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Effects of different salinity levels on water quality, growth performance and body composition of Pacific white shrimp (*Litopenaeus vannamei* Boone, 1931) cultured in a zero water exchange heterotrophic system

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Abstract

The present study was aimed to evaluate the effects of different levels of salinity on water quality, growth performance, survival rate and body composition of Pacific white shrimp in a heterotrophic/biofloc technology (BFT). Shrimp post-larvae with an average
weight of 74.46 mg were cultured in 300 L fiberglass tanks containing 130 L water at a density of 1 post-larva/L. Three treatments including different levels of salinity of 8, 21 and 32 ppt with three replicates were considered. The highest levels of body weight, growth rate, specific growth rate, increase in body length and survival rate were observed at high salinity level (32 ppt). The highest feed conversion ratio (FCR) and the lowest level of feed efficiency were obtained in shrimps cultured at lowest salinity level (P<0.05). Biochemical analysis of shrimp body composition showed an increase in protein, lipid and ash content as the salinity elevated (P<0.05). The zero-water exchange system used in this study had no significant effects on water quality parameters. The results of the present study concluded that high salinity level (32 ppt) improves the growth and survival of the biofloc supplemented Pacific white shrimp in a BFT system.

Key words: Aquaculture, Shrimp, Pacific white shrimp, Salinity, Biofloc

Feed management plays a key role in enhancing the production of aquatic species. The most important goals of sustainable aquaculture are to create a system that reduces the need for non-renewable resources in the nature (Ahmad et al., 2016). High-density production and zero water exchange system in aquaculture is called heterotrophic/biofloc technology (BFT), which is environmentally friendly and can be useful for development of sustainable aquaculture through intensive fish production by recycling of water (Khanjani and Sharifinia, 2020).

Biofloc can be defined as a complex community of organic matter associated with other components, which forms mass suspended particles (Cuzon et al., 2004; Emerenciano et
al., 2012; Emerenciano et al., 2013b) including organic matters (60 to 70%), an inhomogeneous mixture of microorganisms (fungi, algae, bacteria, protozoa, rotifer, nematode) and inorganic materials (30-40%) such as colloids, organic polymers, bivalent ions, salts and dead cells (Chu and Lee, 2004). The diversity of the microorganisms in the biofloc depends on various factors, including the type of carbon source, the salinity level and the farmed species (Ray et al., 2010). The nutritional quality of the biofloc is comparable to that of the concentrate-based diets in terms of protein, lipid, carbohydrate and ash content, which this make it nutritionally valuable as diet in aquaculture. Bioflocs are also rich in terms of vitamins, minerals especially phosphorus, calcium and magnesium (Crab et al., 2010; Crab et al., 2012; Emerenciano et al., 2013a; Hargreaves, 2013). Various studies have reported the enhancing effects of biofloc technology on resistance to diseases, growth, survival and feed conversion ratio of fishes (Azim and Little, 2008; Khanjani et al., 2019; Khanjani et al., 2017).

