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# Growth, survival and feed utilization of Pearl spot, *Etroplus suratensis* larvae co-fed with atremia and micro diets of varying protein levels under controlled conditions

# Sayooj, P.<sup>1, 2</sup>, Vijayagopal, P.<sup>1</sup>, Anusree, V.N.<sup>1</sup>, Prasanth, K.G.<sup>3</sup> and Vijayan, K.K.<sup>4\*</sup>

<sup>1</sup>ICAR - Central Marine Fisheries Research Institute, Kerala, India 682018 <sup>2</sup>Cochin University of Science and Technology, Kerala, India 682022 <sup>3</sup>Department of Aquatic Biology and Fisheries, University of Kerala, Thiruvananthapuram-695581 <sup>4</sup>ICAR - Central Institute of Brackish Water Aquaculture, Chennai, India 600028.

\*E.mail: vijayankk@gmail.com

#### ABSTRACT

Nutrition is a dominant factor influencing larval growth; knowledge of the larval nutritional requirements would contribute to optimising diets and feeding protocols, which improve larval and juvenile quality. Feeding with a limited number of dry feeds as a supplement or replacement of live feeds has led to poor larval nutrition in many fish species. Therefore, the study aimed at (1) evaluating the efficacy of live food organism (Artemia salina) and micro diets in the rearing of 8-day-old (12.5-13 mg) pearl spot, Etroplus suratensis larvae and (2) determining the effects of varying protein levels on growth and survival for appropriate rearing conditions for the larvae. The experiment ended after 30 days of culture and respective groups were compared based on growth parameters and survival. There were 8 treatments in triplicates and four experimental diets were formulated to contain 40%, 45%, 50%, and 55% dietary protein with 9% lipid levels to feed the fish. In the first four treatments, the fish larvae were provided with formulated micro diet only and in the subsequent four treatments, co-feeding was done with artemia for an initial 10 days thereafter, fed only with formulated micro diets. Larvae co-fed using artemia and 50% protein micro diet resulted in significantly better growth in terms of weight gain, feed conversion ratio and specific growth rate than other treatments. The lowest growth occurred in larvae weaned using 40% formulated feed. Better survival was obtained in larvae weaned with co fed diets, while abrupt weaning using 100% dry diets resulted in lower survival (<66%). These results recommend co-feeding E. suratensis larvae using a formulated dry diet and artemia for the successful culture under controlled conditions.

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# 1. Introduction

The pearl spot (Etroplus suratensis), family: Cichlidae, inhabits both freshwater and brackish water ecosystems and has a widespread distribution in India and Sri Lanka (Hora and Pillay, 1962). This species gains popularity as an ornamental fish due to its brilliant coloration and high demand as a food source. This fish shared a good percentage (8-10 percent) of total fish landings in the backwaters (George and Sebastian, 1970), which has been drastically reduced due to overfishing and facing serious extinction in its natural habitat (Padmakumar et al., 2002). The optimum conditions of rearing from the onset of exogenous feeding have not been identified, and one of the main drawbacks is to provide nutritionally adequate food for their larval stage. Larval development and the survival of aquatic animals are among the most important factors for successful aquaculture (Gong et al., 2014). Generally, larval rearing in hatcheries primarily depends on live foods. Live feeds such as rotifers (Brachionus sp.) and brine shrimp (artemia) are considered as excellent food for successful fish larviculture (Baskerville-Bridges and Kling, 2000; Sorgeloos et al., 2001). Live food organisms stimulate larval feeding activity through their movement and release of metabolic wastes and chemicals (e.g. amino acids, peptides and ammonium salts) that act as attractants (Kolkovski et al., 1997). Absolute dependence on live feeds as the source of diet is a major constraint in larval nutrition (Kerdchuen and Legendre, 1994). The continued utilization of live feeds as live and dry diets is likely to bring considerable challenges because of the intensive production techniques as well as the cost of live feed production and labour requirements

along with the highly variable nutritional value of live feeds (Cahu and Zambonino Infante, 2001; Langdon, 2003) and the unreliability of mass cultures illustrate the need to find viable micro diet alternatives (Baskerville-Bridges and Kling, 2000).

Developing a formulated micro diet with a full nutritional complement, good physical and biochemical properties for partial or complete replacement of live food will decrease production costs for larviculture. However, these micro diets must meet the nutritional requirements, and the larval fish should be readily accepted. Defining an effective diet is one of the most important steps towards a successful larval culture (Nasrolahi et al., 2007). The dietary protein requirement of a species is of prime importance in aquaculture because protein influences fish growth and determines the cost of feeding in fish feed. The level of protein utilization by the fish depends on the quantity and quality of dietary protein. (Li and Lovell, 1992; Li et al., 2008).

