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Effect of stocking density and different feed on growth and survival of *Mastacembelus armatus* larvae

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ABSTRACT

The Spiny Eel, *Mastacembelus armatus* which is considered as endangered freshwater fish species deserves immediate attention for commercial scale seed production for farming. Although initial success on the artificial propagation has been achieved provision of suitable stocking density and feed are the bottlenecks in rearing larvae of this fish. Therefore, two experiments were conducted to assess the effects of stocking densities and different feed types on growth and survival of larvae of *M. armatus*. To determine appropriate stocking density, 10 days of old larvae of *M. armatus* were reared for 28 days in bowl system each with 10L capacity. Twelve bowls were divided into 4 treatments namely T-I (2 larvae/L), T-II (4 larvae/L), T-III (6 larvae/L), and T-IV (12 larvae/L) with 3 replications of each. Larvae were fed two times a day with chopped tubificid worms. The result showed significantly ($P<0.05$) better growth in terms of length gain (21.88±1.10 mm and 18.88±0.78 mm) and weight gain (86.30±1.59 mg and 84.95±0.94 mg), SGR (3.06±0.05 and 3.03±0.03), and survival rate (95.00±2.41% and 92.50±2.17%) in T-I and T-II, respectively compared to T-III and T-IV. For feeding trial, 6 trays were used and divided into 3 treatments namely T-I, T-II, and T-III having 2 replications for each and fed three different feeds, chopped tubificid worms, trash fish, and mega feed, respectively. Tubificid worms gave significantly ($P<0.05$) better result in terms of growth (length, 56.96±3.05 mm and weight, 326.65±12.27 mg), SGR (3.01±0.11), and survival rate (95.00±1.56%) over other feeds. From this study, it was found that stocking density at the rate of 4 larvae/L and tubificid worms as live feed are suitable for obtaining desirable level of growth performance and survival of *M. armatus* larvae/fry.

INTRODUCTION

The spiny eel, *Mastacembelus armatus* is a freshwater fish belonging to the family Mastacembelidae under the order Synbranchiformes. It is native of Bangladesh, India, Pakistan, Sri Lanka, Thailand and other parts of South East Asia. This fish is considered as a delicacy and precious food in these countries. In fact, it dwells in rivers, canals, beels, lakes and other floodplain areas adjacent to paddy fields during the rainy season. Being nocturnal carnivores, it feeds on benthic insect larvae, earthworms, black worms, small dead fish, shrimps and some submerged plant materials (Nasar 1997; Narejo 2003). It is an economically important inland water fish having a good taste, high market price, important production potentials and high protein contents (Talwar and Jhingran 1991). The caloric value of eel flesh is as high as 303 Cal/100g compared to 110 Cal/100g in other average fishes (Nasar 1997). In consideration of these, *M. armatus* could be a potential candidate for aquaculture especially in Bangladesh. Although initial success on the induced breeding technique of *M. armatus* (Mollah et al. 2013) had opened a window, larval rearing techniques of this species is not standardized. Therefore, this species is yet to be brought under farming, largely due to unavailability of seed. Nevertheless, it is not possible to collect sufficient quantity of fry and fingerling from wild source for culture of this species.

Feed and stocking densities are two major factors in aquaculture influencing growth, welfare, and health (Ellis et al. 2002; Alcorn et al. 2003). The optimization of growth rates and feed efficiency depends on the quantity of food delivered, feeding method and frequency, quality and composition of the diet (Yang et al. 2003; Erondu et al. 2006). In the larval stage, fish are very susceptible

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to the feed supplied to them, even if other conditions of rearing are properly maintained (Watanabe et al. 1983). At the stage of first feeding, the larvae of many fish species do not accept any formulated feeds, even if it contains balanced amount of nutrients (Rahman et al. 2002). Stocking density directly influences survival, growth, behaviour, water quality and feeding. Generally, the increase in stocking density results in directly increase on the stress condition, causing a reduction in growth rate and food utilization (Sharma and Chakrabarti 1998). On the other hand, fish may not form shoals and may feel unprotected during stocking in very low densities. Consequently, identifying the optimum stocking density for a fish species is an important factor not only to enable efficient management and to maximize production and profitability, but also for optimum husbandry practices (Kristiansen et al. 2004; Rowland et al. 2006).

