

Research Article

The effect of biofloc and clear water at low and high salinity concentration on growth performance and antioxidant response of wild juveniles of Atlantic white shrimp *Penaeus setiferus*

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ABSTRACT. The effect of biofloc (BFT) and clear water (CW) at low (ls) and high-water salinity (hs) in wild juveniles of *Penaeus setiferus* were evaluated. Four treatments were implemented: hsBFT, lsBFT, hsCW, and lsCW. After 45 days, final weight (FW), weight gain (WG), hepatosomatic index (HSI), and survival rate (SR) were evaluated. Antioxidant activity in muscle and hepatopancreas were measured: superoxide dismutase (SOD), catalase (CAT), glutathione-S transferase (GST), lipid peroxidation (LPO), protein oxidation (PO). The FW and SR was low for lsCW ($P < 0.05$), whereas the WG and HSI was high in hsBFT and lsBFT ($P < 0.05$). The integrative biomarker response index (IBR) showed differences between muscle (M) and hepatopancreas (HP). Results showed an increase of SOD and CAT measured from HP for lsBFT treatment. For M, CAT and GST were high only in BFT (regardless of the water salinity). LPO determined from M and HP recorded the high values at hs and ls, respectively. The PO determined in HP showed substantial damage in BFT (regardless of the salinity), whereas the same determination from M was only important for lsCW treatment. When the analyses of variance (ANOVA) were performed, SOD, LPO, and PO were significant in response to salinity ($P < 0.05$). In contrast, for CAT, the higher activity was due to the rearing system and salinity interaction ($P < 0.05$). Results suggest that BFT could diminish the stress by stimulating the antioxidant system to maintain the redox balance through a higher enzyme activity and decrease damage in the proteins and lipids of the cells.

Keywords: *Penaeus setiferus*; biofloc; salinity; antioxidant activity; oxidative damage; biomarker response index

INTRODUCTION

In the southern Gulf of Mexico, *Penaeus setiferus* (Linnaeus, 1767) is exposed to a wide range of environmental pressures, including overfishing, pollution of coastal bays, estuaries, and lagoons with pesticides associated with agriculture, sewage discharge from coastal cities, or the petroleum industry operating in Campeche Bay (Arreguín et al. 1997, Garcia & Vázquez-Bader 1998, Rosas et al. 2004). *P. setiferus*

also presents serious deterioration of the reproductive quality of males (Rosas et al. 2004) in captivity and the natural environment. The indiscriminate global transfer of aquatic animals has been responsible for introducing, establishing, and disseminating many pathogens to new geographic areas (Briggs et al. 2005). In addition, the development of shrimp farms is based on widely domesticated species that are usually exotic to many geographic regions, so it is necessary to develop domestication programs for native species to maintain their sustainability (Gaxiola et al. 2008).

The rearing of *P. setiferus* is an alternative not only for production purposes but also for conservation purposes for itself and other native species through sustainable biotechnologies (Valenzuela 2009). Several experiments have been carried out in the northern Gulf of Mexico, mainly focused on bait production for sport fishing (Sandifer et al. 1993, Samocha et al. 1998, Gandy et al. 2001). In these studies, the growth was slightly less than that of the commonly harvested *P. vannamei* (king prawn; Wakida-Kusunoki et al. 2011); however, *P. setiferus* is less sensitive to viral diseases and low salinities (Sandifer et al. 1993, Chapman et al. 2004). Moreover, *P. setiferus* does not represent an escape risk in the region associated with natural disasters such as floods caused by hurricanes or tropical storms in the Gulf of Mexico (Laramore et al. 2001, Valenzuela et al. 2002).

Salinity is the most important abiotic factor affecting the growth and survival of penaeid shrimp except for temperature (Kumlu et al. 2000). The idea that aquatic organisms exhibit better growth under a constant optimal salinity than under fluctuating conditions seems to be widely accepted (Yuepeng et al. 2010). Several studies mention the advantages of using inland low salinity water, including its low cost of land, less strict environmental protection legislation (Atwood et al. 2003), and the location of shrimp farms at a distance from potentially contaminated coastal waters (Moya et al. 1999). However, these rearing systems may negatively affect the physiology and survival of penaeid shrimp (Jiang et al. 2000).

The performance of *P. setiferus* in captivity may improve with a sustainable alternative that offers a substantial amount of live food, such as biofloc technology (BFT). The BFT rearing system helps to maintain the water quality by providing constant and abundant aeration in the pond to keep organic matter in suspension. Heterotrophic bacteria colonize the waste particles and absorb nitrogen, phosphorus, and other soluble nutrients, maintaining the water quality (Otoshi et al. 2001, Wasielesky et al. 2006). An additional benefit is the presence of microbial protein, filtered by juveniles, along with organic carbon and nitrogenous waste (Avnimelech 2007, Ballester et al. 2007, Azim & Little 2008, Avnimelech & Kochba 2009). The nutritional and probiotic contribution of microorganisms in BFT strengthens the immune response and antioxidant status of shrimp rearing in this condition (Ju et al. 2008, Xu & Pan 2013, Aguilera-Rivera et al. 2014, 2018, 2019a,b, da Silva Martins et al. 2015).

