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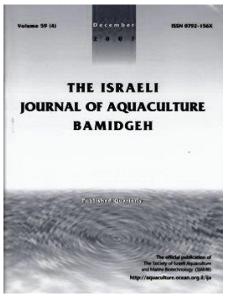

Effect of Replacing Live Food with Formulated Feed on Reproductive Performance of Freshwater Angelfish, *Pterophyllum scalare* (Shcultze, 1823)

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Abstract

The combined effect of live food (LF) and formulated feed (FF) on reproductive performance of *Pterophyllum scalare* was studied. Mosquito larvae were used as live food in addition to formulated feed which contained 50% protein and 12% lipid. The experiment was arranged as a completely randomized design (CRD) with six replicates for each treatment: *P. scalare* were fed with formulated feed and/or live food: T1 (100% FF), T2 (75% FF and 25% LF), T3 (50% FF and 50% LF), T4 (25% FF and 75% LF) and T5 (100% LF). Relative fecundity, egg and oil globule diameter was significantly higher ($P < 0.05$) in T4 and T5 diets whereas inter spawning interval was lower in T3, T4, and T5 diets. Similarly hatching percentages were significantly higher with diets T3, T4, and T5. There were no significant differences observed between treatments on relative hatchling number, hatchling length, and weight. No significant difference was observed for egg and whole body composition of *P. scalare* fed with different diets. However there was a significant difference between treatments on fatty acid profile. It was concluded that live food can be replaced by up to 25-50% formulated feed without compromising reproductive performance of *P. scalare*.

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Introduction

Ornamental fish culture is one of the most popular hobbies in developed countries of the world and is also gaining popularity in many developing markets of the world. The growing interest in aquarium fish has resulted in a steady increase in aquarium fish trade globally. According to FAO (2010), export earnings from the ornamental fish trade in US was worth $1 billion annually and developing countries produced more than 60%. Freshwater cichlids are popular ornamental fish and most of the species are traded worldwide.

The main dietary function is to provide essential nutrients and energy for maintenance of physiological functions, growth, and gonadal development in fish. Most fish breeders use live food to induce breeding in *P. scalare*. It is believed that live food, (which includes worms, insects, and their larvae), induce fish to breed. However live food is not always available. The biggest problem with live food is the possible introduction of diseases into the holding facilities. Comparisons have been made between live food and artificial feed as broodstock diets for *P. scalare* (Degani and Yehuda, 1996), *Xiphophorus helleri* (Kruger et al., 2001; James and Sampath, 2004), *Betta splendens* (James and Sampath, 2002; Mandal et al., 2010) and zebrafish (Markovich et al., 2007). No one has replaced live food with specifically formulated feeds for *P. scalare*. Thus, the aim of the present study was evaluate the effect of partial or total replacement of live feed by formulated feed, on the reproductive performance of *P. scalare*.

Materials and Methods

Eight to nine month old males and females of *P. scalare* (50 fish) weighing 16.43±0.72 g were reared in captivity in the wet laboratory of Department of Aquaculture, College of Fisheries, Ratnagiri, Maharashtra, India. Prior to initiation of experiments, fish were acclimated to the experimental diets for up to four weeks and were allowed to choose their own mates in a community set-up. A pair was identified in a community tank (180x60x80 cm) when it isolated itself in a corner and drove away other fish in the tank; these pairs were transferred to small spawning tanks (38x23x23 cm) and the experiments were initiated after three spawning cycles of each pair.

Mosquito larvae were used as live food (LF). The formulated feed (FF) was given in different combinations with live feed (LF): T1 (100% FF); T2 (75% FF + 25% LF); T3 (50% FF + 50% LF); T4 (25% FF + 75% LF) and T5 (100% LF). The proximate composition of the experimental diets is presented in Table 1 and fatty acid profile is given in Table 2.

Table 1. Proximate composition (g/kg dry weight basis) of formulated feed and mosquito larvae.

Particulars (g/kg DM)	*Formulated feed*	*Mosquito larvae*
Dry matter	964.9	944.3
Crude protein	518.9	498.8
Crude lipid	121.0	141.5
Ash	63.0	173.5
Crude fiber	58	37.2
NFE*	297.1	39.0
Gross energy (KJ/g)**	22.44	16.83

*NFE = 1000 – (Crude protein + Crude lipid + Ash)
**Gross energy, calculated based on 23.9, 39.8 and 17.6 MJ/kg for protein, lipid and NFE, respectively (Schulz et al., 2007)

Table 2. Fatty acid composition (% of total fatty acids) of formulated feeds and mosquito larvae.

