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Nursery rearing of Amur carp (*Cyprinus carpio haematopterus*) fry in floating cages in a tropical large reservoir of India: Influence of stocking density on growth performance and survival

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Abstract

The study was carried out for 90 days in floating net cages installed at Maithon Reservoir, Jharkhand, India, with the aim of assessing the growth performance and survival of Amur carp fry at varied stocking densities. The Amur carp seed (4.38±0.19 cm and 2.08±0.28 g) was raised in galvanised iron (GI) cages at three stocking densities (SD) of 100, 200 and 300 nos. m^3, *i.e.,* SD 100, SD 200 and SD 300 were considered as low, medium and high stocking densities. The stocked fish were fed with commercial floating pellets containing 32% crude protein and 4% crude fat, at a rate of 5-3% of their body weight, twice daily (at 10:00 and 16:00 hrs). The water quality parameters were checked from within the cage and at distances of 50 and 100 m from the cages on a monthly basis. The results of this study demonstrated that stocking density had a significant impact on the growth performance and survival rate of Amur carp. The growth attributes, comprising weight gain (WG), absolute growth rate (AGR) and specific growth rate (SGR), were considerably higher at a lower SD of 100 compared to a higher SD of 300. SD 100 exhibited greater feed efficiency and effective feed utilisation compared to SD 300. No significant difference was observed in condition factor (K) among the treatments. The findings of the current investigation indicated that the stocking densities of 100 and 200 numbers m^{-3} showed no significant variations in terms of feed utilisation, growth, or survival. The results of the present study showed that stocking densities ranging from 100 to 200 numbers m^{-3} may be optimal for the higher growth performance and survival of Amur carp in inland cages. The viability of rearing Amur carp in cages for nursery purposes was investigated for the first time, in this study.

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Introduction

In fish rearing system, stocking density may have a direct or indirect impact on fish growth, productivity, behaviour and health status (El-Sayed, 2002; Kohli, *et al.,* 2002; de Oliveira *et al.,* 2012). In case of under-stocking, there may be underutilisation of the available space which leads to poor production from cages, whereas overstocking may lead to poor survival and production of smaller individuals due to crowding stress (Rahman *et al.*, 2006). Higher stocking densities may suppress fish growth due to factors that include social interactions and water quality degradation, which can affect the fish's feed consumption and feed conversion efficiency (Ellis *et al.,* 2002). Hence, there is a need to optimise the stocking density of the candidate species for successful production from inland cage culture. In India, with the vast area of about 3.5 million ha, covering 15 states, reservoirs form an important source of fish production. With the widespread use of cage culture technology, these reservoirs have the potential to produce large quantity of fish (Sarkar *et al.*, 2018).

Indian freshwater cage culture is dominated by *Pangasianodon hypophthalmus* due to its fast growth and hardy nature but this species has low consumer preference in the domestic market. Therefore, there is a necessity for the diversification of fish species for inland cage culture for augmenting the production and adoptability (Karnatak *et al.,* 2021; Upadhyay *et al.,* 2021). Amur common carp are known for their better growth performance over the current local strain with a mean increase of 27.3%, with great ability to utilise the natural food and better food conversion rate (Basavaraju and Reddy, 2013). Availability of quality seed throughout the year is the major constraint for inland cage culture. Further, the long-distance transport of the fingerlings from distant seed farms to the cage site is uneconomical as it cause heavy mortality due to transportation stress and involves high transportation cost. Poor survival of the seed was also observed in the cages after stocking due to improper acclimatisation in cage environment. Hence inland cages can be effectively used as *in situ* nursery rearing system for raising stocking material for grow-out culture and reservoir stocking within the same environment. This is one of the first studies to investigate the feasibility of rearing Amur carp in floating net cages during the nursery phase, which is a novel approach for producing quality fingerlings on-site. The study aimed to generate baseline data and standardised protocols for the culture of Amur carp in cages, which can facilitate the adoption of this species in inland reservoir cage culture operations.

Materials and methods

Experimental site and set up

The present study was conducted in Maithon Reservoir, Jharkhand, India (23°47'36.1"N 86°49'00.1"E) from May to August 2021. With a water spread area of 10,716 ha. Maithon Reservoir falls into the category of large reservoirs. It is situated on the river Barakar, a tributary of the river Damodar. The experiment was conducted in nine Galvanised Iron (GI) model square cages, which was developed and commercialised by ICAR-CIFRI, Barrackpore, India. The dimension of the cage was 5 x 5 x 2.5 m with an effective water volume of 50 m^3.

