Note

Effect of feeding frequency on growth, feed utilisation and cannibalism in climbing perch *Anabas testudineus* (Bloch 1792) fry

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ABSTRACT

A 35 days experiment was conducted to study the effect of different feeding frequencies on growth and feed utilisation in climbing perch *Anabas testudineus* (Bloch 1792) fry. Four different feeding frequencies in triplicate viz., 2 (T_1), 3 (T_2), 4 (T_3) and 5 (T_4) times a day were evaluated. Fry (144 nos.) of climbing perch were randomly distributed in 12 glass aquaria (50 l) containing 25 l of filtered and well aerated freshwater. Formulated pellet feed was prepared and fed @ 6% body weight to all the experimental groups at different feeding frequencies as mentioned above. At the end of the experiment, fishes were sampled to determine weight gain percentage (WG %), specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER), survival percentage (%) and partial cannibalism rate (%). Results revealed that the WG% (112.80 ± 2.25), PER (1.65 ± 0.03) and SGR (2.15 ± 0.03) were significantly higher ($p < 0.05$) in T_3 group. FCR (1.55 ± 0.03) was lowest in T_3 group. Partial cannibalism rate (%) was significantly higher ($p < 0.05$) in the treatment group T_1 (6.33 ± 0.88) whereas the survival rate (%) was lowest in T_1 (91.66 ± 4.81). The results therefore, indicated that feeding frequency of 4 times a day (T_3) could be the ideal feeding frequency for better growth and feed utilisation during fry rearing of climbing perch.

Keywords: *Anabas testudienus*, Cannibalism, Climbing perch, Feeding frequency, Food conversion ratio, Growth

The climbing perch, *Anabas testudineus* (Bloch 1792) is one of the important indigenous air-breathing fish in India with high market price (₹300-500 kg⁻¹) in the states of Assam, Bihar, Jharkhand, Odisha, Manipur, Tripura and West Bengal (Kumar *et al.*, 2013a). It is popularly known as 'kavai' in Hindi, 'kou' in Odisha, 'koi' in West Bengal and 'kai' in Assam. It has a wide range of geographical distribution due to its exceptional physiological adaptation with respect to air breathing habit and salinity tolerance (Kumar *et al.*, 2012). This fish, due to its air breathing ability and tolerance to extremely unfavourable environmental conditions, is a prominent candidate fish for aquaculture (Sarkar *et al.*, 2005). The possession of accessory respiratory organs enable the species to be farmed at high stocking density (Anantharaja *et al.*, 2017). The fish contains very high amount of physiologically available iron and copper required for haemoglobin synthesis (Kumar *et al.*, 2013b); as well as essential amino acids and easily digestible polyunsaturated fatty acids (Zalina *et al.*, 2012). Due to its air breathing ability and tolerance to adverse environmental conditions, it is a good candidate fish for climate resilient aquaculture.

Feed plays a vital role for culture of any fish species. The larval and young fry stages are very much dependent

on the feed supplied to them. Most fish in fry stage do not accept any formulated feed given initially, but after some days they start accepting the feed, if other conditions of rearing are properly maintained (Watanabe *et al.*, 1983). In case of climbing perch, larvae and young fry feed on phytoplankton and zooplankton and adults feed on crustaceans, worms, insects, algae, soft higher plants as well as organic debris. Sufficient literature is available on feeding of fry of carps and cat fishes (Lovell and Stickney, 1977; Debrowskie *et al.*, 1987; Marte *et al.*, 1991). Till date there is scanty information available on feeding as well as feeding frequency of climbing perch fry. As the breeding protocol for climbing perch using exogenous hormone (GnRHa) has been standardised (Mandal *et al.*, 2016), it is necessary to have knowledge on the proper feeding frequency for mass production of climbing perch seed and also to enhance their growth and feed utilisation. Therefore, the present study was carried out to evaluate the role of different feeding frequencies on growth, feed utilisation and cannibalism in climbing perch fry.

The experiment was conducted over a period of five weeks (35 days) at the Air-breathing Fish Breeding and Culture Unit of Aquaculture Production and Environment Division, ICAR-Central Institute of Freshwater Aquaculture

(ICAR-CIFA), Bhubaneswar, Odisha. The biochemical analysis of the test diet was conducted in the laboratory of Fish Nutrition and Physiology Division, ICAR-CIFA.