The Pacific white shrimp, *Litopenaeus vannamei* belongs to Penaeidae family, widely is grown in the western hemisphere. Due to the ability to maintain osmotic regulation in a wide range of salinity, this species is able to live in water resources with salinity ranges of 5 to 40% (Saoud et al., 2003). The high adaptability and good cultural features have made the white shrimp as an appropriate candidate for farming in different salinities in the United States (McGraw et al., 2002), Thailand, Southeast Asia (Saoud et al., 2003) and Iran. Salinity is one of the important factors, affecting the growth and survival of aquatic species and Penaeidae family (Kumlu et al., 2000; Sharifinia et al., 2019). Slight changes in salinity during the cultivation period have a positive effect on shrimp growth (Mu et al., 2005).
Although adaptive responses to salinity changes are vital for keeping growth and survival at appropriate condition in aquatic species, long-term exposures may adversely affect these parameters (Young et al., 1989). Therefore, physiological adaptation to salinity changes are vital for restoring shrimp stocks in the nature and also its growth, survival and food consumption in rearing conditions (Jaffer et al., 2020). Ponce-Palafox et al. (1997) reported the highest survival of *L. vannamei* juveniles in salinities over 20 ppt, whereas, Briggs et al. (2004) reported the best growth between 10-15 ppt. However, Diaz et al. (2001) reported the low growth and survival of this species at low salinity. Sowers et al. (2005) found that mixed salt and sea salt environments do not effect osmotic regulation in environments with <2 ppt total dissolved salts (TDS). In contrast, Laramore et al. (2001) observed more survival rate of *L. vannamei* at 30 ppt salinity. Although some studies have examined the effects of different salinity levels on growth, survival, oxygen consumption, and immune system of *L. vannamei*, the results are controversial yet (Decamp et al., 2003; Esparza-Leal et al., 2016; Jannathulla et al., 2019; Lin and Chen, 2001; Maicá et al., 2012; Wang and Chen, 2005). Therefore, it seems that more studies are necessary to optimize the salinity in BFT system. Due to the importance of this issue, in present study we examine the effects of different salinity levels on water quality, growth, survival and body composition of the pacific white shrimp fed wet biofloc in a zero water exchange system.

**Material and methods**

**Experimental design**
The experiment was conducted in Kolahi marine shrimp hatchery (KMSH) located at Hormozgan province, east of the Persian Gulf, Iran (Sharifinia et al., 2018). Pacific white shrimp post-larvae (PL18; shrimp that has passed 18 days in the post-larval stage) with a weight of 74.46 ± 6.17 mg and a length of 19.79 ± 0.88 mm (Mean ± SD) were obtained from the KMSH. Twelve polyethylene circular tanks (area of 0.38 m²) were considered for the experiment. Each rearing tank filled with 130 L of sand filtered water, and then 130 PL (1 PL/l) were cultured in each tank and tested for four weeks. Three experimental treatments with three replicates were considered for the present study, which included flocs with no water exchange and different salinity levels of 8 (biofloc treatment with the salinity of 8ppt, PBS1), 21 (biofloc treatment with the salinity of 21ppt, PBS2) and 32 (biofloc treatment with the salinity of 32ppt, PBS3) (Table 1). Dechlorinated and filtered water (with equal salinity for each treatment) was added to the tanks to recover the water lost through evaporation.

At the beginning of the study, the PLs were fed moist bioflocs at a rate of 15% of the body weight, and the feeding rate decreased to 9% as the shrimp grown at the end of the study. Feeding was done three times a day in all treatments (8 a.m., 14 a.m., 20 p.m.). To estimate the amount of bioflocs, a given volume of flocs was initially dried, and the volume of them was estimated based on that. Approximately 10 g of shrimp biomass were cultured in each tank, with 15% of the biomass fed at the beginning of the experiment (1.5 g dry biofloc equivalent to that was given wet biofloc). Wet biofloc was prepared from three 2000 L shrimp tanks based on biofloc. At first, the water of the rearing tanks (containing biofloc) was filtered with 20 µm net. Then, 100 g wet biofloc was dried in three replicates. This work was done every five days to set a clear ratio between wet and dry bioflocs. The properties of the wet biofloc are presented in Table 2 and Fig 1.
Organic matter including commercial shrimp feed containing 42% protein (produced by Hvorash Company, Bushehr, Iran), wheat flour, wheat bran and molasses were used to form bioflocs. The wet bioflocs were prepared in Three 2000-liter containers. Past shrimp feeds were used as a source of nitrogen and carbonaceous organic matter (molasses, flour and wheat bran) as carbon sources. After formation of a certain amount of biofloc, 100 g of the wet biofloc was dried in three replicates and the dried floc was weighted. During the experimental period, the increasing floc-production trend and also water quality was kept through adding the molasses as a carbon source to the tanks. The amount of molasses was calculated assuming that 50% of the carbon would be used by the bacteria to maintain the carbon to nitrogen ratio at 15, (Avnimelech, 2009). Molasses was added to rearing tanks approximately at a rate 50% of the feed input. For aerating and supplying oxygen, three air stones connected to an aeration source were used on the floor of the tanks (Stream HG-1100SB; 1.1 kW – 1.5 horsepower).

