RESEARCH ARTICLE Indian Journal of Animal Research, Volume 56 Issue 8: 1034-1040 (August 2022)

Evaluation of Effect of the Different Stocking Densities on Growth Performance, Survival, Water Quality and Body Indices of Pearlspot (*Etroplus suratensis*) Fingerlings in Biofloc Technology

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ABSTRACT

Background: This experiment was conducted to evaluate the effect of different stocking density on growth performance, survival, water quality and body indices of *E. suratensis* fingerlings for 90 days at PRFF, Pazhaverkadu.

Methods: Three different stocking densities are 100, 200, 300 no of fishes/m^3. The fingerlings of pearlspot, *E.suratensis* with an average body weight of 5.05±0.03 g were stocked at a rate of SD-1 (100/m^3), SD-2 (200/m^3) and SD-3 (300/m^3) culture for a period of 90 days.

Result: The growth parameters were found to significantly decrease with increasing stocking density in the biofloc rearing system. Result of Nitrite and nitrate concentrations were significantly lower in the BFT treatment with lower stocking density than in higher stocking density ($P<0.05$). The highest mean body weight gain recorded in SD1 (42.37±0.04 g) followed by SD2 (29.98±0.08 g) and SD3 (20.85±0.06 g). BFT significantly increased fish specific growth rate and net yield. There was no significant difference in hematology analysis (in terms of white blood cell and red blood cell counts, hemoglobin and hematocrit levels), total superoxide dismutase activity of fish between different stocking density ($P>0.05$). Second-order polynomial regression analysis of weight gain against pearlspot culture under lower stocking stocking density (100/m^3) indicate that biofloc technology culture with lower stocking density can improve the growth performance and immune response and also the maintenance of good water quality and improvement of feed utilization of pearlspot.

Key words: Biofloc, *E. suratensis*, Growth, Stocking density, Water quality parameter.

INTRODUCTION

Aquaculture is the ideal source of animal proteins, which could be produced at the lowest cost and very fast. The global population is expected to reach 9.6 billion by Yr. 2050 and as the demand for animal protein is increasing year by year it is a challenge to provide quality protein by safeguarding its natural resources for future generations. Aquaculture plays a key role in promoting health by providing animal protein as well as generating employment and economic growth. Biofloc technology (BFT) applications are one of the best aquaculture systems and contribute to the achievement of sustainable development and desired objectives for a clean environment (Bossier and Ekasari 2017). Despite this fast growth, the aquaculture industry is facing problems interlinked with the disease outbreak due to high stocking density followed in intensive culture conditions (Pulkkinen *et al.*, 2010 and Hargreaves 2013). Pearlspotis the largest among Indian cichlids, a high-valued food fish endemic to peninsular India and Sri Lanka. Because of their wide salinity tolerance, omnivorous feeding habit and high market price, pearlspot is considered ideal species for culturing in brackish and freshwater. With its increasing demand, the price of the fish variety also soars. Apart from the growing demand from local market, the fish is also exported in large quantity to foreign markets Padmakumar *et al.* (2002). In aquaculture, nearly 50 per cent of the total cost is associated with feed, which is mainly due to the cost of the protein component in commercial diets. By means of biofloc technology, a preferred fish species can be cultured in a closed system without the addition of feed also which will help maintaining water quality in a controlled aquaculture system. Compared to other treatments, biofloc system shows better growth of fishes as well as good quality and it more economical and viable.

Haematological indices are often used to interpret the metabolic and health of status of fish (Bond, 1979; Harikrishnan *et al.*, 2011). The biofloc principle combines the removal of nutrients from the water with the production of

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How to cite this article: Ruby, P., Ahilan, B., Antony, C., Manikandavelu, D., Selvaraj, S. and Moses, T.L.S.S. (2022). Evaluation of Effect of the Different Stocking Densities on Growth Performance, Survival, Water Quality and Body Indices of Pearlspot (*Etroplus suratensis*) Fingerlings in Biofloc Technology. Indian Journal of Animal Research. 56(8): 1034-1040. DOI: 10.18805/IJAR.B-4922.

