

Role of Biofloc in the Growth and Survival of Blue morph, *Pseudotropheus saulosi*

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Abstract

Objective: To evaluate the effect of biofloc on the growth and survival of ornamental cichlid, Blue morph. **Methods/Statistical analysis:** Thousand number of Blue morph were stocked in circular cement tanks of 14000 l capacity in control and biofloc rearing tanks. Various water quality parameters, qualitative and quantitative parameters of biofloc and growth parameters were estimated periodically. The results were statistically analysed using ANOVA. **Results/Findings:** All the water quality parameters were high in Control tank compared to biofloc. Floc volume was higher in biofloc tank (4.4 mg/l). The number of microorganisms present in biofloc was much higher than Control. The survival rate of Blue morph young ones in biofloc treatment was 93% and it was 90.2% in control. All growth parameters except length, weight gain and mean length were significantly significant ($P < 0.01$) among the days of culture as well as between control and biofloc treatment tanks. **Conclusion:** Biofloc increased the growth and survival of Blue morph significantly.

Keywords: Biofloc, Floc Volume, Growth and Survival, Proximate Composition, *Pseudotropheus saulosi*

1. Introduction

Aquaculture is rearing of aquatic organisms for commercial or recreational purposes^[1]. Ornamental fish production is an important component of the aquaculture industry and in recent years, ornamental fishes are receiving increased attention in the Indian export trade. *Pseudotropheus saulosi* is a species of cichlid endemic to Lake Malawi where it prefers areas with rocky substrates. It is classified as a dwarf mbuna and was discovered by Ad Konings in 1989^[2]. It is a maternal mouth brooder. The female holds the eggs in her mouth until the fry are able to swim. This normally takes 2-3 weeks. The fish is endangered in the wild and efforts are currently under way to re-stock Taiwan Reef with captive bred individuals^[3].

Intensive aquaculture industry faces two major problems. The first is water quality deterioration caused by high concentrations of metabolites and the second is the low feed utilization in case of high water exchange system^[4]. Biofloc Technology (BFT) is considered as an advanced and efficient alternative system since nutrients

could be continuously recycled and reused. Intensive BFT for tilapia *Oreochromis niloticus* culture could produce approximately 155 ton/ha/crop^[6]. Use of biofloc as a dietary ingredient in feed preparation is found to enhance the growth rate of fish^[7,8]. In ornamental fish culture, rearing of young ones are the difficult phase where the commercial ornamental fish farmer face a lot of difficulties due to low survival rate, right type of live feed availability, water quality management, incidence of disease, etc.

2. Materials and Methods

Two thousand numbers of Blue morph young ones with an average body weight of 40 ± 0.80 mg were purchased from the commercial ornamental fish farm. They were transported in oxygenated double-layered polythene bags and on arrival to the laboratory they were kept in the FRP tanks (250 litres capacity) with good aeration for one day to monitor their health. No feeding was done for first 24 hours after their arrival. They were acclimatized and released in the control and biofloc treatment tanks.

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The experiment was carried out in outdoor circular cement tanks (14000 l). The rearing system consisted of three similar circular cement tanks, in which one tank was used as control, while the other two were used as biofloc treatment and reservoir tank. All the three tanks were filled with freshwater and was chlorinated with chlorine powder following standard norms [9]. The biofloc treatment tank was added with fertilizer and chemical as per the protocol [10] which is given in Table 1. The ratio of Carbon/Nitrogen (C:N) in the biofloc treatment tanks was monitored regularly and adjusted by adding sugar at the rate of 15 times the total ammonia. The control and biofloc treatment tank were stocked with 1000 numbers of Blue morph youngones. The fishes were fed with commercial pellet feed (Crude protein 32%). Every day, the fishes were fed at 5% of their body weight. The feeding ration was divided into two equal quantities and given twice a day viz. morning and evening.

During experimental culture period, water quality parameters such as Temperature, Dissolved Oxygen, pH, and Total alkalinity were recorded three times a day (morning, afternoon, evening) in addition to ambient temperature in the laboratory following standard procedures. Water temperature was measured using a thermometer with an accuracy of 0.1°C. The pH of the water was measured using the laboratory model Elico pH meter. Modified/Winkler's titration method [11] was followed to estimate the Dissolved Oxygen. Total alkalinity, Total ammonia-N, nitrite-N, Nitrate-N, water hardness, turbidity, and total floc volume (FV) were assessed twice a week following standard methods [11]. Turbidity was measured using a secchi disc twice daily (morning and evening).

