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Gonadal maturation, fecundity and hatching performance of wild caught tiger shrimp *Penaeus monodon* using unilateral eyestalk ablation in captivity

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Abstract

In this study, the effects of eyestalk ablation on maturation, moulting, spawning and hatching rate of wild caught *Penaeus monodon* were investigated. Twenty males (M) and 30 eyestalk–ablated and 30 non–ablated females (F), all individually marked (M: F=1:3) were stocked into two 15–ton capacity (each) maturation tank. All the ablated females moulted in 7.92±0.24 (mean±standard error) days, and first spawning occurred 3.2±0.20 days following eyestalk ablation. While twenty-five ablated females spawned, producing an average of 296,160±26,589 eggs, only eight non–ablated females spawned, producing an average of 195,462±20,565 eggs during the investigation period of 120 days. A significant positive correlation between fecundity and female body weight ($P<0.05$; $P<0.001$) was observed. Throughout the experiment, the average number of spawning per female was 1.8±0.14 for ablated and one for non–ablated females. Multiple spawning (up to three times) occurred within the same moulting cycle in ablated females. Among the ablated *P. monodon* females, 36% spawn once, 48% second time, and 16% third time. The average fertility rate of the eggs was high, ranging between 80 and 90% in non-ablated females, while in ablated females; the range was between 72 and 88% and differ significantly ($p<0.05$) ablated and non ablated females. Average hatching rate ranges between 70 and 80% for ablated and 75 and 82% for non-ablated females ($p<0.05$). From eggs to nauplii production per female was 167,838 for ablated and 127,500for non–ablated females. This study indicates that eyestalk ablation and environmental condition were important inducing tools for re-maturation of spent *P. monodon* that continued supply of seed stock for effective commercial shrimp farming.

Keywords: *Penaeus monodon* shrimp, Moulting, Re-maturation, Spawning, Eyestalk-ablation

Introduction

Penaeus monodon is a marine an enshrine and marine species that is cultured widely for human food (FAO, 2010). *P. monodon*is distributed throughout the Indo-Pacific region. However, its abundance in any given area is seasonal, and its availability to hatcheries depends on the catching of local stock, e.g. by fishing trawlers and local fishing gears. The wild berried female that is ready to spawn in captivity is known as “sourcing”. Until the 1970s, the sourcing of gravid female was the only method known and practiced for inducing penaeid females to spawn in captivity and Bangladesh is no exceptional. Various researchers conducted successful researches on the maturation of penaeid shrimp in captivity through unilateral eyestalk ablation (Santiago, 1977; Aquacop, 1979; Beard and Wickins, 1980; Emmerson, 1983). Noble literature reviews of maturation and reproduction in penaeid shrimp was done by Primavera (1984), Harrison (1990), Bray and Lawrence (1992). Emmerson (1980) and Primavera *et al*., (1980) successfully achieved the reproduction of penaeid shrimps without ablation, through the control of nutrition, temperature, salinity, pH, light and density. Unfortunately, the method applied on small scale; hence, not dependable for commercial use. In Bangladesh, the maturation in the captivity of *P. monodon* was successfully completed at Pioneer Hatchery Limited, Cox’s Bazar in 1994.

With the evidence of WSSV in wild caught *P. monodon* broodstock (Lo *et al*., 1996; de la Pena *et al*., 2007; Sethi *et al*., 2011; Aftabuddin *et al*., 2014), the hatchery industry is gradually becoming more dependent on captive stocks. Significant efforts have been made towards domesticating *P. monodon* over the past two decades (Pratoomchat *et al*., 1993; Makinouchi and Hirata, 1995; Hall *et al*., 2003; Coman *et al*., 2005, 2006). Disease-resistant, hatchery-reared seeds are becoming more popular for shrimp pond

stocking. Practicing the High Health Genetically Improved (HHGI) animals, specific pathogen free (SPF), selective breeding for disease resistance shrimp is a costly and lengthy process (Cock *et al*., 2009; Moss *et al*., 2012). Currently, only SPF populations of Pacific white shrimp, *Litopenaeusvannamei* in Hawaii, USA and Thailand are commercially available on a large scale. However, by definition, SPF shrimps are exclusively free of specifically listed pathogens and SPF shrimps may not be disease free.

