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Efficacy of different types of live and non-conventional diets in endangered clown knife fish *Chitala chitala* (Hamilton-Buchanan) during its early life stages

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Abstract

In the present study, the growth and survival of early life stages (ELS) of *Chitala chitala* were studied in nylon hapa for 28 days, followed by rearing in fibreglass reinforcement plastic (FRP) tanks for a period of 30 days. Ten-day-old ELS of *C. chitala* reared in hapa were fed with three different diets namely Indian Major Carp (IMC) spawn (<8 mm), live tubifex and fresh fish eggs. In the second phase, 28-day-old ELS were stocked in 200-lit FRP tank and supplied four different live diets namely live tubifex worm, chironomous larvae, zooplanktons and mosquito larvae. Fish accepted all types of diets in the experimental rearing period in both the systems. The experiments conducted in hapa showed a higher specific growth rate (SGR), weight gain per cent and survival rate in larvae fed with live tubifex (SGR $= 1.76 \pm 0.02$) than fish eggs (0.77 ± 0.31) and IMC spawn (0.46 ± 0.12). The study carried out in FRP tanks revealed that SGR was higher in ELS fed on chironomous larvae (4.44 ± 0.61), followed by mosquito larvae (3.29 ± 0.40) and live tubifex (3.28 ± 0.36), whereas minimum SGR was recorded with zooplanktons (2.84 ± 0.66). A significant difference ($P<0.05$) in SGR, final mean weight and weight gain (%) was also recorded. The highest mean survival rate (100%) of ELS in an FRP tank was observed in chironomous larvae and zooplanktons, whereas with live tubifex and mosquito larvae the same survival rate (80%) was recorded. The rate of survival of the ELS reared in hapa varied from 65% to 85%. The experiments showed that ELS of *C. chitala* could be reared successfully in hapas and fibreglass reinforcement tanks for attaining better survivability and growth.

Keywords: endangered fish, *Chitala chitala*, diets, early life stages, growth and survival

Introduction

Selection of a new freshwater fish species with a potential value for aquaculture is of tremendous importance for the sustainable development of the aquaculture as well as stock enhancement and conservation programme. In India, the *Chitala chitala* constitutes an important part of riverine fisheries and is considered to be one of the most commercially important and highly priced food fish due to its rarity and delicacy. Recently, this species has been prioritized as a new candidate for fresh water aquaculture (Ponniah & Sarkar 2000; Ayyappan, Raizada & Reddy 2001). They are generally carnivorous and insectivorous in nature but sometimes they feed on crustaceans and planktons and change their feeding habits completely at the early stage and start feeding voraciously on carp fry and aquatic insects (Alikunhi 1957). In recent years, there has been a steady decline in wild stocks in India due to overfishing, habitat degradation and pollution, etc and the species has been categorized to be endangered according to International Union for Conservation of Nature and Natural Resources criterion (Camp 1998). Although a successful captive-breeding protocol and larval rearing under a recirculatory system have been developed by the author and his colleagues (Sarkar, Deepak, Negi, Singh & Kapoor 2006; Sarkar, Lakra, Deepak, Negi, Paul & Shrivastava 2006) for the first time, the suitability and adaptability of successful rearing of

early life stages (ELS) in other simple rearing systems [hapa, fibreglass reinforcement plastic (FRP) tank] have not been determined. Therefore, the development of farmer-friendly rearing techniques is essential for producing seed for aquaculture as well as conservation. This paper presents new information on the feeding, growth and survival rates of ELS of *C. chitala* fed on different live and non-conventional diets in two different rearing systems.

Material and methods

The experiment was conducted in 2003–2004, during the ELS of *C. chitala*. Wild adults were spawned by an induced spawning technique developed by the authors (Sarkar, Deepak *et al.* 2006). The newly hatched larvae were mainly fed on egg yolk, live mixed zooplankton, predominantly consisting of copepods and cladocerans in rectangular nylon hapas fitted in the nursery pond arranged in rows (0.06 ha) for the first 5–7 days before commencement of the experiments.

