

Reproductive performance of freshwater prawn *Macrobrachium rosenbergii* (De Man 1879) broodstocks grown on different diets

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Abstract

An experiment was carried out during April to September 2013 to compare the reproductive performance of freshwater prawn *golda* (*Macrobrachium rosenbergii*) broodstocks fed on diets including 3 commercial (*Mega*-F₁, *SABINCO*-F₂ and *CP*-F₃) and a laboratory formulated test feed (F₄). Post larvae (PL) were collected from the river Kocha of district Pirozpur in April 2012 and nursed in the *hapa* (net cage) then reared in earthen ponds at the Fisheries Field Laboratory, Bangladesh Agricultural University (BAU), Mymensingh. The broods were fed with selective feeds since their PL stage. The feeding was continued till their maturity of both male and female up to berried females. The proximate composition of the feeds, F₁ where crude protein, 33.49%, lipid 5.38%, carbohydrate 30.31%, F₂-crude protein, 26.70%, lipid 5.50%, carbohydrate 34.34%, F₃- crude protein, 38.90%, lipid 8.09%, carbohydrate 25.37% and treatment F₄- crude protein 33.00%, lipid 11.13%, carbohydrate 25.43%. Feeds were supplied at 5% body weight twice at dawn (5:30-6:00 am) and dusk (5:30-7:30 pm) till the end of the experiment. The variation in most water quality parameters, temperature, alkalinity, DO, transparency, TDS, conductivity NO₂⁻-N, PO₄³⁻ and chlorophyll-a were insignificant; while parameters, water depth, pH, NH₃-N and NO₃⁻-N was found to vary significantly (p<0.05) among treatments. Brood observation was carried out by dragging seine net or/and hand picking for eggs in egg chamber remained intact. The male and female ratio estimated 1:2.8, 1: 2.4, 1: 2.4 and 1: 1.7 in the treatments F₁, F₂, F₃ and F₄. The weight of the berried females was 56.33±19.55 g, 85.50±14.85 g, 73.67±23.09 g and 94.50±51.62 g; total weight of eggs was 7.61±1.86g, 7.04±0.69 g, 10.26±1.41g and 6.15±1.20 g in F₁, F₂, F₃ and F₄. The estimated numbers of eggs were 53,889±10,541, 37,555±1,217, 59,640±5,270 and 36,017±7,790 in F₁, F₂, F₃ and F₄ respectively. The weight of berried females with eggs (g), total eggs (g) and estimated number of eggs were significant (p<0.05) among treatments. Females fed with commercial feed F₃ (*CP*) were found to produce the sufficient number of eggs (59,640/female) followed by F₁ (*Mega*). The findings may contribute to the better broodstock management of freshwater prawn to produce of a sufficient number of embryonic eggs for hatchery operation.

Keywords: Freshwater prawn, Broodstocks, Reproductive performance, Different diets

Introduction

Freshwater prawn (*Macrobrachium rosenbergii*, De Man 1879) locally known *golda* is one of the important aquaculture species in Bangladesh. About 75,000 ha land is being used for *golda* culture and it produce about 41,000 Mt in 2012 (DoF 2013). Due to its increasing demand and as a priority sector for economic growth, Bangladesh has its rapid extension of cultural practices. On the question of culture, seed availability is the prime consideration. Since culture practices began in the country, the seed of freshwater prawn *i.e.*, post larvae (PL) were largely collected from wild sources (Ahmed 2008). Due to the rapid extension of cultural practices, in many parts of the world, a huge number of PL were collected from nature (Mohanta 2000) and concerning the biodiversity conservation, however, exerted ban in many countries including Bangladesh. About 80 *golda* hatcheries (DoF 2012) have been established for the last couple decades in the country among which 70 are existing (DoF 2013) and each of them requires about 520 berried females in a productive year (Sardar *et al.* 2013). The majority of the hatcheries are to fulfill their brood requirement from wild sources, while a few are from cultural farms and the number is inadequate. The availability of qualitative berried females is essential for the successful hatchery operation and production of PL. However, development of a large scale brood in captive conditions with proper nourishment is prerequisite for quality seed production. An attempt was taken to develop a suitable feeding regime using locally available commercial and laboratory prepared feeds for the proper nourishment of quality berried females for hatchery operations.

Methods and Materials

The experiment was carried out during April to September 2013 to compare the reproductive performance of freshwater prawn Golda (*Macrobrachium rosenbergii*) broodstocks fed on four diets including 3 commercial (Mega -F₁, SABINCO- F₂ and CP- F₃) and a laboratory prepared test feed (F₄).

Collection of PL

Wild post larvae (PL) were collected from the river Kocha of district Pirozpur in late April 2012 within the location 22°35'521'' N, 90°0052.38''-03'18.52'' E, and 22°29'531'' N, 90°00'536''-01'461'' E (position taken by GARMIN-GPSMAP 765).