Traditionally, the indicators of the success of a culture refer to growth performance, i.e. growth, survival, or feed conversion ratio. However, all these responses and the physiology, immunology, and

biochemical mechanisms depend on the environmental conditions. For example, the immunological processes associated with physiological biochemistry will assist in evaluating the health status (Bachère et al. 1995, LeMoullac et al. 1998). Considering these types of variables would reveal the mechanisms that shrimp use to adapt to the culture medium and identify how such information can achieve optimal results for the species. Some cellular defense mechanisms in crustaceans depend on free radicals during phagocytosis and encapsulation in response to oxidative stress (Smith et al. 2003). Due to changes in environmental conditions, the aerobic metabolism of crustaceans generates reactive oxygen species (ROS) (Muñoz et al. 2000). A certain concentration of ROS is necessary for defense against microbial infections. ROS residues can result in severe cell damage and affect a crustacean's ability to maintain balance. One response mechanism is a lipid-soluble antioxidant system (LSAS), which includes glutathione peroxidase, superoxide dismutase (SOD), and catalase (CAT) (Ren et al. 2009, Zhou et al. 2009, Quiu et al. 2011, Song et al. 2015).

The objective of this study was to evaluate the effect of BFT and clear water (CW) at low and high salinity concentration on growth performance, in addition to the differences between the antioxidant activity and oxidative damage measured in muscle (M) and hepatopancreas (HP) tissues of wild juveniles of *P. setiferus*.

MATERIALS AND METHODS

The experiment was carried out at Unidad Multidisciplinaria de Docencia e Investigación (UMDI), Facultad de Ciencias, Universidad Nacional Autónoma de México, Campus Sisal, located in northeastern coastal region of the state of Yucatán (21°9'55.22"N, 90°1'54.93"W) in Puerto de Abrigo, s/n en Sisal, Municipio de Hunucmá (Yucatán, México).

Subjects of study, capture, and transportation to the laboratory

A total of 350 *Penaeus setiferus* juveniles (5.75 ± 1.96 g) were collected at Laguna de Términos in Campeche, México, and transported at a density of 2.5 ind L⁻¹ in 50 L transparent plastic bags with one third water and two parts O₂. They were acclimatized in a 20 m³ tank with seawater salinity at 25 for 10 days.

Shrimp were separated into two groups to continue acclimation. The first group was acclimated to marine seawater (35) and the second at low-salinity (5). For both cases, salinity was adjusted at two ups over 10 days. At the end of salinity acclimation, the shrimp were distributed in 100 L fiberglass tanks at a density

of 10 shrimp per tank (100 ind m⁻³). The initial wet weight (IW) was recorded using an Ohaus balance (0.1 g of precision).

Experimental design and rearing conditions

The experimental design consisted of four treatments with three replicates per treatment (n = 10 shrimp per treatment): biofloc in high salinity water (hsBFT), biofloc in low salinity water (lsBFT); clear seawater in high salinity (hsCW) and, clearwater in low salinity (lsCW).

Fifty liters (50%) of biofloc inoculum water was added for each tank (from the grow-out system) at a C:N ratio of 20:1 to obtain the BFT (Avnimelech 1999). The tanks were filled to their full capacity (100 L) with filtered marine seawater. During the experimental period, cane molasses was added as a source of organic carbon at a rate of 6 g of carbon (C) per gram of total ammonia nitrogen (TAN) when the ammonium reached 1 mg L⁻¹, as described in Ebeling et al. (2006), Samocha et al. (2007) and Avnimelech & Kochba (2009). In BFT tanks, the water lost by evaporation was replaced in tanks at 2%. For CW treatments at high and low salinity, water was also filtered using a sand filter. Water exchange was adjusted to a daily renewal of 35%.

On day 0, salinity for the four treatments was stabilized at 35 and 5 (for high and low-salinity water, respectively). Feed was given five times per day at 3% biomass; shrimp from each tank were weighed weekly to adjust the amount of commercial feed (35% CP, Malta Clayton; Culiacán, Sinaloa, México). The experiment lasted 45 days.

Water quality parameters

Temperature (T°), dissolved oxygen (DO), and pH were measured daily at 08:00, 16:00, 20:00, 24:00 and 04:00 h with a multiparameter HACH™ (model Sension6-Hq40; Loveland, Colorado, USA) that was calibrated for each salinity value, which was measured daily with a self-compensating refractometer (Fisher-brand™, Fisher Scientific Company; Ottawa, Canada). Every three days, suspended solids in tanks were collected and sedimented using Imhoff® cones (Aquatic Ecosystems Inc.®) (Avnimelech et al. 2008). Water samples were collected for ammonia (NH₄), nitrite (NO₂), and nitrate (NO₃) analyses. Which were performed every week with a HACH™ colorimetric test kit (model NI-SA; Loveland, Colorado, USA) using the manufacturer's method for measuring diluted and full seawater (IOC 1983, APHA 2012). In addition, the chemical composition of hsCW and lsCW treatments was determined: hardness, alkalinity, ions concentration (Na⁺, K⁺, Ca⁺², Mg⁺², Cl⁻, SO₄⁻²), according to

Geochemical and Environmental Quality Laboratory (Sisal, Yucatán) protocols.

Growth performance

At the end of the experiment, all shrimp from the four treatments were weighed and counted to estimate the growth performance (Cruz-Suárez et al. 2009):

$$WG = \left[\left(\frac{\text{average individual final weight} - \text{average individual initial weight}}{\text{average individual initial weight}} \right) \right] \times 100$$

$$HSI = \left(\frac{\text{hepatopancreas (g)}}{\text{individual weight (g)}} \right) \times 100$$

$$SR = \left(\frac{\text{final number}}{\text{initial number}} \right) \times 100$$

where WG: weight gain, HSI: hepatosomatic index, and SR: survival rate.

Sampling

At the end of the experiment, 30 shrimp from each treatment and at the intermolt stage (Corteel et al. 2012) were placed in freezing water at 5.0°C below the initial temperature to decrease their metabolic activity (Pascual et al. 2003). They were transported to the laboratory for analysis. For each shrimp, 100 µL of hemolymph was collected from the ventral sinus using a 30 G x 13 mm syringe preloaded with SIC-EDTA anticoagulant (NaCl 450 mM, KCl 10 mM, HEPES 10 mM, EDTA 10 mM; pH 7.3). Hemolymph was placed in a hematocytometer glass for THC determination (Automated cell counter, model TC20™, BIO-RAD, Philadelphia, PA, USA). A portion of M and HP tissue was stored at -80°C for further analysis.

