



Nitrate level safety to Amazon River shrimp juveniles

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Abstract

The study's objective was to evaluate the nitrate level safety for *Macrobrachium amazonicum* juvenile in the laboratory, a potential native species for culture in Brazil. The experiment consisted of six treatments with six replicates in a completely randomized block design: 0, 250, 500, 1,000, 1,500, and 2,000 mg L⁻¹. Physical and chemical water quality parameters were recorded every 12 h, while the shrimp mortalities in the 24-h interval. Except for nitrate, all physical and chemical water quality parameters remained within the ideal range rearing to this species. No deaths were observed during the first 6 h of exposure range 0-500 mg L⁻¹ concentrations. At 250 mg L⁻¹ N-NO₃⁻, the mortality (10%) started from 48 h. At 500 mg L⁻¹ N-NO₃⁻, shrimp mortalities occurred after 24 h, reaching 60% after 72 h. In the treatments with 1000 and 1500 mg L⁻¹ N-NO₃⁻ concentrations, dead shrimps can be observed after 24 h, with a mortality rate of 78% and 90% of the population in 96 h, respectively. All shrimps exposed at 2000 mg L⁻¹ died in 96 h. The LC50 values obtained decreased with increasing exposure time. Based on LC50 (96 h), the N-NO₃⁻ level safety to *M. amazonicum* is 48.5 mg L⁻¹.

Keywords *Macrobrachium amazonicum* · Nitrate lethal level · Palaemonidae · Toxic effect

Introduction

Brazil has many shrimp native species with great potential for aquaculture exploitation. The Amazon River shrimp *Macrobrachium amazonicum* (Heller 1862) is an endemic species from South America, distributed in the rivers, floodplains, reservoirs, and lakes in tropical and subtropical regions of this continent (Moraes-Valenti and Valenti 2010). This Amazon shrimp has high economic and gastronomic

importance, widely exploited by artisanal fisheries in northern and northeastern Brazil, and indigenous people and Brazilians of all economic groups (Marques and Moraes-Valenti 2012) broadly consume it. In aquaculture, this shrimp has demonstrated fast growth, rusticity, and satisfactory productivity, suggesting an alternative that avoids environmental impacts due to escapes and the establishment of non-native shrimp in the natural environment (Moraes-Valenti and Valenti 2010). In this species, the hatchery has been conducted in intensive

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systems in high stocking densities (100–140 larvae/L) using recirculating aquaculture systems (Moraes-Valenti and Valenti 2010) or aquaponics system using constructed semi-dry wetland with lettuce (Lima et al. 2019).

In aquaponics and RAS, inorganic nitrogen forms are present naturally because of atmospheric deposition and organic matter biological degradation. Ammonium tends to be oxidized to nitrate in a two-step process ($\text{NH}_4^+ \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^-$) by aerobic chemoautotrophic bacteria (Nitrosomonas and Nitrobacter, primarily). This process promotes nitrate accumulation in both systems, although in aquaponics, part of the nitrate can be absorbed by the plants and removed from the system, unlike in RAS. Despite this, in intensive systems, shrimp can be exposed to high concentration of ammonia, nitrite, or nitrate due to the biofilter imbalances. Thus, a high load of nitrogenous compounds and other toxic metabolites may change water quality, impairing the metabolism of organisms cultured (Romano and Zeng 2007, 2009, 2013).

Information on acute nitrate-N toxicity is limited to only a few decapod species such as *Cherax quadricarinatus* (Mead and Watts 1995), *Penaeus monodon* (Tsai and Chen 2002; Romano and Zeng 2009), *Portunus pelagicus* and *Scylla serrata* (Romano and Zeng 2007), *Farfantepenaeus brasiliensis* (Campos et al. 2012), and *Litopenaeus vannamei* (Kuhn et al. 2010; Furtado et al. 2015). Although nitrate will be considered a weak toxicant to crustaceans, in high concentrations in the water, it can accumulate in the shrimp tissue (Tsai and Chen 2002; Cheng et al. 2002; Poersch et al. 2007) or act synergistically with other nitrogenous forms and caused sublethal or lethal effects (Kuhn et al. 2010; Furtado et al. 2015).

The nitrate accumulation in the shrimp tissue can cause indirectly unwanted problems in humans after ingestion, including methemoglobinemia with subsequent blockade of the oxygen-carrying capacity of hemoglobin through their conversion to nitrites in the gut and buccal cavity (Wolfe and Patz 2002). Another potential consequence of ingestion of nitrates is the developing cancers of the digestive tract because it has a contribution to the formation of nitrosamines, which are among the most potent of the known carcinogens in mammals (Song et al. 2015). Despite the potential health risk, Brazil does not have criteria established to an accumulation of nitrate in the tissues of fish and shrimp cultured.

