of fluid while holding the prawn under the water with thumb and index finger, beginning one half to two third down the eyestalk and moving distally, squeezing the eyeball contents outwards, and pinching hard the eyestalk tissue (Bray and Lawrence 1992 and Wyke, 1998).

Experiment I (June – July):

Adult spent P. japonicus females (29.24g b.w., and 53 mm TL), and males (16.15 g b.w., and 131 mm TL) were used in this experiment. The maturation system consisted of four indoor fiberglass tanks, two 400l rectangular and two 500 L rounded tanks, with water depth 0.5m and 0.9 m, respectively. Water exchange was 100% daily and a dark cover was used to prevent the daytime light intensity covered the tanks. Water temperature was 27±1.5° C, and salinity 30 - 34 ppt. Animals were fed at a rate of 21% from the body weight daily with fresh clams, and sardine, at a ratio 1:1. Eighteen females per treatment were randomly distributed into the maturation tanks.

Treatment A consisted of unilateral eyestalk ablated females while treatment B served as a control. Non treated males were used, with 1:2 male / female sex ratios. The prawns were individually marked periodically by clipping, one or more of the four uropda, The study was undertaken for 36 days. Each female was checked daily for ovarian maturation through the exoskeleton according to the key described by Wyke (1998) and Vaca and Alfaro (2000) with slight modification as following:

Stage I the ovary is transparent with no distinguishable outline.
Stage II The ovary is visible as a thin opaque line along the dorsal central axis.
Stage III The ovary is visible as a thick and yellow band.
Stage IV The ovary is turgid, broad and dark brownish yellow.

Quality of spawns was evaluated in terms of number of eggs per spawn, hatching rate, number of nauplii per spawn, metamorphosis rate from nauplius stage to zoea stage, and number of zoea stage per spawn.

Females detected with ripe ovaries were removed to 280 l spawning tanks with moderate aeration, when spawning was detected; 100 ml four sub samples were taken to estimate the number of eggs per individual prawn. Additional samples were taken for microscopic investigation of fertilized and embryonic eggs. They were allowed to hatch in the spawning tanks, hatched nauplii (N3) were counted in 100 ml four sub samples, and the hatching rate was estimated as the number of hatched nauplii to the number of eggs produced per individual prawn.

The nauplii in the spawning tanks were left to zoea stage, the number of zoea and metamorphosis rate from the naplius stage to zoea stage was estimated per individual female. Larval feeding and rearing was completely similar to that

mentioned formerly, animals, which did not spawn, were fed and transferred to clean tank daily. The females were returned to the maturation tank after spawning or when reabsorption of the ovaries was observed.

Serotonin injection (Experiment II. September – October) :

Adult spent *P. japonicus* females (35g b.w., 164 mm TL), and males (23.84g b.w., 148 mm TL) were used in this experiment. The maturation system consisted of two outdoor concrete tanks (4.5 m^2. / each). White fluorescent lamp (18 watt) was used for each tank to provide a photoperiod of 10 h light and 14 h dark. Feeding, water temperature, salinity, depth, and exchange were completely as in experiment I. The experimental brood stock were acclimated to the experimental conditions for at least two weeks, thereafter the molting stages of females were determined by uropod analysis, based on the technique described by Abdel Rahman (1979). Thirty six intermolt females (1:2 sex ratios) were randomly distributed into three maturation (treatment) tanks. Each female was individually eyestalk tagged, using a white plastic instrument (electrical belts) after numbering with a permanent blue marker (Fig. 1).

The first treatment consisted of unilateral eyestalk ablated females, and the second treatment received three injections (days 1, 14 and 28) of 5-HT creatinine sulfate (Sigma, Louis, MO, USA) at 60 µg g^{-1} b.w., while the third one served as control receiving three injections of sterile vehicle solution (NaCl 0.85 %), at the same time. Ovarian maturation, within 42 days, and females at stage III, or IV suspected to spawn were managed as described in the previous experiment.

Ovarian histology:

The ovaries of treated and control females were dissected and fixed in 10% buffered neutral formalin (Ban Croft and Stevens, 1979), for 48 hrs then dehydrated in ethyl alcohol, cleared in chloroform and embedded in paraffin. The paraffin blocks were cut, stained with Haematoxylin and Eosin (Harris, 1900), and examined by light ordinary microscope. Five distinct stages have been identified, according to Medina et al (1996): stage I (previtellogenic), stage II (early vitellogenic), stage III (late vitellogenic), stage IV (mature) and stage V (spent or degenerating).