Fatty acid	*Formulated feed*	*Mosquito larvae*
Saturates		
12:0	0.4	4.88
14:0	2.7	8.15
16:0	16.9	14.47
18:0	3.4	11.42
20:0	0.4	4.35
22:0	1.5	3.22
Total	25.3	46.49
Monosaturates		
16:1n-7	4.1	-
18:1n-9	33.4	17.78
Total	37.5	17.78
n-6 Polysaturates		
18:2n-6	26.8	6.54
Total	26.8	6.54
n-3 Polysaturates		
18:3n-3	1.8	5.66
20:5n-3	3	3.85
22:6n-3	0.3	1.35
Total	5.1	10.86
Total *polyunsaturates*	31.9	17.4
P:S[1]	1.26	0.37
M:S[2]	1.48	0.38
n-3:n-6	0.19	1.66

[1]P:S = Polyunsaturates : saturates.
[2]M:S =Monounsaturates : saturates.

The experiment was arranged in a completely randomized design (CRD) with six replicates for each treatment. Uneaten feed together with about 40% of the water was siphoned out from each container daily. The hatching rate (%), relative fecundity, was measured as the number of hatchlings (n) in relation to the weight of the parents (n/g^{-1}), inter spawning interval (ISI), relative hatchling number, length and weight of free swimming larvae was recorded after each spawning until the fifth consecutive spawn of each pair. On the sixth spawn, the egg and oil globule diameter were estimated by tpsDIG2 software (Rohlf, 2004). The remaining eggs were used for biochemical analysis. At the end of the experiment the broodfish were sacrificed for muscle tissue biochemical analysis.

Each tank (38x23x23 cm) was provided with a spawning slate (11″x3″) that was placed vertically along the wall of the aquarium. After spawning, the eggs were counted, the spawning slate was transferred to a vigorously aerated hatching tank (45x22x30 cm), and one ppm methylene blue was added to prevent the growth of fungi. After 3-4 days the hatchlings reached wriggler-stage and remained attached to the spawning slate. After 6-7 days, the hatchlings consumed their yolk sac and became free swimming. These hatchlings were used to evaluate the hatching performance of the fish.

The proximate analyses of feed ingredients, diets, muscle tissue, and eggs, were carried out according to the standard methods of AOAC (2006). Moisture content was estimated by drying the samples to constant weight at 110ºC in a drying oven (BTI); protein was determined using the Kjeldahl method (Kel Plus-Classic DX, Pelican, India); and crude fat was determined by ether extraction using Soxhlet apparatus (Socs Plus-SCS 2, Pelican, India). Crude fiber was estimated through 1.25% acid subsequent 1.25% alkali digestion using Fibra plus-FES 2, Pelican, India. Ash was determined by combusting fat free samples in a muffle furnace at 550ºC for 5 hours. The egg protein and lipid content was analyzed using Lowry (Lowry et al., 1951) and Folch’s (Folch et al., 1957) methods respectively.

Spawning and hatching performance. The reproductive parameters such as relative fecundity (RF), time interval between each spawning in days, i.e. inter spawning interval (ISI), as well as egg and oil globule diameter were calculated. The mean of the short and long axes diameters were taken as the diameter of the egg (Gunasekera et al., 1995). Egg and oil globule diameter was measured by tpsDIG2 software (Rohlf, 2004).

$$\textbf{a.}\ \text{Relative fecundity (nos.)} = \frac{\text{Total Number of eggs produced}}{\text{Total body weight of female (g)}}$$

The weight of hatchling was estimated using the Top pan electronic precision (0.01 mg accuracy) balance. The length of hatchling was recorded using a measuring scale (0.5 mm fraction). Hatchlings were counted and shifted to another rearing tank. The healthy hatchlings were used to calculate relative hatchling number using formulae as follows,

$$\textbf{a.}\ \text{Hatching percent (\%)} = \frac{\text{Total number of eggs hatched}}{\text{Total number of eggs produced}} \text{x100}$$

$$\textbf{b.}\ \text{Relative hatchling number} = \frac{\text{Total number of hatchling produced}}{\text{Total body weight of female (g)}} \text{x100}$$

Statistical analysis. Experiments were analyzed by analysis of variance (ANOVA) using Statistical Analysis System (SAS 9.2) and difference among means were tested using Tukey’s HSD test (Snedecor and Cochran, 1967). Percent values were transformed into arcsines (Zar, 2005).