Experimental set-up

Two sets of experiments were considered in this study. The first set of experiments of the ELS of *C. chitala* was conducted in rectangular-shaped hapas (nylon make), whereas the second set was carried out in rectangular-shaped FRP tanks. The hapa measured about 7 × 3 × 1.5 ft arranged in different rows in an earthen pond (2 ha, average depth 1 m), and the FRP tanks measured 4.7 × 3.2 × 2.3 ft arranged in rows in the wet laboratory. Artificial hideouts, hollow nylon pipes measuring 50 × 10 cm, bricks arranged like a hut. Each hapa was stocked with 100 no of 7–10-day-old larvae for a period of 28 days, whereas each FRP tank was stocked with 15 no of 25–30-day-old larvae and was kept for 30 days. Before conducting any experiment, the ELS were initially stocked in nylon hapas and FRP and kept for 5 days for acclimatization. Separate hapas and FRP tanks were used for each of the diets and three replicates were considered for each treatment. The larval rearing experiment were conducted in hapas with three different feeds namely Indian Major Carp (IMC) spawn (< 8 mm), live tubifex and fish eggs [*Puntius ticto* (Hamilton-Buchanan), *Labeo bata* (Hamilton-Buchanan)]. Experiments in FRP tanks were conducted with four different feeds namely live tubifex, chironomous larvae, zooplanktons and mosquito larvae. In both the experiments, fish were fed at 10% body weight per day during the first week, 8% per day during the second week and 5% per day during the third and fourth weeks. Rations were given twice daily i.e. one each for morning (10:00 hours) and evening (16:00 hours) feedings. The total length (TL) of larvae stocked initially in hapa ranged from 24.42 ± 1.74 to 27.92 ± 1.94 mm and body weight ranged from 1.35 ± 0.07 to 1.73 ± 0.008 g, whereas in FRP tanks the TL of post larvae ranged from 42.13 ± 4.12 to 45.08 ± 5.78 mm and body weight ranged from 0.46 ± 0.05 to 0.60 ± 0.13 g.

Live tubifex and chironomus larvae were collected from nearby river Gomti close to Lucknow city, Uttar Pradesh, India. Mosquito larvae were cultured outside the wet laboratory whereas zooplanktons were cultured in the laboratory. Each hapa and tank was stocked with healthy individuals during the rearing experiment. Fish eggs were collected by dissecting fresh gravid *P. ticto* (Hamilton-Buchanan) and *L. bata* (Hamilton-Buchanan) (order Cypriniformes, family Cyprinidae) daily, which were cultured in an uncontaminated pond. Eggs were washed with tap water to remove blood vessels before placing them in the experimental sets. In the hapa, sprinklers were fitted from one side for water circulation through an overhead tank. In the FRP tanks, about 50% of the water volume was exchanged each time daily by means of siphoning and refilled, so that the fish were sampled for length and weight increment. Each FRP tank was provided with an aerator. Water exchange was performed in the morning at 10:00 hours before feeding. Every seventh day, 10 post hatchlings were sampled. They were placed in a paper towel in order to absorb water and weighed on an electronic balance to an accuracy of 0.01 mg. The length of the fish was measured by a digital caliper (Mitutoya make) with an accuracy of 0.01 mm. Survival rates were calculated by taking into account the remaining and discarded individuals. The specific growth rate (SGR) was calculated to determine the growth performance during the experimental period according to the following relation: $\text{SGR} = 100\ (\text{Ln}\, W_2 - \text{Ln}\, W_1)/\Delta t$, Weight gain (%) $= W_2 \times 100/W_1$, where W_2, W_1 = final and initial body weight.

Weight gain (%) per day = weight gain (%)/number of days.

The behaviour of the ELS of fish was also observed during the course of the experiment, especially during feeding. The other parameters like weight gain (%) survival (%) were also recorded.

Table 1 Results of larval rearing experiments of *Chitala chitala* in hapa (28 days)

Parameters feed	Initial mean length (mm ± SD)	Final mean length (mm ± SD)	Initial mean weight (g ± SD)	Final mean weight (g ± SD)	Weight gain (%)	Weight gain (%) per day (g ± SD)	SGR (g ± SD)
IMC spawn	24.42 ± 1.74	42.89 ± 2.66	1.73 ± 0.008	1.97 ± 0.21 a	113.87† ± 3.60 a	4.0† ± 0.1 a	0.46† ± 0.12 a
Live tubifex	25.56 ± 1.13	55.10 ± 4.07	1.35 ± 0.07	2.21 ± 0.26 b	163.70† ± 0.73 b	5.8† ± 0.0 b	1.76† ± 0.06 b
Fish eggs	27.92 ± 1.94	44.66 ± 2.91	1.45 ± 0.10	1.80 ± 0.15 a	124.13† ± 13.5 a	4.4† ± 0.4 a	0.77† ± 0.31 a

†Significant difference at $P<0.05$.
Figures in the same column having the same superscript are not significantly different.
SGR, specific growth rate.

