

## Original Article

# Use of a zero-exchange brackish water biofloc system to increase growth, survival, and color intensity of guppy, *Poecilia reticulata* (Peters 1859)

Liyanage Dilini Kaushalya Perera, Udaya Priyantha Kankanamge Epa\*

Department of Zoology and Environmental Management, University of Kelaniya, Dalugama, Kelaniya, Sri Lanka.

**Abstract:** Biofloc technology is based on carbon metabolism and nitrogen immobilizing microbial activities. The present study aimed to maintain water quality and increase the production performance of guppy *Poecilia reticulata* (Peters 1859) in a zero-water exchange brackish water biofloc system. Twenty *P. reticulata* were stocked into each treatment and control tank in triplicate and fed a formulated diet at a rate of 3% of body mass daily. In control-1, 50% water was exchanged weekly following the industrial practice, and no water was exchanged in control-2 and the treatment. Depending on the concentration of total ammonia nitrogen (TAN) in water, rice bran as a carbon source was added to the treatment tanks to keep the C:N ratio at 20:1. Water quality in biofloc treatment and control-1 was within the favorable range for *P. reticulata*. TAN and pH in tanks with zero water exchange were significantly higher ( $P < 0.05$ ). Weight gain, specific growth rate, survival rate, final weight and length of *P. reticulata* in the biofloc treatment were significantly higher ( $P < 0.05$ ) than controls. Fish reared in the biofloc treatment had an intense bright red body and fin color compared to those in the controls. Biofloc technology can be adopted to maintain water quality and enhance the production performance of *P. reticulata* in zero-water exchange brackish water culture systems.

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## Introduction

Biofloc technology (BFT) is getting popular in the aquaculture industry as it improves water quality, reduces pathogens, removes waste, and increases the availability of food for cultured organisms (Azim and Little, 2008; Yusuf et al., 2015; Najdegerami et al., 2016; Mirzakhani et al., 2019; Kishawy et al., 2020). In this technology, bioflocs are formed by aggregating bacteria, fungus, algae, zooplankton, and protozoa together in a matrix with particulate organic matter (Avnimelech and Kochba, 2009; Hargreaves, 2013; Emerenciano et al., 2017; Saha et al., 2022). BFT maintains water quality mainly by controlling heterotrophic bacterial communities over autotrophic microorganisms, using a high carbon-to-nitrogen (C:N) ratio (Avnimelech, 1999; Deocampo et al., 2021). Heterotrophic bacteria can easily take up nitrogenous by-products (Crab et al., 2010) and control inorganic nitrogen accumulation in

aquaculture systems through carbon metabolism and nitrogen-immobilizing microbial processes (Avnimelech and Kochba, 2009).

Most feeds used in semi-intensive aquaculture systems have a C:N ratio of roughly 10:1, whereas bacteria require about 20 units of carbon per unit of nitrogen digested (Avnimelech, 1999; Minabi et al., 2020). As a result, when the C:N ratio in the feed is low, carbon becomes the limiting nutrient for heterotrophic bacteria populations in aquaculture systems (Ekasari, 2008; Asaduzzaman et al., 2009; Saha et al., 2022), and the bacterial population will not expand beyond a certain point due to the limited supply of carbon (Michaud et al., 2006; Panigrahi et al., 2019). Hence, bioflocs are produced in aquaculture systems by adding carbon sources to adjust the C:N ratio range from 15:1 to 20:1 (Emerenciano et al., 2017; Mirzakhani et al., 2019; Kishawy et al., 2020). Preferentially, cheap and

\*Correspondence: Liyanage Dilini Kaushalya Perera  
E-mail: epa@kln.ac.lk

locally available by-products derived from the human and animal food industry are often used as carbon sources in biofloc culture systems. Sources of carbohydrates such as molasses, glycerol, plant meals (i.e., wheat, corn, rice, tapioca, etc.) (Rajkumar et al., 2016; Deocampo et al., 2021), sugar cane bagasse, and chopped hay (Hargreaves, 2013) have been applied to maintain a high C: N ratio and to control N-compounds in the aquaculture systems. Rice bran, one of the most performed carbon sources in the biofloc culture systems (Bakhshi et al., 2018; Kishawy et al., 2020), is one of the cheapest carbon sources in Asia and Sri Lanka.

BFT has been used in brackish and marine shrimp (Decamp et al., 2008; Ju et al., 2008; Emerenciano et al., 2012; Khanjani et al., 2017), freshwater prawn (Crab et al., 2010) and finfish (Avnimelech, 2007; Mahanand et al., 2013; Luo et al., 2014; Ekasari et al., 2015; Yusuf et al., 2015; Najdegerami et al., 2016; Minabi et al., 2020; Saha et al., 2022) aquaculture. However, BFT has rarely been used to improve production performance and maintain water quality in ornamental fish culture systems (Wang et al., 2015; da Cunha et al., 2020; Deocampo et al., 2021).

