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Effects of Ambient Nitrite on Amazon River Prawn, *Macrobrachium amazonicum*, larvae

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Abstract

The effects of nitrite concentration on larval development of Amazon river prawn, *Macrobrachium amazonicum*, were studied in laboratory. In Experiment 1, larvae were reared in 600-mL glass beakers filled with 300-mL water with nitrite concentration of 0, 0.2, 0.4, 0.8 and 1.6 mg/L NO_2-N. In Experiment 2, total ammonia nitrogen (TAN, NH_3-N + NH_4-N) excretion were analyzed in zoea (Z) I, III, VII and IX exposed to 0, 0.4, 0.8 and 1.6 mg/L NO_2-N. In both experiments each treatment was conducted in five replicates. The experiments were carried out in test solutions at 10 salinity, constant temperature 30 C and 12:12 h daylight : darkness regime. Survival, productivity, weight gain and larval stage index decreased linearly with increasing ambient nitrite concentration. However, there was no significant difference among larvae reared at concentration ranging from 0 to 0.8 mg/L NO_2-N by ANOVA in all variables. Individual ammonia-N and mass-specific ammonia-N excretion increased in ZI and ZIX, was almost constant in ZIII and decreased in ZVII from 0 to 1.6 mg/L NO_2-N. The relationship between individual TAN and body mass suggested that 1.6 mg/L NO_2-N stress the larvae. Despite of the effects of nitrite on larvae follow a dose-dependent response and shows large variability among individuals, levels below 0.8 mg/L may be used as a general reference in commercial hatcheries, which should be applied carefully.

Freshwater prawn farming is an aquaculture sector that dramatically increased globally during the past years (Kutty 2005; New 2005; New 2010; FAO 2011). In Brazil, the culture of freshwater prawns is focused on the giant river prawn, *Macrobrachium rosenbergii* (Marques and Moraes-Valenti 2012). Furthermore, a large Brazilian group is working on the culture of an indigenous species, the Amazon river prawn, *Macrobrachium amazonicum*, which has high potential for small-scale farms in the Amazonia (Valenti and Moraes-Riodades 2004; New 2005; Moraes-Valenti and Valenti 2010) and western Pantanal regions (Valenti et al. 2011; Hayd and Anger 2013). This species shows fast larval development, with nine free-swimming zoea stages (Guest 1979) and metamorphosis in 18–21 d in recirculating hatchery systems (temperature 30 ± 1 C and 10 salinity). Larvae have facultative lecitotrophy in the early stages (Anger and Hayd 2009) and feeding habit polytrophic (Araujo and Valenti 2007). In addition, *M. amazonicum* shows high growth and survival rates in nursery phase (Marques et al. 2012) and in semi-intensive grow-out ponds (Moraes-Valenti and Valenti 2007).

Macrobrachium amazonicum hatchery is conducted in intensive systems in high stocking densities (100–140 larvae/L) (Moraes-Valenti and Valenti 2010). Thus, the load of nitrogenous compounds and other toxic metabolites is high and may change water quality, such as increased ammonia (Hayd et al. 2010) and nitrite concentration (Das et al. 2004; Madison and Wang 2006). These inorganic nitrogen forms are the most important problem in

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recirculating aquaculture systems because of toxicity (Chin and Chen 1987; Koo et al. 2005; Romano and Zeng 2007; Hayd et al. 2010). Recirculating water through biological filters lowers ammonia and nitrite levels due to a nitrification process, which convert ammonia to nitrate via nitrite (Timmons et al. 2002; Valenti et al. 2010). Despite of this, in intensive systems, animals can be exposed to high concentration of nitrite due to biofilter imbalances (Tomasso 1994).

In crustaceans, ambient nitrite reduces their thermal tolerance and induces methaemocyanin formation, causes hypoxia in tissues and diminishes the respiration efficiency (Alcaraz and Carnegas 1997; Timmons et al. 2002). Studies on the effects of nitrite on some Crustacea have been reported, such as on juveniles and adults of *Procambarus clarkii* (Gutzmer and Tomasso 1985), *Artemia* nauplii (Chen et al. 1989), early larval stages of *Macrobrachium nipponense* (Wang et al. 2004), larvae of *M. rosenbergii* (Mallasen and Valenti 2006), *Scylla serrata* (Seneriches-Abiera et al. 2007) zoeae and megalops of *Portunus trituberculatus* (Lu et al. 2006), juveniles of *Orithyia sinica* (Koo et al. 2005) and *Portunus pelagicus* (Romano and Zeng 2009), larvae of the blue swimming crab *P. pelagicus* (Liao et al. 2011), and juveniles of *Marsupenaeus japonicus* (Cheng et al. 2013). Nevertheless, the tolerance limits, and the mechanisms of nitrite action in crustacean development and metabolism are almost unknown (Romano and Zeng 2013).

Some authors have recommended levels below 0.1 mg/L NO_2-N for *M. rosenbergii* hatchery (New 1990; Correia et al. 2000; New 2002). Otherwise, it was demonstrated that very high levels around 1–2 mg/L may not stress larvae (Armstrong et al. 1976; Mallasen and Valenti 2006) showing that scientific-based information is really required. Nothing is known for *M. amazonicum*, although identifying security levels of ambient nitrite is very important for hatchery protocols. Therefore, the aim of this study was to investigate the effects of ambient nitrite on the development and nitrogen excretion of *M. amazonicum* larvae under laboratory conditions. Results will contribute to understanding the biology of this species and to developing techniques for commercial production.

