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Growth and survival of neon tetra, *Paracheirodon innesi* (Myers, 1936) fry fed mixed zooplankton, formulated feed and combination thereof

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ABSTRACT

Provision of appropriate food at different larval and post larval stages is an important aspect in success of seed production of neon tetra. The experiment was carried out to evaluate growth and survival of the neon tetra, Paracheirodon innesi reared by employing different methods of weaning fry from live mixed zooplankton to formulated feed. The fry of neon tetra (14.62 ± 0.27 mm initial length, 40.09 ± 2.72 mg initial body weight and 43 days old post hatched) were weaned onto formulated feed from day 1 (D_1), day 6 (D_2), day 12 (D_3), day 24 (D_4). The fry fed exclusively with mixed zooplankton (D_5) served as control. The fry weaned at day 6 (D_2) showed significantly higher length, weight and SGR than weaned at different periods. No mortality was recorded during whole experimental duration within treatments. Neon tetra fry can be weaned on formulated feed from day 6 at higher growth and survival successfully.

Keywords: neon tetra, *Paracheirodon innesi*, weaning, live food, Formulated feed

INTRODUCTION

In recent decades, the global market for ornamental fishes has been growing steadily at the rate of 14% per annum [1]. The management techniques have been optimized for gaining higher growth and production for food fish species through intensive investigations. On the other hand, very little work is carried out on husbandry management of ornamental fishes [2]. While food fish production focuses primarily on the total mass of fish produced [3], ornamental fish sold by number and have to be of a minimum size to be accepted in the market. Therefore, the goal of production is to produce highest number of fish of a given size within shortest possible time [2].

The cost of feed contributes nearly 40-60% of operation cost of most of the aquaculture systems. Rearing of juveniles solely on live food organisms is one of the most expensive processes. The higher cost associated with production of the live food organisms is mainly because of requirement for space, water, infrastructure and labour [4, 5]. Mass production of live prey is time consuming and also it is a source of risk in the hatchery management. On contrary, dry feeds can be economically produced, transferred and stored for longer periods. Moreover, formulated feed have the potential to meet the specific nutritional needs of the larvae, have an appropriate particle size and are of a standard quality [6].

In order to reduce cost of production of ornamental fishes, it is advantageous to wean fish onto formulated diet as early as possible [7]. At the same time, seed stock is the generally an expensive item involved in this farming procedure and the minimization of mortality is another important goal towards increasing farming profitability [8]. Fish fed dry feed grew significantly slower than those fed with live food organisms. Problems associated with the use of dry feed are an increase in size variation, a potential deterioration of water quality and lack of information about the best time and method to wean fish from live food to dry feed. The suitability of combinations of live food and formulated feed and the time at which the feeding of live food can be discontinued, needs to be investigated [9].

Neon tetra, *Paracheirodon innesi* is one of the most popular among the aquarium hobbyist and fetches good price in ornamental fish market. They inhabit the streams of the Rio Putumaya in Peruvian Amazon near Iquitoes and Yarrapa River. In past, during a single month, an average of 1.8 millions of neon tetra, with an estimated value of US$ 1, 75,000 were imported into United States for aquarium trade [10]. Provision of appropriate food at different larval and post larval stages is an important aspect in success of seed production of neon tetra. The aim of this trial to evaluate a method to wean fry of neon tetra from live food to formulated feed which may result in better commercial production of neon tetra under captive conditions.

MATERIALS AND METHODS

Fry of neon tetra, *P. innesi* were obtained from Wet laboratory at College of Fisheries, Ratnagiri, India. The weaning experiment was conducted with five treatments *viz*, formulated feed from day 1 (D_1), day 6 (D_2), day 12 (D_3), day 24 (D_4) and mixed zooplankton (D_5) for entire experimental period which served as a control diet. In case of weaning treatments for example, weaning at day 24 (D_4), the reduction in quantity of mixed zooplankton (numbers of mixed zooplankton were reduced to half of previous density at each reduction) were performed at day 12, 18 and 24. The quantity of mixed zooplankton was provided @ 66 numbers fry^{-1} and after 12 days it was reduced to half i.e. 33 numbers fry^{-1} and formulated feed was provided at the rate of 2% of the biomass. At 18 days it again reduced to half in mixed zooplankton and at day 24 mixed zooplankton was completely replaced by formulated feed. Similarly for weaning treatment D_3 (weaning at day 12), these changes were made at day 6, 9 and 12 and for weaning treatment D_2 (weaning at day 6) at day 2, 4 and 6. The plastic container (capacity 6 liter) containing five liters of freshwater used to rear the fry for experimental duration of 30 days, arranged as per Completely Randomized Block Design with four replicates for each treatment. Fry of neon tetra (length =14.62 ± 0.27 mm, weight = 40.09 ± 2.72 mg and 43 day old post-hatched) were stocked in the experimental rearing containers at the rate of 2 L^{-1}.

