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EFFECTS OF *CYLINDROSPERMOPSIS RACIBORSKII* (CYANOBACTERIA) ON THE SWIMMING BEHAVIOR OF *DAPHNIA* (CLADOCERA)

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Abstract: The present study aimed to test the effects of raw water samples from a eutrophic reservoir and of a saxitoxin-producing strain of *Cylindrospermopsis raciborskii* on the swimming behavior of 2 key herbivore species of *Daphnia*. Two complementary approaches were used, acute bioassays and behavioral assays using an automated movement tracking system for measuring the following activity parameters: swimming time, resting time, distance traveled, and mean velocity. In both assays, animals were exposed to field samples or to toxic filaments in different concentrations and observed for 2 h to 3 h. In the acute bioassays, there was a decrease in the number of swimming individuals during the exposure period and a recovery following removal from toxic algae. A significant relationship was found between median effective concentration and the saxitoxin content of seston ($r^2 = 0.998$; $p = 0.025$) in the acute bioassays with raw water samples. Behavioral assays also showed significant effects in the activity parameters with both field samples and the strain of *C. raciborskii*, with some recovery during the exposure period. Both approaches corroborated previous research on the effects of neurotoxic *C. raciborskii* on the swimming activity of *Daphnia*, and these effects are compatible with the mechanism of action of saxitoxins. The present study showed that activity parameters of aquatic organisms may be a useful tool in the evaluation of sublethal toxicity and detection of neurotoxins in raw water. *Environ Toxicol Chem* 2014;33:223–229. © 2013 SETAC

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INTRODUCTION

Cyanobacteria are prokaryotic organisms that are capable of producing several types of secondary metabolites, such as the cyanotoxins. These are categorized mainly as follows: hepatotoxic cyclic peptides, microcystins (MC) and nodularins (NOD); neurotoxic alkaloids, anatoxin-a (ANTX-a) and saxitoxins (STX); the organophosphate anatoxin-a(s) (ANTX-a(s)); the cytotoxic alkaloid cylindrospermopsin (CYN); and lipopolysaccharides (LPS). Among the typical toxin producers are the planktonic genera such as *Anabaena* (ANTX-a, ANTX-a(s), STX), *Aphanizomenon* (ANTX-a, CYN, STX), *Cylindrospermopsis* (CYN, STX), *Microcystis*, *Nodularia* (NOD), and *Planktothrix* (ANTX-a, MC) [1].

Both MC and NOD are the best-studied toxins regarding both their toxicity and mechanism of action. These toxins act as inhibitors of protein phosphatases 1 and 2A, causing a disruption in the cytoskeleton of hepatocytes, loss of sinusoidal structure, increase in liver weight as a result of intrahepatic hemorrhage, hemodynamic shock, heart failure, and death [1]. Several effects of MC- and NOD-producing cyanobacterial strains on zooplankton have been also reported in the literature such as decreased survivorship, fecundity, and growth and feeding inhibition [2–5].

In contrast, the effects of neurotoxins on aquatic biota have so far been studied little [6]. The alkaloid ANTX-a is a postsynaptic cholinergic nicotinic agonist, acting as a potent neuromuscular blocking agent, binding irreversibly to the acetylcholine receptors and causing staggering, muscle twitching, and gasping in animals and rapid death by respiratory arrest [1]. Among the aquatic invertebrates, rotifers are especially sensitive to neurotoxic strains of *Anabaena flos-aquae* and to pure ANTX-a [7]. The organophosphate ANTX-a(s) acts by inhibiting the activity of acetylcholinesterase, causing hypersalivation and convulsions in mammals and also death by respiration arrest [8]. Reports of the effects of ANTX-a(s) on invertebrates are scarce, but there is evidence that toxic *A. flos-aquae* reduces the survivorship and clearance rates of *Daphnia* and copepods [9].

The STXs are a class of alkaloid neurotoxins with 22 variants also known as paralytic shellfish poisons or toxins. The mechanism of action is the reversible blocking of sodium channels in the neurons, leading to impaired action potential and paralysis of muscles and respiratory arrest, killing mice 2 min to 30 min after intraperitoneal injection [1]. In aquatic invertebrates, STX-producing strains have been reported to affect the fitness and growth potential of *Daphnia* [10,11], the filtering behavior of mussels [12], the thoracic appendage beating rate [13], and the swimming movements of *Daphnia* [14–16].

