



Long-term ammonia toxicity to the pink-shrimp *Farfantepenaeus paulensis*

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ARTICLE INFO

Article history:

Received 15 March 2006

Received in revised form 29 May 2009

Accepted 5 June 2009

Available online 11 June 2009

Keywords:

Ammonia
Chronic toxicity
Farfantepenaeus paulensis
Feeding response
Growth
Metabolism
Shrimp
Predatory activity

ABSTRACT

Juvenile pink-shrimp *Farfantepenaeus paulensis* were exposed (75 days) to NH₃ (0.016–0.287 mg L⁻¹) under static condition with water renewal every 24 h. Experiments were performed at 20 °C, at a water salinity of 15 ppt, and at pH 7.8. Endpoints analyzed were survival, growth and predation rates. After 75 days of exposure, survival was ≥90% in all concentrations tested. However, growth (carapace length and wet body mass) was reduced after exposure to NH₃ concentrations as low as 0.033 mg L⁻¹, while the relative growth (dry body mass and ash content) was reduced after exposure to the highest NH₃ concentration (0.287 mg L⁻¹). Predatory activity was inhibited after exposure to 0.144 or 0.287 mg L⁻¹ NH₃. Post-larvae exposed (75 days) to 0.301 mg L⁻¹ NH₃ under the same experimental conditions also showed a reduced growth (wet body mass) and relative growth (dry body mass). In addition, they showed decreased body lipids content and increased body glycogen and glucose contents. However, no changes in body protein, chitin and uric acid contents were observed. Also, NH₃ did not affect post-larvae feeding response. Altogether, findings suggest that *F. paulensis* reduces its food intake to limit the internal accumulation of nitrogenous waste products when exposed for long time to high levels of ambient ammonia. As a consequence, shrimp show a marked change in energy metabolism, characterized by a decreased content of body lipids paralleled by an increased content of body carbohydrates, resulting in a significant reduction in growth.

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1. Introduction

Ammonia is naturally excreted by most aquatic animals and arises from the degradation of both natural and anthropogenic organic matter entering the aquatic system. If their production exceeds the capacity of absorption, recycling and purification of the aquatic system, accumulation and effects on biota can be observed.

In aqueous solution, total ammonia (T_{amm}) comprises the highly toxic un-ionized form (ammonia; NH₃) and the generally less-toxic ionized form (ammonium; NH₄⁺), which are in equilibrium. The proportions of each chemical species depend mainly on pH, but also on temperature, salinity and pressure (Whitfield, 1974; Russo, 1985). The higher NH₃ toxicity is mainly because of its high diffusion capacity throughout biological membranes (Cooper and Plum, 1987; Barimo and Walsh, 2005). Therefore, the environmental concentration of this nitrogenous compound is of greatest concern because it easily diffuses across the body surface and is accumulated. Internally, the ionized form likely poses the greatest problem because at physiological pH more than 95% of ammonia exists as NH₄⁺.

At high environmental concentrations of ammonia, disparity exists between the response of fish and crustaceans. Up to 300 μM, ammonia (NH₄Cl) stimulates fish growth (Wood, 2004; Barimo and Walsh, 2005). However, such levels of ammonia can be harmful to aquatic crustaceans, including the pink-shrimp *Farfantepenaeus paulensis*, causing several adverse effects such as reduced growth and survival (Chen and Kou, 1992; Wasielesky et al., 1994; Cavalli et al., 1998; Lin and Chen, 2001). Adverse effects are observed in both cultured crustaceans when kept under inappropriate conditions of water treatment or renewal (Montoya et al., 1999) and wild populations in natural environments heavily contaminated with organic matter (Dyer et al., 2003). Despite the bulk of data available on ammonia effects upon growth and survival rates in crustaceans, information on the mechanisms of ammonia toxicity remain poorly understood (Bermudes and Ritar, 2008). Also, evidences on how crustaceans deal with the excess of ambient or internal ammonia are scarce. Colt and Armstrong (1981) hypothesized that animals may reduce or even stop feeding to limit the internal accumulation of nitrogenous waste products when exposed to high concentrations of ambient ammonia. Regnault (1996) proposed that aquatic crabs could reduce their internal level of ammonia storing this compound as glucosamine (or acetylglucosamine) in tissues involved in the chitin synthesis during air exposure. In turn, Durand et al. (1999) proposed that aquatic crabs exposed to air could show an increased synthesis of aminoacids and uric acid as a strategy to reduce the body levels of ammonia.

