Effects of a Saxitoxin-Producer Strain of *Cylindrospermopsis raciborskii* (Cyanobacteria) on the Swimming Movements of Cladocerans

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Received 6 July 2007; accepted 8 July 2007

**ABSTRACT:** This study evaluated the effects of a saxitoxin-producer strain (T3) of the cyanobacteria species *Cylindrospermopsis raciborskii* on the swimming movements of three cladoceran species (*Daphnia gessneri*, *D. pulex*, and *Moina micrura*). Acute toxicity bioassays were designed to access the effects of T3 strain, of a nonsaxitoxin producer strain (NPLP-1) of the same species and of a raw water sample from Funil reservoir (Rio de Janeiro, Brazil), that contained this and other cyanobacteria. In the acute bioassays, animals were exposed to *C. raciborskii* filaments or Funil water for 24–48 h and then transferred to food suspensions without cyanobacterial filaments for a further 48 h. During the exposure time to T3 strain filaments there was a decrease in the number of swimming individuals, with animals showing progressive immobilization. The same effect was observed with Funil water sample. Animals stayed alive on the bottom of the test tube and recovered swimming movements when transferred to food suspensions without toxic cells. This effect was not observed with the strain NPLP-1. The cladoceran *D. pulex* showed to be extremely sensitive to T3 strain and to Funil water containing *C. raciborskii* filaments, showing complete paralysis after 24-h exposure to T3 cell densities of 10^3 and 10^4 cells mL^-1, and after 24-h exposure to only 10% of raw water. However, *D. gessneri* was not sensitive to both T3 and to Funil water, whereas *M. micrura* was intermediate in sensitivity. This is the first report on the effects of cyanobacterial saxitoxins on movements of freshwater cladocerans, showing also difference in sensitivity among closely related *Daphnia* species. © 2008 Wiley Periodicals, Inc. Environ Toxicol 23: 161–168, 2008.

**Keywords:** zooplankton; cladocerans; cyanobacteria; saxitoxins; toxicity bioassays

**INTRODUCTION**

Saxitoxins (STxs) are potent alkaloid neurotoxins that act by blocking sodium ion channel in neurons, impairing impulse propagation, and leading to a lack of stimulus in muscle cells. These toxins were first isolated in marine shellfish contaminated with marine dinoflagellate species (Anderson, 1994), and have been involved in the death of humans (Bricelj et al., 1990). The intoxication syndrome in humans is known as paralytic shellfish poisoning (PSP) and the toxins involved are known as paralytic shellfish toxins (PST) produced by many genera of red tide forming dinoflagellates such as *Alexandrium* sp., *Pyrodinium* sp., and *Gymnodinium* sp. (Darànas et al., 2001), and by some freshwater cyanobacteria such as *Anabaena circinalis* (Negri...
and Jones, 1995), Aphanizomenon flos-aquae (Mahmood and Carmichael, 1986), and Cylindrospermopsis raciborskii (Lagos et al., 1999).

STXs are known to accumulate in several marine organisms such as mussels and in zooplankton, which act as vectors of these toxins to higher trophic levels such as fish and whales (White, 1981; Boyer et al., 1985; Turrif et al., 1995; Teegarden and Cembella, 1996; Turner et al., 2000; Chen and Chou, 2001; Durbin et al., 2002; Hamazaki et al., 2003).

Despite the great number of saxitoxin studies in marine ecosystems, there are few studies that have been done with freshwater organisms. The study of Negri and Jones (1995) demonstrated the accumulation of STXs produced by A. circinalis in freshwater mussels Alathrya condola, and Nogueira et al. (2004) have shown the accumulation of STXs from A. isatschenkoi in D. magna. Since no adverse effects have been reported on these filter-feeders, the accumulation of STXs can be attributed to resistance of these organisms to such toxins.