Japan (\$41.2M), Singapore (\$40.6M), Indonesia (\$34.5M), Thailand (\$34.4M) and Sri Lanka (\$20.97M) were among the top exporters of freshwater ornamental fish in 2021. While many industries incurred significant losses because of the covid-19 outbreak, Sri Lanka's ornamental fish industry achieved record highs of US\$ 20.97M and US\$ 21.74M in 2021 and 2022, respectively (EDB, 2023). Sixty percent of Sri Lankan ornamental fish export consist of *Poecilia reticulata* (Peters 1859), commonly known as the fancy guppy, million fish, or rainbowfish. It is among the world's top 30 most sought-after freshwater ornamental fish species. *Poecilia reticulata* is a tropical live-bearing species that feeds on algal remains, diatoms, invertebrates, plant fragments, mineral particles, aquatic insect larvae, and other sources (Bonatto et al., 2012; Mousavi-Sabet and Eagderi, 2014). However, its diets vary depending on the environmental conditions and food availability in the habitat (Dussault and Kramer,

1981). Under captive conditions, *P. reticulata* is fed on formulated diets with a low protein level of 30% (Dzikowski et al., 2001). To use BFT in guppy farming, the fish must be able to graze and gather suspended flocs, digest and absorb nutrient-rich microbial biomass, and convert it to animal protein. Laboratory experiments confirmed that guppies show 'diet switching' behavior, feeding disproportionately on the more abundant food when offered two choices (Parameshwaran et al., 2001). Whatever the food fish consumes influences in part its body and fin color. As such, there is a great demand for including natural pigments such as carotenoids in aqua feeds to achieve bright coloration in ornamental fish. As bioflocs contain natural carotenoids (Deocampo et al., 2021), studying their effects on the body color of popular ornamental fish species is vital.

One of the significant water quality problems in guppy-rearing systems is the accumulation of toxic inorganic nitrogenous ions ( $\text{NH}_4^+$  and  $\text{NO}_2^-$ ) in water. The common solutions to remove excess nitrogen from the culture system are frequent exchange and the use of different biofilters. These approaches are limited by the environmental regulations that prohibit the release of nutrient-rich water into the environment, the danger of introducing pathogens into the external water and the high expense of pumping vast amounts of water and the high cost taken by biofiltration for treating a large mass of feed residues. Therefore, there is a necessity to develop technologies to reduce or prevent water exchange in guppy-rearing systems. This study was conducted to investigate the impact of zero exchange brackish water biofloc system on the growth, survival, and color of *P. reticulata*.

## Materials and Methods

**Experimental setup:** The experiment was conducted for 85 days using glass tanks with a capacity of 72 L arranged in indoor conditions. The tanks were prevented from direct sunlight by shading since light penetration into the tanks may stimulate the growth of planktonic, filamentous cyanobacteria that may produce toxins or compete with the heterotrophic bacteria. Tanks were filled with brackish water

(salinity-8 ppt) extracted from a coastal well. All the tanks were continuously aerated by an oxygen pump (Model SOBO SB-248A) for 24 hr daily. Triplicate treatment (biofloc) and control tanks (control-1 and control-2) were assigned in a fully randomized design. No water was exchanged in the treatment and control-2 during the experimental period. The water loss due to evaporation in those tanks was only refilled, whereas 50% of the water weekly was exchanged in control-1. No rice bran was added to manipulate C:N ratio in both controls during the experimental period. Control-1 in the present study was utilized to compare the treatment to traditional guppy farming practices. Control-2 was used to evaluate the effects of zero water exchange on the growth performance of *P. reticulata* in a culture system without C:N ratio manipulation.

**Fish stocking and maintenance:** Twenty male guppies of red blonde variety with a mean weight of  $0.16 \pm 0.02$  g and standard length of  $2.0 \pm 0.1$  cm were stocked in each experimental tank. Fish were acclimatized for one week before the commencement of the study. They were fed a commercial feed (with crude protein 56%, crude fat 15%, and crude fiber 0.1%) at a rate of 3% of their body weight daily. The feed ratio was split into two equal portions and provided at 10 am and 3 pm. The feed ratio was adjusted fortnightly according to the variation in fish weight. The feed weight was measured using an electrical balance (Model OHAS CORP, PA4102C). The feed ration was calculated as follows:

$$\text{Feed weight per day} = \text{Mean body weight of fish in a tank} \times \text{Number of fish in relevant tank} \times 3\%$$

**Water quality parameter analysis:** Temperature, dissolved oxygen (DO), salinity, and pH in tank water were measured weekly by a portable multimeter (Model HQ40D). Total ammonia nitrogen (TAN) was measured weekly according to the standard phenate method using a UV-visible spectrophotometer (Model UV-1700, Japan) (APHA, 2012). Biofloc volume in tank water was measured biweekly using Imhoff cones, registering the volume taken by the bio flocs in a 1 L volumetric cylinder after 20 min sedimentation of the enclosure water (Avnimelech and Kochba,

2009).

**C:N ratio in the treatment tanks:** The rice bran collected from a commercial supplier was filtered using an 80  $\mu\text{m}$  mesh sieve to remove the rice husk and impurities. Rice bran was dried in an oven at  $80^\circ\text{C}$  to sterilize the microorganisms for 24 hours. Then it was added weekly to the treatment tanks based on the average TAN in the water to maintain C:N ratio at 20:1. The weight of the carbon source was measured as follows (Serra et al., 2015):

$$\text{Weight of carbon source (g)} = [\text{TAN}] \times \text{C:N} \times \text{EF} \times \text{volume of a tank (L)} \div 1000$$

Where [TAN] = total ammoniacal nitrogen concentration (mg/L), C:N = C:N ratio (1:20), and EF = equivalence factor (rice bran contains 43.36% of carbon; EF value is 2.31).

**Determination of fish growth and feeding performance:** The body weight (electrical balance, Model OHAS CORP, PA4102C) and the standard length of the fish (vernier caliper) were measured fortnightly by randomly collecting five fish from each tank. At the end of the experiment, the growth performance and feed conversion ratio (FCR) of fish were calculated using the following equations (Luo et al., 2014).

$$\text{Survival rate (\%)} = 100 \times (\text{final fish count} / \text{initial fish count})$$

$$\text{Weight gain (g)} = \text{final body weight (g)} - \text{initial body weight (g)}$$

$$\text{Specific growth rate (SGR) (\% /day)} = [(\text{Ln final weight} - \text{Ln initial weight}) \times 100] / \text{Duration of the experiment}$$

$$\text{Feed conversion ratio (FCR)} = \text{Total dry weight of feed (g)} / \text{Total wet weight gain (g)}$$

**Statistical analysis:** All statistical analyses were performed using Minitab software (Version 17). Data were expressed as mean  $\pm$  standard deviation and analyzed by one-way ANOVA after the homogeneity of variance was assessed by the Anderson-Darling Normality test. When significant differences were found, Tukey's pairwise comparison test was used to identify differences among the three treatments. Differences were considered significant at level  $P < 0.05$ .