The study of biology of this species are indispensable and very little attempt has been made in Bangladesh to initiate their breeding and culture. Narejo (2003) and Rahman (2007) did some works on its biology and rearing technique under laboratory condition. Therefore, the immediate protection through the development and standardization of large scale seed production and culture technique of this species is utmost important. Considering the above realities the present research work was carried out to find out the appropriate stocking density and a suitable feed for optimal growth and survival during rearing of *M. armatus* larvae and fry, respectively.

METHODOLOGY

Experimental site

The experiments were conducted in the Mini Hatchery cum Breeding Complex and Wet Laboratory under the Department of Fisheries Biology and Genetics of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh during the period of July to September 2014.

Source of experimental larvae

The larvae used in the experiments were obtained through the successful breeding trial of *M. armatus* according to Mollah et al. (2013). For the breeding trial, the fish were collected and domesticated in indoor cisterns of size 2.33m×1.34m having all facilities i.e., continuous water supply through porous PVC pipes for aeration, inlet and outlet. Mature male and female fish were kept in separate cisterns with constant water flow ensuring proper aeration. The gravid females were treated with PG at the dose of 40 mg/kg body weight. The dose was divided into two volumes (30% and 70%) and injected to the females at 6 h interval. Males were injected at the dose of 10 mg/kg body weight during 2nd injection of female. Ovulated females and males were stripped out for eggs and milt, respectively. To ensure fertilization the sperm suspension was mixed with eggs by gently stirring with a clean and soft feather. During mixing, water was added to the egg-sperm mixture to activate the sperms for fertilizing the eggs. Fertilized eggs were washed several times with clean water to remove the excess milt, blood etc and transferred to mini plastic circular hatchery (50 L capacity) for incubation and hatching. All the incubators received water flow to ensure adequate aeration. Dead eggs were removed after every 3 h and their number was carefully recorded. Upon completion of hatching, the number of hatchlings were also counted and recorded. For complete hatching it took about 55 h for fertilized eggs of *M. armatus* at 27-28°C.

Effects of stocking density on growth and survival of *M. armatus* larvae

Ten days old larvae of *M. armatus* were reared in a bowl system (each with 10 L capacity) (Fig. 1A) for 28 days at the ambient temperature. Continuous water flow from perforated PVC pipe was provided. Twelve bowls were divided into four treatments namely T-I, T-II, T-III and T-IV with 3 replications of each. The stocking density in T-I, T-II, T-III and T-IV was 2 larvae/L, 4 larvae/L, 6 larvae/L, and 12 larvae/L, respectively. In all treatments larvae were fed with cleaned and chopped tubificid worms.

Effects of feed type on growth and survival of *M. armatus* fry

Thirty eight days old fry of *M. armatus* were reared in a tray system (each with 20 L capacity) provided with continuous flow of water from perforated PVC pipe (Fig. 1B) at the ambient temperature. Here, 6 trays were divided into three treatments namely T-I, T-II, and T-III each with 2 replications. Stocking density was 4 larvae/L for all treatments. Larvae were fed with chopped tubificid worms, trash fish (Mola, Dhela, Kachki etc.), and mega feed (special nursery feed) in T-I, T-II, and T-III, respectively.

Method of feeding

The *M. armatus* fry were fed two times a day up to satiation. They were considered satiated when they stopped eating or searching for food. The rearing units were cleaned by siphoning during morning and evening daily and 50% of the total water was exchanged each time. Dead larvae were removed immediately and the number was recorded.

Sampling procedure

Sampling was done every 7 days interval in the morning prior to feeding. The length and weight were recorded by random sampling of ten larvae from each rearing unit by using small scoop net. All the data were recorded in a note book and finally the average length and weight of larvae were calculated according to the treatments on each sampling day. After completion of the experiment, the number of total larvae in each rearing unit was counted separately.

