BOLETIM DO INSTITUTO DE PESCA
ISSN 1678-2305 online version
Scientific Note

LETHAL AND SUBLETHAL EFFECTS OF AMMONIA IN *Deuterodon iguape* (Eigenmann 1907), POTENTIAL SPECIES FOR BRAZILIAN AQUACULTURE

Edison Barbieri[1]*
Rafaela Marina Lenz[2]
América Andrade de Nascimento[2]
Genésio Lopes de Almeida[3]
Larissa Yoshida Roselli[4]
Marcelo Barbosa Henriques[1]

[1]Instituto de Pesca, Agência Paulista de Tecnologia dos Agronegócios – APTA, Secretaria da Agricultura e Abastecimento, Governo do Estado de São Paulo. Av. Professor Wladimir Besnard, s/n°, CP 43, CEP 11990-000, Cananéia, SP, Brasil. E-mail: edisonbarbieri@yahoo.com.br (corresponding author).

[2]Universidade Federal da Fronteira Sul, Rodovia BR 158, s/nº, Zona Rural, CEP 85301-970, Laranjeiras do Sul, PR, Brasil.

[3]Instituto de Pesca, Programa Institucional de Bolsas de Iniciação Científica – PIBIC, Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPQ,

[4]Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, Programa de Pós-graduação em Biodiversidade, Praça Infante Dom Henrique, s/nº, Parque Bitaru, CP 73601, CEP 11380-972, São Vicente, SP, Brasil.

Received: August 16, 2018
Approved: November 29, 2018

ABSTRACT

The cultivation of the lambari, *Deuterodon iguape*, supplies much of the Brazilian market with live bait for sport fishing. The high densities used to maximize production can increase the concentration of ammonia. In order to evaluate the sublethal and lethal effects of different concentrations of ammoniacal nitrogen (non-ionized ammonia plus ionized ammonia), *D. iguape* were exposed to this xenobiotic. The LC_{50} values for 24, 48, 72, 96 h of ammonia-N were 6.17, 5.57, 3.88 and 2.90 mg L^{-1} at 23°C. The LC_{50} values of 24, 48, 72, 96 h of NH_3-N (non-ionized ammonia with nitrogen) were 0.015; 0.013; 0.009; 0.007 mg L^{-1}. The specific oxygen consumption increased at the ammonia-N concentrations tested. The values for the concentrations of 0.1; 0.25; 0.5 and 1.0 mg L^{-1} were: 0.25, 0.33; 0.31 and 0.44 mlO_2 g^{-1} h^{-1}. At the concentration of 1.0 mg L^{-1} of ammonium chloride there was a 41.33% increase in the consumption level in relation to the control. Ammonia excretion also increased with increasing ammoniacal nitrogen concentration. The fish excreted, on average, 0.0320, 0.0364, 0.0368 and 0.0370 mg/g/h of ammonia. Comparing these results with the means of ammonia excretion of the control (0.0 mg L^{-1}), it was observed that these values represent an increase in the excretion of 146%, 177%, 189% and 184%, respectively. After 24 h of exposure to ammonia, the O:N ratio decreased by 43.46%. Our results indicate pronounced metabolic effects and increased toxicity with increasing ammonia concentrations. We recommend avoiding concentrations higher than 0.25 mg L^{-1} NH_3-N in the culture water.

Key words: Lambari; $LC_{50;}$ Nitrogenous compounds; O:N ratio; aquaculture.

EFEITO LETAL E SUBLETAL DA AMÔNIA SOBRE O LAMBARI (*Deuterodon iguape*, Eigenmann 1907), ESPÉCIE POTENCIAL PARA A AQUICULTURA BRASILEIRA

RESUMO

O cultivo do lambari, *Deuterodon iguape*, abastece grande parte do mercado brasileiro com iscas vivas para a pesca esportiva. As altas densidades utilizadas para maximizar a produção podem aumentar a concentração de amônia. A fim de avaliar os efeitos subletais e letais de diferentes concentrações de nitrogênio amoniacal (amônia não ionizada mais amônia ionizada), *D. iguape* foram expostos a este xenobiótico. Os valores de CL_{50} para 24, 48, 72, 96 h de amônia-N foram 6,17; 5,57; 3,88 e 2,90 mg L^{-1} a 23 °C. Os valores de CL_{50} de 24, 48, 72, 96 h de NH_3-N (amônia não ionizada) foram de 0,015; 0,013; 0,009 e 0,007 mg L^{-1}. O consumo específico de oxigênio aumentou nas concentrações de nitrogênio amoniacal testadas. Os valores para as concentrações de 0,1; 0,25; 0,5 e 1,0 mg L^{-1} foram: 0,25, 0,33; 0,31 e 0,44 mlO_2 g^{-1} h^{-1}. Na concentração de 1,0 mg L^{-1} de cloreto de amônio houve aumento de 41,33% no nível de consumo em relação ao controle. A excreção de amônia também aumentou com o aumento da concentração de nitrogênio amoniacal. Os peixes excretaram, em média, 0,0320; 0,0364; 0,0368 e 0,0370 mg/g/h de amônia. Comparando esses resultados com as médias de excreção de amônia do controle (0,0 mg L^{-1}), observou-se que esses valores representam um aumento na excreção de 146%, 177%, 189% e 184%, respectivamente. Após 24 h de exposição à amônia, a relação O: N diminuiu em 43,46%. Nossos resultados indicam efeitos metabólicos pronunciados e aumento da toxicidade com o aumento das concentrações de amônia. Recomendamos evitar concentrações superiores a 0,25 mg L^{-1} de NH_3-N na água da cultura para o *D. iguape*.