Experimental fish, design and cage maintenance

The seed of Amur carp were collected from nearby fish farm and acclimatised in cages for 15 days. The fry of Amur carp (4.38±0.19 cm and 2.08±0.28 g) were stocked at three different stocking densities (SD) *i.e.* 100 numbers m^{-3} (5000 numbers per cage), 200 numbers m^{-3} (10000 numbers per cage) and 300 numbers m^{-3} (15000 numbers per cage) in cages, in triplicate as per Complete Randomised Design (CRD). Fish were treated with $KMnO_4$ (5 mg l^{-1}) and salt (2%) prior to stocking, as a prophylactic measure to prevent occurrence of diseases. The commercial floating pellet feed, which contained 32% crude protein and 4% crude fat, was initially given to the fish in two regimes (10.00 and 16.00 hrs) each day at 5% body weight and was then gradually reduced to 3% based on data on daily food intake and growth. The feed was given in circular plastic feeding trays (35 cm diameter), two numbers placed in each cage. To ensure free water exchange, the nets were cleaned periodically and no antibiotics or other harmful chemicals were used in the cages.

Analysis of water quality parameters

To assess the effect of cage culture on the surrounding reservoir environment, water quality parameters were monitored monthly, both inside and outside the cages. Outside the cage, water was sampled for analysis from two sites *i.e.,* at 50 and 100 m distance. YSI-Pro DSS multi-parameter water quality metre® was used to record the parameters including dissolved oxygen (mg l^{-1}), water temperature (°C), depth (m), specific conductivity (S cm^{-1}), pH and total dissolve solids (mg l^{-1}). Transparency was measured using secchi disc. Titrimetric analytical method was used to analyse total alkalinity, total hardness and other nutrients such as silicates, nitrates and phosphate as per APHA (2012).

Growth performance

Monthly samplings were carried out to assess the growth performance of the fishes. Prior to sampling, the fishes were starved for about 24 h and 30 individuals were randomly collected from each cage by partially lifting the cage net and growth data were recorded by measuring individual length (cm) and weight (g). The growth attributes such as weight gain (WG), absolute growth rate (AGR), relative growth rate (RGR), specific growth rate (SGR), survival (%) and condition factor (CF) were calculated to estimate growth performance using the following formulae:

$$\text{WG (g)} = W_f - W_i$$

$$\text{AGR (g)} = (W_f - W_i)/t$$

$$\text{RGR (\%)} = (W_f - W_i)/W_i \times 100$$

$$\text{SGR (\%/day)} = (\ln W_f - \ln W_i)/t \times 100$$

where, W*i* and W*f* are initial and final weights (g), respectively and t is the duration of the experiment (days).

Feed conversion ratio (FCR) = Total feed intake/Total weight gain of fish

Feed conversion efficiency (FCE) = Total weight gain of fish / Total feed intake

Protein efficiency ratio (PER) = Total weight gain of fish/ Protein intake

Survival rate (%) = (Total number of fish harvested /Total number fish stocked) x 100

Length-weight relationship (LWR) was estimated using the equation $W = aL^b$ where W = Weight of fish in g; L = Length of fish in cm; 'a' and 'b' are intercept and slope of the regression line respectively (Ricker, 1973).

Statistical analysis

One-way analysis of variance (ANOVA) was used to evaluate the experimental data in SPSS (version 22) and the results were given as mean±standard error (SE). Significant differences between means were assessed using Duncan's multiple range tests at 5% level of significance ($p<0.05$).

Results

Water quality parameters

The important water quality parameters *viz.,* dissolved oxygen, pH, conductivity and transparency showed no significant variation ($p>0.05$) among inside and outside the cages (reference sites, 50 and 100 m distance from cage site) (Table 1). During the study period, the water temperature ranged from 27.31-30.5^{0}C, dissolved oxygen ranged from 6.04-6.95 mg l^{-1} at the cage site, and at intermediate (50 m) and reference site (100 m). The pH values ranged from 7.25-8.39 in the three locations. Total alkalinity and hardness ranged from 52.75 to 72 mg l^{-1} and 53 to 67.87 mg l^{-1}, respectively, with mean values showing small variation among the cage and reference sites with no significant difference between different locations. At the cage site, the nutrients such as silicate, phosphate and nitrate were insignificantly ($p>0.05$) high, compared to the reference sites. All the important water quality parameters were found in suitable range for carp culture.