Statistical analysis:

The effect of season and maturity on CL (mm) and the effects of season and CL (mm) on egg, nauplii number and hatch rate were carried out by the analysis of variance (ANOVA), using the GLM procedure of the statistical analysis system (SAS, 1996). The egg and nauplii numbers were log 10 transformed and also the % of maturing & spawning individuals, hatching rate % and metamorphosis % were Arc Sine transformed to normalize the distribution before the analysis (Gomez and Gomez, 1983).

RESULTS

Experiment I (Eyestalk ablation – June) :

Concerning ovarian maturation of *P. japonicus* by using eyestalk ablation technique the results obtained showed a high % of maturing prawns St. (II) 41.67 at

day 35 compared to 7.14 for control group at the same day Table (1). The number of successful spawning was high, 16.67% versus 0.00% in the control group, both after 35 days. On the contrary, survival rate was higher in control group (77.78%) than treated one (66.67%) at the same day Table (1).

Experiment II:

Spent *P. japonicus* was induced to mature by eyestalk ablation as well as by using 5-HT injection. The results showed ovarian development after unilateral eyestalk ablation. The weekly analysis shows a high rate of maturation through days 7, 14, 21, 35 and 42 for ablated shrimp. Moreover, using 60 ug g^{-1} b.w., 5-HT resulted in ovarian maturation starting on the 7th day and generating a low rate of induction during the experimental period.

The rate at which females were induced to mature and spawn under the tested protocols are presented in Table (2). A great proportion of ablated females reached the spawning condition especially at the 14^{th} and 21^{st} days, whereas for the 5-HT injection only 20 –10% of females showed maturation signs, however actual spawning was not achieved in those injected prawns.

Spawning activity and performance:

Eyestalk ablation induced spawning in 7 and 8 females of experiment I & II, during the 35^{th} and 42^{nd} days of experimentation, respectively (Table 1 & 2). The average number of eggs produced per female in the two treatments (Table 3), did not differ significantly (P<o.o5). Hatching eggs (39184± 8210 nauplii) was observed in only four spawned females, during experiment I, whereas zoea stage (31380 ± 11753) was observed only in three cases (Table 3).

Spawning behavior:

Successful mating was not achieved in the space vicinity available in the tanks (400l, 0.5 m water depth, and 500 l, 0.9 m water depth) used in experiment I. This was indicated by the release of unfertilized eggs of certain females after molting. On the other hand, no hatch was observed in experiment II, although all the eggs obtained during the experiment were fertilized. The observed fertilized eggs, hatched nauplii and zoea stages during the initial trial that was carried out earlier in the same outdoor tanks (4.5 m^2, 0.5 m water depth) confirm the possibility of successful mating in this space vicinity.

Wild spawning versus induced spawning:

The data for egg production of females induced by eyestalk ablation was not statistically different in the two induced spawning experiments. Therefore, it was pooled together, and the eggs, nauplii, and zoea numbers, hatchability % and metamorphosis % from nauplii to zoea stage were compared to that produced by wild matured females (spawned under captivity) as shown in Table (4). The mean for egg and nauplii numbers and hatchability % were significantly high (P > 0.05) in case of wild matured prawns, whereas no significant difference (P > 0.05) were observed in the metamorphosis rate from nauplii to zoea stage.

Ovarian histology:

No vitellogenic activity was observed at the 10th day of experimentation for both 5-HT injected and control groups. The ovary has only oogonia and previtellogenic oocytes Fig (2). On the contrary, all the unilaterally eyestalk ablated females had started vitellogenesis, with one female showing the histological features of stage II, where the ovary displayed oocytes at early vitellogenesis and central nucleus with several large nucleoli and significant increase in size (Fig 3). Stage III ovaries (Fig 4) from eyestalk ablated females as well as those females rematured for the second time after ablation, displayed mature oocytes with central germinal vesicle, yolk granules dispersed in the cytoplasm, spindle shaped follicle. Atretic oocytes however, and cortical rods never differentiated in any of the animals examined (Fig. 4). On the opposite, the wild matured females showed typical stage IV, histological features with extensive accumulation of yolk granules in the cytoplasm, clearly differentiated cortical rods and germinal vesicle migrating toward the periphery (Fig. 5 & 6).

