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RESEARCH ARTICLE

Effect of different stocking densities on growth performance, feed utilization, and survival of *Labeo angra* (Hamilton) fry in aquaria

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Abstract. This study was conducted to assess the effect of stocking density on the growth performance, feed utilization, and survival of *Labeo angra* (Hamilton) in nine glass aquaria each containing 30 l of water for 45 days. Three different stocking densities of 1, 1.5, and 2 fry l^{-1} of water (T_1, T_2 and T_3, respectively) were assessed in three replicates each. *L. angra* fry of approximately 0.32 ± 0.10 g weight were stocked and fed with a commercial feed four times daily at a rate of 15–30% body weight. Growth performance in terms of final weight (2.53 ± 0.22 g), weight gain (2.20 ± 0.05 g), percentage of weight gain (567.68 ± 15.25%), average daily gain (4.90 ± 0.11% day^{-1}), and specific growth rate (4.54 ± 0.04% day^{-1}) showed significantly higher values in T_2 compared to the other treatments. Significantly lower feed conversion ratios and the highest protein efficiency ratio were noted in T_2 (1.5 fry l^{-1}) diets. Significantly higher survival was observed in T_2 compared to T_1 and T_3. However, the results indicated that T_2 (1.5 fry l^{-1}) had more positive influence on the enhancement of growth performance, feed utilization, and survival of *L. angra* fry nursing in aquaria.

Keywords: feed conversion ratio, growth parameters, nursing, protein efficiency ratio, survivability

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Introduction

Aquaculture is the farming of fish and other aquatic organisms, with "farming" implying some form of intervention to increase production and some form of private rights of the stock under intervention (Beveridge and Little 2002). The nursing stage is one of the most critical stages to produce quality fingerlings. So, it is important to give the appropriate knowledge of nursing fry to farmers to produce quality fish production. Another important factor that has direct effects on the growth, survival, and production of fishes is to determine the optimum stocking density for each species (Backiel and Lecren 1978). Stocking densities and management measures practiced by nursery pond operators in Bangladesh are not based on scientific knowledge, thus resulting in poor fry growth and survival. To obtain maximum economic returns, it is necessary to stock nursery ponds at optimum stocking densities for optimum growth.

Approximately 260 freshwater fish species and 475 marine species are identified in Bangladesh (DoF 2016). Among the freshwater species, *Labeo angra* is the most important, which is known as angus, agun chokha, angrot, and karsa in Bangladesh. *L. angra* is a species of fish in the family Cyprinidae. The body of this fish is elongated and cylindrical, with a dorsal profile that is more convex than the ventral profile. It has a small mouth with thick, fimbriated, continuous lips, and large eyes that are not visible from the underside of the head. The fish features a pair of short maxillary barbels concealed in a labial fold, and its pectoral fin is as long as the head, while the caudal fin is deeply forked. The lateral line is complete, consisting of 42 moderate scales. In its mature state, the fish exhibits a brownish color along the back, with yellowish sides and abdomen, adorned with a black or bluish stripe along the flanks from the eye to the base of the caudal fin. According to Talwar and Jhingran (1991), the maximum length of *L. angra* is 22 cm. It is a herbivorous freshwater fish that can be found in several habitat types in rivers, lakes, and streams of Sylhet, Mymensingh, Dinajpur, Rangpur and the northern districts of the country (Rahman 1989), and it is found in rare numbers in the Tista River, the Atrai River (Mohonpur), and Chikli Lake in Rangpur. It is also distributed in India, Bangladesh, Nepal, and Burma (Talwar and Jhingran 1991). The species is of commercial importance as a food and sport fish (Froese and Pauly 2011).

L. angra is a delicious native fish in Bangladesh. The abundance of it has also declined drastically, and it was placed on the Red List (IUCN 2015). This might be due to the pollution of water bodies, construction of unplanned dams, the use of current nets, drying up of various water bodies, and destruction of habitats and breeding grounds. In this context, to conserve this fish species, breeding techniques and farming methods are under development for 37 species of native endangered fishes, including *L. angra*, by scientists of the Bangladesh Fisheries Research Institute (BFRI), Freshwater Sub-Station, Saidpur, Nilphamari in 2020. No work has been undertaken to evaluate the effect of different stocking densities on growth performance, feed utilization, or survival of *L. angra* in aquaria. Consequently, this study was undertaken to develop a suitable and economically viable technology for the production of *L. angra* fingerlings in aquaria. The specific objectives were: (i) to determine the effect of stocking density on the growth performance, feed utilization, and survival of *L. angra* in aquaria, and (ii) to evaluate the influence of stocking density on key water-quality parameters during the culture period.

