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Short-term toxicity of ammonia, sodium Hydroxide and a commercial biocide to golden mussel *Limnoperna fortunei* (Dunker, 1857)

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ABSTRACT

Macrofouling bivalves are considered an ecological and technological problem worldwide. Control measures have been researched with *Limnoperna fortunei*, but without success. The aim of the manuscript is to test some alternatives to regulate this harmful invasive mollusk. Mortality and behavioral response (shell gaping) of *Limnoperna fortunei* exposed to three chemical compounds were evaluated. Values for LC_{50} 96 h were: 0.25 (0.24–0.27) mg/L NH_3-N, 11.10 (7.45–16.55) mg/L MXD-100 and 88.51 (74.61–105.01) mg/L NaOH. Reduced gaping was observed beginning at concentrations of 0.31 mg/L (NH_3-N), 100 mg/L (MXD-100) and 160 mg/L (NaOH) and increased above these values. The percentage of individuals gaping after two hours at LC_{50} 96 h differed significantly ($\chi^2=79.9$; DF=3; $p<0.001$) in MXD-100 (50%), NaOH (0%), NH_3-N (96.7%) and the controls (93.3%). This study contributes to the understanding of the relationship between toxicity and behavioral effects of some toxicants in *L. fortunei*.

1. Introduction

Chemical pollution in freshwater ecosystems is an area of concern (Mason, 2002). The spread of invasive species and the use of chemical control in their management results in a continuous load of these compounds, contributing to the increase of the chemical pollution in freshwater (Mackie and Claudi, 2010). Among the invasive species, bivalve mollusks can cause severe economic and environmental damages worldwide. A wide variety of chemical treatments have been used for mitigation of invasive mussels, including sodium hypochlorite, solid calcium hypochlorite, chlorine dioxide, brominated compounds, hydrogen peroxide, potassium permanganate and quaternary amines (Mackie and Claudi, 2010).

Limnoperna fortunei is a very effective ecosystem engineer, altering both ecosystem structure and function (Darrigran and Damborenea, 2011), and it is also a fouling pest for industrial plants that use untreated water, including hydroelectric power plants, which are the main source of electricity in Brazil (Darrigran, 2010; Rolla and Mota, 2010). After its invasion, chemical control of this species became necessary in Brazil. Field trials carried out in Brazil, achieved promising results in long-term treatments of cooling system pipes with sodium hydroxide and with a commercial formulation of tannins and quaternary ammonium produced in Brazil (MXD-100, Maxclean Ambiental e Química S.A, Brazil) (Calazans et al., 2012; Rolla and Mota, 2010). However, there are few laboratory studies examining the efficacy and impacts of chemicals being used to control them.

Tannins are natural polyphenolic compounds known for their anticorrosive, antimicrobial and anti-biofouling properties (Pérez et al., 2007; Qian et al., 2010). These natural compounds are considered eco-friendly alternatives for biofouling control (Pereyra et al., 2011; Pérez et al., 2007; Qian et al., 2010). The MXD-100 is a combination of tannins and quaternary ammonium; the latter are used in the chemical control of biofouling in industrial water systems (Cloete et al., 1998). These non-oxidizing biocides are organically substituted nitrogen compounds that have their action attributed to the electrostatic bond of their positive charge with the negatively charged cell walls. Attempts to use such compounds in the control of mollusks, including *L. fortunei*, were made (Darrigran, et al., 2004, 2007; Mackie and Claudi, 2010; Maroñas

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and Damborenea, 2006), with positive results. Sodium hydroxide (NaOH) is a strong base, able to produce rapid increases in pH, and it has been used in the chemical control of undesirable species (Calazans et al., 2012; TenEyek, 2009). Tolerance limits of several species, including mussels, to pH increases have been investigated and used in control strategies (Bowman and Bailey, 1998; TenEyek, 2009). In aquatic environments as pH increases, the concentration of unionized ammonia (NH_3) form (considered extremely toxic) also increases (Thurston and Russo, 1981). Despite efforts to understand the environmental tolerance limits of *L. fortunei*, no attention was given to its tolerance to ammonia (Darrigran et al., 2011; Oliveira et al., 2010).