Palavras-chave: Lambari; LC_{50}; Compostos nitrogenados; relação O:N; aquacultura.

Abbreviations

Ammonia-N = $NH_4^+ + NH_3$
LC_{50} = Median Lethal Concentration Test
NH_4^+ = ionized ammonia
NH_3: non-ionized ammonia
O:N ratio = Oxygen Nitrogen Ratio
TAN = Total Ammonia Nitrogen

INTRODUCTION

There are few studies on the toxicity and sublethal effects of ammonia for neotropical freshwater fish species in Brazil, justified by the predominant cultivation of exotic species, mainly Nile tilapia (Martinez et al., 2006). For this reason studies that approach the effect of the ammonia in neotropical fishes that have potential for aquaculture are very important.

Several authors have agreed to call NH_4^+ ionized ammonia, and NH_3 non-ionized ammonia, and the sum ($NH_4^+ + NH_3$) is called total ammonia (Arana, 1997). The increase of the concentration of total ammonia in crop systems is one of the most damaging factors in production, being able to reach sublethal or lethal levels in static or recirculation systems (Medeiros et al., 2016). Therefore, it is fundamental to determine the toleration limits of commercially valuable aquatic organisms, in relation to this compound for the technical and economic viability of intensive crops (Damato and Barbieri, 2011).

Among the native species with potential for Brazilian aquaculture, some small species of the Characidae family, popularly known as lambaris, have received more attention due to zootechnical characteristics favorable for production in captivity (Silva et al., 2011a). The lambari *Deuterodon iguape* is an endemic species of small rivers and coastal streams of the Atlantic forest with great market potential, both for human consumption and for use as bait in sport fishing (Silva et al., 2011b). However, there is no information in the literature on the effects of ammonia at different concentrations for this species, although it has already been widely cultivated in Brazil.

Exposure to ammonia in an aquatic environment produces many physiological changes in fish, including changes in their metabolism (Cavero et al., 2004, Barbieri and Doi, 2012). The metabolic rate of an organism is a useful and sensitive indication of their daily energy consumption. Therefore, in aerobic organisms, quantifying the rate of oxygen consumption may be directly associated with the amount of energy released from the food substrate oxidation. Based on the amount of oxygen consumed by an animal over a period of time, it is possible to calculate the energy spent during the same period to maintain its vital processes (Bosisio et al., 2017).

According to Person-Le Ruyet et al. (1995), ammonia concentration above 0.3 mg L^{-1} can be lethal to freshwater fish, although fish such as pirarucu, *Arapaima gigas* tolerate concentrations above 25 mg L^{-1} (Cavero et al., 2004). The effects of lethal ammonia on fish have been studied in *Salmo salar* (Fivelstad et al., 1993), *Arapaima gigas* (Cavero et al., 2004), *Colosoma macropomum* (Croux et al. 2004), *Cirrhinus mrigala* (Das et al., 2004), *Cyprinus carpio* (Tilak et al., 2007), *Oncorhynchus mykiss* (Brinkman et al., 2009), *Stizostedion vitreum* (Bergerhouse, 2011), *Hyphessobrycon callistus* (Damato and Barbieri, 2011), *Rhandia quelen* (Miron et al., 2011), *Piaractus mesopotamicus* (Barbieri and Bondioli, 2013), *Amphiprion ocellaris* (Medeiros et al., 2016) and *Astyanax ribeirae* (Barbieri et al., 2018).

The evaluation of oxygen consumption and ammonia excretion in fish was used, for example, to study the toxic effects caused by nanoparticles (Rezende et al., 2018), saninity (Bosisio et al., 2017), ammonium chloride (Damato and Barbieri, 2011; Barbieri and Doi, 2012), heavy metal (Martinez et al., 2013; Ferrarini et al., 2016; Barbieri, 2007), pesticide (Ruiz-Hidalgo et al., 2016) and a variety of toxic substances (Campos-Garcia et al., 2015).

Particularly in Brazil, the cultivation of *D. iguape* was intensified due to the market demand and the availability of water bodies and their breeding facility. In an intensive culture system, ammonia is a common pollutant resulting from the excretion of domesticated animals and the mineralization of organic debris such as unconsumed food and feces (Medeiros et al., 2016). The accumulation of ammonia can reduce growth, increase oxygen consumption and N-ammonia excretion, and even cause increased mortality (Barbieri and Bondioli, 2013).

In order to guarantee the success of the intensive production of *D. iguape*, determining the toxicity and effects of ammonia are of fundamental importance to verify the sensitivity of this species to parameters of water quality, such as nitrogen residues, since these are limiting factors for the survival and growth of confined fish. Therefore, the objective of the present study was to estimate the toxicity and the ammonia effect on oxygen consumption and ammonia excretion of *D. iguape*, increasing the knowledge concerning the culture of this fish that has excellent market potential in Brazil.

MATERIALS AND METHODS

This research is in accordance with the ethical principles in animal experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and was approved by the Animal Experimentation Ethics Committee of the Fisheries Institute (process number 06/2016).

