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THE CHRONIC TOXICITY OF AMMONIA, NITRITE AND NITRATE ON JUVENILE *Farfantepenaeus brasiliensis* (CRUSTACEA: DECAPODA)*

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ABSTRACT

In general, the adverse effect of a chemical compound present in water varies with the concentration and time of exposure to the compound, the nature of the chemical species and age of the exposed organisms. Thus, nitrogen does not necessarily cause adverse effects on shrimp, but may, instead, promote sub-lethal effects by long-term exposure. Juvenile *Farfantepenaeus brasiliensis* (initial mean weight = 0.61 g ± 0.07) were exposed to sub-lethal concentrations of ammonia (0.44 and 0.88 mg L^{-1}), nitrite (5.30 and 10.60 mg L^{-1}) and nitrate (45.60 and 91.20 mg L^{-1}) corresponding to the safe levels for the species. After 40 days of exposure of juveniles to ammonia, nitrite and nitrate, all groups differed significantly (p<0.05) from the control group regarding the growth and survival. Based on the results, it was determined that the shrimp *F. brasiliensis* was susceptible to nitrogen compounds in concentrations equivalent to supposedly safe levels previously proposed for the specie. Thus, the security levels of ammonia, nitrite and nitrate for pink shrimp juveniles were 0.88 mg L^{-1}, 10.60 mg L^{-1} and 91.20 mg L^{-1}, respectively.

Keywords: nitrogen compounds; performance; pink shrimp

TOXICIDADE CRÔNICA DA AMÔNIA, NITRITO E NITRATO EM JUVENIS DE *Farfantepenaeus brasiliensis* (CRUSTACEA: DECAPODA)

RESUMO

Em geral, o efeito adverso de um composto químico presente na água varia com a concentração, o tempo de exposição ao composto, à natureza do produto químico e a idade das espécies de organismos expostos. Assim, o nitrogênio não necessariamente causa efeitos adversos, mas pode, em vez disso, promover efeitos subletais por meio da exposição em longo prazo. Juvenis de *Farfantepenaeus brasiliensis* (peso médio inicial = 0,61 g ± 0,07) foram expostos a concentrações subletais de amônia (0,44 e 0,88 mg L^{-1}), nitrito (5,30 e 10,60 mg L^{-1}) e nitrato (45,60 e 91,20 mg L^{-1}) correspondente aos "níveis de segurança" para a espécie. Após 40 dias de exposição dos juvenis à amônia, nitrito e nitrato, todos os grupos diferiram significativamente (p<0,05) do grupo controle em relação ao crescimento e sobrevivência. Com base nos resultados, o camarão *F. brasiliensis* foi susceptível aos compostos nitrogenados em concentrações equivalentes aos níveis supostamente seguros anteriormente propostos para a espécie. Assim, os níveis de segurança de amônia, nitrito e nitrato propostos para juvenis de camarão-rosa são 0,88 mg L^{-1}, 10,60 mg L^{-1} e 91,20 mg L^{-1}, respectivamente.

Palavras chave: compostos nitrogenados; desempenho zootécnico; camarão-rosa

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INTRODUCTION

Knowledge of water quality parameters and their maintenance within tolerance limits for a species are essential requirements in any aquaculture system (KINNE, 1976). These factors decisively determine the success or failure of aquaculture (OSTRENSKY and WASIELESKY, 1995).

The nitrogenous waste from the excretion of farmed organisms and feed degradation frequently deteriorate the environment where these organisms are raised (TOMASSO, 1994). The nitrogen compounds can damage the gill tissues and affect oxygen consumption by the farmed organisms and/or cause their death (LIN and CHEN, 2003; KUHN *et al.*, 2010; BARBIERI, 2010; CAMPOS *et al.*, 2012; BARBIERI *et al.*, 2014).

The concentration and time required for a compound to produce an adverse effect vary according to the chemical agent and the type and severity of the effect. Adverse or toxic effects can be produced in the laboratory or in the natural environment through lethal (high concentrations for a short period of time) or chronic (sub-lethal concentrations over a long period of time) exposure to the chemical pollutant (RAND and PETROCELLI, 1985). The sensitivity of the organisms to a toxic substance also may vary according to their stage of development and their state of health (WAJSBROT *et al.*, 1993; COBO *et al.*, 2014).