Materials and Methods

Experimental site

The current investigation was carried out in nine glass aquaria at the hatchery facility of BFRI, Freshwater Sub-station, Saidpur, Nilphamari, in Rangpur division for 45 days (October 10 to November 23, 2023) (Fig. 1).

Design of the experiment

The experiment was performed with three treatments, T_1, T_2 and T_3, each in three replicates, R_1, R_2, and R_3. *L. angra* fry was collected from the nursery pond of the BFRI, Freshwater Sub-station, Saidpur, Nilphamari and brought to the hatchery complex. The fish were acclimatized for one hour before the study began. Thereafter, fish with an average body weight of 0.32 ± 0.10 g and an average total length (measured from the tip of the snout to the tip of the caudal fin) of 3.02 ± 0.34 cm were randomly stocked into the nine aquaria at the three different stocking densities of 1, 1.5, and 2 fry l^{-1} of water. Each aquarium was filled with 30 l of water, and the aquaria contained 30, 45, and 60 *L. angra* fry, which were designated as T_1, T_2 and T_3, respectively (Table 1), each in three replicates.

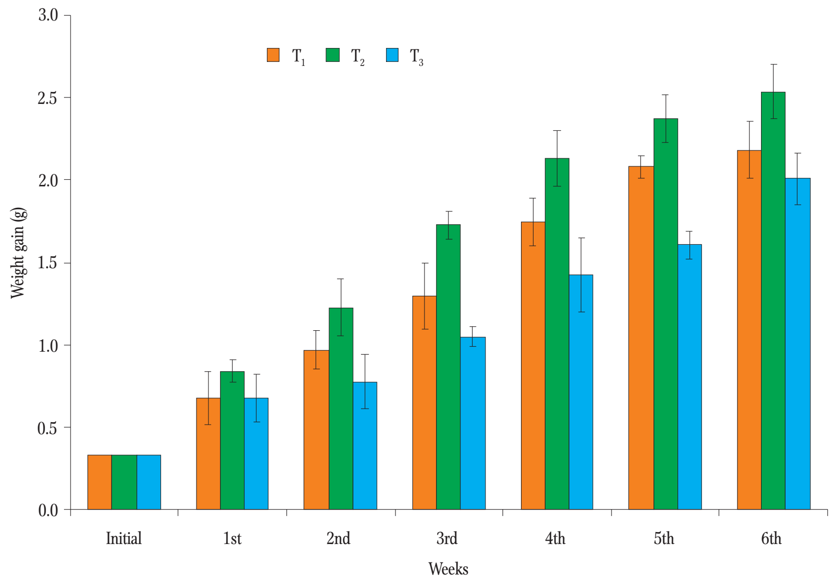

Figure 1. Change in body weight of *L. angra* fry over the course of the 6-week experiment.

Table 1
Design of the culture setup for *L. angra* under different stocking densities in aquaria

Treatment	Replication	Stocking density (fry litre^{-1})
T_1	R_1	1.0
	R_2	1.0
	R_3	1.0
T_2	R_1	1.5
	R_2	1.5
	R_3	1.5
T_3	R_1	2.0
	R_2	2.0
	R_3	2.0

Aquarium preparation and maintenance

Nine aquaria measuring 60 × 30 × 45 cm with a capacity of 30 l of water were used in this experiment. The aquaria were cleaned with a mild salt solution, and all the debris was siphoned out. The aquarium was then filled with clean water. Water from underground was supplied by an electric pump during the experiment. Continuous aeration was provided in each aquarium from an air stone that was connected to a central air compressor. Feces were siphoned out as required. The aquaria were placed in a bright spot with a net covering to prevent the fish from escaping.

Feed selection and feeding strategy

The fish were fed commercial powdered floating feed (Tiger Brand Eon Nursery Feed) four times daily in equal portions (06:00, 12:00, 18:00, and 24:00) for 45 days manually. The nutritional compositions of the experimental diets are presented in Table 2. The feed ration was 30% body weight for the first 15 days, 20% for the next 15 days and 15% for the last 15 days. Halim and Nabi (2022) previously provided commercial pellet feed with 30% protein content at 10 to 25% of fry body weight.