However, adverse effects of STXs in aquatic organisms includes a variety of responses including reduced ingestion rates caused by “physiological incapacitation” (Ives, 1985, 1987), avoidance of toxic cells by chemosensory means in copepods (Huntley et al., 1986; Sykes and Huntley, 1987; Teegarden and Cembella, 1996), reduced somatic growth, size at maturity, and fecundity (Dutz, 1998; Colin and Dam, 2004). Haney et al. (1995) have reported a reduction in the thoracic appendages beating rate and an increase in rejection rate of particles by the postabdomen of D. carinata when exposed to a filtrate of A. flos-aquae and to purified saxitoxin. Nevertheless, to date no other adverse effects of STXs have been reported in freshwater cladocerans.

In this study, we present the first report on the effects of a saxitoxin-producer strain of C. raciborskii on the swimming behavior of freshwater cladoceran species. We also perform bioassays with a nontoxic strain of C. raciborskii and with a water sample from a reservoir with occurrence of this cyanobacterium.

MATERIALS AND METHODS

Origin and Culture of Methods

Two Brazilian strains of C. raciborskii Woloszynska (T3 and NPLP-1) were cultured in ASM-1 medium (Gorham et al., 1964), pH 8.0, 23 ± 2°C, and 40–50 μE m⁻² s⁻¹ and 12:12 h light–dark cycle. The strain T3, isolated from Billings Reservoir, City of São Paulo (SP, Brazil), was firstly reported to produce STXs by Lagos et al. (1999), and its toxins include neosaxitoxin (NEO), decarbamoyl neosaxitoxin (deNEO), saxitoxin (STX), and decarbamoyl saxitoxin (dcSTX) (G. K. Eaglesham, Queensland Health Scientific Services, Coopers Plains, Austrália; pers. comm.). The strain NPLP-1 was isolated from Paranoá Lake, Brásilia D. C. (GO, Brazil), and was nontoxic in mouse bioassay. Both C. raciborskii strains were grown in the form of straight trichomes of >100 μm in length.

Two cladocerans species, D. gessneri Herbt and Moina micrura Kurs, were isolated from an oligotrophic reservoir (Lajes) in Rio de Janeiro State, Brazil, with no occurrence of toxic blooms. A temperate clone of D. pulex Leydig was obtained from Carolina Biological Supply, NC. Cladocerans were cultured in mineral commercial water, with a hardness of 77.2 mg CaCO₃ L⁻¹, pH of 7.4, at 23 ± 2°C and at dim light. For D. gessneri and M. micrura, 20% of filtered (glass fiber membrane, Sartorius AG 37070) water from Funil reservoir was added to mineral water. This filtered reservoir water was free from cyanobacteria and was supposed to supply some organic matter (i.e., vitamins), necessary as a growth factor for these species. Cladocerans were fed Ankistrodesmus falcatus Braun at a density of 1.6 × 10⁴ cells ml⁻¹ (~0.5 mg C L⁻¹).

Acute Toxicity Bioassays

These assays were conducted under the same conditions as the cladocerans cultures, using 30-mL flat-bottom test tubes. The experimental design consisted of two phases: (1) Exposure phase: when animals were exposed to different filament densities of each strain of C. raciborskii plus nutritious alga (A. falcatus) and (2) Recovery phase: when animals were transferred to new suspensions containing only the nutritious alga. The exposure phase usually lasted 48 h, except for D. pulex in the experiment with raw water from Funil reservoir which lasted 24 h. The recovery phase always lasted 48 h. For strain NPLP-1, exposure phase lasted 48 h and there was no recovery phase. There were 10 neonates (<24 h) in each test tube and four replicates per treatment. Every day, during both phases, animals were transferred to new algal suspensions and the number of immobile, dead, or swimming individuals were counted. Controls consisted of animals exposed only to the nutritious alga.

