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Effect of Biofloc, Live Feed, and Formulated Feed on Survival, Growth, and Whole-Body Proximate Composition in Larval Rearing of Pearlspot (*Etroplus suratensis*)

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Abstract

Fish larval rearing plays a critical role in aquaculture and fisheries development, serving as the foundation for successful fish production. One of the most critical aspects of larval rearing is the provision of appropriate feed. This study evaluated the effects of live feed Artemia (LF), formulated feed (FF), and a biofloc (BF) system on the survival, growth, and body proximate composition of Pearlspot (*Etroplus suratensis*) larvae during the rearing phase. The experiment commenced at 20 days post hatch and continued for 60 days. By the conclusion of the trial, the larvae raised in BF system exhibited the significantly highest (p= 0.001) final body weight and specific growth rate (SGR), followed by those fed LF, while FF group showed lowest results. Survival rates, which ranged from 76% to 78.33%, showed no significant differences (p= 0.46) across the treatments. The whole-body proximate composition analysis revealed significant dietary effects, with larvae from BF system exhibiting superior nutritional quality compared to those in the LF and FF groups. These findings highlight the potential of biofloc technology as an effective approach for enhancing the growth performance and nutritional status of Pearlspot larvae. This study provides valuable insights that can be applied to enhance the larval rearing practice of Pearlspot, thereby supporting the development of efficient seed production techniques.

Keywords:
Biofloc technology, Body proximate composition, Nutritional benefits, Survival rate

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Introduction

Larval rearing is a critical phase in aquaculture, serving a vital role in ensuring the survival, growth, and nutritional quality of fish larvae. This phase demands precise care and suitable feeding strategies to meet the nutritional requirements of the larvae, which are highly sensitive and require nutrient-rich diets for optimal development. Feeding fish larvae and fingerlings with natural live food sources, especially *Artemia salina,* has been widely adopted (Das et al., 2012). One of the main benefits of this approach is that live food encourages feeding behavior (Radhakrishnan *et al*., 2020), offers high digestibility, and supplies essential nutrients for proper larval development (Simhachalam et al., 2015; Pan *et al*.,, 2022). However, cultivating live feed can be expensive and often lacks consistent nutritional quality, posing challenges for reliable larval rearing (Cahu *et al*., 2012; Samat *et al*., 2020). The invention of manufactured diets has made it possible to rear valuable fish larvae without relying on live feed. These diets are generally easier to manage and often come with lower production costs (Chong, 2022). Several studies have explored the feasibility of replacing live feed with manufactured diets from the beginning of exogenous feeding (Goncalves *et al*., 2022a; Gonclaves *et al*., 2024). Efforts to fully substitute live feed during the initial phases of larval development have met with only partial success.

However, advancements in aquaculture practices have introduced alternative methods to enhance larval nutrition and growth. Among these, biofloc technology has emerged as a promising option. Biofloc systems are protein-rich ecosystems where microbial flocs provide a continuous and easily accessible source of nutrition (Thilakan *et al*., 2018). This approach not only improves feed availability but also enhances water quality (Van, 2013), creating an ideal environment for larval development. The constant availability of food in the biofloc system minimizes stress and optimizes growth, making it a viable and sustainable alternative to conventional live feed methods. There is limited research available on the larval development of fish within biofloc systems (Ekasari *et al*., 2015; Poli *et al*., 2015).

Pearlspot (*Etroplus suratensis*) is a valuable brackish water species with significant economic importance. Due to high market demand, its production has diversified extensively (Smina *et al*., in press). Currently, there is no available information on cultivating Pearlspot larvae within a biofloc system. Rearing of Pearlspot larvae in a biofloc system could offer a promising alternative to enhance growth performance and survival rates compared to conventional methods. Therefore, this study investigates the potential of biofloc technology as an innovative and effective strategy for Pearlspot larval rearing, while comparing its advantages with traditional live feed and formulated feed approaches, aiming to support sustainable aquaculture practice.

Materials and Methods

Experimental fish and research design

Larvae of Pearlspot (*E. suratensis)* were procured from Aqua One Center, Alappuzha, for the experiment. Up on arrival in the hatchery of Central Marine Fisheries Research Institute, Cochin, Kerala, they were carefully transferred to a rectangular tank (500 L) and left undisturbed the whole night. Fifty percentage of the water was exchanged every 24 h. The larvae were acclimatized to laboratory conditions for one week and fed live Artemia *ad libitum* three times daily.

