Bhutan Journal of Animal Science (BJAS), Volume 8, Issue 1, Page 71-79, June 2024

EFFECT OF DIFFERENT STOCKING DENSITIES ON THE GROWTH PERFORMANCE, SURVIVABILITY AND LENGTH-WEIGHT RELATIONSHIP OF JUVENILE CHOCOLATE MAHSEER, *NEOLISSOCHILUS HEXAGONOLEPIS* (McClelland, 1839)

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ABSTRACT: This study examined the effect of different stocking densities on the growth performance, survivability, and length-weight relationship of juvenile Chocolate mahseer (Neolissochilus hexagonolepis). Three 74m^3 concrete tank (10m × 4.9m × 1.5m) were assigned to treatments: T1(1 juvenile/m^3; n = 74), T2 (2 juvenile/m^3; n = 148), and T3 (3 juvenile/m^3; n = 222) in a completely randomized design. Juveniles were fed twice daily at 2% of their total biomass. The body weight and body lengths were measured at day 0 and after every 30 days for 90 days. Likewise, pH, dissolved oxygen, and temperature of the water were recorded twice daily for all treatments. Survivability reached 100% across treatments. The overall mean percentage weight gain was significantly higher in T1 (26.02 ± 4.52 %) and T2 (26.02±4.99 %) compared to T3 (8.34±1.29 %) (p >.05). The overall mean percentage length gain was significantly higher in T1(12.71±2.17%) followed by T2 (6.49±1.34%) (p < 0.05). Likewise, specific growth rate (%/day) was significantly higher in T1 (0.20±0.04 %) and T2 (0.14±0.04 %) compared to T3 (0.07±0.01 %) (p < 0.05). Overall feed conversion ratio was significantly lower in T1 (6.47) and T2 (6.81) compared to T3 (29.26) (p < 0.05). The juveniles showed different degrees of negative allometry. The allometric form of the length-weight relationship equation was established as $W = e^{-1.62} \times L^{2.01}$ for T1, $W = e^{-3.81} \times L^{2.74}$ for T2, and $W = e^{0.28} \times L^{1.40}$ for T3. Observed water quality parameters did not differ significantly among treatments (p > 0.05). A cross-sectional cost-benefit analysis showed T1 had a better economic benefit than T2 and T3. The study recommends T1 as the optimal stocking density for better growth performance, feed conversion, and economic return.

Keywords: Chocolate mahseer; growth performance; length-weight relationship; stocking density.

1. INTRODUCTION

Bhutan's gradual privatization of the fish industry has gained momentum through warm-water fisheries primarily focusing on carp, and cold-water streams and lakes, emphasizing on trout production. Recent reports cite a total fish production of 193 metric tons (National Statistics Bureau [NSB], 2022). The native Copper/ Chocolate mahseer (*Neolissochilus hexagonolepis)* thrives in the hill streams and tributaries of Mangde, Chamkhar, Kuri, and Dangme rivers (Royal Society for Protection of Nature [RSPN], 2023). It is an omnivore, feeding on algae, crustaceans, small fish, insects, and worms. Their habitat is characterized by rapid streams with rocky bottoms and substrates, strong currents, clear water with high dissolved oxygen (DO) content, and can endure temperatures ranging from 5°C to 25°C (Aafaq 2021). Information on Chocolate mahseer biology, length-weight relationship (LWR), condition factor, reproduction and food and feeding habits from the natural ecosystem, as well as captive conditions, have been reported in other parts of the Himalayas (Gupta 1988; Sanwal et al. 2010; Sarma 2009; Sarma et al. 2010). However, there is limited information on Mahseer habitat use, reproductive biology, migration, age, and growth patterns in Bhutan making it difficult to start culture and conservation efforts under captivity. Moreover, the predominant habitat of Chocolate mahseer like most other Himalayan regions is severely threatened due to dam construction causing river fragmentation, and unfriendly fishing practices such as poisoning and electric fishing (Sarma et al. 2015; Cafasso 2020).

The culture of Mahseer is not popularized in Bhutan despite being abundant in cold water and torrential streams. Ayyappan et al. 2001 and Lepcha et al. 2003 regarded chocolate mahseer as the most preferred indigenous cold-water species for culture in ponds in hilly areas together with exotic carps but the major limitation to its successful culture and commercialization is the lack of infrastructure facilities and availability of low-cost feed (Dash et al. 2020). Further, there is a lack of experimented culture practices such as optimum stocking density and feeding practices under a captive environment which affects survivability, feed conversion efficiency and growth.

