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Effect of stocking density on the growth, body composition, and blood parameters of cage-reared Gangetic mystus catfish (*Mystus cavasius*)

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ABSTRACT

Suitable stocking density of Gulsa tengra (*Mystus cavasius*) was observed by measuring growth performance, production, and survival in nylon net cages for 120 days in Chalan Beel floodplain ecosystem. Fry of Gulsha tengra (5.55 ± 0.49 g in weight) were stocked into 6 × 3 × 2-m (36 m^3) floating net cages with treatments: T_1-50, T_2-100, T_3-150, and T_4-200 fry/m^3 (in triplicates). The stocked fry was fed twice daily with commercial floating pellet containing 32 % protein at the rate of 5 % /fish body weight during the first 2 months and then reduced to 3 % for the next 2 months. The highest weight gain was observed in the lowest density treatment, T_1 (18.70 ± 1.37 g) and the lowest (8.66 ± 0.99 g) in highest density treatment, T_4 (all tests for significance were performed at a 5 % level). The rate of survival varied from 86.6 % and 95.8 %, with T_1 resulting in the significantly highest survival. A better feed conversion ratio was also observed in T_1 (1.75 ± 0.26). Significantly higher protein (16.86 ± 0.36) and fat (2.78 ± 0.02) contents were recorded in the fish body composition in T_1. Water quality parameters (temperature: 26.0 ± 1.0 °C, pH: 7.2 ± 0.2, DO: 6.4 ± 0.1 mg/l, NH_3-N: 0.002 ± 0.001) recorded throughout the study period were within the suitable range for fish culture. The highest red (119.0 ± 4.85 $\times 10^6$ μl) and white blood cell (233.2 ± 16.02 $\times 10^3$ μl), hemoglobin (5.65 ± 0.30 g/dl), total protein (5.50 ± 0.03 g/dl), albumin (1.82 ± 0.06 g/dl), and globulin (3.65 ± 0.15 g/dl) were also observed in T_1 indicating optimum condition. Total and net production per cage were the highest in T_1 with approximately 50 % increase (88.31 ± 6.72 and 59.27 ± 5.79 kg, respectively) compared to T_4. The net income and benefit–cost ratio was increased by approximately 42 % and 30 %, respectively, in T_1. Thus, this study recommended an optimal stocking density of 50 fish/m^3 for Gulsha tengra to achieve a profitable and robust yield in cage culture.

1. Introduction

Stocking density is critical in determining aquaculture's yield capability and revenue. On the other hand, increased stocking density may result in stress and diminish fish robustness (Yadata et al., 2020). Stocking density has a different impact on the growth rate and behavior of fish, determined by a variety of parameters, including the species of fish, the size of the fish, and the culture conditions. For example, species like carps (Hoseini et al., 2019; Karnatak et al., 2021; Chen et al., 2021) and sturgeons (Long et al., 2019; Ni et al., 2016) show a steady decline in growth performance and health as stocking density increases; however, some species like rainbow trout has an optimal stocking density, and growth and health retardation are observed at either high and low densities (Ellis et al., 2002; North et al., 2006; Hoseini et al., 2020a, 2020b). However, several studies have revealed that different stocking densities had no significant effect on beluga, silver catfish, *Rhamdia quelen* (Menezes et al., 2015); olive barb, *Puntius sarana* (Upadhyay et al., 2022). Cage overcrowding is a chronic stressor that might impair body composition and increase physiological stress (Hoseini et al., 2020a). Although neither high nor low stocking densities may be beneficial to some species, such as the Atlantic salmon, *Oncorhynchus mykiss* (Hoseini et al., 2020a), the majority of research has indicated that

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as stocking density increases, the body composition is negatively affected (Vungarala et al., 2021; Aliabad et al., 2022).

Blood parameters and stress levels in fish are associated with their growth regulation (Noor et al., 2021; De et al., 2019). Moreover, stress-induced by stocking density alters the physiological performance of fish; for example, it influences the growth, survival, body proximate composition, and health of farm-raised catfish (Shoko et al., 2016; Refaey et al., 2018; Mazumder et al., 2019). This affects the economics of cage culture, since optimal stocking density is even more effective when the expense and profit of the system are considered (Biswas et al., 2015; Paul et al., 2021; Hossain et al., 2022). The reaction of fish to various stocking densities is species-specific; thus, cost-effective cage culture is highly dependent on the most profitable stocking density with minimum impact on fish physiology and welfare. Stocking density has also been reported to manipulate blood hematology, a crucial parameter for determining the physiological performance of fish (Refaey et al., 2018; Fauji et al., 2018). Besides that, higher stocking density will require more food inputs and also produce more waste. The high stocking density cage needed more feed production, which increased about five times compared to the low stocking density cage (Moura et al., 2016). This will increase the pressure on local ecosystems as the release of wastes in the form of effluents into aquatic ecosystems results in alterations of the receiving water bodies and consequently triggers eutrophication.

