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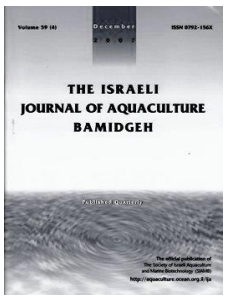

Effect of Dietary Protein and Lipid Levels on the Reproductive Performance and Body Composition of Angelfish, *Pterophyllum scalare* (Schultze, 1823)

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Abstract

A study to determine the effect of increasing levels of dietary protein and lipid on *Pterophyllum scalare* was carried out using twelve semi-purified diets varying in dietary protein (P) and lipid (L) levels (48P8L, 48P12L, 48P16L, 50P8L, 50P12L, 50P16L, 52P8L, 52P12L, 52P16L, 54P8L, 54P12L and 54P16L). Broodstock performance was evaluated based on relative fecundity, fertilization rate, hatching rate, egg size, spawning interval, egg and body composition of *P. scalare* broodstock. Egg production was highest from fish fed with 50P12L, 52P12L and 52P16L diets. Fish fed the 50P12L and 52P12L diets tended to spawn at shorter intervals. Relative fecundity, fertilization rate, hatching rate, egg size and relative hatchling number, were all significantly higher on diets containing 52% protein than at lower levels. There was no significant difference in hatchling length but hatchling weight differed significantly ($P < 0.05$) with dietary protein and lipid levels. The protein content of fish and eggs was also influenced ($P < 0.05$) by dietary protein and lipid levels. It is therefore suggested that in *P. scalare* broodstock fed with 50-52% protein and 12% lipid, reproductive performance, egg and larval quality improved compared with other diets.

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Introduction

Pterophyllum scalare which belongs to the Cichlidae family, is one of the most popular aquarium fish and commands a higher price compared to most freshwater ornamental fish. In spite of the economic importance of *P. scalare* in the ornamental fish trade, there has been neither research nor development of cost-effective feeds for the spawning of this species. Fish breeders rely on specific formulated dry feeds (Mohanta *et al.,* 2011) and live food such as artemia, tubifex, daphnia and mosquito larvae. Availability and production of live food is limited in comparison to formulated dry feeds. It is also essential to formulate broodstock diet for *P. scalare*.

Reproductive performance and seed production of fish are directly related to broodstock nutrition (Izquierdo *et al.,* 2001). Exogenous nutrition of broodfish provides the essential nutrients required for gonadal development and for quality seed production.

Egg yolk is considered a major source of essential nutrients which play an important role in fish nutrition. Protein also acts as a source of amino acids and a reservoir of materials utilized for biosynthetic processes essential for early stages of embryogenesis. There is an optimal protein level for reproductive success and this level is related to growth (De Silva and Anderson, 1995). Several authors have evaluated the effects of dietary protein levels on the reproductive performance of fish (Manissery *et al*., 2001; Khan *et al*., 2004, 2005; Ling *et al*., 2006). However, information on the effect of dietary lipid levels on the reproductive performance of fish is scanty. The present study was conducted to investigate the effect of dietary protein and lipid levels on reproductive performance, egg and larval quality of *P. scalare*.

Materials and Methods

Eight to nine month old male and female *P. scalare* (100 fish) weighing 16.43±0.72g were reared in captivity in the wet laboratory of the Department of Aquaculture, College of Fisheries, Ratnagiri, Maharashtra, India for this experiment. Prior to initiation of experiments, fish were conditioned for four weeks, during which they were acclimatized to the experimental diets 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 (36 pairs).

Twelve experimental diets were formulated to contain four levels of protein (48, 50, 52 and 54 %) and three levels of lipid (8, 12 and 16 %). Data for ingredients and chemical composition are shown in Table 1.

Table 1. Ingredients used and proximate analysis of experimental diets (% DM)