Table 2 Results of larval rearing experiments of *C.chitala* in FRP tanks (30 days)

Parameters feeds	Initial mean length (mm ± SD)	Final mean length (mm ± SD)	Initial mean weight (g ± SD)	Final mean weight (g ± SD)	Weight gain (%)	Weight gain (%) per day (g ± SD)	SGR (g ± SD)
Live tubifex	45.08 ± 5.78	61.92 ± 10.52	0.56 ± 0.10	1.50† ± 0.30 a	267.8† ± 44.3a	8.9† ± 1.4	3.28† ± 0.36 a
Chironomous larvae	42.92 ± 2.23	67.34 ± 10.3	0.48 ± 0.05	1.82† ± 0.80 b	379.1† ± 89.9 b	12.6† ± 6.3	4.44† ± 0.61 b
Zooplanktons	42.13 ± 4.12	55.73 ± 10.3	0.46 ± 0.05	1.08† ± 0.40 a	234.7† ± 99.5a	7.8† ± 9.9	2.84† ± 0.66 a
Mosquito larvae	42.86 ± 2.11	66.01 ± 4.3	0.60 ± 0.13	1.61† ± 0.34 a	268.3† ± 69.4 b	8.9† ± 2.3	3.29† ± 0.40 a

†Significant difference at $P<0.05$.
Figures in the same column having the same superscript are not significantly different.
SGR, specific growth rate.

Water chemistry

The physico-chemical conditions of water including water temperature, pH, dissolved oxygen (DO), total dissolved solids (TDS), and conductivity were measured early in the morning before feeding at a depth of 20 cm using a multiparameter water analysis instrument (model No. Multi 340i, WTW, Weilheim, Germany). Total alkalinity, total hardness and free CO_2 were determined as per APHA (1989). Parameters like nitrate and phosphate were determined on a weekly basis using a spectrophotometer (Spectroquant NOVA 60 Darmstadt, Germany). Ammonia and nitrite concentrations were determined at the same time using Spectrophotometer kits. Dead ELS of *C. chitala* were removed and counted twice a day, simultaneous with water changes, to estimate the percentage of survival rate per 24 h. On the last day of the experiment, all the remaining larvae were individually counted for calculation of the actual survival rate.

Statistical analysis

Analysis of variance (ANOVA) was used to test the effects of the treatments on various growth and feed utilization parameters. Tukey's Multiple-Range test was applied to compare the significance level of the tested treatments. Differences were considered to be significant at $P<0.05$. All data were analysed using SPSS (11.0 version) for windows software program for statistical analysis. Data are presented as treatment means ± standard error of means.

Results

In the present study, both the rearing systems (nylon hapa, FRP tank) were found to be useful with respect to adaptability, water quality, growth and survival of the ELS of *C. chitala*. The different growth parameters of ELS of *C. chitala* reared in nylon hapas and FRP tanks with different diets are shown in Tables 1 and 2 and Fig. 1a–d. The ELS appeared to adapt easily to confinement (hapa, FRP tank) and accepted the diets. In the nylon hapa, they showed schooling and good swimming behaviour and moved on the side of the hapa in the upward direction, and no cannibalism was noticed. However, after feed intake lethargic behaviour was observed. In FRP tanks, they also showed lethargic movement and moved in the middle of the water column. Sometimes, they showed a typical preying habit of swallowing feed with upward