Materials and Methods

Experimental Animals

Larvae were taken from berried females from Crustacean Sector of the Aquaculture Center (Caunesp) in São Paulo State University, Brazil. Broodstock was formed from wild animals captured in Northeast Para, Brazil (01°14′30″S, 48°19′52″W) in 2001. Females carrying eggs in advanced stages of embryonic development (ca. 24 h before hatching) were collected. Animals were disinfected in formaldehyde solution at 20 mg/L for 30 min and then kept in a hatching tank at 70 individuals/m^2, 8 salinity and 30 ± 1 C. It was provided with aeration, heating system and artificial substrates. After hatching, larvae were collected using a 250 μm screen box.

Larval Development Test

The effects of nitrite concentration on survival, productivity, mass gain and larval development were evaluated. The experiment followed a completely randomized design with five nitrite concentrations (treatments) and five replicates. Tested treatments were 0, 0.2, 0.4, 0.8 and 1.6 mg/L NO_2-N. All test solutions were prepared by dissolving sodium nitrite in brackish water (10 salinity), according to APHA (2005). General methodology was adapted from Mallasen and Valenti (2006) and is summarized below.

Larvae were reared in 600-mL glass beakers filled with 300 mL of test solution gently aerated at 10 salinity, constant 30 C, and subjected to 12:12 h daylight : darkness regime. The beakers were placed in water bath inside black trays to minimize light reflections and prevent larvae crowding around luminous points, due to positive phototactism. Fifteen newly hatched larvae were carefully washed out with the test solution, acclimated for 2 h and transferred to each beaker. Larvae were fed freshly hatched *Artemia* nauplii from ZII to ZIV (4–6 nauplii/mL). After this stage, they were fed on inert feed (3.3–13.2 μg/mL, see Mallasen and Valenti 1998 for diet composition) and *Artemia*

nauplii (8–12 nauplii/mL), added to the beakers every day until metamorphosis. Food residues were siphoned 3 h after feeding.

Every 24 h, test solutions were completely replaced with new solution prepared each day for maintaining constant water quality and the tested nitrite concentrations. Temperature and salinity were monitored twice a day (measured with a Yellow Springs Instruments, YSI 63, Yellow Springs, OH, USA), whereas dissolved oxygen (measured with Yellow Springs Instruments YSI 52 oxygen meter) and pH (measured with Yellow Springs Instruments YSI 60 pH meter) were measured three times a week. Ammonia and nitrite levels were determined twice a week before water replacement, according to APHA (2005), using a Hach (Loveland, CO, USA) DR 2000 spectrophotometer.

The experiment was finished when approximately 80% of the larvae of one replicate of any tested treatment metamorphosed to post-larvae. Thus, the surviving animals were counted and observed under stereomicroscope to determine larval stage according to Guest (1979) and weighed. Larval stage index (LSI) was calculated according to Manzi et al. (1977) as follows: $\mathrm{LSI} = (\sum Si \,.\, ni/N)$

In which S_i is the larval stage ($i = 1$–9); n_i, the number of larvae in stage S_i and N, the total number of larvae observed.

Larvae were sampled from the hatchery tank at the same day of stocking to estimate initial dry mass (IDM). At the end of the test, the surviving larvae and post-larvae were used to determine final dry mass (FDM). Mass gain was then given by the difference between FDM and IDM. To determine dry mass, the larvae were briefly rinsed in distilled water, dried with filter paper, placed in pre-weighted cartridges and dried for 48 h at 70 C. Then, they were transferred to a desiccator for 2 h and weighed on a Mettler–Toledo Model AT21 analytical balance (Mettler–Toledo Incorporation, Im Langacher, Greifensee, Switzerland), at the nearest 1 μg. Ten replicates were obtained for each developmental stage.

The data were first subjected to tests of normality (Shapiro–Wilk) and homoscedasticity (Brown–Forsythe). As these assumptions were satisfied, data were subjected to one-way ANOVA (F-test), followed by Tukey test, whenever significant values were obtained, and linear regression analysis to identify significant differences among treatments. All measured values of each variable were entered into the regression analysis and the significance of linear regressions was assessed by parametric ANOVA (Sokal and Holf 1995). Statistics analyses were performed with the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA, version 8.0). Values expressed as percentages were square root arcsine transformed prior to statistical analysis, although they are presented as non-transformed data for easier interpretation. Significance level was set at $P < 0.05$.

Ammonia-N Excretion Test. Ammonia-N excretion was determined in larvae at stages I, III, VII and IX subjected to ambient nitrite concentrations of 0, 0.4, 0.8 and 1.6 mg/L NO_2-N to detect possible alterations in larval nitrogen metabolism when ambient nitrite increases. Treatments were conducted in five replicates. These selected larval stages and nitrite levels were defined from the results obtained for the larval development test.

First, larvae were sampled from 120-L hatchery tanks provided with mechanical and biological filter and artificial heater, filled with 10 salinity brackish water at 30 C. They were acclimated in beakers with water at different nitrite concentration during 2 h. After acclimating, larvae at postmolt–intermolt (A–C) stages (Hayd et al. 2008) were selected and placed into chambers (ca. 30 mL) for 2 h to quantify ammonia-N excretion. General methodology was described in Hayd et al. (2010) and is summarized below.

