

Acute toxicity of neurotoxin-producing *Raphidiopsis* (*Cylindrospermopsis*) *raciborskii* ITEP-A1 (Cyanobacteria) on the neotropical cladoceran *Macrothrix spinosa*

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Abstract

Cyanobacterial blooms are recurrent phenomena in several water bodies, mostly eutrophic. They are considered a public health problem, especially considering harmful species like *Raphidiopsis raciborskii*, a bloom-forming cyanobacteria recorded as a producer of neurotoxic and cytotoxic alkaloids. The present study aimed to assess the acute toxicity of a saxitoxin-producing strain *Raphidiopsis raciborskii* ITEP-A1 on the zooplankton *Macrothrix spinosa*. Cladoceran clones isolated from an eutrophic system with cyanobacterial blooms records and an oligotrophic one with no bloom record; both from Pernambuco (Northeastern Brazil), were tested for *R. raciborskii* ITEP-A1 toxicity. Acute toxicity assays were carried out with newborns (<24h, n=10) and the animals were exposed to three concentrations of cyanobacterial cell biomass ($\mu\text{gC L}^{-1}$). Quantification of saxitoxins (STX and neoSTX) was done by high-performance liquid chromatography coupled with fluorescence detector (HPLC-RF). The EC_{50} (48h) for the Duas Unas and Prata clones were 359 and 189 $\mu\text{gC L}^{-1}$, respectively. Obtained results showed that *M. spinosa* Prata was more sensitive to *R. raciborskii* than Duas Unas, reinforcing the importance of analyzing the previous life history of the test organisms regarding their native environments. However, both clones demonstrated high sensitivity to *R. raciborskii*, which enhances their potential for biomonitoring toxic cyanobacterial blooms in tropical reservoirs.

Keywords: Cyanotoxin, tropical, zooplankton

INTRODUCTION

Widespread pollution from human activity followed by the eutrophication of water bodies has continuously increased the occurrence of cyanobacterial blooms, a worldwide phenomenon and environmental issue. Aquatic environments that are rich in nutrients, particularly nitrogen and phosphorus, are directly connected to the prevalence of blooms, however, it is not the only factor influencing the predominance of cyanobacteria. Rising CO_2 levels and global warming are also believed to favor harmful cyanobacterial blooms (Paerl & Huisman, 2009), increasing their frequency, intensity and duration in many ecosystems all over the world (Huisman *et al.*, 2018).

Some of the most recorded bloom- or mats-forming cyanobacterial genera are *Planktothrix*, *Microseira* (syn.

Lyngbya and *Plectonema*), *Dolichospermum*, *Microcystis* and *Raphidiopsis* (formerly *Cylindrospermopsis*) (Mowe *et al.*, 2015). The latter is commonly found in East and Southeast Asia, Australia and South America, with difference in the type of toxin reported by their isolated strains – for instance, while *Raphidiopsis* in Australia, East and Southeast Asia produces cylindrospermopsins (CYNs), in South America isolated strains from that species are reported to produce saxitoxins (STXs) (Antunes *et al.*, 2015; Burford *et al.*, 2016; Mowe *et al.*, 2015). The later are a group of neurotoxic carbamate alkaloids which act as ionic channel blockers and affect the nervous system, leading to paralysis and death (Wiese *et al.*, 2010). These toxins are also produced by some genus of eukaryotic marine dinoflagellates such as *Alexandrium* which concerns due to forming red tides and compromising seafood in marine and coastal ecosystems (Anderson *et al.*, 2012).

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In freshwater systems saxitoxins are usually associated to *Raphidiopsis raciborskii* (Woloszynska) Aguilera, Berrendero Gómez, Kastovsky, Echenique & Salerno (basionym *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju) (Aguilera *et al.*, 2018) which is an important bloom-forming and toxin-producing filamentous cyanobacteria, predominant in Brazilian aquatic environments (Bouvry *et al.*, 2001; Soares *et al.*, 2013; Guedes *et al.*, 2018). Its presence in water bodies poses risks to endemic organisms by ingestion of cells or absorption of toxin dissolved in water (Costa *et al.*, 2013), as well as might impact public health by compromising water supply and fish.