Results

Spawning and hatching performance of P. scalare fed with different type of feeds. The observed effect of varying levels of formulated feed and live food on average values for relative fecundity, inter spawning interval, egg and oil globule diameter of *P. scalare* is presented in Table 3. The maximum relative fecundity of 59.52±0.36 nos.g^{-1} was observed in T5 diet which was significantly different (P < 0.05) from T1, T2 and T3 diets. Minimum average spawning interval of 12.12±0.23 days was recorded in T4 diet while with the T1 diet, maximum average was 14.70±0.36 days. Significantly higher egg (1.3602±0.0069 mm) and oil globule diameter (1.2243±0.0015 mm) was observed in T5 diet.

Average hatching percent, relative hatchling number, length and weight of *P. scalare* observed due to effect of varying levels of formulated feed and live food is presented in Table 3.0.

Table 3.0 Spawning and hatching performance of *P. scalare* fed with different feed types.

Particulars	*T1*	*T2*	*T3*	*T4*	*T5*
Relative fecundity (nos. g^{-1})	55.89±0.85^{b}	54.50±1.24^{b}	56.60±0.63^{b}	59.10±0.54^{a}	59.52±0.36^{a}
Inter spawning interval (days)	14.70±0.36^{a}	14.57±0.18^{a}	12.72±0.20^{b}	12.12±0.23^{b}	12.17±0.19^{b}
Egg diameter (mm)	1.3262 ±0.0073^{ab}	1.3233 ±0.0075^{ab}	1.3209 ±0.0055^{ab}	1.3426 ±0.0022^{a}	1.3602 ±0.0069^{a}
Oil globule diameter (mm)	1.2003 ±0.0037^{b}	1.2020 ±0.0026^{b}	1.2016 ±0.0021^{b}	1.2193 ±0.0035^{a}	1.2243 ±0.0015^{a}
Hatching percent %	68.08±0.98^{b}	67.43±0.80^{b}	70.18±0.40^{a}	70.90±0.32^{a}	71.75±0.67^{a}
Relative hatchling number	41.46±0.21	41.21±0.57	41.57±0.22	41.57±0.23	41.92±0.56
Hatchling length (cm)	0.52±0.02	0.50±0.00	0.52±0.02	0.53±0.02	0.55±0.02
Hatchling weight (mg)	4.35±0.08	4.33±0.11	4.48±0.08	4.57±0.07	4.50±0.06

Mean values in similar row with different letters are significantly different (Tukey's HSD test, P<0.05).
T_1=100% FF); T_2=75% FF + 25% LF; T_3=50% FF + 50% LF; T_4=25% FF + 75% LF; T_5=100% LF.

Significantly maximum hatching percent of 71.75±0.67 % was observed in T5 diet whereas the minimum of 67.43±0.80 % was seen in the T2 diet. This was higher than in diets T1 and T2. The maximum relative hatchling number 41.92±0.56 was observed in T5 diet and the lowest 41.21±0.57 was seen in T2. Maximum average hatchling length (0.55±0.02 cm) and weight (4.57±0.07 mg) was recorded in T5 and T4 diets respectively. There was no significant difference for relative hatchling number, hatchling length and weight.

Biochemical composition of eggs and muscle tissue of P. scalare. The average moisture, protein and lipid content of eggs of *P. scalare* fed with different levels of formulated feed and live food is given in Table 4. ANOVA test did not show a significant difference (P > 0.05) between moisture, protein, and lipid percent of eggs.

The average values for moisture, protein, lipid, and ash content of muscle tissue of *P. scalare* fed with different levels of formulated feed and live food are given in Table 4. There was no significant difference (P > 0.05) for muscle moisture, protein, lipid, and ash between the different treatments.

Table 4. Proximate composition of eggs (% wet weight basis) and muscle composition (% dry weight basis) of *P. scalare* fed with different feed type.

