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Rematuration of hatchery used wild spawners of *Macrobrachium rosenbergii* (De Man 1879) in captive condition

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Abstract. Successful rematuration and spawning of hatchery used wild spawners (Broodstock) were conducted in the green house of Niribili Hatchery at Cox's Bazar, Bangladesh. After first spawning, ten healthy female spawners having length and body weight ranges of 18.0-22.0 cm and 90.9-105.1 gm respectively were directly stocked (one per each tank) in ten circular fiberglass tanks of 500 L capacity. In addition to first spawning in the maturation tanks, a total of three repeat spawnings were observed from the rematured spawners. Gonadal condition of the experimental spawners (n=10) was fully spent for eight individuals and partially spent for two individuals during stocking in the rematuration tanks. Of all the spawners, 100% spawned for second time, 80% spawned for third time and 50% spawned in the fourth time. Total number of eggs laid by the ten spawners was $60.4x10^4$, out of which fertilized eggs and larvae obtained were $57.5x10^4$ and $49.5x10^4$ respectively in the first spawning, the corresponding rates of fertilization and hatching being 95.05% and 85.08%. In first repeat (rematured) spawning, the total number of eggs and larvae produced by ten spawners were $52x10^4$ and $37x10^4$ and fertilization and hatching rates were 92.76% and 76.89% respectively. Observations limited to the 10 prawns studied indicate that while the fertilization rate of eggs was almost uniform, hatching rate gradually decreased with each consecutive spawning. The recovering spawners were maintained under controlled environmental conditions of water temperature (28.05 ± 0.15 ^oC), salinity (12.0 ± 0.10 gL-1) and DO (6.5±0.05 ppm), and fed on fresh frozen meat of squid, clam, cockle, and crab as well as a combination of those items, at 15% (wet weight) of their body weight, crab meat (97.79%) being the most preferred feed consumed during their rematuration.
Key Words: *Macrobrachium rosenbergii*, rematuration, repeat spawning, gonadal maturation, eggs, zoeae.

Introduction. *Macrobrachium rosenbergii* (De Man 1879), the giant freshwater prawn of tropical and subtropical areas of the Indo-Pacific region, is the biggest and one of the most important commercial prawns available in both fresh and brackish water environments of Bangladesh. Though it has a high demand in the local and foreign markets, its production from the natural waters of Bangladesh is not enough even to fulfill the internal demand. There are more than ten million ponds in Bangladesh covering approximately 172,612 acres of water area, where profitable aquafarming of this prawn is possible for which large-scale seed production in captivity is needed. Ling & Merican (1961) first recommended the use of brackish water for spawning and hatching of *M. rosenbergii*. Ling (1962, 1964, 1969a, 1969b), Fujimura (1966, 1978), Rajyalakshmi (1961), Ibrahim (1962) and Rao (1965) studied the breeding biology of *M. rosenbergii*. With the starting of scientific culture practice, there is very high demand for prawn seed in Bangladesh. Over one billion wild fry were collected from the natural sources in the year 1985, which as reported has increased to over two billion in 1990 (Siddique 1999). In order to cope with the demand for the seed, establishment of hatcheries to supplement the natural supply is being encouraged. The objective of the present study is to find out if rematuration of used wild spawners of *M. rosenbergii* in captivity to facilitate more seeds for stocking, as availability of wild broodstock is likely to be a problem in future.

Material and Method. The experiment was carried out in a commercial hatchery, at the Niribily Hatchery Ltd., situated at Kalatali Hatchery Zone, about 5 km south of Cox's Bazar City in Bangladesh. Maturation and larval rearing units were set up in the "green house" of the hatchery. 10 circular fiberglass tanks of 500 L capacity each for rematuration and 5 tanks of the same size for spawning and hatching purposes were used. Two rectangular tanks of 10 metric tones (MT) each were used for water storage and treatment. Fresh seawater of 30-35 ‰ was pumped through a submersible sand filter placed at the sub-littoral zone in the sea near the hatchery. The pumped seawater was stored initially in the sedimentation tank of 120 MT capacities. After 24 hours of sedimentation water was again pumped to the treatment tanks through sand gravel filter. The filtrate water was treated with 25-30 ppm formalin for six days before use and heated to desired temperature by titanium water heaters. The treated seawater with 28^{o}C was pumped to the rematuration and spawning cum hatching tanks. The seawater was mixed with freshwater made available from ground water source, to get the desired salinity of around 12 ‰ .

Spawners are usually abandoned in commercial hatcheries after first spawning. Ten females having size variation of 90.9 - 105.1 g (mean 98.32 ± 4.08 g) and 10 males of 110.0 - 130.5 g (mean 119.28 ± 5.08 g) of *M. rosenbergii* were collected immediately after first spawning and the average number of eggs and larvae per spawner was recorded. The spawners were immediately stocked in the rematuration tanks at a stocking density of two individuals (a male and a female) per tank at a ratio of 1:1 (Aquacop 1977). The physico-chemical parameters of the water in the rematuration tanks were recorded daily. In the maturation tank, water temperature, salinity, D.O., pH and NO_2-N were 28.05 ± 0.15 ^{0}C, 12.0 ± 0.10 gL^{-1}, 6.5 ± 0.05 ppm, 8.0 ± 0.15 and 0.05 ± 0.03 ppm respectively.