Homogenate preparation

One hundred milligrams of M and HP samples previously-stored at -80°C were cold macerated using 1000 µL of 0.05 M Tris buffer (pH 7.4). Then, 500 µL of the obtained homogenate was separated and centrifuged at 1000 g for 5 min at 4.0°C to determine superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) enzymes. Whereas 500 µL of the non-centrifuged homogenate was used to evaluate lipid and protein oxidation (LPO and PO). All analyses were evaluated using 10 µL of homogenized preparations by triplicate, reading the absorbance in 96-well plates using a spectrophotometer (PerkinElmer®, Waltham, Massachusetts, USA).

Antioxidant activity

SOD activity from M (SOD-M) and HP (SOD-HP) samples were quantified with a commercial kit (Sigma-Aldrich, St. Louis, MO, USA). One unit was defined as

the amount of enzyme required to inhibit 50% of xanthine activity in 20 min, and the absorbance was measured at 450 nm. CAT activity (Hadwan & Abed 2016) was measured from M (CAT-M), and HP (CAT-HP) tissues using a microplate assay. Samples were incubated in two 96-well plates with 100 μ L of phosphate buffer (pH 7.4) or 100 μ L of the same buffer with H₂O₂; both microplates were incubated for 3 min, and the reaction was stopped by the addition of 100 μ L of ammonium molybdate. The absorbance was measured at 450 nm, and the amounts of both enzymes are expressed as U mg protein⁻¹. GST activity in M (GST-M) was evaluated with a microplate method using 190 μ L of Na phosphate buffer; 200 mM reduced glutathione plus 1-chloro-2,4-dinitrobenzene was added to a final concentration of 100 mM, and the absorbance was read at 412 nm after 5 min. The activity was calculated using an extinction coefficient of $E = 9.6 \text{ mM}^{-1} \text{ cm}^{-1}$; all results are expressed in nmol mg⁻¹ protein min⁻¹ (Contreras-Vergara et al. 2007).

The total protein content in the M and HP supernatants was tested using a microplate assay with a dye reagent concentrate (Bio-Rad, Philadelphia, PA, USA). The bovine serum albumin (BSA) solution (EMD Biosciences, Inc., La Jolla, CA, USA) was used as a standard according to Bradford (1976). The absorbance was measured at 595 nm.

Oxidative damage

LPO from M (LPO-M) and HP (LPO-HP) samples were quantified (Fox et al. 1994) using the PeroxiDetect[®] kit (Sigma-Aldrich, St. Louis, MO, USA) after extraction without centrifugation, homogenization with methanol 1:1 (v/v) and centrifugation at an RCF of 10,000 g for 5 min at 27°C. Then, 10 μ L of the extract was placed in two 96-well plates, and 160 μ L of the working solution was added. The samples were incubated for 60 min, and the absorbance was measured at 595 nm. A standard curve was prepared using serial dilutions with 129 μ L of 1 M tert-butyl hydroperoxide (TBOOH) plus 871 μ L of methanol (results expressed in nmol peroxide mL⁻¹).

Moreover, the PO level from M (PO-M) and HP (PO-HP) tissues were evaluated (Mesquita et al. 2014) by adding 100 μ L of dinitrophenyl hydrazine (DNPH) plus 2N HCl combined with 100 μ L of Tris buffer pH 7.4 (blank) or 100 μ L of the homogenized solution, without centrifugation, to Eppendorf[®] tubes, which were incubated for 10 min. Then, 50 μ L of 6 M NaOH was added to the samples and blank. All tubes were centrifuged at an RCF of 10,000 g for 5 min at 27°C. Finally, 150 μ L of each sample and the blank were added to a 96-well plate. The absorbance was read at 450 nm. The results are expressed in nmol mg protein⁻¹.

Integrative biomarker index

Nine biomarkers (SOD-M, SOD-HP, CAT-M, CAT-HP, GST-M, LPO-M, LPO-HP, PO-M, PO-HP) were integrated with a method that combines several biomarker responses into a general "stress index" denominated integrative biomarker response index (IBR) (Beliaeff & Burgeot 2002). The IBR was calculated according to Xie et al. (2016):

- 1) Calculation of mean and standard deviation for each biomarker of the treatments.
- 2) Standardization of data: $Y = (X - m) s^{-1}$, where Y: standardized value of the biomarker, X: the value of each biomarker response, m: mean value of the biomarker.
- 3) The score (S): $S = Y + |\text{min}|$, where $S \geq 0$ and $|\text{min}|$: absolute minimum value of Y for each biomarker.

The score of biomarkers was visualized using a stat plot in which the radial coordinate corresponded to the score. All graphs were produced using Microsoft[™] Excel[™] for Office 365 MSO.

Statistical analysis

All graphs and statistical analyses were conducted using R Statistical Software (R Core Team, 2018). Results were expressed as mean \pm standard error (SE). After the normality of data was verified by the Shapiro-Wilk test and homoscedasticity by the Bartlett test. Subsequently, bifactorial ANOVA of 2 \times 2 was used to determine the interaction between the rearing system (BFT, CW) and low (ls) and high-water salinity (hs). If the primary effect was significant, the ANOVA was followed by Tukey's test. When data were not homogeneous, a Wilcoxon Rank-Sum test was performed to determine differences between treatments. The significance level was set at 0.05.

Ethical approval

The authors followed all applicable international, national, and institutional guidelines for the care and use of animals.