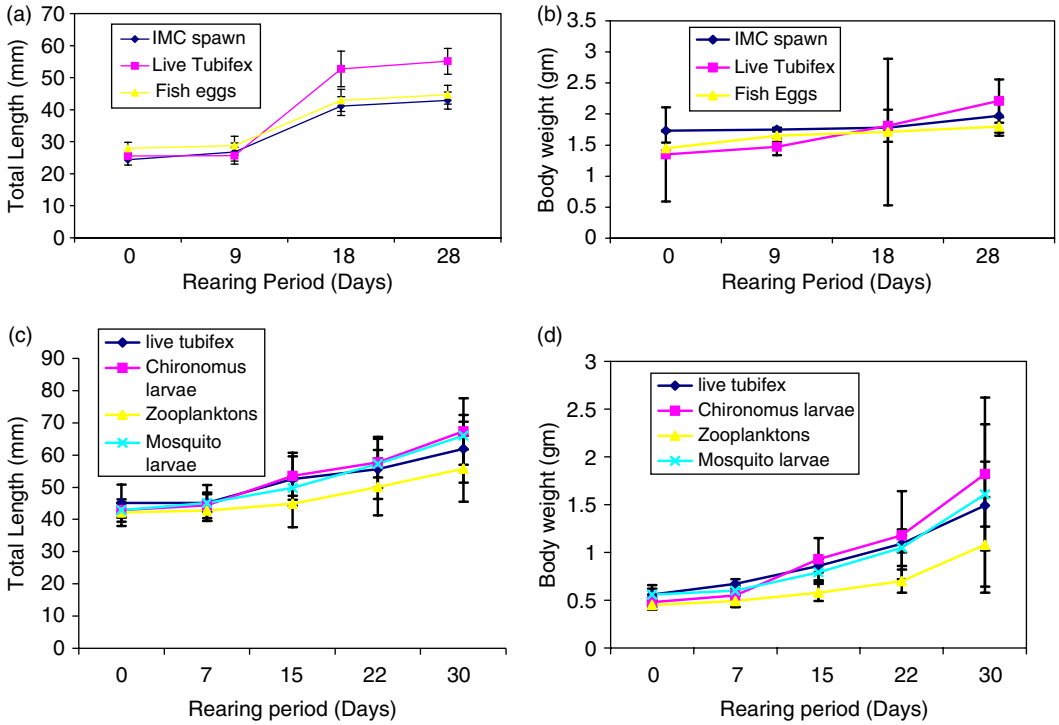

Figure 1 (a, b) Total mean length (mean ± SD) and body weight (mean ± SD) of early life stages (ELS) of *C. chitala* reared in hapa with different diets. (c, d) Total mean length (mean ± SD) and body weight (mean ± SD) of ELS of *C. chitala* reared in fibreglass reinforcement plastic tanks with different diets.

and downward directions in FRP tanks. They also showed good swimming behaviour, and no aggressive behaviour was noticed. The fish became sluggish after feed intake and took shelter inside artificial hideouts in the bottom of the tank. During the experimental rearing, the larvae were negatively phototactic and aggregated in the dark hiding areas of the tanks provided at the bottom.

In the nylon hapa, the larvae fed on live tubifex showed a rapid growth rate and attained the highest final length and final weight as compared with those that were fed fish eggs and IMC spawn. Analysis of variance revealed a significant effect of diet on the growth parameters. The final mean length and final mean weight showed a highly significant difference ($P < 0.05$) in all the diets. Thus, the final mean length and final mean weight showed an increase in body length and body weight in all treatment groups.

The experiment conducted in FRP tanks with four different types of diets (live tubifex, chironomous larvae, zooplanktons and mosquito larvae) indicated that the ELS of *C. chitala* fed on chironomous larvae, mosquito larvae and live tubifex showed a rapid growth rate compared with zooplanktons. ANOVA revealed a significant effect ($P < 0.05$) of diet on the final mean weight increase in all the treatments.

Specific growth rate and weight gain (%)

In the experiment conducted in pond hapas with different types of feeds, a higher value of SGR (g day^{-1}) was observed in larvae fed on live tubifex (1.76 ± 0.02), followed by fish eggs (0.77 ± 0.31) and IMC spawn (0.46 ± 0.12). Analysis of variance showed a significant difference ($P < 0.05$) in all the feeds (fish eggs, live tubifex and IMC spawn). Among other growth parameters, weight gain per cent was higher in live tubifex (163.70 ± 0.73), followed by fish eggs (124.13 ± 13.5) and IMC spawn (113.87 ± 3.60). Analysis of variance performed for weight gain per cent and weight gain per cent per day showed a significant difference ($P < 0.05$) in all the diets.

