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Chronic effects of nitrogenous compounds on survival and growth of juvenile pink shrimp

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Abstract

In response to growing worldwide market demand, intensive shrimp farming, based on high feed, has developed over the past decade. The nitrogenous compounds mainly generated by animal excretion can cause deterioration of water quality and produce chronic or even acute toxicity to aquatic animals. As prevention, theoretical safety levels have been estimated from acute toxicity tests and they are traditionally used to prevent toxic effects on biota. However, are those concentrations of nitrogenous compounds really safe to *Farfantepenaeus paulensis*? The current study aimed to investigate the lethal and sublethal effects of ammonia, nitrite and nitrate to juvenile *F. paulensis* based on safety levels. Each experiment was performed independently in 100 L tanks for 30 days. The survival rates and wet weight of all shrimps were recorded every 10 days. The concentrations tested for ammonia, nitrite and nitrate were respectively: treatment "$T_{1/4}$", a quarter of the safety level (0.91 mg/L TA-N, 2.55 mg/L NO_2^--N and 80.7 mg/L NO_3^--N); treatment "T_{SL}", the safety level (3.65 mg/L TA-N, 10.2 mg/L NO_2^--N and 323 mg/L NO_3^--N); and treatment "T_{2X}", twice the safety level (7.30 mg/L TA-N, 20.4 mg/L NO_2^--N and 646 mg/L NO_3^--N). For *F. paulensis* cultivation, the real safety level for nitrite was estimated to be 2.55 mg/L NO_2^--N. For ammonia and nitrate, the recommended concentrations were <0.91 mg/L TA-N corresponding to 0.045 mg/L NH_3-N and <80.7 mg/L NO_3^--N, respectively.

Keywords: ammonia, aquaculture, nitrate, nitrite, water quality.

Efeito crônico de compostos nitrogenados sobre a sobrevivência e crescimento de juvenis de camarão rosa

Resumo

Em resposta à crescente demanda do mercado mundial, a carcinicultura intensiva tem se desenvolvido ao longo da última década. Os compostos nitrogenados gerados principalmente pela excreção dos animais podem causar a deterioração da qualidade da água e produzir toxicidade crônica ou mesmo aguda para os animais cultivados. Como prevenção, os níveis de segurança teóricos são estimados a partir de testes de toxicidade aguda e são tradicionalmente usados para evitar efeitos tóxicos sobre a biota. No entanto, as estimativas das concentrações dos compostos nitrogenados são realmente seguras para *Farfantepenaeus paulensis*? O presente estudo teve como objetivo investigar os efeitos letais e subletais da amônia, nitrito e nitrato em juvenis de camarão marinho *F. paulensis* com base em níveis de segurança. Cada experimento foi realizado de forma independente em tanques com capacidade de 100 L durante 30 dias. As taxas de sobrevivência e peso úmido de todos os camarões foram registrados a cada 10 dias. As concentrações testadas para amônia, nitrito e nitrato foram respectivamente: "$T_{1/4}$", um quarto do nível de segurança (0,91 mg/L N-AT, 2,55 mg/L de N-NO_2^- e 80,7 mg/L N-NO_3^-); "T_{SL}", nível de segurança (3,65 mg/L N-AT, 10,2 mg/L de N-NO_2^- e 323 mg/L N-NO_3^-); e "T_{2X}", duas vezes o nível de segurança (7,30 mg/L N-AT, 20,4 mg/L de N-NO_2^- e 646 mg/L de N-NO_3^-). Para a criação de *F. paulensis*, o nível de segurança real para nitrito foi estimado em 2,55 mg/L N-NO_2^-. Para amônia e nitrato, concentrações recomendadas foram: <0,91 mg/L N-AT correspondente a 0,045 mg/L N-NH_3 e <80,7 mg/L N-NO_3^-, respectivamente.

Palavras-chave: amônia, aquicultura, nitrato, nitrito, qualidade da água.