DISCUSSION

This is a pioneer report that demonstrates maturation and spawning by serotonin (5-HT) injection in P. japonicus, based on the findings by Fingerman (1997), and Vaca and Alfaro (2000) that 5- HT activated the release of GSH, producing gonad maturation and spawning in *P. vannamei*. Meanwhile successful maturation and spawning by eyestalk ablation in P. japonicus (Table 1) agree with those demonstrated by Liao and Chen (1983) in Taiwan but disagree with Liao (1992) who did not observe spawning.

During the course of this study, ablated *P. japonicus* females started to mature 3 days after ablation and kept at high rates of maturation until the 41st day (6 week) (Table 2). Similar patterns have been previously reported by Chamberlain and Gervais (1984), who obtained spawning activity during 45 days for ablated *P. stylirostris*. Moreover, Simon (1982) reported spawning activity during 6 and 8 weeks for ablated *P. monodon*, in addition, Vaca and Alfaro (2000), displayed high spawning activity during 41 days for *P. vannamei*.

In the present study, regardless of treatment and re-maturation, P. japonicus ablated females (June) generated an average of 114306 ± 4233 eggs per spawn, with approximately 48% hatching rate (Table 6). These values agree with the commercial production of 55000 to 150000 nauplii per spawn for ablated *P. vannamei* (Kawahigashi, 1992), and the number of eggs per spawn was 119000 and 152432 as being reported by Wyban et al., (1992) and Lotz and Ogle (1994) respectively.

The *P. japonicus* females used in this study were allowed to spawn before unilateral eyestalk ablation, meanwhile hatching achieved in eggs obtained from those females ablated in spring which are strongly thought to be their second spawn in this season. On the contrary, there is strong suggestion that those females ablated by the end of September (late season) have spawned during the season,

before spawning again in the laboratory, and then induced to remature and spawn again by unilateral eyestalk ablation.

Therefore, the consecutive spawns in the later group may partially account for the inability of egg produced by those females to hatch as a result of reproductive exhaustion. These findings are in close agreement with those of Lumare (1979), who noticed that females of *P. kerathurus* with several spawning had ovaries that did not fully enlarge and did not have a uniform development.

Moreover, the decrease in hatching rates has been reported in consecutive spawns in the same intermolt period and in subsequent intermolting periods for *P. monodon* (Beard and Wickins, 1980; Hansford and Marsden, 1995), *P. indicus* (Emmerson, 1980), and *P. stylirostris* (Mendoza, 1997). The first two authors as well as Harrison (1990) and Browdy, (1992) suggested that continuous forced reproduction cause exhaustion of the female reserves because of the insufficient time between spawning that allow the shrimp to accumulate sufficient nutrients.

Removal of one eyestalk induced a sooner and higher rate of ovarian maturation and spawning, compared to 5-HT (serotonin) treatment (Table 2). This observation may point out to the role of gonad inhibitory hormone over ovaries, however other factors should be considered.

Although, both serotonin and eyestalk ablation induce hyperglycemia in decapods by the releasing of crustacea hyperglycemic hormone (Keller and Beyer, 1968; Keller et al, 1985; and Fingerman, 1997), the elevated glucose level measured in the hemolymph of *P. vannamei* after 5-Ht injection may have a negative (stress modulator) effect on ovarian maturation (Racotta and Palcios, 1998). Additionally, the inhibitory effect of the 5-HT on Methyl Farnesoate synthesis (a gonad maturation stimulant) (Mattson and Spaziani, 1985) and the stimulation of molt inhibitory hormone (ovulation and embryonic development suppressor) (Chang, 1985) may have a negative effect on ovarian maturation (Wilder and Aida, 1995).