Several studies have been conducted to determine the acute toxicity of nitrogenous waste in *M. amazonicum* as effects of ammonia and nitrite on different life stages of this species (Hayd et al. 2010, 2014; Dutra et al. 2016). However, the published data on the acute toxicity or chronic effects of nitrate in this species are absent, although identifying security levels of ambient nitrate is very important for hatchery protocols and for human health. Therefore, this study aimed to evaluate the median lethal concentration (LC_{50}) nitrate in 96 h for juvenile *M. amazonicum* in the laboratory.

Material and methods

This study used a static experimental method to assess the lethal effect of different nitrate (N-NO_3^-) concentrations on juvenile shrimps. The trial occurred in the Agroforestry Research Center laboratory of Amapá-Embrapa Amapá (Brazil). This study was developed following the principles recommended by the Brazilian College of Animal Experimentation (COBEA) and with the authorization from the Ethics Committee in the Use of Animals of Embrapa Amapá (# 008 - CEUA/CPAFAP). The experiment consisted of six treatments with six replicates each, in a completely randomized block design: 0 (control), 250, 500, 1,000, 1,500, and 2,000 mg L^{-1} , respectively. A total of 180 juveniles with 12.58 ± 1.36 mm total length and 156 ± 44.0 mg wet mass were used. The juvenile shrimps were reared at the laboratory and subsequently transferred to experimental units with useful volume of 2 L, with five shrimps being placed in each rectangular polyethylene tank. They were monitored every hour interval until the 96-h endpoint. Dead shrimps were recorded twice daily and removed after 24, 48, 72, and 96 h of exposure. The first step was creating a stock solution with a concentration of 10,000 mg L^{-1} nitrate (N-NO_3^-) using sodium nitrate (P.A., Synth) that was subsequently diluted to create a range of the desired test using a micropipette. Shrimp juveniles were not fed during experiments, and water was changed each 12 h. At the bottom of each experimental unit, there was one air diffuser (porous stone) to provide water oxygenation. The chosen photoperiod for the experimental room was 12/12-h light/dark cycle, with a 250 lx intensity at the water surface provided by artificial lighting. Determinations of the pH, temperature (T °C), dissolved oxygen (DO), total ammonia (TAN) ($\text{NH}_3 + \text{NH}_4^+$), nitrite (N-NO_2^-), and nitrate (N-NO_3^-) were measured twice a day using the HORIBA U-50 Multiparameter Water Quality Checker and Hanna HI83326 Multiparameter photometer, respectively. The lethal nitrate concentration (LC_{50} with 95% confidence limits) was calculated by Spearman–Kärber estimates (Hamilton et al. 1977).

The statistical analysis was performed through the © BioEstat 5.3 software (Ayres et al. 2007). Afterward the verification over the data's homoscedasticity and normality, a one-way analysis of variance (ANOVA), was performed to check for significant differences in the obtained data. When a significant difference was detected in the treatments ($p < 0.05$), Tukey's test for comparison of means was applied.

Results and discussion

The physical and chemical water quality parameters are presented in Table 1. The temperature, dissolved oxygen, and pH values recorded in all treatments remained within the ideal

Table 1 Average value \pm standard deviation of the physical and chemical water quality parameters during 96-h exposure of *M. amazonicum* under different nitrate concentrations

Parameters	Treatments with N-NO ₃ ⁻ concentration (mg L ⁻¹)*					
	Control 0	250	500	1000	1500	2000
T (°C)	29.01 \pm 1.4a	29.03 \pm 1.6a	28.50 \pm 1.4a	29.04 \pm 1.5a	29.03 \pm 1.6a	29.04 \pm 1.4a
DO (mg L ⁻¹)	6.03 \pm 0.5a	6.05 \pm 0.7a	6.04 \pm 0.6a	6.03 \pm 0.5a	6.04 \pm 0.6a	6.02 \pm 0.7a
pH	7.23 \pm 0.5a	7.25 \pm 0.3a	7.24 \pm 0.3a	7.23 \pm 0.5a	7.26 \pm 0.6a	7.24 \pm 0.3a
TAN (mg L ⁻¹)	0.13 \pm 0.04a	0.15 \pm 0.03a	0.14 \pm 0.05a	0.13 \pm 0.5a	0.12 \pm 0.06a	0.13 \pm 0.05 ^a
NO ₂ ⁻ (mg L ⁻¹)	0.03 \pm 0.02a	0.05 \pm 0.03a	0.03 \pm 0.04a	0.04 \pm 0.03a	0.04 \pm 0.02a	0.03 \pm 0.04a
N-NO ₃ ⁻ (mg L ⁻¹)	0.01 \pm 0.01a	249.25 \pm 10.3b	498.35 \pm 10.3c	1001.23 \pm 20.5d	1499.56 \pm 10.6e	2002.13 \pm 15.7f