1. Introduction

The pink shrimp *Farfantepenaeus paulensis* (Latreille, 1817) is a euryhaline crustacean and commercially important in Brazil. Many studies have been performed in order to introduce it in aquaculture (Abreu et al., 2007; Ballester et al., 2007, 2010; Emerenciano et al., 2007; Henriques et al., 2014; Peixoto et al., 2003, 2004; Tsuzuki and Cavalli, 2000; Wasielesky et al., 2003).

Nowadays, the development of aquaculture techniques resulted in intensification of culture conditions. The feed used in aquaculture systems contain high levels of crude protein. Therefore, the release of nitrogen to the environment has increased. The main sources of these substances are: excretion of cultured organisms and organic matter mineralization (Baldisserotto, 2009; Lazzari and Baldisserotto, 2008; Tomasso, 1994).

Ammonia is the final product of protein catabolism in most aquatic organisms. It occurs naturally in aquatic systems and in aqueous solution in two different chemical forms. The medium becomes more or less toxic based on the concentration of unionized or gaseous ammonia (NH_3) (Wright and Wood, 2012).

Nitrite is the intermediate compound in the bacterial nitrification of ammonia to nitrate in oxidizing environments and the product of the denitrification of nitrate in reducing environments (Thurston et al., 1978). The accumulation of nitrite may degrade water quality, increasing both the consumption of oxygen and excretion of ammonia, reducing animal growth and even mortality (Lin and Chen, 2003). The nitrite binds to hemocyanin, occupying the active site in place of oxygen and causing a transformation to meta-hemocyanin, which is unable to transfer oxygen to the tissues. For this reason, nitrite decreases the amount of oxygen available for tissue oxygenation (Tahon et al., 1988). As a result, hypoxia and hypoxia-related mortality may occur (Chen and Chin, 1988; Chen et al., 1986). Nitrite can also inhibit carbonic anhydrase, a metallo-enzyme that influences branchial ion transport in freshwater fish and crustaceans (Innocenti et al., 2004).

In turn, researchers and farmers view nitrate as a weakly toxic substance. As the product of nitrification, this compound can accumulate in large quantities, especially in closed aquaculture systems (Spieck and Bock, 2005; Thurston et al., 1978) such as Recirculating Aquaculture System (RAS) (Kuhn et al., 2010) and Biofloc Technology (BFT) (Luo et al., 2013). Thus, this substance can cause lethal (Tsai and Chen, 2002) or sublethal (Kuhn et al., 2010) effects to aquatic organisms or act synergistically with other nitrogenous compounds.

The deleterious effects of nitrogenous compounds have been analyzed in penaeid shrimp (Barbieri, 2010; Kir and Kumlu, 2006; Lin and Chen, 2001; Romano and Zeng, 2013). Independent studies were carried out with *F. paulensis* as follows: Ostrensky et al. (1992) examined the toxicity of ammonia in the production of post-larvae; Ostrensky and Wasielesky (1995) have estimated the LC_{50} (24-96 h) of ammonia in different stages of *F. paulensis*; Cavalli et al. (1996) determined the LC_{50} (96 h) of nitrogenous products for pink shrimp adults; and Peixoto (1996) determined the effects of ammonia on the number of spawning events, the fecundity and the hatching rate. In addition, Miranda-Filho et al. (2009) examined the toxic effects of ammonia on the growth of the early stages of *F. paulensis* (nursery phase); Ostrensky (1997) determined the lethal effects of mixtures of ammonia and nitrite; and Castaño (1997) and Sachisida (1997) analyzed the effect of salinity on the acute toxicity of nitrite and nitrate, respectively. From the acute toxicity tests performed with nitrogenous compounds, it is possible to estimate the species-specific safety levels in order to predict toxicity thresholds, as described by Sprague (1971).

Despite these studies, according to Romano and Zeng (2013), theoretical safety levels can underestimate the impact of nitrogenous waste on the growth and physiological condition of crustaceans. So, are the safety concentrations of nitrogenous compounds estimated to *F. paulensis* really safe?