Nursing of PL and rearing broods

The collected PL were carried in the oxygenated polyethylene bags and nursed initially at a density 350 PL m⁻² in the fine meshed (0.5mm) nylon *hapa* (net cage) of size 1 m x1 m x 2.5 m for 30 days in the earthen ponds at the Fisheries Field Laboratory, Bangladesh Agricultural University (BAU), Mymensingh (24°43'32.70" N and 90°25'43.69"E – Google Earth). The juveniles were then reared in 12 earthen ponds, each of 40 m² under 4 treatments and 3 replications each. The juveniles were stocked randomly at a density of 3 m⁻².

Feed and Feeding

The broods were fed with selective locally available 3 commercial (Mega -F₁, SABINCO- F₂ and CP- F₃) pelleted feeds and a laboratory formulated test feed (F₄) since their PL stage. The feeding was continued till their maturity of both male and female to obtain the berried females. The proximate composition of the feeds, F₁ where crude protein, 33.49%, lipid 5.38%, carbohydrate 30.31%; F₂- crude protein, 26.70%, lipid 5.50%, carbohydrate 34.34%; F₃- crude protein, 38.90%, lipid 8.09%, carbohydrate 25.37% and treatment F₄- crude protein 33.00%, lipid 11.13%, carbohydrate 25.43%. Feeds were supplied at 5% body weight, twice at dawn (5:30-6:00 am) and dusk (5:30-7:30 pm) until the end of the experiment.

Monitoring of water quality parameters

The water quality parameters, i.e., water temperature, pH, dissolved oxygen (DO), depth, transparency, total dissolved solids (TDS), conductivity, alkalinity, ammonia nitrogen (NH₃-N), nitrite nitrogen (NO₂-N), nitrate nitrogen (NO₃-N), phosphate (PO₄⁻³) and chlorophyll-*a* were monitored fortnightly. The devices, portable Multiparameter: *HACH sension*TM 156 and *HACH DR 4000* version 2.35 were used in the Water Quality and Pond Dynamics Laboratory, Bangladesh Agricultural University (BAU) for measuring major physical-chemical parameters. Water depth was measured by a meter scale, and total alkalinity was estimated by the titration method using 0.02N H₂SO₄ and Methyl Orange indicator. The chlorophyll-*a* was estimated followed by Vollenweider's equation methods (Stirling, 1985) using a spectrophotometer (SPECTRONIC[®] GENISYSTM 5) at wavelengths 664 and 750 nm.

Examination of berried females

The broods were examined every week during April to September for observing their gonadal developments followed by a collection of berried females from all treatments. Broods were collected either by dragging a seine net or hand picking carefully so that the embryos in egg chamber were remained intact.

Collection eggs from berried females

The berried females were immediately transferred to the plastic buckets containing about 3 liters of pond water which aerated with handy portable dry cell operated aerator. The length and weight of the females were recorded. The eggs were carefully examined on the basis of embryonic development of major three color phases, i.e., orange, yellow and brown was recorded. A portion of yellow and brown eggs (approx. 1-2 g) from each berried female was collected with the help of forceps and scissors and placed in 5 ml plastic vials containing Bouin's fluid.

Assessment of eggs

A small part of collecting eggs were weighted by an electronic balance (METTLER TOLEDO B2002-S) and placed on a watch glass. The egg sample was then washed with distilled water to remove Bouin's fluid and placed in 10 ml of 1% sodium sulfite (Na_2SO_3) solution for half an hour to remove the stickiness of eggs (Fisher *et al.* 1999). The eggs then placed on a glass slide and counted manually under an electric microscope (Olympus BH2). The eggs were measured along their axis length (wide side), and the transverse width (narrow side). The egg size was measured with the help of a kilometer. The number of eggs per mg was counted. Total weight of eggs of a berried female was determined by individual weighing with eggs minus individual without eggs just after hatching.

Analysis of data

One way ANOVA was done among the treatments followed by Duncan PostHoc analysis for multiple comparisons. Statistical analysis was done with the help of software packages MS Excel version 2010 and SPSS version 16 for Windows.