Submitted: 21-04-2022 **Accepted:** 03-06-2022 **Online:** 23-06-2022

microbial biomass, which can be used by the cultured species, *in situ* as additional food source. The optimum C:N ratio in aquaculture system can be maintained by adding different cheap carbon sources and/or reducing protein percentage in feed. In aquaculture, nearly 50 per cent of the total cost is associated with feed, which is mainly due to the cost of the protein component in commercial diets by means of biofloc technology, a preferred fish species can be cultured in a closed system without the addition of feed also which will help maintaining water quality in a controlled aquaculture system. The *in natura* bioflocs are always available to the aquatic organisms as a supplemental food supply (Avnimelech, 1999). They can be ingested and digested by the animals, replacing a significant fraction of the artificial feed (Anand *et al*., 2014; Crab *et al*., 2010; Xu and Pan, 2012). The study focused on evaluation the effect of growth performance of the fish under biofloc-based culture system.

MATERIALS AND METHODS

Prior to experiment, the fishes were graded to select an individual average weight of advanced fry 5.05±0.03 g was used. The fishes were acclimatized in the FRP tanks (4 ton capacity) for 15 days and were fed with commericial feed (CP 32%). Feeding was carried out twice a day at 10.30 am and 4.00 pm during acclimatization. All the FRP tanks (2400 L) were filled with brackishwater. Totally 9 (3 treatments with triplicate) numbers of individual fish tanks were arranged. The experimental set up and biofloc culture tank were given in Fig 2.

Production of biofloc (Jaggery-based BFT)

Protocol for the development of biofloc in the FRP tank.

Day	Activity (Application in g/2.4 ton)
1	Urea -38.4 g, TSP*- 3.6 g, Grain pellet- 144 g, Dolomite - 240 g
2	Tea seed cake 15 ppm
4	Grain pellet - 144 g, dolomite- 240 g
6	Grain pellet - 144 g, dolomite - 240 g
8	Grain pellet - 240 g, sugar - 38.4 g, Tapioca - 240 g
10	Grain pellet - 240 g
12	Tapioca - 240 g

*TSP= Tri super phosphate.

The experimental biofloc tanks were added with fertilizer and chemical as per the protocol described by Avnimelech, (2009). The tanks were left for 15 days to make it favorable for the growth of microbes to develop the biofloc. Vigorous aeration was made to keep the biofloc in constant suspension. C: N ratio of the biofloc treatment tank was monitored regularly and adjusted by adding sugar at the rate of 20 times the ammonia-N content of culture water. Floc samples from the biofloc tanks were collected and examined under microscope to record the presence of microorganisms. This study was approved by ethical committee of Department of Aquaculture, Dr. M.G.R Fisheries College and Research Institute, Tamil Nadu Dr J Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India.

Culture of pearlspot in biofloc tank

The desired range for operation of biofloc system is a settleable solid concentration is 10-15 ml/L. The higher C:N ratio was maintained through the addition of carbohydrate sources (jaggery). Three different stocking densities were followed and each tank was stocked with 100/m^3, 200/m^3 and 300/m^3 respectively in triplicates. The C/N ratio was maintained at 20:1. The water quality parameters such as DO, temperature and pH were measured daily and salinity, BOD, TSS, Nitrite -N, Nitrate-N and TAN were recorded thrice in a week. Fishes were fed with 1.2 mm floating feeds twice daily @ 2% of body weight. The feeding ration was divided into three equal quantities and given thrice a day *viz*., 10.00 am, 2.00 pm and 5.00 pm. The bio-growth parameters were evaluated at the end of the feeding trial. The trial was conducted for a period of 90 days.