Growth performance

The growth, survival and feed utilisation of the stocked fishes, was significantly affected by the stocking density (Table 2). The study revealed that, the growth performance in terms of weight gain (WG), absolute growth rate (AGR), relative growth rate (RGR) and specific growth rate (SGR) decreased significantly ($p<0.05$) with increment in the stocking density. The higher WG was achieved in lower stocking density of SD 100 followed by SD 200 and lowest value was recorded in SD 300. The SGR ranged from 3.29±0.10 (SD 300) to 3.62±0.03 (SD 100). The growth pattern of Amur carp showed that, the average weight of the fish, reared at various stocking densities showed insignificant variations ($p>0.05$) up to 30 days of the culture period. At 60 days and beyond, significant differences were seen ($p<0.05$). (Fig. 1).

Feed utilisation

The FCR value ranged from 2.18±0.11 to (SD 100) to 2.92±0.12 (SD 300) and showed better feed efficiency at lower stocking density of SD 100 with no significant difference from SD 200. The lower FCE and PER were observed at higher stocking density of SD 300. The lower survival was observed at higher density of SD 300 than SD 100. No noticeable differences were observed in growth attributes, survival and feed efficiencies among the stocking densities of SD 100 and SD 200.

Length-weight relationship

The length-weight relationship variables for Amur carp are depicted in Table 3. The length and weight of the species found within ranges from 3.2 - 17.6 cm (TL) and 1.10 - 65.5 g (body weight) for SD 100; 3.9-18 cm and 1.08-75.8 g for SD 200 and 4.0-18.6 cm and 1.26-83.4 g for the SD 300. The length-weight relationship was estimated for each stocking density and the growth pattern is shown in Fig. 2. In the present study, the correlation coefficient, 'R^2' ranged from 0.93-0.96 and the slope or regression coefficient, 'b ranged between 2.92-2.96. Negative allometric growth pattern was observed in all three stocking densities. In the present study,

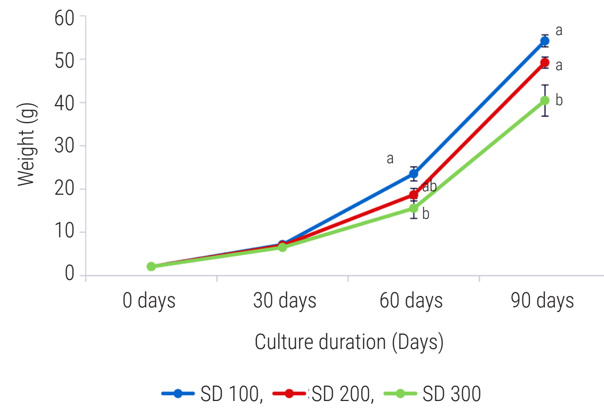

Fig. 1. Growth pattern of Amur carp reared at various stocking densities in cages

Table 1. Important water quality parameters recorded from inside and outside the cages

Water quality parameters	Inside cage	50 m	100 m
Water temperature (^{0}C)	29.40±0.99	29.65±1.02	29.46±1.09
Depth (m)	15.14±1.61	19.12±1.41	19.88±1.65
Transparency (cm)	67.41±21.57	68.89±21.84	68.23±21.27
pH	7.79±0.29	7.87±0.28	7.97±0.28
DO (mg l^{-1})	6.35±0.22	6.44±0.21	6.46±0.27
Specific conductivity	174.22±23.11	175.83±23.02	176.83±22.91
Total alkalinity (mg l^{-1})	64.03±5.80	64.36±5.43	62.5±4.44
Total dissolved solids (mg l^{-1})	110±13.28	107.5±13.43	106.91±12.78
Hardness (mg l^{-1})	61.37±3.85	61.47±4.42	60.87±3.11
Ca^{++} (mg l^{-1})	19.32±1.70	17.75±1.56	17.95±1.36
Mg^{++} (mg l^{-1})	8.06±0.20	7.53±0.31	7.45±0.24
Silicate (mg l^{-1})	10.36±1.96	9.04±2.12	9.08±1.98
Chloride (mg l^{-1})	24.04±0.87	24.27±1.03	24.47±1.23
Nitrate-N (mg l^{-1})	0.29±0.04	0.21±0.03	0.21±0.01
Phosphate-P (mg l^{-1})	0.10 ± 0.01	0.08±0.01	0.09±0.02

