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EFFECT OF STOCKING DENSITY ON GROWTH AND SURVIVAL OF ENDANGERED *MYSTUS BLEEKERI* (DAY, 1877) IN NURSERY SYSTEM

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Abstract: The effect of stocking densities on growth, survival and production of gulsa tengra, *Mystus bleekeri* fry and fingerlings were practiced in a nursery rearing system. The experiment was designed with 3 treatments and 3 replications and conducted for seven weeks in nine earthen nursery ponds of 0.032 ha each. Four-days-old hatchlings were stocked at 0.60 million/ha, 0.80 million/ha and 1.0 million/ha was designated as treatment T_1, T_2 and T_3. At stocking, hatchlings had a mean length and weight of 0.91±0.01 cm and 0.001±0.01g. Hatchlings in all treatments were fed with 32.90% crude protein and 8.80% crude lipid. Physico-chemical parameters (temperature, transparency, pH, dissolved oxygen and total alkalinity) and plankton populations were at the optimum level for fish culture. The highest weight gain (3.16±0.66g) was measured in treatment T_1 and the lowest (2.01±0.88 g) in treatment $T_{3.}$ Final length, final weight and survival of fingerlings followed the similar trends as weight gain. Specific growth rate and feed conversion ratio was significantly better (P<0.05) in treatment T_1 followed by treatment T_2 and $T_{3.}$ Survival rate was also higher (P<0.05) in treatment T_1 than in treatment T_2 and T_3, respectively. Despite this, higher economic returns (Bd Tk. 367733/ ha) were observed for treatment T_1 than from treatment T_2 and T_3.

Keywords: Food conversion rate, Hatchling, Stocking density, Supplementary feed, Survival rate.

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INTRODUCTION

Mystus bleekeri (Day, 1877) is a cat fish under family Bagridae of order Siluriformes. It is commonly known as Gangetic Mystus, which has been reported to be distributed in Bangladesh, India, Pakistan, Nepal, Sri Lanka, Thailand and Myanmar (Day, 1878; Talwar and Jhingran, 1991; Tripathi, 1996; Rahman *et al.*, 2004; Chakrabarty and Ng, 2005). The fish is commonly known as gulsha tengra, usually found in fresh water and is mostly available in rivers (both fast flowing and slow flowing), canals, beels and inundated fields (Roy and Hossain, 2006); also has been reported from tidal rivers and lakes (Talwar and Jhingran, 1991). It contains high nutritious protein, vitamins, minerals and other micronutrients that usually not existing in others (Mazumder *et al*., 2021; Tiwana *et al*., 2007). It has high market demand as food fish with high market price due to good protein content in its flesh (Roy and Hossain, 2006; Siddiqui *et al*., 2010; Ashashree *et al*., 2013 Hossen *et al*., 2014). The species is caught largely in the river Padma and Sumeswari. It is the source of protein to the common people of Bangladesh.

The gulsha tengra is abundantly available in our open water system of rivers, streams, haors and beels of Bangladesh. It normally breeds in streams, rivers and floodplains. However, in very recent year, frequent devastating floods, prolonged draught, siltation and soil erosion in the rivers and reservoirs, dumping of agrochemicals and industrial pollution, construction of flood control measures and drainage structures, indiscriminate and destructive practices is unexpected changed aquatic ecosystem and in its natural habitat. These not only destroyed the breeding grounds but also caused havoc to the availability of brood fish including fry and fingerlings of open water. Recent studies suggest that world wide 20% of all fresh water species are extinct, endangered or vulnerable (Moyle and Leidy, 1992).

It is the right time to save all the species from extinction for biodiversity conservation (Chakraborty *et al*., 2021; Chakraborty and Mome, 2022), ecological balance (Ashok, 2017; Verma, 2018), and sustainable development (Ashok, 2019; Verma, 2021), however, the current species studied can be conserved through development of appropriate breeding, nursing and rearing techniques of spawn, fry and fingerlings of *M. bleekeri*. This technology will prevent this species from extinction and at the same time, the general people will have the opportunity to consume this delicious fish, if the culture practice is developed in both closed and open water bodies. This fish has enormous aquaculture potential and it could be easily grown in fish ponds along with other polyculture species. In order to do so, a large quantity of fry and fingerling would be required, which could be met through successful rearing of fry and fingerlings.