In FRP tanks with four different types of diets, a higher value of SGR was observed in larvae fed on chironomous larvae (4.44 ± 0.61), followed by mosquito larvae (3.29 ± 0.40), live tubifex (3.28 ± 0.36) while the lowest value was observed for zooplank-

tons (2.84 ± 0.66). Analysis of variance of SGR showed a significant difference ($P < 0.05$) in all the diets (live tubifex, chironomous larvae, zooplankton and mosquito larvae). Among other growth parameters weight gain per cent was higher in Chironomous larvae (379.16 ± 89.90) and the lowest in zooplanktons (234.78 ± 99.54). ANOVA performed for weight gain per cent and weight gain per cent per day showed significant difference ($P < 0.05$) in all the diets.

Survival rates

The mean survival rate of the ELS of *C. chitala* fed on three different diets in nylon hapas varied from 65 to 85% on day 28. The results indicated maximum mean survivability in fish eggs (85%), followed by IMC spawn (82%) and live tubifex (65%). However, the mean survival rates of the ELS reared in FRP tanks with fed on four different diets were on the higher side and varied from 80 to 100%, indicating good suitability of the systems. The results indicated maximum mean survivability in chironomous larvae and zooplanktons (100%) and live tubifex and mosquito larvae (80%).

Water chemistry

In nylon hapas, the average air and water temperature were in the range 30 ± 1.1 to 31 ± 2.2 °C respectively. In the present study, the mean DO level in the hapas was 8.0 ± 2.3 mg L^{-1} at 10:00 hours. Also, the water pH was 7.8 ± 0.2 during the rearing period and reached a higher level, whereas the mean free CO_2 was 2.3 ± 0.5 ppm. The results of other water quality parameters were as follows: alkalinity 60.0 ± 5.0 ppm, hardness 200 ± 10.0 ppm, TDS 380 ± 14.14 ppm and conductivity 617 ± 67.63 μmhos cm^{-1}.

The FRP tanks used in the experimental larval rearing were found to be useful with respect to the levels of DO and free carbon dioxide. No marked variation in water quality parameters was observed in the experimental system treated with different diets. All the parameters were under the tolerance limit of fish and they did not exhibit any distress. Temperature plays an important role in larval rearing. Little variation in water quality parameter was observed. The average air and water temperature were in the range 27.94 ± 0.82 and 25.48 ± 0.75 respectively. Dissolved oxygen is the key factor in rearing of larvae because larvae need an optimum level of oxygen to maintain their physiological condition. In the present study, the mean DO level in the FRP tank was 9.65 ± 0.46 mg L^{-1} at 10:00 hours. Also, the water pH was 8.40 ± 0.26 during the rearing period and reached a higher level, whereas the mean free CO_2 was 0.19 ± 0.17 ppm. The results of the other water quality parameters were as follows: alkalinity 61.34 ± 3.44 ppm, hardness 260.73 ± 30.71 ppm, TDS 375.16 ± 3.53 ppm and conductivity 386.10 ± 20.83 μmhos cm^{-1}. Ammonia and nitrite concentrations were in the range 0.002–0.024 and 0.01–0.015 mg L^{-1} respectively.

Discussion

The present study showed differences in the growth, SGR and survival rate of the ELS of *C. chitala* fed the same level of different diets in hapas and FRP tanks. The differences in SGR observed in hapas and FRP tanks with the same feed (live tubifex) may be due to the higher stocking density and smaller sizes of fish experimented in the hapa. However, many factors are related to the feeding such as the production system, type and size of rearing tanks, the size of fish and the quality and quantity of food and stocking density (Mgaya & Mercer 1995). When fish is provided with the appropriate quantity and quality of feed, it has been observed that the growth rate depends on two main factors: voluntary feed intake and assimilation efficiency (Brett 1979; Diana, Kohler & Ottey 1988). In the present study, the fish ration was reduced (10% body weight per day during the first week, 8% per day during the second week and 5% per day during the third and fourth weeks) in the progressive weeks based on the intensity of feeding.