No water exchange was done during the first three days of stocking. After that 50% water in each tank was exchanged daily before siphoning of fecal matter. The temperature was kept around 28^{0}C with the help of immersion heaters. The spawners were fed with fresh frozen flesh of cockle (*Anadara* sp.), blood clam (*Dosinia* sp.), mud crab (*Scylla serrata*) and squids (*Sepia* sp.) either singly or as a mixture in accordance with their consumption capacity. Each feed of five items was given daily to the ten tanks (each tank with one male and one female) in randomising way of each successive day during whole rearing period of 30 days. The total body weight of 20 prawns (10 males and 10 females) was calculated as 2,176 g and the daily feed consumed by the individuals was calculated as 326.5 g (average) showing the 15% (wet weight) at four times a day. The uneaten feed was removed daily from the tanks.

The rematuration tanks were covered with black polyethylene paper together with 'orchid sheet' by at least 90% to minimize the disturbance of the animals. The ovarian maturity was checked daily at the evening by holding them against an underwater flash light beneath the ventral side of the abdomen. Development of eggs was examined with a scoop net. When the color of the eggs became dark grey from yellowish, they were transferred to hatching tank after disinfection with 25 ppm formalin for 90 minutes. Sodium salt of EDTA was added to the hatching tanks at the rate of 2-3 ppm concentration. The water in the hatching tanks was agitated with a stirrer at 10-15 minutes interval until hatching was completed.

Egg samples from the spawners and newly hatched larvae were preserved in 5% neutral formalin. At the end of the study, surviving spawners and males were preserved in 10% formalin and the length and weight were taken from the preserved samples.

Results and Discussion. Gonadal condition of the experimental female spawners (n=10) was 'fully spent' for eight individuals and partially for two individuals during stocking in the rematuration tanks. Total number of eggs laid by ten spawners was 60.4x10^{4} showing an average of 60.4x10^{3} per spawner (SD ± 4.48). The number of fertilized eggs and larvae from these eggs were 57.7x10^{4} (mean 57.5 ± 5.97) and 49.4x10^{4} (mean 49.45 ± 7.55) respectively. Fertilization and hatching rates was 95.05% and 85.08% respectively (Table 1).

Table 1

First spawning of the ten selected female spawners

Spawner's serial	*Spawner's body weight (g)*	*Spawned eggs ($\times 10^3$)*	*Fertilized eggs ($\times 10^3$)*	*Fertilization rate (%)*	*Total of zoeae ($x10^3$)*	*Hatching rate (%)*
1	102.5	65	63	96.92	60	95.23
2	100.2	62	61	98.32	47	77.04
3	95.5	58	55	94.82	52	94.54
4	105.1	70	66	94.28	65	98.48
5	99.2	60	55.5	83.33	40.5	72.97
6	98.5	59	57	96.61	48	84.21
7	95.3	55	54	98.18	45	83.33
8	100.3	65	62	95.38	50	80.64
9	90.9	52	50	96.15	42	84.00
10	95.7	58	56	96.55	45	80.34
Total	983.2	604	575	-	494.5	-
Mean (SD)	98.32 (±4.08)	60.4 (±4.48)	57.5 (±5.97)	95.05	49.45 (±7.55)	85.08

Table 2

First repeat spawning of ten individuals

Spawner's serial	*Interval (days)*	*Spawned eggs ($\times 10^3$)*	*Fertilized eggs ($\times 10^3$)*	*Fertilization rate (%)*	*Total of zoeae ($\times 10^3$)*	*Hatching rate (%)*
1	7	56	51	91.07	39	76.47
2	10	54	51	94.44	37	72.54
3	4	51	47	92.15	36	76.60
4	8	58	52	89.66	39	75.0
5	7	52	49	94.23	35	71.43
6	5	49	45	91.83	33	73.32
7	6	48	46	95.83	37	80.43
8	5	55	51	92.72	39	76.48
9	8	45	42	93.33	35	83.33
10	10	52	48	92.30	40	83.33
Total	-	520	502	-	370	-
Mean (SD)	-	52.0 (±4.57)	50.2 (±3.74)	92.76	37.0 (±2.49)	76.89

Table 3

Second repeat spawning of eight individuals

Spawner's serial	*Interval (days)*	*Spawned eggs ($\times 10^3$)*	*Fertilized eggs ($\times 10^3$)*	*Fertilization rate (%)*	*Total of zoeae ($\times 10^3$)*	*Hatching rate (%)*
1	8	48	43	93.75	16	37.22
2	7	40	38	95.00	15	39.48
3	4	33	32	96.96	15	46.88
4	8	46	40	86.96	16	40.00
5	7	38	36	94.73	14	38.89
6	5	33	32	96.97	15	46.87
7	-	-	-	-	-	-
8	5	46	41	89.13	16	39.03
9	-	-	-	-	-	-
10	10	37	34	91.89	13	38.24
Total	-	321	296	-	120	-
Mean (SD)	-	40.13 (±5.21)	37.0 (±3.10)	90.67	15 (±0.87)	40.83