Due to high-cost involvement in SPF and selective breeding program, almost all shrimp hatcheries in Bangladesh rely on wild broodstock to meet their demand. Currently, 56 *P. monodon* shrimp hatcheries are in Bangladesh among them 39 are running at different places of Cox's Bazar and Satkhira region. As the increased uncertainty and high price of wild brood stock in the Bay of Bengal area, it is of importance to control their reproduction for a regular supply of seeds. For economically and environmentally friendly sustainable hatchery operation, it is crucial to know to what level spent females can re-mature and spawn successively after ablation. Consequently, the rate of re-maturation and the quantity and quality of larvae from re-maturing females in comparison to those from the first spawning is also important. Hence, the present investigation was conducted to assess the effects of eyestalk ablation on the maturity, survival, fecundity and hatching rates of *P. monodon*.

Materials and Methods

This study was carried out from April 2014 to July 2014 in a reputed shrimp hatchery, Cox's Bazar, Bangladesh, where adequate research facilities for the study of hatchery operation and management were readily available. Adult broodstocks (60 female and 20 male) were collected from the south and western part of the Bay of Bengal, e.g. elephant point, bottom of the St. Martin's Island and Kohinur point (Fig. 1) using a fishing trawler namely, Fisher 2. Animals, that are in good condition (i.e. no sign of stress), and minimum of 165mm length, and weight about 50 g (for male), and of 200 mm total length, weight about 65 g (for female), were chosen. Usually, five individuals of broodstocks were transported in a broodstock carrying polythene bag filled with water (1/3) and oxygen (2/3), were used to transport the animals from ships to the hatchery.

