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Md. Samsul Alam
Department of Fisheries Biology and Genetics Bangladesh Agricultural University, Mymensingh 2202 Bangladesh.

Mohammad Nurul Alam
Department of Fisheries Biology and Genetics Bangladesh Agricultural University, Mymensingh 2202 Bangladesh.

Correspondence:
Md. Samsul Alam
Department of Fisheries Biology and Genetics Bangladesh Agricultural University, Mymensingh 2202Bangladesh.
Email: samsul_bau@yahoo.com

Development of the giant freshwater prawn *Macrobrachium rosenbergii* (De Man 1779) broodstock in culture ponds of South-Western Bangladesh: a case study

Md. Samsul Alam and Mohammad Nurul Alam

Abstract
A study was conducted on development of the giant freshwater prawn *Macrobrachium rosenbergii* broodstocks in farmed conditions in Bangladesh. The post larvae (PL) were stocked in nine farms at an average density of 13263 Post larvae/hectare and fed with commercial feed until maturity. For complete maturation and spawning, the male and female prawns were stocked at a ratio of 1 male: 3 females in the month of January and fed with the commercial feed supplemented with cod liver oil. Out of the nine farms, brood prawns were successfully developed in three farms with good fecundity. Overall, the fecundity ranged from 8223 to 56853 with a mean of 30201±3026 and showed strong correlation with the size of the prawn. This study concludes that good quality *M. rosenbergii* broods can be produced in culture ponds by proper stocking, feeding and water quality management.

Keywords: *Macrobrachium rosenbergii*, broodstock, nutrition, stocking density, fecundity

1. Introduction
The giant freshwater prawn (*Macrobrachium rosenbergii* De Man), known as galda in Bangladesh, is naturally distributed in South and South-East Asia, Northern Australia and the Western Pacific Islands [1]. Rearing of freshwater prawn by collecting or trapping juveniles in tidal ponds had long been practiced in the Indian sub-continent and Malaysia [2]. However, with the development of captive breeding and post larvae (PLs) production technologies, the freshwater prawn has appeared as one of the major commercial aquaculture species in many countries such as China, India, Thailand, Vietnam and Bangladesh. *M. rosenbergii* has been proven as a suitable species for commercial aquaculture along with other species in various culture practices [3-6].

In Bangladesh, commercial farming of prawn started in early 1990s but the increasing price of prawn in local and international market has promoted rapid expansion of galda farming in recent years. The area of galda farm was estimated to be 62, 874 ha in 2011 producing 30636 Metric Ton (MT) and in 2010-2011, Bangladesh exported 7020 MT of galda valued at US$96 million (Bangladesh Taka 6.95 billion) [7].

Life cycle of galda requires a short stay in the estuarine waters for larval development. Naturally, after mating the gravid females migrate towards brackish water where the eggs hatch as free-swimming larvae [8] and after passing through the necessary developmental stages, the post-larvae (PLs) move upstream to freshwater area where they live up to their adult life. Galda culture has been expanded in the districts of Khulna, Satkhira, Bagerhat, Jessore, Narail, Gopalgonj and Noakhali due to the availability of seed in the coastal areas [9]. Moreover, there is an established export market in the coastal districts [10]. As a freshwater species however galda also has tremendous potential in the freshwater ponds in the interior districts of the country.

Galda PLs are collected from the brackish water in the Southern region. Since the late 1980s, there have been concerns over the effects of intensive catching of prawn PL [11]. Indiscriminate catching of wild PL with high levels of by-catch (i.e. non-target species caught incidentally) and biodiversity impacts on the coastal ecosystem has provoked imposition of ban on wild PL collection [12].

Due to the scarcity of wild PL supply and simultaneously increased demand, prawn hatchery enterprises have emerged over the last few years and the galda hatcheries has already exceeded 50 [7]. However, the quality of hatchery PL remains a concern for prawn farmers. It is assumed that source and quality of broodstock (i.e. mother prawns or berried females) is an important issue for producing quality fry in hatcheries.

