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Effect of Green Water and Mixed Zooplankton on Growth and Survival in Neon Tetra, *Paracheirodon innesi* (Myers, 1936) during Larval and Early Fry Rearing

S. V. Sanaye, H. S. Dhaker, R. M. Tibile, V. D. Mhatre

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***Abstract*—**Larval rearing and seed production of most of tetra fishes (Family: *Characidae*) is critical due to their small size larvae and limited numbers of spawning attempts. During the present study the effect of different live foods on growth and survival of neon tetra, *Paracheirodon innesi* larvae (length 3.1 ± 0.012mm, weight 0.048 ± 0.00015mg) and early fry (length = 6.44 ± 0.025mm, weight = 0.64 ± 0.003mg and 13 days old) was determined in two experiments. Experiment I was conducted for rearing the larvae by using mixed green water and Infusoria whereas, in Experiment II, early fry were fed with mixed zooplankton, decapsulated *Artemia* cyst and *Artemia* nauplii. The larvae fed on mixed green water showed significant ($p<0.05$) growth and survival when compared to those fed with infusoria. Similarly, the larvae fed with mixed zooplankton exhibited higher growth in terms of length gain (131.98%), weight gain (6658.78%), SGR (14.04%) and survival (95.23%) compared to the other treatments of decapsulated *Artemia cyst* and *Artemia* nauplii. The present study concluded that mixed green water and mixed zooplankton should be used as food for better growth and survival of the larvae and early fry of *P. innesi*, respectively.

Keywords*—**Growth, Mixed Green water, mixed zooplankton, Neon tetra, *Paracheirodon innesi.

I. Introduction

ORNAMENTAL fish plays a significant role in the economics of developed and developing countries both as a foreign exchange earner and as a source of employment [1]. Fishes of family *Characidae*, which inhabiting tropical waters of South America are one of the major groups of ornamental fishes commonly reared worldwide. The best-known representatives and most valuable species of that group is Neon tetra, *Paracheirodon innesi* [2]. The fish is characterized by an iridescent blue-green lateral body stripe that extends from the head to the base of the adipose fin. A broad, shining red stripe begins at the middle of the body and extends posteriorly to the base of the caudal fin [3]. Previously, [4] reported that during a single month, an average of 1.8 million neon tetras with an estimated value of US $ 175 000 were imported by the United States for the aquarium trade while another report indicates that annually 12–15 million neon tetras collected from the wild were exported and this accounts for 80% of the total market of ornamental fish of the Amazon State [5].

Some members of family *Characidae* are known for very limited numbers of reproductive attempts mainly due to their small body size (max. 3cm). The number of such acts in neon tetra (*Paracheirodon innesi*) or cardinal tetra (*Paracheirodon axelrodi*) can be just two or three in their whole life cycle in captivity. In such a case the optimization in feeding, water quality and light conditions plays an important role in larval rearing and seed production [6]. Despite the considerable development in spawning techniques of various ornamental fishes, proper knowledge of larval rearing techniques seems scanty. Lack of proper data as well as documentation or publication of data for larval rearing, could be the main possible reason for less development of the ornamental fish industry in many countries.

Several authors have described improvement in fish larval performance in terms of growth and survival by using micro algae during rearing [7]-[11]. In addition, use of zooplankton as food for various ornamental fish larvae has been investigated [12]-[14]. Zooplankton is considered as a valuable source of various amino acids, fatty acids, minerals and enzymes [15]. Live zooplankton contains enzymes (for e.g. amylase, protease), which play an important role in larval nutrition and are easily digestible [16]. In aquaculture, brine shrimp (*Artemia* spp.) is most commonly used for first feeding of fish larvae. However, the cost of brine shrimp is prohibitive for resource-poor farmers in the developing nations, which has necessitated investigation into alternative feeds [17].

Although there is a high demand for neon tetra, larval and early fry rearing practice is quite difficult to achieve [18]. Over collection from their natural habitat may doom this species to extinction [19]. This situation has led to development and publication of breeding and rearing methods for different aquarium fishes [20]. Research developments in larviculture and early rearing technology have enabled 90% of currently marketed freshwater ornamental fish to be reproduced and cultured successfully throughout the world [21]. However, feed being the major deciding factor in rearing of fishes in controlled conditions, it is necessary to determine the most appropriate food for larval and early fry rearing of *P. innesi*. As mass production of this species is a valuable

Mr. Sushant Sanaye, Mr. H. S. Dhaker, Mr. R. M. Tibile, and Mr. V. D. Mhatre are with the Department of Aquaculture, College of Fisheries, Ratnagiri. Dr. Balasaheb Sawant Konkan Krishi Vidyepeeth, India (Corresponding author: Sushant Sanaye; e-mail-sushant.sanaye@gmail.com).