This study demonstrates the possibility of successful malting of *P. japonicus* in the 2.25m3 outdoors rectangular tanks (4.5m^2, 0.5m water depth). Despite no hatch was observed, all the eggs obtained during the experiment were fertilized. In addition to the observation of fertilized eggs, hatched nauplii and zoea stages during the initial trial that was carried out earlier in the same outdoor tanks before the start of this experiment, confirm the possibility of successful mating in this space vicinity. These results disagree with those obtained by Yano (1992) who concluded that, *P. japonicus* could mate in 25 cubic meter tanks, but not in 3 cubic meter tanks.

Moreover, this work tend to demonstrate higher means of eggs (251419 ± 16395) & nauplii (166287± 11610) production and hatchability % (66.36 ± 2.39) for spawns from wild matured females during June (spring peak), compared to the productivity of captive rematured unilaterally eyestalk-ablated females (Table 4). No significant differences were observed in the metamorphosis rate from nauplii to zoea stage. Similarly, Wyban et al (1987), Bray & Lawrence (1992) and Robertson et al., (1993), under controlled conditions on the mainland, reported lower values where

the mean hatching rates for *P. vannamei* ranged from 32 to 66%. On the other hand, the hatchability % and metamorphosis to zoea I for spawns from wild-caught female did not differ from those of spawns from the same females rematured in captivity (Browdy et al., 1987). Differences are probably caused by age, eyestalk ablation, nutritional status, genetic make up and environmental conditions. Additionally, there is strong circumstantial evidence that a part of the problems seen with captive reproduction is related to a simple inability of current diets to supply the required nutrients as rapidly as required for the gonadal hypertrophy stimulated by eyestalk ablation in nature, an organism would not be anticipated to develop eggs, unless nutrients are available for metabolism, growth and reproduction, in sequence.

Eyestalk ablation accelerates the production of ova, regardless of whether the proper types and balance of nutrients are available, and regardless of whether those ova are capable of fertilization (Bray and Lawrence, 1992). The results obtained from this study suggest abnormal ova development where the histological features of stage III ovaries of eyestalk ablated females (Fig. 4) never differentiated cortical rods, whereas, stage IV derived from wild matured females (Fig 5 & 6) showed typical stage IV histological features with clearly differentiated cortical rods. Since the penaeid cortical rods are thought to be important in early development (Lynn et al., 1991) this may partially explain the inability of eggs obtained from ablated shrimp in experiment II to hatch.
These findings give some evidence that eyestalk ablation causes abnormal ova development, which could be expected to reduce the quality of the offspring (Bray and Lawrence, 1992). While several studies of paenaid shrimp have found no histological distinctions between ovaries produced with or without eyestalk ablation (Lawrence et al., 1979; Teshima et al., 1988 and Michael et al. 2000).

On conclusion, based on our experimental data, a serotonin injection program does not seems to be a practical alternative to eyestalk ablation; however, further research is required to evaluate the duration of the reproductive performance for serotonin treated females, the release of different neurohormones GSH, MIH, CHH, RPDH, and NHH may be controlled by a difference in the threshold of 5-HT; therefore, it would be interested to screen the effect of different 5-HT concentrations on shrimp maturation.

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Table (1): Effects of eyestalk ablation on induction of ovarian maturation and spawning of *P. japonicus*.

Days	*Ablated prawns*									
	No. of prawns	*Degree of ovarian maturation*				*% of maturing Prawns St.(II)*	*% of spawning Prawns St.(III,IV)*	*No. of Successful spawning*		*Survival %*
		Stage I	*Stage II*	*Stage III*	*Stage IV*			*No.*	*%*	
Day 0	18	18	0	0	0	0.00	0.00	0	0.00	100.00
Day 7	13	6	5	2	0	38.46	15.38	2	15.38	72.22
Day 14	13	6	4	3	0	30.77	23.07	2	15.38	72.22
Day 21	13	6	6	1	0	46.15	7.69	1	7.69	72.22
Day 28	12	7	3	2	0	25.00	16.67	0	0.00	66.67
Day 35	12	5	5	2	0	41.67	16.67	2	16.67	66.67
Days	*Control prawns*									
Day 0	18	18	0	0	0	0.00	0.00	0	0.00	100.00
Day 7	16	12	4	0	0	25.00	0.00	0	0.00	88.89
Day 14	16	12	4	0	0	25.00	0.00	0	0.00	88.89
Day 21	15	12	3	0	0	20.00	0.00	0	0.00	83.33
Day 28	15	12	2	1	0	13.33	6.67	0	0.00	83.33
Day 35	14	11	1	2	0	7.14	13.33	0	0.00	77.78

Table (2): Effects of eyestalk ablation and Serotonin injection on induction of ovarian maturation and spawning of *P. japonicus*.