Values are Mean±S.E. (n = 3)

Table 2. Growth attributes of Amur carp reared at various densities in cages

Parameters	SD 100	SD 200	SD 300
Initial weight (g)	2.08±0.28^a	2.08±0.28^a	2.08±0.28^a
Final weight (g)	54.22±1.39^a	49.22±1.28^a	40.45±3.59^b
Weight gain (g)	52.14±1.39^a	47.14±1.28^a	38.37±3.59^b
AGR (g)	0.58±0.01^a	0.52±0.01^a	0.43±0.04^b
RGR (%)	2506.89±66.69^a	2266.51±61.42^a	1844.87±172.81^b
SGR (%)	3.62±0.03^a	3.52±0.03^a	3.29±0.10^b
FCR	2.18±0.11^b	2.35±0.21^b	2.92±0.12^a
FCE	0.46±0.02^a	0.43±0.04^{ab}	0.34±0.01^b
PER	1.65±0.08^a	1.54±0.14^{ab}	1.23±0.05^b
Survival rate (%)	61.33±1.20^a	59.37±1.64^{ab}	54.48±1.91^b
CF (K)	2.57±0.17^a	2.56±0.18^a	2.23±0.22^a

Values are Mean±standard error. Different superscripts of mean values in the same row differ significantly ($p<0.05$). (n = 30 for weight measurements, n = 3 for estimation of WG, AGR, RGR%, SGR%, FCR, FCE, PER, survival rate % and CF)

WG - Weight gain (g); RGR% - Relative growth rate; SGR% - Specific growth rate; FCR - Feed conversion ratio; FCE - Feed conversion efficiency; PER - Protein efficiency ratio; CF - Condition factor

Table 3. Parameters of length-weight relationship (LWR) of Amur carp reared at various stocking densities in cages

Treatments	N	Length (cm)		Weight (g)		LWR variables			Growth pattern
		Minimum	Maximum	Minimum	Maximum	a	b	R^2	
SD 100	103	3.20	17.60	1.10	65.50	1.58	2.92	0.96	A^-
SD 200	103	3.90	18.00	1.08	75.80	1.65	2.96	0.93	A^-
SD 300	103	4.00	18.60	1.26	83.40	1.60	2.92	0.96	A^-

N - Size of sample, a and b - Length-weight relationship parameters, R^2 - Correlation coefficient, A^- - Negative allometry