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business [2] and only limited data is available on breeding [22], [23], [2], during the present study, an attempt was made to determine the food suitable for better production of larvae and early fry of *P. innesi*.

II. Materials and Methods

A. Source Of Fish

The larvae and early fry of neon tetra, *Paracheirodon innesi* were produced in the Wet laboratory of College of Fisheries, Ratnagiri, India according to the method described by [23]. Two follow-up experiments were conducted to evaluate suitability of different live foods on neon tetra larvae and early fry.

B. Experiments

1. Experiment I

In the first experiment, the effect of infusoria and mixed green water was tested on growth and survival of free swimming neon tetra larvae over a period of 13 days. Infusoria culture was achieved by the method described by [24]. The maximum density of infusoria (600 no. ml^{-1}) was obtained in four days. The infusoria culture was maintained by daily replacing 30% of cultured medium with fresh cultured medium. Infusoria culture was dominated with *Paramecium* sp.

Mixed green water was produced in the plastic pool (4' x 2' size) which was filled with 300 litres of freshwater to which 140g of soaked groundnut oil cake powder was added for fertilization. On the third day, fertilized water was inoculated with 50 litres of mixed algae (3.0×10^6 cells ml^{-1}). Three days after inoculation the fresh water turned greenish due to increase in cell density. The term 'mixed green water' indicates a mixed culture of microalgae and other organisms. Qualitative analysis of mixed green water revealed the presence of *Chlorella* sp. (90%) and remaining includes infusorians. Soaked groundnut oil cake was added every third day @ 140g to maintain the mixed green water culture.

The larvae of *P. innesi* (length 3.1 ± 0.012mm, weight 0.048 ± 0.00015mg) were transferred to a plastic container filled with 3 litres of freshwater and stocked @ 10 no. L^{-1}. Larvae were fed with two different types of live foods *viz*, Infusoria and mixed green water. The experiment was conducted for 13 days with 13 replicates for each food. The period of 13 days was chosen on the basis of our previous observations where it was found to be sufficient for shifting to next bigger size prey items. The larvae of neon tetra were fed with mixed green water @ 150,000 to 200,000 algal cells + 1-2 infusorian ml^{-1} and six infusorian ml^{-1} for 13 days. As suggested by [24], 30ml of infusoria culture was directly added into rearing containers. Adjustment in density of algal cells and infusoria was done by counting remaining numbers in rearing water before each feeding. After 13 days of rearing period, growth parameters and survival of larvae were recorded from each larval rearing container.

2. Experiment II

In this experiment, the effect of three different live food organisms on growth and survival of early fry of neon tetra was observed for 30 days. Mixed green water was produced as described in Experiment I and inoculated with mixed zooplankton @ 10 no. ml^{-1}. Mixed zooplankton was dominated with *Daphnia* sp. (60%), *Moina* sp. (30%) and Copepods (10%) in the culture tanks. Mixed zooplankton was harvested by using bolting silk cloth net (360 micron mesh). The harvested mixed zooplankton was washed with one ppm of potassium permanganate ($KMnO_4$) solution and then thoroughly washed with freshwater. Decapsulated *Artemia* cysts and *Artemia* nauplii were obtained by adopting methods of [25] and [26].

The experiment was conducted in plastic containers (Capacity 3 litres) filled with clean freshwater. There were three treatments with seven replicates for each treatment. Early fry of *P. innesi* (length = 6.44 ± 0.025mm, weight = 0.64 ± 0.003mg; 13 days old) were stocked in each plastic container @ 5 nos. L^{-1}. Three treatments *viz*., mixed zooplankton, decapsulated *Artemia* cyst and *Artemia* nauplii were given @ 300 to 350 nos. L^{-1} for feeding. Early fry were fed thrice a day at 07:00, 14:00 and 18:00 hours. Uneaten food and faecal matter was siphoned out and nearly 30 % of water from each container was replaced daily. After 30 days of rearing period, the growth and survival of early fry fed with three different live foods were recorded.

C. Growth Parameters

Total length of each larva was measured by occular micrometer in Experiment I while measurement scale with nearest minimum accuracy of 0.5mm was used in Experiment II. The weight was recorded by using the monopan balance (Dhona 100 DS), Dhona Industries Pvt. Ltd., Kolkata, India) with accuracy of 0.01mg. The growth parameters, such as length gain, weight gain and Specific Growth Rate (SGR) were calculated.

D. Water Quality

Water quality parameters during both experiments such as water temperature, pH, dissolved oxygen, free carbon dioxide, total alkalinity and total hardness were measured weekly by adopting standard methods and average values are presented in Table I.