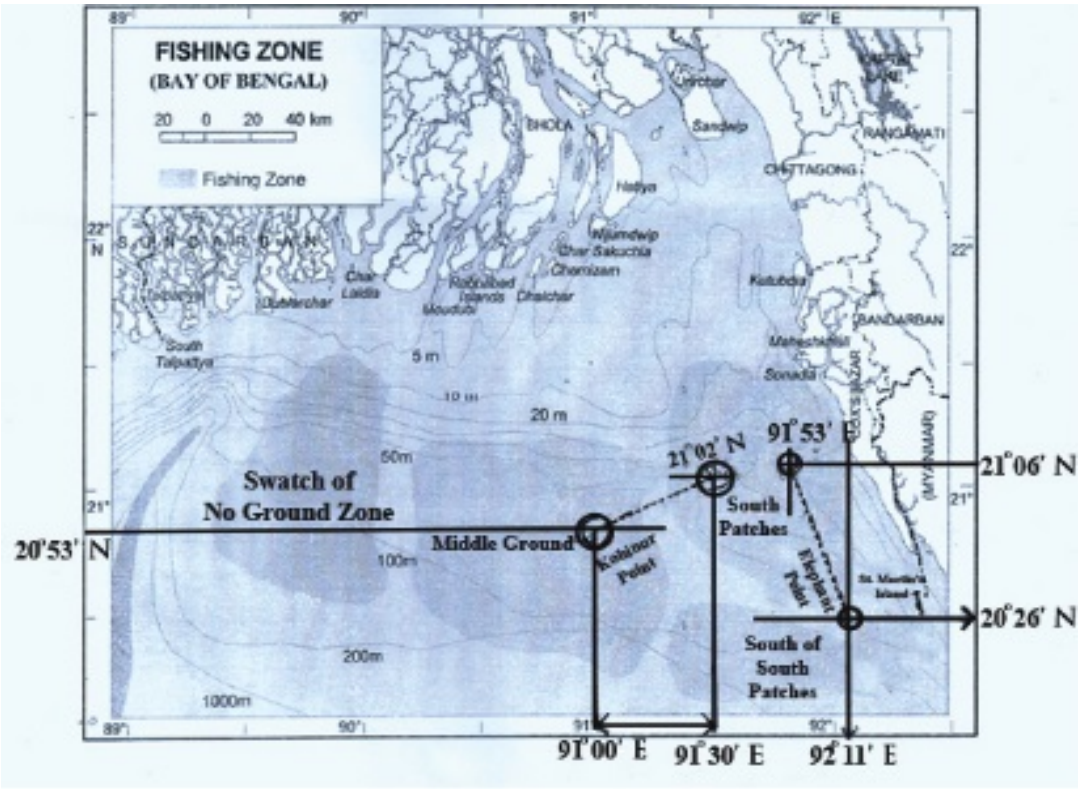

Fig. 1. Present *P. monodon* broodstock trawling area by Bangladeshi shrimp trawlers in the Bay of Bengal

Acclimation and water management

The broodstocks were quarantined with 500 ppm formalin for 2 minutes (dip bath) and acclimated to ambient water conditions at the hatchery in a 15 ton cemented broodstock holding tank. After acclimatization (1/2 days later), the animals were then transferred to the maturation tanks (12 ton cemented) at the density of 6-7 animals /square meter, at 1:3 ratio of male to female. Two hours before, 10 pm EDTA(Ethylene diamine tetra acetic acid, a chelating reagent)and 0.1 ppmtreflan (fungicide) were applied prior to transferring the animals. Oceanic quality seawater was provided at all times as defined as salinity 26 to 32 ppt, temperature 28 to 32^0 C and pH 7.8 to 8.5. Filtered (cartridge filter, 0.5µ) and disinfected (UV treated) seawater was always used. The tank was covered with a black corrugated plastic, and sufficient aeration was provided. The water level in the maturation tanks was kept at the level at 60cm with a constant flow of about 10 liters/minute which provides 200% exchange/day. The water in the maturation spawning and hatching tank was kept with the minimum disturbance.

Feeding management

About 12% of the estimated shrimp biomass was fed to the shrimp and the feeding distributed proportionately with various feeds like squid, clams. The shrimps were fed fresh diet in twice daily at early morning at 6.00 am and late evening at 7.00 pm. The remaining feed and fecal materials were siphoned out in the morning, and the lost water was replaced with fresh seawater. Fresh squids and clams were brought once a week. Skin and all internal organs were removed and rinsed well to eliminate all traces of ink and cutting into small pieces about 1-2cm and stored in a deep freezer for the whole week. According to the requirements of individual tanks, feeds were weighed out.

All tanks were equipped with aeration. For controlling of photoperiod, a balanced light/dark phase was maintained by artificial lighting in the maturation room. The fluorescent lights covered with dark blue acrylic sheets to provide very subdued light similar to the natural habitat of the adult shrimp. For prophylactic treatment at first the water volume of the tanks was lowered up to 3 tons in the reservoir. Formalin was applied at a rate of 50 ppm (two-hour static treatment with no water flow) and the tank flushed out after the treatment.

Induced maturation by unilateral eyestalk ablation

Before ablation, the males and females were segregated in two separate tanks containing 100 liters of seawater. A single female from the tank was removed and hold it in one hand with the head facing away from the body and observed the both eyes for any damage. At first the damaged eye was ablated, otherwise left or right one was ablated. Ablation was carried out unilaterally with a heated surgical forceps (Fig. 2). Povidone iodine (1%) was swabbed on the cutting side (Fig. 3) to prevent any fungal infection. After ablation, females were placed in indoor tanks containing 10 ppmoxytetracycline solution (FDA approved drug) for half an hour and then released to the maturation tank.

Selection of potential spawners

The gradation of maturation was examined externally by shining the light on the side of the abdomen with a waterproof hand light, particularly in the area behind the cephalothorax. Potential spawners were those, which showed thickening of the ovarian wall all along the length of the abdomen. The selected spawners with ripe ovaries was removed and dipped for about 2 minutes in 500-ppm formalin. After formalin bath, the female was rinsed with filtered, disinfected seawater and then placed into the 400-liter spawning tank. The moulting, maturation and spawning of each female were checked daily. For this purpose, the females were marked by pasting various colored plastic labels onto the carapace and making coded cuts in one or more uropods.