In spite of the great majority of commercial farming that is performed with the exotic species *Litopenaeus vannamei*, some native species of marine shrimp, such as *Farfantepenaeus brasiliensis*, have already demonstrated the potential for culture (LOPES *et al.*, 2009). The application of low-cost structures for culturing native shrimp (i.e., cages or pens in natural water bodies) allows the inclusion of low-income communities in this activity (WASIELESKY *et al.*, 2004). JENSEN (2012) recently concluded that *F. brasiliensis* is potentially suited for farming in bioflocs systems with a stocking density of up to 100 shrimp m^{-2} during the nursery stage and up to 75 shrimp m^{-2} during the grow-out stage.

The aim of this study was to evaluate the effects of sub-lethal concentrations of ammonia, nitrite and nitrate on the survival and growth of juvenile *F. brasiliensis* reared under laboratory conditions.

MATERIAL AND METHODS

Juveniles of *F. brasiliensis* were reared at the Marine Aquaculture Station hatchery, Federal University of Rio Grande (FURG), Rio Grande do Sul State, Brazil. The animals were kept in a 1,000 L tank with a controlled temperature and photoperiod (25 ºC and 12L:12D, respectively), under constant aeration and with a salinity adjusted to 28.

The experiments were conducted in 200 L tanks, where thirty juveniles of *F. brasiliensis* (initial mean weight = 0.61 g ± 0.07), at a density of 120 shrimps m^{-3}) were used per experimental unit in all treatments. The experiment consisted of a control group (without adding nitrogen) and six treatments, each with three replicates (21 tanks): two concentrations of ammonia (TAN), two of nitrite (NO_2^-) and two of nitrate (NO_3^-), which are presented in Table 1. These concentrations corresponded to the “safe levels" (SL) for this species (CAMPOS *et al.*, 2012) and half concentrations of those values (50%).

Table 1. Concentrations of ammonia, nitrite and nitrate used in the experiments.

Nitrogen compounds	Treatment	Concentration (mg L^{-1})
Without adding nitrogen	Control	0.0
		0.0
Ammonia	TAN-50%	0.44
	TAN-SL	0.88
Nitrite	NO_2^--50%	5.30
	NO_2^--SL	10.59
Nitrate	NO_3^--50%	45.60
	NO_3^--SL	91.20

The concentrations were obtained from stock solutions prepared with ammonium chloride p.a. (Synth®), sodium nitrite p.a. (Synth®) and sodium nitrate p.a (Synth®).

The experiment lasted 40 days, and the water for each treatment was completely renewed every 48 h to maintain the desired concentrations. The excreta were siphoned daily and aeration was provided constantly. The water temperature was maintained at 25 °C using heaters with a thermostat (Visi-therm) and the salinity was kept at 28.

The water samples were collected daily from experimental units to measure salinity and pH with an optical refractometer (Atago, model 103) and a digital pH meter (DMpH-1, Digimed, precision 0.01), respectively. The concentrations of total ammonia (TAN) ($NH_3 + NH_4^+$), nitrite (NO_2^-) and nitrate (NO_3^-) were analyzed daily, according to the methodologies proposed by UNESCO (1983), BENDSCHNEIDER and ROBINSON (1952) and AMINOT and CHAUSSEPIED (1983), respectively. The oxygen concentrations and temperature were monitored daily with an oximeter (model OXI-315i, WTW).

The shrimp were fed twice a day (08:00 h and 16:00 h) with a commercial ration (Ø of pellets = 1.6 mm; 40% crude protein and 8% lipid) offered in trays at a proportion of 10% of the total biomass of each experimental unit. Every 10 days, 10 juveniles of each experimental unit were weighed with an electronic digital scale (± 0.01 g; SD-Marte®) to analyze the growth and feed adjustment. The mortality was verified every 24 h. Animals were considered dead when they were still and did not respond to mechanical stimuli with a glass cane (LIN and CHEN, 2003) and were immediately removed from the tank. The following performance parameters were determined: survival final (S; %) = (final n / initial n) × 100 (where n = number of shrimp); final mean weight (g) = final biomass of live shrimp / total n; specific growth rate (SGR; % day^{-1}) = 100 × [(ln final weight - ln initial weight) / days of experiment].