RESULTS

Water quality parameters

All water quality parameters for both rearing systems at high and low salinity remained within the recommended levels for shrimp reared through the 45 days of the experiment (Table 1). Due to the low survival in hsCW and lsCW treatments, chemical composition performed in water showed a lower ion concentration of K⁺ and Mg⁺² only for lsCW (Table 2).

Growth performance

The results related to the growth performance (Table 3) of wild *Penaeus setiferus* juveniles after 45 days experiment recorded the higher FW (g) and WG (g) for

Table 1. Water quality parameters for both rearing systems at high and low salinity during 45 days of the experiment. Each value represents the mean \pm standard deviation. DO: dissolved oxygen, hsBFT: biofloc at high salinity, lsBFT: biofloc at low salinity, hsCW: clear water at high salinity, lsCW: clear water at low salinity, n/a: analysis not applicable.

Treatment	DO (mg L ⁻¹)	Temperature (°C)	pH	Salinity	NH ₄ (mg L ⁻¹)	NO ₂ (mg L ⁻¹)	NO ₃ (mg L ⁻¹)
hsBFT	7.1 \pm 0.5	26.5 \pm 0.9	7.4 \pm 0.6	34.7 \pm 1.4	0.4 \pm 0.5	1.4 \pm 1.2	82.4 \pm 25.2
lsBFT	7.3 \pm 0.3	26.4 \pm 1.0	8.6 \pm 0.5	5.9 \pm 2.0	0.7 \pm 0.26	3.9 \pm 2.2	53.5 \pm 18.5
hsCW	7.2 \pm 0.6	26.6 \pm 0.7	8.2 \pm 0.6	35 \pm 0.4	n/a	n/a	n/a
lsCW	7.25 \pm 0.3	26.8 \pm 0.7	7.8 \pm 0.4	5.82 \pm 3.9	n/a	n/a	n/a

Table 2. Chemical composition of the water used for clear water at high salinity (hsCW) and clear water at low salinity (lsCW) treatments during 45 days of the experiment.

	hsCW	lsCW
Hardness (mg CaCO ₃ L ⁻¹)	6361.9	734.5
Alkalinity (mg CaCO ₃ L ⁻¹)	114.8	333.7
Na ⁺ (mg L ⁻¹)	11490	693
K ⁺ (mg L ⁻¹)	432.4	24.7
Ca ⁺² (mg L ⁻¹)	380.4	141.8
Mg ⁺² (mg L ⁻¹)	1396	106.0
Cl ⁻ (mg L ⁻¹)	15800	1200.0
SO ₄ ⁻² (mg L ⁻¹)	1380.21	74

lsBFT, hsBFT, and hsCW treatments ($P < 0.05$). In contrast, the lowest HSI (%) and SR (%) were observed for low-salinity water used for both rearing conditions (BFT, CW) (Table 3).

Antioxidant activity

The activities of SOD, CAT, and GST enzymes measured in M and HP samples are shown (Fig. 1). When the SOD enzyme activity was evaluated with the IBR analyses, the higher activity was recorded in M and HP samples obtained from lsBFT and followed by lsCW treatment. In contrast, the lowest values were observed for hsCW and hsBFT in M and H tissue, respectively (Figs. 1a-b). SOD levels in M samples were similar between treatments ($P > 0.05$) (Table 4). The levels increased significantly when the determination was performed with HP tissues and as a result of the water salinity factor ($P < 0.05$) (Table 4, Fig. 2).

As seen in Figure 1c, the CAT activity measured from M tissue exhibited increase relatives to the BFT treatments (regardless of water salinity, whereas for the HP samples, the higher activity of the enzyme was maintained for hsCW and lsBFT (Fig. 1d). Significant CAT enzyme activity in M was identified for interaction between the rearing system and the water salinity ($P < 0.05$). The higher activity in shrimp reared in lsBFT was observed, whereas for lsCW, and both

rearing systems at hs (hsBFT, hsCW) treatments were statistically similar between them (Table 4, Fig. 3). In Figure 1e, no significant differences in GST activities in M and HP samples were observed by the effect of the rearing system or the water salinity ($P > 0.05$) (Table 4).

Oxidative damage

The oxidative damage related to LPO and PO measured in M, and HP tissues are shown (Fig. 4). As illustrated in Figure 4a, the higher LPO in M samples was related to low salinity in water (regardless of the rearing system), whereas for HP tissue, the higher LPO was recorded for high salinity water treatments (regardless of the rearing condition) (Fig. 4b). Only for HP samples, an increase of LPO was observed ($P < 0.05$) because of the water salinity condition (Table 5, Fig. 5).

Finally, the PO measured in M tissue was higher in lsCW treatment (Fig. 4c), whereas in HP samples, the PO was observed only in BFT treatments (regardless of water salinity) (Table 4, Fig. 4d). The PO exhibited an increase in M and HP samples always in response to the water salinity ($P < 0.05$) (Table 5, Figs. 6a-b).

DISCUSSION

The postlarvae and juveniles of *Penaeus setiferus* can develop much better in euryhaline environments since they have a better capacity for osmotic and ionic regulation than adults, which allows them to adapt to conditions of both high and very low salinity (Castille & Lawrence 1980, Laramore et al. 2001). In this study, water salinity was maintained at 5 and 35, a known range for this species in the coastal lagoons where they were captured (Rozas & Minello 2011). The water quality conditions, in general, were maintained at levels suitable for the culture of penaeid shrimp. The DO remained above 6.0 mL L⁻¹, the temperature ranged between 26 and 27°C. The pH values were maintained in the upper limits, but within what is established for these animals, due to the natural chemical composition of well water in the Yucatan Peninsula, which also has

Table 3. Initial weight (IW), final weight (FW), weight gain (WG), hepatosomatic index (HSI), and survival rate (SR) for wild juveniles *Penaeus setiferus* after 45 days of the experiment. Each value represents the mean \pm standard deviation. According to the ANOVA test, different superscript letters within rows indicate significant differences among treatments ($P < 0.05$). hsBFT: biofloc at high salinity, lsBFT: biofloc at low salinity, hsCW: clear water at high salinity, lsCW: clear water at low salinity. *: rearing system factor, **: salinity water factor.