Stocking density refers to fish concentration at initial stocking, determined by carrying capacity. It influences fish welfare and growth by determining the intensity of fish in a given production system (Ondhoro et al. 2019; Sadek 2013). High stocking density can lead to competition for resources, aggression, elevated stress levels, and compromised immune systems, affecting fish growth and survival rates (Seo & Park, 2022). Additionally, overstocking contributes to poor water quality due to waste accumulation like ammonia and nitrate, posing significant threats to fish health and overall growth (Sumalde et al. 2016; Wanja et al. 2020). However, specific effects of stocking density on chocolate mahseer and other indigenous species remain unstudied in Bhutan. Therefore, this study aims to fill this gap by assessing the optimal stocking density for juvenile chocolate mahseer under captivity and its effects on growth performance, survival rate, LWR and water quality.

2. MATERIALS AND METHODS

2.1 Study area

The study was conducted at the National Research & Development Centre for Aquaculture (NRDCA) in Gelephu under Sarpang District. Gelephu is located on the plains in south-central Bhutan. The study site lies at a latitude of 26°52’11’N and a longitude of 90°29’42” E, at an elevation of 221 meters above sea level. The area experiences minimum, maximum and mean temperature ranges of 14-18°C, 28-31°C, and 5-34°C, respectively (National Center for Hydrology and Meteorology [NCHM], 2021). The experiment was conducted for a period of 90 days from December 2022 to February 2023.

2.2 Experimental setup

Three concrete tanks, each with a volume of $74m^3$ (10m × 4.9m × 1.5m) were used in a completely randomized design (CRD) to assess three different stocking densities designated as treatments: T1 (1 juvenile/m^3; n = 74), T2 (2 juvenile/m^3; n = 148), and T3 (3 juvenile/m^3; n = 222). In total, 444 numbers of juvenile chocolate mahseer were cultured for this experiment. To achieve the desired pH range of 7-9 in the water, 35 g/m^3 of finely powdered calcium carbonate was evenly distributed across the pond bottoms. Organic fertilizer in the form of cow dung slurry was applied at the rate of 2.5/kg/m^3/year to promote plankton growth which will supplement artificial feeding (Thinley et al. 2018). Continuous water flow was maintained and additionally, 30% of the pond water was replaced weekly to maintain the quality of the water. To ensure optimal conditions, stocking was done five days after fertilization.

2.3 Feeding

Fish were fed a commercial grower fish feed manufactured by Manasarowars Agro Trade and Processing Private Limited, West Bengal, India which is specifically formulated for carp. The feed comes in 3.0 mm diameter extruded pellets and contains a minimum of 25 % crude protein, 7% lipid, 40 % carbohydrate, and a maximum of 15 % fibre, 12 % moisture, and 1% mineral/vitamin premix. Feeding was done at 2% of the body weight and divided into two meals (8:00 hrs. and 16:00 hrs.). Feed was given by hand broadcasting.

2.4 Data collection

For evaluating the growth performance of juveniles, different parameters such as body weight (g), body length, mean percentage gain in weight and length, Specific growth rate (SGR) (% per day) and feed conversion ratio (FCR) were used. The body weight and length of all fish were measured at the beginning of the experiment and every 30 days thereafter. Weight was measured to the nearest gram (g) using a digital weighing balance (Eminent company, Model: ISO9001:2024), while body length (from snout to the end of the tail) was measured to the nearest centimeter (cm) on a calibrated length measuring board, ensuring minimal stress to the fish. Daily feed intake was assumed as the total amount of feed given (i.e. 2% of body weight). Casual observations of physical condition were made during the final measurement to detect signs of aggressive activity (e.g., bruises and loss of scales). The following equations were used to calculate the growth parameters:

$$\text{Mean gain in length (cm)} = \text{Mean final length (cm)} - \text{Mean initial length (cm)}$$

$$\text{Mean gain in weight (g)} = \text{Mean final weight (g)} - \text{Mean initial weight (g)}$$

$$\text{Specific Growth Rate (SGR)} = \frac{\text{Log final weight (g)} - \text{Log initial weight (g)}}{\text{Total number of days reared}} \times 100$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Total feed intake (g)}}{\text{Total weight gain (g)}}$$

Water quality parameters, including temperature, DO and pH, were monitored twice daily at 9:00 and 14:00 hours. Temperature readings were taken using a clinical thermometer, DO levels were measured in mg/l using a digital DO meter (Lutrron DO-5509), and pH levels were recorded by a digital pH meter (Hanna HI98100). After 90 days, the fish were harvested by repeated netting, followed by drying of the ponds. The live fishes were counted and survival (%) was calculated and compared among the treatments. A cross-sectional cost-benefit analysis focusing on the 90-day experimental period, considering feed costs incurred and growth rates was done to assess the resulting economic differences between the treatments.