Table 1. Physicochemical water quality parameters (mean±SD) of *Poecilia reticulata* culture water in biofloc treatment and control tanks in an 85-day trial.

Water quality parameter	Biofloc treatment	Control-1	Control-2	The favorable range for <i>P. reticulata</i>
Temperature (°C)	27.79±0.62 <sup>a</sup>	27.72±0.79 <sup>a</sup>	27.60±0.65 <sup>a</sup>	23-28 (Dzikowski et al., 2001)
Salinity (ppt)	8.31±0.03 <sup>a</sup>	8.30±0.02 <sup>a</sup>	8.31±0.02 <sup>a</sup>	Up to 20 (Sirimanna and Dissanayaka, 2019).
pH	7.64±0.26 <sup>b</sup>	7.71 ±0.36 <sup>b</sup>	8.24±0.19 <sup>a</sup>	6.8-7.8 (Parameshwaran et al., 2001)
DO (mgL <sup>-1</sup> )	6.47±0.88 <sup>b</sup>	7.51±0.23 <sup>a</sup>	7.66±0.19 <sup>a</sup>	> 6 (Parameshwaran et al., 2001)
TAN (mgL <sup>-1</sup> )	0.31±0.10 <sup>b</sup>	0.36±0.10 <sup>b</sup>	1.01±0.64 <sup>a</sup>	0-0.5 (Rubin and Elmaraghy, 1977)
Floc volume (mLL <sup>-1</sup> )	1.68±1.75 <sup>a</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	-

Values in the same row with different superscripts are significantly different ( $P<0.05$ ) according to one-way ANOVA following Tukey's pairwise comparison.

Table 2. Growth performance and FCR (mean±SD) of *Poecilia reticulata* in biofloc treatment and two controls at the end of the 85-day trial.

Parameter	Biofloc treatment	Control-1	Control-2
Final weight (g)	1.76±0.14 <sup>a</sup>	0.70±0.03 <sup>b</sup>	0.68±0.02 <sup>b</sup>
Final standard length (cm)	4.13±0.07 <sup>a</sup>	3.05±0.08 <sup>b</sup>	2.53±0.05 <sup>c</sup>
Weight gain (g)	1.35±0.12 <sup>a</sup>	0.27±0.05 <sup>b</sup>	0.27±0.03 <sup>b</sup>
SGR (% day <sup>-1</sup> )	1.71±0.09 <sup>a</sup>	0.57 ±0.12 <sup>b</sup>	0.61±0.07 <sup>b</sup>
FCR	1.02±0.03 <sup>b</sup>	2.50±0.05 <sup>c</sup>	2.78±0.11 <sup>a</sup>
Survival (%)	88.33±2.89 <sup>a</sup>	86.67±2.89 <sup>a</sup>	66.67±7.64 <sup>b</sup>
Gross body and fin color	More intense bright red color	Lesser intense red color	Least intense red color

Values in the same row with different superscripts are significantly different ( $P<0.05$ ) according to one-way ANOVA following Tukey's pairwise comparison test.

## Results

**Water quality:** The mean values with the standard deviation of water quality parameters in the treatment and two control tanks during the experimental period are given in Table 1. The temperature and salinity in experimental tanks varied in a range of 27-29 °C and 8.28-8.35ppt, respectively, during the experimental period of 85 days (Fig. 1). However, salinity in control-2, where 50% water was exchanged fortnightly, fluctuated with time. In contrast, salinity gradually increased in the treatment and control-1. The pH in the control-2 was significantly higher (8.24±0.19) than in the biofloc treatment (7.64±0.26) and control-1 (7.71±0.36). A slightly decreasing trend of pH was observed in the biofloc treatment, while it showed a slightly increasing trend in the control-2. The highest mean DO (7.66±0.19 mgL<sup>-1</sup>) was recorded in the control-2 and the lowest (6.47±0.88 mg L<sup>-1</sup>) was recorded in the biofloc treatment. DO in the biofloc tank decreased gradually during the experimental period, but such a variation was not

observed in both controls.

A slightly decreasing trend of TAN was observed in the biofloc treatment tank, while it showed an increasing trend in the control-2. Mean TAN in biofloc treatment and control-2 was within the favorable range for *P. reticulata*, but control-1 exceeded the favorable range. Biofloc volume in the treatment tanks increased gradually during the experimental period. Floc volume was measured only in the treatment tanks as biofloc was not formed in the two controls during the experimental period.

**Fish growth and feeding performance:** Production performance and feed conversion ratio of *P. reticulata* in the treatment and control tanks are given in Table 2. *Poecilia reticulata* cultured in the biofloc treatment tanks looked healthier and grew faster than those in the control tanks (Fig. 2). The standard length and weight of fish in the treatment were significantly higher ( $P<0.05$ ) than those in the two controls at the end of the experiment. The average weight of fish in the zero-water exchange control-2 did not