TABLE I
Water Quality Variables Recorded during Rearing of Neon Tetra in Experiment I and Experiment II

	Experiment I	Experiment II
Temperature	24 to 25 °C	24 to 25.1 °C
pH	6.8 to 7.2	6.9 to 7.2
dissolved oxygen	7.78 to 7.85 mgL^{-1}	5.7 to 7.0 mgL^{-1}
carbon dioxide	6.19 to 6.05 mgL^{-1}	3.96 to 6.05 mgL^{-1}
total alkalinity	24 to 26.1 mgL^{-1}	32.33 to 38.33 mgL^{-1}
total hardness	28 to 29.5 mgL^{-1}	37.33 to 42.66 mgL^{-1}

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E. Statistical Analysis

Student's *t* test was applied to determine the significance of difference between mean values in Experiment I. Data of experiment II was subjected to ANOVA analysis. Differences were considered significant when $p < 0.05$. Tukey's Multiple Comparison Test was applied to determine significant difference between mean values of the individual treatments. All statistical analysis was performed by using computer based Graphpad Prism 5.02 statistical software and [27].

III. Results and Discussion

The average length gain, weight gain, Specific Growth Rate (SGR) and survival of larvae of *P. innesi* in Experiment I, are given in Table II. The maximum length gain, weight gain, SGR and survival were found in the larvae fed with mixed green water. Significant difference ($p<0.05$) was found between mixed green water and infusoria.

In comparison with the vast published literature on the culture techniques associated with other marine fish and shell fish species, reports on freshwater ornamental fish rearing techniques particularly during early larval stages are few. Survival and growth are found to be higher as a result of introduction of phytoplankton/green water in rearing tanks of numerous fresh and sea water fish species than in clear water. 'Green water' and 'Pseudo green water' techniques described by [28] are now-a-days widely used all over the world for rearing of small fish larvae and crustaceans. According to [29], feeding ability of fish larvae was significantly influenced by the presence of micro algal species in rearing water during their development.

During the present study, mixed green water treatment resulted in maximum length gain (94.66%), weight gain (1120.49%), specific growth rate (19.24%) and survival (80%) of neon tetra larvae after 13 days of rearing period. Reference [11], reported that higher survival was observed in larvae of Atlantic halibut, *Hippoglossus hippoglossus*, fed with green water as compared to clear water. They further observed that the halibut larvae in clear water concentrated at the water surface and near tank walls, whereas in green water the larvae spent most of the time in the water column. In the present study, neon tetra larvae reared in green water were distributed uniformly in water column while in infusoria (comparatively clear water), larvae concentrated near container walls and at bottom. Thus the result of the present experiment showed agreement with [11]. Reference [30] found that, when six different ornamental fishes *viz*., Siamese fighting fish, angel fish, Bosmani rainbow fish, Buenos aires tetra, Redtail shark and Rainbow shark fed with green water, green water + Encapsulan and formulated diet, green water and green water + Encapsulan resulted in higher survival and total length than formulated diet.

According to [31], use of green water improved the survival and growth rate of fish larvae, but the mechanism(s) through which the micro algae act to generate this effect remained unclear. Reference [32] reported that use of algae enhanced larval survival and supported initial larval feeding in Atlantic cod, *Gadus morhua* by increasing prey ingestion. This could be due to the presence of microalgae in the rearing water influencing diffusion of light in rearing tanks, thus enhancing prey capture [11] [33]. The higher growth and survival in neon tetra larvae in the experiment I, might be due to mixed green water rearing medium. The eggs and larvae of neon tetra are photosensitive and die in bright light tank condition [23].The presence of green water with *Chlorella* along with other organisms diffuses light in the rearing container which is an additional benefit for protection from bright light, better prey capture and ingestion of prey resulting in higher growth and survival of larvae of *P. innesi*.

TABLE II
THE AVERAGE LENGTH GAIN, WEIGHT GAIN, SPECIFIC GROWTH RATE (SGR) AND SURVIVAL OF LARVAE OF NEON TETRA, *P. INNESI* FED ON INFUSORIA AND GREEN WATER FOR 13 DAYS
DATA PRESENTED AS MEAN ± S.E

Particulars	Infusoria	Green water
Avg initial length (mm)	3.1 ± 0.012	3.1 ± 0.012
Avg final length (mm)	4.7905 ± 0.031	6.034 ± 0.025
Length gain (%)	54.5325 ± 0.88	94.66 ± 0.71
Avg initial weight (mg)	0.048 ± 0.00015	0.048 ± 0.0001
Avg final weight (mg)	0.4538 ± 0.0048	0.58 ± 0.0034
Weight gain (%)	845.37 ± 8.78	1120.49 ± 6.09
SGR (%)	17.27 ± 0.07	19.24 ± 0.035
Survival (%)	62.82 ± 1.84	80.00 ± 2.35

The maximum length gain, weight gain, SGR and survival of early fry fed with mixed zooplankton in Experiment II is shown in Table III. ANOVA showed significant difference ($p < 0.05$) in length gain, weight gain, SGR and survival of early fry fed on different live food organisms.