After acclimatization, larvae were harvested, and only healthy, uniformly sized individuals were selected for the experiment. The initial length and weight were determined by randomly sampling three sets of 10 larvae from the same population. Three different feeding regimes were tested to evaluate their impact on the performance of Pearlspot over a 60 day period: live Artemia (LF), formulated feed (FF), and biofloc (BF). In this trial, triplicate groups of 50 Pearlspot larvae (average length: 1.15 ± 0.05 cm, average weight: 40.33 ± 10.69 mg) were stocked in nine aquaria (70×56.5×40 cm; total capacity: 158 L) and assigned randomly to the three dietary treatments. Each tank was supplied with filtered de-chlorinated freshwater and equipped with an individual air stone for continuous aeration. A consistent water volume of 100 L was maintained in each tank throughout the experiment. Nylon nets were placed over the tanks to prevent the fish from escaping.

Feed preparation and feeding

The artemia used in this study was sourced from laboratory cultures maintained in-house. Nauplii were hatched by hydrating 2 g of cysts in freshwater for 30 min. After hydration, the cysts were collected and incubated in seawater under vigorous aeration for 24 h. The salinity of the incubation medium was 25 ppt. Hatched artemia nauplii were collected with a 100 µm plankton net and fed chaetoceros algae until use. Before feeding, the required number of nauplii was thoroughly rinsed with freshwater and offered to the larvae *ad libitum*. Feeding was done thrice daily at 8:00 am, 1:00 pm and 6:00 pm.

A formulate artificial feed with 50% protein content (Lipid: 9.31%, ash: 19.39%, nitrogen free extract: 21.44%) was utilized in the study. This feed was procured from the Central Marine Fisheries Research Institute feed mill. Feeding was done thrice daily at 8:00 am, 1:00 pm and 6:00 pm at a rate equivalent to 10% of the total body weight of the cultured organisms. To maintain good water quality, uneaten feed and waste were siphoned out daily from both AF and BF tanks, and 10% of the water was replaced each day.

The BF inoculum was created using the method described by Avinmelech (1999). To prepare the inoculum, 10 mgL^{-1} ammonium sulphate (a nitrogen source) and 200 mgL^{-1} of carbon source (jaggery) were mixed with 20 gL^{-1} of pond bottom soil collected from the Moothakunnam (N10°11.478 ' E076°11.901 ') cage culture site. The mixture was incubated for 24–48 h. After 48 h, the inoculum was ready and transferred to the BF treatment tanks, where adequate aeration was suppllied to maintain the biofloc in suspension. The carbon nitrogen (C/N) ratio was retained at 10:1, using jaggery as the carbon source, assuming it contain 50 % carbon per gram. During the trial, the tanks were fed a formulated floating feed containing 50% crude protein. Feeding was done twice daily at 9:00 am. and 5:00 pm, at a rate equivalent to 5% of the total body weight of the cultured organisms. To promote floc development, 60 mg of the carbon source (jaggery) was provided daily to the tanks. The sludge was removed from the tanks on the 20th and 40th days of the experiment. On the same day, the tank water was replenished to 100 L with fresh water.

Growth performance analysis

The growth parameters of *E. suratensis* were measured by taking their body weight at the completion of the trial. For this, 25 fish were randomly selected using a hand net, gain in length and weight was measured to calculate the weight gain, feed conversion ratio (FCR), specific growth rate (SGR), survival rate percentage and condition factor (K) by the respective calculations:

$$\text{Weight gain (g)} = \text{Final weight} - \text{Initial weight}$$

$$\text{Daily growth rate (g} \cdot \text{ day} - 1) = \frac{\text{Final weight} - \text{Initial weight}}{\text{Days}}$$

$$\text{Specific growth rate (g\%} \cdot \text{day} - 1) = \frac{\ln(\text{Final weight}) - \ln(\text{Initial weight})}{\text{Days}} \times 100$$

$$\text{Survival (\%)} = \frac{\text{Number of fish survived}}{\text{Number of fish stocked}} \times 100$$

$$\text{Condition factor (K)} = \frac{W100}{L^3}$$

Where, W is weight (g) and L is total length (cm).

Biochemical analysis

The proximate analysis of feed and fish was conducted following standard AOAC methodology (2005). The moisture content was determined by oven-drying the sample at 105 °C until a constant weight was reached. Crude protein was analyzed using the Kjeldahl method with a semi-automated Kjeldahl system (FOSS Kjeltec 2300) after acid digestion. The crude lipid content was assessed through ether extraction using a Soxhlet system (FOSS Soxtec2043). Ash content was measured by incinerating the sample in a muffle furnace at 550 °C for 3 h.

