IMPACT OF STOCKING DENSITY ON GROWTH PERFORMANCE OF STUNTED FINGERLINGS

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ABSTRACT: The impact of stocking densities on growth performance of stunted fingerlings was evaluated. Advanced fries of *Labeo rohita* and *Cirrhinus mrigala* each weighing between 0.66 and 0.72 grams were reared in earthen ponds with optimum water quality parameters for 120 days. Three ponds with dimensions of 2442m² (T1), 2176m² (T2), and 2652m²(T3) were adopted considering the existing facilities in the study site. T1was stocked with 50 fries/m², T2 with 60 fries/m², and T3 with 70 fries/m² of *L. rohita* and *C. mrigala* mixed in equal proportion. The fingerlings were fed a commercial sinking pellet with 35% protein at a rate of 2% of their body weight throughout the study. Descriptive statistics, including the mean, standard deviation, and percentages were analyzed for quantitative variables. ANOVA was utilized to assess differences in the growth performance of stunted fingerlings. Treatment T1 resulted in the highest weight gain (7.12±5.09g), and the highest density treatment, T3, showed the lowest weight gain (3.63±3.62g) with all significance tests conducted at the 5% level. Survival rates varied from 44.53% to 84.04% with T1 showing significantly higher survival rate. Similarly, T1 exhibited the best specific growth rate of 1.98 as compared to other two treatments. Therefore, the research observed that a stocking density of 50 fries/m² is optimal stocking density particularly for *L. rohita* and *C. mrigala* to derive a maximum yield in terms of stunted fingerlings.

Keywords: *Cirrhinus mrigala*; *Labeo rohita*; stocking density; stunted fingerlings.

1. INTRODUCTION

Aquaculture, the farming of aquatic organisms, plays a crucial role in meeting the demand for fish and seafood products. Bhutan, a landlocked country nestled in the Eastern Himalayas, is emerging as a noteworthy player in the realm of aquaculture, demonstrating a commitment to sustainable development and environmental conservation. With Bhutan's population steadily increasing, there is a growing need to develop sustainable aquaculture practices to ensure food security and alleviate pressure on natural ecosystems (FAO 2021). The history of aquaculture in Bhutan dates back to the 1960s when the government initiated small-scale fish farming projects to supplement the protein needs of the population. However, it was only in the 1980s that Bhutan began to actively promote and invest in aquaculture as a means of economic diversification and poverty alleviation (FAO 1983).

The primary species being cultivated in Bhutan include Grass carp, Silver carp, Common carp, Rohu, Mrigal, and Catla. To support these efforts, producers rely on the government fishery farm for carp seeds and crucial technical assistance, ensuring successful aquaculture practices (Dorji et al. 2022). Indian Major Carp (IMC) holds significant importance in aquaculture due to its ease of breeding and adaptability to various water conditions (Rahman et al. 2020). The intensive cultivation of IMCs is expanding, particularly in the southern region, to meet rising demand for fingerlings.

Bhutan's aquaculture sector emphasizes these considerations to ensure the successful development and growth of IMCs in closed management systems. Specifically, the National Development Centre for Aquaculture (NDCA), Gelephu and Regional Centre for Aquaculture (RCA), Phuntshothang a government fishery centre spearhead in supplying fingerlings i.e. both ordinary and stunted fingerlings. Annually, two centres dedicate their time and effort in producing stunted fingerlings out of Indian Major Carps (IMCs) i.e. Rohu, *Labeo rohita*, Mrigal, *Cirrhinus mrigala* and *Catla catla* because it was only species left at the centre after distribution of ordinary fingerlings to fish farmers for annual re-stocking.

Rohu (*L. rohita)*, being an osseous fish, possesses inverted bones, resulting in an anatomy resembling an inverted cup. This unique feature facilitates easier filleting for fishermen. Mrigal (*C. mrigala*), with its elongated and flattened body, also exhibits a rich nutrient profile.