Treatment	IW (g)	FW (g)	WG (g)	HSI (%)	SR (%)
hsBFT	5.75 \pm 1.12	9.28 \pm 2.48 ^{a*}	3.53 \pm 0.53 ^{a*}	4.19 \pm 1.17 ^{a**}	56.67 ^{a**}
lsBFT	5.78 \pm 1.26	8.33 \pm 1.62 ^{a*}	2.55 \pm 0.18 ^b	3.35 \pm 0.89 ^b	40 ^b
hsCW	5.23 \pm 0.96	8.08 \pm 3.1 ^{a*}	2.78 \pm 0.97 ^{a*}	4.29 \pm 0.36 ^{a**}	60 ^{a**}
lsCW	5.96 \pm 1.75	4.49 \pm 0.41 ^b	0.28 \pm 0.41 ^b	3.45 \pm 0.55 ^b	26.67 ^b

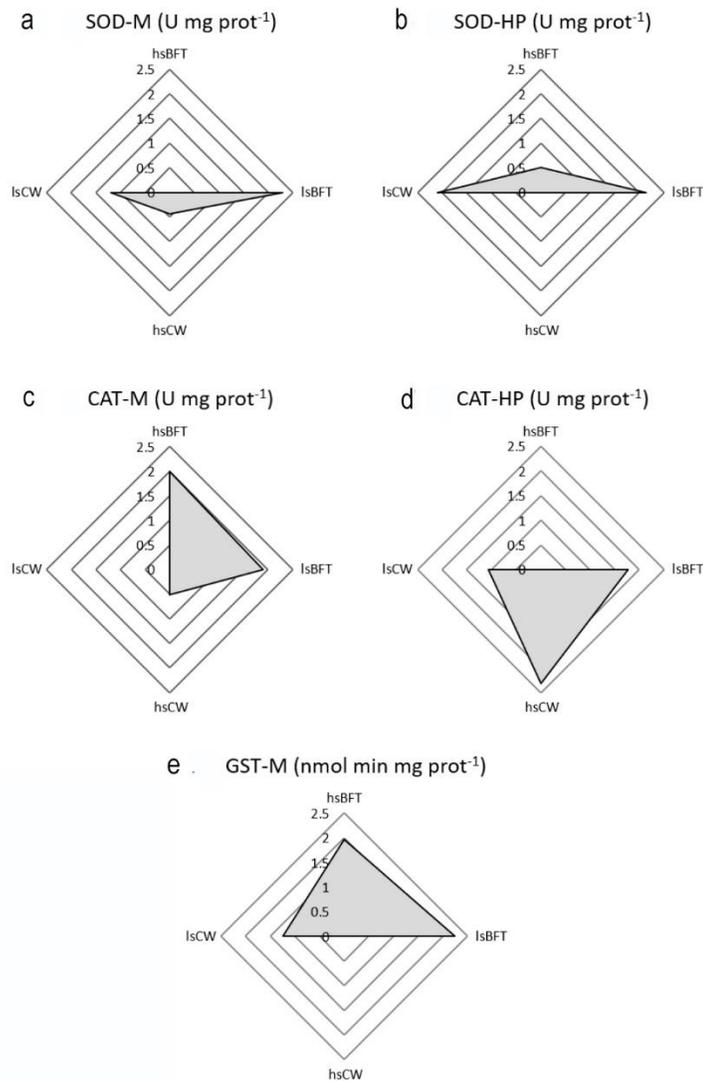


Figure 1. Integrated biomarker response (IBR) and stat plots for the following biomarkers: a) superoxide dismutase measured in muscle (SOD-M), b) superoxide dismutase measured in hepatopancreas (SOD-HP), c) catalase measured in muscle (CAT-M), d) catalase measured in hepatopancreas (CAT-HP), and e) glutathione-S transferase (GST-M) measured in wild juveniles' shrimp *Penaeus setiferus* and after 45 days of the experiment. hsBFT: biofloc at high salinity, lsBFT: biofloc at low salinity, hsCW: clear water at high salinity, lsCW: clear water at low salinity.

Table 4. Superoxide dismutase (SOD), catalase (CAT), and glutathione-S transferase (GST) measured in muscle (M) and hepatopancreas (HP) of wild juveniles' shrimp *Penaeus setiferus*, according to the rearing system (BFT, CW) and water salinity (hs, ls) and after 45 days of the experiment. Each value represents the mean \pm standard deviation. According to the ANOVA test, different superscript letters within rows indicate significant differences among treatments ($P < 0.05$). hsBFT: biofloc at high salinity, lsBFT: biofloc at low salinity, hsCW: clear water at high salinity, lsCW: clear water at low salinity. *: rearing system factor, **: water salinity factor, ***: interaction between factors.