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In light of the above, the present study was performed to generate more data for a better understanding of the long-term ammonia toxicity, as well as of the strategies adopted by aquatic crustaceans to deal with long-term exposure to ambient ammonia. To achieve our goal, the long-term effects of ammonia on survival, growth, predatory activity, feeding response, and body composition were evaluated in *F. paulensis*, a native shrimp from Brazilian coastal and marine waters.

2. Material and methods

2.1. Shrimp collection, cultivation and acclimation

Wild mature *F. paulensis* (Pérez-Farfante, 1967) were captured along the Atlantic coast in Southern Brazil, transferred to the laboratory and maintained at the Aquaculture Marine Station of the “Universidade Federal do Rio Grande” (Rio Grande, RS, Southern Brazil). Larvae obtained in the laboratory were reared in seawater at a salinity of 31 ppt and a temperature of 26 °C until the post-larvae and the juvenile stages, following previously described techniques (Marchiori and Boff, 1983; Marchiori, 1996). Juvenile shrimp were used to evaluate the effect of different concentrations of ammonia on survival, growth and predation rates. Based on results obtained with juveniles, a second experiment was performed using post-larvae to evaluate feeding response and body composition of juvenile shrimp (4.2 ± 0.15 mm body length) after exposure to a single effective ammonia concentration under the same experimental conditions used in the experiments with juveniles.

Before testing, post-larvae and juvenile shrimp were gradually acclimated to seawater at a salinity of 15 ppt and a temperature of 20 °C in plastic tanks (200 L) for at least two weeks. These conditions approximate the mean values registered in summer in the Patos Lagoon estuary (Baumgarten et al., 1995, 2001), where post-larvae and juveniles of *F. paulensis* reside until the sub-adult stage (Marchiori, 1996). A salinity of 15 ppt was obtained by diluting seawater with dechlorinated tap water.

2.2. Experiment with juveniles

2.2.1. Survival and growth tests

For the respective survival and growth tests, juvenile shrimp were maintained in 15 L and 70 L plastic tanks at a density of two shrimp per liter. No sediment was provided to prevent the nitrification process (Allan et al., 1990). The total number of juvenile shrimp used was 1440. Tests were run using standard-static procedures with experimental media being renewed every 24 h. Shrimp were fed once a day until satiation with metanauplii and adults of *Artemia* sp. Experimental media were gently and continuously aerated. Feces, carapaces and uneaten food were daily removed by siphon and approximately 100% of the experimental medium was gently changed. Photoperiod was fixed at 12L:12D.

Five NH₃ concentrations and a control (no ammonia addition) were tested. Nominal concentrations tested were 0.025, 0.050, 0.100, 0.200 and 0.400 mg L⁻¹ NH₃. They were selected to bracket the safe level of NH₃ (0.110 mg L⁻¹ NH₃) to prevent adverse effect on growth of *F. paulensis* juveniles (Ostrensky and Wasielesky, 1995). The tested concentrations were obtained from a stock solution (10 g T_{amm} L⁻¹ as NH₄Cl, Merck, USA). Each treatment was carried out in duplicate.

In each experimental unit, water temperature and salinity (refractometer, Digimed, São Paulo, SP, Brazil) were measured at the beginning and at the end of the daily routine. Also, one water sample (200 mL) from each experimental unit was collected at the beginning and at the end of the daily routine. For each sample, water pH was measured (pH meter, Digimed DMPH-2, São Paulo, SP, Brazil) and raised to 10 by addition of NaOH to convert the total ammonia (T_{amm}) into NH₃. Measurement of the NH₃ concentration was then performed using an NH₃-selective electrode (Orion, 95-12, Sergipe, SE, Brazil)

connected to a multimeter (Minipa EP-2002, São Paulo, SP, Brazil). The NH₃ concentration for each water sample was calculated according to the methodology described by Whitfield (1974) and modified by Ostrensky et al. (1992), considering water pH, temperature, salinity and T_{amm} concentration data for the respective water sample.