Ingredients (%)	*Diets*											
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
Fish meat	20	20	20	20	20	20	20	20	20	20	20	20
Casein[b]	33.6	33.6	33.6	37.8	37.8	37.8	40.2	40.2	40.2	44.4	44.4	44.4
Gelatin[b]	3	3	3	3	3	3	3	3	3	3	3	3
Cod liver oil	2.5	4.5	6.5	2.5	4.5	6.5	2.5	4.5	6.5	2.5	4.5	6.5
Soyabean oil	2.5	4.5	6.5	2.5	4.5	6.5	2.5	4.5	6.5	2.5	4.5	6.5
Dextrin[b]	21.4	19.4	17.4	17.2	15.2	13.2	16.8	14.8	12.8	15.6	13.6	11.6
Vitamin	3	3	3	3	3	3	3	3	3	3	3	3
Mineral mixture[d]	2	2	2	2	2	2	2	2	2	2	2	2
CMC[be]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Cellulose[b]	10.5	8.5	6.5	10.5	8.5	6.58	8.5	6.5	4.5	7.5	5.5	3.5
Proximate composition (% dry weight basis)												
Crude protein	48.1	48.3	47.9	50.3	50.1	50.4	52.3	52.2	52.3	54.3	54.1	55.1
Crude lipid	8.1	12.4	16.8	8.1	12.9	16.6	8.4	12.6	16.5	8.2	12.8	16.5
Crude ash	6.6	6.3	5.6	6.6	6.0	5.9	6.5	6.2	6.6	6.8	6.2	5.9
Crude fiber	4.8	4.5	5.0	4.7	4.7	4.8	4.9	5.1	5.0	4.9	5.2	5.1
NFE[f]	37.2	33.0	29.7	35.0	31.0	27.1	32.8	29.0	24.6	30.7	26.9	22.5
GE (KJ/g)[g]	21.2	22.29	23.36	21.4	22.56	23.42	21.61	22.59	23.4	21.64	22.7	23.69

a. Fish meat powder (Moisture: 6.4 %, Crude protein: 71.06 %, Crude lipid: 7.55 %, Crude ash: 12.03 %, Crude Fiber: 2.13 %, Carbohydrate: 2.73 %),

b. Obtained from Himedia, India,

c. Becosules capsules, product of Pfizer Ltd., India: Vitamin(mg/g): Thiamine mononitrate IP–10mg, Riboflavina IP–10mg, Pyridoxine Hydrochloride IP–3mg, Vitamin B_{12} IP-15mcg, Niacinamide IP–100mg, Calcium Pantothenate IP–50mg, Folic acid IP-1.5mg, Biotin USP-100mcg, Ascorbic acid IP–150mg.

d. Agrimin, product of Virbac Animal Health India Pvt. Ltd.; Mineral (mg/kg): Cobalt – 150mg, Copper – 1200mg, Iodine – 325mg, Iron – 5000mg, Magnesium – 6000mg, Manganese – 1500mg, Potassium – 100mg, Selenium – 10mg, Sodium - 5.9mg, Sulphur - 0.922%, Zinc – 9600mg, DL-Methionine – 1920mg, L-lysine mono-hydrochloride - 4400mg, Calcium - 24%, Phosphorous - 12%.
e. Carboxy Methyl Cellulose,
f. NFE = 100 – (Crude protein + Crude lipid + Ash).
g. Gross energy, calculated based on 23.9, 39.8 and 17.6 MJ/kg for protein, lipid and NFE, respectively (Schulz *et al.,* 2007).

Energy levels of the diets were calculated based on 23.9, 39.8 and 17.6 MJ/kg for protein, lipid and NFE, respectively (Schulz *et al.,* 2007).

Fish meat powder (prepared from *Otolithus sp.*), defatted casein and gelatin were used as sources of dietary protein, and dextrin was used as a carbohydrate source. Cellulose was used as filler while carboxymethyl cellulose (CMC) was used as binder. The ingredients were manually mixed and then blended with lipid (soybean oil and cod liver oil 1:1 v/v) and warm water. A vitamin and mineral mixture was mixed into the slurry. Flakes were prepared by spreading the slurry uniformly on polythene sheets with a brush. The flakes were dried in an oven for 8 hrs at 65ºC. Dry flakes were placed in sealed plastic bags and stored at -20 ºC until use.

Each tank (38x23x23 cm) was equipped with a spawning slate (11”x3”) placed vertically along the wall of the aquarium. After spawning, the eggs were counted and 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 fungus. After 3 to 4 days the hatchlings reached wriggler stage and remained attached to the spawning slate. After 6 to 7 days, the hatchlings had consumed their yolk sac and became free swimming. These were used to evaluate the hatching performance of the *P. scalare*.

Feed and whole body samples were analyzed for proximate composition. Moisture content was estimated by drying the samples at 110ºC in an oven to constant weight; protein was estimated using the Kjeldahl method (total N x 6.25) (Kel plus classic DX, Pelican, India) and total lipid was determined by Petroleum ether extraction using Soxhlet apparatus (SOCS PLUS, Pelican, India). Crude fiber was estimated using 1.25% acid subsequent 1.25% alkali digestion (Fibra Plus FES 2, Pelican, India) and ash was determined by combusting fat free samples in a muffle furnace at 550ºC for 5 h (AOAC, 2006). The egg protein and lipid content was analyzed using Lowry (Lowry *et al.,* 1951) and Folch’s method (Folch *et al.,* 1957) respectively.