Gangetic mystus (*Mystus cavasius*), locally known as Gulsha tengra, has been widely cultured in Bangladesh (Rahman et al., 2016). Several studies have demonstrated the effects of high stocking density on this fish species' overall growth and development in ponds (Rahman et al., 2016; Hosen et al., 2017). The majority of these research, however, have explored the correlation among both stocking density and growth. The currently available data are limited and focused on pond-based culture. Thus, information about the influence of stocking density on the physiological responses of cage-reared Gulsha tengra remains limited.

Floodplain cage culture needs a cost-effective way to stock fish at high densities while achieving the highest cultured fish production. In comparison to overstocking, understocking involves less input and cost.

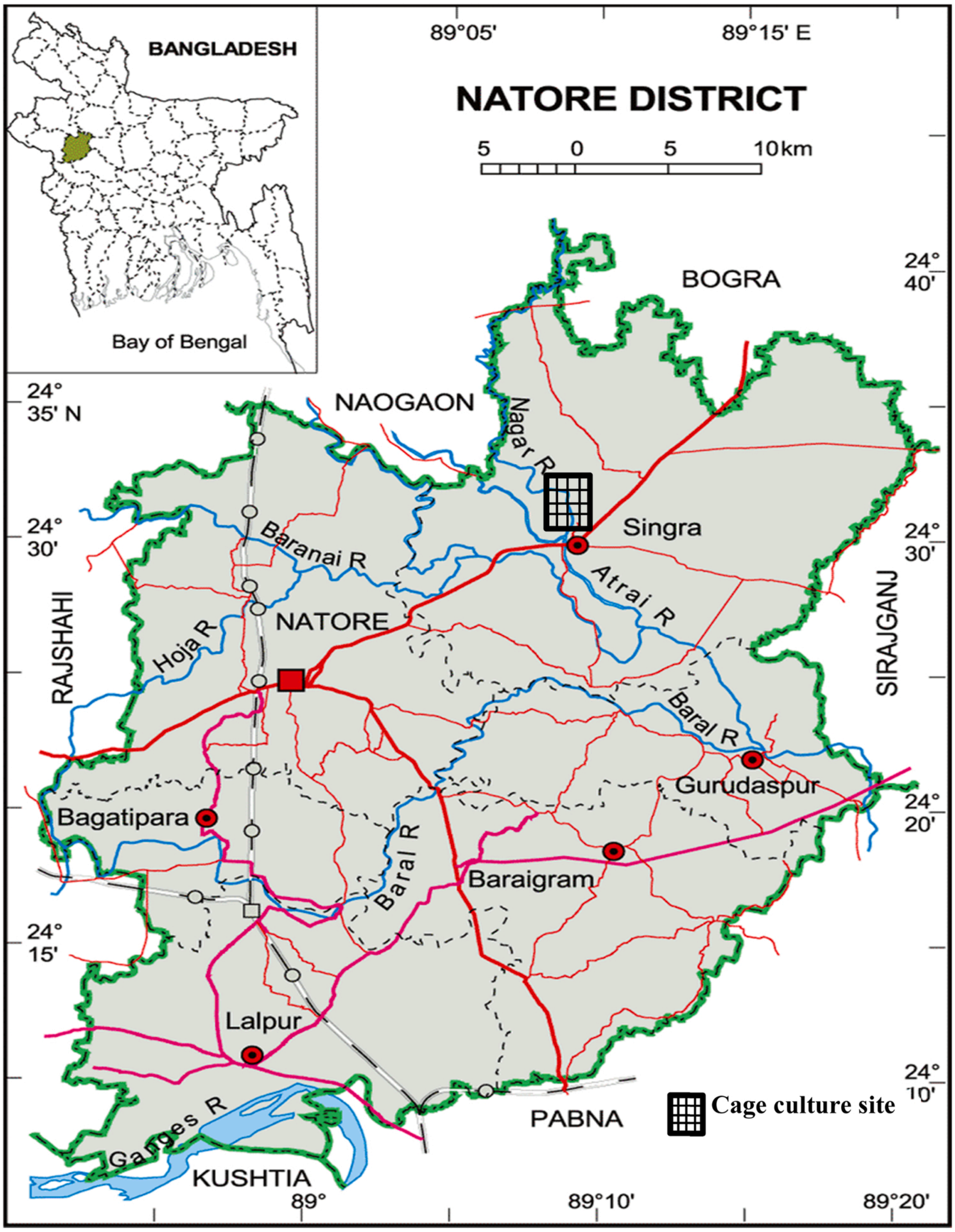

Fig. 1. Location for cage fish farming in Chalan Beel floodplain ecosystem, Atri River at Singra upazila, Natore district, Bangladesh.