The feed intake was higher for the ELS fed on live tubifex as compared with those that were fed with fish eggs and IMC spawn in nylon hapas. This was evident while we observed the daily feed intake, the quantity of unused feeds available in the rearing system and considering the variation in growth. However, in FRP tanks, the feed intake was higher for ELS fed on chironomous larvae, mosquito larvae and live tubifex. In fish, there are a wide variety of feeding characteristics that depend on the use of a range of senses. Reports also indicate that feeding activity may also be induced by a chemical stimulus from the food (Mackie & Adorn 1978), involvement of sensory organs like olfaction (Demsy 1978) and a lateral line system (Montgomery & Saunders 1985). The diet in-

take of fish depends on the size of the prey and predator, quality, density, physical attractiveness and mode of presentation of food (Mathavan 1976; James, Muthukrishnan & Sampath 1993). Chaitanawisuti, Kritsanapuntu and Kathinmai (2001) reported differences in the growth and FCR of juvenile spotted Babylon, influenced by feeding levels in a flow-through seawater system.

The study demonstrated that ELS of *C. chitala* successfully weaned to different diets on an experimental scale. Live tubifex, chironomous larvae and mosquito larvae among live and IMC spawn, fish eggs among non-conventional diets, respectively, proved to be an excellent diet. It was interesting to note that fish eggs served as an effective diet for the initial rearing ELS of *C. chitala* in nylon hapas up to a certain period. The ELS fed on live tubifex acquired an average weight of 2.21 g at the end of the experiment with an SGR of 1.76 g day $^{-1}$. Sarkar, Lakra *et al.* (2006) reported that posthatchlings fed on zooplanktons (copepods); dried daphnia and boiled egg yolk induced lower growth than tubifex worm, fish eggs, chironomous larvae and dry tubifex. The fish eggs (*P. ticto* (Hamilton-Buchanan), *L. bata* (Hamilton-Buchanan), were tested for the first time as a diet of the ELS of *C. chitala,* which interestingly proved to be highly useful in the experimental rearing of ELS of *C. chitala.*

While many authors have studied the effects of different diets on the growth of larval fish (Khan, Jafri, Chadha & Usmani 2003; Kim, Masses & Hardy 1996), however, information was lacking on the rearing of *C. chitala,* except a study by Sarkar, Lakra *et al.* (2006) in an indoor experimental recirculatory system. The tubifex worms have been used as a live feed for nursing European catfish (*Silurus glanis*) on a large scale in Hungery owing to its economic value (Horvath, Tamas & Tolg 1981) and in Mekong catfish (*Pangasius bicourti*) as reported by Hung, Thanh, Cacot and Lazard (1999). In the present study, live tubifex, chironomous larvae, mosquito larvae and also fish eggs may have stimulated the feeding behaviour of ELS of *C. chitala* and increased the acceptance, which supports previous observations (Hart, Hutchinson & Purser 1996; Sarkar, Lakra *et al.* 2006).

In the present study, higher survival (80–100%) of the ELS reared in the FRP tank was observed with live feed (tubifex, chironomus larvae, plankton, mosquito larvae) and 82–85% in unconventional feed (fish eggs and IMC spawn), while ELS fed with live tubifex in nylon hapas had a comparatively lower survival (65%). This could be due to the improved food intake. Sarkar, Lakra *et al.* (2006) reported the highest mean survival rate of (> 80%) of *C. chitala* larvae reared in a recirculatory system fed with live food (tubifex, chironomus larvae, zooplankton) and artificial diet (dry tubifex, *Spirulina,* daphnia), and a medium rate of survival (66–74%) was reported with unconventional feed (boiled egg yolk and fish eggs). In another study, Hung, Tuan, Cacot and Lazard (2002) recorded a survival of up to 92.7% in catfish (*Pangassius bocourti*) larvae fed on live tubifex.

The results of the present study indicate that ELS of *C. chitala* will actively feed on several types of live (live tubifex chironomous larvae, zooplankton, mosquito larvae) and non-conventional feed (IMC spawn and fish eggs). The study also shows that fish eggs may serve as a potential diet for rearing ELS of *C. chitala.* The overall experience suggests that *C. chitala* is suitable for commercial rearing because of its good acclimatization to confinement and good tolerance in the captivity.

In conclusion, the results obtained by the present study indicate the reliability and effectiveness of the rearing in hapas and FRP tanks for rearing of *C. chitala* at an experimental scale. It is important to take the differences in growth into account with the diets treated while preparing feeding strategies to ensure that the diet requirements of the ELS are met. To achieve more survival and better growth of the ELS of *C. chitala,* particular attention should be paid to the dietary composition, optimization of stocking density and improvement in diet presentation.

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