Spawning and hatching performance. Reproductive parameters such as relative fecundity (RF), interspawning interval (ISI) (time interval between each spawning in days) as well as egg and oil globule diameter were calculated. The mean diameters of the short and long axes were taken as the diameter of the egg (Gunasekera *et al.,* 1995). Egg and oil globule diameter was measured by tpsdig2 software (Rohlf, 2004).

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

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

$$\text{Hatching rate (\%)} = \frac{\text{Total number of eggs hatched x 100}}{\text{Total number of eggs produced}}$$

$$\text{Relative hatchling number} = \frac{\text{Total number of hatchlings produced x 100}}{\text{Total body weight of female (g)}}$$

Statistical analysis. Data were analyzed by two-way analysis of variance (ANOVA) using Statistical Analysis System (SAS 9.3) and differences between means were tested by Tukey’s HSD. Percent values were transformed into arcsines (Zar, 2005).

Results

Results of reproductive and hatching performance of *P. scalare* fed on different dietary protein and lipid levels are given in Table 2.

Table 2 Reproductive and hatching performance of *P. scalare* fed with experimental diets.

Particulars	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
Relative fecundity (Nos/g)	28.01 ± 0.65^{a}	35.24 ± 2.27^{b}	30.43 ± 2.94^{a}	53.38 ± 1.04^{d}	59.44 ± 1.38^{e}	56.19 ± 3.04^{d}	46.23 ± 0.80^{c}	62.19 ± 1.50^{e}	58.34 ± 2.51^{e}	40.24 ± 0.97^{b}	40.18 ± 1.19^{b}	37.74 ± 0.94^{b}
Inter spawning interval (days)	24.23 ± 0.61^{a}	25.12 ± 1.18^{a}	24.10 ± 1.53^{a}	20.54 ± 0.48^{b}	14.38 ± 0.56^{c}	15.31 ± 0.78^{c}	13.65 ± 0.66^{c}	12.91 ± 0.25^{c}	13.06 ± 0.22^{c}	14.93 ± 0.76^{c}	16.57 ± 0.96^{c}	15.25 ± 0.90^{c}
Egg diameter (mm)	1.2689 ± 0.0049^{d}	1.2668 ± 0.0023^{d}	1.2823 ± 0.0065^{d}	1.2762 ± 0.0083^{d}	1.3240 ± 0.0142^{c}	1.3296 ± 0.0052^{bc}	1.3099 ± 0.0102^{c}	1.4111 ± 0.0100^{a}	1.4404 ± 0.0088^{a}	1.3738 ± 0.0031^{b}	1.2688 ± 0.0081^{d}	1.2709 ± 0.0131^{d}
Oil globule diameter (mm)	1.1211 ± 0.0155^{d}	1.0930 ± 0.0118^{d}	1.1373 ± 0.0170^{d}	1.1280 ±0.0065^{d}	1.2018 ± 0.0108^{c}	1.2454 ± 0.0090^{bc}	1.1998 ±0.0128^{c}	1.2883 ± 0.0109^{a}	1.3065 ± 0.0076^{a}	1.2692 ± 0.0092^{b}	1.1332 ± 0.0101^{d}	1.1495 ± 0.0092^{d}
Fertiliz. rate (%)	60.44 ± 2.25^{a}	66.19 ± 1.16^{b}	66.54 ±2.17^{b}	71.67 ± 5.15^{bc}	82.59 ±1.19^{d}	74.42 ± 3.10^{bcd}	72.81 ± 1.52^{bcd}	81.42 ± 1.69^{cd}	78.60 ± 1.53^{cd}	75.09 ± 2.44^{bcd}	75.26 ± 1.57^{bcd}	72.62 ± 1.98^{bcd}
Hatching rate	55.54 ± 3.00^{c}	57.93 ± 1.95^{c}	61.94 ± 2.87^{c}	66.29 ± 6.33^{b}	76.84 ± 2.72^{d}	68.16 ± 3.30^{e}	64.93 ± 2.29^{c}	74.98 ± 1.29^{a}	74.75 ± 2.34^{a}	70.76 ± 2.41^{e}	69.99 ± 1.45^{e}	67.16 ± 2.32^{b}
Relative hatchling number	13.52 ± 0.66^{a}	17.45 ± 1.12^{a}	15.75 ±1.49^{a}	29.21 ± 1.62^{c}	36.42 ±0.91^{d}	31.30 ±1.77^{c}	24.81 ±0.47^{b}	37.33 ±1.08^{d}	34.90 ±1.44^{d}	23.05 ±0.66^{b}	22.83 ±0.80^{b}	20.74 ± 0.25^{b}
Hatchling length (cm)	0.48 ± 0.02	0.50± 0	0.50± 0.02	0.50± 0	0.50± 0	0.50± 0	0.51± 0.02	0.52± 0.02	0.52± 0.02	0.53± 0.02	0.5± 0	0.5± 0
Hatchling weight (mg)	3.36 ± 0.06^{f}	3.45 ± 0.04^{f}	3.98 ± 0.08^{e}	4.03 ± 0.09^{e}	4.37 ± 0.05^{d}	4.60 ± 0.07^{c}	5.06 ± 0.06^{b}	5.43 ±0.08^{a}	5.53 ±0.05^{a}	4.88 ±0.13^{b}	4.77 ±0.06^{c}	4.77 ± 0.12^{c}