The survival, wet weight and specific growth rate data were evaluated with an analysis of variance (one-way ANOVA) and validated in terms of the assumptions of the method (Levene Test and Kolmogorov-Smirnov). If significant differences were observed (p<0.05), a Tukey test was applied to determine the moment when a significant toxic effect appeared. The percentage values were arcsine transformed (ZAR, 1996).

RESULTS

The mean values of pH, dissolved oxygen, temperature, salinity and nitrogen compounds registered for each treatment during the experimental period are presented in Table 2.

Table 2. Mean concentration (mg L^{-1}) values of ammonia, nitrite, nitrate, temperature (T; °C), dissolved oxygen (D.O.; mg L^{-1}), salinity and pH in the different treatments during the 40-day experiment. The data correspond to the mean ± standard deviation.

Treatment	Concentration (mg L^{-1})	pH	D.O. (mg L^{-1})	T°C	Salinity
Control	-	8.31 ± 0.13	6.32 ± 0.24	24.96 ± 0.21	28.03 ± 0.50
TAN-50%	0.46 ± 0.05	8.27 ± 0.11	6.30 ± 0.21	24.90 ± 0.23	28.05 ± 0.30
TAN-SL	0.88 ± 0.08	8.32 ± 0.15	6.32 ± 0.21	24.87 ± 0.26	28.00 ± 0.50
NO_2^--50%	5.32 ± 0.18	8.27 ± 0.13	6.36 ± 0.30	24.86 ± 0.27	28.05 ± 0.50
NO_2^--SL	10.59 ± 0.25	8.31 ± 0.16	6.23 ± 0.27	24.88 ± 0.22	28.10 ± 0.40
NO_3^--50%	45.61 ± 0.58	8.30 ± 0.15	6.40 ± 0.22	24.96 ± 0.24	28.15 ± 0.40
NO_3^--SL	91.29 ± 0.36	8.27 ± 0.16	6.36 ± 0.20	24.95 ± 0.23	28.05 ± 0.53

The specific growth rate was significantly higher (p<0.05) in the control when compared with the other treatments. Among the nitrogen treatments, there were no significant differences (p>0.05). The specific growth and survival data are shown in Table 3. The greatest final mean weight and percentage of survival was obtained in the control group and the lowest survival rate was observed with the nitrate treatment (NO_3^--SL).

Table 3. Data (mean ± standard deviation) for final mean weight (g), specific growth rate (SGR; % day^{-1}) and survival (%) of *Farfantepenaeus brasiliensis* juveniles after 40 days of exposure to ammonia (TAN-50% and TAN-SL), nitrite (NO_2^--50% and NO_2^--SL) and nitrate (NO_3^--50% and NO_3^--SL).

Treatment	Final Weight (g)	SGR (%/day)	Survival (%)
Control	1.11 ± 0.27^{a}	1.53 ± 0.15^{a}	77.78 ± 3.85^{a}
TAN-50%	0.94 ± 0.14^{ab}	1.02 ± 0.20^{b}	64.44 ± 3.85^{bc}
TAN-SL	0.90 ± 0.03^{b}	1.01 ± 0.09^{b}	60.00 ± 6.67^{bc}
NO_2^--50%	0.88 ± 0.08^{b}	0.94 ± 0.07^{b}	67.78 ± 1.92^{b}
NO_2^--SL	0.90 ± 0.11^{b}	0.89 ± 0.15^{b}	58.89 ± 1.92^{bc}
NO_3^--50%	0.94 ± 0.05^{b}	0.93 ± 0.03^{b}	67.78 ± 1.92^{b}
NO_3^--SL	0.92 ± 0.04^{b}	1.00 ± 0.04^{b}	56.67 ± 3.33^{c}

Different superscript letters in the same column indicate significantly different means among the treatments (p<0.05).