Carcass composition analysis

To determine the carcass composition of pearlspot fingerlings the fishes were cut into pieces, minced, homogenized and immediately frozen until further analysis. The following parameters were analyzed moisture (%), crude protein (%), crude lipid (%) and ash (%).

Haematological assay

After end of the trial, blood samples were collected from each group of the fishes.The blood (0.5 ml) were drawn from the caudal vein with the help of a sterile 2 ml hypodermal syringe. The syringes were pre-coated with EDTA (2.7%) as an anticoagulant. Before drawing blood, fishes were anesthetized using clove oil. The blood was then transferred to 1 ml EDTA coated vials and used for NBT assay.

Biochemical analysis of hepatic tissue

The superoxide dismuatse (SOD) activity was estimated following the modified method of Misha and Fridovich, (1972). Catalase activity (CAT) was estimated according to the method of Takahara *et al*. (1960).

Determination of VSI, HSI

$$HSI = \frac{\text{Weight of the liver}}{\text{Weight of the fish}} \times 100$$

$$VSI = \frac{\text{Weight of the viscera}}{\text{Weight of the fish}} \times 100$$

Statistical analysis

Oneway analysis of variance (ANOVA) was performed to examine the difference in growth parameters, water quality parameters, biochemical parameters and body indices among the treatments (Level of significance $p<0.05$).

RESULTS AND DISCUSSION

In the present study, the highest mean body weight gain recorded in the lower stocking density (SD1) was 42.37±0.04 g followed by (SD2)-29.98±0.08 g and SD3-20.85±0.06 g. Second-order polynomial regression analysis of weight gain against graded dietary pearlspot culture in biofloc under different stocking density were given in Fig 1. Mahanad *et al*. (2013) reported that effectiveness of biofloc technology in *L. rohita* and observed an increase in growth performance under BFT system compared to control. The growth parameters were found to significantly decrease with increasing stocking density in the biofloc culture system. Significant differences ($p<0.05$) were observed in weight gain and SGR. Schreyer *et al*. (2008) reported that the specific growth rate (SGR) values were higher under biofloc system with lower stocking density than higher stocking density. Rostika *et al*. (2018) reported that carbohydrate addition can result in the production and accumulation of bioflocs which could serve as an important food source for the zooplankton and thus could increase the growth of shrimp. Low growth of fingerlings in higher density rearing may be because of increased competition for food and space (Chakraborty and Mirza 2007). Survival of pearlspot decreased with the increasing stocking density. Similar inverse relationship between survival and stocking density was recorded in burbot juveniles in tank rearing by Wocher *et al*. (2011). Bio-growth parameters of pearlspot culture in biofloc under different stocking density were given in Table 1.

Water quality parameters of *E. suratensis* after 90 days of rearing in the biofloc based system showed a considerable variation. Water quality parameters of *Etroplus suratensis*

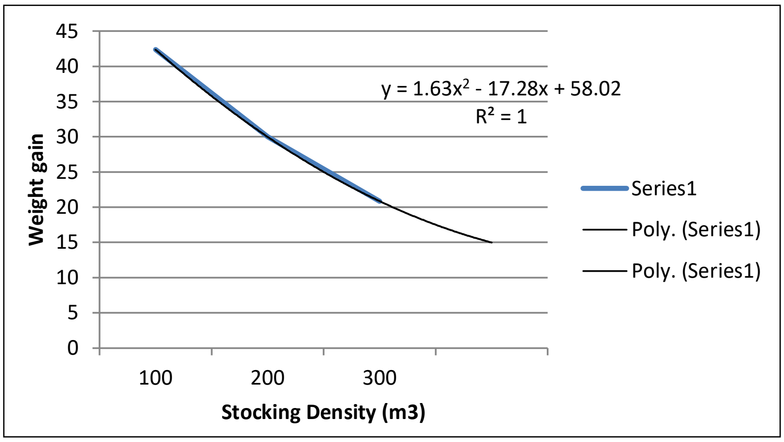

Fig 1: Second-order polynomial regression analysis of weight gain against pearlspot culture in biofloc under different stocking density.