Table 2
Nutritional composition of commercial powdered (finely ground) floating feed (Tiger Brand Eon Nursery Feed) used in the experiment. The gross energy value of Tiger Brand Eon Nursery Feed is 2,800 kcal/kg feed

Component	Diet (%)
Moisture	12.0
Protein	28.0
Fat	7.0
Carbohydrate	30.0
Fiber	6.0
Ash	17.0
Calcium	2.2
Phosphorus	0.8

Water quality assessment

The parameters of water temperature, pH, dissolved oxygen (DO), and total dissolved solids (TDS) were collected from each experimental aquarium from 09:00 to 10:00 and recorded daily with a HANNA HI 98494 pH/EC/DO Multiparameter. Water quality in the aquaria was maintained by siphoning out the bottom debris (fecal matter, uneaten food) with a rubber hose 30 min after feed delivery to maintain proper water quality for fish growth and health. Approximately 30% of water was replaced, and the water level was adjusted by refilling the aquaria with clean water.

Sampling and data collection

The survival of the experimental fish was recorded during the feeding experiment. Individual fish length and weight were measured at the beginning and end of the feeding experiment, whereas bulk weighing was done weekly during the feeding trial to monitor growth and to correct the feed ration. In each replicate, sampling was done randomly with a scoop net. To prevent handling stress and mechanical injury, sampling was done with extreme caution. During sampling, the experimental fish were anaesthetized with clove oil at a dose of 0.02 ml l^{-1}. The fish were returned to their respective aquaria once the data had been collected. The length of the fish was recorded with a steel scale and weight was measured with a digital balance (EK 600i) to adjust the feed ration.

Determining growth performance, feed utilization, and survival of the fish

The growth of *L. angra* fry was monitored at intervals of seven days in each aquarium by sampling randomly with a scoop net. The following formulae were used to estimate growth parameters that were used previously by Akter et al. (2019, 2021):

a) Weight gain (g) = Final weight (g) – Initial weight (g)

b) Percentage of weight gain:

$$\frac{Final\ weight\,(g) - Initial\ weight\,(g)}{Initial\ weight\,(g)} \times 100$$

c) Average Daily Gain (ADG % day^{-1}):

$$\frac{Mean\ final\ weight\ (g) - Mean\ initial\ weight\ (g)}{Initial\ weight\ (g)} \times 100$$

d) Specific Growth Rate (SGR % day^{-1}):

$$100 \times (\ln \text{ the final body weight (g)} - \ln \text{ the initial body weight (g)}) \times \text{the rearing period (days)}^{-1}$$

e) Feed Conversion Ratio (FCR):

$$FCR = \frac{Total\ dry\ feed\ gain\,(g)}{Total\ wet\ weight\ gain\,(g)}$$

f) Protein efficiency ratio (PER):

$$PER = \frac{Wet\ weight\ gain\,(g)}{Total\ protein\ intake\,(g)} \times 100$$

g) Survival Rate (%): $= \dfrac{Final\ number\ of\ fish}{Initial\ number\ of\ fish} \times 100$

Data analysis

All data were tested using one-way Analysis of Variance (ANOVA). Significant results ($P < 0.05$) were further tested using one-way ANOVA followed by Duncan's Multiple Range Test (Duncan 1955) to identify significant differences among means. The data were expressed as mean ± standard deviation (SD), and the statistical analysis was performed using SPSS version 22 and Microsoft Office Excel for Windows.

Results

Water quality parameters

Water quality parameters, including temperature, pH, DO, and TDS were recorded regularly, and ranged between 21.2–24.0°C, 8.09–8.41, 7.98–8.71 mg l^{-1}, and 108–121 mg l^{-1}, respectively, and did not differ significantly ($P > 0.05$) among the three treatments (Table 3).

Table 3
Water quality parameters examined in different treatments of *L. angra* culture of the experiment over 45 days culture periods in tanks. Data presented as mean ± SD (standard deviation)

	Treatment		
Parameter	T_1	T_2	T_3
Temperature (°C)	22.45±0.86	22.38±0.87	22.32±0.78
	(21.20-24.00)	(21.16-23.74)	(21.18-23.22)
pH	8.20±0.08	8.18±0.05	8.19±0.09
	(8.09-8.41)	(8.09-8.25)	(8.07-8.40)
Dissolved Oxygen (mg l^{-1})	8.37±0.24	8.36±0.19	8.30±0.23
	(8.02-8.70)	(8.14-8.65)	(7.98-8.71)
Total Dissolved Solids (mg l^{-1})	112.11±2.72	110.72±1.64	112.11±3.86
	(109-118)	(108-114)	(110-121)