Particulars	*T1*	*T2*	*T3*	*T4*	*T5*
Eggs (% wet weight basis)					
Moisture (%)	58.39±0.50	58.22±0.32	58.46±0.29	58.24±0.34	58.65±0.11
Protein (%)	26.49±0.68	26.69±0.48	26.83±0.58	26.45±0.54	25.88±0.36
Lipid (%)	13.81±0.44	13.94±0.18	14.01±0.35	14.24±0.38	14.26±0.30
Muscle tissue (% dry weight basis)					
Moisture (%)	74.10±1.11	75.23±1.23	74.23±0.93	75.25±1.12	74.10±1.21
Protein (%)	64.49±0.27	62.74±0.28	62.84±0.78	62.73±0.59	62.58±0.50
Lipid (%)	31.60±0.13	32.35±0.33	32.62±0.46	32.64±0.48	32.59±0.45
Ash (%)	2.68±0.08	2.54±0.07	2.67±0.08	2.52±0.04	2.53±0.10

Mean values in similar row with different letters are significantly different (Tukey's HSD test, P<0.05).
T_1=100% FF); T_2=75% FF + 25% LF; T_3=50% FF + 50% LF; T_4=25% FF + 75% LF; T_5=100% LF.

Fatty acid profile of muscle tissue. The fatty acids of muscle tissue of *P. scalare* fed on different levels of formulated feed and live food is given in Table 5.

Table 5. Classification of fatty acid of total lipid in muscles of *P. scalare* broodstock fed on formulated feed and live food.

Fatty acid	*T1*	*T2*	*T3*	*T4*	*T5*
Saturates					
12:0	8.11±0.13^a	8.45±0.23^a	4.34±0.34^b	2.65±0.26^c	2.57±0.29^c
14:0	9.21±0.35^c	9.14±0.47^c	4.88±0.12^b	2.90±0.34^a	2.81±0.11^a
16:0	5.57±0.30^a	5.70±0.52^a	10.44±0.45^b	19.99±0.68^c	20.99±0.49^c
18:0	1.83±0.14^a	1.90±0.12^a	6.90±0.36^b	7.36±0.23^b	7.88±0.42^b
20:0	6.25±0.24^a	5.89±0.20^a	5.22±0.25^b	3.06±0.21^c	2.52±0.18^c
Monounsaturates					
18:1n-9	4.70±0.21^a	5.09±0.15^a	19.09±0.33^b	23.48±0.43^c	24.10±0.37^c
n-6 Polyunsaturates					
18:2n-6	12.20±0.28	11.84±0.33	11.81±0.26	11.66±0.23	11.42±0.19
n-3 Polyunsaturates					
18:3n-3	1.75±0.19^a	1.65±0.08^a	1.92±0.07^a	2.34±0.04^b	2.78±0.10^b
20:5n-3	12.31±0.27^c	11.78±0.24^c	4.42±0.16^b	2.30±0.30^a	2.26±0.13^a
22:6n-3	10.80±0.25^a	10.51±0.35^a	10.41±0.35^a	11.85±0.26^b	12.02±0.22^b

Mean values in similar row with different letters are significantly different (Tukey's HSD test, P<0.05).

Discussion

Various studies have been conducted on the effect of nutrition on growth or reproduction of ornamental fish (Degani and Gur, 1992; Degani and Yehuda, 1996; Kruger et al., 2001; James and Sampath, 2002, 2004; Chong et al., 2004; Ling et al., 2006) but research into the impact of the combined feeding of live food and formulated feed on the reproductive performance of *P. scalare* is rare. Hence, this study was undertaken to examine the effects of feed quality on reproductive performance in marble angelfish, *P. scalare*.

From the different combinations of formulated feed and live food used, the highest relative fecundity and hatching rate was observed in fish fed on live food (mosquito larvae) indicating that the fatty acid composition of the diet influenced the reproductive performance of *P. scalare*. The best reproductive performance was observed in mosquito larvae fed groups (T4 and T5). This could be due to larger amounts of EPA (3.85%) and DHA (3.35%) than in the formulated feeds which contained 3.0% and 0.3% respectively. The balanced composition of lipids and essential n-3 and n-6 fatty acids in the live food might have stimulated gonad development and enhanced relative fecundity and hatching percentage in the angelfish. Similar observations were made for *Betta splendens*, where a mixed diet (50-100% substitution of live food) enhanced reproductive performance of fish (James and Sampath, 2002; 2003). In another study there was no significant difference in the number of eggs spawned in relation to diets (Luquet and Watanabe, 1986).