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

There were significant differences ($P<0.05$) in relative fecundity of fish fed on experimental diets with different protein and lipid levels. Low relative fecundity was recorded in fish fed T1 diet and in fish fed T8 diet it was high.

Fish fed T5 diet exhibited higher ($P < 0.05$) fertilization and hatching rates than fish receiving other diets. With the T5 diet maximum fertilization rate recorded was 82.59±1.19% while hatching rate was 76.84±2.72%. Hatching rate increased with increasing dietary protein levels up to T11 diet, beyond which it did not differ significantly. A similar trend was observed for relative hatchling number.

The average spawning interval recorded ranged from 13-25 days. Minimum spawning interval (12.91±0.25 days) was observed in T8 while, maximum (25.12±1.18 days) was recorded in fish receiving T2 diet. Egg and oil globule diameter increased ($P < 0.05$) with increasing dietary protein and lipid levels up to 520 g/kg protein, beyond which they gradually decreased with increasing lipid levels. The egg size in fish receiving T9 diet was larger than those receiving other diets. There was no significant difference ($P > 0.05$) in the total length of hatchlings produced among the different dietary treatments. Hatchling weight increased ($P < 0.05$) with increasing dietary protein and lipid level.

Chemical composition of eggs and muscle are shown in Table 3. Dietary protein and lipid levels significantly affected body protein while moisture, lipid and ash content were not significantly affected.

Table 3. Chemical composition (% wet weight basis) of eggs and muscle of *P. scalare* fed experimental diets.

Particulars	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
Egg composition												
Moisture	56.97 ±0.35	57.51± 0.31	57.01± 0.22	57.93 ± 0.39	57.25± 0.36	58.12± 0.21	57.33± 0.24	57.17 ±0.20	57.51± 0.32	57.61± 0.27	57.38 ±0.49	57.73 ±0.35
Protein	26.72 ± 0.13^{a}	26.64 ± 0.31^{a}	26.63 ± 0.28^{a}	27.42 ± 0.38^{ab}	27.30 ± 0.36^{ab}	27.17 ± 0.38^{ab}	27.87 ± 0.28^{ab}	27.80 ± 0.19^{ab}	27.45 ± 0.13^{ab}	28.10 ± 0.19^{b}	28.09 ± 0.23^{b}	27.96 ± 0.31^{b}
Lipid	13.26 ±0.25	13.3 ± 0.35	13.47 ±0.32	13.30 ±0.42	13.64 ±0.27	13.68 ±0.11	13.53 ±0.43	13.56 ±0.16	13.60 ±0.12	13.37 ±0.42	13.39 ±0.67	13.40 ±0.31
Body composition												
Moisture	73.98± 0.51	73.15 ±0.76	72.52 ±0.68	72.09± 0.53	71.91± 0.88	72.62± 0.52	72.52± 0.91	72.24± 0.72	71.97± 0.66	72.63± 0.54	72.55 ±0.73	72.32 ± 0.66
Protein	63.43± 0.31^{ab}	63.26 ± 0.17^{a}	63.20 ± 0.29^{a}	63.65± 0.23^{abc}	63.53± 0.20^{abc}	63.45± 0.44^{ab}	64.27± 0.18^{abc}	64.17± 0.15^{abc}	64.10± 0.16^{abc}	64.69± 0.20^{d}	64.54 ±0.19^{c}	64.46 ± 0.13^{bc}
Lipid	31.88± 0.32	32.21 ±0.58	32.41 ±0.55	32.37± 0.54	32.54± 0.27	32.56± 0.49	31.95± 0.14	32.03± 0.22	32.17± 0.34	31.61± 0.17	31.67 ±0.23	31.93 ± 0.32
Ash	2.54 ± 0.07	2.64± 0.15	2.56± 0.06	2.49± 0.07	2.42± 0.07	2.65± 0.09	2.52± 0.08	2.51±0 .11	2.47± 0.09	2.49± 0.05	2.56± 0.10	2.63± 0.09

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

Discussion

It is widely known that a complete broodstock diet is necessary to improve spawning quality and consistency. High seed production demands particular nutrition of broodstock which significantly affects fecundity and survival (Bromage *et al.,* 1992). Thus, the objective of the present study was to examine the effect of protein and lipid levels on reproductive performance of *P. scalare*.