Some studies have shown that cyanobacteria exert a variety of effects on zooplankton, such as decrease in survivorship, growth and reproduction (Costa *et al.*, 2013; Ferrão-Filho *et al.*, 2011; Lüring, 2003; Vilar *et al.*, 2014). These effects, however, vary according to the zooplankton species. Some have developed, throughout life history, the ability to coexist with certain species of cyanobacteria. On the other hand, reports suggest that cyanobacteria might have resistance to predation by zooplankton; specifically when there is dominance of *R. raciborskii* in a water body, there is inhibition of the growth of zooplankton as they have difficulty using the large filaments and colonies of cyanobacteria as food (Leonard & Pearl, 2005; Havens & East 2006).

Although saxitoxins are potent paralyzing alkaloids and feeding inhibitors, studies suggest they present low lethality effects in invertebrates (Ferrão-Filho *et al.*, 2013). The immobilizing effect of the toxin affects the movements of cladocerans by paralyzing their swimming appendages, but once they are still able to filter-feed even with immobilized limbs they do not suffer from starvation nor respiratory arrest. Thus, as observed by Costa *et al.* (2013) and Ferrão-Filho *et al.* (2010), the continuous filtration of food particles whilst paralyzed allows the organisms to survive for long periods. Nevertheless, there is still a lack of research on the effects of STXs on tropical freshwater zooplankton.

Daphnia spp. are among the most commonly used organisms in freshwater quality monitoring programs (ABNT, 2016; USEPA, 2002). Nowadays, most ecotoxicological research in tropical environments are conducted with acclimated exotic cladoceran species from temperate regions, such as *Daphnia similis* Claus, 1876 and *Daphnia magna* Straus, 1820. In the other hand, very few studies are carried out with native ones (Freitas & Rocha, 2011), therefore, there is a growing preference for using native species on ecotoxicological assessments, as they are more representative of the environment and local-species sensitivity.

On that context, *Macrothrix* appears to be a potential organism to be used in ecotoxicological assays, although studies on cladocerans from the family Macrothricidae are still incipient. Available data on sensitivity are on the species *Macrothrix elegans* Sars, 1901 and *Macrothrix flabelligera* Smirnov, 1992 that have shown potential as suitable test-organisms for a variety toxicity tests (Araújo *et al.*, 2008; Moreira *et al.*, 2014; Moreira *et al.*, 2017). These animals'

adaptability, short life cycle and easy maintenance in laboratory facilitate their choice as potential biomonitors for tropical aquatic systems. Species in this genus are described as having an oval body in lateral view, a dilated antennule being identified by the serrations along the dorsal part of its valve (Fuentes-Reinés *et al.*, 2015; Fuentes-Reinés *et al.*, 2018).

Although there is an apparent lack of in-depth studies regarding the tropical species, *Macrothrix spinosa* King, 1853 is reportedly well-distributed in South American water bodies (Fuentes-Reinés *et al.*, 2018; Sousa *et al.*, 2018). *M. spinosa* is often presented as synonym of *Macrothrix squamosa* Sars, 1901. This synonymy is based on superficial characteristics, such as the aspect of the carapace, antennule, and post abdomen (Elmoor-Loureiro, 2007). In contrast, recent studies suggest that *Macrothrix* spp. tend to have continental or regional distribution, which indicates that *M. spinosa* – originally described from Australia, and its congener *M. squamosa* might be different species (Elmoor-Loureiro, 2007; Fuentes-Reinés *et al.*, 2015; Fuentes-Reinés *et al.*, 2018). Regardless, further revision is important to know the diversity of potentially sensitive species on water biomonitoring. Thus, the present study aimed to evaluate the toxicity of a neurotoxin-producing *Raphidiopsis raciborskii* on the cladoceran *Macrothrix spinosa*. Moreover, this is the first report of the effects of a saxitoxin-producing cyanobacteria on that zooplankton species. Therefore, the findings may contribute to the knowledge on the potential of a (sub)tropical zooplankton as a tool on the biomonitoring of freshwaters.

MATERIALS AND METHODS

Cultivation and maintenance of organisms

The cyanobacterial strain used in this study was a saxitoxin-producing *Raphidiopsis raciborskii* ITEP-A1 which was isolated from Riacho dos Paus reservoir located in Arcoverde, countryside of Pernambuco state, Brazil. The culture were maintained in ASM-1 (Gorham *et al.*, 1964) medium under initial pH 8.0, controlled temperature (24 °C ± 1) and 12h dark:light cycle under light intensity (50 µmol m⁻² s⁻¹). Culture samples were collected for acute toxicity tests and toxin analysis during exponential growth phase (about 8 days).