The highest growth rate of juvenile *F. brasiliensis* occurred in the control group when compared to the other treatments. Significant differences were found ($p<0.05$) after 30 days in treatments TAN-SL, NO_2-50% and NO_2^--SL when compared with the control treatment. On day 40, significant differences ($p<0.05$) were observed between the control group and the nitrogen treatments. The increase in the wet weight with the different concentrations of ammonia, nitrite and nitrate are shown in Figure 1.

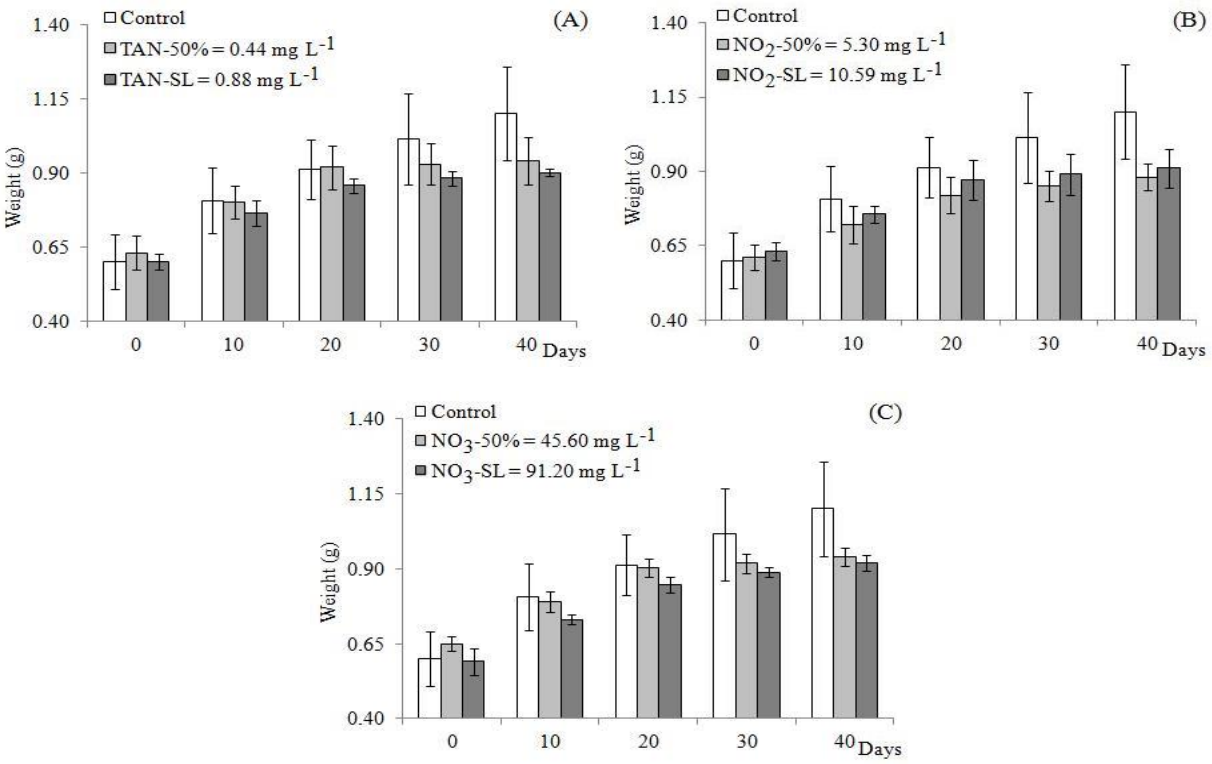

Figure 1. Growth of juvenile *Farfantepenaeus brasiliensis* at different concentrations of ammonia (A), nitrite (B) and nitrate (C) over 40 days. Bars = standard error.