Annually, NDCA provides numerous Cauvery white stunted fingerlings known for their accelerated growth and enhanced survival in farmers' fields. Although, majority of annual fish inputs supply from NDCA and RCA are ordinary fingerlings, farmers consistently expressed their desire and preference for stunted fingerlings due to its rapid growth and increased survival rates as compared to ordinary fingerlings. In this regard, both centres initiated the production of stunted fingerlings with adoption of flexible stocking density based on the availability of advanced fries. Such stocking density practices adopted are not scientific and as a result it may have impacted on the production performance. Thus, this study was designed to determine how varied stocking density affects the growth performance of stunted fingerlings. Moreover, the findings of the study can also serve as a baseline or standard level of stocking density for stunted fingerlings production.

2. MATERIALS AND METHODS

2.1 Study Site

This research was carried out in the government farm, NDCA, Gelephu located at geo-coordinates of 26°52’7.68” N and 90°29’45.25” E in southern Bhutan. A field experiment was conducted between November 2022 to February 2023.

2.2 Pond preparation and stocking of fingerlings

Before introducing advanced fries, the three treatment ponds were prepared by removing debris, leveling, clearing drainage, and implementing potential pest control measures. Required water quality was ensured by testing and aeration, which is crucial for fingerlings’ growth. The earthen ponds received uniform treatments of pond drying, liming, water filling, and manuring.

With confirmation of adequate natural productivity through expert judgement, the ponds were stocked with four months old advanced fries of *L. rohita* and *C. mrigala*. The initial weight and length of the fries (n=399) were recorded individually at initial stocking as reference point for growth indices.

2.3 Feeding management

Fries were fed a balanced diet adjusting for size and water conditions. The feed type used was sinking pellet as a supplementary diet daily, following a 2% per body weight feeding rate (Thinley and Drukpola 2021). For this study, a formulated diet size (Diameter): 1.5 mm%, containing: Lipid % (minimum): 7%, carbohydrate % (minimum): 40%, fiber % (maximum): 10%, moisture % (maximum):12%, mineral/ vitamin premix % (maximum): 1% with a minimum of 35% CP was fed. The fingerlings were fed twice a day as per the feeding practice adopted at NDCA.

2.4 Experimental design

This study was designed with three different stocking density as treatments to test the impact on growth performance of stunted fingerlings. Treatments were 50 fries/m^2 as Treatment 1 (T1), 60 fries/m^2 as Treatment 2 (T2), and 70 fries/m^2 as Treatment 3 (T3). Specifically, the study accounted these two species i.e. *L. rohita* and *C. mrigala* considering the availability of ordinary advanced fries at the time of study for stunting purpose. The additional rationale of the species choice was also for synchronizing with the current practices of the NDCA, Gelephu. The centre commits its time and effort towards stunted fingerlings production from above two mixed species after the offset of breeding season in summer.

Four-month-old advanced fries were 0.66 to 0.72g in weight and 3.67 to 3.97cm in length during the initial stocking period. Advanced fries were collected from NDCA nursing ponds and then initially stocked 122100, 130560 and 185640 numbers into T1, T2 and T3 respectively. For sampling purpose, the sample size was derived by using Yamane's (1967) formula as follows:

$$n = \frac{N}{1+N(e)2}$$

where n is the sample size, N is the population size, 1 is the constant and e is the level of precision/margin of error (0.05). The above formula-based derived sample size is 399 for each treatment. A total of 399 fingerlings each from three treatment ponds were captured randomly, and the body length and body weight were measured and recorded on stocking day (0-day) and at harvest (120-day) for further analysis of growth performance of *L. rohita* and *C. mrigala*. The total length to the nearest centimeter (cm) and total weight to the nearest gram (g) of advanced ordinary fingerlings were measured with a Vernier caliper and a high-precision digital weighing scale, respectively.

The weight gain (g) was calculated using the formula of Hassan et al. (2020) and specific growth rate (SGR), and feed conversion ratio (FCR) were calculated using the method and derivations of Rahman and Arifuzzaman (2021). Following formulae were used to estimate the growth parameters for this study;

1. *Weight gain (g)*

   Weight gain (g) = Final weight of fingerling – Initial weight of fingerling

2. *Specific growth rate (SGR %):*

$$\text{Specific growth rate (\%)} = \frac{(\text{Ln}(Wt) - \text{Ln}(W0))}{T(d)} \times 100$$