Statistical analysis

The results from all treatments were compared using a one-way analysis of variance (ANOVA) (significance was determined at $p<0.05$). Differences among means were determined using Tukey's HSD test. Statistical analyses were performed using the SPSS version 16.

Results and discussion

After 60 days of rearing, the highest values for mean final weight, mean final length, weight gain, and specific growth rate (SGR) were recorded in the BF treatment, followed by the LF treatment, while the lowest values were observed in the FF treatment (Table 1). Statistical analysis revealed that all the above-mentioned growth parameters showed significant differences among the treatments ($p < 0.001$). The mean final lengths of Pearlspot were 3.99±0.05cm in LF, 3.67±0.04cm in FF, and 4.35±0.06cm in BF. Similarly, the highest mean final weight was recorded in BF (2.24±0.08g), followed by LF (1.32±0.06g) and FF (1.12±0.06g). Mean weight gain was also highest in BF (2.20±0.07g), compared to LF (1.28±0.06g) and FF (1.11±0.07g).The results clearly indicate that the use of biofloc significantly improves growth performance compared to live feed or commercial feed during the larval rearing of Pearlspot. Specific growth rates (SGR, %) was significantly higher ($p<0.05$) in BF treatment (7.81 ± 0.03) followed by LF (7.67 ± 0.02), while the lowest SGR was found in FF treatment (7.5 ± 0.02), further demonstrating the advantages of biofloc over other feeding methods. The biofloc system improved the development of Pearlspot larvae. This study is the first to assess the growth performance of Pearlspot larvae in a biofloc system. Previous research has also highlighted the positive impact of biofloc system on the growth of other fish larvae, such as Nile tilapia (Ekasari *et al.,* 2015) and silver catfish (Rhamdia quelen) (Poli *et al*., 2015; Ekasari *et al.,* 2016). The observed growth improvements in these larval fish species are most likely due to increased food availability given by biofloccs. The biofloc provides an extra supply of nitrogen to larvae (Tacon *et al*., 2002; Burford *et al*., 2004; Avnimelech, 2007).

The survival rate of Pearlspot larvae was highest in LF (78.33.0 ± 1.53%) followed by BF (78.00 ± 2.65 %) and lowest in FF (76.33 ± 1.53 %), but it was not statistically significant ($p = 0.46$). Poli *et al*. (2015) and Basen *et al*. (2023) reported high survival rates in silver catfish and gold fish larvae cultured in biofloc system. The reduced growth observed in larvae fed formulated feed is consistent with results from other fish species. For example, larvae of *Heterobranchus longifilis* (Kerdchuen and Legendre, 1994) (African catfish), *Clarias gariepinus* (Hogendoorn, 1980) (African sharptooth catfish), and *Pangasius bocourti* (Hung *et al*., 2002) (Basa catfish) provided trout starter feed had poor survival rates of 32%, 12%, and 67.2 %, respectively.

The condition factor (K) did not exhibit significant difference ($p>0.463$) between the treatments (Figure 1). According to Mozsar *et al*. (2015) a K value below one indicates poor, elongated, and thin body condition. In this study, fish from all treatment groups exhibited good condition, as indicated by condition factor (K) values equal to or greater than 1. This can be attributed to the favorable culture conditions, including abundant food and high water quality, which positively impacted the overall condition of fish. According to Ujjania *et al*. (2012), a condition factor of one or higher is regarded as favorable, indicating