Growth parameters studied

The following parameters were used to evaluate the growth:

i. Length gain (mm) = Average final length - Average initial length

ii. Weight gain (mg) = Average final weight - Average initial weight

iii. Percent length gain =

$$\frac{\text{Average final length - Average initial length}}{\text{Average initial length}} \times 100$$

iv. Percent weight gain =

$$\frac{\text{Average final weight - Average initial weight}}{\text{Average initial weight}} \times 100$$

v. Specific growth rate:

$$SGR\ (\%day) = (\ln W_2 - \ln W_1)/(T_2 - T_1) \times 100$$

(Brown, 1957)

Where, W_2= Final live body weight at time T2

W_1= Initial live body weight at time T1

vi. Survival rate (%) = $$\frac{\text{Number of larvae alive}}{\text{Total number of larvae stocked}} \times 100$$

Statistical analysis

The data obtained from the experiment were treated statistically to see whether the influence of different treatments on the growth (length and weight) of larvae were significant or not. Several morphological characters were analyzed by Microsoft Excel (MS Excel) computer package as descriptive values such as mean and percentage. Determination of linear relationship and correlation coefficient (r) between total length and body weight was performed using MS Excel and SPSS version 11.5.

RESULTS

Effects of stocking density on growth and survival of *M. armatus* larvae

The results on growth in terms of mean initial length, mean length gain, mean initial weight, mean weight gain, percentage of length and weight gain, specific growth rate (SGR %/day) and survival rate (%) were calculated and are shown in Table 1. Ten-days old larvae were reared for 28 days under four different stocking densities (2, 4, 6, and 12 larvae/L of water) to monitor their growth and survival rates. The initial average length in T-I, T-II, T-III, and T-IV were 14.65±0.10, 14.65±0.13, 14.65±0.09, and 14.65±0.19 mm and initial weight were 13.95±0.13, 13.95±0.07, 13.95±0.31, and 13.95±0.28 mg, respectively. After 28 days of experiment, there was significant difference (P<0.05) among the treatments in terms of growth parameters. Significantly higher length gain (21.88±1.10 mm) and weight gain (86.30±1.59 mg) was observed in T-I stocked with 2 larvae/L followed by T-II (18.88±0.78 mm and 84.95±0.94 mg, respectively) and T-III (16.08±0.39 mm and 70.50±2.00 mg, respectively); and the lowest was observed in T-IV (14.30±0.30 mm and 64.40±2.22 mg, respectively) (Table 1). The highest percent length gain and percent weight gain were found 149.35±6.06 and 618.64±18.27, respectively in the treatment stocked with 2 larvae/L of water (Table1). The specific growth rates (%) in different treatments ranged from 2.67±0.09 to 3.06±0.05 (Table 1). Significantly (P<0.05) higher SGR (%) values were recorded in T-I (3.06±0.05) and T-II (3.03±0.03) compared with T-III (2.79±0.09) and T-IV (2.67±0.09) (Table 1). Survival rate of *M. armatus* larvae was also higher in T-I (95.00±2.41%) followed by T-II (92.50±2.17%), but the difference was not significant. However, Table-1 showed that these values differed significantly than those of T-III (86.67±3.42%) and T-IV (67.50±0.70%).

Table 1: Growth performance and survival rate (%) of *M. armatus* larvae during 28 days experiment under four stocking densities