Hence, a suitable culture technology for nursing and rearing of *M. bleekeri* are very important to ensure reliable and regular supply of fingerlings. Improper care and lack of understanding about the biotic and abiotic factors in the rearing system may result in mass mortality of young fry (Jhingran and Pullin, 1985; Chakraborty, 2020). Success in fry nursing depends on a good knowledge of nutritional and environmental requirement of the larvae in the open aquatic ecosystem (Mollah, 1985; Rahman and Arifuzzaman, 2021). In order to maintain the population of this fish as well as its conservation and rehabilitation, development of suitable technology for rearing and nursing of fry and fingerlings is very essential. Growth, survival and production of fry and fingerlings in nursery ponds depend on stocking density, type and quality of supplementary feeds. The present study has been undertaken to develop a practical and economically viable methodology for mass seed production and rearing of *M. bleekeri* under controlled nursery management system.

MATERIALS AND METHODS

Study area and experimental design

The research was carried out at the private nursery ponds of Rupali Hatchery and Fisheries farm, Balia, Phulpur, Mymensingh, Bangladesh. The experiment was conducted for a period of 7 weeks from June to July 2022 in nine earthen nursery ponds with a surface area of 0.032 ha with an average depth of 0.80 m. The ponds were having similar rectangular size, depth, basin conformation, contour and bottom type. The experiment was designed with 3 treatments and 3 replications. Three stocking densities of hatchlings were employed with three replicates each; 0.60 million/ha (treatment T_1), 0.8 million/ha (treatment T_2) and 1.0 million/ha (treatment T_3).

Source of spawn

The wild brood of *Mystus bleekeri* was collected from Someswari River and stocked in the farm of Rupali Hatchery and Fisheries, Balia, Phulpur, Mymensingh. The brood was brought up in the farm and breed in Rupali Hatchery and Fisheries. The spawn was collected from the farm of Rupali Hatchery and Fisheries for nursery study.

Pond preparation, stocking and fertilization

The ponds were dewatered, freed from aquatic vegetation, exposed to full sunlight and had a well designed system of inlet and outlet. After drying, quicklime ($CaCO_3$, 250 kg ha^{-1}) was spread over the pond bottom and liming also during the experimental period. All the ponds were filled with ground water. Five days subsequent to liming, the ponds were fertilized with organic manure (cow dung @ 2470 kg ha^{-1}). Seven days after manuring the pond water was sprayed with dipterex (1.0 ppm) to eradicate harmful insects and predatory zooplankton. The experimental ponds were stocked with 4 days old *M. bleekeri* having an initial length of 0.91±0.01cm and weight of 0.001±0.01 g, respectively. After stocking hatchlings, all the ponds were fertilized with cowdung at the rate of 247 kg/ha, Urea 25.0 kg/ha and TSP 12.5 kg/ha at weekly intervals to stimulate primary productivity.

Supplementary feeding

In order to meet the increasing dietary demand, supplementary feed consisting different ingredient of mustard oilcake, rice bran, wheat bran, and fish meal in 40:20:25:15 proportions was supplied at the rate of 20 kg/million hatchlings/day for the first one week, 24 kg for the second 2 weeks, 28 kg for the third 2 weeks and 32 kg for the fourth two weeks twice daily. Proximate composition of the feeds was analyzed according to AOAC (1984) method, nitrogen free extract (NFE) by subtraction (Castell and Tiews, 1980). Proximate composition (% dry matter) of the supplementary feeds (crude protein, crude lipid, crude fiber, ash and nitrogen-free extract) of experimental feeds was 32.90%, 8.80%, 10.10%, 17.95% and 30.25%, respectively.

Water Quality Parameters

Physico-chemical parameters of pond water were maintained weekly between 9.00 and 10.00 h. Water temperature was recorded using a Celsius thermometer and transparency (cm) was measured by using a Secchi disc of 20 cm diameter. Dissolved oxygen and pH were measured directly using a digital electronic oxygen meter (YSI, Model 58, USA) and an electronic pH meter (Jenway, Model 3020, UK). Total alkalinity was determined by titrimetric method (Clesceri *et al.*, 1989).

Estimation of growth, survival, production and feed utilization

Thirty fish species from in each pond were sampled weekly until the fingerlings stage. Growth in terms of length and weight, Average daily gain (ADG), Specific Growth Rate (SGR) and Food conversion rate (FCR) was estimated. SGR and FCR were calculated according to Brown (1957); Castell and Tiews (1980) and Gangadhara *et al.* (1997). After seven weeks, the fingerlings were harvested by repeated netting, followed by drying the ponds. The fingerlings were counted and weighed. Survival (%) and production (number/ha) of fingerlings were then calculated and compared among the treatments.

Analysis of experimental data

The data were analyzed a one way analysis of variance (ANOVA) using MSTAT followed by Duncan's New Multiple Range test to determine significant difference existed among treatment means (Duncan, 1955; Zar, 1984). A simple cost-benefit analysis was done to estimate the net benefits from different treatments.