Water Quality Parameters

The experiment was conducted in indoor tanks for four weeks with 12-hour light period, 12-hours dark period. A single lamp (metal halide lamp, 400 W) was hung above the tanks as an artificial light source. A light intensity of 1000 lx (ICEL LD - 550 lx meter) was maintained constant during the experiment. The water quality parameters including temperature (Digital Thermometer), pH (pH Lutron 208, pH meter) and dissolved oxygen (DO Lutron 510 Oxygen Meter) was measured two time a day at 8:00 and 16:00. To determine the concentration of suspended materials, 1 liter of water was poured into a conical funnel and left for 30 min to settle (Avnimelech and Kochba, 2009).
To determine the total suspended solids, 100 ml of the water of each tank was filtered with Whatman filter paper No. 42 and dried at 105 °C for 3 h (Xu and Pan, 2012). Ammonia, nitrite, and nitrate were spectrophotometrically measured based on MOOPAM (1999) using a spectrophotometer (Model 9200 Cecil, CE). Total Heterotrophic Bacteria (THB) count in the water was calculated according to standard procedures APHA (2005) and expressed as colony forming units per milliliter (CFU).

**Growth indices**

To growth indices (weight gain, weight gain percent, growth rate, biomass, specific growth rate, and daily growth rate between treatments) were measured at the beginning of the experiment and weekly during the experimental period. The numbers of post-larvae were cultured at the beginning of the experiment and at the end of the experiment to calculate the survival rate. Also, nutritional indices including feed conversion ratio and nutritional efficiency were calculated based on the following formula (Tacon et al., 2002):

- Body weight gain (mg) = final weight - initial weight
- Body length gain (mm) = final length - initial length
- Body weight index (BWI) (%) = [(final weight - initial weight)/initial weight] × 100
- Growth rate (GR) (mg/day) = [(final weight - initial weight)/ days of experiment]
- Total Biomass (g) = (final weight - initial weight) × survival rate × number of shrimp
- Survival rate (SR; %) = (number of individuals at end of testing period/initial number of individuals) × 100.
- Specific growth rate (SGR; %/day) = [(ln final weight - ln initial weight) ×100]/days of experiment
Feed conversion ratio (FCR) = feed consumed (dry weight)/live weight gain (wet weight)

Feed efficiency (FE) (%) = [final weight - initial weight]/ feed consumed ×100

**Biochemical analysis of shrimp body composition**

At the end of the experimental period, 20 shrimps were randomly collected from each salinity treatment. The shrimps were first washed with water and then the head and shell were separated. After that, they were homogenized with a meat grinder and finally, stored at -18 °C until the test time. The chemical composition of the carcass including protein, lipid, dry matter and ash were measured by the AOAC (2005) method.

**Biochemical analysis of bioflocs**

At the end of the experiment period, water samples were collected from each treatment, passed through 20 μm mesh nets (Khanjani et al., 2017), the related biofloc produced was placed in pre-numbered aluminum foil and put in an oven at 102 °C for 24 h to dry. Dry flocs were then stored in a freezer at -18 °C until biochemical analysis. Protein, lipid, dry matter and ash content were measured using the AOAC (2005) method.

**Data analysis**

All data were analyzed by SPSS software version 21. At first, the Kolmogorov-Smirnov test was used to determine the normality of the data. Then, to identify the differences and compare the mean of the treatments, one-way ANOVA and the Duncan multi-domain test at 5% level were applied respectively. All the graphs were drawn with Excel version 2012.