Tissue	Antioxidant activity	Treatment			
		hsBFT	hsCW	lsBFT	lsCW
M	SOD (U mg prot ⁻¹)	2.33 \pm 0.16 ^a	2.32 \pm 0.30 ^a	2.46 \pm 0.25 ^a	2.46 \pm 0.04 ^a
	CAT (U mg prot ⁻¹)	0.85 \pm 0.14 ^b	0.86 \pm 0.16 ^b	1.01 \pm 0.27 ^{a***}	0.73 \pm 0.15 ^b
	GST (nmol min mg prot ⁻¹)	1.30 \pm 0.25 ^a	1.20 \pm 0.27 ^a	1.34 \pm 0.24 ^a	1.27 \pm 0.15 ^a
HP	SOD (U mg prot ⁻¹)	2.57 \pm 0.32 ^b	2.53 \pm 0.20 ^b	2.83 \pm 0.17 ^{a**}	2.74 \pm 0.15 ^{a**}
	CAT (U mg prot ⁻¹)	0.97 \pm 0.59 ^a	0.91 \pm 0.50 ^a	1.23 \pm 0.53 ^a	1.13 \pm 0.62 ^a

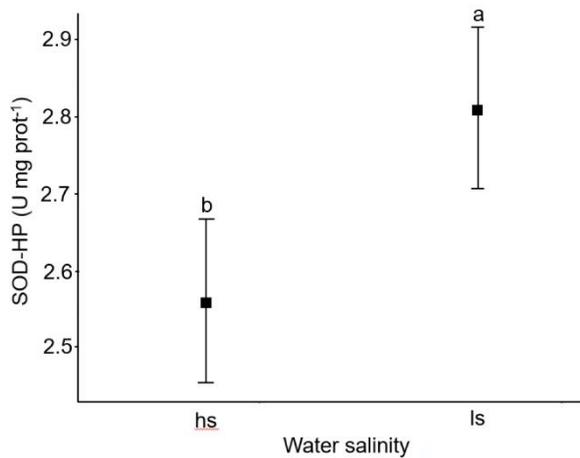


Figure 2. Principal factor effect by bifactorial ANOVA of 2 \times 2 followed by Tukey's test for superoxide dismutase (SOD) enzyme in hepatopancreas (HP) of wild juveniles' shrimp *Penaeus setiferus*, according to the water salinity (high, low) and after 45 days of the experiment. hs: high salinity (35), ls: low salinity (5).

a high level of hardness and alkalinity (Van Wyk & Scarpa 1999, Laramore et al. 2001, Rozas & Minello 2011). Ammonium, nitrite, and nitrate in BFT treatments were maintained within the normal limits for this type of rearing system (Lin & Chen 2001, Campos et al. 2012, da Silva Martins et al. 2015, Prangnell & Castro 2016, Samocha et al. 2017, De Souza et al. 2019).

Shrimp from high salinity water (hs) treatments showed higher weight and percentage of HSI during the experiment (hsBFT and hsCW). It could indicate that animals in low salinity spent a greater amount of energy to stay alive (routine metabolism); this corroborated with the low food consumption, as they have the lowest survival rate (40 and 27% for lsBFT and lsCW, respectively). The low survival and growth of treatments at low salinity water could be affected by the potassium

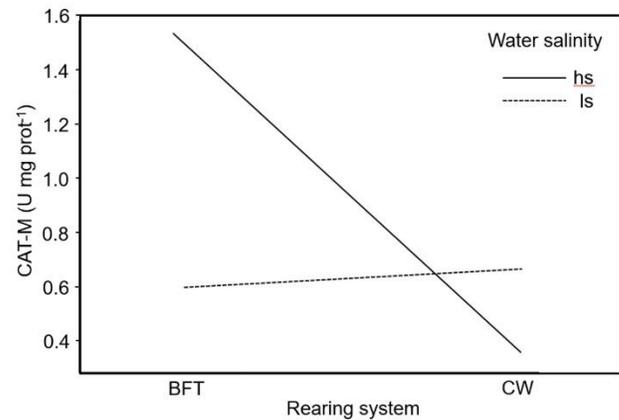


Figure 3. Interaction obtained by bifactorial ANOVA of 2 \times 2 followed by Tukey's test for catalase (CAT) enzyme activity in muscle (M) of wild juveniles' shrimp *Penaeus setiferus*, according to the rearing system (BFT, CW) and water salinity (hs, ls) and after 45 days of the experiment. hs: high salinity (35), ls: low salinity (5), BFT: biofloc, CW: clear water.

concentration in the water and magnesium's lesser degree (Davis et al. 2002, Saoud et al. 2003, McNevin et al. 2004). For this reason, the addition of both elements in low salinity water could enhance the survival and growth of postlarvae (Davis et al. 2005, Roy et al. 2007).

The HSI values were significantly higher ($P < 0.05$) for treatments with high salinity (hs), probably attributed to a better capacity to store and assimilate nutrients, which can be reflected in better nutritional and physiological conditions and, therefore, a higher increase in weight. It is most likely that in the low-salinity water treatments (ls), the nutrients were being used by the shrimp to maintain their daily metabolism and survival requirements rather than being used for growth. In addition, it is important to mention that wild shrimp *P. setiferus* for CW treatments presented diffi-