Parameters	T-I (2 larvae/L)	T-II (4 larvae/L)	T-III (6 larvae/L)	T-IV (12 larvae/L)
Initial length (mm)	14.65±0.10^{a}	14.65±0.13 ^{a}	14.65±0.09^{a}	14.65±0.19^{a}
Final length (mm)	36.53±0.92^{a}	33.53±0.56^{b}	30.73±0.73^{c}	28.95±0.83^{d}
Length gain (mm)	21.88±1.10^{a}	18.88±0.78^{b}	16.08±0.39^{c}	14.30±0.30^{d}
Length gain (%)	149.35±6.06^{a}	128.87±2.68^{b}	109.76±2.90^{c}	97.61±4.23^{d}
Initial weight (mg)	13.95±0.13^{a}	13.95±0.07^{a}	13.95±0.31^{a}	13.95±0.28^{a}
Final weight (mg)	100.25±2.83^{a}	98.45±2.15^{a}	84.45±2.39^{b}	78.35±3.65^{c}
Weight gain (mg)	86.30±1.59^{a}	84.95±0.94^{b}	70.50±2.00^{c}	64.40±2.22^{d}
Weight gain (%)	618.64±18.27^{a}	608.96±4.65^{a}	505.38±13.60^{b}	461.65±13.21^{c}
SGR (%/day)	3.06±0.05^{a}	3.03±0.03^{a}	2.79±0.09^{b}	2.67±0.09^{b}
Survival rate (%)	95.00±2.41^{a}	92.50±2.17^{a}	86.67±3.42^{b}	67.50±0.70^{c}

Figures in the same row having different superscripts are significantly different (*P*<0.05).

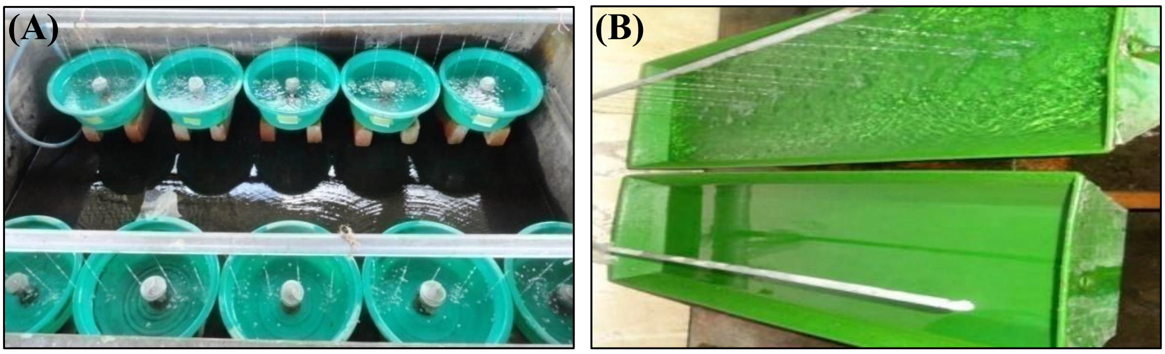

Figure 1. Rearing systems of larvae and fry of *M. armatus* in bowl (A) and tray (B), respectively

Effects of feed type on growth and survival of *M. armatus* fry

Different growth parameters i.e., mean initial length, mean length gain, mean initial weight, mean weight gain, percentage of length and weight gain, specific growth rate (SGR %/day) and survival rate of *M. armatus* fry reared with 3 different types of feeds were calculated and is presented in Table 2. In this case, growth in terms of length and weight was significantly different among the three treatments. The mean final length (mm) was significantly (P<0.05) higher in T-I (56.96±3.05) than in T-II (45.97±1.14) and T-III (30.78±0.90) (Table 2). The highest growth (in weight in mg) was observed in T-I (326.65±12.27) followed by T-II (156.10±3.18) where fry were fed with tubificid worms collected from natural sources and trash fish, respectively (Table 2). The lowest growth was observed in T-III (95.90±1.13) where fry were fed with mega feed. The percent length gains of *M. armatus* fry in T-I, T-II, and T-III were 186.57±4.37, 150.57±3.14, and 100.82±2.74, respectively (Table 2). The percent weight gains were 599.91±22.88, 286.68±3.93, and 176.12±4.26 in T-I, T-II, and T-III, respectively. The highest percent length gain (186.57±4.37) and weight gain (599.91±22.88) obtained in T-I was significantly (P<0.05) different from those of T-II and T-II (Table 2). The specific growth rates of *M. armatus* fry in different treatments were also different. Significantly (P<0.05) higher SGR values were observed in T-I (3.01±0.11) compared with T-II (2.09±0.09) and T-III (1.58±0.03) (Table 2). The survival rate (%) was significantly (P<0.05) higher in T-I (95.00±1.56) where fry were fed with chopped tubificid worms, than in T-II (88.00±2.29) and T-III (73.00±1.37) (Table 2).