2.5. Data Analysis

Descriptive statistics for growth parameters were presented in means and standard error of the mean (SEM). One-way Analysis of variance (ANOVA) was used to compare body weight, body length, percentage gain in weight and length, and SGR between the treatments following assumption tests such as the normality test using the Shapiro-Wilk test and Bartlett's test for homoscedasticity. Tukey's HSD was used for the pairwise comparison of means following one-way ANOVA. Kruskal Walli's non-parametric test with Bonferroni correction was used to check differences in mean ranks of FCR between the treatments. The length-weight relationship (LWR) equation $W = aLb$ (Le Cren, 1951) was used to estimate the relationship between the weight (W) and length (L) of the fish. Using the linear regression of the log-transformed equation: $log\ (W) = log$ (a) $+$ b $log\ (L)$, the parameters a and b were calculated with 'a' representing the intercept and 'b' the slope of the relationship. To establish LWRs to treatments (stocking density) that can affect b, b was assessed and

Table 1: Mean weight (g) and length (cm) and final weight and length gain percentage in three treatments (mean ± SEM).

Treatment	Initial (Day 0)	30 days	60 days	90 days	Percentage gain
Body weight (g)					
T1	83.62± 2.64[a]	86.9±3.14[a]	92.47±3.05[a]	98.62±2.33[a]	26.02±4.52[a]
T2	85.57±2.54[a]	87.92±2.56[a]	90.88±2.48[a]	94.53±2.26[a]	26.42±4.99[a]
T3	88.04±0.65[a]	89.55±0.75[a]	91.99±0.80[a]	94.2±0.85[a]	8.34±1.29[b]
Body length (cm)					
T1	19.68± 0.37[a]	20.67±0.27[a]	21.12±0.25[a]	21.64±0.18[a]	12.71±2.17[a]
T2	19.91±0.19[a]	20.43±0.19[a]	20.52±0.18[a]	20.92±0.68[a]	6.49±1.34[b]
T3	20.91±0.07[a]	20.48±0.73[a]	20.43±0.87[b]	20.77±0.09[b]	-0.47±0.55[c]

interpreted by individual treatments (Ondhoro et al. 2019). When applying this formula, *b* may deviate from the "ideal value" of 3 that represents an isometric growth and when *b* is less than 3, fish become slimmer with increasing length, and growth will be negatively allometric. When *b* is greater than 3.0, fish become heavier showing a positive allometric growth and reflecting optimum conditions for growth (Ricker and Carter, 1958). Data was recorded in Microsoft Excel and was analyzed using IBM- SPSS (Statistical Package for Social Sciences) version 26 except for LWR analysis and graphs, which were done using RStudio version 4.3.2. The significance level for all the analyses was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Growth Performance and Survivability

The growth analysis included a comparison of mean initial body weight and length, monthly weight and length, as well as final percentage gain in weight and length (Table 1). Juveniles held in lower stocking densities showed higher weight and length gain than the ones with higher densities. The mean percentage of weight gain was significantly higher in T1 (26.02 ± 4.52 %) and T2 (26.02±4.99 %) compared to T3 (8.34±1.29 %) ($p > 0.05$). In terms of body length, there was a significant difference ($p < 0.05$) in body length across treatments at 60 days and 90 days as shown in Table 1. The overall mean percentage length gain was also significantly higher in T1(12.71±2.17%) followed by T2 (6.49±1.34%). In contrast, T3 showed a negative percentage length gain (-0.47±0.55%). A similar relation between growth and stocking density was observed in different mahseer (Aksungur et al. 2007; Ullah et al. 2018) and other species (El-Sayed 2002; Latifah et al. 2021; Rahman et al. 2005; Seo and Park 2022). Stocking densities did not affect the survival rates of juvenile chocolate mahseer in this study as there was a 100 % survival rate in all the treatments. Moreover, the lack of physical injury to fish suggests non-aggressive competition among the juveniles.

