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Short Communication

Effects of ammonia and nitrite on food consumption of the Amazon River prawn *Macrobrachium amazonicum* (Heller, 1862) postlarvae

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ABSTRACT. Experiments were carried out to study the effects of ammonia and nitrite on food consumption of *Macrobrachium amazonicum* postlarvae. Postlarvae (average weight of 0.0625 g) were exposed during 21 days to four concentrations of total ammonia (0, 1.05, 2.1 and 4.2 mg. NH_3+NH_4^+ L^{-1}) and four concentrations of nitrite (0, 0.075, 0.15 and 0.30 mg $N\text{-}NO_2^-$ L^{-1}). After the exposure period, six prawns per treatment were maintained individually in 250 mL experimental units to analyze the food consumption as a function of the amount of food offered and the leftovers during a 24 h period. The food consumption presented significant alterations for prawns exposed to all nitrite concentrations and at concentrations of twice the recommended safety level for total ammonia. According to the results obtained, high concentrations of total ammonia and nitrite affect the food consumption of *M. amazonicum* adversely, influencing the species performance in culture systems.

Keywords: *Macrobrachium amazonicum*; water quality; nitrogenous compounds; nutrition; aquaculture

The Amazon River prawn, *Macrobrachium amazonicum* (Heller, 1862), has shown great aquaculture potential in subtropical and tropical regions (Moraes-Valenti & Valenti 2010, Marques & Moraes-Valenti 2012). However, environmental factors are considered determinants for successful prawn cultivation (Boyd & Tucker 2012), and the development of modern aquaculture techniques led to the intensification in cultures of different species. There is an increasing trend in generating nitrogen compounds in these systems (Ballester et al. 2017).

In closed systems, especially in a modern hatchery, nursery, and grow-out systems that use water recirculation and high stocking densities, there is an accumulation of inorganic compounds, especially ammonia (Ballester et al. 2017), and nitrite (Furtado et al. 2016). These compounds are commonly related to the mortality of crustaceans in production systems; ammonia non-ionized form is demonstrably toxic (Armstrong et al. 1978) and influences negatively growth, feeding, survival, and susceptibility to diseases and parasites in prawns and other aquatic organisms (Daniels et al. 1992, Mugnier & Justou 2004). Nitrite is a common toxic substance in culture systems, and the stress may affect hemocyanin synthesis and energy metabolism, resulting in the prawn's death (Li et al. 2019). Moreover, even if they do not cause mortality, they can directly affect growth and food consumption (Tomasso 1994, Campos et al. 2013, Maicá et al. 2018).

In rearing systems, nitrogen residues are common pollutants of the environment, with the excretion of organisms and degradation of food waste being the main sources of these substances (Boyd & Tucker 2012). Diets used in aquaculture contain high protein levels and, as they are digested or degraded, the release of nitrogen compounds increases (Tomasso 1994). Ni-

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trogen compounds occur naturally in aquatic systems; however, if concentrations reach high levels, they may cause mortality or affect the growth of reared organisms (Boyd & Tucker 2012).

Knowledge about food consumption is fundamental for food management to avoid excessive administration, which compromises water quality and insufficient supply of food that affects growth (Soares et al. 2005). Although studies have shown that food consumption varies according to the weight of prawns, different results among species and experimental conditions have been described (González-Peña et al. 2002, Campos et al. 2013, Maicá et al. 2018).

Prawn farms have used feed trays to reduce feed costs and maintain adequate water quality for the development of farmed organisms rather than following feeding tables. However, such practice does not prevent the detriment of water quality, and, in both cases, it is essential to know how water quality influences food consumption (Wasielesky Jr. et al. 2003, Campos et al. 2013). Therefore, this study aimed to evaluate the effects of total ammonia and nitrite on the food consumption of *M. amazonicum* postlarvae.

The experiment was carried out at the Laboratory of Prawn Culture, located at the Federal University of Paraná - Palotina Sector (UFPR by its acronym in Portuguese), and experimental organisms were obtained from the Aquaculture Center of UNESP - CAUNESP / Jaboticabal.

M. amazonicum postlarvae (average weight of 0.0625 g) were exposed during 21 days to four concentrations of total ammonia (control: 0; A: 1.05; B: 2.1; and C: 4.2 mg NH_3+NH_4^+ L^{-1}) and four concentrations of nitrite (control: 0; D: 0.075; E: 0.15; and F: 0.30 mg N-NO_2^- L^{-1}). These values corresponded to control, half the safety level, safety level, and twice the safety level, as determined by Dutra et al. (2016a,b). The total ammonia levels were obtained by adding appropriate volumes of the stock solution of NH_4CL (1000 mg L^{-1}). Nitrite levels resulted from the addition of appropriate volumes of the stock solution of $NaNO_2$ (250 mg L^{-1}).