Animals used for experimental purpose were the hatchery produced climbing perch fry of an average individual weight of 0.225 ± 0.02 g. An indoor rearing system was utilised for the study. The system consisted of 12 glass aquaria of 50 l capacity holding 25 l seasoned bore well water. The tanks were divided into four treatments based on feeding frequency *viz.*, treatment T_1 (2 times a day), T_2 (3 times a day), T_3 (4 times a day) and T_4 (5 times a day), each having three replicates. One hundred forty four fry of climbing perch were randomly distributed in 12 tanks @ 12 fry per tank. Before starting the experiment, the tanks were disinfected with potassium permanganate solution ($KMnO_4$). All the tanks were cleaned and siphoned every day in the morning before feeding the fry, and replenished with clean and seasoned bore well water to maintain water level in the tank.

Ingredients such as fish meal, soyabean meal, groundnut oil cake (GNOC), maize flour, fish oil, vitamin-mineral mixture (Kalvimin Forte) and carboxy methyl cellulose (CMC) were used for feed preparation. All the required ingredients were weighed accurately in an electronic balance (Table 1). All the dry ingredients except vitamin-mineral mix were mixed manually to form homogeneous mixture. Then water was added to form dough and the dough was wrapped in a wet cloth and transferred to an aluminum container which was then placed in a pressure cooker for steaming for half an hour. The steamed dough was taken out and cooled further and to this vitamin-mineral mixture along with fish oil were added and mixed properly. The dough was then pressed through a hand pelletiser to get uniform size pellets and was initially fan dried on a polythene sheet. The feed was then transferred to a tray and kept in a hot air oven overnight for complete drying at 50-60°C and later

crumbled manually, packed in an airtight jar and labeled according to the treatment.

The proximate composition of experimental diet was analysed by AOAC (1990) method. Dry matter was estimated by oven drying the samples at 105°C overnight. Crude protein percent was calculated by estimating nitrogen content by micro-Kjeldahl method and multiplying with a factor 6.25. Ether extract was determined by solvent extraction with petroleum ether, at 40-60°C, for 10-12 h. Total ash content was determined by incinerating the sample at 650°C for 6 h and crude fiber by acid digestion (1.25%) followed by alkali digestion (1.25%).

Gross energy in the diet was calculated using Bomb Calorimeter (Parr, model 1341; Parr Instrument Company, Moline, IL, USA). The ingredients composition and proximate composition of the test diet are shown in Table 1.

After acclimatisation in the experimental tanks, *A. testudineus* fry were fed manually with the test diet at 6% of their total body weight at different feeding frequencies such as T_1 - 2 times a day (at 08:00 and 17:00 hrs), T_2 - 3 times a day (at 08:00, 12:00 and 17:00 hrs), T_3 - 4 times a day (at 08:00, 11:00, 13:00 and 17:00 hrs), T_4 - 5 times a day (at 08:00, 11:00, 13:00, 15:00 and 17:00 hrs).

For the fish in each experimental group, the means of weight gain% (WG%), specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and survival rate were calculated as: weight gain % ($WG\%$) = $(W_f - W_i) / W_i \times 100$ where W_i and W_f are the initial and final weight of all fish in each glass aquaria. $SGR = [(\ln W_f - \ln W_i) / t] \times 100$, where t is the number of experimental days. $FCR = \text{Feed given} / \text{Weight gain of animal}$. $PER = \text{Gain in body mass (g)} / \text{Protein intake (g)}$.

Table 1. Ingredient and proximate composition of the test diet

Ingredient composition of the test diet		Proximate composition of the test diet	
Ingredients	% (Dry matter)	Nutritional parameters	Proximate composition (%)
Fish meal	40	Dry matter	94.3
Ground nut oil cake	20	Crude protein	39.15
Soya bean meal	15	Ether extract	7.13
Maize flour	20	Crude fiber	9.81
Carboxy methyl cellulose	1	Ash	10.15
Vitamin-mineral mix.*	2	Nitrogen free extract	43.57
Fish oil	2	Energy (k cal g ⁻¹)	39.76

*Vit. A - 2,00,000 IU, Vit. B2 - 80 mg, Vit. B12 - 0.24 mg, Vit. D3 - 40,000 IU, Vit. E - 32 IU, Calcium pantothenate -160 mg, Calcium - 32 g, Phosphorous - 6 g, Manganese - 1.1g, Iodine - 40 mg, Iron - 300 mg, Zinc - 600 mg, Copper - 80 mg, Cobalt - 20 mg

After completion of the feeding experiment, the survival rate of climbing perch fry was calculated as: Total number of fry survived/Total number of fry stocked * 100.

