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Influence of different live food organisms on the reproductive performance of female *Trichogaster leerii*

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Abstract
The brood stock management is of prime concern in a hatchery system as the production of viable and quality seed is largely dependent upon the broodstock management practices. In the present study, brood stock of *Trichogaster leerii* was fed with live food alone or in combination. The experiment was conducted in all glass aquarium tank (60x30x30 cm, 54-Lcapacity) filled with water up to a level of 15 cm. The adult females of pearl gourami (length 5.80 ± 0.06 cm and weight 1.9207 ± 0.08 g) were fed with different live foods such as mosquito larvae (T_1), *Artemia* (T_2), earthworms (T_3) and combinations of the above food viz. mosquito larvae + *Artemia* (T_4), mosquito larvae + earthworm (T_5) and *Artemia* + earthworm (T_6). The fishes were fed three times up to satiation. The results indicated higher growth rate and reproductive performance in adult females of pearl gourami fed with mosquito larvae, as compared to other test diets.

Keywords: Mosquito larvae, *Artemia*, number of spawn, pearl gourami

Introduction
The world trade of ornamental fish has been estimated around 5 billion US$ with an annual growth rate about 10% per year [1]. The largest volume of fishes (approximately 95%) in the aquarium trade is from farm raised which are mainly from South East Asia and the United States. The main markets are USA, UK, Belgium, Italy, Japan, China, Australia and South Africa. Singapore and other East Asian countries account for 80% of the global trade of aquarium fishes. India's contribution in world trade of ornamental fishes is around INR 4.0 crores only [2]. About 90% of Indian aquarium fish export is from Kolkata, followed by 8% from Mumbai and 2% from Chennai. The internal ornamental fish trade of India is estimated to be of the order of INR 15 crores and having the annual growth rate 14%.
All gouramis belong to the suborder Anabantoidei and are commonly known as the labyrinth fishes [3, 4]. Labyrinth fishes are only found in tropical African and Asian region. The natural distribution of Labyrinth fishes is in China, Korea, Sumatra, India, Malaya Peninsula, Thailand, Indonesia and tropical African countries [3-6]. Generally the *Trichogaster* spp. inhabits the thickly vegetated areas of rivers, canals, ditches, lakes and swamps to avoid predation from birds and fish. There are 50 species belonging to genus *Trichogaster*. Pearl Gourami is an exotic, popular aquarium fish and fetches high price in the Indian market. Unless the technology of breeding of any species is developed, it is difficult to conserve the biodiversity as well as adoption of the species on commercial scale for export.
The natural diet of *T. leerii* is composed of different species of invertebrate [7]. However no investigation was carried out on scientific lines to determine the effect of different live food on reproductive performance of pearl gourami. Hence, the present study was carried out with the objectives, to study the reproductive performance of Pearl Gourami, *Trichogaster leerii* fed with different live food organisms.

Materials and Methods
In the present study, *Trichogaster leerii* (Bleeker, 1852) commonly known as pearl gourami was used as test animal (length 5.70 ± 0.07 cm and weight 1.8208 ± 0.09 g) to study it's rhythm of reproductive performance using different live foods.

Culture of live food organisms

1. Mosquito larvae: The plastic pool (120cm dia. x 60 cm height) was filled with 300L freshwater and 2 kg sugarcane bagasse. The pool was kept separate in undisturbed area of wet laboratory. On the third day onwards, mosquito larvae were developed in pool. 3-4 g of mosquito larvae of 5-6 mm size was harvested daily. The larvae were thoroughly washed in flowing water in small net (mesh size 1 mm) before feeding to the fish.

2. Earth worms: Earth worm culture was carried out in wet laboratory [8].

3. *Artemia* culture: For culturing *Artemia*, the natural seawater (35.5 g L^{-1}) was used. Culture was carried out in 350 L circular tanks [9].

Experimental procedure

An experiment was conducted for 30 days to observe the influence of different live food organisms on reproductive performance of pearl gourami. Experiment was conducted on adult female of *T. leerii* by feeding with different types of live foods. Glass aquaria were arranged as per Completely Randomized Design (CRD) [10] with six treatments having four replicates for each diet. Water quality parameters such as temperature, pH, dissolved oxygen, free carbon dioxide, total alkalinity and total hardness were measured by following standard methods [11, 12] and were maintained throughout the experimental period (Table 1). Adult female of pearl gourami (length 5.80 ± 0.06 cm and weight 1.9207 ± 0.08 g) were stocked in each experimental glass aquaria at the rate of one number per tank. The six test diets viz. T_1: Mosquito larvae; T_2: *Artemia;* T_3: Earth worm; T_4: combination of mosquito larvae and *Artemia;* T_5: combination of mosquito larvae and earth worm; T_6: combination of *Artemia* and earth worm.