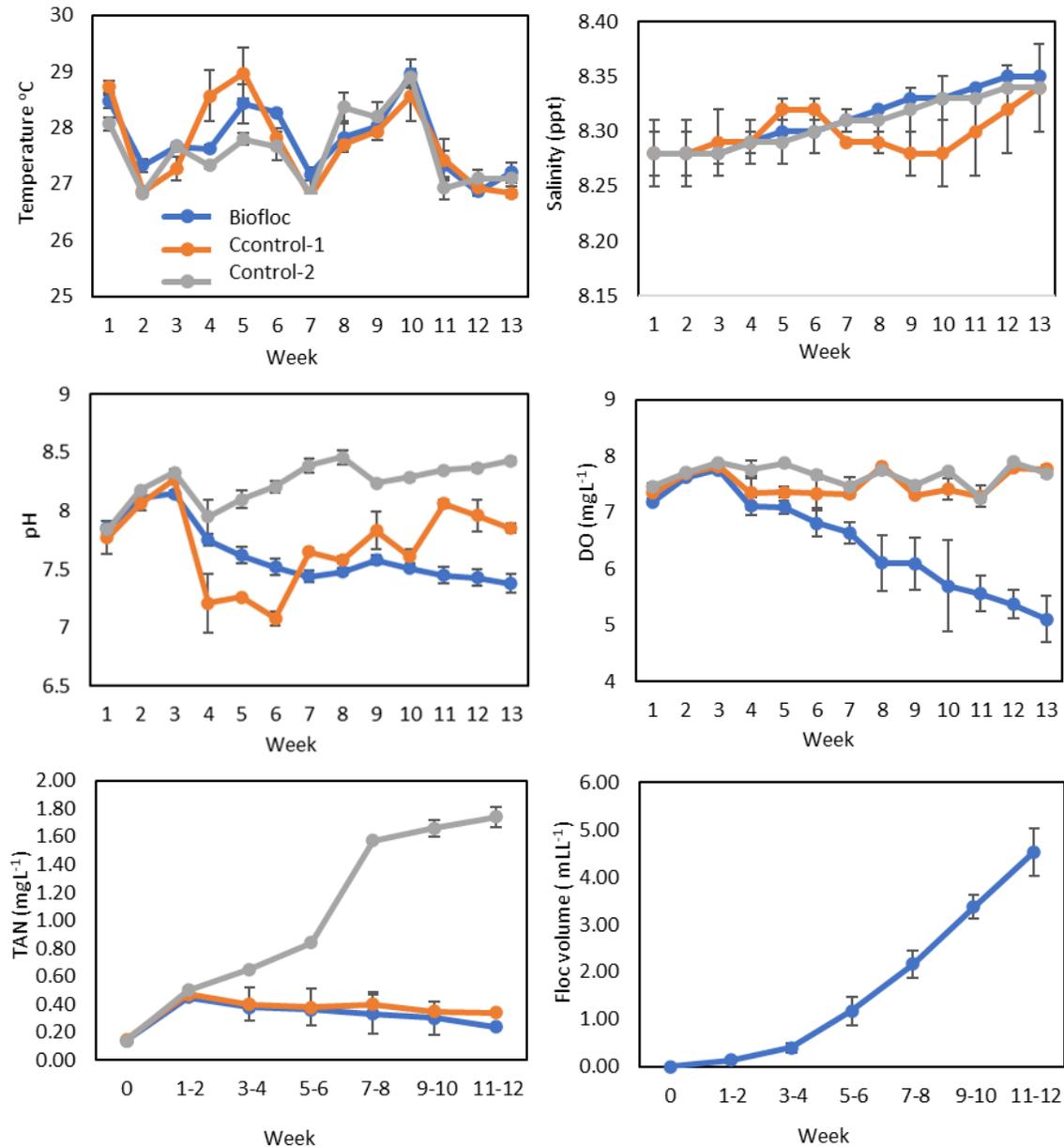


Figure 1. Variation of physicochemical water quality parameters (mean $\pm$ SD) of *Poecilia reticulata* culture water in biofloc treatment and control tanks in an 85-day trial.

significantly differ ( $P>0.05$ ) from those in the control-1. However, the final standard length of *P. reticulata* in the control-2 was significantly lower ( $P<0.05$ ) than those in the treatment and control-1. The final weight gain and %SGR of *P. reticulata* in the biofloc treatment were significantly higher than those in both controls at the end of the experiment ( $P<0.05$ ). FCR among the biofloc treatment and two controls differed significantly ( $P<0.05$ ). The mean FCR recorded in the biofloc treatment was  $1.02\pm 0.03$ , at the optimum level for fish reared in an intensive aquaculture system.

FCR was significantly higher in both controls, while control-2 with zero water exchange had the highest mean FCR ( $2.78\pm 0.11$ ). The percentage survival of *P. reticulata* in the biofloc treatment and control-1 with weekly water exchange was significantly higher than in control-2 with zero water exchange.

The fish in the biofloc treatment showed a more intense bright red color in the body and fins than in both controls (Fig. 3). The fish in control-2 showed the most diminutive low body and fin color compared to biofloc treatment and control-1.