Luna-Figueroa *et al*. [11] evaluated the effect of different protein concentration in the feed for neon tetra and they had concluded that rates of growth were higher in fish fed with diet having 53.5% protein level. On that respect a formulated flake feed having 53.3% crude protein level was prepared using locally available ingredients which shown in Table 1 along with its proximate composition. Fishes were fed thrice a day at 08.00, 14.00 and 18.00 h. Uneaten food was siphoned out and nearly 30% of water from each container was exchanged daily. Water Temperature, pH and dissolved oxygen, were measured daily and ranged 25-26.5°C, 6.8-7.2, and 5.7-6.5 ppm, respectively. Alkalinity, total hardness was examined weekly and ranged 32.71-39.58 ppm, 43.58-52.66 ppm respectively. After rearing period of 30 days, fry from each replicate were measured for length and weight and Specific Growth Rate (SGR) was calculated (mg/day) as ln final wt (mg) - ln initial wt (mg)/day. Survival (%) was calculated as 100 x (no. survived fish)/(no. initial fish).

Table 1: Percentage of ingredients and proximate composition of the formulated dry feed (g/100 g) used to rear fry neon tetra, *P. innesi*.
Values expressed as % dry weight, ± S.E

Ingredients (g/100 gm)		**Proximate composition**	
Crocker fish meat powder	19.32	Crude protein	53.3 ± 0.024
Acetes powder	19.32	Crude fat	7.43 ± 0.039
Squid meal	19.32	Moisture	10.12 ±0.003
Ground nut oil cake powder	19.32	Nitrogen free extract	14.87 ±0.032
Egg white	19.32	Total ash	14.31 ±0.003
Tapioca floor	1.70		
Milk powder	1.70		
Agar powder	2.00		

Data were analyzed using one-way analysis of variance. Tukey's multiple comparison test was employed to determine significant difference between mean values of the individual treatments at $P = 0.05$. All statistical analysis was performed by using Windows based Graphpad Prism 5.02 statistical software.

RESULTS AND DISCUSSION

During this experiment, fry of *P. innesi* were weaned from mixed zooplankton to the formulated feed. Neon tetra fry were weaned at 6 days resulted in maximum length gain (48.38%), weigh gain (239.20%), SGR (4.05%) (Table 2) and no mortality were recorded after 30 days of rearing period. There was no significant difference (P>0.05) in growth and survival of fry were weaned at 24 days, 12 days and fed with complete formulated feed. However, the results significantly differed (P<0.05) from control (mixed zooplankton). Maximum length gain (48.38%) and SGR (4.05%) was recorded in fish weaned at 6 days and Tukey's multiple comparison reveled that it was significantly higher (P<0.05) from mixed zooplankton, fish weaned at 12 days and weaned by 24 days.

The time at which neon tetra can be weaned from live food to formulated feed had not been previously established. Moreover, early weaning induced a high mortality. Weaning at a larger size improved the survival rate but temporarily lowered fish growth after the transfer of larvae to a dry feed. Considering the poor growth rates obtained by offering the fish dry feed from hatching onwards, it appears more appropriate to feed the newly- hatched larvae with live organisms or mixed diets (live + dry foods) up to a developmental stage allowing successful weaning of the larvae with dry feed. In that respect neon tetra fry previously fed with mixed zooplankton for one month were weaned to dry formulated feed.

Cole *et al.* [12] and Cole & Haring [13] proposed to wean Lemon tetra, *Hyphessobrycon pulchripinnis* and Serpae tetra, *Hyphessobrycon serape*, respectively, using a combination of brine shrimp and fine powdered feed (55 % crude protein level) for first two weeks. This study concluded that the fry can be weaned after initial two weeks from live food to formulated diet but this study could not provide scientific weaning protocol.