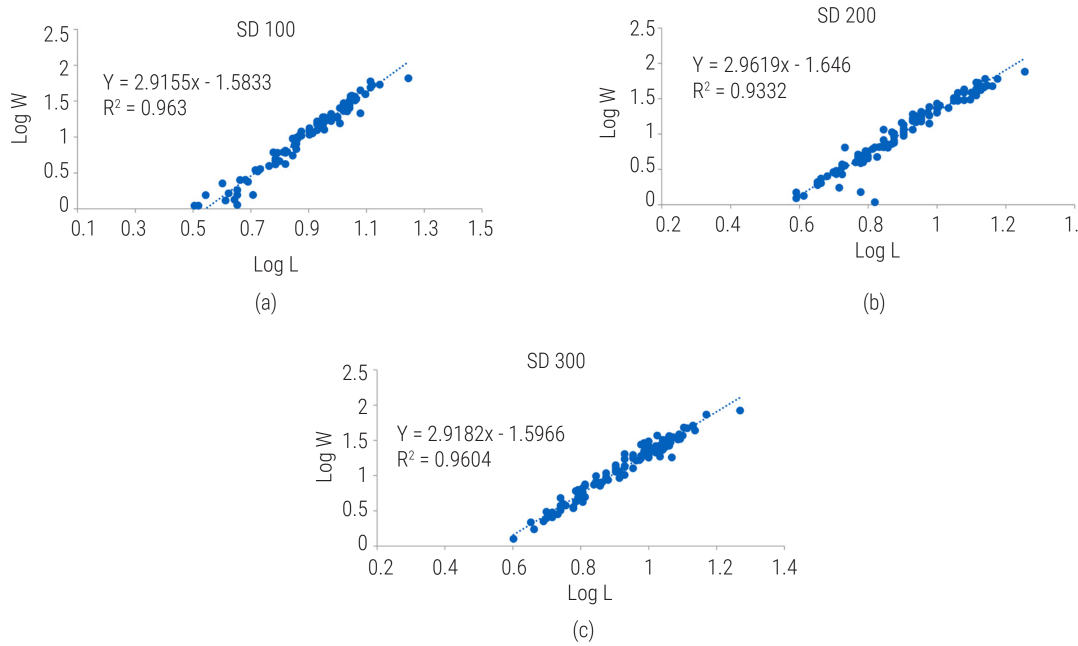

Fig. 2. Length-weight relationship of Amur carp at various stocking densities

the condition factor in different stocking densities ranged from 2.23±0.22 (SD 300) to 2.57±0.17 (SD 100). The K value reduced with increment in the stocking density but no significant difference (p>0.05) was observed among the treatments (Table 2).

Discussion

The results of the current study revealed that, all the water quality parameters were in the suitable range for fish culture (Boyd, 1982; Beveridge, 1996) throughout the study period. The physico-chemical parameters indicated that the cage culture operation had negligible impact on reservoir environment, as no significant variation was recorded in the water quality at cage site and reference sites (at 50 and 100 m away from cage). This could be attributed to small-scale cage culture operation undertaken during the study and proper feed management in relation to the reservoir's large size (Lianthuamluaia *et al.,* 2019). Similar kind of observations have been previously reported in reservoirs (Devi *et al.* 2015; Karnatak *et al.*, 2021; Upadhyay *et al.*, 2022; Ramteke *et al.,* 2023) and wetlands (Yengkokpam *et al.*, 2020).

Optimisation of stocking density for the candidate fish species, for inland cage culture is the critical factor for developing an efficient cage culture system, as overcrowding may negatively affect the growth performance of the fishes (Swain *et al.*, 2022). In the current study, Amur carp grew to an average weight of 40.45 to 54.22 g in nursery cages over a 90-days culture period at stocking densities ranging from 100 to 300 numbers m^{-3}. Singh *et al.*, (2020), reported that, amur carp achieved growth of 30.83 g in 500 l FRP tank in 90 days at stocking density of 20 fish per tank whereas, in small poly-lined ponds in hilly region of India, it grows up to 7.84 g in 10 weeks at a stocking density of 3 lakhs fry ha^{-1} (Das *et al.,* 2020). The growth of Amur carp in nursery cages was comparatively higher than that recorded from tank and pond systems. In the current study, an inverse relationship was derived between stocking density and fish growth performance, with increase in stocking density resulting in decrease in final body weight, WG, AGR, RGR and SGR. Increased stocking density altered the individual's space availability, which affects social interactions and increases stress on the fish, possibly resulting in decreased growth and feed efficiency (Ellis *et al.,* 2002; Biswas *et al.*, 2015; Yengkokpam *et al.*, 2020). A similar effect of stocking density on the growth attributes, in different culture systems was reported by several researchers for Nile tilapia *Oreochromis niloticus* (Gibtan *et al*., 2008; Chakraborty *et al.*, 2010); Atlantic salmon *Salmo salar* (Liu *et al.*, 2017); rohu *Labeo rohita* (Chattopadhyay *et al.*, 2013; Swain *et al.*, 2022); striped catfish, *P. hypophthalmus* (Chowdhury *et al*., 2020; Ramteke *et al*., 2023); bata *Labeo bata* (Yengkokpam *et al.*, 2020; Karnatak, *et al.*, 2021) and olive barb *Puntius sarana* (Upadhyay *et al.,* 2022). In contrast, studies by various authors showed no specific relationship between stocking density and growth characteristics in Asian river catfish *Pangasius bocourti* (Jiwyam, 2011); sutchi catfish *Pangasius* *sutchi* (Rahman *et al.,* 2006) and African catfish *Clarias gariepinus* (Hengsawat *et al.,* 1997). The effect of stocking density may differ depending on the mode of culture system, species, stocking size, growth phase, nutritional and health status of the fish (Tolussi *et al.,* 2010). In present study, no significant variation was observed in growth trend of Amur carp up to 30 days of culture and a noticeable variation was observed beyond that.This suggests that phase rearing of Amur carp may be beneficial for better growth as it can be cultured at higher density for up to 30 days of duration and thereafter thinning of the stock can be done. Similar observation was reported in *L. bata* raised up to 60 days (Karnatak *et al.*, 2021) and in *Ompok bimaculatus* raised up to 120 days (Karnatak *et al.*, 2021a) at high stocking density, where no significant variations were observed in growth performance.