The broods used in the hatchery are collected directly either from the rivers or from ponds/ghers. To meet the demand of the prawn hatcheries it is essential to maintain a steady supply of good quality brood. The present study was based on extension and training project for the brood bank farmers in developing quality galda broods by improving genetic and nutritional management implemented by Winrock International, Bangladesh. We report here a partial success of galda brood development in selected private farms in Bangladesh.

2. Materials and methods

The study was conducted for a period of seven months from August 2009 to February 2010.

2.1 Selection of farms

Nine private farms were selected from Satkhira, Bagerhat, Khulna, Narail and Jessore districts for the study (Figure 1). The names of the farms, location, size of the brood rearing bonds, source of post larvae, stocking density and feeding are shown in Table 1. PLs were collected from adjacent rivers and reared for a period of three months from August to November, 2009 in the maturing pond.

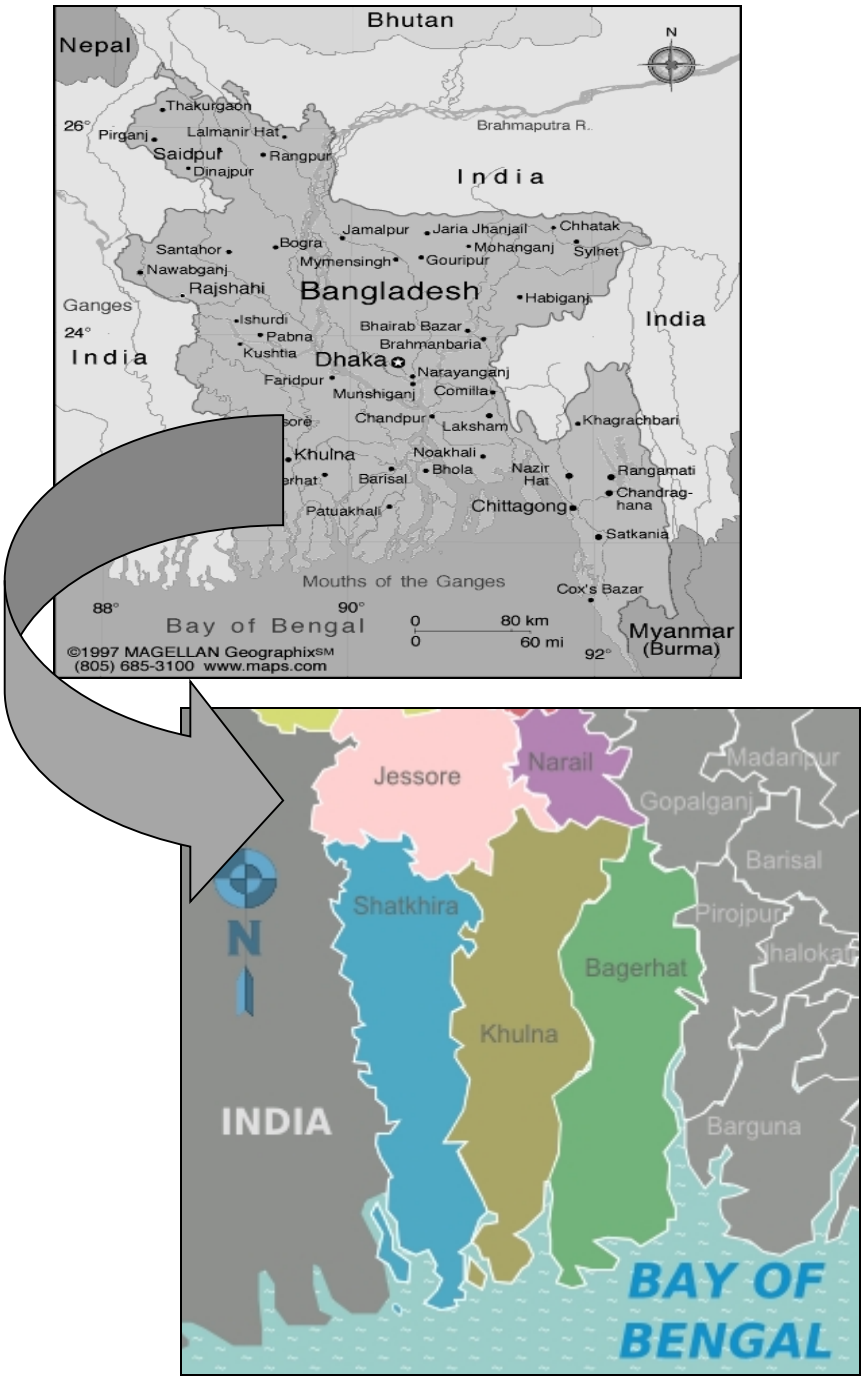

Fig 1: Map of Bangladesh (above). The farms are located in the districts of Satkhira, Bagerhat, Khulna, Narail and Jessore (below).

2.2 Stocking of male and female brooders in brood maturation pond

For maturation, the male and female broods were stocked together in single pond in each farm at a sex ratio of 1 male: 3 females. Commercial feed of Mega with a trade name of "Prawn Finisher" (35% protein, 4% lipid, and 6% crude fibre) supplemented with 5 ml cod liver oil per kg feed was provided. Out of the nine farms, aeration/water undulation facilities with motorized blower/aerator were available only in Anamika farm, Kaligonj, Satkhira.

2.3 Water quality parameters

Different physico-chemical parameters such as water temperatures, p^H, dissolved oxygen (DO) and alkalinity were recorded fortnightly from October 2009 to February 2010. Temperature of the pond water was recorded with a Celsius thermometer and p^H was determined by a direct reading digital p^H meter (Jenway, model, 3020). The dissolved oxygen was measured by a portable digital DO meter (YSI, model 58) and the total alkalinity was estimated by using a HACH Kit (DR-2010) following manufacturer's instructions.