Gordon *et al*. [14] weaned Clown fish *Amphiriprion percula* larvae from the enriched *Artemia* to the formulated dry feed. At the end of 32 days, juvenile *A. percula* can readily accept and benefit nutritionally from a formulated dry feed. Duray and Bagarinao [15] shown that there is feasibility of gradual weaning even younger milk- fish larvae (two week old) in hatcheries, using formulated diets. Al-Harbi and Siddiqui [16] had concluded that, *Artemia salina* nauplii (for first week) and 34% protein dry feed (for next three weeks) could be successfully used for feeding *C. carpio* larvae.

Table 2: Growth of *P. innesi* weaned from live food organisms to formulated feed for 30 days.

Particulars	Formulated feed (D_1)	Weaning at day 6 (D_2)	Weaning at day 12 (D_3)	Weaning at day 24 (D_4)	Mixed zooplankton (D_5)
Initial length (mm)	14.37± 0.23	14.25 ± 0.14	14.87 ± 0.37	14.62 ± 0.23	15 ± 0.35
Final length (mm)	20.6 ± 0.10	21.13 ± 0.14	20.45 ± 0.13	20.32 ± 0.13	20.15 ± 0.10
Initial weight (mg)	38.27± 2.36	36 ± 1.96	42.25 ± 3.47	40.6 ± 2.22	43.32 ± 3.59
Final weight (mg)	116.1± 0.86	120.95 ± 1.07	114.5 ± 1.66	112.25 ± 0.32	110.85± 1.25
Gain in length (mm)	6.43	6.88	5.58	5.7	5.15
Gain in weight (mg)	77.83	84.95	72.25	71.65	67.53
Length gain (%)	43.41 ± 2.13	48.39 ± 2.02	37.79 ± 4.12	39.08 ± 2.22	34.56 ± 3.32
Weight gain (%)	206.51 ± 17.22	239.2 ± 19.93	177.45 ± 26.28	179.21 ± 16.63	162.12 ± 25.21
SGR (%)	3.71 ± 0.19	4.05 ± 0.19	3.35 ± 0.31	3.40 ± 0.19	3.16 ± 0.30
Survival (%)	100	100	100	100	100

Here we adopted gradual weaning protocol for neon tetra and similar observations were recorded in the present study which revealed that fish weaned by day 6 (i.e. Reduction in mixed zooplankton for first 6 day and complete formulated feed (53.3% crude protein) for remaining 24 days) showed maximum length gain (48.38%), weight gain (239.20%) and higher SGR (4.05%) than fish fed only mixed zooplankton and only formulated feed for experimental duration.

Kaiser *et al*. [17] weaned gold fish, *Carassius auratus* to dry feed at day 12, significantly differed from fish weaned at day 6 and only dry feed. But this group was grown slower than treatments weaned at day 24 and fed only *Artemia*. They revealed that gold fish juveniles can be weaned step wise at day 24 from live food organisms without causing any significant reduction in growth and survival.

Freshwater species can be fed formulated diets as early as mouth opening [18]. However, obtaining feeds that satisfy the nutritional needs of larvae is difficult since mechanisms of digestion and absorption, as well as nutritional requirements, change during larval development [19]. Although inert diets are well ingested at the early stage, larvae can die with guts full of food, suggesting that they are unable to digest compound diets [18]. Luna-Figueroa et al. (2001) evaluated effect of different protein levels on the juveniles of *P. innesi*. The weight and total length gain as well as rates of growth were higher in fish (P<0.05) fed with diet having 53.5% protein level. In the present study, it was observed that neon tetra fry weaned by using formulated feed having crude protein level of 53.3 % showed better results than mixed zooplankton. Fry were weaned at day 6 and fed only formulated feed resulted in maximum length and weight gain and higher SGR.

During weaning, it is crucial that mortality through delayed development of active feeding behavior, and starvation is kept to a minimum, while maintaining decent growth rates [7]. However, considering the importance to wean fry as quickly as possible to on formulated diet, to reduce the cost but also to shorten the weaning period and therefore decrease the chance of mortality, the mixed-diet treatment seems to be preferable for weaning larval stages of fish [20].

CONCLUSION

In conclusion, it was observed that weaning method significantly affected the average growth of the fish at day 30. The growth of neon tetra fry weaned onto formulated feed at day 6 and fed only formulated feed having crude protein level of 53.3% was significantly higher (P<0.05) than fry weaned at day 12 and day 24 and mixed zooplankton. Neon tetra fry can be weaned to formulated feed at 49 days (43 days post hatch and 6 days for weaning) from live food with higher growth and survival.

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