Table 1. Fertilizers and chemicals added in the biofloc tank

DAY	ACTIVITY (Application in g/ 14,000lit)
1	Urea -22.4g, TSP-2.8g, fish feed pellet-84g, dolomite- 140g
2	Tea seed cake -15ppm (14mg)
4	Fish feed pellet -84g, dolomite- 140g
6	Fish feed pellet -84g, dolomite- 140g
8	Fish feed pellet-140g, molasses-22.4g, kaolin- 140g
10	Grain pellet-140g
12	Kaolin- 140g

*TSP-Triple Super Phosphate

The total floc volume was the measure of biofloc in treatment tank which was determined by adopting the method using Imhoff cones [12]. One ml of biofloc sample was taken by micropipette and microscopically analysed using LCD projected monitor (10X and 40X). All the samples were analysed and identified immediately in live condition [13,14]. A sample of 100 numbers of Blue morph was taken to assess the total length in millimetre (mm) and average body weight in milli gram (mg). Growth was assessed by measuring the length and weight of ten individual from control and biofloc rearing experimental tanks by random sampling with scoop net once in 10 days for 135 days and other growth parameters were assessed following [15,16,17].

Biofloc samples were collected regularly and the concentrate were dried at room temperature and then preserved in a refrigerator. Dry biofloc samples were analysed for proximate analysis. The ash content of dry sample was determined after burning it in a muffle furnace at 550° C and afterwards the ash was cooled and weighed. The crude protein content was determined by micro Kjeldahl method and lipid was estimated using Soxhlet apparatus [18]. The moisture content was assessed using hot air oven [18]. The results were analysed statistically using ANOVA and regression coefficient. The level of significance at $P < 0.05$ and $P < 0.01$ were considered for validation [19].

3. Results

The mean value of water temperature, pH, DO, total alkalinity, ammonia -N, nitrate- N, nitrate -N in biofloc tank was 27.2°C, 7.8, 3.52 mg/l, 153mg/l, 52.45 µgat-N/l, 1.55 µgat-N/l, 341 µgat-N/l while in control it was 25.7°C, 8.0, 3.77 mg /l, 160mg/l, 69.96 µgat-N/l, 52.45 µgat-N/l, 263 µgat-N/l respectively which was higher than values noticed in biofloc tank. The mean secchi disc readings recorded in biofloc tank was 31.2 cm which was lower when compared with the values of treatment and control tank (40.97cm). Similarly the mean value of water hardness recorded in experimental biofloc and control tanks were 606 mg/l and 617 mg/l respectively.

Microorganisms like *Chlorella sp*, *Anabaena sp*, *Spirogyra sp*, *Microcystis sp*, *Stephanodiscus sp*, *Cosinodiscus sp*, *Paramecium sp* alone were noticed in Control tank whereas the biofloc treatment encouraged the growth of *Aphanothece sp*, *Chroococcus sp*, *Desmodesmus sp*, *Chlorella sp*, *Oscillatorias sp*, *Anabaena sp*, *Spirogyra*

sp, *Microcystis* sp, *Staurastrum* sp, *Trachelomonas* sp, *Spirulina* sp, *Tychonema* sp, *Merismopedias* sp, *Triceratium* sp, *Cymbella* sp, *Gomphonema* sp, *Cocconeis* sp, *Tabellaria* sp, *Coscinodiscus* sp, *Navicula* sp, *Stephanodiscus* sp, *Amphipro* ras, *Nitzschia* sp, *Chaetoceros* sp, *Cyclotella* sp, *Stentor* sp, *Paramecium* sp, *Cyclidium* sp, *Peranema* sp, *Petalomonas* sp, Nematode or round worm, *Chaetonotus* sp, and *Cyclops* sp, and bacteria like organisms.

Temporal variation of floc volume in biofloc during the experimental is depicted in Figure 1. The floc volume ranged from 1.2 ml to 8.0 ml/l in biofloc treatment tank where it ranged from 0.01 to 0.45 ml /l in control tank. The mean value of floc volume was higher in biofloc tank (4.4mg/l) and lower in control (0.21 ml/l).