**Results**
The values (Mean ± SD) of water physicochemical parameters including temperature, dissolved oxygen, pH, ammonia, nitrite, and nitrate, the level of settle-able solids, the total suspended solids, and the water transparency during the experiment are presented in Table 3. There were no significant differences in temperature and dissolved oxygen between treatments in the morning (P< 0.05). The lowest (5.68 ± 0.30 mg. L\(^{-1}\)) and highest (6.27 ± 0.19 mg. L\(^{-1}\)) content of dissolved oxygen were observed in treatment PBS3 in the afternoon and treatment PBS1 in the morning, respectively (P<0.05).

There were significant differences in the value of pH and nitrate between the treatments, PBS1 and PBS3 (P <0.05). The highest (3.78 mg. L\(^{-1}\)) and lowest (1.60 mg. L\(^{-1}\)) concentration of NO\(_2^-\) were observed in PBS3 and PBS1 treatments, respectively (P <0.05). There were not significant differences in the levels of ammonia and nitrate between treatments (P >0.05). The variation of ammonia, nitrite, and nitrate in treatments are shown in Figure 2 A, B, C. No significant differences were observed in the level of the settle-able solids (SS), total suspended solids (TSS) and transparency between the treatments (P>0.05). The amounts of recorded SS, TSS and water transparency during the experiment period are shown in Figure 3 A, B, C.

Results of the growth indices (Mean ± SD) are presented in Table 4. The highest amount of final weight (661.62 mg), final length (36.2 mm), body weight gain (587.1 mg), weight gain percent (788.42 %), growth rate (20.96 mg), increase of biomass (69.67 g), specific growth factor (7.8% per day) were found in treatment PBS3, which showed a significant difference compared to other treatments (P <0.05). The highest level of feed conversion ratio (3.84) and the lowest nutrient efficiency (25.99%) obtained in treatment with a salinity of 8 ppt, which showed significant differences compared to various treatments. The values (Mean ± SD) of
the survival rate of treatments were given in Table 4. The highest survival rate (91.28%) was found in salinity of 32 ppt, which was significantly different from other treatments (P <0.05).

The values of the biochemical composition (in terms of dry weight) of carcass are presented in Table 5. There was a significant difference in protein, lipid, and ash content of white shrimp body between treatments of 8 and 32 ppt salinity (P <0.05). In addition, the amount of protein and lipid of the floc decreased with increasing salinity, but the amount of ash increased.

**Discussion**

**Water quality**

Water quality is very important for maintaining the health of aquatic species and can act as a limiting factor (Kamrani et al., 2016; Sharifinia, 2015; Sharifinia et al., 2015). The levels of environmental factors obtained in the present study were in the optimal range for the growth and production of Pacific white shrimp. The lowest amount of dissolved oxygen was obtained in the BFT treatment with the salinity of 32 ppt. This decrease in oxygen content can be attributed to the high amount of bioflocs and associated oxygen consumed by microbial communities (De Schryver and Verstraete, 2009; Khanjani et al., 2019). In addition, it should also be noted that high water salinity reduces the solubility of dissolved oxygen in water (Weiss, 1970). Furthermore, previous studies indicated that the addition of carbon to water resulted in a temporary reduction in the concentration of dissolved oxygen and an increase in microbial metabolism due to the decomposition of organic matter (De Schryver and Verstraete, 2009).

The level of pH was significantly different among treatments and the lowest level was observed in the BFT treatment with the salinity of 8 ppt. Ma et al. (2009) reported that the
addition of molasses carbohydrate to BFT system can cause an increase in the activity of microbial communities and respiration, and also the formation of lactic acid by heterotrophic bacteria as a result of molasses decomposition. In the present study, lower concentration of ammonia was observed in the treatment with highest salinity. Decamp et al. (2003) found that in ammonia-free systems, the amount of ammonia is significantly affected by salinity. In addition, a reverse relationship has been observed between water salinity and the rates of \( \text{NH}_3^+ \) excreted by Penaeid shrimp (Maicá et al., 2014; Perez-Velazquez et al., 2008). 