Cylindrical plastic containers with approximately 30 mL sealed with silicon tablets (Lemos and Phan 2001; Lemos et al. 2003) were used as chambers to incubate animals for ammonia-N excretion measurement. Chambers were individually identified and the exact volume was gravimetrically determined. An orifice of 1.5 mm in the center of the cover enabled the elimination of air bubbles from inside the chamber during closure. Chambers were filled with brackish water prepared

TABLE 1. *Age, dry mass (DM), number of individuals, and biomass : volume ratio in the chambers used to determine total ammonia nitrogen excretion during zoea I, III, VII and IX stages of* Macrobrachium amazonicum *at 30 ± 1 C and 10 salinity.*[a]

Stage	Age[b]	DM (μg)	Individuals/ chamber	Biomass: volume (μg/mL)
Zoea I	0.5	60.0 ± 1.2	12	24.0
Zoea III	3	86.3 ± 1.7	6	17.3
Zoea VII	9	483.0 ± 8.5	3	48.3
Zoea IX	14	700.3 ± 19.2	2	46.7

[a]Results are expressed as mean values ± SD. Number of replicates = 10.
[b]Days after hatching.

using natural seawater and Milli-Q (Millipore) freshwater. The number of individuals used inside the chamber was determined according to individual dry mass (Table 1). The biomass : volume ratio (B:V) was calculated by dividing the total dry mass of individuals by the chamber volume (Table 1). ZI, III, VII and IX were kept in the sealed chambers samples for 2 h. Brackish water (10 salinity) without animals was used as control. Samples and controls were kept in water bath at 30 ± 1 C. After incubation, the tablet was removed, and water was sampled through a plastic cannula by chemically calibrated glass syringes (syringes plus plastic nozzles).

Variation in total ammonia-N (TAN = unionized plus ionized ammonia as nitrogen) contents was calculated by the difference between values obtained in sample (with animals) and control (no animals) units at each tested nitrite concentration. For TAN analysis, the method described by Koroleff (1983) in separate water samples was used. TAN excretion was expressed as individual (μg TAN/individual/h) and dry-mass specific (μg TAN/mg DM/h) rates. Salinity effect on TAN readings was corrected using the factor 1.06 (Koroleff 1983).

Individual dry mass (DM) was determined by groups of 10 larvae at ZI, ZIII, ZVII and ZIX with eight replicates. Individuals were gently rinsed with distilled water, dried with filter paper and separated prior to mass determination. After 48 h at 70 C, dry samples were weighed on a Mettler Toledo AT21 analytical balance, at the nearest 1 μg.

The data were subjected to analyses of detection and exclusion of outliers (Statistica software, v. 6.0, Chicago, IL, USA) with coefficient 1.5. Total excluded data was always lower than 20% of data obtained for each stage. The test was a 4 × 4 factorial design with four nitrite concentrations and four larval stages. For each treatment, five replicates were performed. Normality was tested using the Shapiro–Wilk test and Homocedasticity by Levene's (using SAS 9.0 software). As these assumptions were satisfied, differences among means were tested by two-way ANOVA (F-test) followed by Duncan's multicomparison test. Data of the ammonia excretion, in μg TAN/individual/h and dry mass (in μg) obtained in 0, 0.8 and 1.6 mg/L NO_2-N were logarithmically transformed and then subjected to linear regression analysis (Statistica Software, v.6). Slopes were compared to 1 using a t-test, according to Zar (1999). Differences were considered significant at $P < 0.05$.

Results

Larval Development Test

Mean water temperature was 30.0 ± 0.5 C, dissolved oxygen was 7.0 ± 0.5 mg/L and the pH ranged around 7.8 ± 0.2 TAN (NH_3-N + NH_4-N) mean value was 0.4 ± 0.1 mg/L and nitrite (NO_2-N) was very close to the nominal values of the treatments (generally, the difference was lower than 10%). Values obtained did not differ among the replicates.

Macrobrachium amazonicum larvae developed until metamorphosis at 0, 0.4, 0.8 and 1.6 mg/L NO_2-N. Survival, productivity, mass gain and LSI decreased linearly with increase ambient nitrite concentration (Fig. 1). In concentration 0 mg/L NO_2-N, mean survival was 88.0 ± 5.6%, productivity 26.8 ± 2.4 PL/L, mass gain 627.6 ± 4.6 μg and LSI 8.8 ± 0.1 (Fig. 1). In 1.6 mg/L NO_2-N all mean values were much lower and survival was 65.6 ± 3.0%, productivity 9.4 ± 2.8 PL/L, mass gain 545.0 ± 14.6 μg and LSI 8.20 ± 0.01 (Fig. 1). However, there were no significant differences, by ANOVA, in all parameters at concentration ranging from 0 to 0.8 mg/L