The number of live and dead shrimp was recorded every 24 h. Shrimp were considered dead when they failed to move even when gently stimulated with a glass pipette. Dead shrimp were removed to prevent fouling. Survival was recorded over 75 days. In fact, shorter periods of exposure to levels of ammonia similar to those employed in the present study were shown to induce significant effects on growth of both post-larvae (Wasielesky et al., 1994) and adults (Cavalli et al., 1998) of *F. paulensis*. Furthermore, the exposure period (75 days) adopted in the present study corresponds to approximately 50% of the time expended by this shrimp species in the Patos Lagoon estuary (Rio Grande, RS, Southern Brazil) until it reaches the sub-adult stage.

Ammonia effects on growth were assessed in 10 juvenile shrimp randomly sampled from each concentration tested at the beginning of the experiment and every 15 days up to 75 days of exposure. The following endpoints were considered: carapace length, wet and dry body mass, and whole body ash, organic matter, inorganic matter, and water content. Carapace length (mm) and wet body mass (mg) were measured according to Neiva and Mistakidis (1966), using a caliper and an electronic scale (Sartorius, Santo André, SP, Brazil), respectively. Shrimp were dried (48 h) in an oven (60 °C), weighed (dry body mass), placed (2 h) in a muffle furnace (550 °C) and then weighed (ash body content) (AOAC, 1965). Using simple proportion calculation, it was possible to determine the percentage of organic and inorganic contents with respect to the wet body mass using dry and ash body content data, respectively. Whole body water content was determined based on wet and dry body mass data.

2.2.2. Predation test

Five juvenile shrimp were randomly sampled from each experimental unit at the end of the exposure period (75 days) and kept under the respective experimental conditions for further 24 h. During this period, they were not fed.

To access the predatory activity, each juvenile shrimp was individually kept in a 250-mL plastic flask containing 200 mL of the respective experimental medium and 25 nauplii of *Artemia* sp. All nauplii used in the tests were randomly collected from the same culture batch reared in our laboratory. Juvenile shrimp were allowed to predate for 1 h and then removed from the test flask. The number of nauplii remaining in the test flask was counted and the predatory activity expressed as the number of *Artemia* consumed per shrimp in 1 h. As five juvenile shrimp were collected from each of the two replicates of the survival and growth tests, the predatory activity was individually measured in ten shrimp for each experimental condition.

2.3. Experiment with post-larvae

2.3.1. Feeding response test

Based on the results obtained in the experiment with juveniles, fifteen post-larvae were kept under control conditions (without addition of ammonia to the water) or exposed to a single ammonia concentration (nominal = 0.400 mg L⁻¹ NH₃) for 75 days, as described for the experiment with juveniles. After the exposure period, surviving shrimp were individually tested for the feeding response induced by L-isoleucine, following the same procedures previously described for *F. paulensis* (dos Santos Fo, 1983; Santos et al., 2000). Feeding response data was expressed in percentage of positive responses to the inducer. Tests were run in duplicate.

After the feeding response test, five shrimp from each replicate ($n = 10$ per experimental condition) were randomly collected and individually blotted dried in paper filter, weighed (dry and wet mass; mg) and frozen (–20 °C) until analysis of body composition.

2.3.2. Body composition analysis

Whole shrimp were thawed, homogenized in distilled water and centrifuged (10,000g; 10 min; 4 °C). For each sample, both supernatant and pellet were collected and separately stored in 1.5 mL microcentrifuge tubes. Supernatant was used for determination of whole body total proteins, uric acid, total lipids, glycogen, glucose, and chitin concentrations. Pellet was used only for chitin determination. All measurements were done using spectrophotometric techniques.

Total proteins were measured using a commercial reagent kit based on the Biuret method (Proteínas Totais®, Doles Reagentes, Goiânia, GO, Brazil). Uric acid concentration was determined using a commercial reagent kit (Ácido úrico®, Labtest Diagnóstica, Lagoa Santa, MG, Brazil) based on the uricase and peroxidase enzymatic assay. Total lipids were measured using a commercial reagent kit (Bioclim, Quibasa Química Básica, Belo Horizonte, MG, Brazil) and following the procedures described by Schmitt and Santos (1993). Glycogen and glucose concentrations were determined using a commercial reagent kit (Glicox®, Doles Reagentes, Goiânia, GO, Brazil) based on the glucose oxidase method and following the procedures described by Nery and Santos (1993). Chitin concentration in both pellet and supernatant was measured following the procedures described by Tsuji et al. (1969) and using D-glucosamine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) as standard. Results from both pellet and supernatant were combined. All results were expressed in mg g⁻¹ wet mass.