The proximate composition of biofloc produced in the biofloc treatment tank is given in Table 2. The length, weight and other growth parameters during the rearing of Blue morph in biofloc treatment (Figure 2) and control tanks are given in Table 3 and 4. It is apparent that all the growth parameters expecting length and weight gain and mean length and weight gain were significantly different ($P < 0.01$) among the days of culture as well as between control and biofloc treatment tanks. The survival rate of Blue morph young ones in biofloc treatment was 93% and in control it was 90.2%.

4. Discussion

In the present study, the water temperature in the biofloc tank ranged from 23.2^o C to 31.8^o C whereas in Control it varied from 23.1^o C to 29.8^o C. [20] stated that this temperature range was optimal for mineralisation of organic nutrients into available form. This temperature range was in accordance with Craig and Helfrich [21] who stated that water temperature in the range of 20-25^o C could produce stable flocs. The pH value in the biofloc tank fluctuated from 7.4 to 8.3 which fall in line with Chen *et al.* [22] who

Table 2. The proximate composition of biofloc (dried) produced in the biofloc treatment of *P. Saulosi*

Days of Culture	Proximate Composition				
	Crude Protein (%)	Crude lipid (%)	Moisture (%)	Carbohydrate (%)	Total Ash (%)
40	15.25±2.58	0.68±0.07	6.12±0.20	21.70±0.56	56.25±1.95
80	24.15±2.53	0.72±0.07	5.82±0.19	19.83±0.54	49.48±1.99
120	20.46±2.63	0.48±0.07	5.42±0.20	21.22±0.57	52.42±1.99

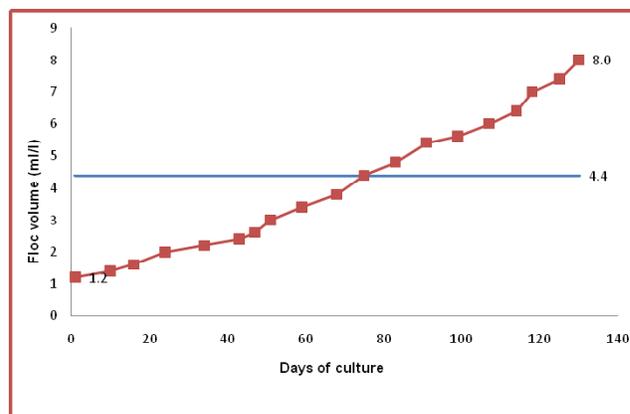


Figure 1. Temporal variation of average floc volume recorded in the biofloc tank during rearing of *P. saulosi*

recommended pH range of 7-9 for the best performance of nitrifying bacteria.

The average values of dissolved oxygen in biofloc treatment tanks and control were 3.52 mg/l and 3.77 mg/l respectively. From the results it could be observed that dissolved oxygen level in biofloc tank is lower when compared to Control. Similar results were obtained by other researchers who stated that heterotrophic microbial population consumed high level of dissolved oxygen in biofloc systems [23,24,25]. The value of total alkalinity was lower in biofloc system. In BFT fish rearing system, the pH level may decrease due to the reduction in the alkalinity level and the increase of dissolved carbon dioxide [26,27,28,29].

In the present study the mean value obtained in biofloc tank was 52.45 µg.at-N/l which was lesser than Control (69.96 µg.at-N/l). The reason for the low value may be attributed to the addition of carbon in the biofloc tank. It is documented that the ammonia-N generated from the faecal pellet and uneaten fish feed was effectively removed by carbon addition [30,31,32]. During the experiment, addition of carbon to the biofloc tank was made at C:N ratio of 15:1. The amount of nitrogen regenerated increased with low C:N level of organic substrate and there was no regeneration of ammonia when C:N ratio level of organic substrate was more than 15:1 [33]. Hence low value of ammonia was obtained in biofloc tank.