Two clones of the cladoceran *M. spinosa* isolated from Prata and Duas Unas reservoirs (Pernambuco, Brazil) were used in this study. Duas Unas is known as a eutrophic water body with previous report of cyanobacterial blooms (Bittencourt-Oliveira *et al.*, 2014; Lorenzi *et al.*, 2018). On the other hand, Prata reservoir is an oligotrophic water body with no previous cyanobacterial bloom report (Almeida *et al.*, 2012).

Zooplankton samples were collected with plankton net (60 µm mesh-size) in the littoral zone of the waterbodies. Animals were anaesthetized with carbonated water (1:20 v/v), sorted and disposed in Petri dish where parthenogenetic females of *M.*

spinosa were isolated under stereoscope and kept in laboratory during several generations. Adults of *M. spinosa* ranged from 0.943–0.961 mm. Animals were cultured in artificial RT culture medium (Tollrian, 1993) with 20% mineral water enriched with commercial humic extract Microbe Lift® 0.1% (approximately 2.25 mg L⁻¹ dissolved carbon content). They were kept under a controlled temperature of 24 ± 1 °C, 12h dark:light cycle and fed by mixed cell suspension of green algae *Selenastrum capricornutum* Printz and *Ankistrodesmus stipitatus* Komárková-Legnerová at a final biomass 1 mgC L⁻¹.

Cyanobacterial cell biomass measurements

R. raciborskii ITEP-A1 biomass (µgC L⁻¹) was estimated according to Rocha & Duncan (1985) using the mean trichome volume (µm³) (Hillebrand *et al.*, 1999) after measuring 50 units, and estimated using trichome density from Fuchs-Rosenthal chamber counting.

Acute toxicity test

To evaluate *R. raciborskii* ITEP-A1 impact on cladocerans' mobility both clones of *M. spinosa* were exposed to different biomass concentrations, with maximum concentration defined after preliminary acute toxicity test.

Tests were carried out with 10 newborns (<24h) of each *M. spinosa* clone in triplicates. The neonates were put in test tubes containing 25 mL of different concentrations of *R. raciborskii* ITEP-A1 biomass: 150, 300 and 500 µgC L⁻¹, and a control which consisted of cyanobacteria-free culture media. Test organisms were exposed to cyanobacterium for 48h. After incubation period, the number of immobile individuals in each concentration as well as in the control was recorded for estimative of half maximal effective concentration (EC₅₀ 48h) using the Trimmed Spearman-Kärber method (Hamilton *et al.*, 1977).

Bibliographic survey of cladoceran sensitivity to saxitoxin-producing cyanobacteria

It was performed a bibliographic survey about published articles on acute toxicity assay experiments testing the effects of saxitoxin-producing cyanobacteria on cladocerans. Previous toxicity studies, published between 1990 and September 2019, were consulted in the database Web of Science using the following keywords and combinations: *bioassay and cyanobacteria* (456 articles); *ciano* and toxicity and Cladocera* (16 articles); *neurotoxicity and cyanobacteria* (73 articles); *saxitoxin and cyanobacteria and Cladocera* (3 articles); *Macrothrix and toxicity* (6 articles).

Aiming to compare our results to the available literature on cladoceran sensitivity to saxitoxin-producing cyanobacteria, the publications were filtered based on the following criteria: 1) the study assessed the acute toxicity of saxitoxin-producing *R. raciborskii* on Cladocera; 2) animals were exposed to intact cyanobacterial cell (or fresh biomass); 3) half maximal

effective concentration values in cell density (cell mL⁻¹) or biomass (µgC L⁻¹).

Saxitoxins extraction and analysis by HPLC-RF

Saxitoxins extraction from cell biomass were performed using 0.5 M acetic acid. After centrifuging (22,000x g), supernatant was collected and filtered through 0.22 µm Millex (Millipore) prior to injection. Chromatographic analyses were performed on a Shimadzu HPLC using a silica-based reverse phase column and separations were carried out under specific mobile phases for non-sulfated saxitoxins (saxitoxin and neosaxitoxin) (hereafter STX and neoSTX) coupled to post-column oxidation as described in (Oshima, 1995). Fluorescent saxitoxins derivatives were detected using a Shimadzu RF-10Ax1 fluorometric detector with excitation at 330 nm and emission at 390 nm. Toxins were identified and quantified by comparison with known retention times and integrated areas of standards, respectively. The standards of saxitoxins were purchased from Institute for Marine Bioscience, National Research Council of Canada (Halifax, Canada). Data were shown as total saxitoxin (neo-saxitoxin and saxitoxin) quota per biomass unit (ngSTX_{total}/µgC).