In the current study, stocking density had a significant impact on feed utilisation and efficiency in terms of FCR, FCE and PER. Lower stocking densities of SD 100 and SD 200 resulted in higher feed utilisation and efficiency. Fish compete for food and space at higher stocking densities, resulting in lower growth and feed utilisation (Li and Brocksen, 1977; Ellis *et al.*, 2002; Ouattara *et al.*, 2003; Gibtan *et al.,* 2008). The crowded environment triggered an increased energy demand, which altered the metabolism and decreased appetite and food intake to combat the stress condition, leading to a reduction in the available energy for growth (Karnatak *et al.*, 2021; Swain *et al.*, 2022). In the present study, the stocking density significantly affected the survival rate in different treatments, with lower survival observed at higher density of SD 300, which recorded 12.57% lower survival rate than SD 100. However, higher mortality rate was reported initially for the first two weeks after stocking, which could be attributed to the transportation and handling stress. Once the fishes were acclimatised properly, the mortality was drastically reduced.

The length-weight relationship of fish is an important tool for assessing the general well being and nutritional status of the fish species in growth studies (Pepin, 1995; Sheikh and Ahmed, 2018). In the present study, the b value ranged from 2.92 to 2.96, which indicated negative allometric growth of the fishes (Pauly, 1984). Juvenile fishes invest more energy in length than in weight, which may be different for adult stage (Possamai *et al.,* 2019). According to Le Cren (1951) and Chauhan (1987), fish usually do not maintain the same shape or body conformance across their various life phases. Negative allometric growth pattern have been reported in *Channa punctata* (Ali *et al.*, 2002; Haniffa *et al*., 2006); *C. maurulius* (Dua and Kumar, 2006); *C. striatus (*Khan *et al.*, 2011) and in *Clarias gariepinus* (Okomoda *et al.,* 2018).

The condition factor (K) is an important tool to assess the physiological wellbeing of fish in relation to growth performance, health and nutritional prosperity of fish in different culture environment (Ofor and Pepple, 2012; Datta *et al.*, 2013; Karnatak *et al.*, 2021). K value close to one indicates overall fitness of fish (Blackwell *et al.*, 2000). In the current study, the K value from 2.23 to 2.57 and was insignificantly higher at lower stocking density, indicating that the fishes were in better condition. Several other studies also reported better condition of the fishes at lower densities (Fatima *et al.*, 2018; Ondhoro *et al.*, 2019; Shao *et al.*, 2019; Karnatak *et al.*, 2021). The condition factor of Amur carp varied from 1.32-1.79 in pond system (Soranganba and Singh, 2019) and that of common carp from 1.48-4.97 in open waters (Al-jebory *et al.,* 2018; Das *et al.,* 2019). In the present study, K values were >1 indicating, the fishes were in good physiological condition in the culture system.

Availability of quality seed throughout the year is the major constraint for inland cage culture. Producing quality fingerlings on-site can increase profitability by lowering seed and transportation costs, as well as mortality. Hence, inland cages can be effectively used as *in situ* nursery rearing systems for raising stocking material for grow-out culture. Amur carp was selected as candidate species for inland cage culture for species diversification of cage culture. This is the first of its kind study to assess the feasibility of rearing Amur carp in inland cages during nursery phase. The results of the current study showed that, the fishes stocked at 100-200 nos. of fry m^{-3} can be considered as optimum for nursery phase rearing in cages based on their growth attributes, survival and feed utilisation. The information generated in the present study will serve as baseline for the standardisation of stocking density of Amur carp for further development of package of practices for inland cage culture in Indian reservoirs.

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