Two experiments were performed with strain T3. Experiment 1 was performed with D. pulex only, and Experiment 2 was performed with both D. gessneri and M. micrura. Although exposure concentrations were based on cell densities (10–10 000 cells ml⁻¹), toxin concentrations varied between experiments since cyanobacterium culture was used in different phases (12 days apart). The experiment with strain NPLP-1 was performed at the same time as Experiment 2 with strain T3.

Another toxicity bioassay was carried out with a raw water sample from Funil Reservoir (40 km² size and 70 m maximum depth), located near Resende City (22° 30’ S e 44° 45’ W, RJ, Brazil). This reservoir was built for water supply and electricity generation purposes, but it is also
used for recreation, fishing and for the regulation of floods in the surrounding areas. Its drainage basin is highly impacted because of erosion processes and discharge of domestic and industrial wastes. The large input of nutrients has turned this reservoir hypereutrophic, with recurrent blooms of cyanobacteria. The water sample was taken close to the dam in August 2003 (spring). This assay had the same experimental design as the previous assays except that treatments with raw water consisted of dilutions of this sample in mineral water.

Toxin Analysis

For STXs, 1 mL from C. raciborskii cultures or 10 mg of the lyophilized raw water (2 L) sample were extracted with 1 mL of HCl 0.1 N (1:1 v/v) for 1 h. Samples were then centrifuged at 10 800 \( \times g \) for 10 min and the supernatant stored at \(-18^\circ C\) until analyzed. Before the analysis, samples were oxidized with periodate solution according to Lawrence and Ménard (1991), and then analyzed by high performance liquid chromatography (HPLC) using a Shimadzu/CLASS VP apparatus with fluorescence detector (RF-10A XL), adjusted to 330 nm of excitation and 400 nm of emission, using a 20-\( \mu \)L loop, reverse column Merck LC-18 (Lichrocart\( ^{R} \) 150 mm \( \times \) 4.6 mm \( \Phi \), 5 \( \mu m \)). The mobile phase consisted of acetonitrile:ammonium formate 0.1 M (v/v), adjusted to pH 6.0, with a gradient varying from 0 to 1% in the first 15 min and from 1 to 4% in the last 15 min at a flow rate of 1 mL min\(^{-1}\). Standard solutions of saxitoxin (STX), neosaxitoxin (NeoSTX), and gonyautoxins (GTX 1 and 4 e GTX 2 and 3) were purchased from the National Research Council (NRC), Institute for Marine Biosciences (Canada), and solutions of C toxins (C1 and C2) and B toxin (B1) were kindly provided by Dr. Y. Oshima, Tohoku University, Japan. Those standard of toxins were used to identification and quantification of toxins present in the sample, by direct comparison to standards carried through the oxidation periodate procedure and the sum of all peak areas of toxins found was expressed as concentration equivalents of STX.

Microcystins were analyzed from 1 L of raw water sample of the reservoir, filtered through borosilicate filters (Sartorius), extracted three times with butanol:methanol:water (5:20:75 v/v/v). The extract was evaporated to 1/3 of its initial volume and then passed through a C18 cartridge (500 mg/6 mL Bond-Elut, Varian). This cartridge was washed and eluted with distilled water, with methanol 20% and then with methanol 100%. This last fraction was dried and stored at \(-20^\circ C\) for subsequent analyses. The microcystin analysis was performed by HPLC (Shimadzu/CLASS VP with UV detector PDA M10A VP), using a Lichrospher 100 RP-18 column (150 \( \times \) 4.6 \( \times \) 5 \( \mu m^3 \) Merck), and a 100-\( \mu L \) loop. Chromatography was conducted under isocratic conditions as follows: mobile phase acetonitrile and 20 mM of ammonium acetate in pH 5.0 (28.72 v/v) for 10 min, at a flow rate of 1 mL min\(^{-1}\), UV detection at 238 nm, and the spectrum of absorbance of each peak was analyzed between 190 and 300 nm. A standard of microcystin-LR (Sigma) was used as an external standard for microcystin quantification. The concentration of microcystin was estimated by the sum of all peak areas presenting an absorption spectrum 95% of similarity index with the standard microcystin-LR and was expressed as microcystin-LR equivalents, as described in Chorus and Bartram (1999).