The main objective of the experiment was to evaluate the effects of different micro diets with ascending levels of protein for maximizing growth and larval survival of E. *suratensis* when used within various co-feeding weaning protocols aimed at minimizing the use of artemia.

#### 2. Materials and Methods

# 2.1 Source of fish larvae and experimental facility

Eight-day-old (8 dph) *Etroplus suratensis* larvae were obtained from Azhikode Hatchery, Thrissur, Kerala, transported and acclimated for 7 days at CMFRI wet lab

facilities. Larvae were transferred into 24 nos of, 20 L rectangular glass tanks (initial density: 50 larvae L<sup>-1</sup>, mean weight 12.5-13  $\pm$  0.02 mg). The facilities were aerated throughout the 30 days study period. Water conditions were as follows: temperature 28.4  $\pm$  0.4 °C, salinity 20 ppt, pH 8.0  $\pm$  0.2, dissolved oxygen 7.5  $\pm$  1.3 mg L<sup>-1</sup>and 50% daily water renewal done in the system.

# 2.2 Ingredients and formulation of experimental diets

Dietary treatments consisted of live food (*Artemia salina*) and four micro diets were formulated to contain 40, 45, 50, 55 g kg<sup>1</sup> protein levels, with a constant lipid of 9 g kg<sup>1</sup> and tested in triplicates. As per the formulations, all ingredients were weighed, ground, mixed, mechanically extruded, dried at 60-65°C in hot air oven, and packed air-tight until use. For feeding the dried pelleted feed were ground in a grinder and then sorted with sieves (150, 200, 250  $\mu$ m mesh) to obtain the desired particle size. The composition of the ingredients and the formulated feeds are presented in Table 1.

# 2.3 Mass production of artemia

Artemia cysts (INVE aquaculture nutrition, High HUFA  $430\mu$ ) were incubated and hatched under optimal conditions according to the manufacturers protocol. After 24 h newly hatched artemia nauplii were collected using a 100  $\mu$ m sieve. The artemia were washed with 25 ppt water. About 50 numbers of artemia were fed four times a day for each treatment and provided to larvae without any further enrichment.

#### 2.4 Dietary treatments and feeding

The experiment was performed under eight different treatments. In the first four treatments, the diets: Diet 1 (D1) larvae were fed with 40% dietary protein micro diet only, diet 2 (D2) 45% dietary protein micro diet, diet 3 (D3) 50% dietary protein micro diet and diet 4 (D4) 55% dietary protein micro diet were provided and in the subsequent four treatments, co-feeding was done with live feed (artemia)

Table 1.	Ingredie	nts used	and	proxin	nate cor	nposition	of
the expe	rimental	diets (g	/kg) (	on dry	matter	basis	

1		2					
Ingredients (g/kg)	D40	D45	D50	D55			
Marine protein mixture	320	370	410	650			
Soy Flour	320	370	410	210			
Wheat Flour	240	140	60	40			
Sardine Oil	60	60	60	40			
Lecithin	3	3	3	3			
Vitamin C	5	5	5	5			
Vitamin Mixture	20	20	20	20			
Mineral Mixture	30	30	30	30			
Antifungal	1	1	1	1			
Antioxidant	1	1	1	1			
Proximate composition of experimental diets a (dry							
matter basis) (g/100g)							
Dry matter	95.57	96.13	96.02	95.81			
Crude Protein	40.82	45.67	49.31	54.85			
Crude Lipid	9.02	9.09	9.24	9.12			
Crude Ash	12.03	14.18	19.47	/ 19.7			
Nitrogen free extract	37.86	30.95	21.8	15.72			
(NFE) <sup>b</sup>							
Gross energy (MJ kg <sup>-1</sup> )	° 11.92	12.74	13.33	13.41			

<sup>a</sup> Values are mean of triplicate analysis.

<sup>b</sup>Nitrogen-free extract (calculated by difference).

for initial 10 days and formulated micro feed alone subsequently, Diet 5 (D5) live artemia and 40% dietary protein micro diet, Diet 6 (D6) live artemia and 45% dietary protein micro diet, Diet 7 (D7) live artemia and 50% dietary protein micro diet, Diet 8 (D8) live artemia and 55% dietary protein micro diet. Each feeding experiment was conducted in triplicate. Prior to feeding, the aeration was interrupted for at least 10 minutes. Pre weighed portions of each of the formulated diet were taken and fed to the fish larvae *ad libitum* four times a day between 08:00 h and 19:00 h. The artemia nauplii were added at a density of 3.0 nauplii mL<sup>-1</sup> four times per day.