Results and Discussion

Water quality parameters measured in the experiment were shown in Table 1. Water temperature, transparency, DO, total alkalinity, TDS, conductivity, $\text{NO}_2\text{-N}$, PO_4^{3-} and chlorophyll-*a* did not vary among treatments, while depth, pH, $\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$ varied significantly ($p < 0.05$) during the experimental period. The mean values of water parameters of all four treatments were, temperature $30.57 \pm 1.35^\circ\text{C}$, depth 99.83 ± 7.74 cm, transparency 34.45 ± 9.00 cm, TDS 134.05 ± 38.26 mg/L, conductivity $276.47 \pm 77.21 \mu\text{S/cm}$; while properties like, DO 3.71 ± 1.11 mg/L, pH 8.55 ± 0.59 , total alkalinity 139.31 ± 31.55 mg/L, $\text{NH}_3\text{-N}$ 0.21 ± 0.10 mg/L, $\text{NO}_2\text{-N}$ 0.01 ± 0.01 , $\text{NO}_3\text{-N}$ 0.02 ± 0.03 and PO_4^{3-} 1.15 ± 0.94 mg/L were estimated, while the chlorophyll-*a* was obtained $11.44 \pm 10.42 \mu\text{g/L}$ in all the treatments. The parameters were within the optimal ranges for prawn culture and agreed to Mohanta, 2000; Habashy *et al.*, 2012; the range of pH between 7-8.5 and alkalinity 50- 150 mg/L was reported by D'Abramo *et al.* 2006, conductivity was within the range described by Bhatnagar and Devi (2013). In wild populations of *M. rosenbergii*, spawning occurs in temperature between 29 and 30.5°C mentioned by Rao (1991) which is a little bit lower than present findings.

The cultural features of the broodstocks developed on different feeds are shown in the Table 2. The survival of prawns in the broodstock was estimated at the end of the experiment and was significant ($p < 0.05$) among treatments. It seems to be lower survival of the broods were directly related to their cultural duration as well on the efficacy of the feed regimes. The highest survival in treatment F_3 (46.11%), followed by F_1 (39.72%) and F_2 (37.22%) while lowest survival in treatment F_4 (24.72%) was estimated. The survival of females 50-60% and for males 20-30% of broodstocks was reported by Tidwell *et al.* (2005). The first berried females were obtained minimum at 295 days and maximum 315 days since the PL stocked in the experiment in different treatments. The male female ration was estimated 1:2.8, 1:2.4, 1:2.4 and 1:1.7 in the treatments F_1 , F_2 , F_3 and F_4 , respectively.

The ultimate aim of the experiment was to assess the performances through numbers of eggs produced by the berried females in broodstocks those were developed under three commercial and one laboratory prepared feed. The results are analyzed and showed in the Table 3. The obtained weight of the berried females with embryonic eggs 56.33 g, 85.50 g, 73.67 g and 94.50 g were significantly varied ($p < 0.05$) among treatments F_1 , F_2 , F_3 and F_4 respectively. The total mean weights of embryonic eggs were 7.61 g, 7.04 g, 10.26 g and 6.15 g, in the treatments F_1 , F_2 , F_3 and F_4 respectively, was also found statistically significant ($p < 0.05$). The embryonic egg sizes of both the axis length (wide side) 0.51, 0.62, 0.60, 0.56 mm and the transverse width (narrow side) 0.47, 0.57, 0.51, 0.48 mm were measured in the treatments F_1 , F_2 , F_3 and F_4 respectively. The embryonic egg sizes were found significantly varied ($p < 0.05$) both in axis length and transverse width among treatments, however eggs sizes were almost similar to Habashy *et al.* (2012). The estimated numbers of eggs were $53,889 \pm 10,541$, $37,555 \pm 1,217$, $59,640 \pm 5,270$ and $36,017 \pm 7,790$ in treatments F_1 , F_2 , F_3 and F_4 , respectively.

Table 1. Water quality parameters of the broodstocks rearing earthen ponds during the experiment period

Treatments	F ₁ Mean ±SD	F ₂ Mean ±SD	F ₃ Mean ±SD	F ₄ Mean ±SD	Total Mean ±SD	Remarks
Water temp. (C°)	30.54 ±1.55	31.11 ±0.96	30.77 ±1.42	30.58± 1.48	30.57 ±1.35	NS
Depth (cm)	97.09 ^{ab} ±5.78	100.09 ^{ab} ±5.85	101.32 ^a ±6.18	98.91 ^b ±7.74	99.83 ±7.74	*
Transparency (cm)	39.12 ±11.23	38.73 ±9.09	36.06 ±10.94	37.17 ±7.70	34.45 ±9.00	NS
DO (mg/L)	3.97 ±1.05	3.70 ±1.21	3.69 ±1.21	3.63 ±1.02	3.71 ±1.11	NS
pH	8.73 ^a ±0.67	8.60 ^{ab} ±0.71	8.57 ^{ab} ±0.73	8.28 ^a ±0.60	8.55 ±0.59	*
Alkalinity (mg/L)	139.62 ±34.97	142.35 ±30.75	136.82 ±29.63	139.24 ±31.79	139.51 ±31.55	NS
TDS (mg/L)	132.84 ±38.68	141.93 ±39.22	130.75 ±35.23	130.68 ±40.32	134.05 ±38.26	NS
Conductivity (µS/cm)	276.60 ±79.47	289.26 ±75.45	272.26 ±72.66	267.76 ±82.67	276.47 ±77.21	NS
NH ₃ -N (mg/L)	0.17 ^b ±0.07	0.19 ^{ab} ±0.08	0.23 ^a ±0.13	0.23 ^a ±0.10	0.21 ±0.10	*
NO ₂ -N (mg/L)	0.01 ±0.01	0.01 ±0.00	0.01 ±0.01	0.01 ±0.02	0.01 ±0.01	NS
NO ₃ -N (mg/L)	0.01 ^b ±0.03	0.03 ^{ab} ±0.03	0.03 ^a ±0.03	0.02 ^{ab} ±0.03	0.02 ±0.03	*
PO ₄ ⁻³ (mg/L)	1.04 ±0.92	1.15 ±0.96	1.21 ±0.97	1.20 ±0.93	1.15 ±0.94	NS
Chlorophyll-a (µg/L)	10.99 ±14.18	10.17 ±7.32	14.82 ±10.45	9.79 ±8.28	11.44 ±10.42	NS