Statistical analysis

To identify any significant difference in the mean number of affected individuals, the one-way analysis of variance (ANOVA) was used with *post hoc* Dunnett test in order to ascertain the lowest-observed-adverse-effect level. All statistical analyses were performed using the software SigmaPlot 11.0 and attending the basic premises of the above-mentioned tests.

RESULTS

Cyanobacterial measurements and toxin content

The average length and width of filament obtained was, respectively, 78.38 (±38.91) µm and 2.99 (±0.58) µm resulting in an average trichome volume of 550.35 µm³ and biomass value of 108,988.601 µgC L⁻¹. The Total saxitoxin concentration normalized by trichome biomass was 0.2 ngSTX_{total} µgC⁻¹.

Acute toxicity of *R. raciborskii* on *M. spinosa*

In the acute bioassays, the effects of *R. raciborskii* ITEP-A1 characterized by paralysis of swimming appendages was noticed in both clones. After 24h of exposure to the cyanobacterium, all replicates already presented immobile organisms in all concentrations of biomass (data not shown). After 48h of exposure that number grew, as the effects of the cyanobacteria affected in more individuals. The immobilization effect was higher with the higher concentrations of cyanobacterial biomass (500 µgC L⁻¹) (Figure 1).

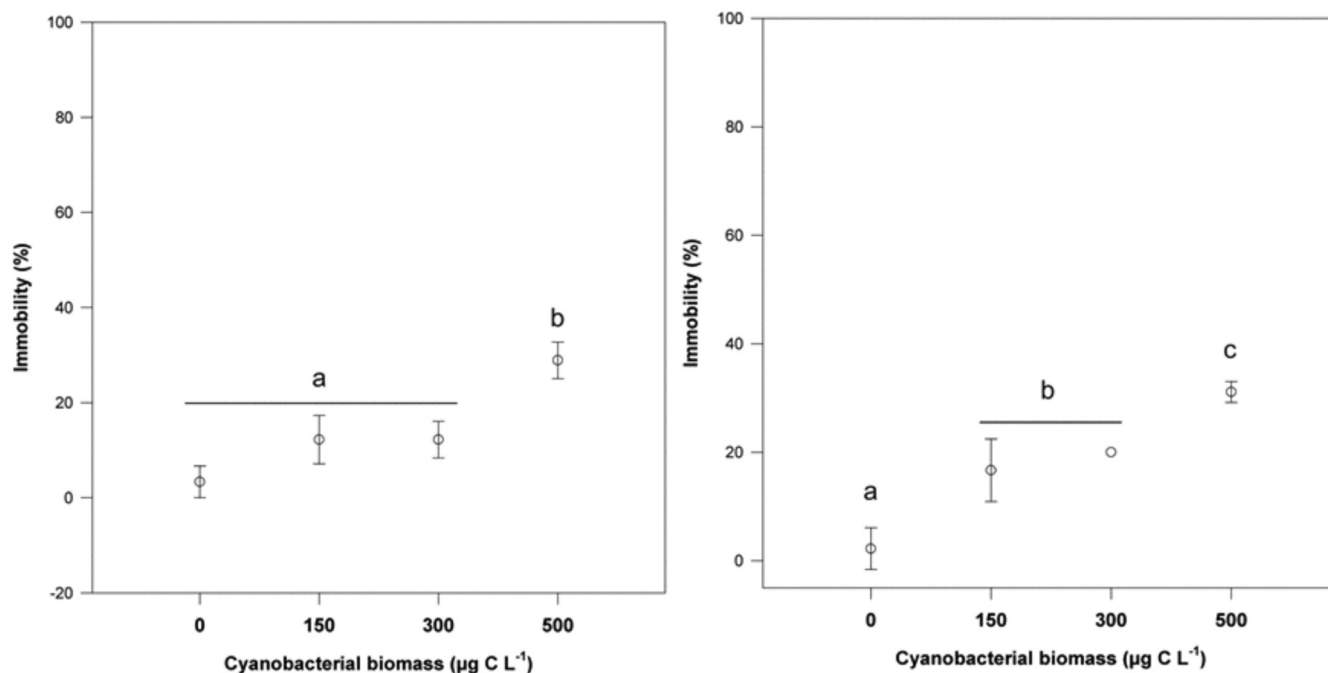


Figure 1. Dose-response of *Macrothrix spinosa*, clones (A) Duas Unas and (B) Prata under acute exposition to toxic *Raphidiopsis raciborskii* ITEP-A1. Letters = Significant difference compared to the control (Dunnett, $p < 0.05$), representing the lowest-observed-adverse-effect.

The lowest-observed-adverse-effect concentrations were equal to or above $150 \mu\text{g C L}^{-1}$ for Prata and $300 \mu\text{g C L}^{-1}$ for Duas Unas *M. spinosa* clones (Dunnett, $p < 0.05$). As showed in Table 1, EC_{50} (48h) for Duas Unas was $359 \mu\text{g C L}^{-1}$, approximately two times higher than the EC_{50} (48h) for Prata, evidencing that individuals originated from Prata reservoir show more sensitivity to *R. raciborskii* ITEP-A1 than the organisms from Duas Unas reservoir.

Bibliographic survey

When analyzed the bibliographic survey it was found 456 articles when used the terms *bioassay and cyanobacteria*, and 73 articles to *neurotoxicity and cyanobacteria*. The most specific the keywords, less articles were found: *cyano* and toxicity and cladocera* (16 articles); *saxitoxin and cyanobacteria and cladocera* (3 articles); *Macrothrix and toxicity* (6 articles). After screening, only 3 papers out of >400 publications reported data that fitted the established criteria and presented EC_{50} values that were then compared to those of the present study, as summarized in the Table 2.