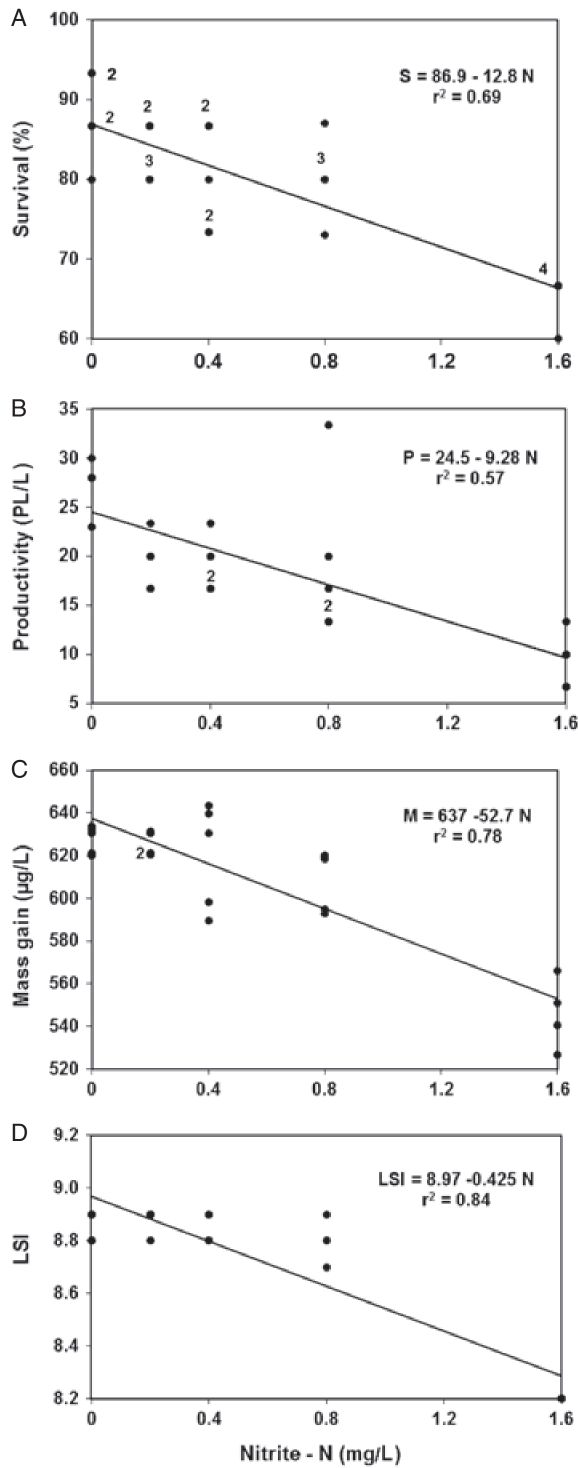

FIGURE 1. *Relationship between survival rate (S) (* $N = 25$, $F = 15.59$, $P < 0.001$*) (A), productivity (P) (* $N = 25$, $F = 10.91$, $P < 0.001$*) (B), mass gain (M) (* $N = 25$, $F = 26.64$, $P < 0.001$*) (C) and larval stage index (LSI) (* $N = 25$, $F = 138.87$, $P < 0.001$*) (D) and nitrite concentration (N). Figures over data-points indicate the number of identical values.*

NO_2-N, whereas they were significantly lower at 1.6 mg/L NO_2-N (Fig. 1).

Ammonia-N Excretion Test

Individual DM increased throughout the ontogenetic development (Table 1). The stages ZI, ZVII and ZIX showed significant differences in individual and mass-specific ammonia excretion between 0 and 1.6 mg/L NO_2-N (Fig. 2). Individual ammonia-N and mass-specific ammonia-N excretion increased in ZI and ZIX, was almost constant in ZIII and decreased in ZVII from 0 to 1.6 mg/L NO_2-N.

The relationships between *M. amazonicum* individual TAN excretion and dry mass at 0, 0.8 and 1.6 mg/L NO_2-N were described, respectively, by the log adjusted allometric equations log E = −2.72 + 0.714 log DM ($N = 24$; $F = 205$; $P < 0.0001$; $r^2 = 0.91$), log E = −2.95 + 0.798 log DM ($N = 19$; $F = 174$; $P < 0.0001$; $r^2 = 0.91$) and log E = −4.12 +1.28 log DM ($N = 18$; $F = 154$ $P < 0.0001$; $r^2 = 0.90$), respectively, in which E = Individual TAN excretion per unit time (µg TAN /individual/h), DM = body dry mass (µg), a and b are constants. The slopes obtained in 0 and 0.8 mg/L NO_2-N were significantly lower than 1 ($t = 4.32$; $P < 0.001$ and $t = 2.78$; $P < 0.01$, respectively), whereas the slope value for 1.6 mg/L NO_2-N was significantly higher than 1 ($t = 3.17$; $P < 0.01$).

Discussion

Complete larval development of *M. amazonicum* occurred in ambient nitrite from 0 to 1.6 mg/L NO_2-N and the dry mass increased during development from ZI onward. Nonetheless, increasing ambient nitrite up to 1.6 mg/L NO_2-N delayed larval development and reduced survival, productivity in PL and mass gain. Besides, there was large variation among replicates. The decrease in all variables was proportional to nitrite concentrations, showing a dose-dependent response. The same pattern was observed for *M. rosenbergii* larvae reared in salinity 12, but in much higher ambient nitrite concentrations, that is, between 0 and 16 mg/L NO_2-N (Mallasen and Valenti 2006). As salinity and other water parameters are similar to the ones used in this study, it suggests that *M. amazonicum* may be more susceptible to nitrite in the environment than *M. rosenbergii* larvae.

The increase in the time to metamorphosis and decrease in mass gain is a generalized response for decapod larvae submitted to pollutants (Sastry 1983). It may occur due to the direct toxic effect of nitrite or physiological adaptations to neutralize nitrite toxicity. Conversely, increasing mortality, as observed in the present work, suggests incapability of many individuals from the same population to adapt to the high environment nitrite concentration. Physiological adaptation may trigger the tradeoff mechanism to drive energy and materials from larvae development and grow to metabolic adjustment to a nitrite-induced stress or to a detoxification mechanism. These processes may cause the reduction on growth and metamorphosis delay observed in *M. amazonicum* in the present work. Endogenous conversion of nitrite in nitrate was observed in *Astacus astacus* (Jensen 1996) and *Penaeus monodon* (Cheng and Chen 2002), whereas *P. clarkii* may accumulate very high concentrations of nitrite in hemolymph (Gutzmer and Tomasso 1985). Further research should be performed to investigate the existence of mechanisms to detoxification in *M. amazonicum*.