For these reasons, the present research was carried out in order to study the estimated safety levels for nitrogenous compounds (ammonia, nitrite and nitrate) on the survival and growth of juvenile *F. paulensis* and also define real safety levels for commercial pink-shrimp production.

2. Materials and Methods

Juvenile shrimps (170 ± 45 mg), obtained from local laboratory reproduction, were acclimated for 15 days under the conditions to be used in the toxicity tests. The tests were performed independently and based on the same methodology. Each toxicity test was performed in 100-L tanks of experimental medium with a 12 h photoperiod, constant aeration, temperature 25 °C and salinity 15 ppt (obtained from seawater pumped and adjusted with municipal freshwater free of chlorine). Three replicates were used per treatment (n=30).

Safety levels were used to establish the chronic concentrations for the three nitrogenous compounds. They were based on the application factor proposed by Sprague (1971), which corresponded 10% of the LC_{50} (96 h) and were estimated as follows: ammonia = 3.87 mg/L TA-N based on the work of Ostrensky and Wasielesky (1995), nitrite = 10.2 mg/L NO_2^--N based on the work of Castaño (1997) and nitrate = 323 mg/L NO_3^--N based on the work of Sachisida (1997). According to these data, the concentrations used in the experiments for ammonia, nitrite and nitrate were respectively: treatment $T_{1/4}$, a quarter of the safety level (0.91 mg/L TA-N, 2.55 mg/L NO_2^--N and 80.7 mg/L NO_3^--N); treatment T_{SL}, the safety level (3.65 mg/L TA-N, 10.2 mg/L NO_2^--N and 323 mg/L NO_3^--N); and treatment T_{2X}, twice the safety level (7.30 mg/L TA-N, 20.4 mg/L NO_2^--N and 646 mg/L NO_2^--N).

The reagents used for the preparation of experimental media for chronic tests were as follow: ammonium chloride (NH_4Cl) (Synth), sodium nitrite ($NaNO_2$) (Synth) and sodium nitrate ($NaNO_3$) (Synth). The tests were performed

in triplicate. In each replicate, 30 juvenile shrimps (90 per treatment) were fed daily with MR35 (Nestlé-Purina®, Brazil) and fresh food (fish and crab) until apparent satiation. The salinity, temperature and pH in all solutions were recorded daily. These variables were monitored and used to determine the proportions of ionized (NH_4^+) and un-ionized ammonia (NH_3) in the experimental media. These proportions were calculated from the concentrations of total ammonia used in the various treatments based on the equations in Whitfield (1974) and Bower and Bidwell (1978) as adapted by Ostrensky et al. (1992).

Eighty percent daily renewals were performed to maintain the physicochemical properties of the water. The total duration of the test was 30 days. At the 15th day of experimental period, shrimps were transferred to clean tanks to prevent the attachment of nitrifying bacteria (e.g. *Nitrobacter* and *Nitrosomonas*) that would cause significant variation in the concentrations of nitrogenous compounds due to the nitrification process.

Every 10 days during the chronic test, the survival rates and wet weight of all shrimp tested were recorded. The data on survival, wet weight and final biomass were subjected to an analysis of variance (ANOVA). The data analysis was conducted in view of the assumptions of this method. The Tukey test was applied if the toxic effects observed showed significant differences ($P<0.05$).

3. Results

The mean concentrations of ammonia, nitrite and nitrate and the physicochemical variables data were recorded (Table 1).

The survival of *F. paulensis* exposed to ammonia was the lowest ($P<0.05$) in T_{2X}. Based on the results of survival and final weight, the total biomass data were calculated for each treatment and showed a significant decrease with increasing level of exposure to ammonia (Table 2).

Regarding nitrite, survival rates of *F. paulensis* varied between 15.7% and 91.0%. Control and $T_{1/4}$ survival was significantly higher ($P<0.05$) than T_{SL} and T_{2X} (Table 3). Only shrimps exposed to T_{2X} had lower growth ($P<0.05$) compared to other treatments tested. Final biomass ranged from 1.46 g to 11.49 g, and was significantly lower in groups exposed to T_{SL} and T_{2X} (Table 3).