DISCUSSION

In the present study a tropical zooplankton species *M. spinosa* showed to be sensitive to ecologically relevant concentrations of saxitoxin-producing cyanobacteria, regarding its potential on biomonitoring cyanobacterial blooms in tropical water bodies.

When comparing to previous toxicity studies of STX-producing *R. raciborskii* strains, the EC_{50} (48h) values to *M.*

spinosa clones Duas Unas $359 \mu\text{g C L}^{-1}$ ($\sim 3.930 \text{ cell mL}^{-1}$) and Prata $189 \mu\text{g C L}^{-1}$ ($\sim 2.607 \text{ cell mL}^{-1}$) were both similar or dissonant to those with standard cladocerans, such as *D. similis*. Also, although *M. spinosa* has not yet been standardized regarding acute toxicity tests, the species presents similarities with standard test-organisms. For instance, data presented by Zagatto *et al.* (2012) demonstrated different sensitivities in relation to our findings – *D. similis* exposed to *R. raciborskii* T2 strain showed less sensitivity when compared to our data, while for the T3 strain the daphnid showed more sensitivity. The same was reported by Ferrão-Filho *et al.* (2014), where EC_{50} values for *D. similis* exposed to the strain *R. raciborskii* CYRF-01 evidenced more sensitivity than our findings. In contrast, when analyzed the data showed by Ferrão-Filho *et al.* (2010), the EC_{50} values for *Daphnia pulex* and *Moina micrura* Kurz, 1875 exposed to CYRF-01 were not so far off from our data, with *D. pulex* exhibiting near equal sensibility to CYRF-01 as *M. spinosa* Prata did to ITEP-A1. Therefore, these data showed us that sensitivity of different cladoceran species or clones to STX-producing cyanobacteria can vary, either by intrinsic differences on tolerance or because different strains may display differences in their toxin profile and total amount of metabolites, which affects the relative toxicity. Also, we cannot disregard the potential

Table 1. Half maximal effective concentration values (EC_{50} – 48h) and 95% confidence intervals (CI) estimated for both clones of *M. spinosa*.

Test organism	EC_{50} –48h (95% CI) $\mu\text{g C L}^{-1}$
<i>Macrothrix spinosa</i> (Duas Unas)	359.27 (323.37 – 399.16)
<i>Macrothrix spinosa</i> (Prata)	188.99 (73.83 – 483.75)

Table 2. Half maximal effective concentration values (EC_{50}) obtained from the literature of cladocerans exposed to saxitoxin-producing strains of *Raphidiopsis raciborskii*.