Acute toxicity assays

The acute toxicity of ammonia-N and NH_3-N in lambaris *D. iguape* was evaluated in the laboratory of the Fisheries Institute (SP) exposed to different concentrations of these chemicals for a period of up to 96 h. A total of 120 fish were used, with a mean body weight of 6.5 ± 0.6 g, obtained from a local fish farmer.

During the experiment, dechlorinated supply water, previously filtered using a 0.25 µm pore cellulose ester filter was used with the aid of a vacuum pump. Three replicates of groups of 5 individuals were exposed to each of the following concentrations of ammonia-N: 0.0; 0.1; 0.5; 1; 2.5; 5; 10 and 15 mg L^{-1}. The experiments were carried out in glass tanks with a volume of 50 liters each in a

static system, aerated by blowers connected to porous stones. The photoperiod was kept in the 12/12 light/dark cycle.

The 10,000 mg L^{-1} stock solution of ammonia-N at was prepared with ammonium chloride (Synth, Brazil), and then diluted to the desired concentrations of ammonia-N. The actual ammonia-N concentration for the test solution ranged from 0.1 to 15 mg L^{-1}. Experimental concentrations of ammonia-N were 0.1, 0.5, 1, 2.5, 5, 10 and 15 mg L^{-1}. Actual concentrations of ammonia-N in the test solutions were measured using the method described by Greenberg (1995). The concentration of NH_3-N (non-ionized ammonia) was calculated according to the equations of Khoo et al. (1977) based on pH 6.70 and temperature 23 °C. NH_3-N concentrations were 0.0002, 0.001, 0.002, 0.006, 0.012, 0.024 and 0.036 mg L^{-1}, respectively (Khoo et al., 1977).

During the experiment, the fish were fed a diet of 40% crude protein and 12% lipids, once daily (4:00pm) at 5% of their body weight per day. The water temperature was maintained at 23 ± 0.5 °C, the dissolved oxygen was at 6.8 ± 0.5 mg L^{-1}, the pH ranged from 6.70 to 6.75, and these parameters were measured with the YSI Pro Plus multiparameter equipment (USA).

Dead fish were removed daily from the tanks and counted after 24, 48, 72 and 96 h of exposure, death was presumed when the fish were immobile with paralyzed opercula and showed no response to mechanical stimuli.

Routine metabolism

Another fifty lambaris (*D. iguape*), 6.57 (± 0.4) g, were used for measurements of routine metabolism using sealed respirometers (Barbieri et al., 2015). The respirometers were manufactured in our laboratory, with an acrylic tube and PVC lids. Ten fish were exposed to the following concentrations of ammonium chloride (0, 0.05, 0.1, 0.5 and 1 mg L^{-1}) for 24 hours. After this period the oxygen consumption was measured. The pH and oxygen concentration of the test solution at the different concentrations of ammonium chloride were also measured, and the range of pH values was between 6.75 to 6.90. The range of oxygen values was between 6.35 to 6.55 mg L^{-1}.

Prior to initiating the experiments, the animals were kept on the respirometer with continuous circulation of water for at least 90 minutes to attenuate the handling stress. Then the water supply was suspended and the vent was closed so that the fish could consume the oxygen in the known volume of water for a period of one hour. The respirators were protected by a barrier to isolate the animals from movement in the laboratory. The difference between the oxygen concentrations determined at the beginning and at the end of the confinement was used to calculate the consumption during the period. To minimize the effect of low oxygen concentration and accumulation of metabolites on metabolism, the duration of the experiment was regulated so that the oxygen concentration at the end of the experiments never fell below 70% of its initial concentration. Dissolved oxygen was determined by the Winkler method (Winkler, 1888).

To obtain the desired concentration of ammonia, the required volume of the main substance (10,000 mg L^{-1} ammonia-N stock solution prepared with ammonium chloride) was calculated for each volume of aquarium and adjusted with an aid (micropipet) at the end of acclimatization. Once the substance was added to the aquarium, after 15 minutes the fish were placed and kept for 24 h until the metabolism experiments were started. After this procedure, the water was sampled at the beginning and end of the oxygen consumption analysis. Total ammonia nitrogen (TAN) was considered as values of ammonia-N (non-ionized plus ionized ammonia) and NH_3-N (non-ionized ammonia) (mg L^{-1}).

Statistical analyses

The median lethal concentrations (LC_{50} with 95% confidence limits) of ammonia-N and NH_3-N were calculated at 24, 48, 72 and 96 h using the Trimmed Spearman-Karber method (Hamilton et al., 1977).

The specific oxygen consumption and ammonia excretion data were analyzed for normality of distribution using the Shapiro-Wilk test and homoscedasticity of variances using the Levene test. Since the results were normal and homoscedastic, the differences between the means of the treatments were evaluated through analysis of variance (ANOVA), with a significance level of $p<0.05$.

RESULTS

Mortality

The percentage mortality of *D. iguape* exposed to ammonia-N and NH_3-N at each 24-hour interval is shown in Table 1. No fish died in the control. As expected, the higher the concentration of ammonia to which the fish were exposed, the higher the observed

Table 1. Mortality rate (%) of *Deuterodon iguape* exposed to various ammonia-N and NH_3-N concentrations at different exposure times and its median lethal concentration (LC_{50} with 95% confidence limits) calculated by Spearman-Karber Estimates, for the temperature of 23°C.