Fig 2: Experimental set up and biofloc culture tank.

Table 1: Bio-growth parameters of pearlspot culture in biofloc under different stocking density.

Variable	SD-100/m^3	SD-200/m^3	SD-300/m^3
Mean Initial weight (g)	5.33±0.01^a	5.12±0.01^a	5.25±0.05^a
Mean final weight (g)	47.7±0.03^a	35.1±0.06^b	26.1±0.01^c
Mean weight gain (g)	42.37±0.04^a	29.98±0.08^b	20.85±0.06^c
Daily growth rate (g/day)	0.53±0.01^a	0.39±0.05^b	0.29±0.06^c
Specific growth rate (%)/day	1.63±0.11^a	1.59±0.29^b	1.62±0.17^a
Survival rate (%)	98±0.19^a	92±0.21^b	80±0.11^c

Values in the same row with different superscript differ significantly ($p<0.05$) between the treatments.

between different stocking density were given in Table 2. The concentrations of dissolved oxygen in all the tanks were recorded in the range of 5.9-6.7 mg/l. The results showed significant variation (P<0.05) among other treatments. SD1 had significantly higher DO (6.7 mg/l) than that of SD2 (6.3 mg/l) and SD3 (5.9 mg/l). The water temperature of the different stocking density groups ranged from 28.1°C to 28.6°C during the experimental period. The mean pH ranged from 7.71 to 8.2. The lower pH values observed in the biofloc tanks were result of high respiration rates by large quantities of microorganisms. The BOD in SD1 was found to be 1.53 mg/l which was significantly lower than SD2 and SD3. Higher BOD in biofloc may due to higher microbial density (Azim *et al*., 2008). The concentration of TAN differ significantly (p<0.05) between the treatments. The highest mean value of 0.092 mg/l and the lowest mean value of 0.07 mg/l and 0.055 mg/l were recorded in SD3, SD2 and SD1 respectively. The concentration of nitrate-N among the treatments varied significantly. However, the mean value of higher concentration of NO3-N (0.25 mg/l) was observed in the higher stocking density followed by lower stocking density. The hepatic tissue of pearl spot showed decreased activities of SOD. The analyzed hepatic tissue of pearl spot showed decreased activities of CAT. The reduced activity of SOD and CAT may reflect inability of liver cells to eliminate the hydrogen peroxide. In the present study, Superoxide dismutase activity was significantly affected by the increasing stocking density. Haematological and stress parameters of *Etroplus suratensis* between different stocking density after the 90 days of experiment were given in Table 3 and 4.

Table 2: Water quality parameters of *Etroplus suratensis* between different stocking density.

Parameters	SD-100/m^3	SD-200/m^3	SD-300/m^3
DO (mg/l)	6.7±0.07^a	6.3±0.06^b	5.9±0.09^c
Temperature (°C)	28.1±0.31^a	28.6±0.11^a	28.3±0.17^a
pH	7.71±0.04^a	8.12±0.02^a	8.2±0.08^a
BOD (mg/l)	1.53±0.06^a	1.72±0.09^b	1.93±0.04^c
Nitrate-N (mg/l)	0.20±0.04^b	0.23±0.31^a	0.25±0.02^a
Nitrite-N (mg/l)	0.21±0.02^c	0.26±0.12^{ab}	0.28±0.04^a
TAN (mg/l)	0.05±0.01^b	0.07±0.01^{ab}	0.092±0.03^a

Values in the same row with different superscript differ significantly (p<0.05) between the treatments.