2.4 Estimation of fecundity and egg sizes of gravid prawn

For estimating fecundity, gravid prawns were randomly collected and taken to the laboratory of the Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh. The weight of the female and the weight of eggs were recorded during the time of collection. For counting the eggs by gravimetric methods, an aliquot of the eggs were weighed using a Metler electronic balance and counted. The total number of eggs per female was calculated by multiplying the total weight of the eggs. The sizes of the eggs were measured by using micrometer fitted in a binocular microscope.

2.5 Statistical analysis

The data on weight of berried females, weight of eggs/female, number of eggs/female, no. number of eggs/g female and egg size were subjected to one way analysis of variance (ANOVA) implemented in Excel 2010 program followed by multiple comparison. The correlation between the size of females and the number of eggs/female was also estimated using the same program

Table 1: The area, stocking density, source and feeding strategy in the nine brood banks under study

S. No	Names and addresses	Size of pond (ha)	Stocking density/ha	Source of PL	Feeding
1	Anamika Bagda Golda Hatchery Kaligonj, Satkhira, Syed Mehedi	0.65	12500	River	Mega Feed + cod liver oil (5 ml/kg feed)
2	Selim Jahangir Kaligonj, Satkhira	0.40	12500	Anamika hatchery	-do-
3	Shahnewas Paikpara Bagerhat	0.80	15000	Baadkhalibeel	-do-
4	Batiagata, Khulna Dipok Kumar Mandal	0.60	12500	River Shibsha	-do-
5	Durgapore, Narail Sanjoy Kumar Saha	0.40	15000	River Shibsha	-do-
6	Maidar Char Narail Ujjal Gazi	0.40	15000	River Shibsha	-do-
7	Mulia, Narail Ramesh Biswas	0.25	12500	River Shibsha	-do-
8	Manirampur Jessore Nesar Ali	0.40	11250	River Joymoni	-do-
9	Manirampur Jessore Moksed Ali	0.16	13125	River Joymoni	-d0-
	Mean±SE	0.45±0.06	13263±463		

3. Results and discussion

3.1 Water quality parameters

The physico-chemical parameters of the pond-water are presented in Table 2. The range and mean values of water temperature, p^H, dissolved oxygen and alkalinity were similar in all the farms and within the suitable range for galda farming. However, one exception was observed in alkalinity of the ponds under Satkhira districts (143±4.23) which was the highest among all the farms under five districts.