Exposure time (h)	Ammonia-N (mg L^{-1})								LC_{50} of ammonia-N (mg L^{-1})	LC_{50} of NH_3-N (mg L^{-1})
	0.0	0.1	0.5	1	2,5	5	10	15		
24	0.0	0.0	0.0	0.0	0.0	20	100	100	6.16 (5.33-7.10)	0,015 (0.013-0.017)
48	0.0	0.0	0.0	0.0	6.66	26.66	100	100	5.57 (4.61-6.73)	0.013 (0.011-0.016)
72	0.0	0.0	0.0	13.33	26.66	40	100	100	3.88 (2.90-5.19)	0.009 (0.007-0.012)
96	0.0	0.0	0.0	20	33.33	66.66	100	100	2.90 (2.13-3.94)	0.007 (0.005-0.009)

mortality. The actual concentrations of ammonia in the test solutions were within 5% of the nominal concentrations. No fish died in solutions of 0.1 and 0.5 mg L^{-1} of ammonia when exposed for 96 hours. The survival rates of *D. iguape* were significantly different for the different interactions: ammonia-N concentration and exposure time.

Mean lethal concentration

The LC_{50} values of ammonia-N and NH_3-N at different exposure times for *D. iguape* are shown in Table 1. At 24, 48, 72 and 96 h, the N-LC_{50} ammonia values were 6.16, 5.57, 3.88 and 2.90 mg L^{-1}, and NH_3-N were 0.015, 0.013; 0.009 and 0.007 mg L^{-1} at 23 °C, respectively. The LC_{50} values of ammonia-N and NH_3-N noticeably decreased during the first 72 hours.

Oxygen consumption

Fish exposed to ammoniacal nitrogen for 24 hours showed an increase in the specific oxygen consumption. The specific oxygen consumption at all of the concentrations of ammonia-N increased. The oxygen consumption values for the concentrations of 0.1; 0.25; 0.5 and 1.0 mg L^{-1} of ammonia were, 0.25, 0.33, 0.31 and 0.44 mlO_2 g^{-1} h^{-1}, respectively. With the concentration of 1 mg L^{-1} of ammonium chloride there was a 41.33% increase in the metabolic level in relation to the control. Using the Tukey statistical test ($p < 0.05$), it was found that only at the concentration of 1 mg L^{-1} ammonia-N was a statistical difference in relation to the control (Figure 1).

Ammonia excretion

Ammonia excretion increased with increasing ammoniacal nitrogen concentration. The samples were incubated at 23 °C (Figure 2) and excreted 0.032, 0.0364, 0.0368 and 0.037 mg/g/h of ammonia on average at concentrations of 0.1, 0.25, 0.5 and 1.0 mg L^{-1} of ammonia for 24 hours. Comparing these results with the means of ammonia excretion of the control (0.0 mg L^{-1}), it was observed that these values represent an increase in the metabolic level of 146%, 177%, 189% and 184% in relation to the control. Using the Tukey statistical test ($p < 0.05$), the mean values of ammonia excretion for 0.25, 0.5 and 1.0 mg L^{-1} ammonia-N concentrations were different in relation to the control.

O:N ratio

The O:N ratio for *D. iguape* under exposure to different concentrations of ammonia-N decreased significantly with increasing ammonia concentration. After 24 h of exposure to ammonia-N, the O:N ratio decreased by 43.46% (Figure 3).

DISCUSSION

Toxicity tests are desirable in cultures of aquatic organisms, since only chemical and physical analyses of water are not sufficient to estimate their sublethal and lethal effects on these organisms (Key et al., 2007). The toxicity of ammonia-N to fish has already been studied, for example, by several authors (Brinkman et al., 2009; Bergerhouse, 2011; Damato and Barbieri, 2011; Miron et al., 2011). The results of these studies confirm that ammonia-N and NH_3-N are toxic for fish as well as for *D. iguape*, an ecologically and economically important organism in Brazil.

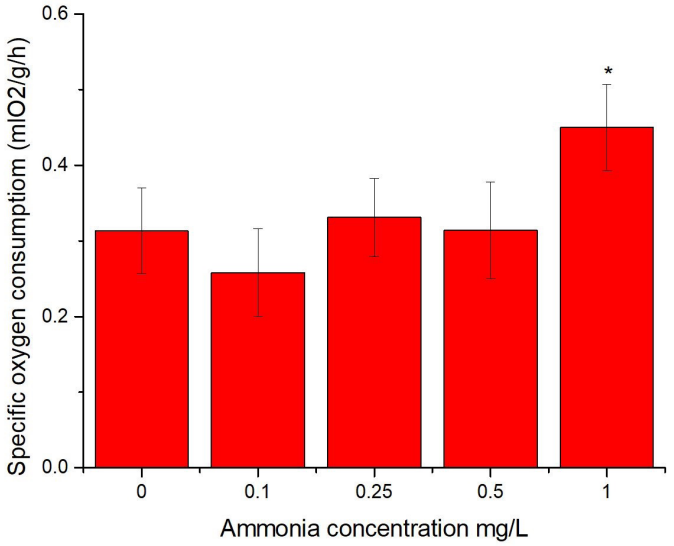

Figure 1. Specific oxygen consumption of *Deuterodon iguape* in relation to the Ammonia concentration. The columns represent the means (n=10) and the bars are their respective standard deviations. The asterisk indicates the groups that presented statistical difference in relation to the control.