RESULTS AND DISCUSSION

Water Quality Parameters

Mean levels of physico-chemical parameters over the seven-week nursing of fry and fingerlings are presented in table 1. The mean water temp. in treatment T_1, T_2 and T_3 were not statistically significant ($P>0.05$). The temperature of the experimental ponds was within the acceptable range for nursery ponds that agrees well with the findings of Haque *et al.* (1994) and Bhagde *et al.* (2020). Mean Secchi disk transparency differed significantly ($P<0.05$), increasing from T_1 to T_3. Transparency was consistently higher in T_3, possibly due to the reduction of the plankton population (Haque *et al.,* 1998). The mean dissolved oxygen (DO) was not significantly different ($P>0.05$) but decreasing from treatment T_1 to treatment T_3. Fluctuation of dissolve oxygen concentration might be attributed to growth, feed utilization and production of fish growths (length and weight) of fry (Boyd, 1982). The mean pH of the different treatment decreased from T_1 to T_3 but did not differ significantly ($P>0.05$). The pH value agrees well with the findings of Chakraborty *et al.* (2003) and Rahman and Rahman (2003). Total alkalinity was decreased from treatment T_1 to treatment T_3 but differ significantly ($P<0.05$). Despite these variations, water quality parameters in all the experimental ponds were within the normal range for fish culture. Alkalinity levels indicate productivity of the ponds was medium to high (Bhuiyan, 1970). Higher total alkalinity values might be due to higher amount of using lime.

Growth, feed utilization and production of fish

Weekly growths (length and weight) of fingerlings are shown in figure 1. The increase in length and weight was the highest in T_1 followed by T_2 and T_3. Growth and production parameters of fingerlings are shown in table 2. The initial length and weight of spawns, stocked in all the ponds were same. The fish in T_1 treatment showed the highest gain in both length and weight over T_2 and T_3 treatment, where stocking density of spawn was 0.60, 0.80 and 1.0 million ha^{-1} in treatment T_1, T_2 and T_3. However, the mean final length and weight of fingerlings in different treatments were significantly different ($P<0.05$). The highest weight gain was in T_1 and lowest in T_3. SGR in T_1 was significantly higher than T_2 and T_3, and was significantly different ($P<0.05$). In this experiment, crude protein levels (32.90% dry weight) in supplementary feeds are very near the dietary protein of 31.00% for the optimal growth of *Labeo rohita* (De Silva and Gunasekera, 1991). The low fish growth at T_3 may be deterioration of water quality as well as the competition for feed and space. (Haque *et al.*, 1994; Islam *et al.*, 1999; Rahman and Rahman, 2003; Chakraborty *et al.*, 2006; Chakraborty and Mirza, 2007; Chakraborty, 2021; Rahman *et al.*, 2022).

FCR was significantly lower in T_1 (1.55±0.01) than T_2 (1.76±0.01) and T_3 (1.99±0.02). Therefore, SGR and FCR were best for fish in T_1 where lowest number of hatchlings (0.60 million/ha) was reared. The FCR values reported in the present study are lower than the values reported by Das and Ray (1989) and Islam (2002). De Silva and Davy (1992) stated that digestibility plays an important role in lowering the FCR value by efficient utilization of food. Digestibility, in turn, depends on daily feeding rate, frequency of feeding, and type of feed used (Chiu *et al.,* 1987). However the lower FCR value in the present study indicates better food utilization efficiency. The highest survival rate was also observed in T_1 and the lowest in T_3. There was a significant variation ($P<0.05$) in the survival rate

Table 1: Physico-chemical characters of water in the nursery ponds during the experimental period.

Parameters	Treatments		
	T_1	T_2	T_3
Temperature $(0)^C$	28.74±2.11 (26.15-30.15)	28.66±2.14 (26.20-30.10)	28.62±2.45 (26.22-30.15)
Transparency (cm)	24.18±3.18a (23.30-31.20)	29.05±4.04b (24.55-34.80)	35.10±4.33c (28.40-37.02)
pH	7.82±0.57 (7.22-8.66)	7.80±0.46 (7.40-8.76)	7.92±0.68 (7.42-8.94)
Dissolved oxygen (mgL^{-1})	5.15±0.56 (4.44-6.22)	4.98±0.66 (3.55-5.15)	4.22±0.88 (3.40-5.22)
Total alkalinity (mgL^{-1})	134.04±7.04a (126.33-140.05)	129.21±7.08b (124.61-130.05)	124.14±6.44c (118.55-130.05)

Figure in the same row having the same superscript are not significantly different (P>0.05). Figure in the parenthesis indicates the range.

in *Mystus bleekeri* fry among different treatments. The reason for reduced survival rate in these treatments was due to higher stocking density of fry as well as competition for food and space in the experimental ponds. Similar results were obtained by Haque *et al*. (1994), Rahman and Rahman (2003), Chakraborty *et al*. (2003), Chakraborty and Mirza (2007), Chakraborty (2021) and Arifuzzaman *et al.* (2022) for fry and fingerlings of various catfish, carp and barb species.