Table 2: Physico-chemical parameters of the pond waters recorded during October 2009 to February 2010.

Paramaters	Temperature (ºC)		pH		Dissolved Oxygen		Alkalinity	
District	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE
Khulna	11-25	17.33±1.56	7.5-8.0	7.8±0.08	5.1-6.8	6.16±0.17	90-130	109±3.78
Satkhira	11-25	18.55±2.03	7.5-8.5	8.05±0.11	5.0-6.7	5.5±0.19	120-160	143±4.23
Bagerhat	17-27	18.55±2.13	7.5-8.5	7.85±0.10	5.4-6.8	6.22±0.14	90-135	110±3.82
Jessore	16-26	18.11±1.99	7.5-8.5	7.8±0.11	5.1-7.0	5.8±0.17	100-130	113±3.67
Narail	11-27	18.56±1.91	7.5-8.5	8.05±0.12	5.0-5.8	5.32±0.08	100-130	115±2.68

The data of the farms under the same district have been pooled

3.2 Stocking and feeding management

The major objective of the study was to develop mature galda brood in the farmed conditions. Stocking density and feeding management are the most important aspects of brood stock development. As far as galda is concerned, most researches on broodstock nutrition have concentrated on the quantitative and qualitative requirements of lipid. Since the quality of eggs is primarily dependent on the lipid content of feed particularly of highly unsaturated fatty acids (HUFA), special attention was given to the level and quality of lipid while preparing feed for galda brood stock. The dietary lipid level in prawn diets can be as low as 5% provided the lipid source contains sufficient levels of essential fatty acids. There is a dietary requirement for HUFAs although in very small quantities. Both n-3 and n-6 HUFAs at dietary levels of 0.075% are known to increase weight gain and feed efficiency remarkably. In addition, both 18:2n-6 and 18:3n-3 fatty acids are also required [13]. Juvenile *M. rosenbergii* require >20 carbon unit polyunsaturated fatty acids at a dietary level of approximately 0.1% [14]. Most studies have shown that a lipid content of around 9- 10% should be provided in the brood stock feed of which 50% should be of animal origin such as squid oil or cod liver oil [15]. But the feeds used in Bangladesh contain only 4% lipid which is insufficient for proper growth and gonadal development. Therefore, to supplement the required level of highly unsaturated fatty acids, we suggest the farmers to add 5 ml cod liver oil per kg of food. HUFA also has positive impact on growth of juvenile prawn. For example, Parakarma *et al.* [16] observed that the post larvae fed with cod liver oil enriched feed had the highest weight gain (225.72±9.05%), highest specific growth rate (2.95±0.07%), and highest survival (77.14±4.28%) as well as low food conversion ratio (0.87±0.03) compared to the post larvae fed with control feed.

Out of nine brood bank farms, three farms were able to produce gravid females. One farm was closed prematurely due to heavy mortality. No eggs were found in the abdomen/legs of broods in five brood banks, four in Jessore/Narail and one in Satkhira districts. Inadequate management particularly water quality and feeding, may be attributed for poor gonadal development of galda. For example, paddy has been cultivated in the galda pond/gher keeping the prawn in a narrow canal resulting in stressed condition in Narail and Jessore districts.

3.3 Fecundity and egg size

The weight of the sampled berried females of three farms ranged from 31 to 83 g and the number of eggs ranged from 8223 to 56853. The mean weight of gravid females, mean weight of eggs/female, mean number of eggs/ females, mean number of eggs/g female and egg size observed in the three farms producing gravid females are shown in Table 3. A photograph of two berried galda of Botiaghata farm is shown in Figure 2. The mean weight of gravid females of Botiaghata