2.4. Data analysis

Data for water chemistry and biological endpoints were expressed as mean ± SE, except for the feeding response data that were expressed in percentage. For data from the experiment with juveniles, one-way analysis of variance (ANOVA) followed by the Duncan's test was used to detect possible significant differences among treatments for each time of exposure. Predatory activity data were log-transformed to meet the ANOVA assumptions for data normality and homogeneity of variances (Zar, 1999). For data from the experiment with post-larvae, mean values were compared using the Student *t* test for independent samples, except for the feeding response data that were analyzed using the Chi-square test. All statistical analyses were performed using Statistica software (StatSoft, Inc., Tulsa, OK, USA). In all cases, significance level adopted was 95% ($\alpha=0.05$).

3. Results

3.1. Experiment with juveniles

For the same time of exposure, no variation in water temperature and pH was observed in each experimental unit at the beginning and at the end of the daily routine. Also, no difference was observed between the two replicates for each experimental condition. Therefore, only one daily mean value was calculated for each experimental condition. These mean values were compared over the exposure period (75 days). No difference was observed between the different times of exposure for the same experimental condition or between treat-

Table 1

Nominal and measured NH₃ concentrations for the different treatments tested.

Nominal concentration	Mean measured concentration
0 = control	0.016 ± 0.0005 ^a
0.025	0.033 ± 0.0010 ^b
0.050	0.047 ± 0.0015 ^c
0.100	0.078 ± 0.0028 ^d
0.200	0.144 ± 0.0045 ^e
0.400	0.287 ± 0.0088 ^f

Data are expressed as mean ± SE. Values are expressed in mg L⁻¹ NH₃. Different letters indicate significant ($P<0.05$) different mean values.

Table 2

Biological endpoints measured in *Farfantepenaeus paulensis* juveniles kept under control conditions for up to 60 days.

Time (days)	Biological endpoints			
	Carapace length (mm)	Wet body mass (mg)	Dry body mass (mg)	Ash body content (mg)
0	6.4 ± 0.13 ^a	158.0 ± 7.51 ^a	39.5 ± 2.19 ^a	6.3 ± 0.57 ^a
15	7.1 ± 0.13 ^b	218.3 ± 12.35 ^b	53.5 ± 3.22 ^b	8.3 ± 0.41 ^b
30	8.1 ± 0.22 ^c	342.2 ± 28.92 ^c	85.4 ± 7.27 ^c	13.3 ± 1.09 ^c
45	9.3 ± 0.22 ^d	536.0 ± 40.63 ^d	140.7 ± 11.52 ^d	20.4 ± 1.64 ^d
60	10.0 ± 0.10 ^e	638.2 ± 39.12 ^e	166.9 ± 11.83 ^e	23.7 ± 1.67 ^e

Data are mean values ± SE ($N=10$). Different letters indicate significant ($P<0.05$) different mean values.

ments at the same exposure time (data not shown). Therefore, only one mean value of water temperature (19.8 ± 0.08 °C; $N=450$) and water pH (7.84 ± 0.006; $N=450$) was calculated for the whole period of exposure.

For NH₃ concentration, a daily mean value was calculated based on the data obtained for the two water samples collected for each replicate, i.e., one at the beginning and one at the end of the daily routine. Based on these values, no difference was observed between replicates (data not shown). Therefore, only one mean value was calculated for each exposure time and experimental condition over the time of exposure. Since no difference was observed between daily mean values, an overall mean value was calculated for each