# 2.5 Sample collection

At the beginning of the experiment, thirty fishes were sampled to analyse proximate whole body composition. After the experiment fishes in each treatment were individually weighed, and fishes from each tank were used for further analysis.

# 2.6 Chemical analysis

Crude protein, crude lipid, moisture and ash in diets and whole body were determined by standard methods (AOAC, 2005). Moisture was determined by oven-drying at 105°C until constant weight. Crude protein (N×6.25) was determined by Kjeldahl method after acid digestion using a semi-automated Kjeldahl System (FOSS Kjeltec 2300). Crude lipid was determined by ether-extraction method using a Soxhlet System (FOSS Soxtec2043). Ash content was determined by incinerating the sample in muffle furnace at 550°C for 24 h.

# 2.7 Water quality management

The experimental tanks were cleaned daily, and the water was partially (2/3) replaced before the first feeding schedule in the morning. Temperature, dissolved oxygen and pH were monitored once per day. Total Ammonia-N and Nitrite-N were recorded every week and determined spectrophotometrically following the Nessler and diazotization methods respectively.

# 2.8 Growth performance and survival determination

Performance of fish on test micro diet was assessed by final body weight (FBW), total length (TL), weight (%WG), percent survival (%), Feed conversion ratio (FCR), specific growth rate (SGR) based on the following standard formulae.

Weight gain % (WG) =100 (final weight- initial weight)/ initial weight

Specific growth (SGR) = 100 (ln final weight-In initial weight)/no of days of feeding trial

Feed conversion ratio

(FCR) = Feed intake / Weight gain

Survival % = 100 (final no. of fish harvested /initial no of fish stocked)

# 2.9 Data analysis

The experimental data were statistically analysed using the Statistical Package for the Social Sciences (SPSS) version 16. Comparison between two treatments was made using Duncan's multiple range test (DMRT). Comparison among all the treatments was made by one-way ANOVA. Comparisons were made at 5% probability level.

 $<sup>^{\</sup>rm c}$  Gross energy, calculated based on 0.017, 0.0398 and 0.0237 MJ/g for carbohydrate, lipid and protein, respectively

#### 3. Results

# 3.1 Physicochemical parameters of water

The water quality parameters like temperature, pH, dissolved oxygen (DO), ammonia nitrogen and nitrite nitrogen were recorded. Dissolved oxygen (DO) and pH ranged from 6.25 to 7.09 mg/L and 6.9 to 7.3 respectively. The total ammonia and nitrite levels varied from 0.19 to 0.44 mg/L and 0.03 to 0.15 mg/L, respectively. Water temperature varied from 27 to 28.7 °C. The values were in tolerable limits for larviculture.

#### 3.2 Growth parameters

The body growth parameters are given in Table 2. The weight gain percentage was significantly different (p<0.05) among the various treatment groups. The highest weight gain percentage was recorded in the D7 group (50% protein + artemia) ( $662.56 \pm 3.46$ ), followed by the other groups, and the lowest growth was recorded in the D1 group ( $429.56 \pm 11.42^{a}$ ). The highest specific growth rate (SGR) was found in the D7 group and the lowest SGR was found in the D1 group, which was significantly (p<0.05) different from other experimental groups. The feed conversion ratio (FCR) of different experimental groups varied significantly (p<0.05). Better FCR value was observed in D7 group followed by other groups. On the 30 dph the treatment groups showed no significant difference in total length (TL) (210.476  $\pm$  26.70). The highest survival (%) was found in

# the D7 group and the lowest in D1 group (p < 0.05).

# 3.3 Whole body proximate composition

The final mean carcass composition of pearl spot larvae maintained on different experimental diets is presented in Table 3. Among the different feeding schedules, highest (p<0.05) protein and lipid content were recorded in the D7 group, whereas the lowest in the D1 group.

# 4. Discussion

The larval period is extremely important stage in fish species in their life history (Wang et al., 2005). The digestion of dietary protein in larval fish has been known to differ from that in juvenile fish (Kolkovski, 2001). Therefore, the study was designed to see the effects of different dietary protein levels on the pearl spot larvae.