Table 3: Fecundity parameters and egg size recorded during the present study

Farm	Weight of gravid female (g)	Weight of egg (g)	Number of eggs per female	Number of eggs /g female	Egg size (mm)
Botiaghata	78.8±1.23^{a} (67-83)	10.06±0.57^{a} (7.5-11.56)	42646±1347^{a} (37940-46668)	536±28^{a} (470-696)	0.53±0.02^{a} 0.52-0.57
Anamika	53.5±1.28^{b} (37-64)	4.69±0.58^{b} (2.97-6.41)	35977±1686^{a} (13290-56853)	614±85^{a} (359-888)	0.54±0.02^{a} (0.42-0.54)
Paikpara	33.7±0.75^{c} (31-42)	3.22±0.17^{b} (2.31-3.84)	11979±728^{b} (8223-15074)	316±14^{b} (265-358)	0.56±0.02^{a} (0.49-0.54)

Different superscripts in the same column indicate significant differences (P<0.001)

(Khulna) farm was found to be the highest followed by the Anamika farm (Satkhira) and Paikpara farm (Bagerhat) respectively. The mean weight of eggs/female of Botiaghata was significantly higher than those of Anamika and Paikpara farms (P<0.001). The mean number of eggs/female and the mean number of eggs/ g female of the Botiaghata and Anamika farms were significantly higher than those of the Paikpara farm (P<0.001). The egg size varied from 0.42 to 0.56 mm. No significant difference was observed in egg size of the three farms. The total number of eggs per female, number of eggs per unit weight (g) of female and egg size found in the present study are greater than those reported by [17]. Fecundity of *M. rosenbergii* varies considerably with the age, size and stage of maturity. A strong correlation was observed between the number of eggs and weight of females (r^2=0.70). However, no correlation was observed between the size of females and size of eggs (r^2=-0.06). Habashy [18] also reported strong correlation between the size of the female *M. rosenbergii* and the fecundity. Proper feeding with balanced diet and maintaining proper stocking density may help increase the fecundity as well as quality of the eggs which has a positive impact on larval survival and growth. Fecundity can be as high as 80,000 to 100,000 eggs in mature females while first broodstock may be around 5,000 to 20,000 [19]. Ratnayake *et al.* [20] reported fecundity ranging from 24171 to 34294 in *M. rosenbergii* reared under varied sex-ratio in Srilanka. In the Botiaghata farm, we found two types of prawns- with bright orange and deep brown colored eggs (Figure 2). The number of eggs in the female with brown colored eggs was relatively lower compared to those containing orange colored eggs. This is attributed to release of some eggs from the pleopods due to delay in harvesting. Therefore, it is suggested that the female broods should be harvested as soon as the eggs are seen in the abdomen inside the pleopods before turning the color from orange to brown.

Fig 2: Brood galda of Batiaghata brood bank owned by Dipok Kumar Mandal. Both orange and brownish eggs are seen in two different brood galda. The hatchery operators prefer to purchase the brood with brown-colored eggs. Orange color eggs are newly fertilized eggs while the brown color eggs bear advanced embryo.

3.4 Optimum ratio of male and female

As far as genetic diversity is concerned the sex ratio of the brood should be 1:1. But at this moment in Bangladesh the broods are not being recycled in the hatchery. The PL produced in the hatchery are used only for grow out as table prawn. All the brood bank operators informed us that they had collected PL from rivers for stocking in the brood pond. In this case considering the economics of brood rearing it is better to stock 1 male: 3 females. The males are territorialistic in nature. Therefore, the females may get more access to feed if the number of males is kept low. For spawning one male is sufficient for three females. There is no optimum stocking density in the outdoor culture of prawn. The stocking density depends on several factors such as water quality, presence or absence of aerators, presence or absence of artificial shelters and overall management. Considering the conditions observed a stocking density of 10000 prawns per hectare [1] is recommended.

4. Conclusion

We have presented a case study that describes the brood stock development practices of the giant freshwater prawn in the pond conditions. This study confirmed that it was possible to produce good quality gravid females in ponds by maintaining proper stocking density, water quality and providing balanced feed supplemented with sufficient amount of unsaturated fatty acid containing oil. The study also confirmed that a sex ratio of 1 male: 3 females was sufficient for successful fertilization of eggs of the gravid females.

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