Where,

W0 (g) = the weight in grams at the beginning of the period

Wt (g) = the weight in grams at the end of the period

t (d) = period, expressed in number of days

Ln = natural logarithm

3. *Survival rate (%)* = (Final total numbers/Initial total numbers) X 100

2.5 Water quality

Regular monitoring of fish and pond conditions was implemented, addressing issues promptly through assessment of water quality. Routine monitoring and evaluation of water quality and its environment ensured optimal conditions, including vegetation control and predator management. Reduction or overall water loss in ponds was periodically compensated and partial water exchange was done to control the growth of algal blooms. Water temperature and DO were measured using a handheld Hanna Multiprobe meter (model Hi-9142, Hanna

Instruments Inc., USA) while pH was measured using a handheld Hanna meter (model Hi-98127, Hanna Instruments Inc., USA).

2.6 Data collection and analysis

The biomass in each tank was recorded at initial stocking, and final harvest. Similarly, feed intake was recorded daily. Likewise, authors recorded morbidity and mortality cases on daily basis during feeding time and also during scheduled monitoring and evaluation phase. Data on water quality was also collected twice once in morning and once in evening.

Both qualitative and quantitative data collected were processed and analyzed using Statistical Package for Social Sciences (SPSS) version 23.0. Descriptive statistics such as mean, standard deviation and percentages were used for quantitative variables. ANOVA was used to test difference in growth performance of stunted fingerlings.

3. RESULTS AND DISCUSSION

3.1 Impact of stocking densities on body weight, SGR and survival rate

The growth performance results of a four-month experiment on advanced fries have been presented in Table 1. The highest mean growth was observed in T1 (7.12±5.09g) followed by T2 (6.4 ±5.17g) and T3 (3.63±3.62g). Mean values for monthly weight gain and average daily growth showed significant differences among treatments ($p < 0.05$). The mean weight of the fish increased linearly in all treatments regardless of the stocking densities. A gradually decreasing pattern of growth was observed by increasing the stocking density which confirms about the optimal stocking density specific to *L. rohita* and *C. mrigala* for stunting purpose (Table 1). The findings evident that the optimum stocking density of 50 fries/m^2 derives better growth performance of stunted fingerlings studied strictly on NDCA's farm management level. Thus, based on this finding, a stocking density of 50 fries/m^2 is the optimal stocking density to arrest fish from attaining proper growth and development due to the high density and minimum food available achieving maximum survival rate. Das (2021) also observed a similar pattern of growth in their stocking density trial on the influence of tank shading and rearing density on growth during stunted juvenile production of rohu, *L. rohita* (Hamilton, 1822). Other authors, Babu et al. (2019) also reported significant compensatory growth patterns, economic usage of feed, and enhanced survival rates with better yields in marine finfishes such as Snubnose pompano and Mangrove red snapper. As per the same author, the compensatory growth pattern in the aquaculture and fisheries sector is an innovative 21st-century technology adopted by fish farmers owing to its multifaceted advantages. Specifically, for the above two marine finfishes, a stocking density of 50 fries/m^3 for stunting fingerlings was found ideal.

The overall SGR value of this study ranged from 1.35 to 1.98 (Table 1). When compared between groups, SGR of all three treatments is not statistically significant ($p>0.05$). Similar observations on SGR of amur common carp (*Cyprinus carpio*) and mrigal (*C. mrigala*) with major carp in polyculture system were also reported for *L. rohita* and *C. mrigala* by Verma and Mandal (2018). Baruah et al. (2020) in their study on growth parameters of *L. rohita* fed with various feeding rate recorded SGR range of 0.87 to 1.50 which is consistent with this finding indicating that how well the fish are growing under specific conditions of stocking density. Higher SGR values typically suggest that the fish are healthy and are being reared in a favorable environment. Although no considerable variations were established among the mean value of SGR, these values were decreased by increasing the level of stocking density indicating that fish are stressed in higher stocking density (Table 1). SGR value is evident that a stocking density of 50 fries/m^2 had higher SGR as compared to 60 fingerlings/m^2 and 70 fingerlings/m^2.