Table 4
Growth performance, feed utilization, and survivability in different treatments of *L. angra* over 45-day cultured periods in aquaria. Data presented as mean ± SD (standard deviation), obtained from three replicate tanks (n = 3). Data with different superscripts in the same row indicate significant differences (P < 0.05)

	Treatment		
Parameter	T_1	T_2	T_3
Initial average body weight (g)	0.32±0.10	0.32±0.10	0.32±0.10
Final average body weight (g)	$2.18±0.17^b$	$2.53±0.22^a$	$2.00±0.06^c$
Initial average length (cm)	3.02±0.34	3.02±0.34	3.02±0.34
Final average length (cm)	$5.23±0.31^b$	$5.41±0.30^a$	$5.13±0.40^c$
Weight gain (g)	$1.85±0.01^b$	$2.20±0.05^a$	$1.68±0.01^c$
Percent weight gain (%)	$461.62±3.50^b$	$567.68±15.25^a$	$408.08±3.50^c$
Average daily gain (% day^{-1})	$4.12±0.02^b$	$4.90±0.11^a$	$3.73±0.03^c$
SGR (% day^{-1})	$4.21±0.01^b$	$4.54±0.04^a$	$4.02±0.01^c$
FCR	$3.00±0.32^b$	$2.41±0.18^c$	$3.98±0.27^a$
PER	$1.20±0.13^b$	$1.49±0.12^a$	$0.90±0.06^c$
Survival rate (%)	$83.33±6.66^a$	$85.92±4.62^a$	$72.22±4.19^b$

Growth performance, feed utilization, and survival

The results of the study of *L. angra* fry in the aquaria are presented in Table 4 and Figures 1 and 2. Generally, growth parameters such as weight gain (2.20 ± 0.05 g), percentage of weight gain (567.68 ± 15.25%), SGR (4.54 ± 0.04% day^{-1}), feed utilization parameters such as FCR (2.41 ± 0.18) and PER (1.49 ± 0.12), and survival rates (85.92 ± 4.62%) were density dependent and significantly improved (P < 0.05) in treatment 2 (1.5 fry l^{-1}) compared to the other treatments.

Discussion

Growth, feed efficiency, and feed consumption of fishes are normally governed by a few environmental factors (Fry 1971). Among environmental factors, water quality parameters play a significant role in fish biology and in the productivity and well-being of

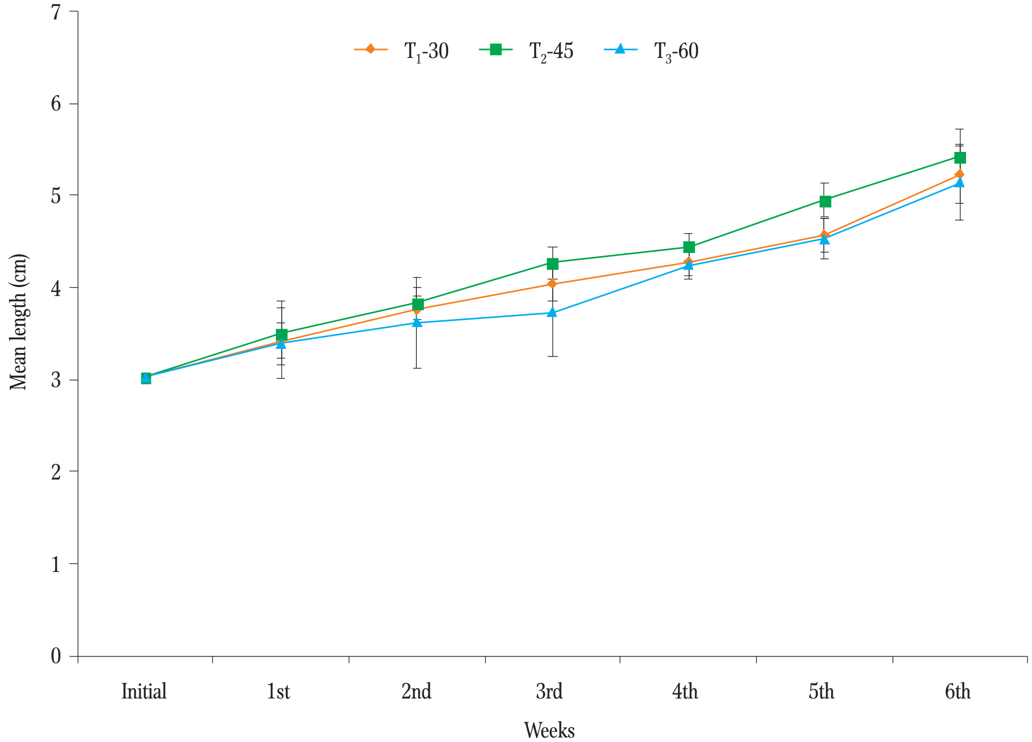

Figure 2. Weekly increases in *L. angra* fry length (cm) during the study period.

the culture system (Hassan et al. 2025). Good water quality is undoubtedly a prerequisite for fish growth and survival.