Adult females were fed three times a day (07:00, 12:00 and 17:00 h) up to satiation (about 8-10% of body weight daily). Nearly 30% of water from each aquarium was exchanged daily.

Spawning, egg hatching and spawn production of *T. leerii*:

All glass aquarium tanks ($60 \times 30 \times 30$ cm, 54 L capacity) were filled with water up to a level of 10 cm. Thermocol sheet (9cm x 12cm) was placed on the water surface as a shelter for construction of bubble nests by males [6].

Matured males (length 6.10 ± 0.06 cm and weight 2.1207 ± 0.07 g) were placed in the morning hours (8.00 am) in each tank to construct bubble nests. Females were introduced on the same day in the evening hours (4.00 pm). The nest was prepared by males underneath the floating material. In present experiment, the fishes spawned in 2 to 3 days. After spawning, the females were removed from the tank to protect it from the male's aggression. Egg hatching was observed after 24 hours of spawning. The young one produced after two days of hatching was referred as spawn. These spawn were counted for observing reproductive performance.

Analysis

Growth parameters: At the beginning and end of each experiment, the fishes were counted from each replicate and their individual length and weight were recorded. Weight of each fish was taken on mono-pan electric (Sartorius, BS 224S) balance having an accuracy of 0.01 mg, maximum 220g. For this, each fish larvae was kept on blotting paper in order to remove excess moisture from the body. Then each blotted fish larvae was taken on plastic paper and weight was recorded.

1. $\text{Length gain (\%)} = \frac{(\text{Final length} - \text{Initial length})}{\text{Initial length}} \times 100$
2. $\text{Weight gain (\%)} = \frac{(\text{Final weigth} - \text{Initial weigth})}{\text{Initial weigth}} \times 100$
3. $\text{Specific Growth Rate (\%)} = \frac{(\text{Log Wt} - \text{Log Wo})}{dt} \times 100$

Where, W_t = Final weight; W_o = Initial weight; dt = Rearing period in days

Proximate composition

Samples were analyzed for moisture, crude protein (Kjeldhal method using KEL PLUS-CLASSIC DX), crude lipids (Lipid extraction method by using SOX PLUS system), ash and carbohydrates content [13]. Nitrogen free extract was estimated as the difference between the sum of the other constituents and the original dry weight of the sample. Gross energy (GE) values of test diets were calculated based on 0.24, 0.40 and 0.17 kJ g^{-1} for protein, lipid and carbohydrate respectively [14]. The proximate composition of different live food organisms is given in Table 2.

Data obtained from the experiments for reproductive performance and growth parameters were analysed by one way ANOVA. Significant difference was indicated as $P < 0.05$, Student's Newman Keul (SNK) multiple range test was used to determine the significant difference between the treatments [10, 15].

Results and Discussion

An experiment was conducted to observe the reproductive performance of female pearl gourami, *T. leerii* fed with different live food organisms. Number of spawn produced by each female, Growth parameters such length and weight gain of females and the average Specific growth rate (SGR) of female for rearing period of 28 days is presented in Table 3.

The live food organisms have immense importance in hatchery phase because of their small size, easy digestibility, readily acceptability and less water quality deteriorating nature [9]. The live food organisms such as infusorians, rotifers, various stages of *Artemia*, cladocerans like *Moina* sp. and *Daphnia* sp., tubifex and insect larvae such as mosquito larvae, bloodworms are extensively used for feeding the larvae and brood fishes in ornamental fish hatcheries. The success of hatchery and the quality of seed largely depend upon proper brood stock management [16]. Provision of proper nutrition by adopting right feeding strategy is one of the key aspects of brood fish conditioning during pre and post spawning management. Providing natural diet to brood fishes improve the health status during captive rearing. Use of live food in pre-spawning broodstock management could enhance growth rate, faster gonadal development and produced quality larvae [17]. Gonadal development of fishes belonging to genus *Trichogaster* is linked to variety of live food organisms [18]. In the present study, broodstock of *T. leerii* was fed with live food organisms in the form of mosquito larvae, earthworms and *Artemia* etc. either singly or in combination.

Importance of live food in aquarium fish management has been scientifically proved by various workers. Better

reproductive performance of angel fish, *Pterophyllum scalare* fed with mosquito larvae were reported by many researchers [16, 17, 19]. In the present study too, higher growth rate and reproductive performance was found in adult females of pearl gourami fed with mosquito larvae (T_1), as compared to other test diets.