Spawning and Hatching

After spawning, the spent females were removed from the tanks by a scoop net and returned to the maturation tank. The tank water (spawning) was drained, and the eggs were passed through a 350 µ hand net that retains feces and they were collected on a 150 µ hand net in a bucket. Before transferring the eggs to the hatching tanks, they were washed thoroughly with running seawater at least for 10 minutes.

Egg Counting

The water level in the harvesting bucket had filled to a known volume i.e. 10 liters. A 5 ml inverted pipette was used to obtain the sample from the bucket and 2 ml of the sample were withdrawn and released the sample into a petridish. Then the eggs were counted with naked eye against a white back –ground. Sampling was performed three times and making average. The eggs were calculated by using the formula:

$$\text{Total No. of eggs} = \frac{\text{Mean no. of eggs in the sample}}{\text{Sample volume (ml)}} \times \text{Total amount of water in the bucket (ml)}$$

Fig. 2. Unilateral eye stalk ablation of *P. monodon* at the investigated hatchery

Fig. 3. Addition of 1% Povidone iodine near the ablated area.

Egg evaluation

Detailed egg evaluation was carried out after 10.00 am in the morning under a compound microscope. This allowed complete development of egg to nauplii so that fertile and unfertile eggs were easily identified. Fertilized eggs were easily determined by the symmetrical nature of cell divisions. Unfertilized eggs appeared as opaque or dark brown sphere, cell divisions very irregular. About 100 eggs counted, as they were fertile or unfertile. Percent of fertilization was determined by using the following formula.

$$\% \text{ Fertilization} = \frac{\text{No. of Fertilized eggs}}{\text{Total no. of eggs counted}} \times 100$$

Preparation of hatching tanks and stocking

After counting, the eggs in the harvesting bucket were transferred to hatching tanks containing one ton of water. The eggs were spread on the tank water gently and evenly. The aeration in the tank was adjusted to a minimum level. Before transferring the eggs to the hatching tanks, they were again treated with 10ppm EDTA and 0.05 ppm treflan and 2.0 ppm Oxytetracycline.

Hatching: After spawning the nauplii were hatched out in about 12-15 hours under normal circumstances (temperature 28 -32 and salinity 29 -34 ppt).The nauplius reached the 6th sub stage N1-N6 within 30-36 hours.

Hatching rate of nauplii: The hatching rate was determined by using the following formula: $H\% = \frac{A}{B} x$ 100; H = hatching rate, the A = total number of nauplii and B = total number of eggs (Chen, 1979). The nauplii were also allowed to develop to the zoea, mysis and post larval stage for a further 7-10 days.

Microsoft Excel and one–way ANOVA in BioStat v5, Analyst Soft Inc.-statistical analysis program for Windows, carried out statistical analysis i.e mean, standard deviation, standard error and p-value.

Results and Discussion

Male *P. monodon* become mature in captivity and induced maturation mainly concerns females. The initial weights, the total length of each female shrimp showed in Tables 1 and 2. The average weight of ablated females (88.2±3.12 g) was about 8.67 g lower than that (96.87 ±3.65 g) of non–ablated females, the difference was notable. At the end of the experiment, the condition of ablated females was poorer than that of non–ablated females. Black spots, loss of steadiness and missing appendages were observed in the ablated females.

Table 1. Moulting, spawning, number of eggs, hatching and fertility of ablated females of *P. monodon* used in the experiment