(*- Significant at p<0.05, NS- Not significant)

Table 2. The features of broodstocks developed under three commercial and one laboratory prepared feeds

Parameters	Treatments	F ₁	F ₂	F ₃	F ₄
Stocking density of randomly selected male and female juvenile (no/m ²)		3	3	3	3
Total number of stocks		120	120	120	120
Survival % (mean ±SD)		39.72 ^{ab} ±7.23	37.22 ^{ab} ±9.29	46.11 ^a ±9.71	24.72 ^b ±10.69
The first appearance of berried females since PL stocked (day)		295	300	300	315
Obtained male and female ratio		1:2.8	1: 2.4	1: 2.4	1: 1.7

Significant at p<0.05

Table 3. Performance of berried females of producing eggs under broodstocks developed under different feeds

Parameters	Treatments	F ₁ (Mean ±SD)	F ₂ (Mean ±SD)	F ₃ (Mean ±SD)	F ₄ (Mean ±SD)
Weight of the berried females (g)		56.33 ^{ab} ±19.55	85.50 ^{ab} ±14.85	73.67 ^a ±23.09	94.50 ^b ±51.62
Total weight of embryonic eggs (g)		7.61 ^b ±1.86	7.04 ^b ±0.69	10.26 ^a ±1.41	6.15 ^c ±1.20
Axis length of eggs (mm x 0.01)		0.51 ^c ±0.02	0.62 ^a ±0.06	0.60 ^b ±0.03	0.56 ^{bc} ±0.03
Transverse length of eggs (mm x 0.01*)		0.47 ^b ±0.01	0.57 ^b ±0.05	0.51 ^a ±0.03	0.48 ^c ±0.04
Numbers of eggs		53,889 ^a ±10,541	37,555 ^b ±1,217	59,640 ^a ±5,270	36,017 ^b ±7,790

Significant at $p < 0.05$; *- calibrating factor at 10x magnification

The eggs number depends on the size of the prawn. However, the number of eggs obtained in the present study was between 36,017 and 59,640 among the treatments was more or less similar to the range reported Nandal and Pickering (2005) of 3,000-80,000 (average No. 41,500), 10,000-30,000 (av. No. 20000) stated by New and Singholka (1982) while 5,000-20,000 (av. No. 12,500) mentioned by New (2002). Reproductive performances of broods might vary on the basis of locations (Nhan *et al.*, 2009; Sardar *et al.*, 2013), Yen and Bart (2008) mentioned that brood developed in saline condition showed better performances. However, in the present study, variation of brood performance on producing eggs was observed in the same batch of population when nourished them with different feed regimes in freshwater condition.

In the present experiment, selected feeds were administered to the prawn of the same batch and single environmental condition. It was evident that egg number varies with the proximate composition of feeds. However, suitable commercial feeds might be available to develop broodstocks in the country that could ensure the numbers of berried female potential for production maximum number of eggs. Further experiments could be done to enriched ready feed adding cod liver oil and/or selective vitamins to increase the potentiality of broodstocks that may support the freshwater prawn hatcheries of producing required number post larvae as well to ensure conservation of the natural broodstocks resources.

Conclusion

Female freshwater prawns fed with commercial feed F₃ (CP shrimp feed- crude protein, 38.90%, lipid 8.09%, carbohydrate 25.37%) was found to produce the maximum number of eggs (59,640/female) followed by F₁ (Mega shrimp feed- crude protein 33.49%, lipid 5.38%, carbohydrate 30.31%). The findings may contribute to better management of mass scale broodstock of freshwater prawn, and help to produce of sufficient number embryonic eggs for hatchery operation.

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