In this experiment, partial cannibalism was encountered among the fry of different experimental groups. Generally in partial cannibalism, cannibals attack the pectoral fin, caudal fin, abdomen region and the eyes of the prey.

Water quality parameters estimated (APHA, 1992) during the experimental period were: temperature - 23.0-28.0°C; pH - 7.4-7.6; alkalinity - 108-136 mg l⁻¹; hardness - 104-144 mg l⁻¹; dissolved oxygen - 4.0-6.0 mg l⁻¹; ammonia - 0.02-0.1 mg l⁻¹; nitrite - 0.03-0.06 mg l⁻¹ and nitrate - 0.1-0.3 mg l⁻¹.

All the data were analysed using one-way analysis of variance (ANOVA) using SPSS V. 15 software followed by use of Duncan's new multiple range tests to compare differences at p≤0.05 level. Results are presented as means ± standard error of the mean.

At the end of the experimental period of 35 days WG% in *A. testudineus* fry was highest (p<0.05) in T₃ group (112.88±2.25%) and lowest in T₄ group (83.61±1.13%). Similarly SGR was highest (p<0.05) in T₃ (2.15 ±0.03) group compared to other treatment groups (Table 2). The FCR was lowest in the treatment group T₃ (1.55 ± 0.03) whereas it was highest in T₂ (2.187 ± 0.08). PER is widely used for evaluating the quality of protein in the feed. PER was highest in T₃ group (1.65 ± 0.03) in comparison to other treatment groups (Table 2). In the treatment groups T₃ and T₄, survival rate of the climbing perch fry was 100% whereas in the treatment group T₁ and T₂ survival rate was 91.66% and 97.22%, respectively (Table 2).

The growth and survival of fish larvae/fry are highly influenced by feed given to them. For successful culture of any fish, suitable feed is one of the most important prerequisites. Non-availability of quality feed is one of the vital factors that affect commercial fish culture. In the present study, the test feed was prepared from locally available ingredients. The test diet had 39.15% crude protein and it was prepared based on the nutrient requirement of this species at fry stage (Hossain *et al.*,

2012). Better weight gain percentage, FCR and SGR were observed in T₃ group where feeding frequency was 4 times a day. Growth and production of fish generally depend on the daily feed consumption, quality of feed and feeding frequency, (Mookerjee and Mazumdar, 1946). In the present study, growth of fishes increased with feeding frequency and the best growth was observed in group where feeding frequency, was 4 times a day (T₃ group) and beyond this feeding frequency, growth performance reduced. This result is in agreement with James and Sampath (2004) who found growth enhancement in *Betta splendens* when fed three times daily for 77 days. Meer *et al.* (1997) reported that red pacu, *Colossoma macropomum* fed five times a day showed better growth and nutrient utilisation.

In this study, SGR was significantly higher (p<0.05) in T₃ group where feeding frequency was 4 times a day and lowest in T₄ group where the experimental animals were fed @ 5 times a day. PER was also highest in T₃ group in comparison to other treatment groups. Doolgindachabaporn (1994) found that FCR in *A. testudineus* ranged from 1.8-3.0. Potongkam (1972) reported that FCR of climbing perch fed on trash fish and pellet were 2.07 and 1.89 respectively. In this study, FCR was lowest in T₃ group (1.55±0.03) which indicates better feed utilisation efficiency.