A direct toxic effect of nitrite may be related to oxygen transportation. Nitrite oxidizes hemocyanin in methaemocyanin (Tahon et al. 1988), which impairs the binding with oxygen and may decrease blood oxygen transport to tissues. Reduction of oxyhaemocyanin occurred in *M. rosenbergii* (Chen and Lee 1997a, 1997b) and some penaeid shrimp (Chen and Cheng 1995a, 1995b, 1996, 2000; Cheng and Chen 1999) exposed to high environment nitrite. Although it is still controversial if high ambient nitrite-N concentration may induce gas exchange disruptions in crustaceans (Gutzmer and Tomasso 1985; Romano and Zeng 2013), in the present work, dose-dependent response was observed, which may be related to a time-sensitive physiological function, such as oxygen transport (Sowers et al. 2004). Thus, the reduction in the capacity of hemolymph to carry oxygen to the tissues may cause

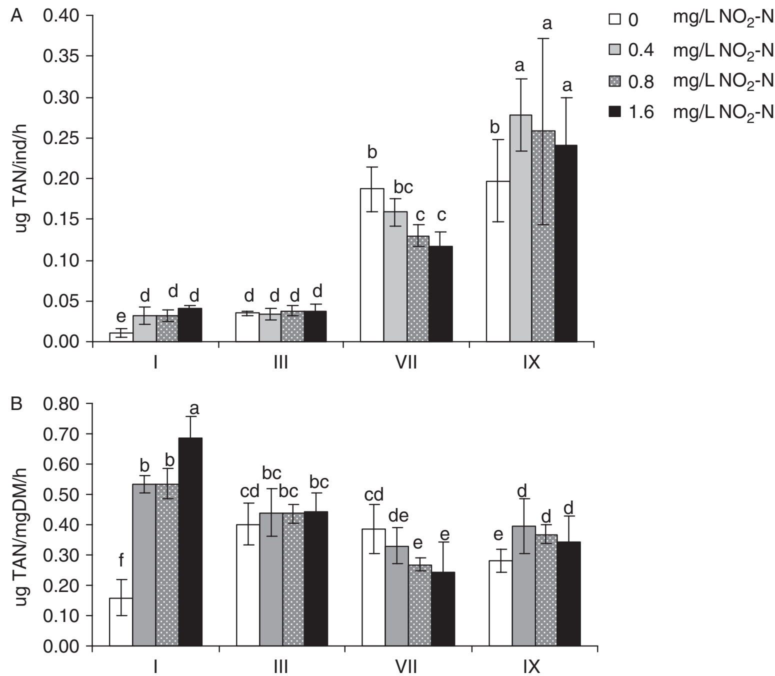

FIGURE 2. *Individual total ammonia nitrogen (TAN) excretion rates (A) and mass-specific rates of TAN excretion (B) during the larval stages of* Macrobrachium amazonicum *exposed to different nitrite concentrations. Results are expressed as means ± SD. (ind = individual, DM = dry mass, I = zoea I, III = zoea III, VII = zoea VII and IX = zoea IX. Statistical comparisons were made among all values regardless the different larval stages. Different letters denote significant differences (* $P < 0.05$*).*

stress in *M. amazonicum* subjected to high environmental nitrite.

Increasing ambient nitrite affected ammonia excretion demonstrating changes in larvae nitrogen metabolism. The effect varied during larval development. Ammonia excretion increased with nitrite concentration in Z I. The mean obtained at 0 mg/L NO_2-N is compatible with the value obtained by Hayd et al. (2010) in normal conditions. Therefore, the higher values obtained at 0.4, 0.8 and 1.6 mg/L NO_2-N are probably due to nitrite effect. This indicates that ambient nitrite should increase protein catabolism in this lecithotrophic phase of *M. amazonicum* (Anger and Hayd 2009; Anger and Hayd 2010) to obtain energy for physiological adaptation to nitrite stress. In Z III, ammonia excretion did not change with nitrite concentration. In this stage, larvae start exogenous feed and fed on freshly hatched *Artemia* nauplii (Araujo and Valenti 2007). Therefore, larvae may change metabolic process and do not use proteins as the main energy source. A similar result was observed in *Ma. japonicus* fed with *Artemia* (Lemos and Rodriguez 1998). In Z VII, ammonia excretion decreased with nitrite concentration. It indicates that protein catabolism decreased. This stage is characterized by a large ingestion of inert diet (Araujo and Valenti 2007), which may increase the lipid or carbohydrate as source of energy. In Z IX, ammonia excretion significantly increased from 0 to 0.4 mg/L NO_2-N. It indicates that ambient nitrite increased protein catabolism again. The changes in catabolism of proteins during ontogenetic development may be due to changes in the feed habit of the larvae, which provide different substrate energetic. It may mask the response to environment nitrite concentration.

The slope obtained for individual excretion/dry weight relationship at 0 and 0.8 mg/L NO_2-N was 0.714 and 0.798, significantly lower than 1. It is almost similar

to values obtained for *M. rosenbergii* larvae (0.904) (Stephenson and Knight 1980) and juveniles (0.628) (Nelson et al. 1977). However, the slope was obtained from the same relationship at 1.8 mg/L NO_2-N was 1.23, significantly higher than 1. In several studies with decapod larvae, slope values above 1 were associated with physiological stress (Anger 2001). Therefore, our results indicated that 1.6 mg/L NO_2-N produces physiological stress, which cause changes in excretion patterns. The same was shown for larvae of the shrimp, *Palaemon serratus*, subjected to stress of temperature and salinity (Yagi et al. 1990), and larvae of the crab, *Cancer irroratus*, (Johns 1981) and lobster, *Homarus americanus*, larvae (Capuzzo and Lacaster 1979) exposed to unfavorable temperatures.