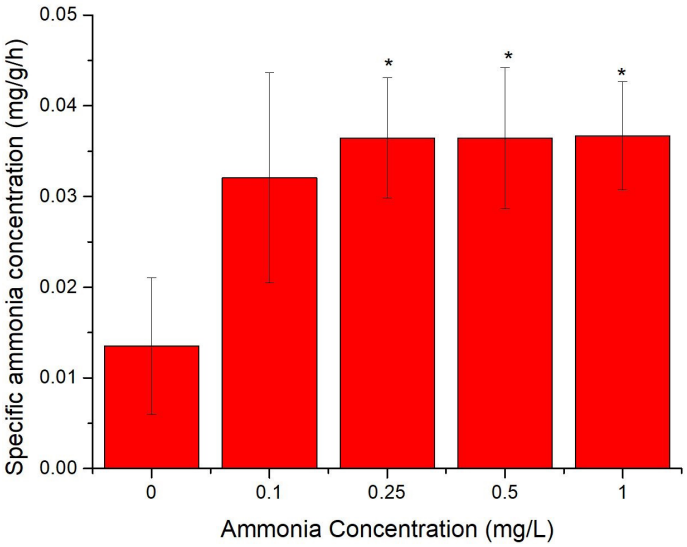

Figure 2. Ammonia excretion of *Deuterodon iguape* in relation to the increase in ammonia concentration. The columns represent the means (n=10) and the bars are their respective standard deviations. The asterisk indicates the groups that presented statistical difference in relation to the control.

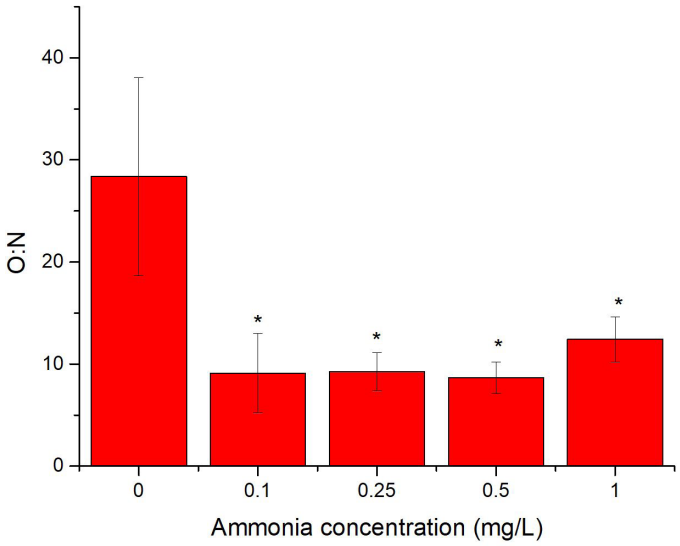

Figure 3. O:N (mean ± SE, n=6) of *Deuterodon iguape* exposed to various Ammonia concentrations. The bars are their respective standard deviations (n=10), and * above the bars indicate significant differences (P <0.05).

Toxic effects of ammonia on various freshwater teleost fishes have already been studied by Brinkman et al. (2009) and Bergerhouse (2011). In different neotropical fish species, Martinez et al. (2006) showed that ammonia-N LC_{50} (24 h) was 5.21 mg L^{-1} for *Astyanax altiparanae*, 6.88 mg L^{-1} for *Piaractus mesopotamicus*, and 5.82 mg L^{-1} for *Prochilodus lineatus*. Studies on the effects of ammonia on *A. altiparanae*, *P. mesopotamicus* and *P. lineatus* demonstrated that the LC_{50} (24 h) of ammonia-N was 5.21 mg L^{-1} for *A. altiparanae*, 6.88 mg L^{-1} for *P. mesopotamicus* and 5.82 mg L^{-1} for *P. lineatus*. The values reported for LC_{50} (24h) of NH_3-N varied from 0.66 mg L^{-1} for *A. altiparanae*, 0.85 mg L^{-1} for *P. mesopotamicus* and 0.74 mg L^{-1} for *P. lineatus*.

The lambari *D. iguape* showed sensitivity to ammonia, presenting the lowest LC_{50} value of 6.16 (5.33 - 7.10) mg L^{-1} for ammonia-N and 0.015 (0.013 - 0.017 mg L^{-1}) in 24 hours of exposure. It should be noted, however, that the fish tested were adults. According to Walker et al. (1996), there is evidence that larger fish are less susceptible than smaller specimens. The effects of size according to Martinez et al. (2006) may be the result of the following factors: i) smaller individuals of a given species present greater body surface area for absorption, in relation to body mass; ii) small individuals have higher respiratory rates.

The toxicity of N-ammonia in *D. iguape*, as expected, increased with time of exposure. The tolerance of *D. iguape* to ammonium-N notably decreased by 9.57%, 37.01% and 52.92% after 48, 72 and 96 h compared to the LC_{50} in 24 hours. Similar results with another lambari species were obtained by Martinez et al. (2006), who observed that the tolerance of *A. altiparanae* decreased with time of exposure. LC_{50} (24h) for freshwater fish in general is 0.82 mg L^{-1} NH_3 (Person-Le Ruyet et al., 1995), and for *D. iguape*, in the present study, it was 0.015 mg L^{-1}.