In most of the fish species used in fish farming, the best survival and growth results in larval rearing are usually obtained with natural foods, preferably live or with a combination of live food and dry diet (Evangelista et al., 2005; Wang et al., 2005; Policar et al., 2007). Feeding live natural zooplankton or artemia to the fish larvae exploits the inherent predatory nature of the larva to catch mobile, live food particle. Our observations on co-feeding weaning protocols as used in experiments of the *E. suratensis* with artemia and formulated micro diet in experiments (D5–D8) simultaneously used to allow a fast and efficient change

Table 2. Growth parameters Etroplus suratensis larvae in the different experimental diets

Parameters	DI	D2	D3	D4	D5	D6	D7	D8
Initial mean weight (mg)	12.57±0.2	12.60±0.3	13.40±0.2	13.10±0.2	12.89±0.4	12.54±0.2	12.53±0.3	12.52±0.3
at day 8								
Final mean weight (mg)	63.90±3.1 <sup>b</sup>	$70.00 \pm 37.^{d}$	$73.23 \pm 2.9^{a}$	72.87±2.6c	75.57±2.7a	93.03±3.4 b	95.37±2.9 c	91.01±3.3 d
at day 30								
Mean weight gain (mg)	51.33±11.42 <sup>a</sup>	57.40±17.79 <sup>a</sup>	59.83±3.97 <sup>ьс</sup>	59.77±2.27 <sup>ьс</sup>	62.68±13.49°	$80.49 \pm 4.19^{d}$	82.85±13.45 <sup>d</sup>	78.49±3.66ª
SGR	$5.81{\pm}~0.8^{\rm a}$	6.12± 0.11 <sup>b</sup>	$6.07 \pm 0.2^{bc}$	$6.13 \pm 0.2^{bc}$	$6.32 \pm 0.8^{\circ}$	$7.16 \pm 0.20^{d}$	$7.25 \pm 0.13^{d}$	$7.0 \pm 0.17^{d}$
FCR	2.55± 0.2°	$2.48 \pm 0.2^{\mathrm{a}}$	$2.37 \pm 0.4^{b}$	$2.37 \pm 0.3^{d}$	2.22± 0.2°	$2.12 \pm 0.3^{b}$	$1.93 \pm 0.3^{\mathrm{a}}$	$2.13 \pm 0.2^{\text{b}}$
% Survival	$66.00 \pm 1.15$	$^{a}68.67 \pm 2.42^{bc}$	70.67± 2.17°	$66.67 \pm 2.20^{bc}$	$76.00 \pm 1.58^{d}$	$77.67{\pm}2.33^{\text{d}}$	$80.87{\pm}\ 2.59^{\circ}$	$77.00 \pm 1.86^{d}$

Values are the mean  $\pm$  SE of triplicate groups of 20 fishes.

a, b,c,d Values in the same row with different letters differ significantly (P < 0.05)

D1, 40% dietary protein micro diet, D2, 45% dietary protein micro diet, D3 50% dietary protein micro diet, D4, 55% dietary protein micro diet; D5, 40% dietary protein micro diet + artemia, D6, 45% dietary protein micro diet + artemia, D7, 50% dietary protein micro diet + artemia, D8, 55% dietary protein micro diet + artemia

SGR, specific growth rate; FCR, food conversion ratio.



Specific growth rate (SGR)

**Fig. 1.** Mean weight gain (mg) of *Etroplus suratensis* larvae in the different experimental diets. D1, 40% dietary protein micro diet, D2, 45% dietary protein micro diet, D3 50% dietary protein micro diet, D4, 55% dietary protein micro diet; D5, 40% dietary protein micro diet + artemia, D6, 45% dietary protein micro diet + artemia, D8, 55% dietary protein micro diet + artemia, D8, 55% dietary protein micro diet + artemia

**Fig. 2.** SGR (specific growth rate) of *Etroplus suratensis* larvae in the different experimental diets. D1, 40% dietary protein micro diet, D2, 45% dietary protein micro diet, D3 50% dietary protein micro diet, D4, 55% dietary protein micro diet; D5, 40% dietary protein micro diet + artemia, D6, 45% dietary protein micro diet + artemia, D7, 50% dietary protein micro diet + artemia, D8, 55% dietary protein micro diet + artemia



**Fig. 3.** FCR (feed conversion ratio) of *Etroplus suratensis* larvae in the different experimental diets. D1, 40% dietary protein micro diet, D2, 45% dietary protein micro diet, D3 50% dietary protein micro diet, D4, 55% dietary protein micro diet; D5, 40% dietary protein micro diet + artemia, D6, 45% dietary protein micro diet + artemia, D7, 50% dietary protein micro diet + artemia, D8, 55% dietary protein micro diet + artemia