Because of the presence of Anabaena sp. in the reservoir water, lyophilized water sample (2 L) was used to detect anatoxin-a(s), a potent acetylcholinesterase (AChE) inhibitor (Henriksen et al., 1997). In addition, the anatoxin-a(s) producer strain ITEP-024 (A. spiroides; Molica et al., 2005) was used as a positive control. Both lyophilized water sample and the ITEP-024 strain were extracted with 0.5–1.0 mL ethanol:acetic acid 1 N (20:80 v/v) for 3–4 h, and centrifuged at 12 400 \( \times g \) for 10 min, being the supernatant utilized in an AChE activity inhibition assay according to Nunes-Tavares et al. (2002) [adapted from Ellman et al. (1961)]. Membrane fractions rich in AChE were obtained from the electric tissue of Electrophorus electricus (Somlo et al., 1977). The method is based on the measurement of the rate of production of thiococholine as acetylthiocholine (ATCh) that is hydrolyzed at 25°C in the presence of 5-5’’-dithiobis-2-nitrobenzoic acid (DTNB), producing its yellow anion, which can be detected at 412 nm (Hitachi spectrophotometer model U-3300). Control consisted of Tris-HCl 50 mM pH 8.0, MgCl\(_2\) 5.0 mM, ATCh 0.4 mM, DTNB 0.125 mM, and 10.0 mg of total protein. Specific activity was expressed in micromole of ATCh hydrolyzed per minute per milligram protein. Protein concentration was determined according to the method of Lowry et al. (1951).

Data Analysis

For testing the effect of the C. raciborskii strains and reservoir water on cladocerans’ swimming activity, during the exposure and recovery phases, a two-way ANOVA was performed, determining mobility as the dependent variable and species and concentration as independent variables. We also used one-way ANOVA for testing differences between controls and treatments with each strain and reservoir water in the mortality rates at the end of each experiment. The statistical software utilized was Systat V.9.0, 1998 (SPSS, Chicago, IL).

RESULTS

STXs concentration varied between experiments, being about 10 times higher in Experiment 2 (Table I). No STXs were found in NPLP-1 strain.
In bioassays with the strain T3, we observed a rapid immobilization of animals in the presence of toxic filaments (Fig. 1). The symptom was characteristic of neurotoxicity, with animals showing immobilization after a few hours (beginning after ∼30 min) but staying alive on the bottom of the test-tube during the exposure phase. When transferred to fresh nutritious food suspensions without toxic filaments, some animals recovered movements and started swimming again after a few hours (1–2 h; data not shown), with most recovering after 48 h (Fig. 1). Species responded differently to the toxic filaments, with D. pulex being more sensitive than the other two species. Although immobilization in D. pulex started at 10 cells mL⁻¹ (0.000846 ng eq STX L⁻¹), this effect was shown by M. micrura only at 1000 cells mL⁻¹ (0.937 ng eq STX L⁻¹). Nevertheless, D. gessneri mobility was not affected at all by the strain T3.

The ANOVA performed on the data of the exposure phase showed a significant effect of species and concentration on the mobility of cladocerans, and also a significant interaction between these factors (Table IIA).

Mortality rates of animals exposed to strain T3 ranged from 0 (control) to 20% for D. pulex, 10% for D. gessneri, and 5% for M. micrura during the experiment, but no significant differences were found between controls and treatments (Table IIB).