Medeiros et al. (2016) exposed juveniles of *Amphiprion ocellaris* to six concentrations of ammonia ranging from 0.23 to 1.63 mg L^{-1} NH_3-N. The values of LC_{50}-24, LC_{50}-48, LC_{50}-72 and LC_{50}-96h were estimated at 1.06, 0.83, 0.75 and 0.75 mg L^{-1}, respectively. Histopathological changes were more evident according to the increase of ammonia in fish exposed to 0.57 mg L^{-1} of NH_3-N.

Studies concerning the effects of ammonia-N on the respiration of teleost fish showed an increase in the rates of oxygen consumption as the ammonia concentration increased (Smart, 1978; Arana, 1997; Ip and Chew, 2010; Barbieri and Doi, 2012; Zeitoun et al., 2016). Exposure to different concentrations of non-ionized ammonia caused a 3.3-fold increase in oxygen consumption in *Salmo gairdneri* (Smart, 1978). In the present study, a 1.41-fold increase in the oxygen consumption of *D. iguape* was recorded, with a41.33% increase in the metabolic level. Despite the regulatory capacity of the fish, the rate of oxygen consumption was actually increased after *D. iguape* had been exposed to high concentrations of ammonia-N. Similar results were also observed for *Hyphessobrycon callistus* (Damato and Barbieri, 2011). Respiratory compromise in fish resulting from exposure to stressors has also been studied (Brydges et al., 2009; Bilberg et al., 2010), and it has been concluded that oxygen consumption usually increases when fish are acutely exposed to stressors. Zeitoun et al. (2016), studying the effect of NH_3-N on *Oreochromis niloticus*, also found an increase in oxygen consumption and opercular ventilation rate in fish exposed for 48 hours.

The ammonia excretion of *D. iguape* acclimatized at 23 °C varied as a function of the increase of ammonia concentration. There was an increase in excretion when exposed to a concentration above 0.25 mg L^{-1}. Ammonia is one of the final products of catabolism, especially of amino acids. The increase in ammonia excretion reflects the increase in amino acid catabolism (Damato and Barbieri, 2011), even at the highest concentrations used in this work. Fish can eliminate metabolic ammonia through gill diffusion, active transport with sodium, and by transforming ammonia into less toxic products such as urea. The increase in ammonia concentration reduced its excretion in *Carassius auratus* (Arana, 1997) and *Salmo salar* (Fivelstad et al., 1993), different from our results in which excretion increased after 24 hours of exposure. In studies with *Rachycentron canadum*, Barbieri and Doi (2012) also reported an increase in ammonia excretion for this fish as it increased its concentration in the medium.

The O:N ratio has been widely used to determine the nature of oxidized metabolic substrates in aquatic animals under conditions of stress. An increase in the O:N ratio represents an increase in the catabolism of lipids and carbohydrates (Zhang et al., 2017). In the present study, the O:N ratio decreased from 28.2 to 12.4 with increased exposure to ammonia. These data demonstrate the changes in strategies of energy use (Zhang et al., 2017). Under a higher concentration of ammonia, more proteins were oxidized to maintain the metabolism of *D. iguape*. Both the increase in ammonia excretion and the consumption of oxygen in *D. iguape*, shows that this fish increased metabolism by expending energy to maintain homeostasis. This energy could be used for example for other metabolic functions such as growth; which is very important in aquaculture.

CONCLUSION

This study aimed to determine the acute toxicity of ammonia and its effects on the metabolic rates of *D. iguape*. Our results indicated distinct metabolic effects and increased toxicity with increasing ammonia concentrations. We recommend avoiding concentrations higher than 0.25 mg L^{-1} NH_3-N in the culture water. Future work should be carried out with the objective of investigating the chronic effects on metabolism, growth and reproduction, and health status, which are also important parameters for the commercial cultivation of *D. iguape*.

ACKNOWLEDGEMENTS

This research has been financed by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) under the research project number 2015/24742-1.

REFERENCES

Arana, L.V. 1997. Princípios químicos da qualidade da água em aquicultura. Florianópolis: Editora da UFSC. 166p.

Barbieri, E. 2007. Use of metabolism and swimming activity to evaluate the sublethal toxicity of surfactant (LAS-C12) on *Mugil platanus*. Brazilian Archives of Biology and Technology, 50(1): 101-112. **http://dx.doi.org/10.1590/S1516-89132007000100012**.

Barbieri, E.; Bondioli, A.C.V. 2013. Acute toxicity of ammonia in Pacu fish (*Piaractus mesopotamicus*, Holmberg, 1887) at different temperatures levels. Aquaculture Research, 46(3): 565-571. **http://dx.doi.org/10.1111/are.12203**.

Barbieri, E.; Doi, S.A. 2012. Acute toxicity of ammonia on juvenile Cobia (*Rachycentron canadum*, Linnaeus, 1766) according to the salinity. Aquaculture International, 20(2): 373-382. **http://dx.doi.org/10.1007/s10499-011-9467-3**.

Barbieri, E.; Ferrarini, A.M.T.; Rezende, K.F.O.; Martinez, D.S.T.; Alves, O.L. 2018. Effects of multiwalled carbon nanotubes and carbofuran on metabolism in Astyanax ribeirae, a native species. Fish Physiology and Biochemistry, 44: 1-10. PMid:30276578.