The survival rates of *F. brasiliensis* juveniles exposed to ammonia, nitrite and nitrate during the experiment are expressed in Figure 2. No significant differences were observed ($p>0.05$)

among treatments TAN-50%, TAN-SL and the control from day 0 to 30. However, at the end of the experiment, treatments TAN-50% and TAN-SL differed significantly (p<0.05) from the control group (Figure 2a).

Among treatments NO_2^--50%, NO_2^--SL and the control, there were no significant differences (p>0.05) on days 0, 10 and 30. On day 20, there were significant differences (p<0.05) between the control and groups NO_2^--50% and NO_2^--SL. After 40 days, all of the groups differed significantly (Figure 2b).

On days 0 and 10, no significant differences were observed (p>0.05) between treatments NO_3^--50%, NO_3^--SL and the control. Significant differences (p<0.05) between the control and groups NO_3^--50% and NO_3^--SL appeared on day 20; after 40 days, there were significant differences among all groups (Figure 2c).

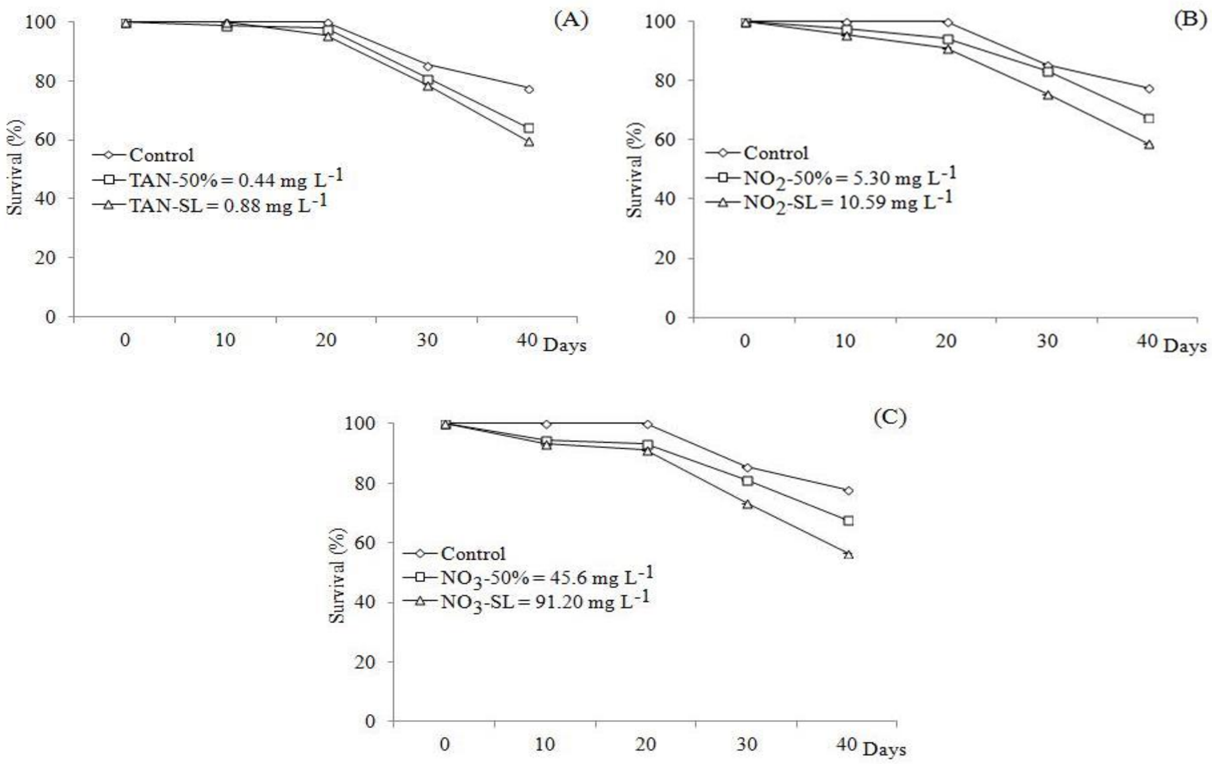

Figure 2. Mean survival values of *Farfantepenaeus brasiliensis* juveniles exposed to different concentrations of ammonia (A), nitrite (B) and nitrate (C) over 40 days.