Lee *et al*. (2002) reported that biological indices such as HSI and VSI are estimated to identify the energy reserved nutritional state of the fish. Lunger *et al*. (2006) reported that the reduction of the HSI index and VSI index is attributed to the lower growth rate of the animals. Wang *et al*. (2015) reported that *Carassius auratus* has high HSI index which was improved by the biofloc. Narra *et al*. (2015) reported that HSI index is used as a biomarker to spot the liver diseases in fishes. High HSI index was found in biofloc cultured fish species (Ekasari *et al*., 2015; Goodall *et al*., 2016; PaezMartinez *et al*., 2003). Supplementary effect of probiotics may positively influenced the HSI index of *E. suratensis* as described by earlier authors (Munir *et al*., 2016a; Mohammadi *et al*., 2016). The significant difference was exhibited between the lower and higher density. HSI and VSI of *E. suratensis* culture under different stocking density after the 90 days experiment were given in Table 5. In the present study *E. suratensis* reared in lower stocking density showed higher percentage of protein compared to higher stocking density. Carcass composition of pearlspot reared in biofloc under different stocking density were given in Table 6. The findings of present study are in agreement with results reported by De Silva and Perera, 1976 in mullet, Hamed *et al*., 2016 in silver pompano, Kumar *et al*., 2017 in pangassius and Hasbullah *et al*., 2018 in Nile tilapia.

Total heterotrophic bacterial count was higher in biofloc tank due to continuous growth of bacterial population with proper aeration and carbon source (jaggery) which

Table 3: Haematological parameters of *Etroplus suratensis* between different stocking density after the 90 days of experiment.

Haematological parameters	SD-100/m^3	SD-200/m^3	SD-300/m^3
Haemoglobin (gm dl^{-1})	7.89±0.12^a	7.83±0.18^a	7.53±0.23^b
Total WBC count (10^6 cells/mm^3)	14.12±0.11^a	13.16±0.22^b	12.15±0.14^c
RBC (10^6 cells/mm^3)	1.98±0.01^a	1.77±0.06^b	1.38±0.05^c
PCV (%)	32.61±0.08^a	28.73±0.03^b	21.72±0.43^c
MCV (Fl)	162.61±0.21^a	156.74±0.31^a	144.80±0.32^b
MCH (pg)	61.20±0.86^a	55.80±0.12^b	51.72±0.14^c
MCHC (gm %)	99.72±0.21^a	91.80±0.12^b	84.46±0.20^c

Values in the same row with different superscript differ significantly (p<0.05) between the treatments.

Table 4: Stress parameters of *Etroplus suratensis* between different stocking density after the 90 days of experiment.

Biochemical analysis	SD-100/m^3	SD-200/m^3	SD-300/m^3
CAT (U/ mg protein)	0.354±0.004^a	0.423±0.006^b	0.930±0.007^c
SOD (U/mg protein)	1.61±0.076^a	1.65±0.034^b	1.69±0.087^c

Values in the same row with different superscript differ significantly (p<0.05) between the treatments.

