**International Journal of Ecology and Environmental Sciences**
www.ecologyjournal.in
Online ISSN: 2664-7133; Print ISSN: 2664-7125; Impact Factor: RJIF 5.28
Received: 25-06-2020; Accepted: 10-07-2020; ; Published: 26-07-2020
Volume 2; Issue 3; 2020; Page No. 79-82

# Reproductive performance of female trichogaster leerii fed with enriched artemia

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**Abstract**
In ornamental fish culture unpredictable and variable reproductive performance is an important limiting factor for successful mass production of juvenile. Pearl gourami, *Trichogaster leerii* is one of the most popular aquarium fish among the aquarists because of its compatibility with other species. Finding of reproduction study of this species can be successfully applied to many other gouramis. Thus, attempt was made to observe the effect of enriched live food organism diets on reproductive performance of *T. leerii*. Experiment was conducted in 60x30x30cm glass tank having 54-Lcapacity, filled with water up to a level of 15 cm. The adult females of pearl gourami (length 5.70 ± 0.07 cm and weight 1.9417 ± 0.11g) were fed with different enriched on grown *Artemia viz*. emulsion of cod liver oil and ground nut oil cake, *spirulina* powder, EPA/DHA medium. The maximum number of spawn 1874 ± 25.26 were produced by each female fed with *Artemia* enriched with emulsion of cod liver oil and ground nut oil cake. Therefore, use of enriched *Artemia* with emulsion of cod liver oil and ground nut oil cake for better reproductive performance of brood stock of *T. leerii* is recommended.

**Keywords:** artemia enrichment, cod liver oil, groundnut oil, epa/hha, reproductive performance, gourami

## Introduction
Tropical ornamental species, such as pearl gourami, *Trichogaster leerii* (Bleeker, 1852) along with other gouramis are very much popular among the aquarists (Mathur *et al.*, 2006 [15], Ayyapan, 2011; Joshi, 2016) [12]. The special possession of the labyrinth organ makes this fish to survive in oxygen depleted waters (Cole *et al.*, 1999) [7]. Pearl gourami is native to the waters of Thailand (The Malayan Peninsula), Sumatra and Borneo (Axelrod, 1967) [3]. In ornamental fish hatcheries, live food such as *Artemia*, mosquito larvae, blood worm, tubifex worm, earth worm, cladocerans etc., are used to mature the brood fishes (Shelar *et al.*, 2014) [19]. The reproductive performance of *T. leerii*, with respect to the effect of different live food has been undertaken during this study to improve spawn production. The natural diet of *T. leerii* is composed of different species of invertebrate (Degani and Gur, 1992) [9]. However no investigation was carried out on scientific lines to determine the effect of different live food on reproductive performance of pearl gourami. The objective of the present experiment was to study the reproductive performance of Pearl Gourami, *Trichogaster leerii* fed with enriched *Artemia*.

## Material and methods
### Experimental Animal
*Trichogaster leerii* (Bleeker, 1852) commonly known as pearl gourami (length 5.70 ± 0.07 cm and weight 1.8208 ± 0.09 g).

### Distinguishing characters of male and female
Male gourami has elongated body, with long and pointed dorsal fin. Their body colour is brighter than female with a black dotted lateral line. During breeding, mouth to throat portion turns into red.
Female gourami has bulgy and curvy body. Dorsal fin was short and rounded. Dull body colouration than males. No dotted lateral line in females was observed as in case of males. Culture of *Artemia* was carried out in 350L circular tank using natural seawater. *Artemia* at the rate of five nauplii $mL^{-1}$ were stocked. An artificial diet containing ragi flour and yeast was used. It is mixed with 500mL seawater and screening to a particle size of 50µm. Daily feed ratios were chosen so as to maintain the transparency of the culture media between 15 and 20 cm. *Artemia* culture were used after 5 days.