The initial length and weight of spawn stocked in all the ponds was the same, 0.91±0.01 cm and 0.001±0.01 g. It is evident from the data that the fry attained an average size of 4.01±0.48 cm in length and 3.16±0.66 g in weight in treatment T_1 with lowest stocking density of 0.60 million ha^{-1}, while the fry attained an average size of, 3.51±0.52 cm in length and 2.57±0.78 g in weight with 0.80 million/ha density and 2.85±0.61cm in length, 2.01±0.88 g in weight in T_3 with 1.0 million/ha density (Fig. 1 and 2).

This is clearly indicated that maximum growth in length and weight was attained at the lower stocking density of 0.60 million/ha with the growth gradually decreasing with increase in density, showing a negative correlation between density and growth.

Table 2: Growth performance, survival and production of *Mystus bleekeri* fry or fingerlings after seven weeks of rearing.

Parameters	Treatments		
	T_1	T_2	T_3
Initial length (cm)	0.91±0.01 (0.88-0.93)	0.91±0.01 (0.88-0.93)	0.91±0.01 (0.88-0.93)
Final length (cm)	4.01±0.48^a (0.88-5.84)	3.51±0.52^b (0.88-4.12)	2.85±0.61^c (0.88-3.55)
Initial weight (g)	0.001±0.01 (0.0009-0.003)	0.001±0.01 (0.0009-0.003)	0.001±0.01 (0.0009-0.003)
Final weight (g)	3.16±0.66^a (0.0009-4.80)	2.57±0.78^b (0.0009-3.74)	2.01±0.88^c (0.0009-2.88)
Net weight gain (g)	3.159±0.38^a (2.88-4.12)	2.569±0.40^b (1.96-3.77)	2.009±0.44^c (1.22-3.11)
Average daily gain(g)	0.075±0.01^a (0.055-0.086)	0.061±0.01^b (0.048-0.072)	0.048±0.01^c (0.034-0.061)
Specific growth rate	2.74±0.05^a (2.07-3.55)	2.25±0.06^b (1.94-2.96)	1.66±0.06^c (1.58-2.04)
Survival rate (%)	71.01±2.10^a (67.62-72.22)	61.08±2.31^b (57.14-64.20)	51.14±2.35^c (47.18-53.77)
FCR	1.55±0.01^a (1.32-1.88)	1.76±0.01^b (1.60-1.80)	1.99±0.02^c (1.90-2.22)
Production#	4,26,060±1091.05^c (4,08,840-4,34,416)	4,88,640±1344.04^b (4,73,201-4,90,853)	5.11,400±1731.50^a (4,97,860-5,38,011)

Figure in the same row having the same superscript are not significantly different (P>0.05).
Figure in the parenthesis indicates the range.
Total number of fingerlings harvested after 7 weeks.
Average daily gain (g) = (mean final weight-mean initial weight)/time interval (days)
Specific growth rate (SGR) = (Ln mean final weight - Ln mean initial weight)/time interval (days) × 100
FCR (Food conversion ratio) = Total diet fed (kg)/ total wet weight gain (kg)

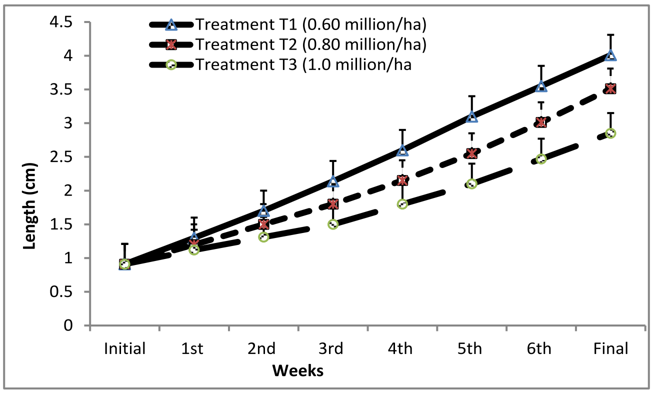

Fig.1: Weekly mean length (cm) gain of fry *Mytus bleekeri* under different densities.