The mean value of nitrite-N in biofloc tank was 1.55 µg.at-N/l which was very low when compared to Control (52.45 µg.at-N/l). Contradictory to this the mean value of nitrate-N in biofloc tank was higher (341 µg.at-N/l) than Control (263 µg.at-N/l). When the nitrite-N and nitrate-N values of biofloc system were compared, the nitrite-N

Table 3. Length, weight and other growth parameters during *P. saulosi* rearing in biofloc treatment tank

Days of culture	Total length(mm)	Body weight(mg)	Length gain (mm)	Weight gain (mg)	Mean length gain (mm)	Mean weight gain (mg)	Specific growth rate
1	9.6±0.19	40±0.80	----	----	----	----	----
10	17.3±0.34	570±11.4	7.7±0.15	530±10.6	0.77±0.01	53±1.06	26.6±0.53
23	23.4±0.46	730±14.6	6.1±0.12	160±3.2	0.46±0.009	12.3±0.24	12.65±0.25
34	36.8±0.73	1090±21.8	13.4±0.26	360±7.2	1.21±0.02	32.7±0.65	9.7±0.19
44	39.6±0.79	1140±22.8	2.8±0.05	50±1.0	0.28±0.005	5±0.10	7.61±0.15
55	42.6±0.85	1650±29.0	3±0.06	510±6.2	0.3±0.006	51±0.62	6.76±0.13
64	46.5±0.93	2670±53.4	3.9±0.07	1020±24.4	0.43±0.008	113.3±2.71	6.5±0.13
75	53.5±1.07	3350±67.0	7±0.14	680±13.6	0.63±0.01	61.8±1.23	5.90±0.11
85	59±1.18	4250±85.0	5.5±0.11	900±18	0.55±0.01	90±1.80	5.49±0.10
95	62.1±1.24	4470±89.4	3.1±0.06	220±4.4	0.31±0.006	22±0.44	4.96±0.09
106	66.5±1.33	5180±103.6	4.4±0.08	710±14.2	0.4±0.008	64.5±1.29	4.59±0.09
116	68.3±1.36	5800±116.0	1.8±0.03	620±12.4	0.18±0.003	62±1.24	4.29±0.08
126	70.2±1.40	6570±131.4	1.9±0.03	770±15.4	0.19±0.003	77±1.54	4.05±0.08
135	77.8±1.55	7140±142.8	7.6±0.15	570±11.4	0.84±0.01	63.3±1.26	3.84±0.07

Table 4. Length, weight and other growth parameters during *P. saulosi* rearing in control tank

Days of culture	Total length(mm)	Body weight(mg)	Length gain (mm)	Weight gain (mg)	Mean length gain (mm)	Mean weight gain (mg)	Specific growth rate
1	9.6±0.19	40±0.80	----	----	----	----	----
10	10.4±0.20	410±8.20	0.8±0.01	370±7.4	0.08±0.001	37±0.74	23.3±0.4
23	17.7±0.34	580±11.6	7.3±0.14	170±3.4	0.56±0.01	13±0.26	11.65±0.23
34	25.2±0.50	640±12.8	7.5±0.15	60±1.2	0.68±0.01	5.45±0.10	8.17±0.16
44	28.1±0.56	800±16.0	2.9±0.05	160±3.2	0.29±0.005	16±0.32	6.81±0.13
55	37.1±0.74	1450±33.0	9±0.18	650±17.0	0.9±0.01	65±1.70	6.52±0.13
64	40±0.80	2010±40.2	2.9±0.05	560±7.2	0.32±0.006	62±0.80	6.12±0.12
75	43.1±0.86	2570±51.4	3.1±0.06	560±11.2	0.28±0.005	50.9±1.01	5.56±0.11
85	44.1±0.88	2800±56.0	1±0.02	230±4.6	0.1±0.002	23±0.46	5.0±0.1
95	51±1.02	3600±72.0	6.9±0.13	800±16	0.69±0.01	80±1.60	4.73±0.09
106	55±1.10	4000±80.0	4±0.08	400±8.0	0.36±0.007	36.3±0.72	4.3±0.08
116	58.8±1.17	4400±88.0	3.8±0.07	400±8.0	0.38±0.007	40±0.80	4.03±0.08
126	61.2±1.22	4500±90.0	2.4±0.04	100±2.0	0.24±0.004	10±0.20	3.75±0.07
135	62.4±1.24	5100±102	1.2±0.02	600±12.0	0.13±0.002	66.6±1.33	3.8±0.07

was less than nitrate- N. The result was in accordance with authors who reported that reduction in nitrite -N could be due to nitrification as corroborate by the rise in the level of nitrate-N as well as by denitrification^[34,35]. Aerobic denitrifiers significantly reduce nitrite-N by denitrification under aerobic and higher C:N conditions.