Nevertheless, under- and over-stocking are economically unviable in a commercial culture (Rahman et al., 2016). Therefore, this study sought to analyze the effect of various stocking densities on the growth, body composition, and blood parameters of Gulsha tengra reared in Chalan Beel floodplain, Bangladesh, for 120 days. The results may help to determine the optimal stocking density to ensure profitability and enhance fish growth and physiological performance, thereby improving the efficiency of Gulsha tengra cage culture.

2. Materials and methods

2.1. Study location and cage installation

A total of 12 cages built with nylon netting (mesh size 5 mm) were setup in a single row within the Chalan Beel floodplain ecosystem, the largest wetland in Bangladesh (Fig. 1). Individual cages measuring 36 m^3 (6 m × 3 m × 2 m) with 0.5-mm cage liners were used. At the time of stocking, the cages were fixed to blocks (three per block) and equipped with cage liners to act as a nursery for the fry. After two months, the liners were detached from the cages as the fish grew bigger. This detachment was also performed to prevent clogging, which might be an obstacle to adequate water exchange. The cages were kept floating in water keeping about 3 m distance from the bottom mud. Most of the dislodged fouling was removed by water movement but some dislodged fouling was manually removed. All cages were thoroughly scrubbed with a hard nylon brush on a two-weekly basis to remove any fouling as described by dos Santos et al. (2020). Cages were maintained free of fouling during the experimental period. The fish were fed using an automatic feeding device positioned over the top of each cage. Two feeding intervals were used: one during the day at 8 am and another during the night at 5 pm. Individual feeding devices were allocated to each rearing tank under automatic feeding.

2.2. Experimental design

Around 53,000 Gulsha tengra fry were acquired from a private hatchery and transferred to a fish hatchery, where they were acclimated to the cage environment, installed within the same experimental site. The fish were then allocated to four distinct stocking densities (T_1-50, T_2-100, T_3-150, and T_4-200 fish/m^3; in triplicates). These stocking densities were determined using a Bangladesh aquaculture policy brief suggesting that fry stocking densities in cage culture range from 50 to 250 fish/m^3 (DoF, 2016). The mean initial body weight (IBW; g/fish) of all fish in each cage was determined collectively, resulting in an overall mean IBW of 5.55 ± 0.49 g/fish. Commercial floating pellets (Mega Feed Co. LTD, Dhaka, Bangladesh) comprising 32 % crude protein, 5 % ash, 10 % crude lipid, and 10 % moisture were fed to the fish during the acclimatization and entire rearing periods. Regular feeding was offered at the rate of 5 % /fish of body weight during the initial two months, followed by a reduction to 3 % for the next two months. The fish were fed twice daily. The fish were sampled (10 fish/cage) and weighed at 1-month intervals (a total of 4 sampling periods); their growth was measured, and the daily feed was adjusted accordingly. At the final harvest, 100 fish were collected from each cage; their mean final growth and production rate were measured, and the economics were calculated accordingly.

2.3. Fish growth performance

After the experimental period, the mean final body weight (FBW, g/fish) was determined for 100 fish sampled from each cage. The growth performance was measured using the following formulae:

$\text{Weight gain (g)} = \text{Mean FBW} - \text{Mean IBW}.$

$\text{Weight gain}(\%) = \frac{\text{FBW(g)} - \text{IBW(g)}}{\text{IBW(g)}} \times 100$

$\text{Average daily weight gain} = \frac{\text{FBW} - \text{IBW}}{\text{Cultureperiod}}$

$\text{Specific growth rate, SGR}(\%/\text{day}) = \frac{\ln[\text{FBW} - \ln[\text{IBW}]}{\text{Cultureperiod}} \times 100$

$\text{Survival rate}(\%) = \frac{\text{No.offishharvested}}{\text{No.offishstock}} \times 100$

$\text{Food conversion ratio} = \frac{\text{Weight of feed fed}}{\text{Fish weight gain}}$

Total production (Kg/cage) = Fish biomass at harvest.

Net production (Kg/cage) = Fish biomass at harvest – Fish biomass at stocking.

The coefficient of variation (CV%) weight was assessed to determine the heterogeneity in fish size and was calculated as CV = standard deviation in body weight / average body weight (g).

2.4. Water quality parameters

During the experimental period, water quality parameters were monitored every week. The water temperature was measured using a thermometer at a depth of 20 cm; the dissolved oxygen concentration was determined using an oxygen meter (YSL, Norwich, UK), and the pH was evaluated using a pH meter (Orion, USA). NH_3-N, unionized ammonia–nitrogen was measured using hach kits (Hach Co., Loveland, Colorado). Table 1 summarizes the mean values (± standard deviation [SD]) and the values were within the acceptable range for Gulsha tengra culture (Hosen et al., 2017).