In the present study, the highest levels of nitrate and nitrite were found in the BFT treatment with the salinity of 32 ppt, which may indicate the activity of heterotrophic and chemoautotroph bacteria and subsequent absorption and nitrification processes. In higher salinity, it seems that the activity of the heterotrophic and chemoautotroph bacteria increases. Salinity is one of the most important factors affecting the development and growth of heterotrophic bacteria and the nitrification process (Timmons et al., 2002). Various studies have reported an increase in nitrate concentration by increasing salinity levels in a zero water exchange system for Pacific white shrimp (Decamp et al., 2003; Maicá et al., 2014). Lin and Chen (2003) found an inverse relationship between the amount of salinity and nitrite toxicity in Pacific white shrimp farms, where the toxicity of nitrite reduced with increasing of salinity. The optimum concentration of nitrate for the cultivation of Pacific white shrimp in seawater is less than 1 mg. L\(^{-1}\) and in water with salinity near 2\%, is reported 0.45 mg. L\(^{-1}\) (Van Wyk and Scarpa, 1999). Therefore, there is probably an increasing pattern in the N-NO\(_3\) concentration as the salinity increases. However, this parameter was less than concentration reported for this species (\( \leq 60 \text{ mg. L}^{-1} \)) (Van Wyk and Scarpa, 1999). Decamp et al. (2003) and Maicá et al.
(2014) also confirmed an elevation in the level of N-NO₃ as the salinity increased in a zero water exchange system for L. vannamei.

In this study, the concentration of SS and TSS increased as the salinity increased. Similar results were observed in the study of Maicá et al. (2014). In the study of Decamp et al. (2003), the highest concentration of suspended solids was obtained at the highest salinity (36 ppt), confirming the effect of salinity on the concentration of biofloc. Similar results were also found in the study of Håkanson (2006) and Avnimelech (2007), where the TSS increased by elevating of salinity levels. Increases in amount of suspended solids reduce the transparency of the water, as the transparency reduced in the tank with increasing of salinity and suspended solids in the present study. In the present study, the concentrations of SS were measured 5, 5.14 and 5.56 ml. L⁻¹ and TSS values 129.6, 135.7 and 159.8 mg. L⁻¹. A concentration of <300 mg. L⁻¹ was reported for an intensive cultural system of shrimp larvae by Mishra et al. (2008). Furthermore, Serra et al. (2015) stated that various carbon sources can affect TSS levels during the nursery and growing stages of Pacific white shrimp in the BFT system.

**Growth performance**

The results of this study showed a better growth for shrimps at salinity of 32 ppt. Wasielesky et al. (2006) reported a positive relationship between growth parameters and food protein in the BFT system. In the present study, optimal and expected growth was not observed in all treatments, indicating the biofloc probably has not been a complete diet for Pacific white shrimp. However, it may improve growth along with artificial diets (Khanjani et al., 2016; Khanjani et al., 2017). Also, the growth of shrimps improved with increasing of salinity and density of bioflocs, indicating the Pacific white shrimp probably has used bioflocs more efficient in higher salinities. Many studies have been investigated the effects of different
salinity levels on growth and survival of *L. vannamei*. Saoud and Davis (2005) reported that the higher salinities (50‰) enhance survival and growth of *L. vannamei*. Laramore et al. (2001) also obtained the higher survival rate for *L. vannamei* at 30 ppt salinity. Ponce-Palafox et al. (1997) reported that *L. vannamei* survives and grow better in salinities more than 20 ppt. In contrast to above studies, Jaffer et al. (2020) found that *L. vannamei* prefers lower salinities. Generally, increase of salinity causes an elevation in the natural products and subsequent growth and development of bioflocs (Maicá et al., 2014).