After the chronic exposure period, the individual food consumption of six postlarvae, randomly selected from each treatment, was analyzed. Prawns were individually placed in 250 mL containers with the respective solutions of nitrogenous compounds; they were kept for 24 h without food. After this period, the media was completely renewed with the corresponding solutions of nitrogenous compounds to keep the total ammonia and nitrite concentrations at the required levels for each experiment. Prawns were fed with a pre-weighed commercial diet (40% crude protein, 8% crude fat, 600 μm pellets), and the feeding rate was 10% of the prawn biomass. During the experimental period, the following water quality parameters were monitored: dissolved oxygen (Oximeter, Alfakit, AT 160), temperature (Thermometer, digital Inconterm), and pH (pHmeter, Luca, 210). Total ammonia concentrations were checked by the colorimetric indophenol method (Koroleff 1976), and nitrite concentrations were determined by the colorimetric method of the Griess reaction (Baumgarten 1996).

After 24 h, the experimental media were siphoned and filtered in a 30 μm mesh, which was subsequently washed to eliminate feces remains. The feed remains were scraped and placed on laminated paper and then oven-dried at 60°C until reaching constant weight (Campos et al. 2013).

Five samples of known weight were placed in an oven at 60°C, until reaching constant weight, to determine the percentage of dry matter of the commercial diet used in the experiment, using a digital electronic scale (AY220, Marte®, precision of 0.01 g). Samples were weighed again, and humidity was determined by the difference between the feed weight before and after drying. Diet leaching was determined in five replicate samples in glass beakers without prawns and with constant aeration. A known feed weight was used, and after 24 h, the experimental media were siphoned and filtered through a 30 μm mesh. The feed remains were scraped and placed on laminated paper and then in an oven at 60°C until a constant weight was reached.

The dry matter consumption of each prawn was determined by the difference between the amount of feed initially provided and the leftovers, considering the initial moisture content, feed leaching, and losses during the siphoning and washing process. The dry matter consumption was calculated using the following formula (Campos et al. 2013):

$$DMC = ((Rprovided \times 0.928) - Rdry) \times 0.55 / \text{weight of prawn}$$

where: DMC: dry matter consumption (g of feed g of $prawn^{-1}$ d^{-1}); Rprovided; amount of feed provided; 0.928 = 92.8% = initial dry matter content of the feed; Rdry: amount of feed after drying; 0.55 = 55.0% = percentage of dry material after the leaching test.

At the beginning and end of the food consumption test, temperature (analog mercury thermometer), dissolved oxygen (AT 170 digital oximeter, Alfakit®), and pH (Yellow Springs pH meter, YSI®) were measured.

Results of water quality variables and prawn food consumption were tested for normality and homoscedasticity of data (Sokal & Rohlf 2012). Since these

Table 1. Mean ± standard deviation of total ammonia (control, A, B and C) and nitrite (control, D, E and F) in the water of the treatments applied to analyze food consumption of *Macrobrachium amazonicum* postlarvae.

	Treatment	Concentration (mg L^{-1})
Ammonia	Control	0.01 ± 0.02
	A	1.05 ± 0.12
	B	2.11 ± 0.07
	C	4.23 ± 0.08
Nitrite	Control	0.02 ± 0.01
	D	0.08 ± 0.10
	E	0.15 ± 0.11
	F	0.30 ± 0.26

assumptions were satisfied, data were subjected to One-Way ANOVA followed by Tukey's test ($\alpha = 0.05$).

During the food consumption test, the mean values ± standard deviation of pH, temperature (°C), and dissolved oxygen (mg L^{-1}) in the experimental units were: 8.22 ± 0.08, 26.8 ± 0.3, and 7.2 ± 0.6, respectively; no significant differences were found among treatments. Total ammonia and nitrite concentrations remained close to the concentrations desired for the chronic toxicity test (Table 1).

For *M. amazonicum* postlarvae exposed to total ammonia, food consumption ranged from 0.0133 to 0.0181 g g^{-1} d^{-1} (Fig. 1) and was affected by the highest ammonia concentration, with treatment C being significantly different ($P < 0.05$) compared to the others. For prawns exposed to nitrite, food consumption ranged from 0.0077 to 0.01690181 g g^{-1} d^{-1} and was significantly affected ($P < 0.05$) in all treatments when compared to the control; treatment F differed significantly from treatments D and E (Fig. 1).