2.5. Body composition analysis

Body composition of experimental fish was analysed at the beginning and the termination of the feeding trial. For each time, 10 fish/cage were collected and frozen before the analysis. The analytical methods used for the whole-body proximate analysis of protein, lipid, moisture, and ash contents followed those recommended by the Association of Official Analytical Chemists (1998). The whole fish body was then dried at 105 °C for moisture content determination. Following acid digestion, the protein content was measured as nitrogen using the automatic Kjeldahl distillation unit (VELP Scientifica, UDK 149, Italy) (Simon et al., 2014). After performing Soxhlet extraction using petroleum ether (60 °C) for the solvent, the lipid percentage was measured gravimetrically. The ash content was determined after 6 hrs of ignition in a muffle furnace (550 °C). For each parameter, all analysis were performed in triplicate.

2.6. Blood hematological analysis

After the final harvesting, five fish/cage were randomly sampled for blood analysis. The fish were anesthetized with 25 mg/L tricaine methane sulphonate (MS-222). Blood was drawn from the caudal vein and then transferred to sterile tubes and stored in a slanting position for 3 h. At 4 °C, samples were centrifuged for 10 min at 5000 rotations per minute (rpm). Total erythrocyte and leucocyte counts were determined using the red blood cell (RBC) and white blood cell (WBC) diluting fluids. About 20 µl of blood was mixed with 4000 µl of the diluting fluid in a clean test tube. Drabkins Fluid (Qualigens Chemicals) was used to determine the hemoglobin (Hb) content in blood following the cyanmethemoglobin method (Abdollahi et al., 2016). The absorbance was spectrophotometrically measured at 540 nm, and the final concentration was calculated by comparing it with the cyanmethemoglobin standard (Qualigens Chemicals). The Hb content was then calculated using the following formula: Hb (g/dl) = [OD (T) / OD (S)], where OD (T) represents the test absorbance, and OD (S) represents the standard absorbance. The total protein and albumin contents were measured using commercial kits (Biodiagnostic Co., Egypt). The globulin content was calculated by subtracting the albumin value from the total protein. Using Crest Biosystems® kits, total cholesterol, triglycerides, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels were measured via atomic absorption spectrophotometry.

Table 1
Water quality parameters (mean ± standard deviation) of experimental cage water at four different stocking densities.

Parameters	T_1	T_2	T_3	T_4
Temperature (°C)	26.86 ± 1.10^a (25.36–27.44)	27.16 ± 1.03^a (26.33–28.65)	27.01 ± 1.45^a (25.36–28.52)	27.07 ± 1.18^a (26.85–28.76)
pH	7.27 ± 0.04^a (7.20–7.33)	7.33 ± 0.11^a (7.25–7.50)	7.38 ± 0.16^a (7.16–7.42)	7.46 ± 0.12^a (7.33–7.51)
DO (mg/l)	6.46 ± 0.14^a (6.12–6.53)	6.54 ± 0.08^a (6.48–6.60)	6.42 ± 0.13^a (6.22–6.52)	6.55 ± 0.09^a (6.42–6.61)
NH_3-N (mg/l)	0.002 ± 0.001^a (0.000–0.003)	0.002 ± 0.001^a (0.000–0.003)	0.002 ± 0.002^a (0.000–0.004)	0.003 ± 0.001^a (0.000–0.004)

Values in each same row having different superscripts are significantly different ($P < 0.05$).
Values in the parenthesis indicates range of the parameters.
T_1: 50 fish/m^3; T_2: 100 fish/m^3; T_3: 150 fish/m^3; and T_4: 200 fish/m^3.

2.7. *Economic analysis*

The prices are stated in Bangladesh Taka, the currency of Bangladesh. All data were obtained from the Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh, and matched with wholesale market prices. Economic analysis was performed using the following equation introduced by Asaduzzaman et al. (2010):

$$R = I - (FC + VC + Ii)$$

Where, R = net return, I = income from fish sale, FC = fixed/common costs, VC = variable costs, and Ii = interest on inputs.

The benefit–cost ratio (BCR) was defined as follows: BCR = Total income / Total input cost.

2.8. *Statistical analysis*

The Statistical Analysis Software (SAS) software package (Version 9.4 of the SAS System for Windows 10) was used to perform statistical analysis, which comprised the one-way analysis of variance test. The data were presented as means with SD. Duncan's multiple range test was used to compare the differences detected, with significance set at $P < 0.05$.

3. Results

3.1. *Growth performance*

The growth of Gulsha tengra in terms of the FBW was uniform during the initial sampling period. However, it showed a tendency to increase gradually and differed significantly ($P < 0.05$) across the stocking densities (Fig. 2).