In the present study, the feed conversion ratio of 3.84, 2.99 and 2 were obtained in PBS1, PBS2 and PBS3 treatments, respectively. The feed conversion ratio of 0.95, 1.03, 1.11, 1.61 have been respectively reported for Pacific white shrimp with different densities of 1500, 3000, 4500, and 6000/m² post larvae in the biofloc system (Wasielesky et al., 2013). In the study of Serra et al. (2015), in the larval stage of *L. vannamei*, the feed conversion ratio was 1.12 to 0.89 and the specific growth rate was 11.17 to 11.45% per day. However, in the growing stage, the feed conversion ratio was 3.25 to 2.03 and the specific growth rate was 0.30 to 0.43 g per week in different treatments. In the present study, feed conversion ratio and feed efficiency improved significantly by increasing the salinity. Decamp et al. (2003) and Maicá et al. (2014) reported that increasing salinity levels can lead to improvement of feed conversion ratio in *L. vannamei* juveniles.

In this study, feed utilization was improved with an increase in salinity level. The weight of shrimp reared at 32 ppt increased compared to those cultured at 8 and 21 ppt. In general, the survival in zero water exchange systems with bioflocs is high (Kuhn et al., 2008; McAbee et al., 2003; Wasielesky et al., 2006). However, the survival of marine shrimps cultured at low
salinities varied depending on water ionic composition, species, age, reduction rate of salinity and final salinity (Cheng et al., 2006; McGraw et al., 2002). In this study, shrimp survival was significantly affected by salinity, where mortality increased as the salinity decreased. Similar findings were reported by other researchers (Decamp et al., 2003; Jayasankar et al., 2009; Laramore et al., 2001; Maicá et al., 2014; Maicá et al., 2012).

**Biochemical composition**

In this study, the biochemical composition of the shrimp body changed by salinity and bioflocs. The most important factors affecting the body biochemical composition of fish are the nutritional status, age, sex, life stage, and environmental conditions (Brey et al., 2010; Brosset et al., 2015; Correia et al., 2003). Many studies have reported that variations in the shrimp biochemical body composition in the biofloc system are probably related to the biochemical composition of bioflocs (Izquierdo et al., 2006; Ju et al., 2008; Tacon et al., 2002). In the present study, protein, lipid, and ash content of the shrimp body increased as the salinity increased. In the study of Khanjani et al. (2017), the shrimp body protein (75.45-75.86%), lipid (7.36-7.54%) and ash (11.18- 11.36% of dry weight) in a biofloc system were in consistent with the results of the present study. Tacon et al. (2002) found that the high levels of ash in the body of Pacific white shrimp is likely due to the availability of large amounts of minerals and metal elements in bioflocs. The ash content was also increased in BFT system designed by Martins et al. (2017) due to the consumption of alkaline compounds such as calcium, magnesium and sodium ions and following increases in bacterial mass of the biofloc. Protein and lipid content of bioflocs decreased with an increase in the salinity level, but the ash content enhanced. Furthermore, the protein (28.9-31.8%), lipid (0.85-1.02%) and ash (27.85-37.94%) content of bioflocs were similar to the values obtained from Khanjani et al.,
The biochemical composition of bioflocs such as protein, lipid, fiber, and ash was reported to be 35, 1, 15 and 15%, based on dry weight (Mahanand et al., 2013). In various studies, the amount of protein in the bioflocs has been reported by about 35% (Mahanand et al., 2013; Tacon et al., 2002; Wasielesky et al., 2006). Generally, the biochemical composition of the bioflocs in the BFT system changes upon type of carbon source, the C:N ratio, water salinity, the source and intensity of light and different feeding levels (Abbaszadeh et al., 2019; Crab et al., 2010; Ekasari et al., 2010; Khanjani et al., 2019). The results of the present study showed that bioflocs in zero water exchange system are consumed as a part of shrimp diet by Pacific white shrimp. Salinity was also confirmed as a factor affecting growth performance, biochemical quality of the shrimp body and bioflocs.