DISCUSSION

In the present study the effect of stocking density and different feed on the growth and survival of *M. armatus* larvae or fry were conducted and observed that the growth performance and

Table 2: Growth performance and survival rate (%) of *M. armatus* fry fed with chopped tubificid worms (T-I), trash fish (T-II), and mega feed (T-III) during 28 days experiment

Parameters	**T-I** (Tubificid worms)	**T-II** (Trash fish)	**T-III** (Mega feed)
Initial length (mm)	$30.53±0.58^a$	$30.53±0.81^a$	$30.53±0.55^a$
Final length (mm)	$87.49±2.25^a$	$76.50±1.07^b$	$61.31±2.71^c$
Length gain (mm)	$56.96±3.05^a$	$45.97±1.14^b$	$30.78±0.90^c$
Length gain (%)	$186.57±4.37^a$	$150.57±3.14^b$	$100.82±2.74^c$
Initial weight (mg)	$54.45±0.25^a$	$54.45±0.55^a$	$54.45±0.13^a$
Final weight (mg)	$381.10±9.07^a$	$210.55±8.73^b$	$150.35±6.09^c$
Weight gain (mg)	$326.65±12.27^a$	$156.10±3.18^b$	$95.90±1.13^c$
Weight gain (%)	$599.91±22.88^a$	$286.68±3.93^b$	$176.12±4.26^c$
SGR (%/day)	$3.01±0.11^a$	$2.09±0.09^b$	$1.58±0.03^c$
Survival rate (%)	$95.00±1.56^a$	$88.00±2.29^b$	$73.00±1.37^c$

Figures in the same row having different superscripts are significantly different (*P*<0.05).

and feed types, respectively. Stocking densities and ration size are two important factors affecting growth and survival of fish (Hernández et al. 2001; Salas-Leiton et al. 2008; Saoud et al. 2008). In the trial of stocking density, the highest growth performance (gain in both length and weight) was shown by the larvae of treatment I followed by treatment II and III where 20, 40, and 60 larvae were reared in each bowl, respectively and the lowest in treatment IV where 120 larvae were reared for a period of 28 days. Growth in length and weight was significantly different among the four treatments. The SGR values of treatment I and II were not significantly different; however, these values were significantly different from those of treatment III and IV. However, the difference between the SGR values of treatment III and IV was significant (P<0.05). The present observation agreed with the findings of Farid et al. (2009) who observed increased growth performance and survival rate with decreased stocking density for *Macrognathus aculeatus* post-larvae/fry. Near about similar SGR value (4.19±0.14) was reported by Saha et al. (1995) in stinging catfish (*Heteropneustes fossilis*) fry. Masud and Rahman (1997) observed that SGR were generally higher in fish stocked at a smaller size and larger fish exhibited low values of SGR. This result supports the present findings. The difference between T-II (92.50±2.17%) and T-III (86.67±3.42%) and between T-III (86.67±3.42%) and T-IV (67.50±0.70%) were significant (P<0.05). Ronald et al. (2014) recorded a similar finding for Nile tilapia (*Oreochromis niloticus*) where they observed a negative correlation between stocking density and growth rate. They found that increase of the stocking density of Nile tilapia fry beyond 2670 fry/m^3 significantly affected survival and growth of fry. Survival was significantly (P<0.05) lowest with high stocking densities, 87% at 4000 fry/m^3 and 82.9% at 5330 fry/m^3. In another study the fry of red tilapia (*Oreochromis mossambicus* × *O. niloticus*) were reared for 60 days in hapa (4.5×2.4×1 m^3) at the stocking densities of 200 (T1); 250 (T2), and 300 (T3) fry/hapa (Daudpota et al. 2014). They determined highest growth in T1 (49.80 g) while lowest (29.09 g) in T3. Survival was significantly (P<0.01) different among treatments and highest survival (100%) was attained in T1 with lower stocking density, followed by T2 (98%) and T3 (95%).