The nonsaxitoxin producer strain NPLP-1 did not cause any effect on the swimming movements of cladocerans. However, mortality rates with this strain ranged from 5 to 15% for D. pulex, 0–20% for D. gessneri, and 0–5% for M. micrura during the experiment, but no significant differences were found between controls and treatments (Table IIB). The water sample from the Funil reservoir included C. raciborskii (5350 cells mL⁻¹), present as straight trichomes, as well as two other potentially toxic cyanobacteria, Anabaena sp. (2317 cells mL⁻¹) and Microcystis sp. (14770 cells mL⁻¹), present as spiral trichomes (>100 µm), and colonies (variable size), respectively.

Reservoir water contained 24.6 ng eq STX L⁻¹ (Table III) and the concentration of microcystins in reservoir water was 1.34 µg eq MC-LR L⁻¹. Also, the reservoir water sample caused 28% inhibition of the AChE activity relative to control. The strain ITEP-024, however, caused a 100% inhibition in the AChE activity. Specific activities for control, reservoir water, and ITEP-024 strain were 5700, 4150, and 0 µmol ACh hydrolyzed per minute per milligram protein, respectively.

There was also a pronounced effect of the reservoir water sample on the swimming movements of cladocerans (Fig. 2). The effect was even faster in D. pulex, with virtually all animals being immobilized after 24-h exposure to only 10% (2.5 ng eq STX L⁻¹) of reservoir water. For this reason we decided to transfer the individuals of D. pulex to cyanobacteria-free medium after 24-h exposure. The

<table>
<thead>
<tr>
<th>Cell Density (cells mL⁻¹)</th>
<th>Experiment 1 (August 28, 2003)</th>
<th>Experiment 2 (September 9, 2003)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. pulex</td>
<td>D. pulex</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.000846</td>
<td>–</td>
</tr>
<tr>
<td>100</td>
<td>0.00846</td>
<td>0.0937</td>
</tr>
<tr>
<td>1000</td>
<td>0.0846</td>
<td>0.937</td>
</tr>
<tr>
<td>10000</td>
<td>0.846</td>
<td>9.37</td>
</tr>
</tbody>
</table>
same effect was experienced by *M. micrura* only with 50% (12.3 ng eq STX L\(^{-1}\)) after 48-h exposure and with 100% (24.6 ng eq STX L\(^{-1}\)) of reservoir water after 24 h. As in the assay with T3 strain, the animals recovered their movements after being transferred to fresh nutritious food suspensions. Species rank in sensitivity was the same as in the previous bioassay, with *D. pulex* being the most sensitive and *D. gessneri* not being affected by the reservoir water.

The results of the two-way ANOVA for the exposure to reservoir water showed a significant effect of species and concentration on mobility of cladocerans, and a significant interaction between these factors (Table IVA). During the exposure to reservoir water no mortality was observed for *D. gessneri*, whereas mortality rates ranged from 20 to 50% for *D. pulex* and from 25 to 35% for *M. micrura* (not significantly different from controls for both species; Table IVB). There was also some mortality in the controls for *D. pulex* and *M. micrura* (<10% for both species).

**TABLE III.** Percentage of raw water from Funil reservoir, cell densities of *C. raciborskii* and saxitoxins concentrations in experiment 3

<table>
<thead>
<tr>
<th>Raw Water (%)</th>
<th>Cell Density (Cells mL(^{-1}))</th>
<th>Saxitoxins (ng eq STX L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>535</td>
<td>2.5</td>
</tr>
<tr>
<td>25</td>
<td>1337</td>
<td>6.2</td>
</tr>
<tr>
<td>50</td>
<td>2675</td>
<td>12.3</td>
</tr>
<tr>
<td>100</td>
<td>5350</td>
<td>24.6</td>
</tr>
</tbody>
</table>


**DISCUSSION**

Our results show strong evidence that STXs can cause a decrease in the swimming movements of some species of freshwater cladocerans. The effect is suggestive of a neurotoxicosis, with rapid immobilization and rapid recovery after the animals were removed from the water medium containing toxic filaments. This effect, however, is dependent on the test-organism (cladoceran species), on the density of toxic filaments in the water and also on the exposure time. The fact that cladocerans did not show any alteration in the swimming behavior in the presence of the nonsaxitoxin-