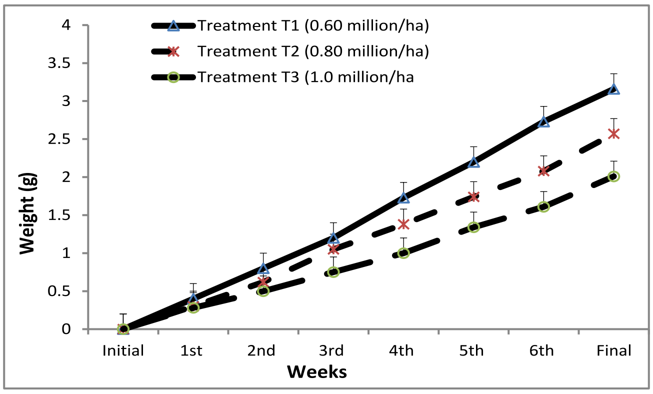

Fig. 2: Weekly mean weight (g) gain of fry *Mystus bleekeri* under different densities.

Table 3: Cost and benefits from the nursing of *Mystus bleekeri* fingerlings in ha^{-1} earthen nursery ponds for a period of 7 weeks.

Item	Amount Tk/ha/week $^{-7}$			Remarks
	Treatment T_1(Tk)	Treatment T_2(Tk)	Treatment T_3(Tk)	
Total return (TR) [b]	596484[a]	537504[b]	409120[c]	Price is related with size and weight
a. Variable cost:				
1. Price of hatchlings	72000	78000	83000	
2. Feed (Tk. 40.00/kg)	90040	92850	95436	
3. Fertilizer	5112	5112	5112	
4. Human labour cost (Tk. 400.00/day)	10000	10000	10000	
5. Chemicals	7840	7840	7840	
6. Miscellaneous	5000	5000	5000	
Total variable cost (TVC)	189992	198802	206388	
b. Fixed cost:				
1. Pond rental value Tk. 80.00/dec./yearly.	19760	19760	19760	Tk. 80.00/dec./yearly. 10% interest according to BKB, Bangladesh
2. Interest of operating capital	18999	19880	20639	
Total fixed cost (TFC)	38759	38840	38898	
Total cost (TC=TVC+TFC)	228751[a]	237642[b]	245286[c]	
Gross margin (GM= TR-TVC)	406492[a]	338702[b]	202732[c]	
Net return (TR-TC)	**367733**[a]	**299862**[b]	**163834**[c]	

[a]1 US$ =Tk. 70.00
BKB= Bangladesh Krishi (Agricultural) Bank
Figures with different superscripts in the same row varied significantly (P<0.05).
Figures in the parenthesis indicate range.
[b]Sale price fingerlings Tk. 1.40/piece (T_1), Tk. 1.10/piece (T_2) and Tk. 0.90/piece (T_3).

The mean productions (number/ha) of fingerlings were 4,26,060, 4,88,640 and 5.11,400 in treatment T_1, T_2 and T_3, respectively. Production in number was higher in treatment T_3 and lowest in treatment T_1. The causes of production might include competition for food, space and habitat of fry (Islam *et al.,* 1999; Islam, 2002; Chakraborty *et al.,* 2006 and 2019; Chakraborty, 2021). This is clearly indicated that maximum growth in length and weight was attained at the rich sustainable nursery management and supplying various rich qualities feeds supplied, showing a negative correlation between nursery management and feed, and growth. Similar results were obtained by Rahman and Rahman (2003), Chakraborty *et al.* (2006) and Phuong *et al.* (2007) for fry of various pangas, carp and barb species. However, production of fingerlings differ significantly ($P<0.05$) among the three treatments (Table 3). On the other hand, cost of production in treatment T_1 was consistently lower than those treatments T_2 and T_3 (Table 3). Highest net benefit ($Tk.ha^{-1}$) was obtained in treatment T_1 (3,67,733) followed by T_2 (2,99,862) and T_3 (1,63,834) in that order, which is very similar study of Ahmed *et al.* (2010) and Belton *et al.* (2017).

Finally, it can be concluded that the survival, growth, production of *M. bleekeri* fingerlings were inversely related to the stocking densities of hatchlings. In all respects, stocking density of 0.60 million hatchlings ha^{-1} may be advisable for rearing of gulsha tengra fingerlings for 7 weeks nursing. Production of adequate quality seeds through application of our present findings might be extremely helpful towards the protection of *Mystus bleekeri* from extinction as well as for its conservation and rehabilitation.

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