Dietary protein level influences the reproductive performance of ornamental fishes [14, 20] and in table fish varieties [21]. Similarly, an increase in the protein percentage from 13-18% to a higher level of 32-49% showed accelerated growth of aquarium fishes viz. dwarf gourami *Colisa lalia* [22]; pearl gourami *Tricogaster leerii* [17]; Siamese fighting fish *Betta splendens* [20]. The level of crude protein (48.6%) in mosquito larvae was in accordance with the range as observed in the above mentioned studies [16]. Among the live foods used in the present study, higher preference to mosquito larvae by the fish was also noticed as compared to other test diets.

Table 1: Water quality parameters during rearing of female *T. leerii*

Water Quality Parameters	Mean observed value
Temperature (°C)	25.75 ± 0.48
pH	7.12 ± 0.03
Total hardness (mg L^{-1})	42.50 ± 1.71
Total alkalinity (mg L^{-1})	38.25 ± 0.85
Dissolved oxygen (mg L^{-1})	3.87 ± 0.13
Free carbon dioxide (mg L^{-1})	27.75 ± 0.48

Values expressed as ± S.E. of mean.

Table 2: Proximate composition (% dry weight basis) of different live food

Diet	Treatments	Moisture	% of dry matter				GE ** (kJ/g)
			Crude protein	Crude lipid	Ash	NFE*	
Mosquito larvae (ML)	T_1	80.00 ± 0.50	48.60 ± 0.10	18.10± 0.01	9.00 ± 0.10	24.30 ± 0.21	23.035 ± 0.29
Artemia (A)	T_2	88.70 ± 0.20	54.05 ± 0.02	13.20 ± 0.20	16.08 ± 0.47	16.67 ± 0.02	21.085 ± 0.24
Earthworms (EW)	T_3	85.30 ± 0.10	57.23 ± 0.02	11.80 ± 0.20	10.80 ± 0.20	20.17 ± 0.02	21.884 ± 0.26
ML:A (1:1)	T_4	84.35 ± 0.10	51.32 ± 0.02	15.65 ± 0.20	12.76 ± 0.20	20.27 ± 0.02	22.060 ± 0.24
ML:EW (1:1)	T_5	82.65 ± 0.50	52.91 ± 0.02	14.95 ± 0.01	9.9 ± 0.20	22.24 ± 0.02	22.459 ± 0.19
A:EW (1:1)	T_6	87.00 ± 0.20	55.64 ± 0.02	12.50 ± 0.20	13.66 ± 0.47	18.20 ± 0.02	21.485 ± 0.26

Where,

* NFE = (100) – [Crude protein (%) + Crude lipid (%) + Ash (%)]

**GE = (Protein × 0.24) + (Lipid × 0.40) + (Carbohydrate × 0.17) (kJ/g)

Values expressed as% dry weight, ± S.E: Standard error of mean n = 3

Table 3: Average initial length, final length and length gain of female of *T. leerii* fed on different live food for rearing period of 28 days

	T_1	T_2	T_3	T_4	T_5	T_6
Average number of spawn/ female	1740.50±66.05^a	1363.16±29.85^b	494.33±36.82^c	827.33±50.01^d	452.33±48.40^c	603.00±114.91^d
Average initial length (cm)	5.70 ± 0.16	5.80 ± 0.16	5.80 ± 0.15	5.70 ± 0.21	5.80 ± 0.13	5.80 ± 0.19
Average final length (cm)	6.10 ± 0.16	6.06 ± 0.18	6.17 ± 0.13	6.03 ± 0.22	6.10 ± 0.12	6.03 ± 0.22
Average length gain (%)	5.86 ± 0.63^a	3.90 ± 0.68^b	5.35 ± 1.71^a	4.88 ± 1.02^a	5.36 ± 1.64^a	3.61 ± 0.97^b
Average initial weight (g)	1.8935 ± 0.23	2.0060 ± 0.14	2.1094 ± 0.23	1.8601 ± 0.23	1.8280 ± 0.06	1.9016 ± 0.22
Average final weight (mg)	2.3176 ± 0.25	2.5233 ± 0.19	2.5457 ± 0.27	2.5182 ± 0.29	2.0289 ± 0.12	2.1096 ± 0.23
Average weight gain (%)	29.81 ± 4.83^a	26.34 ± 6.16^a	21.04 ± 5.81^b	20.27 ± 2.36^b	10.82 ± 4.36^c	11.58 ± 3.31^c
SGR (%)	0.0092 ± 0.001^a	0.0081 ± 0.0018^a	0.0066 ± 0.0018^b	0.0065 ± 0.0007^b	0.0035 ± 0.0013^c	0.0038 ± 0.0010^c

Mean values in similar row with different letters are significantly different (SNK, $P<0.05$).

Conclusion

This is evident from the significant level of reproductive performance and considerably higher level of growth performance. Results indicated that mosquito larvae served as a best diet for better growth and reproductive performance.

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