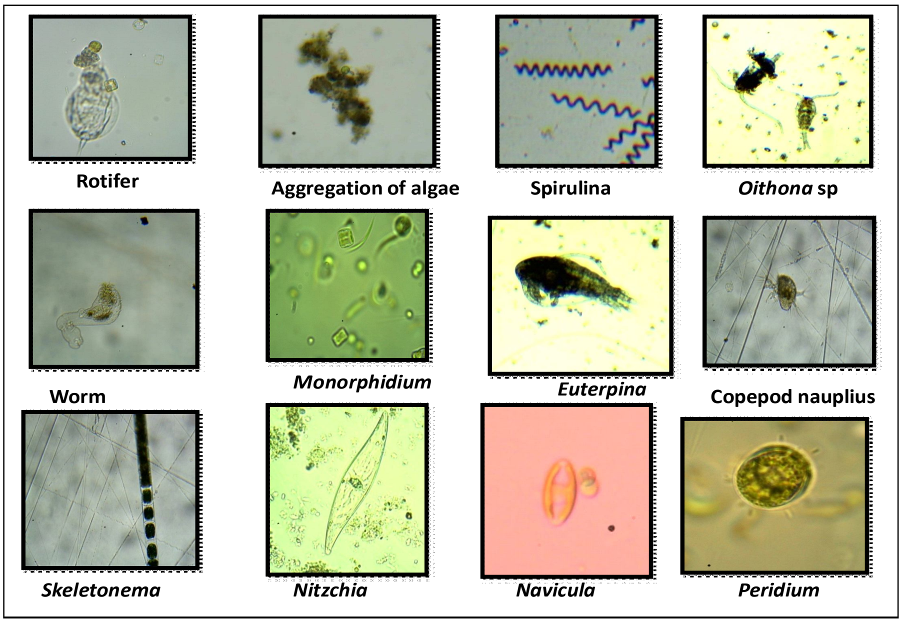

Fig 3: Composition of phytoplankton and zooplankton associated with biofloc.

Table 5: HSI and VSI of *E. suratensis* culture under different stocking density after the 90 days experiment.

Variable	HSI	VSI
SD -100/m^3	1.98±0.08^a	3.59±0.05^a
SD-200/m^3	1.84±0.04^b	2.32±0.02^b
SD-300/m^3	1.71±0.01^c	2.08±0.08^c

Values in the same row with different superscript differ significantly (p<0.05) between the treatments.

Table 6: Carcass composition of pearlspot reared in biofloc under different stocking density.

Parameters	SD-100/m^3	SD-200/m^3	SD-300/m^3
Moisture (%)	71.91±0.19^a	73.92±0.11^b	75.91±0.19^c
Crude lipid (%)	2.31±0.19^a	2.42±0.19^b	2.95±0.19^c
Crude protein (%)	20.89±0.31^a	18.07±0.10^b	15.89±0.31^c
Ash (%)	3.91±0.16^a	3.28±0.12^a	3.91±0.16^a

Values in the same row with different superscript differ significantly (p<0.05) between the treatments.

helps to breakdown the organic matter into heterotrophic bacterial biomass this results agreed with findings of De Schryver *et al.* (2008). The present study was observed composition of phytoplankton and zooplankton associated with biofloc were given in Fig 3. Elaiyaraja *et al.* (2020) reported that the jaggery supported the growth of fungi, yeast and bacteria. Jaggery based biofloc technology encouraged the ammonia immobilization and improved water quality through bacterial assimilation and nitrification process. Abbaszadeh *et al.* (2019) reported that the carbon source used is mainly a by-product that is derived from either the plant or animal food industry and is locally available. Pinto (2020) reported the study on 120 days of *L. vannamei* biofloc culture, reported 92, 81 and 75% survival with stocking of 150, 300 and 450 shrimps/m^2, respectively and detected no significant changes in FCR when feeding *L. vannamei* with different percentages of CP diets. Further in the present study C:N ratio of 20:1 provided better growth performance and survival of pearlspot. Increasing stocking density in biofloc system resulted in the decreased growth of pearlspot. Low growth of fingerlings in higher density rearing may be because of increased competition for food and space.

CONCLUSION

Tank based indoor seed rearing system is advantageous to pond system for higher production and easier harvest. It is very important to find out optimal stocking density for any aquaculture practice to reduce the pressure on resources and maximize the production and profitability. To make any aquaculture venture profitable, there is a need to reduce production costs and increase profitability. The key deciding factors for the aquaculture industry to sustain in the long-run are feed cost and environmental protection. The growth parameters were found to significantly decrease with increasing stocking density in the biofloc rearing system and also noticed positive changes in haematological parameters of pearlspot, *E. suratensis* at lower stocking density. It was concluded that the biofloc culture under lower stocking density (100/m^3) is notably promoting growth performances of *E. suratensis*.

ACKNOWLEDGEMENT

The authors are thankful to the TNJFU, Tamil Nadu for providing facility to carry out this work.

Conflict of interest

The authors declare that they have no conflict of interest.

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