DISCUSSION

The water temperature during the experiment remained within the adequate range of 24 to 32 °C for the growth of penaeid shrimp (VAN WYK and SCARPA, 1999). The true safe levels for different species of farmed aquatic organisms may differ markedly from those obtained in short-term tests (TOMASSO, 1994). The safe levels proposed by this method, used by CAMPOS *et al.* (2012), can be subject to errors in the estimation of the concentrations that are effectively chronic to *F. brasiliensis*, as evidenced by the results of this study.

High concentrations of nitrogen compounds (ammonia, nitrite and nitrate) affect physiological processes that are important to aquaculture activities (MONTOYA *et al.,* 1999). Among these processes, affected osmoregulation and respiration result in low food consumption, low specific growth rates and even mortality of the shrimp (WASIELESKY, 2000; KUHN *et al.,* 2010, BARBIERI 2010; CAMPOS *et al.,* 2012; BARBIERI *et al.*, 2014). During the period of molting, shrimp can be more sensitive to the different effects of the nitrogenous compounds, such as ammonia, nitrite and nitrate, which are also more toxic during this period (WASIELESKY, 2000).

Ammonia is the end product of protein catabolism for most aquatic organisms (KINNE, 1976) and is present in the aquatic environment in the ionized (NH_4^+) and non-ionized form (NH_3), which can spread through cell membranes. The process of ammonia ionization is regulated by the pH level such that the percentage of NH_3 increases and the percentage of NH_4^+ decreases in relation to increasing pH levels (FROMM and GILLETTE, 1968). In this context, after a 30-day exposure to ammonia, the survival of the shrimp in the treatments TAN-50% and TAN-SL did not show significant differences in comparison with the control group. However, during this period, the growth in wet weight demonstrated that a concentration as low as the safe level, as determined by CAMPOS *et al.* (2012), caused growth retardation.

High levels of ammonia can be harmful to crustaceans and cause various adverse effects such as reduced growth and survival (LIN and CHEN, 2001; BARBIERI, 2010; COBO *et al.*, 2014). WASIELESKY *et al.* (1994) confirmed that the growth rates of *Farfantepenaeus paulensis* (post-larvae) were significantly reduced in ammonia concentrations of 0.07 to 0.14 mg L^{-1}. MIRANDA-FILHO *et al.* (2009) studied juveniles of the same species in pre-nursery and nursery stages for 75 days and observed reduced predation activity and growth.

Nitrite is the intermediate compound in the bacterial nitrification of ammonia into nitrate (in oxidizing environments) or the production of nitrate denitrification (in reducing environments) and is toxic to aquatic organisms, causing mortality in culture systems (BROWNELL, 1980; TSAI and CHEN, 2002). The ionization process of nitrite is also mediated by pH levels, so that the percentage of nitrous acid (HNO_2) increases with decreasing pH levels. Introduction of high concentrations of nitrite into the aquatic environment may lead to hemolymphatic problems because its toxic action targets the process of oxygen transport, converting hemocyanin into metahemocyanin, which is unable to carry oxygen to the tissues (GROSS, 2004). This process decreases the amount of oxygen available for metabolism (TAHON *et al.*, 1988) and leads to hypoxia and significant mortality (CHEN *et al.*, 1986; BARBIERI *et al.*, 2014).

The results of the present study demonstrated that juveniles of *F. brasiliensis* only showed significant reduction in weight gain after 30 days of exposure. However, after 20 days of exposure to nitrite, the survival of the shrimp in treatments NO_2^--50% and NO_2^--SL showed significant differences in relation to the control group. GROSS (2004) reported growth retardation and mortality in *L. vannamei* in farms located in Israel, which could be attributed to the high concentrations of nitrite (8 mg L^{-1}). CHEN and CHEN (1992) exposed juvenile *Penaeus monodon* to nitrite concentrations that ranged between 2 and 20 mg L^{-1} for 60 days. The shrimp exposed to 4, 8 and 20 mg L^{-1} of nitrite showed significantly lower weight gain. WASIELESKY (2000) studied juvenile *F. paulensis* and verified a significant reduction in weight gain at a concentration of 20.4 mg NO_2^- L^{-1} (equivalent to twice the safe level) after the 30-day trial. In addition, this species showed a mortality rate of 61% at a nitrite concentration of 10.2 mg L^{-1}. However, in spite of the high mortality rate, the growth of the shrimp that survived was not significantly affected by nitrite. According to the author, this fact is likely related to the greater requirement for oxygen during the process of molting. This suggests that shrimp that manage to pass through the molting stage (ecdysis) have relatively normal weight gain for a period of time even under these elevated nitrite concentrations.