According to nitrate toxicity test, the lowest ($P<0.05$) survival rate (40.0%) was observed in the T_{2X}. Other survival rates varied between 70.2 and 88.9%, showing no significant differences ($P>0.05$). The final biomass of *F. paulensis* significantly decreased with increasing nitrate concentrations (Table 4), presenting chronic effects on *F. paulensis* (Table 4).

4. Discussion

Recently, several authors have conducted studies to determine the acute toxicity of various nitrogenous compounds to aquatic cultured organisms (Barbieri, 2010; Romano and Zeng, 2013). The median lethal concentrations (LC_{50}) for penaeid shrimp were estimated in short-duration tests (acute toxicity tests) [e.g. *Penaeus monodon* (Fabricius, 1798); *Marsupenaeus japonicus* (Bate, 1888); *Fenneropenaeus indicus* (H. Milne-Edwards, 1837); *Farfantepenaeus chinensis* (Osbeck, 1765); *Litopenaeus vannamei* (Boone, 1931); *Litopenaeus schmitti* (Burkenroad, 1936); *F. paulensis*, etc)]. Miranda-Filho and Costa (2015) have also published

Table 1. Nominal concentrations of ammonia (mg/L TA-N), nitrite (mg/L NO_2^--N) and nitrate (mg/L NO_3^--N) used in the experiments.

Toxic Compounds	Treatments		
	A	B	C
Ammonia	0.91	3.65	7.30
Nitrite	2.55	10.2	20.4
Nitrate	80.7	323	646

Test	Treatments	Physicochemical parameter		
		Temperature	pH	Dissolved Oxygen
Control		25.1 ± 0.9	8.13 ± 0.21	7.53 ± 1.4
Ammonia	A	25.0 ± 0.7	8.01 ± 0.26	7.19 ± 2.1
	B	25.2 ± 0.8	8.12 ± 0.24	7.55 ± 1.5
	C	25.0 ± 0.8	8.10 ± 0.28	7.51 ± 1.7
Nitrite	A	25.8 ± 1.1	8.12 ± 0.34	7.25 ± 1.3
	B	25.5 ± 0.7	8.16 ± 0.21	6.88 ± 1.6
	C	25.4 ± 0.7	8.12 ± 0.17	7.15 ± 1.6
Nitrate	A	25.3 ± 0.6	8.08 ± 0.24	7.10 ± 1.9
	B	25.2 ± 0.9	8.06 ± 0.21	6.89 ± 2.0
	C	25.9 ± 0.9	8.11 ± 0.10	7.30 ± 1.5

Treatment (A) a quarter of safety level; treatment (B) safety level; treatment (C) twice the safety level. Temperature (°C), pH and mean concentration of dissolved oxygen (mg/L) in different treatments in 30-day trials with nitrogenous compounds using *Farfantepenaeus paulensis* juveniles. Data are mean ± SD.

Table 2. Survival (number of animals and percentual), final weight (mg) and biomass (g) of shrimp *Farfantepenaeus paulensis* exposed to different concentrations of ammonia (mg/L TA-N/NH_3-N). Data are based on three replicates (mean ± SD).

Treatment	Survival	Survival (%)	Final weight	Final biomass
Control	26.7^A	88.9^A	431^A	11.49^A
	(26.1-27.3)		(409-453)	(10.81-12.18)
$T_{1/4}$ 0.91/0.0453	28.0^A	93.3^A	340^B	9.52^B
	(27.0-29.0)		(304-376)	(8.65-10.40)
T_{SL} 3.65/0.2363	27.7^A	92.2^A	260^B	7.18^C
	(26.1-29.3)		(233-287)	(6.79-7.58)
T_{2X} 7.30/0.4453	15.7^B	52.2^B	265^B	4.15^D
	(15.1-16.3)		(228-302)	(3.53-4.77)

Identical letters in columns indicate statistically equal means (P>0.05).