Barbieri, E.; Medeiros, A.M.Z.; Henriques, M.B. 2015. Oxygen consumption and ammonia excretion of juvenile pink shrimp (*Farfantepenaeus paulensis*) in culture: temperature effects. Marine and Freshwater Behaviour and Physiology, 49(1): 19-25. **http://dx.doi.org/10.1080/10236244.2015.1108057**.

Bergerhouse, D.L. 2011. Lethal effects of elevated pH and ammonia on early life stages of walleye. North American Journal of Fisheries Management, 12(2): 356-366. **http://dx.doi.org/10.1577/1548-8675(1992)012<0356:LEOEPA>2.3.CO;2**.

Bilberg, K.; Malte, H.; Wang, T.; Baatrup, E. 2010. Silver nanoparticles and silver nitrate cause respiratory stress in Eurasian perch (*Perca fluviatilis*). Aquatic Toxicology, 96(2): 159-165. **http://dx.doi.org/10.1016/j.aquatox.2009.10.019**. PMid:19923013.

Bosisio, F.; Rezende, K.F.O.; Barbieri, E. 2017. Alterations in the hematological parameters of Juvenile Nile Tilapia (*Oreochromis niloticus*) submitted to different salinities. Pan-American Journal of Aquatic Sciences, 12: 146-154.

Brinkman, S.F.; Woodling, J.D.; Vajda, A.M.; Norris, D.O. 2009. Chronic toxicity of ammonia to early life stage rainbow trout. Transactions of the American Fisheries Society, 138(2): 433-440. **http://dx.doi.org/10.1577/T07-224.1**.

Brydges, N.M.; Boulcott, P.; Ellis, T.; Braithwaite, V.A. 2009. Quantifying stress responses induced by different handling methods in three species of fish. Applied Animal Behaviour Science Abbreviation, 116(2-4): 295-301. **http://dx.doi.org/10.1016/j.applanim.2008.09.003**.

Campos-Garcia, J.; Martinez, D.S.; Alves, O.L.; Leonardo, A.F.; Barbieri, E. 2015. Ecotoxicological effects of carbofuran and oxidised multiwalled carbon nanotubes on the freshwater fish Nile tilapia: Nanotubes enhance pesticide ecotoxicity. Ecotoxicology and Environmental Safety, 111: 131-137. **http://dx.doi.org/10.1016/j.ecoenv.2014.10.005**. PMid:25450925.

Cavero, B.A.S.; Pereira-Filho, M.; Bordinhon, A.M.; Fonseca, F.A.L.; Ituassú, D.R.; Roubach, R.; Ono, E.A. 2004. Tolerância de juvenis de pirarucu ao aumento da concentração de amônia em ambiente confinado. Pesquisa Agropecuária Brasileira, 39(5): 513-516. **http://dx.doi.org/10.1590/S0100-204X2004000500015**.

Croux, P.; Julieta, M.; Loteste, A. 2004. Lethal effects of elevated pH and ammonia on juveniles of neotropical fish *Colosoma macropomum* (Pisces, Caracidae). Journal of Environmental Biology, 25(1): 7-10. PMid:15303698.

Damato, M.; Barbieri, E. 2011. Determinação da toxicidade aguda de cloreto de amônia para uma espécie de peixe (Hyphessobrycon callistus) indicadora regional. O Mundo Saúde, 35(4): 401-407.

Das, P.C.; Ayyappan, S.; Jena, J.K.; Das, B.K. 2004. Acute toxicity of ammonia and its sub-lethal effects on selected haematological and enzymatic parameters of mrigal, *Cirrhinus mrigala* (Hamilton). Aquaculture Research, 35(2): 134-143. **http://dx.doi.org/10.1111/j.1365-2109.2004.00994.x**.

Ferrarini, A.M.T.; Rezende, K.F.O.; Barbieri, E. 2016. Use of swimming capacity to evaluate the effect of mercury on Poecilia vivipara (Poecilídeos) according to salinity and temperature. Journal of Marine Biology & Oceanography, 5(4): 1-5. **http://dx.doi.org/10.4172/2324-8661.1000163**.

Fivelstad, S.; Kallevik, H.; Iversen, H.M.; Møretrø, T.; Våge, K.; Binde, M. 1993. Sublethal effects of ammonia in soft water on Atlantic salmon smolts at a low temperature. Aquaculture International, 1(2): 157-169. **http://dx.doi.org/10.1007/BF00692618**.

Greenberg, A.E. 1995. Standard methods for the examination of water and wastewater. 19th ed. Washington: Amer Public Health Assn.

Hamilton, M.A.; Russo, R.C.; Thurston, R.V. 1977. Trimmed spearman-karber method for estimating median lethal concentrations in toxicity bioassays. Environmental Science & Technology, 11(7): 714-719. **http://dx.doi.org/10.1021/es60130a004**.

Ip, Y.K.; Chew, S.F. 2010. Ammonia production, excretion, toxicity, and defense in fish: a review. Frontiers in Physiology, 1: 134-20. **http://dx.doi.org/10.3389/fphys.2010.00134**. PMid:21423375.

Key, P.; Chung, K.; Siewicki, T.; Fulton, M. 2007. Toxicity of three pesticides individually and in mixture to larval grass shrimp (*Palaemonetes pugio*). Ecotoxicology and Environmental Safety, 68(2): 272-277. **http://dx.doi.org/10.1016/j.ecoenv.2006.11.017**. PMid:17204326.