Test organism	Parameters	EC_{50} ($\mu\text{gC L}^{-1}$)	EC_{50} (cell mL^{-1})	Reference
<i>Macrothrix spinosa</i>	Immobilization - 48 h	359.27 (323.37 – 399.16)	3,930	Present study
<i>Macrothrix spinosa</i>	Immobilization - 48 h	188.99 (73.83 – 483.75)	2,607	Present study
<i>Daphnia similis</i>	Immobilization - 48 h	-	302.56×10^3	Zagatto <i>et al.</i> (2012)
<i>Daphnia similis</i>	Immobilization - 48 h	-	0.218×10^3	Zagatto <i>et al.</i> (2012)
<i>Daphnia similis</i>	Immobilization - 2 h	31.5 (13.8 – 48.0)	-	Ferrão-Filho <i>et al.</i> (2014b)
<i>Moina micrura</i>	Immobilization - 2–3 h	-	8,905	Ferrão-Filho <i>et al.</i> (2010)
<i>Daphnia pulex</i>	Immobilization - 2–3 h	-	2,133	Ferrão-Filho <i>et al.</i> (2010)

variation among experimental designs. Those differences, even small, allow some variation in sensitivity.

Saxitoxin-producing cyanobacteria are usually reported as affecting swimming behavior of freshwater zooplankton by decreasing mobility and activity parameters (time spent swimming and resting, distance traveled, and mean velocity) (Ferrão-Filho *et al.*, 2008; 2014b). Saxitoxins can exert acute effects on zooplankton such as paralyze limbs, inhibit thoracic appendages beating, besides reducing the post-abdominal rejection of undesirable particles (Ferrão-Filho *et al.*, 2008; 2014b). Most recently Ferrão-Filho & Silva (2020) evidenced that neurotoxic cyanobacteria can reduce *Daphnia*'s heartbeat. Indeed, that result is in line with saxitoxins effects once these toxins also block calcium channels (Wiese *et al.*, 2010) which are abundant in the cardiac muscle.

Bibliographic survey allowed to evidence that most of data produced on STX-producing cyanobacteria impact on zooplankton focused on chronic effects such as growth, survivorship and reproduction (Soares *et al.*, 2009; Costa *et al.*, 2013; Ferrão-Filho *et al.*, 2014a; Ferrão-Filho *et al.*, 2019). Therefore, the lack of investigations regarding the acute toxicity of STX-producing cyanobacteria to freshwater zooplankton, especially those of the Macrothricidae family, limits a more in-depth, extensive comparison in our study. Moreover, once STX-producing *R. raciborskii* is up to date reported to South America, most of the studies concentrates in this area, although still incipient. However, considering that *R. raciborskii* is an opportunistic and potentially-toxic species which has dominated in waterbodies from different regions and more recently spread to temperate zones, it is important to produce data on its early impacts on aquatic biota in order to predict future environmental outbreaks as well as subsidize monitoring programs on water quality.

Besides zooplankton sensitivity, cyanobacterial toxicity also depends on the composition of secondary metabolites, such as known cyanotoxins and other non-characterized

molecules, as well as their specific cell content. Several of these secondary metabolites are shown to be toxic to plants, invertebrates and vertebrates (Huisman *et al.*, 2018) and, considering the same strain can produce more than one toxic metabolite, it becomes difficult to link the adverse effects to one single known toxin (Ferrão-Filho *et al.*, 2011).

There are substantial differences between the sensibilities of different bioindicator species (Ferrão-Filho *et al.*, 2017). Body length (surface area/volume ratio), having a more selective diet and metabolic differences are some of the characteristics interfering on the effects of toxicants in the organism, and can be limiting factors when choosing a bioindicator species for water monitoring assessments (Costa *et al.*, 2013; Ferrão-Filho *et al.*, 2011; 2014; Gustafsson *et al.*, 2004). However, geographical differences also play a part in a species' sensitivity (Araújo *et al.*, 2008). In some studies, autochthonous tropical species have demonstrated higher sensitivity to certain compounds than their temperate counterparts (Moreira *et al.*, 2014; Moreira *et al.*, 2017), which leads to an increasing interest in the use of native species in ecotoxicological assessments, as the results tend to be more representative of local impacts (Araújo *et al.*, 2008). Despite this, water quality assessments in tropical regions are still mostly based on methods developed in temperate regions, which may diminish the ecological relevance of results (Araújo *et al.*, 2008; Moreira *et al.*, 2014).