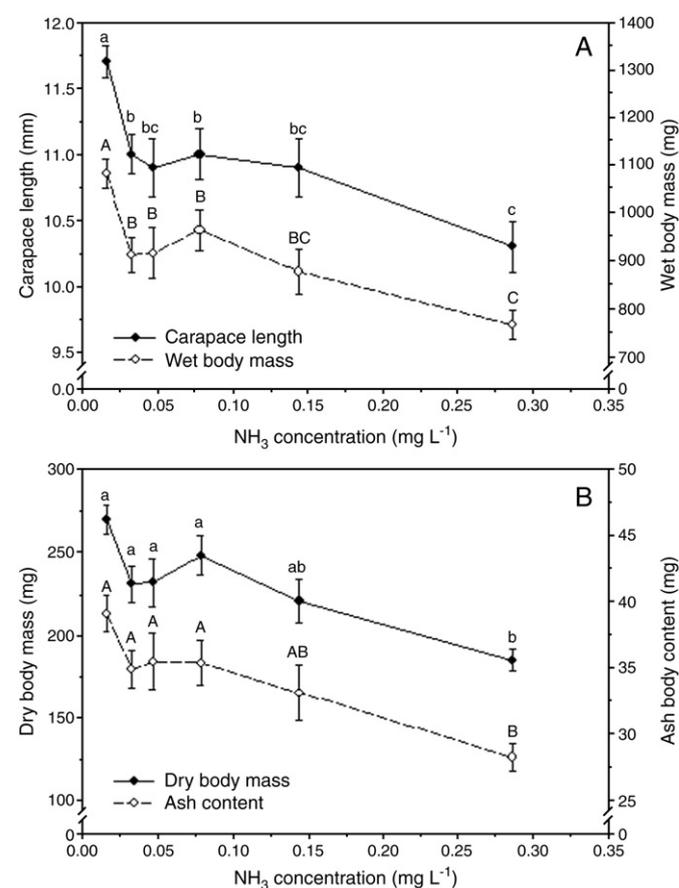


Fig. 1. Biological endpoints measured in *Farfantepenaeus paulensis* juveniles after 75 days of exposure to different NH₃ concentrations. (A) Carapace length (●; left axis) and wet body mass (○; right axis). (B) Dry body mass (●; left axis) and ash body content (○; right axis). Data are expressed as mean ± SE ($N=10$). Different letters indicate significant different ($P<0.05$) mean values between treatments for each endpoint analyzed.

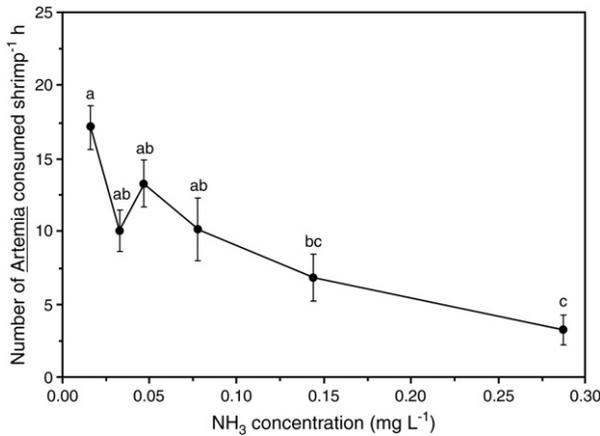


Fig. 2. Predatory activity of *Farfantepenaeus paulensis* juveniles after 75 days of exposure to different NH₃ concentrations. Data are expressed as mean \pm SE ($N = 10$). Different letters indicate significant different ($P < 0.05$) mean values between treatments.

experimental condition considering the whole period of exposure ($N = 75$). The actual mean measured concentrations were deviated by 6 to 32% of the nominal concentrations. The mean NH₃ concentration in the control treatment was 0.016 mg L⁻¹ NH₃ (Table 1).

In all treatments, mortality was negligible ($\leq 10\%$) after 75 days of exposure. Growth of control shrimp over the experiment corresponded to 82.8% and 585.4% when measured as carapace length and wet body mass, respectively.

There were no differences between treatments for any biological endpoint measured prior to day 75, including at day 0, i.e., at the beginning of the experiment (data not shown). The mean value for control shrimp at each exposure time is shown in Table 2. However, a growth inhibition (carapace length and wet body mass) was observed in shrimp exposed to NH₃ for 75 days, with respect to those kept under control conditions. Mean carapace length was shorter by 6.8, 6.0, 6.8 and 12.0% at 0.047, 0.078, 0.144 and 0.287 mg L⁻¹ NH₃, respectively. Mean wet body mass was lower by 15.8, 15.4, 11.0, 19.0 and 29.3% at 0.033, 0.047, 0.078, 0.144 and 0.287 mg L⁻¹ NH₃, respectively (Fig. 1A).