The toxicity of a chemical compound to a species is usually assessed in terms of mortality and behavioral responses (Maroñas and Damborenea, 2006; USEPA, 2008; Soares et al., 2009). Thus, the aims of the present study were to evaluate the short-term toxicity to *L. fortunei* in terms of the median lethal concentration (LC_{50}) and behavioral effects of three chemical compounds: NH_3, MXD-100 and NaOH. These data may provide insights that are helpful for developing control strategies in the wild and for laboratory rearing of this species for additional studies.

2. Materials and methods

Golden mussels were collected by hand from Bela Vista Reservoir (Foz do Iguaçu, PR, Brazil). Individuals were acclimatized to laboratory conditions for 15 days, in 200 L aquaria containing dechlorinated tap water at 12:12 h light:dark cycle. During this period, constant aeration was provided and animals were fed daily with live algae *Scenedesmus* sp. and *Ankistrodesmus* sp. The physicochemical parameters in aquaria were monitored daily using an optical oximeter (ProODO; YSI), a pH-meter (HI 3221; HI 1131B; Hanna) and an ammonia selective electrode (HI 4101; Hanna). Tap water was dechlorinated with sodium thiosulfate in order to maintain the mussels in the laboratory and to prepare all the tested solutions. The aquaria were maintained under the following conditions: 20.03 ± 2.98 °C, pH 7.7 ± 0.39, 7.63 ± 0.67 mg/L dissolved oxygen and total ammonia-N (TA-N)≤0.6 mg/L.

Two days prior to the experiment a group of 1000 mussels, 21–26 mm in shell length, were placed in an aquarium in a incubator and maintained at 25 ± 2 °C, 12:12 h light:dark cycle and kept in dechlorinated water with constant aeration. Specimens, after 24 h, that did not attach to the containers and that which did not present extended siphon, were excluded. After 24 h, groups of 11 individuals were randomly distributed in 60 glass jars (500 mL) containing 300 mL of dechlorinated water each, with constant aeration. Jars with all specimens attached were selected for the tests.

Three independent semi-static short-term bioassays (96 h) with daily solution renewal were conducted, in triplicates (each of 11 individuals). Each test consisted of a control group with dechlorinated water only and an experimental group with 5 to 7 different concentrations: TA-N (5, 10, 20, 40 and 80 mg/L), MXD-100 (0.05, 0.5, 1, 10, 100 and 500 mg/L) and NaOH (40, 80, 160, 240, 320, 400 and 800 mg/L). Concentrations selected to the trials were based on preliminary range-finding tests. No food was provided during the tests.

Stock solutions were prepared with distilled water and the respective chemical compounds: ammonium chloride (NH_4Cl) (Merck, Darmstadt, Germany, 99.8% purity); MXD-100 (Maxclean Ambiental e Química S.A, Brazil) and NaOH (Merck, Darmstadt, Germany, 99% purity). Nominal concentrations used in experiments were prepared by diluting the stock solutions with dechlorinated water. The five nominal concentrations of TA-N (5, 10, 20, 40 and 80 mg/L) were prepared and the real TA-N concentrations (3.39, 7.37, 16.82, 34.47 and 73.74 mg/L) were determined using the Ammonia-Selective Electrode Method (APHA, 2005). Unionized ammonia concentrations were calculated according to the methodology described by Emerson et al. (1975), considering water pH and temperature and TA-N concentration data for the respective water sample (Table 1). The concentrations tested were NH_3-N (0.14, 0.21, 0.31, 0.50 and 0.72 mg/L).

Before the daily solution renewal, physicochemical parameters were monitored (dissolved oxygen=ProODO-YSI; pH and temperature=pH/ ISE-meter-HI3221-Hanna and TA-N=HI4101-Hanna). Mortality and behavior (shell gaping) were recorded at 24 h intervals and dead individuals were removed from the jars twice a day, to prevent water degradation.

Solutions were replaced with dechlorinated water and monitoring of physico-chemical parameters, mortality and behavior were continued for an additional 48 h, with conditions maintained as previously described. At the end of this 48 h recovery interval, mussels that remained closed were individually inspected and those exhibiting ciliary beats in the gills were considered alive.