The obtained results also revealed that the two clones of *M. spinosa* isolated from different locations were differently affected by *R. raciborskii* metabolites (Table 1). Curiously, the clone isolated from the eutrophic lake with previous occurrence of cyanobacterial blooms seems to be more tolerant to *R. raciborskii*. It is suggested that *M. spinosa* Duas Unas which has previously experienced cyanobacteria, may have evolved tolerance along generations. Chislock *et al.* (2019) evidenced that clones of *Daphnia pulex* from eutrophic lakes tend to have higher growth rates and survival when

fed toxic cyanobacteria than populations from oligotrophic environments where cyanobacteria are less abundant. Besides species-specific traits, intraspecific (clonal) particularities should also be taken into consideration. In our study we used two clones of the same species, however the differences in tolerance to toxic dietary cyanobacteria could be linked to their dietary history in their respective sampling locations as hypothesized by Hairston *et al.* (2001).

In addition, small-size zooplankton generally represents the size spectra predominant under cyanobacterial dominance. Studies show that different body length among *Daphnia* spp. clones, as well as predation rate, promote different responses to filamentous cyanobacteria (Gilbert *et al.*, 1990; Spitze, 1992). However, when considering the changes in zooplankton structure along bloom establishment, clonal differences are probably as large as those among species, and therefore as important for community structure (Hietala *et al.*, 1995).

According to Ortiz-Rodríguez *et al.* (2012), exposure of the parental generation to certain substances results in more tolerance from future generations. Gustafsson *et al.* (2004), for instance, revealed that parts of *D. magna* populations that had been pre-exposed to toxic cyanobacteria *Microcystis aeruginosa* had a higher tolerance manifested as higher survivorship even under cyanobacterial food than those populations that were not pre-exposed. Results from Sarnelle *et al.* (2005) also indicated that *D. pulicaria* clones isolated from eutrophic lakes were less inhibited by toxic *Microcystis* than *D. pulicaria* isolated from oligotrophic lakes.

This phenomenon is suggested as a result of natural selection driving adaptation to cyanobacteria producing toxic or inhibitory compounds (Sarnelle *et al.*, 2005). When facing water bodies with recurring toxic cyanobacterial blooms, selection may act on zooplanktonic grazers and favor those that evolve resistance to the compounds and are best able to survive, grow, and reproduce (Ferrão-Filho *et al.*, 2011; Hairston *et al.*, 2001; Sarnelle *et al.*, 2005).

In *Daphnia*, many adaptive responses, such as responses to dietary changes in the environment, are maternally transferred to the next generation (Schwarzenberger *et al.*, 2013). Hairston *et al.* (1999) revealed these short-term evolutionary adaptations in *Daphnia* to an hepatotoxin-producing cyanobacterial strain, but no studies have reported this effect stimulated by saxitoxin-producing cyanobacteria, hence the need of future studies to thoroughly analyze these transgenerational effects on the latter.

Nevertheless, it is likely that a disparity in sensibility between Duas Unas and Prata clones of *M. spinosa* comes from the fact that Duas Unas Reservoir is a eutrophic environment, with recorded *R. raciborskii* blooms occurrence, while Prata lake does not show any recorded bloom. It is presumed that previous exposition to cyanobacterium and its metabolites resulted in transgenerational adaptations, justifying the neonates from Prata lake being two times more sensible than those from Duas Unas.

The obtained results reinforce the importance of analyzing whether the test organisms had previous contact

with toxicants in their natural environments, seeing that a correlation can be made between tolerance of toxic cyanobacteria by *M. spinosa* and prevalence of cyanobacteria in their habitat. Difference in sensitivity between species and clones of the species is definitely important to factor in when choosing test-organisms in ecotoxicological assays, once transgenerational heritage (rapid evolutionary events) seemingly has great influence in bioindicators' responses to toxicants. Besides, the use of ecologically-relevant test-subjects benefits tropical ecotoxicology, as it prospects tropical standardization and the responses are more relevant to the regions. Furthermore, despite of the difference among clones, native Macrothricidae have shown to be sensitive model organisms and have the potential for biomonitoring toxic cyanobacterial blooms in tropical reservoirs.

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