Sl. No.	Female individual Body wt. (g)	Length (mm)	Days of first spawning after eye stalk ablation	Days between eyestalk ablation and moulting	Days between moulting and spawning	Spawning frequency	Avg. No. of eggs	Fertility (%)	Hatching (%)
1	65	190	5	8	-	1	108,300	85	80
2	65	190	5	8	-	1	105,200	88	80
3	70	200	5	7	-	1	115,400	82	70
4	70	200	4	7	4	2	106,600	75	70
5	75	210	4	8	-	1	128,200	78	75
6	75	210	4	8	5	2	119,300	80	72
7	75	212	4	9	4	2	125,500	75	70
8	80	215	4	8	-	1	142,200	77	75
9	80	220	3	7	-	2	130,400	72	70
10	80	220	3	9	-	2	136,500	74	70
11	85	225	2	10	-	2	165,000	75	70
12	85	225	2	10	-	3	174,000	72	70
13	85	225	2	10	8	3	168,500	78	70
14	90	230	3	9	-	2	182,500	75	70
15	90	230	2	9	-	3	195,000	75	72
16	90	230	2	8	-	3	185,300	74	70
17	95	235	3	8	-	2	175,600	75	70
18	95	235	3	8	-	2	200,200	78	75
19	95	235	4	7	-	1	188,000	85	80
20	100	240	3	7	6	2	215,200	75	72
21	105	242	2	8	-	2	208,500	78	75
22	105	242	2	6	-	2	198,200	72	75
23	110	245	3	7	-	1	230,600	82	74
24	120	250	3	6	-	1	240,500	80	72
25	120	250	3	6	-	1	250,200	84	75

Of 30 ablated *P. monodon* females, 36% had spawned once, 48% had a second spawning and 16% a third spawning (Table 3). From the onset of the experiment, regardless of ablation treatment, all females moulted in 7.92±0.24 days when mating occurred. Following ablation, it was possible to achieve the first spawnings within 3.2±0.2 days. As shown in Tables 1 and 2, only eight spawning was detected in the non–ablated females in comparison to 45 spawnings for ablated females. The ablated females spawned more than one time, and average spawning was 1.8±0.14, producing an average of 296,160±26,589 eggs per female while only eight non–ablated females spawned once each producing only an average of 195,462±20,565 eggs. The highest number of eggs (273,200) was produced by a non-ablated female weighted 110 g (Table 2). Multiple spawning (up to three times) within one moulting period were also observed (Table 1). A similar 4-6 spawns/molt cycle for *P. monodon* was found by Beard and Wickins (1980) and Hillier (1984). In contrast, non-ablated females had a lower maturation and spawning rate than ablated females of *P. monodon*. The average maturation time for non-ablated wild *P. monodon* female was high, ranging between 25 and 40 days (Table 2). It is also reported that the maturation time is minimum for 10 days up to 2.7 months in non-ablated wild females (Santiago,1977; Primavera, 1978; Aquacop, 1979; Emmerson, 1983). This extended time (1-2 months) probably required to develop the eggs fully matured during a reproductive cycle.

Table 2. Moulting, spawning, number of eggs, hatching and fertility of non-ablated females of *P. monodon* used in the experiment

Sl. No.	Individual Body wt. (g)	Length (mm)	Days between Stocking and spawning	Days between moulting and spawning	Spawning frequency	Avg. No. of eggs	Fertility (%)	Hatching (%)
1	85	225	25	-	1	122,300	82	75
2	85	225	27	-	1	132,200	80	75
3	90	230	32	-	1	155,400	85	82
4	95	235	25	-	1	193,600	85	78
5	95	235	29	-	1	185,200	84	80
6	105	240	32	-	1	236,300	90	82
7	110	245	37	-	1	265,500	82	78
8	110	245	40	-	1	273,200	80	75

Table 3. Moulting, spawning and egg production of ablated and non–ablated *P. monodon* broodstock

	Ablated	Non-ablated
Average Initial weight (g)	88.2±3.12	96.87±3.65
Average number of eggs/female	296,160±26,589	195462±20,565
Average number of spawns/female	1.8±0.14	1
Average fertility (%)	77.76±0.89	83.50±1.16
Average hatching (%)	72.88±0.68	78.12±1.05
Average days between ablation and moulting	7.92±0.24	-
Average nauplii production	167,838	127,500

±= mean standard error.

Fig. 4 shows the fertilized and unfertilized eggs of *P. monodon* while Fig. 5 shows the newly hatched nauplii. The average percentage of egg fertility was 77.76±0.89(%) and 83.5±1.16(%) for ablated and non-ablated females which differ significantly ($P<0.05$). The average hatching percentages also vary significantly ($P<0.05$) from each other i.e. ablated (72.88±0.68 %) and non-ablated (78.12±1.05 %). The average number of nauplii produced per ablated female (167,838) was pointedly higher than that (127,500) of non-ablated females, this is because of multiple spawning of the single female.