Days	Ablated group									
	No. of prawns	Degree of ovarian maturation				% of maturing Prawns St. (II)	% of spawning Prawns St. (III,IV)	No. of Successful spawning		Survival %
		Stage I	Stage II	Stage III	Stage IV			No.	%	
Day 0	12	12	0	0	0	0.00	0.00	0	0.00	100
Day 7	12	5	7	0	0	41.67	0.00	0	0.00	100
Day 14	10	0	3	7	0	30.00	70.00	2	20.00	83.3
Day 21	8	2	0	5	1	0.00	75.00	3	37.50	66.7
Day 28	8	1	4	3	0	50.00	37.50	2	25.00	66.7
Day 35	8	2	4	2	0	50.00	25.00	0	0.00	66.7
Day 42	8	2	3	3	0	37.50	37.50	1	12.50	66.7
Days	*Injected group*									
Day 0	12	12	0	0	0	0.00	0.00	0	0	100
Day 7	12	10	2	0	0	16.67	0.00	0	0	100
Day 14	10	6	2	2	0	20.00	20.00	0	0	83.3
Day 21	10	6	3	1	0	30.00	10.00	0	0	83.3
Day 28	8	7	1	0	0	12.50	0.00	0	0	66.7
Day 35	8	8	0	0	0	0.00	0.00	0	0	66.7
Day 42	8	8	0	0	0	0.00	0.00	0	0	66.7
	Control group									
Day 0	12	12	0	0	0	0.00	0.00	0	0	100
Day 7	12	11	0	0	0	0.00	0.00	0	0	100
Day 14	11	8	2	0	0	20.00	0.00	0	0	91.67
Day 21	11	8	2	1	0	20.00	10.00	0	0	91.67
Day 28	9	9	0	0	0	0.00	0.00	0	0	75.00
Day 35	9	9	0	0	0	0.00	0.00	0	0	75.00
Day 42	9	9	0	0	0	0.00	0.00	0	0	75.00

Table (3): Differences in eggs, nauplii, zoea numbers, hatchability %, and etamorphosis % as an effect of ablated *P. japonicus* broodstock (LSM ± SE), for experiments I & II.

Item	Experiment I		Experiment II	
	No.	Mean ± SE	No.	Mean ± SE
Egg number	7	11346 ± 15325 a	8	115044 ± 14335 a
Nauplii number	4	39184 ± 8210	--	---
Hatchability %	4	47.61 ± 3.38	--	---
Number of zoea	3	31380 ± 11753	--	---
Metamorphosis %	3	75.91 ± 9.24	--	---

Means in the same row with similar letter are not significantly different (P > 0.05).

Table (4): Differences in eggs, nauplii, zoea numbers, hatchability %, and metamorphosis % as an effect of wild and ablated *P. japonicus* broodstock (LSM ± SE).

Item	Wild prawns		Ablated prawns	
	No.	Mean ± SE	No.	Mean ± SE
Egg number	8	251419 ± 16395 a	15	114306 ± 4233 b
Nauplii number	8	166287 ± 11610 a	4	39184 ± 8210 b
Hatchability %	8	66.36 ± 2.39 a	4	47.61 ± 3.38 b
Number of zoea	8	145492 ± 12466 a	3	31380 ± 11753 b
Metamorphosis %	8	87.68 ± 5.66 a	3	75.91 ± 9.24 a

* Means in the same row with similar letter are not significantly different (P > 0.05).