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Table 1. Specifications of the treatments used in this study

| Treatments | Salinity levels (ppt) | Culture volume (L) | Density (ind./L) | Replicate | Water exchange  |
|------------|-----------------------|--------------------|-----------------|-----------|----------------|
| PBS1       | 8                     | 130                | 1               | 3         | No exchange    |
| PBS2       | 21                    | 130                | 1               | 3         | No exchange    |
| PBS3       | 32                    | 130                | 1               | 3         | No exchange    |

Table 2. Features of used wet biofloc for rearing Pacific white shrimp in this experiment

| Feed                                      | Wet biofloc biomass                           |
|-------------------------------------------|----------------------------------------------|
| Type of carbon for biofloc formation      | Molasses + wheat flour, bran                 |
| Total Heterotroph bacteria (THB)          | 46±0.13 × 10⁵ until 3.36±0.15 × 10⁷ (CFU/ml) |
| Dry matter (%)                            | 19.25±0.94                                   |
| Crude protein(% DW)                       | 29.23±0.8                                    |
| Crude lipid (% DW)                        | 0.9±0.07                                     |
| Ash (% DW)                                | 37.4±0.57                                    |
| The size of biofloc (µm)                  | 107±5 until 703±27                           |

Total Heterotrophic Bacteria was estimated according to standard procedures (APHA, 2005) and expressed as colony forming units (CFU).
Table 3. Values of some water quality parameters during the experiment period (Mean ± SD)

| Parameters          | PBS1         | PBS2         | PBS3         |
|---------------------|--------------|--------------|--------------|
| T a.m. (°C)         | 30.49 ± 0.19a| 30.51 ± 0.32a| 30.54 ± 0.29a|
| T p.m. (°C)         | 31.34 ± 0.17a| 31.38 ± 0.26a| 31.39 ± 0.21a|
| DO a.m. (mg. L⁻¹)   | 6.27 ± 0.19a | 6.19 ± 0.17a | 6.10 ± 0.22a |
| DO p.m. (mg. L⁻¹)   | 6.03 ± 0.34b | 5.87 ± 0.26ab| 5.68 ± 0.30a |
| pH a.m.             | 8.12 ± 0.05b | 8.18 ± 0.02ab| 8.22 ± 0.11a |
| pH p.m.             | 8 ± 0.04b    | 8.09 ± 0.05ab| 8.12 ± 0.03a |
| Salinity (ppt)      | 8.56 ± 0.46  | 21.61 ± 0.46 | 32.88 ± 0.84 |
| NH₃⁺ (mg. L⁻¹)      | 0.46 ± 0.19a | 0.35 ± 0.24a | 0.23 ± 0.20a |
| NO₂⁻ (mg. L⁻¹)      | 1.60 ± 0.46b | 2.46 ± 1.04ab| 3.78 ± 1.98a |
| NO₃ (mg. L⁻¹)       | 2.12 ± 0.65a | 2.62 ± 1.25a | 3.7 ± 1.68a  |
| SS (ml. L⁻¹)        | 5 ± 1.7a     | 5.14 ± 1.6a  | 5.56 ± 1.91a |
| TSS (mg. L⁻¹)       | 129.6 ± 50.29| 135.7 ± 48.41| 159.8 ± 59.82a|
| Transparency (cm)   | 20.98 ± 6.13a| 20.82 ± 6.31a| 19.46 ± 6.84a|