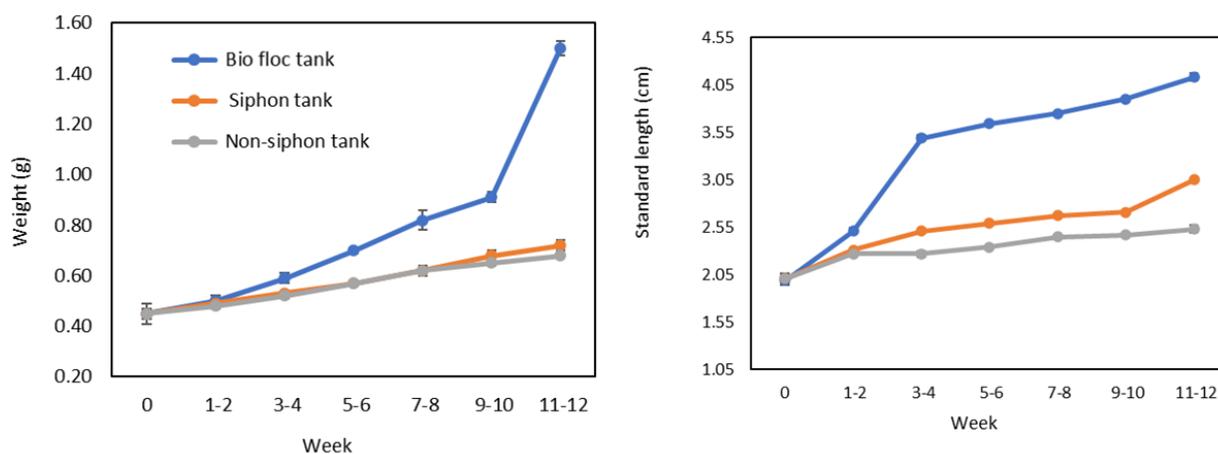


Figure 2. Variation of standard weight and length (mean $\pm$ SD) of *Poecilia reticulata* in biofloc treatment and control tanks in an 85-day trial.



Figure 3. Variation of the body and fin color of *Poecilia reticulata* after 85-day trial (A) - Biofloc treatment (B) Control-1 with water exchange, (C) Control-2 with zero water exchange.

## Discussions

According to the results, the production of bioflocs by manipulating the C:N ratio enhances the production performance and color of *P. reticulata* in a zero-water exchange brackish water culture system. The development of bioflocs and different water exchange practices applied in the experiment affected the physicochemical parameters in *P. reticulata* rearing water except for the temperature and salinity. The temperature in the experimental tanks varied in a narrow range of 27.72-27.79°C due to tropical weather in Sri Lanka. This higher stable temperature may have provided a conducive environment for heterotrophic bacterial growth when a high C:N ratio was maintained in the treatment tanks. Even though there was no significant variation in salinity in the experimental tanks, salinity eventually increased in the biofloc treatment and control-2 due to a lack of water exchange over the experimental period. In control-1, salinity fluctuated due to weekly water exchange from the beginning to the end of the experiment. The DO in the biofloc treatment gradually decreased with time and was significantly lower than in the two controls. Increasing biofloc volume

followed by accelerated microbial activity may have been attributed to low DO in the treatment compared to the controls. During organic matter decomposition, microorganisms consume a high amount of oxygen to maintain their metabolic activities (Avnimelech, 2009; Hargreaves, 2013). In some studies, the DO concentrations could not even be maintained at a desirable level in biofloc tanks until the end of the production cycle, especially in high concentrations of suspended solids (Van Wyk et al., 1999). However, the 24 hr aeration provided in the present experiment was sufficient to supply a favorable DO concentration to *P. reticulata* and microorganisms in both treatment and controls.

The pH in the biofloc treatment decreased gradually, as observed for *Liptopenaeus vannamei* (Boone 1931) (Khanjani et al., 2017) and *Oreochromis niloticus* (Linnaeus 1758) (Mirzakhani et al., 2019) when cultured in the biofloc production systems using different carbon sources. The significantly increased pH in the control-2 (8.24 $\pm$ 0.19) than that of the biofloc treatment (7.64 $\pm$ 0.26) and the control-1 (7.71 $\pm$ 0.36) indicates the inability to maintain water quality at the optimum level with zero

water exchange in *P. reticulata* culture systems. TAN also gradually increased in control-2 due to zero water exchange, while the same was within a very low range in both biofloc treatment and control-1. Even though biofloc treatment and control-2 were zero water exchange systems, a higher rate of carbon metabolism and nitrogen immobilization by heterotrophic bacteria could have occurred only in the treatment tanks. This is evidenced by significantly low TAN and higher floc volume in the treatment compared to control-2. According to Lim (2018), platy, *Xiphophorus maculatus* (Günther 1866) biofloc culture systems produced less nitrogenous waste than non-bio floc culture systems.

The increasing trend of floc volume in the treatment tanks during the experimental period may be attributed to the zero-water exchange, high amount of organic substances, high growth rate of heterotrophic bacteria, and a subsequent increase in microbial biomass (Khanjani et al., 2017; Deocampo et al., 2021). The biofloc formation may be supported by mechanical aeration, which promotes the mixing and distribution of rice bran and other particles in the treatment tanks. If a high concentration of total suspended solids develops in a biofloc system, it could reduce water quality with time, affecting species respiration and changing the composition of the organisms that make up flocs (Hargreaves, 2013). However, such an issue may not arise in *P. reticulata* culture as the fish matures and grows up to marketable size within a short period of 2-3 months.

The survival of *P. reticulata* in the zero-water exchange control-2 was significantly lower (67%) than that of biofloc treatment (88%) and control-1 (87%). The higher fish survival in the biofloc treatment in the present study agrees with Ekasari et al. (2015) and Wang et al. (2015) who recorded high survival rates of fish reared in biofloc systems. Similarly, Harini et al. (2016) indicated that blue morph cichlids, *Pseudotropheus saulosi* (Konings 1990), reared in a biofloc culture system, had higher survival and production rates. The significantly low survival of *P. reticulata* in the control-2 may be related to comparatively higher TAN and pH levels

recorded from the tank water. The presence of un-ionized ammonia, the toxic form, increases as pH rises, which causes ammonia to become more ionized (Schneider et al., 2005; Ip and Chew, 2010). In addition, the higher temperature of tank water may have caused a significant elevation in ammonia toxicity (Ip and Chew, 2010). Thus, the present study shows the inability to maintain the culture system of brackish water fancy guppy with zero water exchange without involving any process to reduce nitrogenous waste products.