**Table 3.** Proximate composition of the whole body of *Etroplus* suratensis larvae in different experimental groups (g/kg on wet weight basis  $\pm$ SE)

Treatments	Moisture	Crude protein	Crude Lipid	Crude Ash
Dĺ	76.13±1.09 <sup>b</sup>	14.27±0.23ª	$1.87 \pm 0.18^{b}$	2.16±0.31ª
D2	76.78±1.16 <sup>b</sup>	13.97±0.18 <sup>b</sup>	$1.88 \pm 0.15^{a}$	2.14±0.45°
D3	$75.04{\pm}1.89^{a}$	15.27±0.32°	2.07±0.21 <sup>b</sup>	2.18±0.41ª
D4	76.64±1.46°	14.25±0.16 <sup>a</sup>	1.98±0.42°	2.17±0.75 <sup>b</sup>
D5	76.99±2.03 <sup>b</sup>	15.32±0.24 <sup>b</sup>	$1.99 \pm 0.19^{b}$	2.16±0.15ª
D6	76.19±1.73ª	15.79±0.13 <sup>b</sup>	2.11±0.36 <sup>b</sup>	2.19±0.38ª
D7	76.29±1.23°	16.22±0.21 <sup>b</sup>	2.20±0.29ª	2.15±0.39°
D8	76.79±1.41 <sup>b</sup>	15.91±0.11°	2.19±0.21°	2.14±0.18°

Values are the mean  $\pm$  SE of triplicate groups of 20 fishes. <sup>a,b,c</sup> values in the same row with different letters differ significantly (P < 0.05).

over period onto dry micro diets from live feed (Daniels and Hodson 1999; Koven, et al., 2001). This method has been found to achieve higher growth and survival than feeding either live feeds or micro diets on their own (Wang et al., 2009).

At the end of the larval rearing period (30 days) in this study, the lowest weight and SGR were found in the group of larvae fed with micro diet (D1). In micro diet group (D5) to (D8). The co-fed groups had the higher larval survival and growth rate. These results are almost similar to those obtained in Clarias macrocephalus (Evangelista et al., 2005), Barbus barbus (Policar et al., 2007) when comparing larvae fed live foods and micro diets. During the experiment, the larvae fed with micro diet grew considerably less than larvae co fed with artemia. This result shows that larvae initially need to receive live food to maintain their high growth (Rust, 2002). The low survival and growth rates of larvae fed only on artificial diet could result from non or poor use of the artificial dry food during the first weeks of life due to the ineffectiveness of their developing gut. It was reported that the inappropriate use of artificial diets by fish larvae come mainly from a lack of equipment in digestive enzymes, particularly proteolytic (Evangelista et al., 2005; Policar et al., 2007).



**Fig. 4.** (%) Survival of *Etroplus suratensis* larvae in the different experimental diets. D1, 40% dietary protein micro diet, D2, 45% dietary protein micro diet, D3 50% dietary protein micro diet, D4, 55% dietary protein micro diet; D5, 40% dietary protein micro diet + artemia, D6, 45% dietary protein micro diet + artemia, D7, 50% dietary protein micro diet + artemia, D8, 55% dietary protein micro diet + artemia

In the present study the increment of protein levels from (400 g kg<sup>-1</sup> to 550 g kg<sup>-1</sup>) in the diets influenced a significant improvement in growth, percent body weight gain, SGR, FCR, survival and body indices in *Etroplus suratensis* larvae. This might be why the body weight gain, SGR, FCR values varied significantly in response to the dietary protein content. These increased differences have also been observed in *Cyprinous carpio* (Ogino and Saito, 1970) and *Sparus auratus* (Santinha et al., 1996).

Whole-body composition is often used to indicate fish quality (Zhang et al., 2011). In the present study, the body lipid and protein content was significantly higher in the co-fed diet (D7). Higher body protein content in the treatment groups implies that by co-feeding with live feeds, the ingested micro diets were converted more effectively into structural protein, resulting in maximum growth and survival, a desirable aspect in fish farming.

# 5. Conclusion

The present study indicated the feasibility of producing *E.* suratensis fingerlings for stock enhancement or grow-out purposes. During the larval rearing period, better growth and survival rates were found when fishes were fed with live feeds for the first week, and feeding micro diet alone had a positive effect on the growth rate of larvae. These results revealed that co feeding can be considered as a suitable method for maximum growth, body composition, and best feed utilization for *E. suratensis* fingerlings.

#### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships

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