### Enrichment media
1. $T_1$ : *Artemia* without enrichment
2. $T_2$: Ground nut oil cake and Cod liver oil: 1.5g Ground nut oil cake (as emulsifier) soaked with 5mL cod liver oil and homogenized with 100mL of sea water. After being well homogenized, 10mL of this mixture was used per liter of enrichment medium for six hour (Narciso *et al.*, 1999) [17].
3. $T_3$: ***Spirulina*****:** 3g of commercial *Spirulina* powder (Dried cells of *Spirulina* sp. Algae, Aquaculture grade) homogenized with 100mL sea water. 10mL of this mixture was used per litre of enrichment medium for six hour (Ritar *et al.*, 2004) [18].
4. $T_4$: EPA/DHA enrichment media: 3g of EPA/DHA capsules (Contain 3:2 EPA: DHA, Cadvion-03, Merck limited, India) homogenized with 1g Egg yolk in 100mL seawater. 10mL of this mixture was used per litre of enrichment medium for six hours (Watanabe *et al.*, 1983 [21]. and Ritar *et al.*, 2004) [18].

**Enrichment procedure**

Artemia were enriched in 500mL beaker containing 400mL sea water with density of eight number of Artemia mL $^{-1}$ and 10mL L$^{-1}$ enrichment media for six hour. In all treatment Artemia were aerated vigorously (Ritar *et al*., 2004) [18].

**Experimental design**

Glass aquarium ( $60 \times 30 \times 30$ cm, 54-L capacity) was filled with water up to a level of 15cm. Water quality parameters were analyzed by using methods given by APHA (2005) [2]. and Boyd (1981) [5]. and, and were maintained throughout the experimental period (Table 1).

An experiment was conducted for 30 days to observe influence of different enriched *Artemia* on reproductive performance of *T. leerii*. Experiment was conducted on adult female of *T. leerii* by feeding with enriched *Artemia*. Glass aquaria were arranged as per Completely Randomised Design (CRD) with four treatments having five replicates for each. Adult female of pearl gourami (length 5.70 ± 0.07 cm, weight 1.9417 ± 0.11 g) were stocked in each experimental glass aquaria at the rate of one number per tank. The four treatments were as follows –

$T_1$: *Artemia* without enrichment served as control

$T_2$: *Artemia* enrich with GOC soaked with cod liver oil

$T_3$: *Artemia* enriched with *Spirulina powder*

$T_4$: *Artemia* enrich with EPA/DHA media

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

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

| Water Quality Parameters | Mean observed value |
|---|---|
| Temperature (°C) | 26.24 ± 0.25 |
| pH | 7.17 ± 0.07 |
| Total hardness (mg L$^{-1}$) | 43.25 ± 2.06 |
| Total alkalinity (mg L$^{-1}$) | 35.00 ± 1.08 |
| Dissolved oxygen (mg L$^{-1}$) | 4.00 ± 0.20 |
| Free carbon dioxide (mg L$^{-1}$) | 30.24 ± 0.64 |

Values expressed as ± S.E. of mean.

**Reproductive performance of *T. leerii***

The glass tanks of $60 \times 30 \times 30$ capacity were filled with water up to 10 cm level. Thermocol sheet (9cm x 12cm) was placed on the surface of each tank which helps in construction of bubble nest to males. In present study, matured males (length 5.80 ± 0.08 cm, weight 1.9615 ± 0.10 g) were released in the glass aquarium tank in the morning hours (8.00 am) for construction of 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. The fishes spawned within 2 to 3 days. After spawning the female was 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 is referred as spawn. These spawn were counted for observing reproductive performance.

**Performance indicators**

At the beginning and end of each experiment, the fish were counted from each replicate and their individual length and weight were recorded. The average value of weight and length gain was calculated for analysis of growth parameters. Total length of fish was measured from tip of mouth to tip of caudal fin with the help of foot rule having a least count of 0.5 mm. Weight of each fish was taken on mono-pan electric balance having an accuracy of 0.01 mg, maximum 220g.

Fish, and test diets were weighed in an electrical mono-pan electric balance (Sartorius, BS 224S) to an accuracy of 0.01 mg. The following formulae were used to estimate the length gain, weight gain and specific growth rate.