In this study, the weight gain, specific growth rate (SGR), survival rate, final weight, final standard length and body color were higher, and the feed conversion ratio (FCR) was at the lowest in *P. reticulata* in the biofloc treatment. The positive effects of the application of biofloc technology on growth performance and FCR of cultured organisms have been reported for *O. niloticus* (Azim and Little, 2008; Mirzakhani et al., 2019), *L. vannamei* (Khanjani et al., 2017; Panigrahi et al., 2019), *Labeo rohita* (Hamilton 1822) (Mahanand et al., 2013; Ahmad et al., 2016), *Carrasius auratus* (Linnaeus 1758) (Wang et al., 2015) and *Cyprinus carpio* (Linnaeus 1758) (Bakhshi et al., 2018). However, Bakhshi et al. (2018) stated that no significant difference was observed between the control and biofloc treatments with different carbon sources regarding the final weight, final productivity, weight gain, and SGR of common carp fingerlings. According to Dauda et al. (2018), the biomass gain, SGR, and FCR of *Clarias gariepinus* (Burchell 1822) were similar among the control and biofloc treatments with different C:N ratios. Probably these results may relate to the species' inability to harvest bioflocs developed in the culture systems effectively. Bioflocs consumption by fish depends most on the fish species and their feeding habits, along with floc size and density (Avnimelech, 2007; Deocampo et al., 2021).

The higher growth and low FCR of *P. reticulata* in biofloc treatment may be attributed to the microbial flocs that could be a supplemental food source available at any time. Bacterial flocs, once ingested, provide protein (essential amino acids),

polyunsaturated fatty acids and different vitamins and minerals for the fish (Azim and Little, 2008; Luo et al., 2014; Wang et al., 2015). Bioflocs could reduce FCR by providing bioactive compounds, such as amino sugar, carotenoids, phytosterol, chlorophyll, and bromophenols (Ju et al., 2008; Crab et al., 2010). Furthermore, by developing microbial flocs, the residual feeds and wastes are recycled and the feed nutrients are reutilized by fish, resulting in improved growth and feeding performance in the biofloc systems (Avnimelech, 2006, 2007; Hargreaves, 2013; Luo et al., 2014). The exogenous enzyme sources and poly- $\beta$ -hydroxybutyrate (a biodegradable polymer) produced by bacteria in bioflocs (De Schryver et al., 2010; Bakhshi et al., 2018) stimulate and improve digestibility in the intestine, increasing nutrient digestibility and enhancing fish growth (Michaud et al., 2006; Crab et al., 2007; Emerenciano et al., 2013; Bakhshi et al., 2018). Directly consuming rice bran containing dietary fiber ( $\beta$ -glucan, pectin, gum),  $\gamma$ -oryzanol, and ferulic acid (Jayadeep et al., 2009) may have further increased the growth of *P. reticulata*. According to the results of the present study, like tilapia and shrimp (Hargreaves, 2013), guppies are also highly efficient in utilizing bioflocs as supplementary feed in aquaculture systems.

In the present study, a more intensive bright red color in the skin and fins was observed in the biofloc tanks compared to the fish in the two control tanks. The highest intensity of skin color of fish in biofloc treatment might be due to the diet that fish consumed during the experimental period. The bioflocs contained various bioactive compounds, including carotenoids (Ju et al., 2008; Xu and Pan, 2013; Deocampo et al., 2021), which may have contributed to the high skin color intensity in *P. reticulata* in the biofloc culture system. Similar results have been reported in previous studies on using biofloc technology to improve the skin pigmentation of *X. maculatus* (Lim, 2018), *C. auratus* (da Cunha et al., 2020), and pink shrimp (Emerenciano et al., 2013). According to Sefc et al. (2014), the red, orange, and yellow colorations of fish can be enhanced by dietary carotenoids produced from biofloc consumption.

As water quality in biofloc treatment in the present study was within the permissible range without any limiting effect on the performance of *P. reticulata*, biofloc technology with zero water exchange can be used instead of typical water exchanged culture systems in brackish water fancy guppy farming. With sustainability and efficiency at its core, biofloc technology is an environmentally friendly aquaculture approach that offers promise for future *P. reticulata* farming applications.

### Conclusion

The biofloc technology can successively be adopted to improve the production performance and body color of *P. reticulata*. Adding rice bran to manipulate the C:N ratio in the water improves water quality in the zero-exchange brackish water *P. reticulata* culture systems. Biofloc development using rice bran lowers the feed conversion ratio and increases the growth rate, body color, and survival rate of *P. reticulata*.

**Ethical statement:** All the experimental protocols and procedures involving fish were performed in accordance with the standard operating procedures of the Ethics Review Committee, University of Kelaniya (Protocol Number: 27-2956).

### References

- American Public Health Association (APHA). (2012). Standard methods for the examination of water and wastewater. 2<sup>nd</sup> edition, American Public Health Association, Washington.
- Asaduzzaman M., Wahab M.A., Verdegem M.C.J., Mondal M.A., Azim M.E. (2009). Effects of stocking density of freshwater prawn *Macrobrachium rosenbergii* and addition of different levels of tilapia *Oreochromis niloticus* on production in C:N controlled periphyton based system. *Aquaculture*, 286: 72-79.
- Avnimelech Y. (2007). Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. *Aquaculture*, 264: 140-147.
- Avnimelech Y. (1999). Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture*, 176: 227-235.
- Avnimelech Y., Kochba M. (2009). Evaluation of nitrogen