Regarding relative growth, measured as dry body mass or ash body content, a difference was detected only for shrimp exposed for 75 days to the highest ammonia concentration tested (0.287 mg L⁻¹ NH₃). Mean dry body mass and ash content were lower by 31.3% and 27.9%, respectively (Fig. 1B). In contrast, no NH₃ effects on whole body water, organic and inorganic contents were observed, even after 75 days of

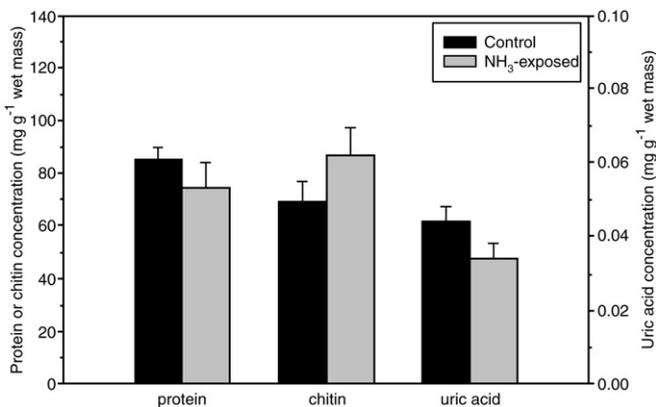


Fig. 3. Body content of proteins, chitin and uric acid in *Farfantepenaeus paulensis* post-larvae exposed to NH₃ (0.301 mg L⁻¹ NH₃) for 75 days. Data are expressed as mean \pm SE ($N = 10$). * indicates significant different ($P < 0.05$) mean values between treatments for each parameter analyzed.

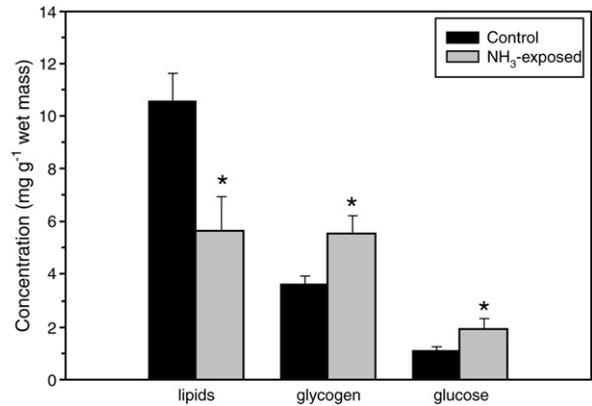


Fig. 4. Body content of lipids, glycogen and glucose in *Farfantepenaeus paulensis* post-larvae exposed to NH₃ (0.301 mg L⁻¹ NH₃) for 75 days. Data are expressed as mean \pm SE ($N = 10$). * indicates significant different ($P < 0.05$) mean values between treatments for each parameter analyzed.

exposure to NH₃ concentrations as high as 0.287 mg L⁻¹ (data not shown). The overall mean (\pm SE) percentage values for control shrimp were 85.1% (± 0.12), 75.6% (± 0.12) and 14.9% (± 0.11), respectively.

The predatory activity of *F. paulensis* juveniles was not affected until after 60 days of NH₃ exposure. For control shrimp, the mean number (\pm SE) of Artemia predated per hour after 15, 30, 45, and 60 days of NH₃ exposure was 0.78 (± 0.35), 2.36 (± 0.61), 3.74 (± 0.67), and 3.99 (± 0.89), respectively. However, an inhibition of the predatory activity was observed after 75 days of exposure to 0.144 or 0.287 mg L⁻¹ NH₃ (Fig. 2).

3.2. Experiment with post-larvae

Based on the results obtained with juveniles, post-larvae were kept under control conditions or exposed to the highest ammonia concentration tested in the first experiment (0.4 mg L⁻¹ NH₃). As described for the experiment with juveniles, only one mean value of water temperature (20.1 ± 0.07 °C; $N = 150$) and water pH (7.80 ± 0.01 ; $N = 100$) was calculated for the whole period of exposure. NH₃ concentration was determined as described for the experiment with juveniles. Also, an overall mean value was calculated for the control (0.012 ± 0.002 mg L⁻¹ NH₃) and experimental (0.301 ± 0.0077 mg L⁻¹ NH₃) conditions considering the whole period of exposure ($N = 75$). The actual mean measured concentration deviated by 25% of the nominal concentration tested.

After 75 days of test, mortality corresponded to 13.3 and 26.7% in control and ammonia-exposed shrimp, respectively. Wet and dry body mass of ammonia-exposed shrimp (456 ± 36 and 120 ± 24 mg, respectively) was lower than that of control shrimp (613 ± 44 and 157 ± 22 mg, respectively). A 25.6 and 23.6%-reduced growth was observed, respectively.