The CV% of weight among the stocking densities did not vary significantly at the beginning of sampling. However, the heterogeneity in fish weight varied significantly during the second sampling, and the fish in T_4 were relatively more heterogeneous than those in other treatments (Fig. 3).

At the final sampling, the FBW weight gain, average daily gain, and SGR were the highest at T_1 compared with those in the other treatments (Table 2). Less utilization of feed by fish in T_4 due to stocking density related stress also triggered a significant decline in the feed conversion ratio (FCR). Total and net productions were also significantly higher at T_1 (88.31 ± 6.72 and 59.27 ± 5.79 kg, respectively) and was lower at T_4 (40.89 ± 2.10 and 30.95 ± 2.37 kg, respectively).

3.2. *Body proximate composition*

The fish in T_4 had the lowest crude protein and highest lipid contents than those in the other treatments. Stocking density induced stress had caused an approximately 20 % reduction in protein and 21 % increase in lipid content, respectively. On the other hand, fish in T_1 had the highest protein with lowest lipid contents. However, no significant difference in

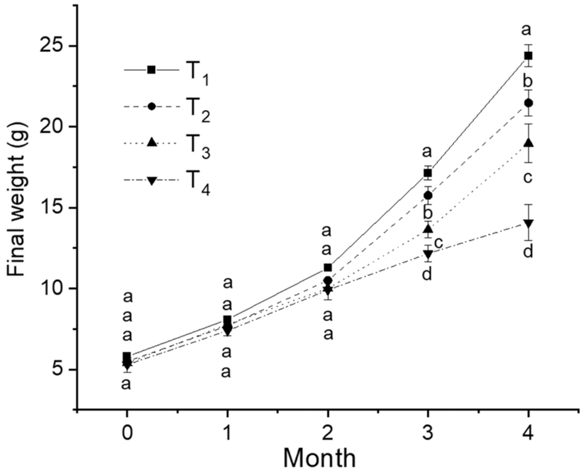

Fig. 2. Growth increment of Gulsha tengra at different sampling period. Means among treatments with different letters at a particular sampling period indicate significance ($P < 0.05$). Solid line indicating T_1, dash line indicating T_2, dot line indicating T_3, and dash dot line indicating T_4.

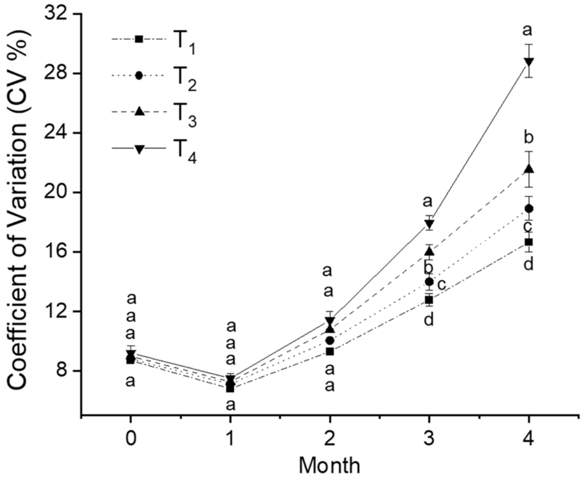

Fig. 3. Coefficient of variation (CV) of weight of Gulsha tengra at different sampling period. Means among treatments with different letters at a particular sampling period indicate significance ($P < 0.05$). Solid line indicating T_1, dash line indicating T_2, dot line indicating T_3, and dash dot line indicating T_4.

Table 2
Growth and production performance (mean ± standard deviation) of Gulsha tengra at four different stocking densities.

Parameters	T_1	T_2	T_3	T_4
Initial weight (g)	5.68 ± 0.56^{a}	5.58 ± 0.55^{a}	5.53 ± 0.42^{a}	5.42 ± 0.45^{a}
Final weight (g)	24.38 ± 1.26^{a}	21.47 ± 1.22^{b}	18.97 ± 1.99^{c}	14.08 ± 0.78^{d}
Weight gain (g)	18.70 ± 1.37^{a}	15.90 ± 0.89^{b}	13.44 ± 1.77^{c}	8.66 ± 0.99^{d}
% weight gain	332.97 ± 47.08^{a}	286.92 ± 25.92^{b}	243.39 ± 29.77^{c}	161.97 ± 32.36^{d}
ADG (G)	0.16 ± 0.01^{a}	0.13 ± 0.01^{b}	0.11 ± 0.02^{c}	0.07 ± 0.01^{d}
SGR (%/day)	1.22 ± 0.09^{a}	1.13 ± 0.05^{b}	1.03 ± 0.07^{c}	0.80 ± 0.10^{d}
Survival rate (%)	95.84 ± 0.85^{a}	92.88 ± 1.61^{b}	88.92 ± 3.35^{c}	86.66 ± 1.73^{d}
FCR	1.75 ± 0.26^{c}	2.04 ± 0.15^{c}	2.52 ± 0.30^{b}	4.09 ± 0.55^{a}
Total production (kg)	88.31 ± 6.72^{a}	85.38 ± 3.94^{a}	69.83 ± 4.66^{b}	40.89 ± 2.10^{c}
Net production (kg)	59.27 ± 5.79^{a}	50.31 ± 3.48^{b}	47.45 ± 5.75^{b}	30.95 ± 2.37^{c}

Values in each same row having different superscripts are significantly different ($P < 0.05$).