- uptake and excretion by tilapia in bio floc tanks, using  $^{15}\text{N}$  tracing. *Aquaculture*, 287: 163-168.
- Azim M.E., Little D.C. (2008). The biofloc technology (BFT) in indoor tanks: water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 283: 29-35.
- Bakhshi F., Najdegerami E.H., Manaffar R., Tukmechi A., Farah K.R. (2018). Use of different carbon sources for the biofloc system during the grow-out culture of common carp (*Cyprinus carpio*) fingerlings. *Aquaculture*, 484: 259-267.
- Bonato K.O., Delariva R.L., Silva J.C. (2012). Diet and trophic guilds of fish assemblages in two streams with different anthropic impacts in the northwest of Paraná, Brazil. *Zoologia*, 29: 27-38.
- Crab R., Avnimelech Y., Defoirdt T., Bossier P., Verstraete W. (2007). Nitrogen removal techniques in aquaculture for a sustainable production. *Aquaculture*, 270: 1-14.
- Crab R., Chielens B., Wille M., Bossier P., Verstraete W. (2010). The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. *Aquaculture Research*, 41: 559-567.
- da Cunha L., Bese, K.P., Ha N., Uczay J., Skoronski E., Fabregat T.E.H.P. (2020). Biofloc technology (BFT) improves skin pigmentation of goldfish (*Carassius auratus*). *Aquaculture*, 522: 735132.
- Dauda A.B., Romano N., Ebrahimi M., Teh J.C., Ajadi A., Chong C.M., Karim M., Natrah I., Kamarudin M.S. (2018). Influence of carbon/nitrogen ratios on biofloc production and biochemical composition and subsequent effects on the growth, physiological status and disease resistance of African catfish (*Clarias gariepinus*) cultured in glycerol-based biofloc systems. *Aquaculture*, 483: 120-130.
- De Schryver P., Sinha A.K., Kunwar P.S., Baruah K., Verstraete W., Boon N., De Boeck G., Bossier P. (2010). Poly- $\beta$ -hydroxybutyrate (PHB) increases growth performance and intestinal bacterial range-weighted richness in juvenile European sea bass, *Dicentrarchus labrax*. *Applied Microbiology and Biotechnology*, 86: 1535-1541.
- Decamp O., Moriarty D.J., Lavens P. (2008). Probiotics for shrimp larviculture: A review of field data from Asia and Latin America. *Aquaculture Research*, 39: 334-338.
- Deocampo Jr. J.E., Fenol J.T., Yerro E.B.S., Pakingking Jr. R.V., Caipang C.M.A. (2021). Biofloc technology (BFT): a promising approach for the intensive production of ornamental fish. *Poeciliid Research*, 11: 18-24.
- Dussault G.V., Kramer D.L. (1981). Food and feeding behavior of the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). *Canadian Journal of Zoology*, 59: 684-701.
- Dzikowski R., Hulata G., Karplus I., Harpaz S. (2001). Effect of temperature and dietary L-carnitine supplementation on reproductive performance of female guppy (*Poecilia reticulata*). *Aquaculture*, 199: 323-332.
- Ekasari J. (2008). Bioflocs technology: the effect of different carbon sources, salinity and the addition of probiotics on the primary nutritional value of the bioflocs. *Aquaculture Research*, 44: 643-654.
- Ekasari J., Zairin J.M., Putri D.U., Sari N.P., Surawidjaja E.H., Bossier P. (2015). Biofloc-based reproductive performance of Nile tilapia *Oreochromis niloticus* broodstock. *Aquaculture Research*, 46: 509-512.
- Emerenciano M., Cuzon G., Goguenheim J., Gaxiola G. and Aquacop. (2012). Flocculation contribution on spawning performance of blue shrimp *Litopenaeus stylirostris*. *Aquaculture Research*, 44: 75-85.
- Emerenciano M., Gaxiola G., Cuzon G. (2013). Biofloc technology (BFT): a review for aquaculture application and animal food industry. *Biomass Now-cultivation and Utilization*, 7: 301-328.
- Emerenciano M.G.C., Martínez-Córdova L.R., Martínez-Porchas M., Miranda-Baeza. (2017). Biofloc technology (BFT): a tool for water quality management in aquaculture. *Water quality*, 5: 92-109.
- Export Development Board (EDB) (2023). Aquarium Fish Export Performance, Sri Lanka 2013-2023. <https://www.srilankabusiness.com/aquarium-fish/aquarium-export-performance.html>. Retrieved 05/19/2023.
- Hargreaves J.A. (2013). Biofloc production systems for aquaculture. Stoneville, MS: Southern Regional Aquaculture Center. 11 p.
- Harini C., Rajagopalasamy C.B.T., Kumar J.S.S., Santhakumar R. (2016). Role of biofloc in the growth and survival of blue morph, *Pseudotropheus saulosi*. *Indian Journal of Science and Technology*, 9: 1-7.
- Ip Y.K., Chew S.F. (2010). Ammonia production, excretion, toxicity, and defense in fish: A Review. *Frontiers in Physiology*, 4: 134.
- Jayadeep A., Vasudeva S., Sathyendra Rao B.V., Srinivas A., Ali S.Z. (2009). Effect of physical processing of