Khoo, K.H.; Ramette, R.W.; Culberson, C.H.; Bates, R.G. 1977. Determination of hydrogen ion concentrations in seawater from 5 to 40°C: Standard potentials at salinities from 20 to 45‰. Analytical Chemistry, 49(1): 29-34. **http://dx.doi.org/10.1021/ac50009a016**.

Martinez, C.B.R.; Azevedo, F.; Winkaler, E.U. 2006. Toxicidade e efeitos da amônia em peixes neotropicais. In: Cyrino, J.E.P.; Urbinati, E.C. (Orgs.). Tópicos especiais em biologia aquática e aquicultura. Jaboticabal: Sociedade Brasileira de Aquicultura e Biologia Aquática. p. 81-95.

Martinez, D.S.T.; Alves, O.L.; Barbieri, E. 2013. Carbon nanotubes enhanced the lead toxicity on the freshwater fish. Journal of Physics: Conference Series, 429: 012043. **http://dx.doi.org/10.1088/1742-6596/429/1/012043**.

Medeiros, R.S.; Lopez, B.A.; Sampaio, L.A.; Romano, L.A.; Rodrigues, R.V. 2016. Ammonia and nitrite toxicity to false clownfish *Amphiprion ocellaris*. Aquaculture International, 24(4): 985-993. **http://dx.doi.org/10.1007/s10499-015-9965-9**.

Miron, D.S.; Becker, A.G.; Loro, V.L.; Baldisserotto, B. 2011. Waterborne ammonia and silver catfish, *Rhandia quelen:* suvival and growth. Ciência Rural, 42(2): 349-353. **http://dx.doi.org/10.1590/S0103-84782011000200028**.

Rezende, K.F.O.; Bergami, E.; Alves, K.V.B.; Corsi, I.; Barbieri, E. 2018. Titanium dioxide nanoparticles alters routine metabolism and causes histopathological alterations in Oreochromis niloticus. Boletim do Instituto de Pesca, 44(2): e343. **http://dx.doi.org/10.20950/1678-2305.2018.343**.

Ruíz-Hidalgo, K.; Masís-Mora, M.; Barbieri, E.; Carazo-Rojas, E.; Rodríguez-Rodríguez, C.E. 2016. Ecotoxicological analysis during the removal of carbofuran in fungal bioaugmented matrices. Chemosphere, 144: 864-871. **http://dx.doi.org/10.1016/j.chemosphere.2015.09.056**. PMid:26421626.

Ruyet, J.P.L.; Chartois, H.; Quemener, L. 1995. Comparative acute ammonia toxicity in marine fish and plasma ammonia response. Aquaculture (Amsterdam, Netherlands), 136(1-2): 181-194. **http://dx.doi.org/10.1016/0044-8486(95)01026-2**.

Silva, N.J.R.; Lopes, M.C.; Fernandes, J.B.K.; Henriques, M.B. 2011a. Caracterização dos sistemas de criação e da cadeia produtiva do lambari no Estado de São Paulo. Informações Econômicas, 41(9): 17-28.

Silva, N.R.; Lopes, M.C.; Gonçalves, F.H.A.S.B.; Gonsales, G.Z.; Henriques, M.B. 2011b. Avaliação do potencial do mercado consumidor de lambari da Baixada Santista. Informações Econômicas, 41(12): 5-13.

Smart, G. 1978. Investigation of the toxic mechanisms of ammonia to fish – gas Exchange in rainbow trout *Salmo gairdneri* exposed to acutely lethal concentrations. Journal of Fish Biology, 12(1): 93-104. **http://dx.doi.org/10.1111/j.1095-8649.1978.tb04155.x**.

Tilak, K.S.; Veeraiah, K.; Raju, J.M. 2007. Effects of ammonia, nitrite and nitrate on hemoglobin content and oxygen consumption of freshwater fish, *Cyprinus carpio* (Linnaeus). Journal of Environmental Biology, 28(1): 45-47. PMid:17717984.

Walker, C.H.; Hopkin, S.P.; Sibly, R.M.; Peakall, D.B. 1996. Principles of ecotoxicology. Taylor & Francis, Londres.

Winkler, L.W. 1888. Methods for measurement of dissolved oxygen. Berichte der Deutschen Chemischen Gesellschaft, 21(2): 2843-2854. **http://dx.doi.org/10.1002/cber.188802102122**.

Zeitoun, M.M.; EL-Azrak, K.E.D.M.; Zaki, M.A.; Nemat-Allah, B.R.; Mehana, E.-S.E. 2016. Effects of ammonia toxicity on growth performance, cortisol, glucose and hematological response of Nile Tilapia (*Oreochromis niloticus*). Aceh Journal of Animal Science, 1(1): 21-28. **http://dx.doi.org/10.13170/ajas.1.1.4077**.

Zhang, C.; Yu, K.; Li, F.; Xiang, J. 2017. Acute toxic effects of zinc and mercury on survival, standard metabolism, and metal accumulation in juvenile ridgetail white prawn, *Exopalaemon carinicauda*. Ecotoxicology and Environmental Safety, 145: 549-556. **http://dx.doi.org/10.1016/j.ecoenv.2017.07.075**. PMid:28797960.