Table 3. Survival (number of animals and percentual), final weight (mg) and biomass (g) of shrimp *Farfantepenaeus paulensis* exposed to different concentrations of nitrite (mg/L NO_2^--N). Data are based on three replicates (mean ± SD).

Treatments	Survival	Survival (%)	Final weight	Final biomass
Control	26.7^A	88.9^A	431^A	11.49^A
	(26.1-27.3)		(409-453)	(10.81-12.18)
$T_{1/4}$ (2.55 mg/L)	27.3^A	91.0^A	438^A	11.93^A
	(25.2-29.4)		(408-468)	(11.73-12.13)
T_{SL} (10.2 mg/L)	11.7^B	39.0^B	381^A	4.45^B
	(10.17-13.23)		(373-389)	(3.83-5.07)
T_{2X} (20.4 mg/L)	4.7^C	15.7^C	311^B	1.46^C
	(3.55-5.85)		(288-334)	(1.07-1.84)

Identical letters in columns indicate statistically equal treatment means (P>0.05).

Table 4. Survival (number of animals and percentual), weight (mg) and biomass (g) of juvenile pink shrimp *Farfantepenaeus paulensis* exposed to different concentrations of nitrate (mg/L NO_3^--N). Data are based on three replicates (mean ± SD).

Treatments	Survival	% Survival	Final weight	Final biomass
Control	26.7^A	88.9^A	431^A	11.49^A
	(26.10-27.30)		(409-453)	(10.81-12.18)
$T_{1/4}$ (80.7 mg/L)	21.7^A	72.2^A	323^B	7.00^B
	(21.10-22.30)		(304-341)	(6.44-7.57)
T_{SL} (323 mg/L)	21.0^A	70.2^A	259^B	5.45^B
	(17.00-25.00)		(253-265)	(4.36-6.53)
T_{2X} (646 mg/L)	12.07^B	40.0^B	275^B	3.26^C
	(10.07-14.07)		(212-338)	(2.61-3.90)

Identical letters in columns indicate statistically equal treatment means (P>0.05).

a compilation of LC_{50} for fish and crustaceans. However, Tomasso (1994) states that the actual safety levels for different species can often vary quite substantially from the values obtained from short-duration tests.

In the present study, chronic concentrations of nitrogenous compounds tested were based on safety levels. However, according to the results, this approach may be misleading and can undermine aquaculture activities. For example, the survival of shrimp at the security level (T_{SL}) after 30 days of ammonia exposure showed no differences compared with the control condition. However, the results in wet weight and final biomass showed that even a concentration as low as 0.91 mg/L TA-N (total ammonia as nitrogen), corresponding to ¼ of the security level (Ostrensky and Wasielesky, 1995), caused a decrease in the growth rate. The results for the nitrite treatments showed that the weight gain, corresponding to the safety level concentration (Castaño, 1997) (T_{SL}), did not differ statistically from that observed in the control treatment. However, the survival of *F. paulensis* was significantly affected. Moreover, as observed for ammonia, the survival of the shrimps exposed to nitrate at the safety level concentration (Sachisida, 1997) was similar to that found in the control. However, the growth in weight and final biomass were reduced by nitrate exposure.

In this context, the weight gain of penaeid shrimp has provided a practical method to allow researchers to define sublethal effects caused by nitrogenous compounds in these organisms. Thus, Chen and Kou (1992), working with juvenile *M. japonicus*, found that after 60 days, this species showed growth reductions between 12.8 and 36.6% in ammonia concentrations ranging between 5 and 30 mg/L TA-N