- commercial de-oiled rice bran on particle size distribution, and content of chemical and bio-functional components. *Food Bioprocess Technology*, 2: 57-67.
- Joya Saha M.A., Hossain M., Mamun Al., Islam M.R., Alam M.S. (2022). Effects of carbon-nitrogen ratio manipulation on the growth performance, body composition and immunity of stinging catfish *Heteropneustes fossilis* in a bio floc-based culture system, *Aquaculture Reports*, 25: 101274.
- Ju Z.Y., Forster I., Conquest L., Dominy W. (2008). Enhanced growth effects on shrimp (*Litopenaeus vannamei*) from inclusion of whole shrimp floc or floc fractions to a formulated diet. *Aquaculture Nutrition*, 14: 533-543.
- Khanjani M.H., Sajjadi M.M., Alizadeh M., Sourinejad I. (2017). Nursery performance of Pacific white shrimp (*Litopenaeus vannamei* Boone, 1931) cultivated in a biofloc system: the effect of adding different carbon sources. *Aquaculture Research*, 48: 1491-1501.
- Kishawy A.T.Y., Sewid A.H., Nada H.S., Kamel M.A., El-Mandrawy S.A.M., Abdelhakim T.M.N., El-Murr A.E.I., Nahhas N.E., Hozzein W.N., Ibrahim D. (2020). Mannan oligosaccharides as a carbon source in Biofloc boost dietary plant protein and water quality, growth, immunity and *Aeromonas hydrophila* resistance in Nile tilapia (*Oreochromis niloticus*). *Animals*, 10: 1724.
- Lim Y.H.F. (2018). Performance of freshwater ornamental fish in a biofloc system reared at various stocking densities. Major Project Report, Diploma in Veterinary Technology, Temasek Polytechnic, Singapore. 128 p.
- Luo G., Gao Q., Wang C., Liu W., Sun D., Li L., Tan H. (2014). Growth, digestive activity, welfare, and partial cost-effectiveness of genetically improved farmed tilapia (*Oreochromis niloticus*) cultured in a recirculating aquaculture system and an indoor biofloc system. *Aquaculture*, 422: 1-7.
- Mahanand S.S., Moulick S., Rao P.S. (2013). Optimum formulation of feed for rohu, *Labeo rohita* (Hamilton), with biofloc as a component. *Aquaculture International*, 21: 347-360.
- Michaud L., Blancheton J.P., Bruni V., Piedrahita R. (2006). Effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. *Aquacultural Engineering*, 34: 224-233.
- Minabi K., Sourinejad I., Alizadeh M., Ghatrami E.R., Khanjani M.H. (2020). Effects of different carbon to nitrogen ratios in the biofloc system on water quality, growth, and body composition of common carp (*Cyprinus carpio*) fingerlings. *Aquaculture International*, 28: 1883-1898.
- Mirzakhani N., Ebrahimi E., Jalali S.A.H., Ekasari J. (2019). Growth performance, intestinal morphology and nonspecific immunity response of Nile tilapia (*Oreochromis niloticus*) fry cultured in biofloc systems with different carbon sources and input C: N ratios. *Aquaculture*, 512: 34-45.
- Mousavi-Sabet H., Eagderi S. (2014). First record of *Poecilia reticulata* Peters, 1859 (Cyprinodontiformes, Poeciliidae) from natural freshwaters of Iran. *Poeciliid Research*, 4: 19-23.
- Najdegerami E.H., Bakhshi F., Lakani F.B. (2016). Effects of biofloc on growth performance, digestive enzyme activities and liver histology of common carp (*Cyprinus carpio* L.) fingerlings in zero-water exchange system. *Fish Physiology and Biochemistry*, 42: 457-465.
- Panigrahi A., Sundaram M., Chakrapani S., Rajasekar S., Syama Dayal J., Chavali G. (2019). Effect of carbon and nitrogen ratio (C: N) manipulation on the production performance and immunity of Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) in a biofloc-based rearing system. *Aquaculture Research*, 50: 29-41.
- Parameshwaran K., Edirisinghe U., Dematawewa C.M.B., Nandasena K.G. (2001). Effect of live and formulated feeds on larval growth and survival of guppy (*Poecilia reticulata*) reared in indoor tanks. *Aquaculture Research*, 483: 120-130.
- Rajkumar M., Pandey P.K., Aravind R., Vennila A., Bharti V., Purushothaman C.S. (2016). Effect of different biofloc systems on water quality, biofloc composition, and growth performance in *Litopenaeus vannamei* (Boone, 1931). *Aquaculture Research*, 47: 3432-3444.
- Rubin A.J., Elmaraghy G.A. (1977). Studies on the toxicity of ammonia, nitrate and their mixtures to guppy fry. *Water Research*, 11: 927-935.
- Schneider O., Sereti V., Eding E.H., Verreth J.A.J. (2005). Analysis of nutrient flows in integrated intensive aquaculture systems. *Aquacultural Engineering* 32: 379-401.
- Serra F.P., Gaona C.A., Furtado P.S., Poersch L.H., Wasielesky W. (2015). Use of different carbon sources for the biofloc system adopted during the nursery and grow-out culture of *Litopenaeus vannamei*. *Aquaculture International*, 23: 1325-1339.
- Sefc K.M., Brown A.C., Clotfelter E.D. (2014). Carotenoid-based coloration in cichlid fishes.

Comparative Biochemistry and Physiology Part A. Molecular and Integrative Physiology, 173: 42-51.

- Sirimanna S.R., Dissanayaka C. (2019). Effects of culture conditions on growth and survival of *Poecilia sphenops* and *Poecilia reticulata*. International Journal of Aquatic Biology, 7: 202-210.
- Van Wyk P., Davishodgkins M., Laramore R., Main K.L., Mountain J., Scarpa J. ( 1999). Farming marine shrimp in recirculating freshwater systems. Florida Department of Agriculture and Consumer Services, Tallahassee. 21 p.
- Wang G., Yu E., Xie J., Yu D., Li Z., Luo W., Qiu L., Zheng Z. (2015). Effect of C:N ratio on water quality in zero-water exchange tanks and the biofloc supplementation in feed on the growth performance of crucian carp, *Carassius auratus*. Aquaculture, 443: 98-104.
- Xu W.J., Pan L.Q. (2013). Enhancement of immune response and antioxidant status of *Litopenaeus vannamei* juvenile in biofloc-based culture tanks manipulating high C/N ratio of feed input. Aquaculture, 412-413:117-124.
- Yusuf M.W., Utomo N.B.P., Yuhana M. (2015). Growth performance of catfish (*Clarias gariepinus*) in biofloc-based super intensive culture added with *Bacillus* sp. Journal of Fisheries and Aquatic Science, 10: 523-525.