Table 3
Body proximate composition (mean ± standard deviation) of Gulsha tengra at different four stocking densities.

Parameters	T_1	T_2	T_3	T_4
Moisture (%)	76.51 ± 0.69^{a}	77.33 ± 0.52^{a}	77.87 ± 0.45^{a}	77.79 ± 0.59^{a}
Protein (%)	17.86 ± 0.36^{a}	16.76 ± 0.24^{b}	15.38 ± 0.57^{c}	13.50 ± 0.67^{d}
Lipid (%)	2.18 ± 0.06^{a}	2.3756 ± 0.06^{b}	2.56 ± 0.12^{c}	2.78 ± 0.02^{d}
Ash (%)	5.00 ± 0.31^{a}	5.06 ± 0.11^{a}	5.09 ± 0.05^{a}	5.12 ± 0.03^{a}

Values in each same row having different superscripts are significantly different ($P < 0.05$).

the ash and moisture contents of fish muscle was noted among the treatments, while some numerical increment was evident (Table 3).

3.3. Blood parameters

Most of the blood parameters studied, including RBC/erythrocyte, WBC, and Hb contents of Gulsha tengra, demonstrated the highest value in T_1 (Table 4). However, increasing the stocking density resulted in a significant reduction in the values of these parameters. The data showed that total protein, albumin, and globulin contents were the highest in the stocking density of T_1, whereas the lowest value were recorded in the stocking density of T_4. Serum cholesterol and triglyceride levels augmented at the higher stocking density of T_4. This pattern was also observed in the levels of ALT and AST.

Table 4
Blood parameters (mean ± standard deviation) of Gulsha tengra at four different stocking densities.

Parameters	T_1	T_2	T_3	T_4
RBC (× 10^6 µl)	119.00 ± 4.85^{a}	108.20 ± 7.09^{b}	52.60 ± 4.39^{c}	24.00 ± 2.35^{d}
WBC (× 10^3 µl)	233.20 ± 16.02^{a}	225.20 ± 5.54^{a}	183.60 ± 7.99^{b}	141.20 ± 11.28^{c}
Hemoglobin (g/dl)	5.65 ± 0.30^{a}	4.97 ± 0.27^{b}	4.22 ± 0.05^{c}	3.22 ± 0.02^{d}
Total protein (g/dl)	5.47 ± 0.03^{a}	5.41 ± 0.04^{b}	4.59 ± 0.17^{c}	4.03 ± 0.14^{d}
Albumin (g/dl)	1.82 ± 0.06^{a}	1.80 ± 0.03^{a}	1.43 ± 0.06^{b}	1.17 ± 0.04^{c}
Globulin (g/dl)	3.65 ± 0.15^{a}	3.61 ± 0.13^{a}	3.16 ± 0.06^{b}	2.86 ± 0.08^{c}
Total cholesterol (mg/dl)	200.40 ± 3.05^{d}	207.80 ± 2.95^{c}	215.40 ± 1.14^{b}	220.60 ± 2.30^{a}
Triglycerides (mg/dl)	147.80 ± 5.67^{c}	158.60 ± 2.70^{b}	168.80 ± 4.76^{a}	173.40 ± 1.82^{a}
AST (U/L)	26.20 ± 1.30^{c}	30.00 ± 0.71^{b}	33.20 ± 1.30^{a}	34.00 ± 1.58^{a}
ALT (U/L)	30.00 ± 1.58^{c}	33.20 ± 2.39^{b}	34.40 ± 2.70^{ab}	36.40 ± 2.07^{a}

Values in each same row having different superscripts are significantly different ($P < 0.05$).

3.4. Profitability

The highest total and net productions were obtained from the fishes in T_1 with a stocking density of 50 fish/m^3 (Table 2). Increased fish accumulation in T_4 significantly increased the feed cost and, subsequently, the total cost. The feed cost accounted for a major cost percentage of the total cost, ranging from 34 % (T_1) to 95 % (T_4). The net income was the highest, while BCR was the lowest for T_1 (Table 5).