(0.35 and 2.1 mg/L NH_3-N), respectively. These authors found that juvenile *M. japonicus* exposed to 0, 5, 10, 20 and 30 mg/L TA-N (0, 0.35, 0.70, 1.40 and 2.10 mg/L NH_3-N) had reduced intermolt periods estimated as 21.9, 19.2, 17.7, 14.6 and 10.3 days, respectively. However, these findings did not represent an increase in growth rate. They occurred in response to the stressful experimental treatments. Chen and Lin (1992a) found a decrease in growth in juvenile *P. monodon* at 4, 8 and 20 mg/L TA-N after 30 days of exposure. These authors, working with juvenile *Farfantepenaeus penicillatus* (Alcock, 1905), estimated that the concentration that reduced the growth rate to 50% of that in the control group (at 56 days) was 12.65 mg/L TA-N (Chen and Lin, 1992b). In the present study, *F. paulensis* juveniles showed a reduction in weight growth of 21.1, 39.7 and 38.5% compared with the control at concentrations of 0.91, 3.65 and 7.30 mg/L TA-N, respectively. These results indicate that *F. paulensis* is more susceptible to ammonia than the other species cited.

Laboratory studies have shown different chronic effects of ammonia in *F. paulensis* according to age, sex, stage of maturation and methodology of research applied. For example, Cavalli et al. (1998) analyzed the effect of ammonia on adults of *F. paulensis* reporting lower growth rates in females in the treatment containing 6.86 mg/L TA-N. However, this effect was not observed in males. In post-larvae of *F. paulensis*, Wasielesky et al. (1994) confirmed significant reductions in the growth rates of the shrimp at concentrations of 2, 3 and 4 mg/L TA-N (0.07, 0.11 and 0.14 mg/L NH_3-N). Miranda-Filho et al. (2009) found that juvenile *F. paulensis* showed reduced growth at concentrations of 1.86, 3.32, 6.27 and 13.3 mg/L TA-N (0.05, 0.1, 0.2 and 0.4 mg/L NH_3-N), at the end of a 75-day experiment.

Wickins (1976) working with *Penaeus semisulcatus* (De Haan, 1844), *M. japonicus*, *Litopenaeus occidentalis* (Streets, 1871), *Litopenaeus setiferus* (Linnaeus, 1767) and *L. schmitti* has indicated that the maximum acceptable level in cropping systems for penaeids would be 0.1 mg/L NH_3-N. However, the results obtained in the present study, combined with others cited above, indicate that the maximum acceptable level of ammonia proposed by Wickins (1976) is not suitable for *F. paulensis* because chronic effects on the growth of shrimp species have been found at concentrations below that level.

Studies of the chronic effects of nitrite on penaeid shrimp are scarce. In addition, most previous studies examine effects of nitrite on growth or sublethal concentrations determined from short-term tests. For example, Chen and Cheng (1996) and Cheng and Chen (1998) observed changes in acid-base balance, ammonia excretion and osmoregulation; and increase of hemolymph urea and decrease of arginine, respectively, in *M. japonicus* exposed to nitrite concentrations between 0.03 and 0.065 mg/L. Chen et al. (1990) estimated a safety level of 10.6 mg/L NO_2^--N for juvenile *P. monodon*. Working with adults and juveniles of *F. paulensis*, Cavalli et al. (1996) and Ostrensky (1997) estimated safety levels of 10.94 and 10.2 mg/L NO_2^--N, respectively. These results were similar to those obtained by Chen et al. (1990) with juveniles of *P. monodon*.