The Tukey's multiple comparison test revealed that all growth parameters and survival of early fry fed on mixed zooplankton were significantly different ($p < 0.05$) from larvae fed with decapsulated *Artemia* cysts and *Artemia* nauplii. In the present study, mixed zooplankton treatment resulted in the maximum length gain (131.98%), weight gain (6658.78%), SGR (14.04%) and survival (95.23%) of early fry after 30 days of rearing period.

Live *Artemia* nauplii have been used as a feed for most fish seed hatcheries worldwide for a long time [25], [34]. However, the product of *Artemia* is becoming more and more expensive in national and international markets. In contrast, cladocerans can be easily obtained from natural aquatic environments or fish farming ponds with lower costs in India and can be mass cultured easily (S. R. Sane, Pers. Comm.). In freshwater ornamental fish culture, *Moina* used to be the most common and plentifully available live food organism for feeding young fish larvae in the industry [13].

Reference [12] fed fry of angel fish, *Pterophyllum scalare* with cladocerans and *Artemia nauplii* and observed that use of cladocerans showed better growth and survival than those fed with *Artemia nauplii*.

TABLE III
THE AVERAGE LENGTH GAIN, WEIGHT GAIN, SPECIFIC GROWTH RATE (SGR) AND SURVIVAL OF EARLY FRY OF NEON TETRA, *P. INNESI* FED ON DIFFERENT LIVE FOOD ORGANISMS FOR 30 DAYS

Particulars	Treatments		
	Mixed zooplankton	**Decapsulated *Artemia* cyst**	***Artemia* nauplii**
Avg initial length (mm)	6.41± 0.032	6.45 ± 0.016	6.45 ± 0.019
Avg final length (mm)	14.87 ± 0.046	13.13 ± 0.086	14.70 ± 0.059
Length gain (%)	131.98 ± 0.96[a]	103.06 ± 0.13[b]	128.01 ± 0.33[c]
Avg initial weight (mg)	0.62 ± 0.0048	0.64 ± 0.0021	0.64 ± 0.0027
Avg final weight (mg)	42.23 ± 0.71	28.86 ± 0.85	39.39 ± 0.95
Weight gain (%)	6658.78 ± 105.11[a]	4400.99 ± 129.26[b]	6040.5± 160.97[c]
SGR (%)	14.04 ± 0.057 [a]	12.68 ± 0.099 [b]	13.71 ± 0.085 [c]
Survival (%)	95.23 ± 3.15[a]	26.66 ± 1.25[b]	67.61 ± 7.89[c]

[abc] Mean values having different superscripts in a row differ significantly ($p<0.05$).
Data presented as mean ± S.E

The growth and survival of *Clarias anguillaris* larvae were similar commercial dry diet ($p > 0.05$) when fed with pure and mixed zooplankton [14]. [35] reared *C. gariepinus*, *Heterobranchus bidorsalis*, and 'Heteroclarias' (hybrid of *H. bidorsalis* male and *C. gariepinus* female) on the cladoceran, *Moina dubia*, commercial dry diet and *Artemia* nauplii, better growth and survival were obtained when fed mixed zooplankton and commercial dry diet than with other treatments. In the present study, better growth and survival is found in early fry fed with mixed zooplankton.

According to [28] decapsulated *Artemia* cysts produce larvae of superior quality in ornamental fish. The highest growth values were obtained in *C. gariepinus* larvae fed decapsulated *Artemia* cyst [17]. Reference [36] found that Goldfish larvae and juveniles grew faster and had a higher survival when fed on decapsulated *Artemia* cysts than on instar I *Artemia nauplii* or a mixed live/dry diet of *Artemia* nauplii and dry food. However in the present study, feeding decapsulated *Artemia* cyst resulted in least growth and survival of early fry of *P. innesi*. Significant results found by above authors were possibly due to choice of fish species which grow bigger in size and also produce larvae of big size. On the other hand neon early fry are of relatively small size with small mouths which restricted them to feeding on and digesting decapsulated cyst.

IV. CONCLUSION

In present investigation, better growth and survival of neon tetra larvae and early fry was found when fed with mixed green water and mixed zooplankton respectively. Results obtained from present studies provide proper protocol for rearing neon tetra larvae, which consider as critical phase in seed production of neon tetra. Further mixed green water and mixed zooplankton are easily producible in seed production units and cheap compared to other *Artemia* products. Therefore, we recommended to use mixed green water and mixed zooplankton for mass production of this valuable ornamental fish species in particular.

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