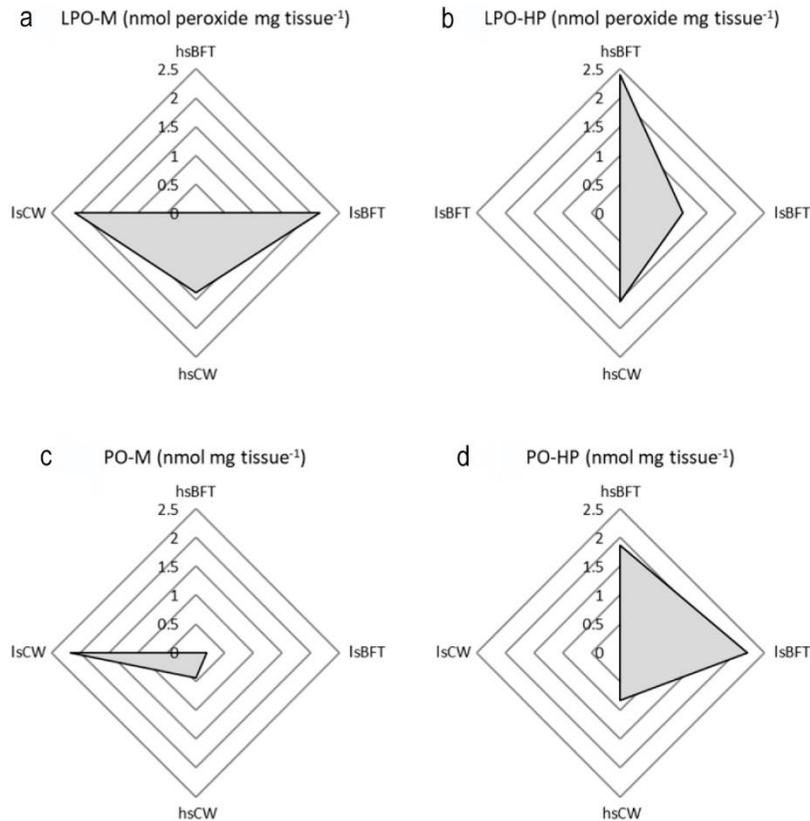


Figure 4. Integrated biomarker response (IBR) and stat plots for the following biomarkers: a) lipid peroxidation measured in muscle (LPO-M), b) lipid peroxidation measured in hepatopancreas (LPO-HP), c) protein oxidation measured in muscle (PO-M), and d) protein oxidation measured in hepatopancreas (PO-HP) of wild juveniles' shrimp *Penaeus setiferus* and after 45 days of the experiment. hsBFT: biofloc at high salinity, lsBFT: biofloc at low salinity, hsCW: clear water at high salinity, lsCW: clear water at low salinity.

Table 5. Lipid peroxidation (LPO) and protein oxidation (PO) measured in muscle (M) and hepatopancreas (HP) of wild juveniles' shrimp *Penaeus setiferus*, according to the rearing system (BFT, CW) and water salinity (hs, ls) and after 45 days of the experiment. Each value represents the mean \pm standard deviation. According to the ANOVA test, different superscript letters within rows indicate significant differences among treatments ($P < 0.05$). hsBFT: biofloc at high salinity, lsBFT: biofloc at low salinity, hsCW: clear water at high salinity, lsCW: clear water at low salinity. *: rearing system factor, **: water salinity factor.

Tissue	Oxidative damage	Treatment			
		hsBFT	hsCW	lsBFT	lsCW
M	LPO (nmol peroxide mg tissue ⁻¹)	0.63 \pm 0.31 ^a	0.76 \pm 0.35 ^a	0.84 \pm 0.41 ^a	0.83 \pm 0.48 ^a
	PO (nmol mg tissue ⁻¹)	3.89 \pm 0.32 ^{a**}	3.94 \pm 0.32 ^{a**}	3.33 \pm 0.38 ^b	1.34 \pm 0.02 ^b
HP	LPO (nmol peroxide mg tissue ⁻¹)	1.17 \pm 0.31 ^{a**}	1.00 \pm 0.21 ^{a**}	0.91 \pm 0.20 ^b	0.70 \pm 0.14 ^b
	PO (nmol mg tissue ⁻¹)	1.77 \pm 0.57 ^b	1.99 \pm 0.34 ^b	2.37 \pm 0.41 ^{a**}	2.64 \pm 0.47 ^{a**}

culties in acclimatizing to a fixed change of salinity and a constant flow of water in this rearing system.

BFT rearing systems have been demonstrated to increase the growth, survival, and reproductive capacity of shrimp. According to Emerenciano et al. (2012) and Ekasari et al. (2013), BFT contains a wide range of nutrients, particularly proteins, of microbial origin

(Tacon et al. 2002, Wasielesky et al. 2006, Kuhn et al. 2008). Which stimulates the immune and antioxidant system of shrimp (Xu & Pan 2013, Cardona et al. 2015, Zhao et al. 2016, Aguilera-Rivera et al. 2018) and even functions as a natural probiotic (Castex et al. 2010, Aguilera-Rivera et al. 2014, Soltes-Ferreira et al. 2015).

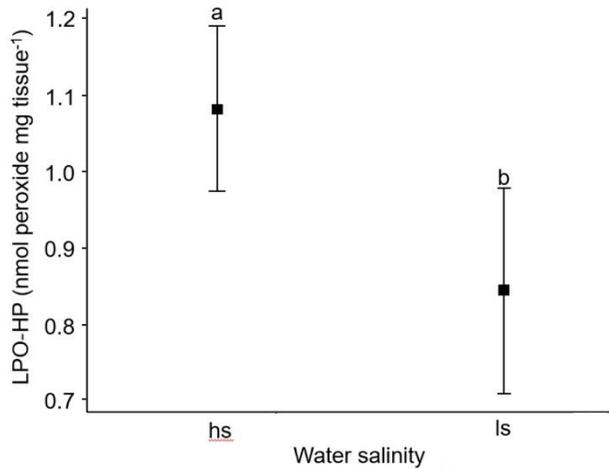


Figure 5. Principal factor effect by bifactorial ANOVA of 2×2 followed by Tukey's test for lipid peroxidation (LPO) produced in hepatopancreas (HP) of wild juveniles' shrimp *Penaeus setiferus*, according to the water salinity (high, low) and after 45 days of the experiment. hs: high salinity (35), ls: low salinity (5).

The lowest growth occurred in the lsCW treatment, along with the lowest SR (%). The difficulty of the wild juveniles to acclimatize to low salinity conditions with constant water exchange was notable.