Similarly, the impact of varied stocking density was also observed in terms of morbidity and mortality that caused 15.96%, 45.78%, and 55.47% rearing mortality in T1, T2, and T3. As a result, the survival rate recorded was 84.04%, 54.22%, and 44.53% for T1, T2, and T3 respectively with an overall mean survival rate of 60.93% which was statistically significant among treatments (Table 1). A high survival rate in T1 indicates good fish health and effective stocking densities. It also suggests that the fish are not significantly stressed and are resistant to diseases at specific stocking densities.

3.2 Water quality

The mean temperature values recorded in the study pond during the study period were 25.24 to 27.26 (T1), 26.10 to 28.00 (T2), and 26.31 to 28.73.17 (T3). The mean ± standard deviation values for physicochemical parameters of water in different treatments are presented in Figure 1. For this study, the water temperature range recorded was within the recommended range of 20°C to 33°C (Manam and Quraishi 2024). However, in temperate regions, winter brings temperatures too low for the rapid development of warmwater aquaculture species and their associated food organisms. As a result, management practices such as feeding and fertilizing are typically reduced or halted during the winter in temperate climates. Temperature significantly affects both chemical and biological processes, with the rates of these reactions doubling for every 10°C rise in temperature. This means that aquatic organisms consume twice as much dissolved oxygen (DO) at 30°C compared to 20°C. Consequently, the DO requirements of aquatic animals become more crucial in warmer water than in cooler environments. Temperature also influences chemical treatments in ponds, as fertilizers dissolve more rapidly in warm water, and the oxygen consumption rate by decaying organic matterincreases. Even a slight temperature change of 3°C or 4°C can induce stress or mortality in aquatic animals. Insufficient DO is recognized as a limiting factor, and its inadequate availability in water can impede

Table 1: Growth performance and survival rate of carp cultured in varied stocking densities

Treatment(s)	No. at Initial Stocking	No. at Final Harvest	Body Weight(g)		Weight Gain(g)	SGR	Survival Rate (%)
			Initial Mean ± SD	Final Mean ± SD			
T1	122100	102615	0.66 ± 0.43	7.12 ± 5.09	6.46	1.98	84.04
T2	130560	70785	0.71 ± 0.32	6.44 ± 5.17	5.73	1.84	54.22
T3	185640	82672	0.72 ± 0.34	3.63 ± 3.62	2.91	1.35	44.53