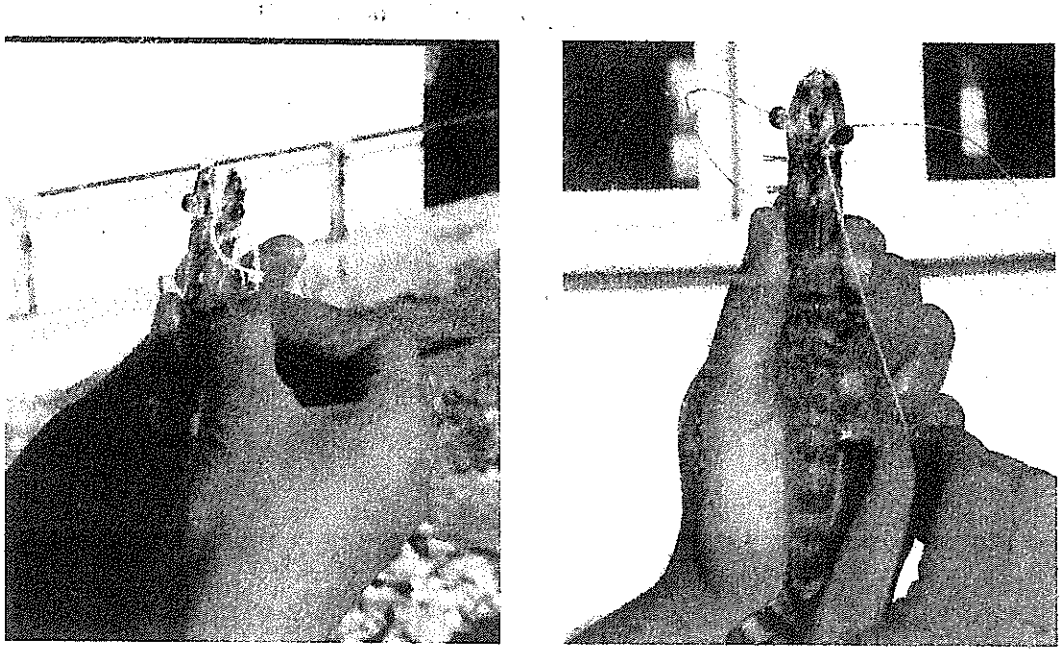

Fig. (1): Eyestalk tagging of female *P. japonicus*.

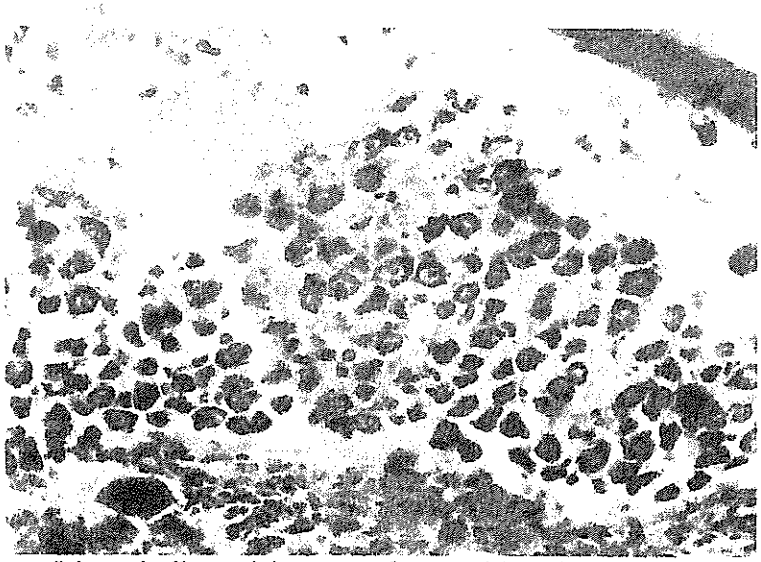

Fig. (2): Stage I (previtellogenic) ovary of non-ablated and serotonin injected *P. japonicus* showing oogonia (O) and primary oocytes (PO). H&E stain. X1400.