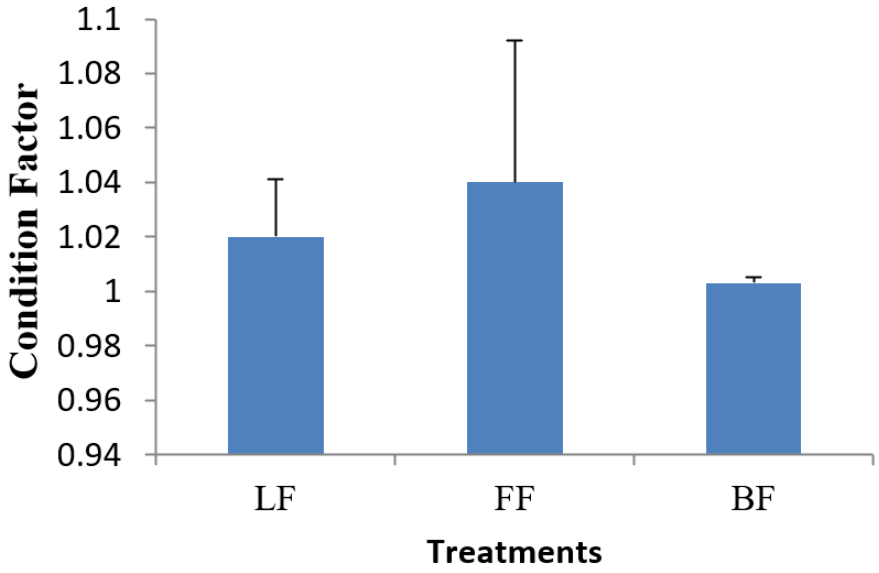

Figure 1. Mean relative condition factor of Pearlspot larvae fed different experimental diets in indoor tanks for 60 days. LF- Life Feed Artemia, FF-Formulated Feed, BF- Biofloc

Table 2. Whole-body proximate composition (on % dry matter basis) of Pearlspot larvae fed different experimental diets in an indoor tank for 60 days.

Parameters	Treatments		
	LF	**FF**	**BF**
Dry matter	96.91 ± 0.44^{a}	96.78 ± 0.50^{a}	97.46 ± 0.29^{a}
Moisture	3.09 ± 0.44^{a}	3.22 ± 0.50^{a}	2.54 ± 0.29^{a}
Crude protein	48.56 ± 0.38^{b}	46.33 ± 0.50^{a}	49.16 ± 0.08^{c}
Crude fat	17.73 ± 0.08^{b}	14.18 ± 0.24^{a}	17.68 ± 0.37^{b}
Crude ash	13.69 ± 0.10^{b}	13.88 ± 0.17^{b}	12.02 ± 0.08^{a}
AIA	0.81 ± 0.02^{c}	0.68 ± 0.01^{b}	0.59 ± 0.01^{a}

Note: The data represent the means ± standard deviation (SD) of three replicates. Means with different superscripts differ significantly ($p<0.05$) across rows. LF- Life Feed Artemia, FF-Formulated Feed, BF- Biofloc

adequate feeding and a suitable environmental conditions.

After 60 days of feeding trial, significant variations were observed in the whole-body crude protein, fat, ash and Acid Insoluble Ash levels among the different dietary treatments (Table 2). The dry matter and moisture content showed no significant difference ($p >0.05$) among the dietary treatments. The larvae fed with biofloc exhibited the highest crude protein and crude fat levels, while ash and AIA were lowest in relation to those receiving LF and FF diets. Significantly lower levels of protein and higher level of crude ash was found in FF. AIA was substantially greater in fish fed live feed than in the other treatments. The findings of this study suggest that the nutritional status of larvae in BF tank was generally superior to those in the LF and FF tanks. Biofloc has been reported to provide additional vital components, such as macronutrients, micronutrients and bioactive compounds (Ju *et al*., 2008 ; Ekasaei *et al*., 2010; Xu and Pan, 2012), which may contribute to enhancing the nutritional value of fish larvae. Similar findings regarding enhanced nutritional content in Pearlspot fingerlings were reported by Ray et al. (2011) and Jackqulinwino et al. (2024). Xu and Pan (2012) also noted that variations in proximate composition can affect the nutritional value, sensory qualities, and shelf life of *Litopenaeus vannamei* (Pacific white shrimp). Furthermore, the presence of other microorganisms within the biofloc system helps to regulate pathogenic bacteria (Michaud et al., 2006)

Conclusion

In the current study, the larviculture of Pearlspot in biofloc rearing system was found to enhance the growth and body composition. Further research is required to understand the impact of biofloc on larval health. The incorporation of BFT not only enhances water quality through the microbial breakdown of organic matter but also contribute to a higher nutrient profile in the water, which supports larval development. However, additional studies are required

to gain deeper insights in to the long-term effects of BFT on the growth, survival, and nutritional composition of the fish, particularly in relation to the biofloc's microbial communities and their influence on the larvae. Additionally, future studies should explore the suitability of various carbon sources and the optimal Carbon to Nitrogen (C/N) ratios for maximizing the efficiency of biofloc systems, ensuring their adaptability to different environment and farming conditions.

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