Perhaps the constant flow of water prevented them from adapting early on to captivity conditions. According to Castille & Lawrence (1980) and Rozas & Minello (2011), animals move continuously within different salinity gradients and do not remain in only one. Due to the low survival for seawater treatments (lsBFT and lsCW), the ionic content of the water was tested, showing a very low concentration of K^+ and Mg^{+2} ions. Probably affected the metabolism of shrimp by the inactivation of the Na^+/K^+ -ATPase enzyme and the osmoregulatory capacity of cells (by the low levels of K^+), or producing an inefficient enzyme activation, cellular regeneration, and carbohydrate assimilation (by the low levels of Mg^{+2}) (Roy et al. 2007, 2010). For this reason, some studies suggest the addition of potassium and magnesium fertilizers to increase the concentration of both elements for inland shrimp farms (Boyd & Thunjai 2003).

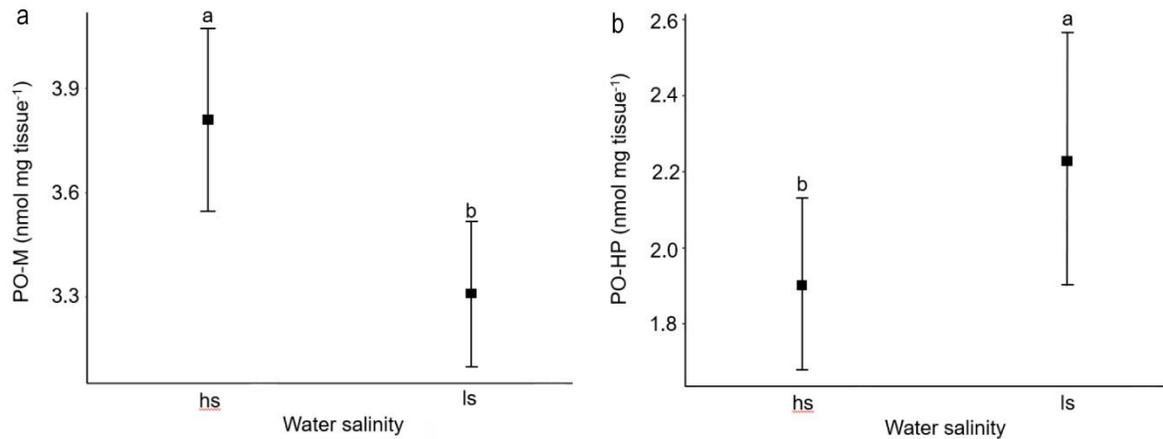


Figure 6. Principal factor effect by bifactorial ANOVA of 2×2 followed by Tukey's test for protein oxidation (PO) produced in a) muscle (M) and b) hepatopancreas (HP) of wild juveniles' shrimp *Penaeus setiferus*, according to the water salinity (high, low) and after 45 days of the experiment. hs: high salinity (35), ls: low salinity (5).

The antioxidant system has a crucial function in the balance of ROS production and control of oxidative stress. This system is regulated by enzymatic (such as SOD and CAT) and non-enzymatic (such as GST) mechanisms, which helps to prevent the damage in cells due to the excess of ROS in tissues (Ren et al. 2015). In this study, SOD, CAT, and GST were measured in muscle and hepatopancreas samples, observing differences for some of these antioxidant enzymes between tissues analyzed. In the case of SOD, the IBR index showed that muscle and hepatopancreas obtained

the higher activity of the enzyme for treatments at low salinity (lsBFT and lsCW), confirming this tendency with the ANOVA analyses only for hepatopancreas tissue and for both treatments ($P < 0.05$). Although IBR showed differences between tissues for the CAT activity, ANOVA recorded interaction between rearing system and salinity factors and maintaining a higher activity for lsBFT, followed by hsBFT, hsCW, and lsCW treatments ($P < 0.05$). SOD and CAT have an important role in protecting organisms against the oxidative stress produced by ROS. Furthermore, the

SOD enzyme helps to prevent the formation of the lipid peroxidation initiator and the transformation of superoxide radicals (O_2^-) to H_2O_2 and O_2 (Tao et al. 2013), whereas the CAT enzyme is responsible for transforming these radicals into water (H_2O) and molecular oxygen (O_2) (Cardona et al. 2015).

It has been demonstrated that some microbial communities in the BFT rearing system can influence the redox state in shrimp (Ju et al. 2008, da Silva Martins et al. 2015). In this study, the higher LPO and PO in hepatopancreas and muscle of shrimp reared at high and low salinity, respectively ($P < 0.05$), could be related to a failure of the antioxidant system to counteract free radicals (Castex et al. 2010, Ren et al. 2015). Although high salinity treatments recorded a percentage of survival less than 80% (57% for hsBFT, 60% for hsCW), the weight gain and HSI were higher in comparison to the treatments reared at low salinity (lsBFT, lsCW), which reflected the higher LPO and PO values when the IBR index was performed. These results suggest that the oxidative damage could be different between tissues analyzed due to changes in the antioxidant composition in muscle and hepatopancreas (Da Silva Martins et al. 2015). It is possible to assume that the tissue factor affected LPO and PO values recorded because the water salinity also played an important role in the results

CONCLUSION

The results obtained in this study suggest that shrimp in BFT showed higher growth and adaptability to the rearing system conditions than those reared at CW (regardless of the water salinity). In addition, the low survival and growth for treatments at low salinity resulted from a low potassium and magnesium concentration in water. For this reason, this study supports the criteria to add these elements as a fertilizer to increase the freshwater quality used for inland shrimp farms. The nutritional and microbiological characteristics of BFT could have reduced stress, stimulating the antioxidant system through a greater activity of the enzymes in penaeid shrimp. Furthermore, the methodology used in this study suggests the use of muscle and hepatopancreas tissues to determine the antioxidant composition and possible oxidative damage in penaeid shrimp. It was possible to observe a significant increase of oxidative damage only in shrimp reared in clear water than those reared in BFT; using this methodology.

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