*Values reported are mean \pm SD for six replicates of the physical and chemical water quality parameters. Value with a different letter in superscript shows a significant difference ($p < 0.05$)

range rearing of *M. amazonicum* (Moraes-Valenti and Valenti 2007; Preto et al. 2008; Marques et al. 2010). The nitrate values were significantly different ($p < 0.05$) among the treatments, as planned in the experimental design. The other water quality parameters that were monitored did not present significant differences among the treatments ($p > 0.05$) throughout the 96-h exposure. Therefore, we can assume that the mortality recorded in this study should be attributed to the different nitrate concentrations (Table 2).

Numerous studies have reported that nitrate is a least harmful nitrogenous compound, but it can cause sublethal or lethal effects in different organisms or act synergistically with other nitrogenous forms, and it is therefore important to study its toxic effects in different species (Cheng et al. 2002; Camargo et al. 2005; Furtado et al. 2015). Nitrate can be a weak toxicant to crustaceans, but in high concentrations in the water, nitrate can accumulate in the shrimp hemolymph and tissue increasing directly with exposure time (Cheng et al. 2002). In this work, neither was observed during the first 6 h of exposure range 0–500 mg L⁻¹ N-NO₃⁻ concentrations. At 250 mg L⁻¹ N-NO₃⁻, the mortality started from 48 h, with a loss of 10% of the individuals during the experiment. At 500 mg L⁻¹ N-NO₃⁻, dead shrimps were observed after 24 h of trial, with a mortality rate of more than 60% after 72 h. In the treatments with 1000 and 1500 mg L⁻¹ N-NO₃⁻ concentrations, dead shrimps can be observed after 24 h, with a mortality rate of

78% and 90% of the population in 96 h, respectively. All shrimps tested at 2000 mg L⁻¹ N-NO₃⁻ were dead after 96 h. In fact, nitrate toxicity to *M. amazonicum* and other aquatic invertebrates increases with increasing nitrate concentrations and exposure times (Tsai and Chen 2002; Alonso and Camargo 2003; Camargo et al. 2005; Furtado et al. 2015). The toxic nitrate action occurs due to the pigment's conversion that carries oxygen (hemoglobin, hemocyanin) into forms that are unable to carry oxygen (methemoglobin) (Camargo et al. 2005). However, nitrate has low permeability in the gills, because the nitrate reception in aquatic animals can be more limited than the reception of ammonia and nitrite (Cheng et al. 2002; Alonso and Camargo 2003).

The LC₅₀ values obtained in this work decreased with increasing exposure time, from 1817.12 mg L⁻¹ N-NO₃⁻ for 24 h, 885.58 mg L⁻¹ N-NO₃⁻ for 48 h, 663.98 mg L⁻¹ N-NO₃⁻ for 72 h, and 484.87 mg L⁻¹ N-NO₃⁻ for 96-h exposure (Table 3). Based on LC₅₀ (96 h), the L⁻¹ N-NO₃⁻ level safety to *M. amazonicum* is 48.5 mg L⁻¹. This value is the maximum limit to which this species can be submitted in cultivation without prejudice in its production. Sublethal or lethal effects may occur by high nitrate concentrations, and it is therefore important to study its toxic effects in different crustacean species (Muir et al. 1991; Poersch et al. 2007). In marine shrimps, concentrations above 60 mg L⁻¹ N-NO₃⁻ already begin to exhibit some deleterious effects (Vanwyk and Scarpa 1999).