For successful *L. angra* culture, the water should be freshwater, well-oxygenated, and free from excessive pollutants. Ideal water quality parameters include a neutral to slightly alkaline pH (approximately 7.0–8.0), moderate temperatures (20–30°C), adequate dissolved oxygen levels (above 5 mg l^{-1}), low salinity (below 2 ppt), and safe limits of heavy metal concentrations as are recommended by WHO/FAO for optimal *Labeo* spp. growth (Chakraborty and Mirza 2007, Hossain 2014, Masood et al. 2023). The results of this research revealed that all the water quality parameters, namely temperature, pH, DO, and TDS, did not differ significantly among the treatments and were within acceptable ranges that were appropriate for the growth and well-being of *L. angra* fry. Ideal water quality parameters ensure that fishes are subjected to the least amount of stress, which, in turn, enhances growth (Boyd 1982, Ntsama et al. 2018). Similar findings regarding temperature, pH, DO, and TDS were also reported by Roy et al. (2002) for common carp (*Cyprinus carpio* L.) and small indigenous fish polyculture; Chakraborty and Mirza (2007) for endangered bata (*Labeo bata* (Hamilton)) fry; Rahman and Marimuthu (2010) for the endangered native fish climbing perch (*Anabas testudineus* (Bloch)); Priyadarshini et al. (2011) for common carp (*C. carpio*) fry; Kohinoor et al. (2012) for the stinging catfish (*Heteropneustes fossilis* (Bloch)); Nath et al. (2014) for Asian catfish (*Clarias batrachus* (L.)) fry; and Hossain (2014) for silver carp (*Hypophthalmichthys molitrix* (Val.)), mirror carp (*C. carpio*), and Roho labeo (*Labeo rohita* (Hamilton) fry nursing.

Stocking density is a vital parameter that may affect directly the growth of fishes and their feed utilization (Backiel and Le Cren 1978). The outcomes of this study clearly demonstrated that the stocking density significantly affected the growth performance, feed utilization, and survival of *L. angra* fry reared at different stocking densities in aquaria.

Growth in terms of weight gain (g) and SGR (% day^{-1}) of *L. angra* fry was significantly higher in T_2 where the stocking density (1.5 fry l^{-1}) was medium compared to those of T_1 and T_3 and in which stocking densities were 1 fry l^{-1}, 2 fry l^{-1}, respectively. Stocking densities that are too low can negatively affect the efficiency of rearing social and/or territorial fish species because of competition for food, increased appetite, and stressful conditions (Kozłowski and Piotrowska 2024). Consequently, the fish in T_3 had limited area and feed for the higher number fish stocked (Rahman and Marimuthu 2010), and this ultimately affected the community and increased stress. As a result, the fish expended more energy to relieve the stress that hindered their ability to develop soundly, which is in agreement with the study by Schreck (1982), who observed that stressed animals expend more energy to maintain homeostasis, which subsequently causes growth depression. The higher weight gain and SGR in the medium stocking density might have been due to the better availability and utilization of food. Similar findings also have been reported in *Labeo calbasu* (Hamilton) (Rahman et al. 2004) where stocking density was maintained at 172,900 individuals/ha, 148,200 individuals/ha, and 123,500 individuals/ha, while the initial length and weight were 1.00 ± 0.02 cm, and 0.013 ± 0.008 g, respectively; in the critically endangered *Tor putitora* (Hamilton) (Rahman et al. 2005) where stocking density was maintained at 0.6 million/hectare, 0.8 million/hectare, and 1 million/hectare, while the initial length and weight were 1.18 ± 0.04 cm and 0.012 ± 0.003 g, respectively; in *A. testudineus* (Rahman and Marimuthu 2010) stocking density was maintained at 1.0 million/hectare, 1.2 million/hectare, and 1.4 million/ha, while the initial length and weight were 0.44 ± 0.05 cm and 0.14 ± 0.05 mg, respectively, and in *C. carpio*, 1758 (Halim and Nabi 2022) at a stocking density maintained at 494 fry/m^2, 556 fry/m^2, and 618 fry/m^2, and the initial length and weight were 0.30 ± 0.05 cm and 0.28 ± 0.06 g, respectively.