To find out suitable feed for the fry of *M. armatus*, another experiment was carried out in the tray system with three different feeds i.e., chopped tubificid worms, trash fish, and mega feed. Growth in terms of length and weight were significantly (P<0.05) higher in T-I (56.96±3.05 mm and 326.65±12.27 mg) as well as in T-II (45.97±1.14 mm and 156.10±3.18 mg) compared with T-III (30.78±0.90 mm and 95.90±1.13 mg). The percent weight gains of *M. armatus* fry were 599.91±22.88%, 286.68±3.93%, and 176.12±4.26% in treatments I, II, and III, respectively. Rahman (2007) carried out an experiment on growth and survival rate of the spiny eel (*M. armatus*) fry in different treatments (shelters) and demonstrated that the weight gain (%) varied significantly (P<0.05) from 542.50±2.60% to 1233.75±2.17%, which supports the present findings. Here significantly (P<0.05) higher SGR values were recorded in T-I (3.01±0.11) and T-II (2.09±0.09) compared with T-III (1.58±0.03). Rahman (2007) observed that the SGR (%) of the mud eel (*Monopterus cuchia*) and spiny eel fry varied significantly (P<0.05) from 3.48±0.04% to 4.00±0.01% and 2.86±0.02% to 3.59±0.01%, respectively among the treatments, which were more or less similar to the present study. Also the survival rate of larvae in T-I (95.00±1.56%) was significantly (P<0.05) higher than those of T-II (88.00±2.29%) and T-III (73.00±1.37%). This result also agreed with the findings of Rahman (2007) for mud eel fry where the survival rates ranged from 85.00±1.15% to 90.00±1.15%. In another study, Mahmood et al. (2004) assessed the suitability of four different larval feeds viz. *Artemia nauplii*, tubificid worms, rotifer powder, and zooplankton for larvae/fry of climbing perch, *Anabas testudineus* for a period 28 days. The larvae fed tubificid worms had significantly highest (P<0.05) growth (percent length gain 237.80±2.09, percent weight gain 2040.10±17.82, specific growth rate 13.61±0.01) and survival (61.0±2.0%), followed by *Artemia nauplii*, zooplankton and rotifer powder and they suggested tubificid worms as best larval feed for climbing perch up to stockable size. In this study, the survival rate obtained in *M. armatus* fry fed tubificid worms (95.00±1.56%) was the highest while the survival rate shown by the fry fed trash fish (88.00±2.29%) was also acceptable. On the other hand, fry fed mega feed showed significantly (P<0.05) lower growth and survival. The results from studies with other fish larvae also support the findings of the present study. Fermin and Boliver (1991) reported that the specific growth rate of *Clarias macrocephalus* larvae fed live feed was higher than those fed non-live food. Ghyeas (1998) studied the effects of three feeds viz., tubificid worms, a formulated feed and a commercial nursery feed on growth and survival of *Heteropneustes fossilis* larvae and found growth and survival of larvae fed tubificid worms to be best. The higher growth and survival rate of *M. armatus* fry fed live feed (tubificid worms) might be due to greater ability of fry to efficiently synthesis protein from live feed. On the other hand, the lower growth and survival rate of the fry fed non-live feeds (mega feed) was perhaps associated with deficiency of some essential components, such as amino acids and fatty acids. Dabrowski (1984) and Dabrowski et al. (1987) reviewed the feeding of fish larvae, and pointed out that fish larvae are susceptible to dietary deficiency in more spectacular ways than juveniles and adults and they noted that amino acids in live foods are catabolized at a lower rate and therefore used to a greater extent for protein synthesis than amino acids from artificial diets.

CONCLUSION

The findings of the present study, therefore, recommended that stocking density at the rate of 4 larvae/L and tubificid worms as live food are suitable for nursing of *M. armatus* larvae or fry. Development of such larvae/fry rearing technique of *M. armatus* will allow the hatchery operators and fish farmers to go for nursing, culture and ultimately making the fish available to the consumers.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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