Weight gain (%) = final weight - initial weight/ Initial weight x 100;

Length gain (%) = final length – initial length/ initial length x 100;

$$\text{Specific Growth Rate (\%)} = \frac{(\text{Log Wt - Log Wo})}{\text{dt}} \times 100$$

[Where, $W_t$ = Final weight; $W_o$ = Initial weight; dt = experimental period in days]

**Proximate composition**

Artemia enriched with different media were analyzed for moisture, crude protein (Kjeldhal method using KEL PLUS-CLASSIC DX, Pelican, India), crude lipids (Lipid extraction method by using SOX PLUS system, Pelican, India), ash and carbohydrates content by using method given in AOAC (2006) [1]. in Nutrition Laboratory of College of Fisheries, Ratnagiri. Nitrogen free extract were 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 (Chong *et al.*, 2004) [6]. The proximate composition of different live food organisms is given in Table 2. Data obtained from the experiments for growth parameters and reproductive performance were analysed by one way ANOVA. Significant difference was indicated as $P < 0.05$, Student's Newman Keul multiple range test was used to determine the significant difference between the treatments (Snedecor and Cochran 1967 [20]; Zar, 2005) [22].

**Table 2:** Proximate composition (% dry weight basis) of different test diets

| Diet | Treatment | Water | % of dry matter | | | | GE ** (kJ/g) |
|---|---|---|---|---|---|---|---|
| | | | Crude protein | Crude lipid | Ash | NFE | |
| *Artemia* | $T_1$ | 88.5 ± 0.20 | 55.82 ± 0.02 | 14.11 ± 0.20 | 16.7 ± 0.20 | 13.37 ± 0.17 | 21.458 ± 0.27 |
| Cod liver oil + Ground nut oil cake | $T_2$ | 87.70 ± 0.20 | 55.70 ± 0.15 | 16.30 ± 0.15 | 17.14 ± 0.03 | 10.86 ± 0.02 | 21.310 ± 0.24 |
| Dry *Spirulina* powder | $T_3$ | 88.70 ± 0.20 | 54.05 ± 0.02 | 13.20 ± 0.20 | 16.08 ± 0.47 | 16.67 ± 0.02 | 21.73 ± 0.26 |
| EPA/DHA medium | $T_4$ | 88.40 ± 0.20 | 56.13 ± 0.03 | 15.70 ± 0.30 | 18.13 ± 0.03 | 10.04 ± 0.03 | 21.085 ± 0.27 |

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

## Results and Discussion

An experiment was conducted to observe the reproductive performance of female pearl gourami, *T. leerii* fed with different enriched *Artemia*.Proximate composition of Artemia infused with different agents is given in Table 2. The reproductive parameters such as number of spawns produced by a female, growth parameters such as initial length and weight, final length and weight, length and weight gain, Specific growth rate (SGR) of females are shown 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 (Lavens and Sorgeloos, 1996). 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 the brood stock management. 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. Degani and Yehuda (1996) [10]. indicated that use of live food in pre-spawning broodstock management could enhance growth rate, faster gonadal development and produced quality larvae. The natural diet of *T. leerii* is composed of different species of invertebrates including insect larvae (Degani and Gur, 1992) [9]. and therefore this fish is selected for assessing the reproductive performance using natural live food organisms. Gonadal development of fishes is linked to variety of live food organisms (Degani, 1991) [8]. An enriched *Artemia* as live food diet was also tried in an attempt to assess its reproductive performance in term of spawn production. Better reproductive performance of angel fish, *Pterophyllum scalare* fed with mosquito larvae were reported by Degani and Yehuda (1996) [10], Meshram (1997) [16], Shelar *et al*. (2014) [19]. and Shelar *et al.* (2014) [19]. Importance of live food in aquarium fish management has been scientifically proved by various workers. The development of freshwater ornamental fish culture industry has been hampered by the lack of availability of suitable live foods for feeding the fish at the various production stages (Lim *et al.*, 2003) [14]. The bigger and older on-grown *Artemia* could be a good alternative live food for use in the hatchery of ornamental fish. Culture of *Artemia* up to bigger size by use of cheap agricultural by-products is possible and advantageous because of its nonselective feeding behaviour and faster growth characteristics (Dhont and Lavens, 1996) [11]. The continuous and nonselective feeding behaviour of on-grown *Artemia* also makes the organism an ideal booster diet, as its nutritional quality could be tailored to suit requirements of the fish through bioencapsulation. Use of on-grown *Artemia* for feeding to achieve better growth performance in Discus juveniles have been demonstrated by Lim *et al.* (2003) [14]. A simple method for production of on-grown *Artemia* was described by Lim *et al.* (2003) [14], following which, a laboratory scale system was designed and used for production of *Artemia* for feeding the brood stock. Due to higher nutritive content, ease in culture along with enrichment to improve nutritional quality, *Artemia* was used as a live feed for the present experiment.