Positive feeding response induced by L-isoleucine was not different in control ($65.4 \pm 11.6\%$) and ammonia-exposed shrimp ($63.6 \pm 9.1\%$). Also, no difference was observed in the body content of proteins, chitin and uric acid between control and ammonia-exposed shrimp (Fig. 3). However, a lower body content of lipids (decrease of 46.1%) and higher body contents of glycogen (increase of 53.0%) and glucose (increase of 70.5%) were observed in ammonia-exposed shrimp when compared to the levels found in control shrimp (Fig. 4).

4. Discussion

Data on acute toxicity and effects of ammonia on shrimp kept under laboratory conditions are largely available in the literature. However, long-term ammonia toxicity is not so well documented. As

discussed below, findings from the present study help to better understand the mechanisms involved in long-term ammonia toxicity, as well as the possible strategies adopted by shrimp to deal with the high levels of ambient ammonia.

The mean NH_3 concentrations observed in the control treatments in the present study (0.016 and 0.012 mg L^{-1} NH_3 with juveniles and post-larvae, respectively) is similar to that found in summer (0.012 mg L^{-1} $\text{NH}_3 = 0.61 \text{ mg L}^{-1}$ NH_4^+ at the water temperature, salinity and pH employed in the present study) in areas non impacted by organic matter in the Patos Lagoon estuary (Baumgarten et al., 1995; Niencheski et al., 2004). This finding indicates that control conditions adopted in laboratory are quite similar to those found in summer at the Patos Lagoon estuary.

Mean NH_3 concentrations measured in the different treatments ranged from 0.033 to 0.301 mg L^{-1} NH_3 , deviating 6 to 32% from the nominal concentrations tested. These deviations could be related to water pH variation (Whitfield, 1974; Erickson, 1985; Ostrensky et al., 1992), ammonia stability in aqueous environment (Ostrensky, 1991), and changes in the excretory rate of shrimps exposed to higher ammonia concentrations (Wickins, 1976; Chen et al., 1993).

The low mortality observed in *F. paulensis* juveniles in the present study appears to be a normal response of shrimps from the Penaeidae family to ammonia exposure (Wasielesky et al., 1994; Cavalli et al., 1998; Lin and Chen, 2001). In turn, the higher mortality rate observed in post-larvae exposed to ammonia is in agreement with previous data showing a higher ammonia sensitivity of post-larvae compared to juvenile *F. paulensis* (Ostrensky et al., 1992; Ostrensky and Wasielesky, 1995).

Regarding growth, data from the present study demonstrated that the long-term ammonia exposure inhibited the shrimp growth expressed as either wet weight gain or carapace length increase in both post-larvae and juveniles. It is interesting to note that the effect on wet weight was more marked than on carapace length in juveniles. A similar response was observed in *F. japonicus* juveniles exposed to ammonia (Chen and Kou, 1992). These findings suggest that ammonia has a higher inhibition effect on physiological processes involved in body mass increase than on those related to molting. This could be related to the infrequent molting of the exoskeleton versus the continual accumulation of wet body mass.

The lack of change in water, organic and inorganic content in juvenile shrimp exposed to NH_3 for 75 days indicates that shrimp growth inhibition would be related to a general effect of ammonia on shrimp energy metabolism. In fact, NH_3 has shown to have a negative effect on the oxidative metabolism (Lehninger, 1990). The observed change in body composition of *F. paulensis* post-larvae after long-term exposure to a high concentration of ambient ammonia (0.301 mg L^{-1} NH_3) corroborates this idea. This change was characterized by a marked reduction in body lipid content paralleled by a significant increase in body carbohydrate (glycogen and glucose) content. This finding suggests a higher use of carbohydrates as energy source to cope with the stress induced by the long-term NH_3 exposure. In this case, the increased carbohydrate levels would be explained by a higher gluconeogenesis rate, having glycerol from lipid oxidation as substrate for glucose synthesis.