In the present study, the mean value of floc volume recorded in biofloc and control tanks were 4.4 and 0.21 ml/l respectively which was in accordance with Azim and Little^[5] who calculated the feed available in the BFT system in terms of volume and according to him one cubic centimetre of floc volume contain 14 mg floc on

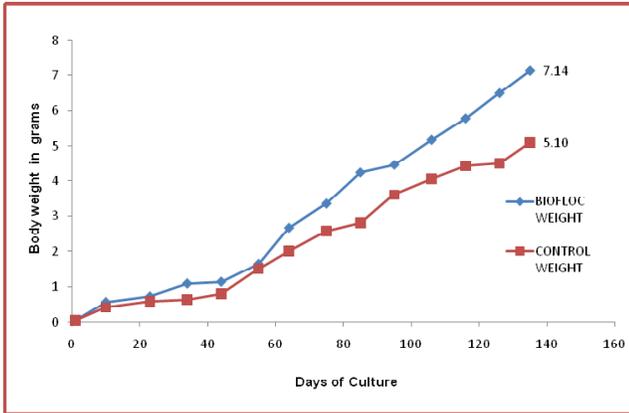


Figure 2. The average body weight of Blue morph in control and biofloc tanks during rearing of *P. saulosi*

dry weight basis and a relatively low reading of 5 ml/l is equivalent to an amount of 700 kg dry matter per hectare. The present result about microorganisms falls in line with De Schryver *et al.* [36] who emphasised the importance of filamentous organisms which helps in bridging the different floc farming components. Similarly Azim and Little [5] recorded three groups of organisms consisting of Protozoa, Rotifer and Oligochaeta in the biofloc system.

The proximate composition of biofloc analysed during the experiment is presented in Table 2. The crude protein content of biofloc ranged from $15.25 \pm 2.58\%$ to $24.15 \pm 2.53\%$. Anand *et al.* [37] recorded more or less similar level (24.30%) of crude protein in biofloc. He also recorded higher level (3.53%) of crude lipid in biofloc which was very high when compared with the present value ($0.48 \pm 0.07\%$ to $0.72 \pm 0.07\%$). The ash content recorded in the present study ($49.48 \pm 1.99\%$ to $56.25 \pm 1.95\%$) was much higher (31.98%) than that recorded by Rostika [38]. In contrast to the results of present study, Juet *et al.* [39] recorded very high level of protein (53.5%), moderate level of crude lipid (3.53%) and lower level of ash content (7.5%). Audelo Naranjo *et al.* [40] opined that the variation in the proximate composition of biofloc was usually based on the dominating living organisms present in the biofloc such as chlorophyll dominated biofloc (26.34%) and bacteria dominated biofloc (38%). At the end of 135th day, Blue morph young ones reared in biofloc tank reached 7.14 ± 142.8 g in weight and 77.8 ± 1.55 mm in length (Table 2). The young ones reared in Control had obtained lower weight (5.1 ± 102 g) and length (62.4 ± 1.24 mm) as shown in Table 3. The higher fish production due to biofloc feeding by fish has been recorded by many authors [41]. In biofloc

system, growth increment of 40% was observed. Azim and Little [5] registered 45% higher net fish production in BFT tanks than in the control tanks. The specific growth rate (SGR) of Blue morph reared in the biofloc treatment was 26.3% which was low when compared with Control (23.3%). The Suresh and Lin [42] and Faizullah [43] recorded 93.56% and 91.8% survival in Tilapia and goldfish biofloc culture system respectively. In line with Suresh and Lin and Faizullah survival rate of 93% was registered in the rearing of blue morph, *Pseudotropheus saulosi* in biofloc treatment and control 90.2% survival was recorded in the control.

5. Conclusion

The rearing of young ones of Blue morph could be undertaken using biofloc technology for better survival rate and higher growth rate. This technology of Blue morph rearing could be made as a cutting edge technology for the benefit of ornamental fish farmers so as to increase aquarium fish production.

6. Acknowledgement

Tamil Nadu Fisheries University is greatly acknowledged for providing financial support to carry out the work in the Institute.

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