In systems of culture LIN and CHEN (2003) verified that nitrite concentrations equal to or higher than 25.7 mg L^{-1} and a salinity of 35 can reduce the growth of *L. vannamei*. FURTADO *et al.* (2011) did not observe significant effects of nitrite on *L. vannamei* in the treatments with 3.1 and 4.3 mg L^{-1}. While MAICÁ *et al.* (2011) worked with different salinities in the bioflocs system and verified that the highest nitrite levels occurred at salinities of 2 and 25. They also observed that there was mortality of the shrimp when the nitrite concentrations exceeded the safe levels for *L. vannamei* juveniles ($\leq$ 1 mg L^{-1}) (VAN WYK and SCARPA, 1999) between 20 and 30 days. The highest mortality rates and the highest nitrite concentrations that occurred at a salinity of 2 suggest that the nitrite level is one of the main factors that cause mortality. BARBIERI *et al.* (2014) showed the inverse relationship between salinity and nitrite toxicity for juveniles of *Litopenaeus schmitti* and emphasized that the toxicity increases when animals are exposed to a hyposmotic conditions.

Nitrate is the least toxic nitrogen compound for aquatic organisms including Penaeidae (VAN WYK and SCARPA, 1999), although its study is important because it can produce lethal or sublethal effects on different organisms or act synergistically with other nitrogenous substances (KUHN *et al.,* 2009). KUHN *et al.* (2010) showed that *L. vannamei* can be cultivated under a salinity of 11 with 220 ppm of nitrate for a period of six weeks, while nitrate levels of 435 ppm were not safe for these shrimp. The growth rates of the shrimp *F. paulensis* exposed to 80.7 mg L^{-1} of nitrate were significantly lower in relation to the control treatment (WASIELESKY, 2000). In the present study, the growth and survival of *F. brasiliensis* juveniles exposed for 40 days to concentrations of 45.6 and 91.2 mg L^{-1} of nitrate were negatively affected in comparison with the control group. In this context, this study confirms the results of the authors mentioned above and emphasizes the greater sensitivity of *F. brasiliensis* to high levels (90 mg L^{-1}) of nitrate compared with that of the white shrimp *L. vannamei*.

According to FRIAS-ESPERICUETA *et al.* (1999), the interaction between nitrogenous compounds and shrimp production is an important consideration for farmers. There is no relevant accumulation of nitrogenous compounds in semi-intensive farming systems with low stocking densities and high rates of water renewal. Conversely, super-intensive systems with biofilter or bioflocs technology and minimal water renewal rates feature a natural process of nitrate accumulation in the system (> 100 mg L^{-1}) (POERSCH *et al.,* 2007, FURTADO *et al.,* 2011). SOUZA *et al.* (2014) verified ammonia and nitrite levels of 6.0 and 10 mg L^{-1}, respectively, at the stage of biofloc formation in the culture of *F. brasiliensis*. EMERENCIANO *et al.* (2012) found that juvenile *F. brasiliensis* reared in a bio-flocs technology (BFT) system showed higher final weight and weight gain than juveniles cultivated in clear water and highlighted the potential of this species in super-intensive systems with minimal water renewal.

CONCLUSIONS

The results of this study emphasize the sensitivity of this species to nitrate as the worst survival results were shown in the treatment at the supposed safe level. Thus, special care should be taken to control the concentrations of nitrogen products in *F. brasiliensis* farming because they can significantly affect the final output of this species in aquaculture systems.

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