Carlos (1988), Chua and Teng (1978) and Goldan *et al.* (1997) showed that feeding frequency had significant effect on the survival rate of different fish species such as *Aristichthys nobilis*, *Epinephelus tauvina* and *Sparus aurata*. Mookerjee and Mazumdar (1946) in a study with climbing perch with prepared feeds (containing 35-45% protein) observed survival rate ranging from 75-89%. Doolgindachabaporn (1994) also recommended that the feed containing 38.6% protein as the best feed formula in terms of growth and survival for *Anabas* fry. Mookerjee and Mazumdar (1946), tested the performance of different diets containing 30, 34.7, 39.5, 44.1 and 48.9% protein on dry weight basis and reported that 39.5% protein is optimum in the diet for commercial rearing of *A. testudineus*. Climbing perch is an excellent candidate for aquaculture as this species can adapt itself and if necessary can change its preference to certain food items,

Table 2. Effect of different feeding frequencies on growth and other nutritional parameters of *Anabas testudineus* fry

Treatments	Initial weight (g)	Final weight (g)	Weight gain (g)	WG %	SGR	FCR	PER	Survival rate (%)
T ₁	2.63±0.0 ^a	4.83±0.0 ^b	2.20±0.0 ^b	83.95±2.88 ^b	1.74±0.0 ^b	2.01±0.16 ^b	1.29±0.0 ^b	91.66±4.81
T ₂	2.67±0.0 ^a	4.82±0.0 ^b	2.14±0.0 ^b	89.44±7.46 ^b	1.82±0.1 ^b	2.187±0 ^b	1.17±0.0 ^b	97.22±2.78
T ₃	2.80±0.0 ^a	5.96±0.0 ^a	3.16±0.0 ^a	112.80±2.2 ^a	2.15±0.03 ^a	1.55±0.03 ^a	1.65±0.03 ^a	100
T ₄	2.76±0.07 ^a	5.07±0.16 ^b	2.31±0.09 ^b	83.61±1.13 ^b	1.73±0.01 ^b	2.09±0.03 ^b	1.22±0.02 ^b	100

Values in the same column with different superscripts differ significantly (p<0.05)

depending on the availability of items in a particular environment. In this study, mortalities occurred during the last phase of the experiment because of the injuries caused by partial cannibalism in lower feeding frequency groups. But this problem was negligible in groups where feeding frequency was more. During the experimental period, water quality was monitored at weekly intervals and found to be in ideal range for the growth of climbing perch fry.

Partial cannibalism was highest in the treatment group T_1 where the fry were fed 2 times a day and lowest in the treatment group T_4 where feeding frequency was 5 times a day. However, in the treatment groups T_3 and T_4 partial cannibalism was minimal (Fig.1).

Cannibalism is considered as one of the major problem under high density fish culture since the mid-1970s. Larval and juvenile sibling cannibalism occur

Fig. 1. Effect of different feeding frequencies on partial cannibalism of *A. testudineus* fry

in important cultured species such as yellowtail (*Seriola quinqueradiata*), turbot (*Scophthalmus maximus*), eels (*Anguilla anguilla*), seabass (*Dicentrarchus labrax*) and gilthead bream (*Sparus aurata*) (Chaudhuri and Tripathi, 1979; Smith, 1979; Kentouri, 1980; Degani and Levanon, 1983). Cannibalism is a behaviour manifested under conditions of food shortage. In larvae and fry rearing stage main cause of cannibalism is associated to food availability. Katavic *et al.* (1989) reported that cannibalism can be reduced by increasing the feeding frequency. The inverse relationship between the feeding rate and cannibalism is also reported in *Gadus morhua* (Folkvord, 1991). In the present study, partial cannibalism in climbing perch fry was highest where feeding frequency was low *i.e.*, 2 times a day and lowest in the treatment group where feeding frequency was more *i.e.* 4 times a day. In several fish species, including *Clarias gariepinus*, a decrease in cannibalism has been reported with increased food availability (Fox,

1975; Polis, 1981; Hecht and Pienaar, 1993). A similar trend has been shown for other cultured marine finfish including greater amberjack, *Seriola dumerili* (Miki *et al.*, 2011) and Japanese flounder, *Paralichthys olivaceus* (Dou *et al.*, 2000). However, partial cannibalism could be controlled by increasing feeding frequency and suitable size grading during seed rearing.

In conclusion, feeding frequency of 4 times a day was found optimal for better growth and feed utilisation in climbing perch fry. Further, at this feeding frequency cannibalism was also minimum and therefore, it is suggested to feed climbing perch fry 4 times a day for better growth and survival during seed rearing.

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