In order to investigate the chronic effects of nitrite, Chen and Chen (1992) exposed juvenile *P. monodon* to concentrations ranging between 2 and 20 mg/L NO_2^--N for 60 days. In the shrimp subjected to 4, 8 and 20 mg/L NO_2^--N, the body weight gain was significantly lower. These authors estimated the median effective concentration (EC_{50}) (60 days) for *P. monodon* as 17.4 mg/L NO_2^--N. In turn, Armstrong et al. (1976) found a 35% growth reduction of *Macrobrachium rosenbergii* (De Man, 1879) prawn exposed to a concentration of 1.8 mg/L NO_2^--N for 30 days. Wickins (1976) concluded that chronic exposure to 6.2 mg/L NO_2^--N can cause a decrease of 50% in the shrimp growth. The current study found that juvenile *F. paulensis* only showed a significant decrease in weight at a concentration of 20.4 mg/L NO_2^--N (twice the safety level) after 30 days of testing. Moreover, *F. paulensis* showed a mortality of 61% at a concentration of 10.2 mg/L NO_2^--N, while *P. monodon*, even if exposed for a longer period than *F. paulensis*, showed high survival up to a concentration of 8 mg/L NO_2^--N (Chen and Chen, 1992). In contrast, despite the high mortality rate of juvenile *F. paulensis* at a concentration of 10.2 mg/L NO_2^--N, the growth of the shrimps that survived was not affected by nitrite. It is probable that this result is related to the increased need for oxygen during the molting process. The presence of nitrite is harmful during molting because it interferes with oxygen uptake. As previously mentioned, nitrite transforms hemocyanin into meta-hemocyanin, decreasing the delivery of oxygen to the tissues. It is suggested that the shrimp during molting stage (ecdysis) shows a relatively normal weight gain for a period of 30 days, as seen at the concentration of 10.2 mg/L NO_2^--N.

Nitrate is considered a weak toxicant to crustaceans and in high concentrations in the water, nitrate can accumulate in the shrimp tissue (Cheng et al., 2002). Colt and Armstrong (1981) stated that the lethal levels for short-term exposure varied between 1,000 and 3,000 mg/L NO_3^--N. Wickins (1976) estimated that concentrations of up to 200 mg/L NO_3^--N would not affect the growth of penaeid shrimp. Moreover, Colt and Armstrong (1981) stated that the growth of the shrimp *M. rosenbergii* decreased by 50% at a concentration of 180 mg/L NO_3^--N and Spotte (1979) reported that approximately 20 mg/L NO_3-N would be the acceptable level of nitrate for seawater culture. Kuhn et al. (2010) demonstrated that 200 mg/L NO_3^--N at salinity 11 ppt would be safe to juveniles *L. vannamei*. However, in the current study, juvenile *F. paulensis* exposed to 80.7 mg/L NO_3^--N, presented lower growth rates compared to control indicating that this species is more sensitive to nitrate than other crustaceans.

Ammonia, nitrite and nitrate are substances that generally do not reach high concentrations in the natural environment. Nevertheless, in high density aquaculture systems, nitrogenous compounds can reach high levels. Chen et al. (1988) reported that concentrations of 46.11 mg/L TA-N (0.84 mg/L NH_3-N), 0.83 mg/L NO_2^--N and 1,795 mg/L NO_3^--N

were reached in superintensive cultures of *F. penicillatus*. Yont et al. (1996) found 0.86 mg/L NH_3-N in an intensive culture of *P. monodon*. Furthermore, Chen and Wang (1990) measured concentrations between 0.01 and 2.0 mg/L NO_2^--N in cultures of *P. monodon* and *F. penicillatus*. In addition, Muir et al. (1991) reported the frequent occurrence of concentrations between 10 and 20 mg/L NO_3^--N in penaeid culture. In this study, chronic effects on the survival and/or growth of the shrimp *F. paulensis* occurred from exposure to ammonia and nitrate concentrations equivalent to ¼ of the safety levels for this species. Moreover, nitrite was toxic at concentrations equivalent to the proposed level of security.

According to what has been described, particular caution must be taken to control the concentrations of nitrogenous compounds during shrimp production in captivity. These findings confirm that experiments involving long-term exposures are needed to produce valid estimates, as previously suggested by Tomasso (1994).

5. Conclusion

The present study showed that the safety level as proposed by Sprague (1971), based on acute-toxicity tests, must be tested in order to prove if the concentrations are really safe. For pink shrimp *F. paulensis*, the proposed level of security for nitrite was 2.55 mg/L NO_2^--N. The safety levels for ammonia and nitrate were not determined and concentrations of less than 0.91 mg/L TA-N (corresponding to 0.045 mg/L NH_3-N) and 80.7 mg/L NO_3^--N, respectively, are recommended for the production of *F. paulensis*.

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