![Fig. 2. Percent mobility during the exposure phase to raw water sample from Funil reservoir: 0–24 h for *D. pulex* and 0–48 h for *M. micrura*; and during the recovery phase: 24–72 h for *D. pulex* and 48–96 h for *M. micrura*. The arrow represents the beginning of the recovery phase. Controls consisted of animals exposed to only mineral water with food and treatments consisted of animals exposed to different dilutions of raw water in mineral water with food.](Image)
TABLE IV. (A) Results of two-way ANOVA from the acute toxicity experiment with the Funil reservoir water. (B) Results of one-way ANOVA for testing differences between controls and treatments with reservoir water.

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Effects on mobility at the end of exposure period</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>2</td>
<td>10285.3</td>
<td>531.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Concentration</td>
<td>3</td>
<td>498.4</td>
<td>124.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Species × concentration</td>
<td>6</td>
<td>107.7</td>
<td>9.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>36</td>
<td>27.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B) Effects on mortality rates at the end of the experiment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. pulex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration</td>
<td>4</td>
<td>870.0</td>
<td>2.42</td>
<td>0.094</td>
</tr>
<tr>
<td>Error</td>
<td>15</td>
<td>360.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. micrura</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration</td>
<td>3</td>
<td>891.7</td>
<td>0.915</td>
<td>0.463</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>975.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

producer strain NPLP-1 also corroborates the hypothesis that STXs are the cause of immobilization.

The possibility that microcystins present in the raw water sample from Funil reservoir could have caused the immobilization is less likely since other studies have reported effects of these toxins other than in swimming behavior of cladocerans (DeMott et al., 1991; Ferrão-Filho et al., 2000; Rohrlack et al., 2005). The effect observed is more compatible with the mechanism of action of neurotoxins than with that of hepatotoxins. On the other hand, anatoxin-a(s) is a potent AChE inhibitor having also effects on neural cells (Mahmood and Carmichael, 1986; Henriksen et al., 1997). Therefore, the slight inhibition of AChE by the reservoir water sample in the inhibition assay indicates that anatoxin-a(s) could be implicated in the immobilization of animals. However, due to its big size and spiral shape, *Anabaena* filaments were probably inedible to most cladocerans tested (Porter and Orcut, 1980; DeBernardi and Giussani, 1990), and thus it is unlikely that anatoxin-a(s) alone have caused the immobilization of cladocerans in reservoir water. However, we can not exclude the possibility that some smaller colonies of *Microcystis* and fragments of *Anabaena* filaments have been eaten by cladocerans. Thus, the strongest immobilization of both cladocerans and the higher mortality observed during the bioassay with reservoir water may be attributed to the higher concentration of STXs in reservoir water (about 2.6–29 times higher than the maximum concentrations of T3 strain) and to a synergistic effect of other cyanobacterial toxins.

Most of the studies done with STXs are in marine systems (Landsberg, 2002). Some studies have shown the effect of dinoflagellates’ STXs on the feeding behavior and reproduction of marine copepods (Ives, 1985; Huntley et al., 1986; Sykes and Huntley, 1987; Teegarden and Cembella, 1996; Dutz, 1998). However, no other study has demonstrated that STXs can exert inhibitory effects on the swimming movements of freshwater cladocerans.