3.2 Specific growth rates (%/day) and Feed conversion ratio

Higher stocking density resulted in a lower SGR in juvenile chocolate mahseer (Table 2). The overall SGR was significantly higher in T1 (0.20±0.04 %) and T2 (0.14±0.04 %) compared to T3 (0.07±0.01 %) ($p < 0.05$). Anderson et al. 2002 and Latifah et al. 2021 reported that fish growth cannot be optimal if the stocking density is too high because it has exceeded the carrying capacity of the waters. The SGR of weight and length decreases at higher stocking densities because the space for movement will be narrower. This can cause stress for fish due to strong competition for food (Aksungur et al. 2007; Latifah et al. 2021). Latifah et al. (2021) reported that under stress conditions there is also a reallocation of metabolic energy for growth and reproduction into activation energy to improve homeostasis such as respiration, movement and tissue repair. In addition, the study also explained that energy obtained from feed is used as maintenance energy and the remaining is used for growth. However, the stress caused by an increase in stocking density will increase the maintenance energy requirement thus leading

Table 2: SGR (%/day) and FCR at different stages of the study in the three treatments (mean ± SEM)

Treatment	Day 0 – 30 days	30 – 60 days	60 – 90 days	Overall
SGR (%/day)				
T1	0.10±0.01[a]	0.23±0.15[a]	0.28±0.13[a]	0.20±0.04[a]
T2	0.10±0.01[a]	0.12±0.13[b]	0.19±0.11[a]	0.14±0.04[a]
T3	0.05±0.03[a]	0.09±0.03[b]	0.08±0.04[b]	0.07±0.01[b]
FCR				
T1	7.87±2.14[a]	4.76±0.93[a]	6.79±1.71[c]	6.47±1.64[a]
T2	6.35±1.68[a]	6.30±0.94[a]	7.78±1.44[a]	6.81±2.57[a]
T3	47.18±24.23[b]	18.52±3.99[b]	22.10±5.42[b]	29.26±5.78[c]

***Values with different superscripts within the column differ significantly ($p < 0.05$)

to lower SGR.

FCR was also favourable in lower stocking densities (Table 2). The overall FCR in T1 (6.47±1.64) and T2 (6.81±2.57) was significantly lower compared to T3 (29.26±5.78) ($p < 0.05$). FCR in T1 and T2 remained relatively stable throughout the experiment (Table 2) and that could mean optimum stocking density for feed utilization. T3 showed significantly higher FCR throughout the period (Table 2) which could be due to stronger competition for feed compared to T1 and T2. The FCR values observed for all the treatments are higher than those obtained in different mahseer species by Hossain et al. 2002 and Islam and Tanaka (2004) as well as within the range reported by Ullah et al (2021) for juvenile mahseer. High FCR values can also be attributed to other factors including feed quality and feeding method, temperature variations, rearing units and size of fish (Aksungur et al. 2007). Further, Sarma et al. 2015 also reported phytoplankton and zooplankton as a major gut content in fry to juvenile stages of chocolate mahseer and found to affect FCR. Phytoplankton and zooplankton growth in the tanks and subsequent gut analysis was not done in our study which could also explain the high FCR observed.

3.3. Length Weight Relationship (LWR)

The LWR is a useful tool in assessing fish growth. The allometric form of the equation determined from Logarithmic LWR is given in Table 3. The value of "*b*" obtained for T1 (2.01), T2 (2.74) and T3 (1.40) showed significant deviation ($p < 0.001$) from the widely accepted value $b = 3$. If the *b*-value is equal to 3.0, growth is isometric, meaning they retain body shape, and specific gravity will also remain unchanged during their lifetime (Lagler 1966; Wootton 2012). Hence, this growth pattern in fish will follow the cube law. But if the *b*-value is less than 3.0 as in our study, fish become slenderer as they increase in length; therefore, growth will be negatively allometric (Mazumder et al. 2016). In this study, juvenile chocolate mahseer under different stocking densities showed different degrees of negative allometry. Le Cren 1951 and Mazumder et al. 2016 reported that the relationship between the length and weight of fish may depart from the ideal value (3.0) / cube law because fish normally do not retain the same shape or body conformity across different growth stages and the specific gravity of tissues does not remain the same. Hence the actual relationship differs significantly from the cube law. While Subba et al. (2018) reported a regression coefficient (*b*) value close to 3.0 for Chocolate mahseer in Nepal, deviations occurred due to sex, season, fluctuation in environmental conditions and nutritive conditions. Our study observed further variations in the negative allometric relationship across different stocking densities (Figure 1) but this cannot be solely attributed to differences in stocking densities (Ondhoro et al. 2019). Figure 1 shows the logarithmic relationships between

Table 3: Logarithmic and allometric LWR equation for juvenile chocolate mahseer in three different treatments.