* In each row, the averages with at least one similar letter do not differ significantly at the 5% level (P>0.05). PBS1: Salinity level 8 ppt, PBS2: Salinity level 21 ppt, PBS1: Salinity level 32 ppt
Table 4. Growth performance of Pacific white shrimp fed with bioflocs affected by different levels of salinity after four weeks of experiment period (Mean ± SD)

| Parameters                  | PBS1          | PBS2          | PBS3          |
|-----------------------------|---------------|---------------|---------------|
| Final weight (mg)           | 381.2 ± 59.57 | 468 ± 36.17   | 661.62 ± 65.5 |
| Final length (mm)           | 31.1±1.37     | 33.1±2.4      | 36.2±2.08     |
| Weight gain (mg)            | 306.76± 59.57 | 393.5±36.17   | 587.1±65.5    |
| Length gain (mm)            | 11.31 ± 1.37  | 13.31±2.4     | 16.41±2.08    |
| Body weight index (%)       | 411.96± 79.99 | 528.48±48.57  | 788.42± 86.84 |
| Growth rate (mg/day)        | 10.95± 1.49   | 14.05±1.04    | 20.96±2.07    |
| Total Biomass (g)           | 31.08± 3.76   | 42.24±3.12    | 69.67±6.89    |
| SGR (%/day)                 | 5.83± 1.02    | 6.56± 0.55    | 7.8± 0.77     |
| Feed conversion ratio       | 3.84±1.23     | 2.99±0.32     | 2± 0.18       |
| Feed efficiency (%)         | 25.99± 4.56   | 33.35±2.64    | 49.75± 5.42   |
| Survival rate (%)           | 77.95± 2.06   | 82.56±1.86    | 91.28± 2.73   |

* In each row, the averages with at least one similar letter do not differ significantly at the 5% level (P>0.05). PBS1: Salinity level 8 ppt, PBS2: Salinity level 21 ppt, PBS3: Salinity level 32 ppt
Table 5. Biochemical composition (in terms of dry weight) of the Pacific white shrimp and the biofloc obtained by the effect of different levels of salinity in the zero water exchange system after four weeks of the experiment period (Mean ± SD, N = 3)

| Treatment | Dry matter (%) | Crude protein (% DW) | Crude lipid (% DW) | Ash (% DW) |
|-----------|----------------|----------------------|--------------------|------------|
| **Shrimp** |                |                      |                    |            |
| PBS1      | 24.4± 0.5\(^a\) | 74.14± 0.14\(^a\) | 6.52± 0.08\(^a\)  | 9.75± 0.34\(^a\) |
| PBS2      | 24.65± 0.6\(^a\) | 74.89± 0.2\(^b\)   | 6.85± 0.04\(^b\)  | 12.23± 0.17\(^b\) |
| PBS3      | 25.1± 0.15\(^a\) | 75.12± 0.04\(^b\)  | 7.16± 0.12\(^c\)  | 12.44± 0.2\(^b\) |
| **Bioflocs** |               |                      |                    |            |
| PBS1      | 22.3± 0.9\(^a\) | 31.8± 0.84\(^b\)   | 1.02± 0.05\(^b\)  | 27.85± 1.4\(^b\) |
| PBS2      | 21.25± 0.7\(^a\) | 30.39± 0.7\(^ab\)  | 0.85± 0.04\(^a\)  | 36.43± 0.57\(^a\) |
| PBS3      | 21.9± 0.55\(^a\) | 28.9± 1.04\(^a\)   | 0.86± 0.07\(^a\)  | 37.94± 0.8\(^a\) |

* In each column, the averages with at least one similar letter do not differ significantly at the 5% level (P >0.05). PBS1: Salinity level 8 ppt, PBS2: Salinity level 21 ppt, PBS3: Salinity level 32 ppt
Figure 1. Wet biofloc for feeding shrimp at different treatments
Figure 2. Values of NH$_3^+$ (A), NO$_2^-$ (B), and NO$_3^-$ (C) in tanks of Pacific white shrimp cultivated at different salinity levels during the rearing period (28 days)
Figure 3. Values of settled solids, SS (A), Total suspended solids, TSS (B), and water transparency (C) in tanks of Pacific white shrimp cultivated at different salinity levels during the rearing period (28 days).