The only study dealing with effects of STXs in cladocerans was that of Haney et al. (1995), who reported a reduction in the thoracic appendage beating rate and an increase in rejection rate of particles by the postabdomen of *D. carinata* when exposed to a filtrate of a saxitoxin-producing strain of *A. flos-aquae* and to purified STX. As in our study, they showed that animals recovered thoracic appendages beating and postabdominal rejection rates after the medium containing STXs was changed to control medium. Similarly, they found also the same effect using filtered water from a pond with a bloom of *A. flos-aquae*. Differently, they did not use intact filaments during their bioassays. Also, they showed a significant effect of incubation time of *A. flos-aquae* medium in these parameters, suggesting that products released by this cyanobacterium were responsible by the effects on *D. carinata*. The fact that purified STX caused inhibition of thoracic appendage beating, which was rapid and reversible and comparable in strength to the inhibition caused by the pond water containing *A. flos-aquae*, lead the authors to conclude that these chemicals play an important role in the feeding behavior of freshwater herbivores such as *Daphnia*. Because of the mixed responses (inhibition of thoracic appendages and stimulus of postabdomen) of *Daphnia* they also concluded that these chemicals act as a chemosensory cue causing behavioral change in feeding activities rather than as a direct inhibition of motor activity.

Our results, however, strongly suggest that chemicals produced by *C. raciborskii* do act as a direct inhibitor of motor activity, as shown by the inhibition effect on mobility of cladocerans. This effect is probably caused by STXs produced by this strain, since the other nonsaxitoxin-producing strain of *C. raciborskii* did not cause any effect on mobility. The mechanism involved in such effect is in agreement with that described in squid and crayfish giant axons when exposed to STX, leading to blocking of sodium channels and interference with nerve impulse conduction, with such effect being completely reversible (Adelman et al., 1982). Thus, in cladocerans, its is likely that STXs are acting on the nerves cells that control the muscles of the second antennae, leading to inhibition of nerve impulse conduction and paralysis of this appendage.

Therefore, contrary to the conclusions of Haney et al. (1995), our results suggest a toxicosis response rather than a behavioral response, since the response in cladocerans mobility was proportional to the density of toxic cells during both the exposure and recovery phase. Although we did not measure the feeding activity of our animals, we observed that they kept thoracic beating during the experiments. However, we cannot exclude the possibility of a behavioral inhibition of the feeding activity. This inhibition would be, however, beneficial for the animals since they would decrease the ingestion of toxic filaments. This
behavior would have an adaptative value for cladocerans living in aquatic environments with occurrence of toxic blooms, which would favor the capacity of rapid sensing and response to these chemical cues (Haney et al., 1995).

Resistance of freshwater zooplankton to toxic blooms of cyanobacteria have been observed in some studies (DeMott and Moxter, 1991; Ferrão-Filho and Azevedo, 2003) and local adaptation (resistance) of historically exposed copepod populations to STXs -producing dinoflagellates have been reported (Colin and Dam, 2004). Copepods have generally been reported as selective filter-feeders while cladocerans are considered nonselective (DeMott, 1990) and are indeed able to discriminate between toxic and nontoxic cyanobacteria (DeMott and Moxter, 1991). However, data on food ingestion of Notodiaptomus inheringi exposed to a natural phytoplankton from Funil reservoir showed that this copepod preyed efficiently on small colonies of Microcystis and also on C. raciborskii (Panoso et al., 2003). Two of the cladocerans tested (D. gessneri and M. micrura) are commonly found in Brazilian reservoirs, with D. gessneri being the most frequently found in Funil reservoir. The resistance shown by D. gessneri to C. raciborskii may explain the coexistence between these species in Funil reservoir. Although we cannot measure if the resistance of D. gessneri is behaviorally or physiologically mediated, this suggests that different susceptibilities of cladocerans to STXs may be of adaptative value, shaping the structure of plankton communities in freshwater lakes.

The rapid and sensitive response of cladocerans, especially D. pulex, to C. raciborskii chemicals in water can be of great value in biomonitoring of water bodies, especially public water supply reservoirs dominated by these cyanobacteria. The chemical analyses of cyanotoxins are expensive, requiring sophisticated equipment and highly qualified technicians. Thus, specific standardized bioassays with these organisms for the detection of STXs in water samples should be developed to ensure, in a first instance, if water is free of these toxins, before a chemical analysis can be made.

REFERENCES