Treatment	R^2	Logarithmic LWR equation	Allometric LWR equation
T1	0.81	$Log(W) = -1.62 + 2.01 \times Log(L)$	$W = e^{-1.62} \times L^{2.01}$
T2	0.78	$Log(W) = -3.81 + 2.74 \times Log(L)$	$W = e^{-3.81} \times L^{2.74}$
T3	0.45	$Log(W) = 0.28 + 1.40 \times Log(L)$	$W = e^{0.28} \times L^{1.40}$

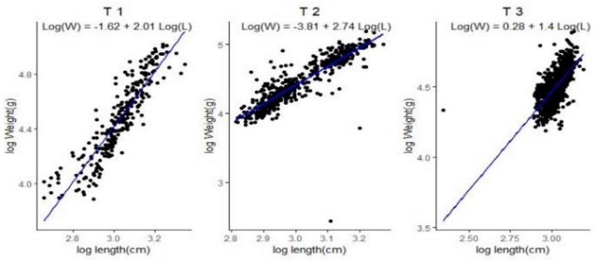

Figure 1: Scatter plot of natural log of weight against the natural log of length

the length and weight of juvenile cholate mahseer reared in different stocking densities.

3.4. Water quality parameters

There were no significant differences in observed water quality parameters (temperature, DO and pH) among the treatments ($p > 0.05$) indicating that increasing stocking density did not cause deterioration in observed water quality. The temperature range was recorded within the required range of 20 to 25°C for cold water species (Boyd, 2018).

Similarly, pH values (6.5-8.5) and the concentration of DO (6.86-7.35 mg/L) were also found to be within the ideal range required for the fish. Figure 2 shows the mean temperature, DO and pH for all treatments combined.

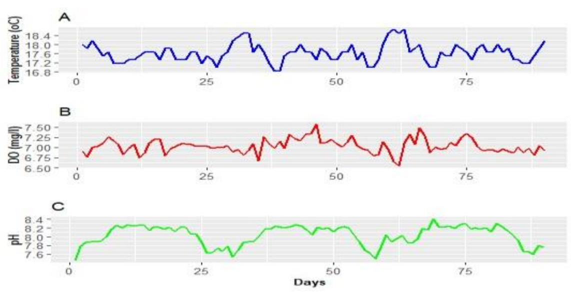

Figure 2: A time series plot of mean daily water (A) temperature (^{o} C), (B) dissolved oxygen content (mg/l) and (C) pH level

3.5. Economic analysis

The cost-benefit analysis focused only on the 90-day experimental period, considering feed costs incurred and growth rates to assess the resulting economic differences. T1 showed better economic benefits compared to T2 and T3. Table 4 provides a cross-sectional view of the cost-benefit analysis between treatments within this timeframe.

4. CONCLUSION & RECOMMENDATIONS

The study concludes that despite achieving a 100% survivability rate across different stocking densities, growth performance varied significantly. Both weight and length gain were significantly higher in lower stocking

Table 4: A cross-sectional cost-benefit analysis of 90-day period of juvenile chocolate mahseer in three different treatments.

Parameter	T1	T2	T3
A. Total no. of fish in each group	74	148	222
B. Cost of 1 kg feed (Nu)	45	45	45
C. Average initial weight (g)	83.62	85.57	88.04
D. Average final weight (g)	98.62	94.53	94.2
E. Average weight gain/fish (g)	15	8.96	6.16
F. Feed consumed in 90 days (kg)	11.63	23.38	36.66
G. Total feed cost for 90 days	523.35	1052.10	1649.7
H. Total weight gain in 90 day/treatment (g) (A* E)	1110	1324.48	1365.52
I. Selling price for fish	500	500	500
J. Total revenue (Nu) from 90 days (H/1000*I)	Nu. 555	662.24	682.76
K. Net benefit (Nu) (J - G)	31.65	-389.76	-966.94

density (T1). Likewise, specific growth rate and feed conversion ratio were also optimum in lower stocking density (T2). The length-weight relationship showed different degrees of negative allometry. Additionally, economic analysis showed better return in lower stocking density. The study recommends 1 juvenile/m^3 as the optimum stocking density for juvenile chocolate mahseer. Future studies can be aimed at understanding the effect of stocking density on later growth stages which will necessitate extended research periods.

Acknowledgments

The authors would like to acknowledge the guidance and support of NRDCA, Gelephu.

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