Table 4

Third repeat spawning of five individuals

Spawner's serial	*Interval (days)*	*Spawned eggs ($\times 10^3$)*	*Fertilized eggs ($\times 10^3$)*	*Fertilization rate (%)*	*Total of zoeae ($\times 10^3$)*	*Hatching rate (%)*
1	4	42	-	-	-	-
2	4	37	-	-	-	-
3	-	-	-	-	-	-
4	6	41	-	-	-	-
5	6	35	-	-	-	-
6	-	-	-	-	-	-
7	-	-	-	-	-	-
8	5	40	-	-	-	-
9	-	-	-	-	-	
10	-	-	-	-	-	-
Total	-	195	-	-	-	-
Mean (SD)	-	39 (±2.24)	-	-	-	-

Three consecutive (repeat) spawning in each female spawner were observed. Of all the reused spawners, 100% spawned in the first, 80% in the second and 50% in the third repeat spawning. Fertilization and hatching success were observed up to the second repeat spawning. Data on spawning, egg fertilization and hatching of the repeat spawning together with that of the first spawning are provided in tables 1-4.

Intervals (recovery period) was measured the gap between two successive spawning of ova that were deposited in the ventral abdominal pouch of the females and the days were counted when one set of ova was 'fully spent' from the pouch until the next set of ova were again deposited in the same pouch. All the ova from the ovary were not released at a time, already matured set released first though the residual sets were being matured during the period in the ovary and released as second and third sets afterwards. In this experiment intervals between two consecutive repeat spawning were 4-10, 7-13 and 4-6 days in the first, second and third repeat (rematured) spawning respectively (Tables 2-4). In first repeat spawning, the total number of eggs laid by ten spawners, their fertilized eggs and larvae were $52x10^4$ (mean 52.0 ± 4.57), $50.2x10^4$ (mean 50.2 ± 3.74) and $37x10^4$ (mean 37.0 ± 2.49) and fertilization and hatching rates were 92.76% and 76.89% respectively (Table 2). In second repeat spawning, the total number of spawned eggs, fertilized eggs and larvae produced by eight spawners were $32.1x10^4$ (mean 40.13 ± 5.21), $29.6x10^4$ (mean 37.0 ± 3.10) and $12x10^4$ (mean 15 ± 0.87) and fertilization and hatching rates were 90.67% and 40.83% respectively (Table 3). In third repeat spawning, though the number of eggs laid by five spawners was $19.5x10^4$ (mean 39.0 ± 2.24), the eggs were not fertilized and hatched (Table 4). Among five feed items (squid meat, clam meat, cockle meat, crab meat and mixed feed), crab meat was highly preferred (97.79%) by the spawners followed by combination of all items (96.34%).

Conclusions. The spawners of *M. rosenbergii* used in hatcheries are found suitable for successful rematuration and spawning. It has been found that after first spawning the spawners are able to spawn some more times and in this study the broodstocks responded three times for repeat spawning and two times for fertilization and hatching of eggs. Intervals between two consecutive spawning required were 4-10, 7-13 and 4-6 days in second, third and fourth spawning respectively. Though the fertilization rate of eggs was almost uniform but hatching rate gradually decreased with each consecutive repeat spawning from first repeat to third repeat spawning and consequently, the number of eggs per spawner also decreased from $52x10^3$ in first repeat spawning to $39x10^3$ in third repeat spawning (Tables 2-4). Total eggs and larvae produced in three repeat spawnings were $103.6x10^4$ and $49x10^4$ respectively. Highest fertilization rate was observed in the first (92.76%) and the lowest in the third repeat spawning (0%). It was found that hatching rate gradually decreased in the repeat spawning, which varied from 76.89% in the first to 0% in the third repeat spawning (Tables 2-4).

From the present study, it has been found that both temperature and feed have strong effect on the early gonadal maturation and spawning of *M. rosenbergii*. Datta (1978) observed in the Karnafully River Estuary that the breeding and spawning of *M. rosenbergii* occurs during summer months of April to September. He added that temperature and high protein rich diet are the main criteria for growth and maturation of the species. During the present investigation, berried females were produced in the hatchery under captive condition by controlling the temperature at optimum level (around 28^0C) and giving protein rich feed. Ling (1964) and Rao (1965) mentioned that both temperature and food, acting together with salinity are the important factors for successful breeding of *M. rosenbergii*. Khan (1997) stated that temperature and food could stimulate the artificial propagation of freshwater prawns. Ling & Merican (1961) opined that the spent individuals of *M. rosenbergii* that spawned once earlier but left uncaught, spawn again after a period of several months. They found that a second spawning of some individuals occurred within a period of 4-5 months in captivity when they are provided with optimum food and other environmental conditions. Raman (1967) stated that the frequent availability of spent individuals with advanced maturity stages of gonads is available in extreme lower reaches during the late breeding season. As the

present result is the outcome of only ten female spawners, it may be concluded that further study could fulfil the requirement of the present knowledge.

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