Studies of food consumption are important to determine survival and growth in adverse conditions (e.g. when organisms are submitted to higher levels of nitrogen compounds). Most of the energy originating from food can be channeled for metabolism and survival, while a smaller part would be available for growth and reproduction (Wong et al. 1993).

The toxic effects of ammonia in postlarvae have been observed in several species of prawns. However, few studies reported these effects in *M. amazonicum* (Dutra et al. 2016a). In the present study, adverse effects were only observed with concentrations of twice the safety level of total ammonia for *M. amazonicum*. Previous studies with other species have shown reduced larval development (Mallasen & Valenti 2005), higher mortality (Ostrensky & Wasielesky Jr. 1995), and changes in physiological processes, such as higher oxy-

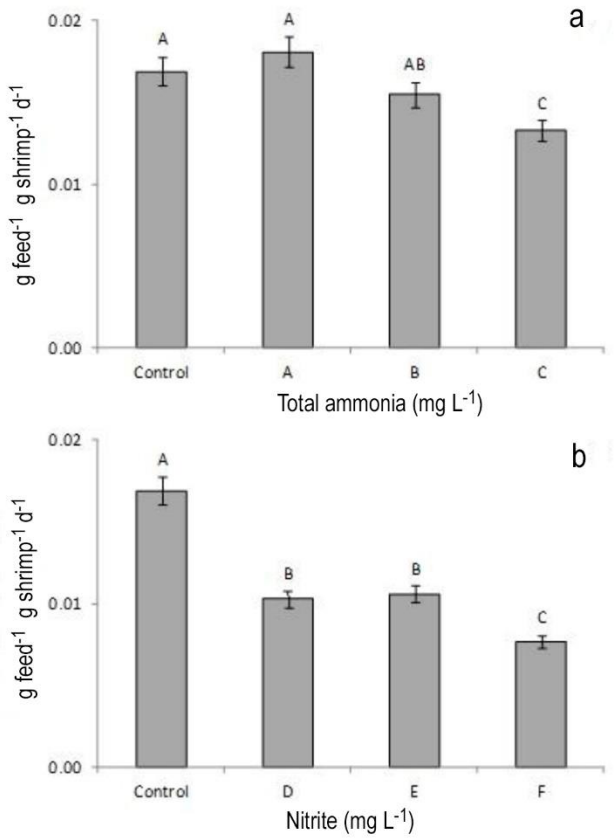

Figure 1. Means ± standard deviation of food consumption of *Macrobrachium amazonicum* postlarvae exposed to different concentrations of a) total ammonia (A, B and C) and b) nitrite (D, E and F). Different superscript letters indicate statistical differences among treatments ($P < 0.05$).

gen consumption (Barbieri 2010) and increase in nitrogen excretion (Romano & Zeng 2013).

Additionally, at high concentrations, the nitrogen compounds may affect physiological processes of the cultured organisms such as osmoregulation and respiration, resulting in low food consumption, low specific growth rate, or even mortality of the prawns (Kuhn et al. 2010, Furtado et al. 2016). Wasielesky Jr. et al. (2003) found no adverse effects on food consumption, exposing *Farfantepenaeus paulensis* for 15 days at concentrations of 0.91, 3.65, and 7.30 mg L^{-1} of total ammonia. On the other hand, Miranda-Filho et al. (2009), analyzing the effect of ammonia on juveniles of the same species in pre-nursery and nursery phases during 75 days, observed a reduction in predation activity and growth.

Evaluating the effects of nitrite concentration on food consumption of *F. paulensis*, Wasielesky Jr. et al. (2003) observed a negative relationship even with only 15 days of exposure. In the present study, for prawns

exposed to nitrite, food consumption was negatively affected in all treatments compared to the control. The action of nitrite on the respiratory pigments and the capacity of oxygen uptake and transport in the hemolymph could be responsible for the decrease in the food consumption rates since they reduce the aerobic metabolism of the prawns (Wasielesky Jr. et al. 2003). Therefore, lower food consumption may compromise growth rates and crop productivity, resulting in financial losses for producers.

As used in this experiment, nitrogen compounds affected food consumption rates of *M. amazonicum* postlarvae and may impact production results. It is also important to highlight that prawns were more sensitive to nitrite than ammonia concentrations.

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