Considering the fundamental importance of lipids in life cycle of fish and as a main source of metabolic energy, in the present study, enrichment of on-grown *Artemia* was carried out by allowing them to feed on emulsion of cod liver oil and ground nut oil cake ($T_2$), *Spirulina* powder ($T_3$), EPA/DHA medium ($T_4$) was carried out. These direct methods of enrichment were also demonstrated by Watanabe *et al.* (1983) [21]. with improvement of the dietary value of live foods containing substantial amount of n-3 HUFA or fat-soluble vitamin. Use of enriched *Artemia* for feeding different species of fishes was carried out by various authors. Narciso *et al*. (1999) [17]. studied the HUFA content and DHA/EPA improvements of *Artemia* sp. with commercial oils from animal and plant origin for different enrichment periods. The oil emulsion from animal origin enhanced the HUFA levels in enriched *Artemia* than that of plant origin. Among the sources, sardine oil was the minimum and squid oil was maximum in terms of the HUFA content and the DHA: EPA ratio. The result indicated that the enrichment could be carried out up to 33 h attaining effective ratio of DHA: EPA along with HUFA. However, biochemical and bacterial profiles of juvenile *Artemia* were examined by Ritar *et al.* (2004) [18]. and indicated that enrichment of juvenile *Artemia* for 6 h was the most appropriate. Considering this, enrichment of *Artemia* was carried through emulsion of EPA/DHA medium, dry *spirulina* powder, cod liver oil and ground nut oil cake for 6hrs duration. The result of the present study revealed higher reproductive performance in term of spawn production of *T. leerii* fed with *Artemia* enriched with cod liver oil and ground nut oil cake emulsion ($T_2$) than that of other enrichment media.Results of the present study showed relatively better growth and reproductive performance using enriched *Artemia* with cod liver oil and ground nut oil cake can be said to be suitable live food organism for achieving better growth and reproductive performance in *T. leerii.* The laboratory based system of culturing *Artemia* developed in the present study to produce on-grown *Artemia* on regular basis can be effectively use to get continuous supply of this precious live food organism.

**Table 3.** Average number of spawn produce by a female, initial length, final length, length gain, initial weight, final weight, weight gain and SGR of female of *T. leerii* fed on different enriched *Artemia* for rearing period of 28 days

| | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
|---|---|---|---|---|
| Average number of spawn produce by a female | 1318.50±52.27$^a$ | 1874.50±25.26$^c$ | 1400.66±40.97$^b$ | 1264.67±42.87$^a$ |
| Average initial length (cm) | 5.8 ± 0.19 | 5.7 ± 0.15 | 5.6 ± 0.14 | 5.8 ± 0.14 |
| Average final length (cm) | 6.1 ± 0.27 | 6.1 ± 0.21 | 5.9 ± 0.08 | 6.1 ± 0.20 |
| Average length gain (%) | 4.46 ± 1.52$^a$ | 7.30 ± 0.92$^b$ | 5.58 ± 1.74$^a$ | 5.47 ± 1.42$^a$ |
| Average initial weight (g) | 1.8718 ± 0.21 | 1.9605 ± 0.24 | 1.8296 ± 0.22 | 2.1047 ± 0.22 |
| Average final weight (mg) | 2.1732 ± 0.21 | 2.5712 ± 0.26 | 2.2720 ± 0.26 | 2.4207 ± 0.22 |
| Average weight gain (%) | 16.97 ± 5.09$^a$ | 34.47 ± 7.75$^c$ | 25.17 ± 5.02$^b$ | 15.97 ± 3.46$^a$ |
| SGR (%) | 0.00543 ± 0.0016$^a$ | 0.0102 ± 0.0022$^c$ | 0.00786 ± 0.0015$^b$ | 0.00521 ± 0.0011$^a$ |

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

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