In crustaceans, it is reported that exposure to high levels of ambient NH_3 affects osmoregulation (Lin et al., 1993), inducing to an increased energy expenditure associated with processes involved in ionic and osmotic regulations (Spaargaren, 1982; Young-Lai et al., 1991; Chen and Cheng, 1993b,c; Chen et al., 1993). However, NH_3 exposure did not seem to affect osmoregulation in *F. paulensis*. This statement is based on the fact that NH_3 did not significantly affect water and inorganic contents in juvenile shrimp. This lack of NH_3 effect on osmoregulation could be related to the fact that experiments were performed on shrimp acclimated to water at salinity 15 ppt, a condition where *F. paulensis* juvenile is osmoconforming its body fluids (Wasielesky et al., 1995).

Also, the total amount of energy available for shrimp growth exposed to ammonia could be decreased due to an increased expenditure associated with a possible switch from ammoniotelic to uricotelic pattern as a mechanism of ammonia detoxification (Chen and Cheng, 1993a,b,c; Chen et al., 1994; Chen and Lin, 1995). However, the strategy to convert ammonia into uric acid or aminoacids to reduce the internal levels of ammonia, as proposed by Durand et al. (1999), seems not to be used by *F. paulensis*. This statement is based on the fact that body contents of uric acid and proteins were not significantly altered in post-larvae exposed to a high concentration of NH_3 ammonia for 75 days. The strategy to reduce internal levels of ammonia by storing this nitrogen compound as glucosamine or acetylglucosamine (Regnault, 1996), the substrate for chitin synthesis, also seems not to be used by *F. paulensis*. In fact, no change in the body content of chitin was observed in post-larvae exposed to NH_3 .

In addition to a possible increased amount of energy required to cope with the stress induced by chronic exposure to ambient NH_3 , the total amount of energy available for shrimp growth could be also decreased as a result of the negative effect of NH_3 on food intake. Short-term exposure (15 days) to up $\sim 0.15 \text{ mg L}^{-1}$ NH_3 was reported to have no significant effect on food intake in *F. paulensis* (Wasielesky et al., 2003). However, data on predatory activity of juveniles in the present study showed that long-term exposure (75 days) to 0.144 mg L^{-1} NH_3 or a higher concentration induced a significant decrease in food intake, leading to a likely reduced amount of energy available for the whole shrimp metabolism. At this point, one should assume that the reduced predatory activity could be an indirect effect of NH_3 associated with its negative impact on juvenile shrimp growth. In fact, a significant positive relationship between carapace length ($y = 2.21x - 15.93$; $R^2 = 0.86$) or wet body weight ($y = 0.0123x - 2.4375$; $R^2 = 0.95$) and predatory activity was observed in control juvenile shrimp. However, the negative effect of NH_3 on growth of juveniles only explains $\sim 50\%$ of the reduction in predatory activity of shrimp exposed for 75 days to the highest NH_3 concentration tested (0.287 mg L^{-1} NH_3). Therefore, other causes must be implicated in the observed response.

Data from the experiment with post-larvae indicate a lack of a direct and negative effect of NH_3 on shrimp ability to sense and locate the presence of food in the water. This statement is based on the fact that the feeding response of post-larvae to the major inducer of feeding in *F. paulensis*, i.e. the aminoacid L-isoleucine (dos Santos Fo, 1983), was not modified by long-term exposure to a high ambient NH_3 concentration (0.301 mg L^{-1}). Unfortunately, data generated does not allow us to evaluate a possible negative NH_3 effect on the ability of shrimps to move and capture the prey.

In light of the above, the observed long-term effects of NH_3 on *F. paulensis* growth could be explained only by considering the reduced food intake, a response of shrimp to limit the internal accumulation of nitrogenous waste products when exposed to high levels of ambient ammonia, as early proposed by Colt and Armstrong (1981). This hypothesis would be in line with the possible occurrence of an alkaline tide followed by a higher rate of ammonia excretion in crustaceans, as described for fish after voluntary feeding (Buckling and Wood, 2008). Unfortunately, the occurrence of this phenomenon has never been evaluated in crustaceans. Therefore, future experiments need to be performed to confirm the proposed hypothesis.

Acknowledgments

The authors would like to thank Dr. Ronaldo O. Cavalli, Dr. Márcio R. Milani and Nérile Troca da Cunha from Universidade Federal do Rio Grande (Rio Grande, RS, Brazil) for their assistance during this work. The authors gratefully acknowledge the financial support from the Brazilian National Research Council (CNPq). Adalto Bianchini is a research fellow from the Brazilian CNPq (# 300906/2006-4).

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