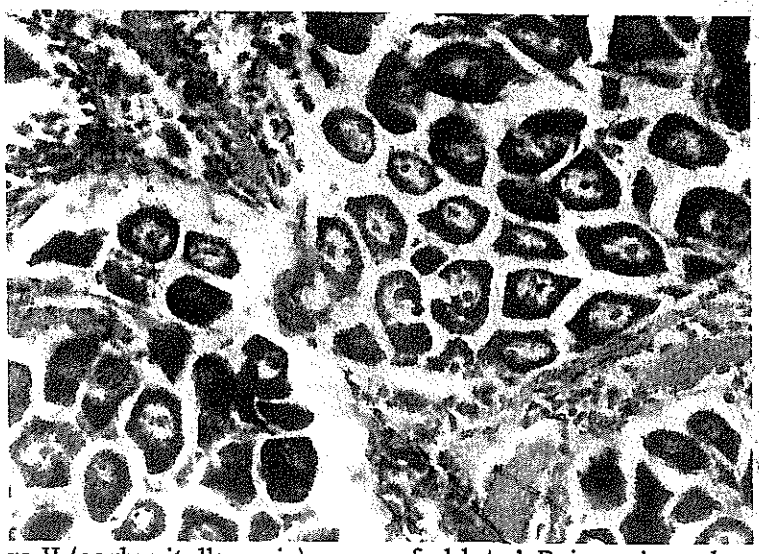

Fig. (3): Stage II (early vitellogenic) ovary of ablated *P. japonicus* showing primary oocytes. Nucleus (N) with multiple nucleoli (NE). H&E stain. X1400.

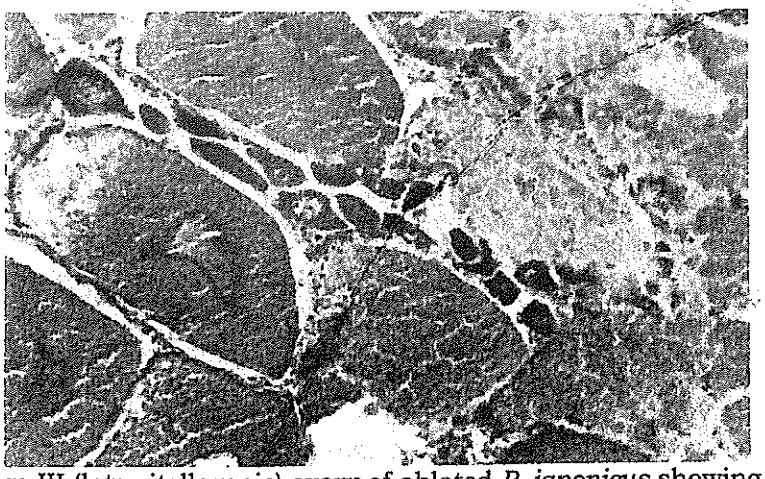

Fig. (4): Stage III (late vitellogenic) ovary of ablated *P. japonicus* showing nearly ripe oocytes with germinal vesicle (GV) and atretic oocytes (AO). H&E stain. X1400.

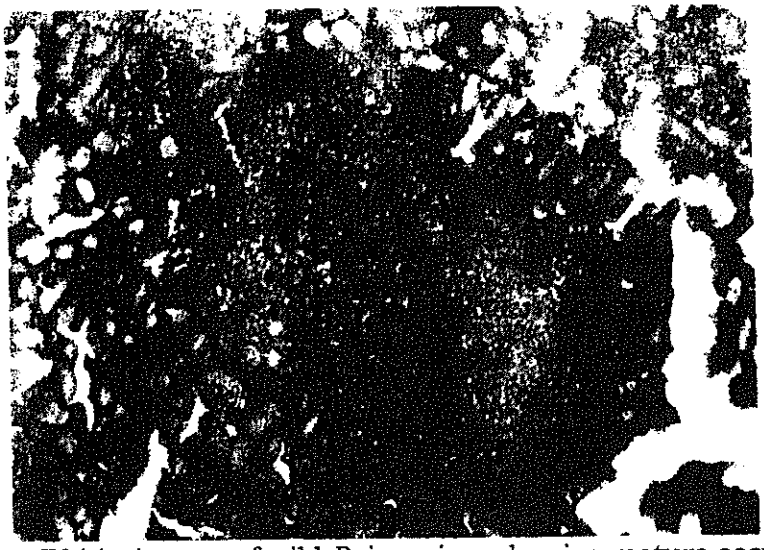

Fig. (5): Stage IV (ripe) ovary of wild *P. japonicus* showing mature oocyte with yolk granules (YG) and cortical rods (CR). H&E stain. X1400.

Fig. (6): Stage IV (ripe) ovary of wild *P. japonicus* showing mature oocyte with yolk granules (YG), cortical rods (CR) and germinal vesicle (GV) migrating toward the periphery. H&E stain. X700.