4. Discussion

The optimum stocking density of Gulsha tengra in cage culture is crucial for better growth, physiology, and overall development of the fish. The present study aimed to investigate the effects of stocking density on the growth, blood parameters, body composition, and total production of Gulsha tengra in cage culture. Water quality is an important factor that is often compromised during cage culture, as the stocking density is high in this type of fish culture (Moura et al., 2016). However, the different stocking densities assessed in the present study did not impact the water quality parameters as the cages were installed in a running water system. As a result, wastes generated from fecal materials of fish and uneaten feed washed away from the culture cages. Even though the water quality parameters were within the acceptable ranges for the culture of this tropical fish (Maucieri et al., 2019; Akhi et al., 2020), when cultured at high density, fish might show aggressive behaviors that demand high energy costs and reduced fish growth.

CV% differed significantly across the stocking densities during the study period, and more heterogeneity was observed in T_4. Size heterogeneity at a higher stocking density indicates the suppression of fish growth because of limited space as fish were fed with floating pellets. The feeding behavior can be directly observed when fish are fed with floating pellets. At T_4, the fish showed aggressive behavior as competing for food. The competition for food and space increased as the fish biomass per unit volume increased which resulted in increased consumption of energy and altering metabolism. The lower growth rate at higher stocking density could have resulted from intense antagonistic behavioral interaction, competition for food and living space leading to stress and lower growth rates (Pakhira et al., 2015). Previous studies have suggested that a higher stocking density decreases food consumption and cause subsequent reduction in growth (Millán-Cubillo et al., 2016; Aliabad et al., 2022). In the present study, the overall growth performance of Gulsha tengra was higher at a lower stocking density (T_1) and lower at a higher stocking density (T_4). Costa et al. (2017) reported similar observations, suggesting an inverse relationship between stocking density and the growth of cage-reared tilapia. The fish at the highest stocking density in T_4 experienced extreme crowding, which influenced fish growth; this finding was consistent with that of Ronald et al. (2014), Moniruzzaman et al. (2015), and Enache et al. (2016). The overcrowding of fish in T_4 also influenced the survival rate, as reported by Moura et al. (2016) in their study that indicated the negative influence of higher stocking density on the survivability of Nile tilapia. This was also observed in the carp *Labeo rohita* and catfish *Ompok bimaculatus* (Biswas et al., 2015; Debnath et al., 2016). FCR varied significantly across the stocking densities assessed in this study; better growth performance was observed in T_1 with the lowest stocking density. FCR measures a fish's ability to convert feed into meat. The findings of this study are consistent with Asase et al. (2016), where the FCR in

Table 5

Economic performance (mean ± standard deviation) in Bangladesh Taka, BDT of Gulsha tengra at four different stocking densities.

Parameters	Unit price (BDT)	T_1	T_2	T_3	T_4
Feed cost	55/kg	3431.48 ± 254.39^d	6550.60 ± 538.35^c	9454.40 ± 385.22^b	$12,252.30 \pm 681.53^a$
Fry cost	1.70/pieces	3062.50	6125.00	9187.50	12,250.00
Cage cost	–	2000.00	2000.00	2000.00	2000.00
Labor cost	400/months	1600.00	1600.00	1600.00	1600.00
Total cost	–	$10,093.98 \pm 254.39^d$	$16,275.60 \pm 538.35^c$	$22,241.90 \pm 385.22^b$	$28,102.30 \pm 681.53^a$
Total income	–	$35,326.52 \pm 2687.13^a$	$34,914.02 \pm 2332.53^a$	$29,882.91 \pm 1378.80^b$	$20,447.29 \pm 1050.43^c$
Net income	–	$18,638.42 \pm 1970.21^a$	1780.61 ± 1709.24^b	$13,084.62 \pm 2747.27^c$	$10,353.31 \pm 1189.36^d$
BCR	–	2.03 ± 0.13^a	2.14 ± 0.10^b	1.59 ± 0.12^c	1.06 ± 0.07^d

Values in each same row having different superscripts are significantly different ($P < 0.05$).

tilapia culture increased with declining stocking density. On the other hand, lower FCR values at lower stocking densities suggest that fish perform better at extracting nutrients from feed and converting them to meat (Alhassan et al., 2018).

Blood parameters were significantly affected by the difference in stocking density in this research. The RBC, WBC, and Hb contents of fish blood decreased with increasing stocking density. A study conducted by Mazumder et al. (2019) demonstrated a similar reduction in the RBC, WBC, and Hb contents of the silver carp cultured at high stocking density. Thus, stocking density affects the fish welfare in Gulsha tengra, indicated by the biochemical parameters. Other important blood parameters such as albumin, total protein, and globulin contents were also depleted with augmented stress conditions at higher stocking densities. Moreover, higher plasma lipid indicates tissue damage and health deterioration (Hoseini et al., 2018a, 2018b). An increase in ALT and AST indicates tissue damage due to stress, supported by higher plasma lipids (Mirghaed et al., 2017). In contrast, lower stocking density minimizes the stress conditions and actively mobilizes the triglycerides to cope with the extra energy balance. Similar trends in cholesterol and triglyceride variations with stocking density were also observed in Nile tilapia in a study conducted by Wu et al. (2018).