This study demonstrates that *P. monodon* mature and reproduces in captivity with or without unilateral eyestalk ablation. However, eyestalk ablation increases the number of spawnings and correspondingly the number of eggs and nauplii produced per female in comparison to non–ablated females. It is assumed that this is due to the lower level of GIH (gonad-inhibiting hormone) and MIH (moult inhibiting hormone) in the hemolymph of the eyestalk ablated females (Dall *et al*., 1990). Browdy and Samocha (1985) also reported a much lower number of spawnings in the control females than in eyestalk–ablated females. The ablation method (cutting the eyestalk) used in the present study appears to be successful.

Only five of the ablated females, which died on the second day of the experiment, were due to handling stress rather than ablation. Only 26.6% of the non-ablated females matured and spawned during the 120–day experimental period. Besides, the fecundity of these females was high compared to that of eyestalk–ablated females.

Eyestalk ablation leads to expectable maturation and spawning in penaeids. However, it is generally accepted that over–stimulation of reproduction in the broodstock ablated in captivity reduces the reproductive performance (low fecundity, low fertility and hatching rate) of the females of various penaeid species (Lumare, 1979; Bray and Lawrence, 1992). The present results show a decline in the reproductive performance in the broodstock of ablated *P. monodon*, confirming the results of Browdy and Samocha (1985) for the same species. The ablated *P. monodon* had lower fecundity, fertility and hatching rates in comparison to non–ablated females in the present study. A similar declining result was observed in a single intermolt cycle in *P. monodon* (Beard and Wickins, 1980) and *P. indicus* (Primavera *et al*., 1982). However, eyestalk ablation and coding the females by cutting one or more uropods lead to deterioration of broodstock quality (loss of balance, melanised regions, missing appendages). It could limit the use of broodstock, the necessitating replacement that is more frequent. Hence, management of environmental factors (i.e. temperature, photoperiod and diet) may be used to stimulate maturation and spawning in *P. monodon*.

The fertility rate of eggs obtained in the current study indicates that the male-female: ratio of 1:3 is adequate for *P. monodon*. Alava and Primavera (1979) reported that this as the best ratio for *P. monodon* with closed thelycum. Browdy and Samocha (1985) obtained 86.7% spermatophore transfer in *P. semisulcatus* with a male: female ratio of 1: 2.6. Hence, stocking a small number of males make more efficient using of the maturation tanks. Repeated spawnings within the same moult cycle are quite common in *P. monodon*. One mating is enough to fertilise the eggs up to 3–4 spawnings without considerably lowering the fertilisation.

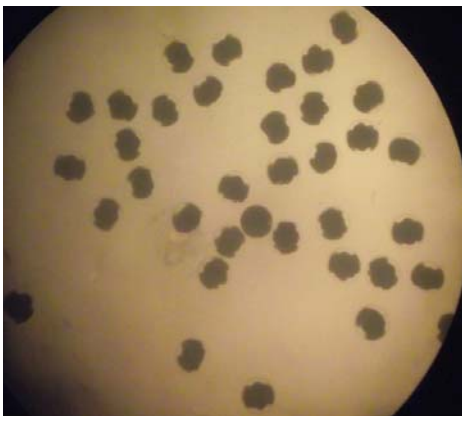

Fig 4. The fertilized (symmetrical cell divisions) and unfertilized eggs (round, cell divisions not clear) of *P. monodon*

Fig 5. Newly hatched nauplii of *P. monodon*

Conclusion

This study demonstrates that *P. monodon* females can readily mature and spawn in captivity with eyestalk ablation under the environmental conditions of the present study. Following ablation, it is possible to achieve the first spawnings within only 3.2 days. The ablation technique for re-maturation of spent *P. monodon* can be used successfully instead of costly selective breeding program to supply the seeds enabling shrimp farms to start their production in all seasons under the tropical condition of Bangladesh.

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