FCR and PER are dietary intake indicators that are used to describe the rate of growth and feed utilization ability of farmed fish (Amin et al. 2005). Treatment 2 (1.5 fry l^{-1}) showed the most efficient FCR (2.41 ± 0.18) and PER (1.49 ± 0.12) values compared to the other treatments. This could be because fish in T_2 utilized the feed most efficiently in less crowded environments because of the smaller ration size, and higher digestibility, which is relevant to the findings of Mou et al. (2018) who reported improved FCR values at lower stocking densities while nursing *Mystus vittatus* (Bloch) fry. On the other hand, T_3 exhibited inefficient FCR (3.98 ± 0.27) and PER (0.90 ± 0.06) values that could have resulted from a stressful environment caused by a higher stocking density, which, in turn, increased competition for food and space thus making fish less efficient in their feed utilization. The FCR values of the present study are lower than those reported by the previous researchers (Reddy and Katro 1979, Islam 2002, Islam et al. 2002, Rahman et al. 2005). This might be because of proper utilization and higher digestibility of smaller size ration, which, in turn, increase growth performance at a lower stocking density. An increasing trend of FCR values was observed with the increasing ration size in Indian major carp (*L. rohita, Catla catla* (Hamilton) and *Cirrhinus mrigala* (Hamilton)), an air-breathing catfish (Clariidae) (Das and Ray 1989), and common carp (Ghosh et al. 1984) fed with supplementary feed. De Silva and Davy (1992) reported that digestibility played an important role in lowering the FCR value by the efficient utilization of foods. The digestibility of fish feed depends on many factors such as daily feeding rate, its frequency, and the type of feed used (Chiu et al. 1987). In addition, PER indicates the efficiency of utilization of dietary protein. Similar to the present findings, the highest PER value was found at the medium stocking density (1.5 fry l^{-1}) when climbing perch (*A. testudineus*) were reared in cages (Habib et al. 2015).

Previous studies have shown that stocking densities that are both lower and higher than optimum can induce stress in fishes (Brown et al. 1992, Jorgensen et al. 1993, Barlaya et al. 2021). Therefore, optimum stocking density must be evaluated for individual fish species.

Significantly higher survival rates were obtained with the fish in T_2 (85.92 ± 4.62%), where the stocking density was medium compared to T_1 (83.33 ± 6.66%) and T_3 (72.22 ± 4.19%). The causes for the decreasing survival rates in higher stocking densities for the fry of reba carp, *Gymnostomus ariza* (Hamilton), are competition for food and space in experimental ponds (Rahman et al. 2009). These results are similar to those previously reported by Uddin et al. (1988), Saha et al. (1989), Haque et al. (1993), Haque et al. (1994), Kohinoor et al. (1994), Rahman et al. (2005), Asadujjaman et al. (2013) during fry rearing experiments of various indigenous and exotic carp and barb species. In contrast to the present investigation, Arifin et al. (2017) showed stocking density had no indicatory or direct effect on barb, *Barbonymus balleroides* (Val.), survival. Similarly, Kunda et al. (2021) indicated that survivability of Nile tilapia did not vary significantly along with stocking density. The differences in the results among various studies might stem from the species being cultured, the culture periods, and the conditions, the stocking densities, the feeds used, and feeding rates and frequency, etc.

Conclusion

Finally, it can be concluded that the growth, feed utilization parameters and survival of *L. angra* fry showed an increasing trend up to T_2, thereafter it showed a decreasing trend. The results should be treated with caution because the stocking densities and the difference in densities between the treatments were low. Further studies should be conducted using larger numbers of individuals. Based on the results of the experiment, farmers could be advised to nurse *L. angra* fry for 45 days at medium stocking densities (1.5 fry l^{-1}) to achieve higher quality fingerling production for 45 days in aquaria, which will ultimately help to improve their economic status and livelihoods. Standardizing stocking density with economic profitability of *L. angra is* required before this culture technology is disseminated widely to farmers.

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Author contributions. A. conducted the experiment, data collection, analysis, and manuscript writing. M.N.A. oversight and leadership responsibility, mentorship, and manuscript editing. S.K.S. M.S. co-supervisor, planning, design and execution of the research activity, and manuscript editing. A.A. experimental set-up, data collection and analysis. M.H.S.D. helped in conducting the experiment, data collection, and analysis.

Conflicts of interest. The authors declare no conflict of interest.

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