Type of diet significantly affected the percentage of hatching, and duration between spawns, but not the number of eggs per spawn in *P. scalare (*Degani and Yehuda, 1996). The short reproductive cycle of asynchronous fish might result in continuous oogenesis in adult females, therefore availability of the right type of diet is very important (Degani and Yehuda, 1996). During oogenesis, the oocytes synthesize vitellogenin (Degani et al., 1997) and in teleosts, oogenesis and spawning are regulated by the gonadotropin hormone (GtH). It is possible that live feed may contain some microelements essential for the secretion of GtHs. Another possibility is that the diet affects steroid production which is also important for oogenesis and the reproductive cycle (Degani and Boker, 1992).

Despite the relatively small quantity of live feed given in T3 diet, there was a significant improvement in the hatching percent when compared to fish fed only formulated feed. No benefits were observed in relative fecundity, inter spawning interval, hatching percent, and relative hatchling number when formulated feed was replaced with 25% live food. In our study presence or absence of mosquito larvae was more important than the relative proportion of protein and lipid. This is supported by other reported observations (Degani and Yehuda, 1996).

Mosquito larvae may contain enzymes that enhance fish appetite, aid fish digestion, feed intake, growth, and reproductive performance. The developing embryo depends on nutrients present in egg yolk. The large size, active movement, and high protein (49.88%), and lipid (14.15%) content of mosquito larvae could have a stimulatory effect on the predatory responses of *P. scalare* which enhances relative fecundity, hatching rate, egg, and larvae quality. These results are supported by other studies for *P. scalare* (Degani and Yehuda, 1996; Ortega-Salas et al., 2009).

There was a 30% increase in fry of *Xiphophorus hellleri* fed daily with a commercial diet supplemented with live *Daphnia spp*. (Kruger et al., 2001). This corresponds with our results. However in another study flake feed for maximum fry production of zebrafish (*Danio rerio*) was recommended (Markovich et al., 2007). This indicates that different fish species may have specific nutritional requirements for reproduction.

In the present study EPA and DHA played an important role in the reproductive performance of *P. scalare*. This is may be due to readily available n-3 HUFA for ovary development. Neural tissue, especially the eyes, forms a relatively large proportion of the total body mass of embryonic fish requirements, 22:6n-3, therefore any deficiency of this component during embryogenesis and larval development can lead to poor hatching rate. Another important function of (n-3) PUFA is their ability to produce eicosanoids, which are important in stress reaction and adaptation (Sargent et al., 1990).

In the present study, reproductive performance in terms of relative fecundity and hatching percentage of T3 was markedly improved compared to T1 and T2 but was lower than the groups fed with mosquito larvae T5 (100%) and T4 (75%) although there was no significant difference between the groups T3, T4 and T5 for hatching percentage and inter spawning interval. This improvement could possibly be due to the more balanced supply of both DHA and EPA. In addition, the amount of essential fatty acids in eggs correlates with inter-spawning interval, hatching rate, egg, and larvae quality (Blaxter, 1969).

Our study revealed a strong correlation between 16:00 and 18:1n-9 fatty acids with relative fecundity and hatching performance (hatching percentage and relative hatchling number) the reason being that that saturated and monounsaturated fatty acids are quantitatively more important than (n-3) PUFA in providing metabolic energy for egg development (Sargent, 1995).

Parameters used in assessing egg quality also include egg and oil globule diameter (Bromage et al., 1992). A number of environmental factors as well as size of brooders have been shown to influence fecundity and egg size. As fish size increases so does fecundity and diameter of eggs. The present study showed that in *P. scalare* fed with T4 and T5 diets, the egg size, and oil globule diameter was significantly larger than T1, T2, and T3. Egg size is an indicator of improved viability of the larvae (Blaxter, 1988). The difference in the size of the oil globule (table 3) did not have a significant effect on the hatching rate of the eggs.

The combined effect of formulated feed and live food significantly influenced the reproductive and hatching performance as well as egg and muscle composition of *P. scalare.* No significant differences were found for body protein, fat, and ash content among the treatments. It can be concluded that up to 50% live feed can be replaced with formulated feed for *P. scalare*, without any adverse effect on the reproductive performance of fish.

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