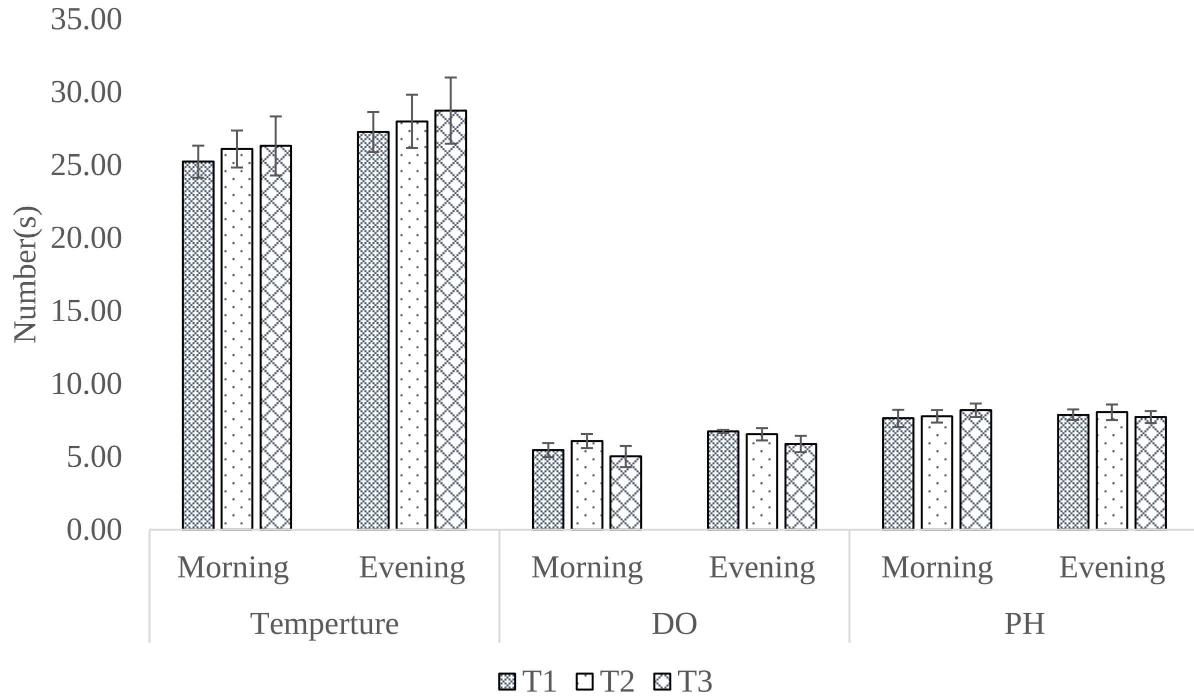

Figure 1: Water quality parameters recorded during the study period

the activities of cultivated biota, as stated by the Ekubo and Abowei (2011).

The DO levels in the study pond during the study period ranged from 5.46 to 6.74 in T1, 6.08 to 6.54 in T2, and 5.02 to 5.88 in T3 (Figure 1). The DO ranges in this study were found within the recommended limits of 5-10mg/L for freshwater fish (Ekubo and Abowei 2011). Specifically, the optimal DO range recommended for carp culture in fish ponds is between 5.00 to 7.00 mg/L (Mallya 2007). A study on general relationship between water quality and aquaculture performance in ponds by Boyd in 2017 reported similar DO range indicating tolerability by Rohu and Mrigal. Das (2004) reported that DO content reduced in all ponds with the progress of culture period and control ponds recorded significantly lower DO compared to the treatment ponds. In control ponds, DO reduced from initial 5.8 mg/L to final 2.0 mg/L compared to the narrow reduction of 5.5 to 3.8 mg/L in treatment ponds. However, this study recorded optimal range of DO indicating that the water supplied to the treatment ponds are well aerated due to constant monitoring and evaluation on flow of water from the inlets.

The pH values reflected in Figure 1 were 7.64 to 7.88 (T1), 7.78 to 8.05 (T2), and 7.72 to 8.19 (T3) for this entire study period. The optimal pH range for carp culture in ponds is between 6.5 and 9.0 (Samad et al. 2017) which is consistent with the findings of this study. pH directly influenced the toxicity level of ammonia, nitrite and hydrogen sulfide, thus, Ph is a critical parameters to observed specially for juveniles (Saharia 2023). Similar pH range indicating tolerability by Rohu and Mrigal was also reported by Rajeshkumar and Balusamy (2017) in their study on the effect of different fish feeding methods on growth performance and fish yield in composite fish culture system. pH levels below four or above 11 are considered extreme and can be lethal for fish

(Bhatnagar and Devi 2013). Therefore, it can be deduced that the observed pH levels in this rearing nursery ponds are within the normal range, enabled the better growth and health of fish for this study.

Therefore, based on the above water quality results of temperature, DO and pH, it can be concluded that all water quality parameters of the experimental ponds were within the productive range.

4. CONCLUSION

Inappropriate stocking density potentially poses a major challenge in the production of stunted fingerlings at the farm level to produce quality fingerlings. In this regard, there is a knowledge gap in the field on the adoption of appropriate stocking density for fries to produce quality stunted fingerlings. To bridge this gap, the present study provided a geographically disaggregated analysis to assess the impact of stocking density in the production of stunted fingerlings. Exploiting the varied high stocking densities as three treatments, the results evidenced that at the study site, 50 fries/m^2 is the most optimum SD to consider while producing stunted fingerlings at least at the study site. According to statistical analysis in this study, SD higher than 50 fries/m^2 confronts low survival rates owing to high morbidity, mortality rates, and low SGR. The aquaculture of stunted fingerlings can offer several benefits for fish farmers. It can help overcome the challenges of limited fingerling availability and high production costs, ultimately leading to higher yields and profitability. However, the success of this approach requires adherence to best management practices, including proper pond management, regular monitoring of water quality, and re-stocking with fingerlings of the same species. Access to quality fingerlings, market demand, and genetic quality are crucial for success. Using stunted fingerlings in aquaculture can improve fish production’s efficiency and sustainability while providing economic opportunities for communities.

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