Table 1
Physicochemical parameters of the test solutions.

Treatment	DO (mg/L)	Temperature (°C)	pH
Control	7.48	21.47	7.73
NaOH 40 mg/L	7.41	22.80	11.24
NaOH 80 mg/L	6.71	24.10	11.43
NaOH 160 mg/L	7.02	24.0	11.86
NaOH 240 mg/L	6.98	24.0	12.07
NaOH 320 mg/L	7.51	24.1	12.26
NaOH 400 mg/L	7.33	24.2	12.34
NaOH 800 mg/L	7.45	22.27	13.04
MXD-100 0.05 mg/L	7.01	24.6	7.97
MXD-100 0.5 mg/L	7.38	23.9	7.99
MXD-100 1 mg/L	7.60	23.8	7.94
MXD-100 10 mg/L	7.41	24.0	7.94
MXD-100 100 mg/L	7.18	24.0	7.9
MXD-100 500 mg/L	7.31	22.7	8.2
NH_3-N 0.14 mg/L	7.34	24.8	7.88
NH_3-N 0.21 mg/L	7.49	23.5	7.73
NH_3-N 0.31 mg/L	7.63	23.5	7.56
NH_3-N 0.50 mg/L	7.52	23.5	7.44
NH_3-N 0.72 mg/L	7.28	23.5	7.27

DO: dissolved oxygen.

An additional experiment was carried out in order to observe the behavior of *L. fortunei* exposed to the LC_{50} 96 h of each chemical compound. Individuals were acclimatized and selected as described above. Thirty individuals per treatment (0.25 mg/L NH_3-N, 11.10 mg/L MXD-100, 88.51 mg/L NaOH and control) were placed in wells containing 10 mL of solution, one individual per well (6-well polystyrene tissue culture plate). The number of individuals with gaping was recorded every 10 min, for 2 h.

Median lethal concentration values and the confidence limits were calculated using the Trimmed Spearman–Karber method (Hamilton et al., 1977). One-way ANOVA (Zar, 2009) was used to test the effects of the concentration in the proportion of opened/closed behavior in the first 24 h. Post-hoc comparisons were made by inferences using 95% confidence interval. A Chi-square test was used to compare differences in the proportion of gaping in the LC_{50} 96 h concentration for each treatment.

3. Results and discussion

As anticipated, mortality increased with increasing chemical concentrations and exposure times. The infrequent mortality recorded during the 48 h recovery period did not alter significantly the LC_{50} values, thus, we used only the mortality recorded during the 96 h exposure to the chemical compounds for analysis. No deaths were recorded in the control groups. The confidence limits of the LC_{50} do not overlap (Table 2), indicating a significant difference in the toxicity of the chemical compounds tested with NH_3 < MXD-100 < NaOH. There were significant differences in the proportion of gaping valves among concentrations of NH_3-N ($F_{5;12}=17.5$; $p<0.001$), MXD-100 ($F_{6;14}=4.9$; $p=0.006$) and NaOH ($F_{7;15}=10.3$; $p<0.001$). Compared to controls, significant differences were recorded beginning at 0.31 mg/L of NH_3-N (Fig. 1), 100 mg/L of MXD-100 (Fig. 2) and 160 mg/L of NaOH (Fig. 3). After 24 h, behavioral changes (decrease in the percentage of gaping) were observable only at the higher concentrations. However, the concentrations that are generally used in industrial plants to control *L. fortunei*, did not cause behavioral changes. Behavioral responses should be considered when choosing among the control strategies available (i.e. continuous or intermittent treatment) (Mackie and Claudi, 2010; Maroñas and Damborenea, 2006; Rolla and Mota, 2010). Bivalves have a limited behavioral repertoire and valve gape is one of the most important and easily measurable behaviors (Frank et al., 2007; Hégaret et al., 2007; Di Fiori et al., 2012). The behavioral response of *L. fortunei*, exposed to the LC_{50} 96 h of each chemical compound for two hours differed significantly ($\chi^2=79.9$; $DF=3$; $p<0.001$). In MXD-100, 50% of the mussels had shell gaping compared with 0% in NaOH. These