Table 2 Percent mortality (%) of *Macrobrachium amazonicum* exposed to various nitrate concentrations for 96 h

Exposure time (h)	Treatments with N-NO ₃ ⁻ concentration (mg L ⁻¹)					
	Control - 0	250	500	1000	1500	2000
24	0.0	10.0	27.0	33.0	43.0	53.0
48	0.0	10.0	33.0	50.0	60.0	73.0
72	0.0	10.0	57.0	60.0	70.0	90.0
96	0.0	10.0	63.0	77.0	93.0	100

Table 3 Estimated LC₅₀ (mg L⁻¹) values and their 95% confidence limits for N-NO₃⁻ were calculated by Spearman–Kärber estimates

Exposure time (h)	LC ₅₀ of N-NO ₃ ⁻ (mg L ⁻¹)	95% confidence limits	
		Lower	Upper
24	1817.12	1230.18	2684.10
48	885.58	623.10	1258.62
72	663.98	533.07	827.04
96	484.87	400.41	587.14

At concentrations above 105 mg L^{-1} , nitrate caused protein reduction in *Marsupenaeus japonicus*, while *Litopenaeus vannamei* can be grown at a concentration of up to $220 \text{ mg L}^{-1} \text{ N-NO}_3^-$ without detriment to the species, being lethal only from $435 \text{ mg L}^{-1} \text{ N-NO}_3^-$ (Furtado et al. 2015). Similar to the marine shrimps, *M. amazonicum* with high nitrate concentrations have lethal effects. In this work, the toxicity to this shrimp increased with increasing nitrate concentrations and exposure times. Concentrations of $250 \text{ mg L}^{-1} \text{ N-NO}_3^-$ showed to be lethal in 10% of population after 24 h of exposure, and exposed to above $1500 \text{ mg L}^{-1} \text{ N-NO}_3^-$ shrimps can have its survival compromised after 24 h into 43% and after 96 h into 93%, indicating that this chemical compound should be also monitored (Table 2). In above $1500 \text{ mg L}^{-1} \text{ N-NO}_3^-$ concentration, immediate intervention with partial water exchange is required.

The increase in the storage density and biomass, in fact, reflects an increase of ammonia, nitrite, and in the nitrate concentration. However, this increase can be controlled using efficient biofiltration systems. According to Lima et al. (2019), ammonia, nitrite, and in the nitrate concentration were controlled in aquaponics system using semi-dry wetland and this system was satisfactorily efficient like water treatment biofilters at the densities to $120 \text{ shrimp's m}^{-2}$, removing the main pollutants from shrimp culture water.

The concentration and time required for a substance to produce an adverse effect vary according to the chemical agent and the type and severity of the effect (Campos et al. 2012; Campos et al. 2015). Adverse or toxic effects can be produced in the laboratory or in the natural environment through lethal (high concentrations for a short period) or chronic (sublethal concentrations over a long period) exposure to the high concentrations of nitrogen compounds (ammonia, nitrite, and nitrate) (Campos et al. 2015). Traditionally, the nitrogenous compound's safety level in many species has been estimated by multiplying the value LC_{50} (96 h) by a factor 0.1 (Cobo et al. 2012). In the present study, the “safety level” for juveniles of *M. amazonicum* was $48.5 \text{ mg L}^{-1} \text{ N-NO}_3^-$, which is the maximum limit to which this species can be submitted without prejudice to its cultivation ($\text{LC}_{50} 96 \text{ h} \times 0.1$). The N-NO_3^- concentration “safety level” for *M. amazonicum* juveniles was inferior to observed in penaeid species as *Litopenaeus vannamei* supporting $200 \text{ mg L}^{-1} \text{ N-NO}_3^-$ at salinity 11 ppt (Kuhn et al. 2010) and *Farfantepenaeus paulensis* (Pérez-Farfante 1967) exposed to $80.7 \text{ mg L}^{-1} \text{ N-NO}_3^-$ at salinity 15 ppt (Wasielesky et al. 2017). Our results corroborate the idea that freshwater shrimps are more sensitive to nitrate toxicity than marine shrimps as a likely consequence of increasing water salinity over nitrate ion tolerance reported in the literature (Tsai and Chen 2002; Camargo et al. 2005; Kuhn et al. 2010; Furtado et al. 2015). However, it suggests that sublethal chronic studies are necessary to corroborate that under the estimated safe concentrations, the physiological

status of *M. amazonicum* is not compromised, supporting good commercial production.

Conclusion

Nitrate concentrations above 250 mg L^{-1} may cause lethal effects in *M. amazonicum*. An immediate intervention should be taken before having concentrations above twice the safety level, about $100 \text{ mg L}^{-1} \text{ N-NO}_3^-$. Nitrate concentrations above $1500 \text{ mg L}^{-1} \text{ N-NO}_3^-$ are critical and cause lethal effects in this species requiring immediate water exchange. Juveniles of *M. amazonicum* can be farmed in safety level with $48.5 \text{ mg L}^{-1} \text{ N-NO}_3^-$.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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