The proximate body composition of Gulsha tengra was also analyzed to confirm their nutritional status at each stocking density. The results indicated that fish in the T_4 group contained significantly the lowest protein with highest fat than fish in the other groups. The possible explanation is that the fish utilized excessive metabolic energy to sustain regular metabolic activity. Oké and Goosen (2019) and Rahman et al. (2020) discovered that stocking densities could affect the body composition of African catfish (*Clarias gariepinus*) and Gangetic mystus (*Mystus cavacius*). When the density was greater than 50 fish/m3, we observed a significant effect of low protein and high fat. Likewise, rainbow trout efficiently convert protein into energy for growth and adaptability to extreme conditions such as high stocking density (Refaey et al., 2018). Net energy for production (NEp) in teleosts comprises growth, body fat accumulation, and reproduction (Liu et al., 2016). Excessive energy was converted to body fat reserves inside the high-density group in a crowded environment with poor performance. The higher lipid levels suggested that oxidative stress could cause hepatic dysfunction and hepatotoxicity in the fish, which was supported by a study in largemouth bass, which was further investigated using histology and revealed significant injury in the liver tissue (Pérez-Jiménez et al., 2017). The liver plays an important role in metabolic homeostasis and coordinates body metabolism responding to different dietary lipid levels and has been proposed as an important index for the diagnosis of liver function and damage. This is also supported by a previous study suggesting that high dietary lipid levels cause stress in fish and significantly reduce immunity (Zhou et al., 2020). Additionally, significant increases in fat content within the high-density group may indicate fat accumulation which could lead to health deterioration in fish. According to Zhao et al. (2019), high stocking density can result in fatty decomposition of common carp (*Cyprinus carpio*). Fish are categorized according to their level of stress and physiological state, which are frequently indicated by biochemical blood indicators, which provide essential parameters to determine fish health (Birnie-Gauvin et al., 2017).

Operational costs increased because of higher stocking density, expanding the total cost in T_4. The highest total and net income were incurred from T_1 and the lowest from T_4. It is observed that T_1 (50 fish/ m^3) was more economically supported by high growth, better blood parameters, and body composition. During the study period, with an increase in stocking density, the total and net productions reduced significantly; this finding was consistent with carps and arapaima (de Oliveira et al., 2012). Fish reared at a stocking density higher than the optimum density experienced limited production because of excessive competition for feed and available space (Kibria and Haque, 2018).

In contrast, extremely low stocking densities lead to declined feed utilization efficiency and poor growth because of the lack of competition for feed or social hierarchy, according to Abaho et al. (2020) in their study involving tilapia culture. Difficulty in tracing food particles causing reduced feed consumption or uneaten food being flushed with drainage water leads to diminished feed utilization efficiency (Sarker et al., 2014). Such food waste can increase the production costs, and the gross yield at such low densities is insufficient to compensate for the cost. Apart from the economic benefit, the low stocking density reduces the discharge of waste from aquaculture operations on a continuous basis, which eventually leads to eutrophication as well as the destruction of natural ecosystems in the surrounding water. Controlled waste production strategies are necessary to maintain sustainable aquaculture into the future, as the long-term sustainability of fish culture systems relies on their ability to reduce their waste outputs. Therefore, an optimum stocking density for fish culture must be determined to ensure economic viability. The present study thus proposes an optimum stocking density for Gulsha tengra cage culture to maximize food and space utilization while minimizing stress and energy expenditure, securing robust fish growth.

5. Conclusion

A stocking density of 50 fish/m^3 may be optimal for cage reared Gulsha tengra juvenile. This was supported by higher growth, acceptable blood parameters, and beneficial body composition at a lower stocking density (50 fish/ m^3). Additionally, a better net income and BCR were observed at the stocking density of 50 fish/m^3. Furthermore, feed contributed to a major percentage of the total cost, ranging from 34 % to 44 %. Therefore, additional studies are warranted to reduce the feed cost by exploring periodic feeding restrictions to ensure sustainability and better economic benefits from the cage culture of Gulsha tengra in floodplain ecosystems.

CRediT authorship contribution statement

MASJ wrote the draft; JA conducted the experiment and collected data, **MAH** analysed the data and drafted the manuscript; **MAH** revised the manuscript and supervised the student; **NMN** edited the draft; **SKD** edited, reviewed, and finalized the manuscript. All authors also critically reviewed the manuscript for intellectual content and gave final approval

for the manuscript to be published.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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