In the present experiment the lowest (48%) level of dietary protein with all three lipid levels showed lowest relative fecundity, egg size, and hatching rate while at 50 and 52% protein, reproductive performance of *P. scalare* was significantly better. Fish fed T10, T11 and T12 diets had significantly poorer relative fecundity and egg size but hatching rate did not change. The possible explanation may be that once the optimum dietary protein and lipid requirement of fish was met they did not utilize excess protein and lipid available in the diet. In *Oreochromis niloticus,* spawning frequency increases with dietary protein level. It is possible that when fish were fed a diet containing 48% dietary protein, energy was insufficient for the maintenance of normal body functions.

Dietary protein level influences relative fecundity (De Silva and Radampola, 1990; Chong *et al.,* 2004; Khan *et al.,* 2004, 2005). Similar observations were made for *Xiphophorus helleri* where maximum fry production was recorded with 30% protein with 12 and 16% lipid diet (Ling *et al.,* 2006). These observations concur with results of the present study where maximum relative fecundity was observed at T5, T6, T8 and T9. Protein requirements of *P. scalare* were relatively higher than the *X. helleri* as *P. scalare* is considered an omnivore and its diet includes plankton, larvae of insects, and crustaceans, worms, and plants. (Soriano-Salazar and Hernandez-Ocampo, 2002).

There was no significant difference in mean fecundity of *Poecilia reticulata* at varying protein levels (Dahlgren, 1980). On the contrary, varying dietary protein levels can influence fecundity in dwarf gourami (*Colisa lalia*) and bighead carp (*Arisfichthys nobilis*) respectively (Shim *et al.,* 1989); Santiago *et al.,* 1991). Similar observations were recorded in the present study where maximum relative fecundity was observed in T5, T8 and T9 diets. Thus, it is possible that the nutrient requirements during spawning season vary with the species.

In the present study, hatching percentage was significantly higher in the T5 diet whereas lowest hatching performance was observed in fish fed the lowest protein level (48%) with 8, 12 and 16% lipid levels. Similarly with lower dietary protein and lipid levels, fish continued to breed but hatchability was low (Khan *et al*., 2004). However, varying dietary protein levels did not affect hatchability of *Labeo rohita* (Khan *et al.,* 2005).

In the present study egg diameter of *P. scalare* differed significantly between treatments. Maximum egg diameter was recorded in T9 diet. Egg diameter was affected by varying dietary protein levels in *Cyprinus carpio* and *C. idella* (Mannisery *et al.,* 2001; Khan *et al.,* 2004). Contradictory observations were made by Dahlgren (1980) and Khan *et al*. (2005) who reported that dietary protein levels did not influence egg diameter.

Broodstock diet did not influence offspring growth (Chong *et al*., 2004). These observations did not agree with results of the present study. In the present study, hatchling length of *P. scalare* did not differ with varying dietary protein and lipid levels but hatchling weight was significantly different.

In the present study, there was no significant difference in egg moisture and lipid content due to varying dietary protein and lipid, while egg protein content differed significantly. Similar observations were made for *L. rohita* (Khan *et al.,* 2005).

Body protein content in *P. scalare* varied with dietary protein and lipid intake, however, lipid and moisture contents remained unaffected. Similarly muscle protein and moisture content was affected by dietary protein intake while ash and lipid content were not affected (Khan *et al.,* 2004). It was noted that there was low moisture and high protein in the muscle of broodstock *O. niloticus* fed high protein (Gunasakera *et al.,* 1997). However, there are very few studies on the effect of dietary protein and lipid levels on body composition of broodstock.

CONCLUSION

Results of the present study indicated that varying dietary protein and lipid levels significantly influence relative fecundity, egg and oil globule diameter, fertilization rate, hatching rate, inter spawning interval, relative hatchling number, egg and body protein of *P. scalare*. Therefore T5 and T8 diets would be optimum for improved reproductive performance, egg and larval quality of *P. scalare*.

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