In conclusion, unsuitable ambient nitrite concentrations reduce survival and growth of *M. amazonicum* larvae, delay metamorphosis, and change excretion metabolism. This negatively impacts commercial hatcheries by enlarging the culture cycle and decreasing productivity and individual size of post-larvae. It certainly increases production costs and reduces profit. Thus, indications on a safe value, to be used as a toxicity threshold, may be very useful. Nevertheless, the effects of nitrite on larvae follow a dose-dependent response and show large variability among individuals. This variability may be higher among individuals of different populations. These traits make it difficult to set up an effective maximum value of nitrite concentration for general application. Although in this article we demonstrate that the deleterious effect of nitrite is proportional to the concentration, no significant differences by ANOVA were observed for nitrite concentrations from 0 to 0.8 mg/L, nor metabolism stress measured by the relationship dry mass/ammonia excretion. Thus, this value (0.8 mg/L) may be used as a general reference in commercial hatcheries, which should be applied with careful consideration.

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Literature Cited

Alcaraz, G. C. and C. Carnegas. 1997. Temperature tolerance of *Penaeus setiferus* post larvae exposed to ammonia and nitrite. Aquatic Toxicology 39:345–353.

Anger, K. 2001. Pages 405 *in* The biology of decapod crustacean larvae. Crustacean issues, Volume 14. A.A. Balkema Publishes, Lisse, The Netherlands.

Anger, K. and L. Hayd. 2009. From lecithotrophy to planktotrophy: ontogeny of larval feeding in the Amazon River prawn *Macrobrachium amazonicum*. Aquatic Biology 7:19–30.

Anger, K. and L. Hayd. 2010. Feeding and growth in early larval shrimp, *Macrobrachium amazonicum*, from the Pantanal, Southwestern Brazil. Aquatic Biology 9:251–261.

APHA (American Public Health Association, American Water Works Association and Water Pollution Control Federation). 2005. Standard methods for the examination of water and wastewater, 21st edition. American Public Health Association, Washington, DC, USA.

Araujo, M. C. and W. C. Valenti. 2007. Feeding habit of the Amazon river prawn *Macrobrachium amazonicum* larvae. Aquaculture 265:187–193.

Armstrong, D. A., M. J. Atephhenson, and A. W. Knight. 1976. Acute toxicity of nitrite larvae of the giant Malaysian prawn *Macrobrachium rosenbergii*. Aquaculture 9:39–46.

Capuzzo, J. M. and B. A. Lacaster. 1979. Larval development in the American lobster, changes in metabolic activity and the O:N ratio. Canadian Journal of Zoology 57:1845–1848.

Chen, J. C. and S. Y. Cheng. 1995a. Changes of oxyhaemocyanin and protein levels in the haemolymph of *Penaeus japonicus* exposed to ambient nitrite. Aquatic Toxicology 33:215–226.

Chen, J. C. and S. Y. Cheng. 1995b. Hemolymph oxygen content, oxyhemocyanin, protein levels and ammonia excretion in the shrimp *Penaeus monodon* exposed to ambient nitrite. Journal of Comparative Physiology 164:530–535.

Chen, J. C. and S. Y. Cheng. 1996. Haemolymph osmolality, acid–base balance, and ammonia excretion of *Penaeus japonicus* Bate exposed to ambient nitrite. Archives of Environmental Contamination and Toxicology 30:151–155.

Chen, J. C. and S. Y. Cheng. 2000. Recovery of *Penaeus monodon* from functional anaemia after exposure to sublethal concentration of nitrite at different pH levels. Aquatic Toxicology 50:73–83.

Chen, J. C. and Y. Lee. 1997a. Effects of nitrite on mortality, ion regulation and acid–base balance of *Macrobrachium rosenbergii* at different external chloride concentrations. Aquatic Toxicology 39:291–305.

Chen, J. C. and Y. Lee. 1997b. Effects of nitrite exposure on acid–base balance, respiratory protein, and ion concentrations of giant freshwater prawn *Macrobrachium rosenbergii* at low pH. Archives of Environmental Contamination and Toxicology 33:290–297.

Chen, J. C., K. J. Chen, and J. M. Liao. 1989. Joint action of ammonia and nitrite on *Artemia nauplii*. Aquaculture 77:329–336.

Cheng, S. Y. and J. C. Chen. 1999. Hemocyanin oxygen affinity and the fractionation of oxyhemocyanin and deoxyhemocyanin for *Penaeus monodon* exposed to elevated nitrite. Aquatic Toxicology 45:35–46.

Cheng, S. Y. and J. C. Chen. 2002. Study on the oxyhemocyanin, deoxyhemocyanin, oxygen affinity and acid–base balance of *Marsupenaeus japonicus* following exposure to combined elevated nitrite and nitrate. Aquatic Toxicology 61:181–193.

Cheng, S. Y., L. W. Shieh, and J. C. Chen. 2013. Changes in hemolymph oxyhemocyanin, acid–base balance, and electrolytes in *Marsupenaeus japonicus* under combined ammonia and nitrite stress. Aquatic Toxicology. 130–131:132–138.

Chin, T. S. and J. C. Chen. 1987. Acute toxicity of ammonia to larvae of tiger prawn, *Penaeus monodon*. Aquaculture 66:247–253.

Correia, E. S., S. Suwannatous, and M. B. New. 2000. Flow-through hatchery systems and management. Pages 52–68 in M. B. New and W. C. Valenti, editors. Freshwater prawn culture: The Farming of *Macrobrachium rosenbergii*. Blackwell Science, Oxford, UK.

Das, P. C., S. Ayyappan, J. K. Jena, and B. K. Das. 2004. Nitrite toxicity in *Cirrhinus mrigala* (Ham.), acute toxicity and sub-lethal effect on selected haematological parameters. Aquaculture 235:633–644.

FAO – Food and Agriculture Organization of the United Nations. 2011. Yearbook of fishery statistics, summary tables. Data and statistics unit. FAO http//www.fao.org, Rome, Italy.

Guest, W. C. 1979. Laboratory life history of the palaemonid shrimp *Macrobrachium amazonicum* (Heller, 1862) (Decapoda, Palaemonidae). Crustaceana 37(2):141–152.

Gutzmer, M. P. and J. R. Tomasso. 1985. Nitrite toxicity to the crayfish *Procambarus clarkii*. Bulletin of Environmental Contamination and Toxicology 34:369–376.

Hayd, L., K. Anger, and W. C. Valenti. 2008. The moulting cycle of larval Amazon River prawn *Macrobrachium amazonicum* reared in the laboratory. Nauplius 16(2):55–63.

Hayd, L. and K. Anger. 2013. Reproductive and morphometric traits of *Macrobrachium amazonicum* (Decapoda, Palaemonidae) from the Pantanal, Brazil, suggests initial speciation. Revista de Biologia Tropical 61(1):39–57.

Hayd, L., D. Lemos, and W. C. Valenti. 2010. Ontogenetic variation in ammonia excretion during the early life stages of the Amazon River Prawn, *Macrobrachium amazonicum*. Journal of the World Aquaculture Society 41:107–115.

Jensen, F. B. 1996. Uptake, elimination and effects of nitrite and nitrate in freshwater crayfish (*Astacus astacus*). Aquatic Toxicology 34:95–104.

Johns, D. M. 1981. Physiological studies on *Cancer irroratus* larvae. Effects of temperature and salinity on survival, development rate and size. Marine Ecology Progress Series 5:75–83.

Koo, J. G., S. G. Kim, J. H. Jee, J. M. Kim, S. C. Bai, and J. C. Kang. 2005. Effects of ammonia and nitrite on survival, growth and moulting in juvenile tiger crab, *Orithyia sinica* (Linnaeus). Aquaculture Research 36:79–85.

Koroleff, F. 1983. Determination of ammonia. Pages 150–157 *in* K. Grasshoff, M. Ehrhardt, and K. Kremling, editors. Methods of seawater analysis, 2 edition. Verlag Chemie, Weinheim, Germany.

Kutty, M. N. 2005. Towards sustainable freshwater prawn aquaculture – lessons from shrimp farming, with special reference to India. Aquaculture Research 36:255–263.

Lemos, D. and A. Rodriguez. 1998. Nutritional effects on body composition, energy content and trypsin activity of *Penaeus japonicus* during early postlarval development. Aquaculture 160:103–116.

Lemos, D. and V. N. Phan. 2001. Ontogenetic variation in metabolism. biochemical composition and energy content during the early life stages of *Farfantepenaeus paulensis* (Crustacea, Decapoda, Penaeidae). Marine Biology 138:985–997.

Lemos, D., R. L. V. Jorge, and V. N. Phan. 2003. Simultaneous measurements of oxygen consumption and ammonia – N excretion in embryos and larvae of marine invertebrates. Comparative Biochemistry and Physiology 136A:321–328.

Liao, Y. Y., H. H. Wang, and Z. G. Lin. 2011. Effect of ammonia and nitrite on vigour, survival rate, moulting rate of the blue swimming crab *Portunus pelagicus* zoea. Aquaculture International 19:339–350.

Lu, G. T., Z. Z. Wang, H. P. Wang, and J. Y. Yuan. 2006. Acute toxic effects of $NaNO_2$ on *Portunus trituberculatus* Larvae. Journal of Zhejiang Ocean University (Nature Science) 25(3):244–248.

Madison, B. N. and Y. S. Wang. 2006. Haematological responses of acute nitrite exposure in walleye *Sander vitreus*. Aquatic Toxicology 79(1):16–23.

Mallasen, M. and W. C. Valenti. 1998. Comparison of artificial and natural, new and reused, brackish water for the larviculture of the freshwater prawn *Macrobrachium rosenbergii* in a recirculating system. Journal of the World Aquaculture Society 29(3):345–350.

Mallasen, M. and W. C. Valenti. 2006. Effect of nitrite on larval development of giant river

prawn *Macrobrachium rosenbergii*. Aquaculture 261(4):1292–1298.

Manzi, J. J., M. B. Maddox, and P. A. Sandifer. 1977. Algal supplement enhancement of *Macrobrachium rosenbergii* (De Muan, 1879) larviculture. Journal of the World Mariculture Society 8:207–223.

Marques, H. L. and P. M. C. Moraes-Valenti. 2012. Current status and prospects of farming the giant river prawn (*Macrobrachium rosenbergii* (De Man 1879) and the Amazon river prawn *Macrobrachium amazonicum* (Heller 1862) in Brazil. Aquaculture Research 43:984–992.

Marques, H. L. A., H. P. Barros, M. Mallasen, M. V. Boock, and P. M. C. Moraes-Valenti. 2012. Influence of stocking densities in the nursery phase on the growth of *Macrobrachium amazonicum* reared in net pens. Aquaculture 358–359:240–245.

Moraes-Valenti, P. M. C. and W. C. Valenti. 2007. Effect of intensification on grow-out of the Amazon River prawn *Macrobrachium amazonicum*. Journal of the Word Aquaculture Society 38(4):516–526.

Moraes-Valenti, P. M. C. and W. C. Valenti. 2010. Culture of the Amazon River Prawn *Macrobrachium amazonicum*. Pages 485–501 *in* M. B. New, W. C. Valenti, J. H. Tidwell, L. R. D'Abramo, and M. N. Dutty, editors. Freshwater prawns: biology and farming. Wiley-Blackwell, Oxford, UK.

Nelson, S. G., D. A. Armstrong, A. W. Knight, and H. W. Li. 1977. The effects of temperature and salinity on the metabolic rate of juvenile *Macrobrachium rosenbergii* (Crustacea: Palaemonidae). Comparative Biochemistry and Physiology 56A:533–537.

New, M. B. 1990. Freshwater prawn culture: a review. Aquaculture 88:99–143.

New, M.B. 2002. Farming freshwater prawns, a manual for the culture of the giant river prawn *Macrobrachium rosenbergii*. FAO Fisheries Technical Paper 428, Rome.

New, M. B. 2005. Freshwater prawn farming, global status recent research and a glance at the future. Aquaculture Research 36:210–230.

New, M. B. 2010. History and global status of freshwater prawn farming. Pages 1–11 *in* M. B. New, W. C. Valenti, J. H. Tidwell, L. R. D'Abramo, and M. N. Dutty, editors. Freshwater prawns: biology and farming. Wiley-Blackwell, Oxford, UK.

Romano, N. and C. Zeng. 2007. Acute toxicity of sodium nitrate, potassium nitrate and potassium chloride and their effects on haemolymph composition and gill structure of early juvenile blue seimmer crabs, *Portunus pelagicus*. Environmental Toxicology and Chemistry 26:1955–1962.

Romano, N. and C. S. Zeng. 2009. Subchronic exposure to nitrite, potassium and their combination on survival, growth, total haemocyte count and gill structure of juvenile blue swimmer crabs, *Portunus pelagicus*. Ecotoxicology and Environmental Safety 72(4):1287–1295.

Romano, N. and C. S. Zeng. 2013. Toxic effects of ammonia, nitrite, and nitrate to decapod crustaceans: a review on factors influencing their toxicity, physiological consequences, and coping mechanisms. Reviews in Fisheries Science 21(1):1–21.

Sastry, A. N. 1983. Pelagic larval ecology and development. Pages 214–283 *in* D. E. Bliss, editor. The Biology of Crustacea. Academic Press, New York, USA.

Seneriches-Abiera, M. L., F. Parado-Estepa, and G. A. Gonzales. 2007. Acute toxicity of nitrite to mud crab *Scylla serrata* (Forsskal) larvae. Aquaculture Research 38(14):1495–1499.

Sokal, R. R. and F. J. Holf. 1995. Biometry. Freeman, New York, New York, USA 887.

Sowers, A., S. P. Young, J. J. Isely, C. L. Browdy, and J. R. Tomasso. 2004. Nitrite toxicity to *Litopenaues vannamei* in water containing low concentrations of sea salt or mixed salts. Journal of the World Aquaculture Society 35:445–451.

Stephenson, M. J. and A. W. Knight. 1980. Growth, respiration and caloric content of larvae of the prawn, *Macrobrachium rosenbergii*. Comparative Biochemistry and Physiology 66A:385–391.

Tahon, J. P., D. Van Hoof, C. Vinckier, R. Witters, M. Deley, and R. Lontie. 1988. The reaction of nitrite with hemocyanin of *Astacus leptodactylus*. Biochemistry Journal 249:891–896.

Timmons, M. B., J. M. Ebeling, F. W. Wheaton, S. T. Summerfelt, and B. J. Vinci. 2002. Recirculating aquaculture systems, 2 edition. Northeastern Regional, Aquaculture Center.

Tomasso, J. R. 1994. Toxicity of nitrogeneous wastes to aquaculture animals. Reviews in Fisheries Science 2(4):291–314.

Valenti, W. C. and P. M. C. Moraes-Riodades. 2004. Freshwater prawn farming in Brazil. Global Aquaculture Advocate 7(4):52–53.

Valenti, W. C., W. H. Daniels, M. B. New, and E. S. Correia. 2010. Hatchery Systems and Management. Pages 55–85 *in* M. B. New, W. C. Valenti, J. H. Tidwell, L. R. D'Abramo, and M. N. Kutty, editors. Freshwater Prawns: Biology and Farming. Wiley-Blackwell, Oxford, UK.

Valenti, W. C., L. A. Hayd, M. P. Vetorelli, and M. I. E. G. Martins. 2011. Economic analysis of amazon river prawn farming *Macrobrachium amazonicum* to the markets for live bait and juveniles in Pantanal, Brazil. Boletim do Instituto de Pesca 37(2):165–176.

Wang, W., A. Wang, Y. Zhang, Z. Li, J. Wang, and R. Sun. 2004. Effects of nitrite on lethal and immune response of *Macrobrachium nipponense*. Aquaculture 232:679–686.

Yagi, H., H. J. Ceccaldi, and R. Gaudy. 1990. Combined influence of temperature and salinity on oxygen consumption of the larvae of the pink shrimp, *Palaemon serratus* (Pennant) (Crustacea, Decapoda, Palaemonidae). Aquaculture 86:77–92.

Zar, J. H. 1999. Biostatistical analysis. Prentice-Hall Inc, Eaglewood Cliffs, New Jersey, USA.