The cyclic guanidinic alkaloid CYN has a completely different mechanism of toxicity, being a protein synthesis inhibitor, with a major impact on liver cells, but also on other organs such as kidneys, spleen, intestine, thymus, and heart in vertebrates, in agreement with the more general concept of cytotoxicity [8]. The CYN-producing strains can cause reduced survivorship and growth, as well as damage to the midgut and diverticula epithelium of *Daphnia* [17,18].

Lipopolysaccharides are produced by all genera of cyanobacteria and cause inflammatory reactions in contact with any tissue. Few studies have reported the effects of LPS in aquatic organisms [19–21]. Some studies have, however, reported a protective effect of LPS against MC toxicity on *Daphnia* [21,22].

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The nonprotein amino acid neurotoxin β-*N*-methylamino-L-alanine has recently been found in a broad diversity of free-living cyanobacteria from freshwater, brackish, and marine environments [23]. It has been linked to neurodegenerative diseases such as amyotrophic lateral sclerosis and Alzheimer's disease in humans [23]. It has also been shown that this neurotoxin has the potential to biomagnify in the aquatic food chain [24]. However, its effects on aquatic biota have never been demonstrated.

In Brazil, several genera of toxin-producing cyanobacteria occur in lakes and reservoirs, and studies have reported the occurrence of MC, STX, and ANTX-a(s) [25–28]. Although all these toxins are potentially capable of causing harmful effects to zooplankton species [7,9,15], these effects can hardly be attributed to specific toxins in nature because environmental samples may contain more than 1 species of cyanobacteria and class of toxin [27]. Nevertheless, study of toxic strains isolated from the same environment makes it easier to relate the observed effects to the toxins present in environmental samples [16].

Considered as an invasive species originally from the tropics, *Cylindrospermopsis raciborskii* have been registered in several aquatic habitats worldwide [29–31], and in Brazil it is dominant or codominant in part of the year or even year round [26,32,33]. Previous studies have indicated a seasonal pattern in the phytoplankton community in the eutrophic Funil Reservoir (Rio de Janeiro, Brazil), with changes in dominance between *Microcystis* and filamentous cyanobacteria, among them *Anabaena circinalis* and *C. raciborskii* [32].

Cyanotoxins have long been hypothesized to be a chemical defense against grazers (i.e., microcystins against *Daphnia* [34]). Although this hypothesis has been mainly directed toward microcystins and much of the research to date has been dedicated to this toxin, some evidence also points to strong grazer-impairing effects of other toxins, such as STX [15,16]. The aim of the present study was to test the hypothesis that natural water samples containing different classes of cyanotoxins as well as an STX-producing strain of *C. raciborskii*, isolated from the same reservoir, affect the swimming activity of 2 *Daphnia* species: *D. pulex* and *D. similis*, both previously known to be sensitive to STX-producing *C. raciborskii* strains [15,16,35]. To test this hypothesis, we used 2 approaches: 1) acute toxicity bioassays specially designed to detect the effects of neurotoxins [16], and 2) behavioral assays using an automated movement tracking system, which is able to track and measure continuously the swimming activity parameters of *Daphnia*.

MATERIALS AND METHODS

Daphnia cultures

Two *Daphnia* species were used in the experiments: *D. similis*, obtained from cultures of the Labtox-Biorio at Federal University of Rio de Janeiro (Rio de Janeiro, Brazil), and *D. pulex*, obtained from Carolina Biological Supply. Parthenogenetic females of both species were maintained in 2-L beakers filled with commercial mineral water (Minalba) at 23 °C, with a 12-h/12-h light/dark cycle (dim light), and fed daily the green algae *Ankistrodesmus falcatus* at a concentration of 0.5 mg C L^{-1}.

Cyanobacterium culture

A strain of *C. raciborskii* (CYRF-01) was isolated from the Funil Reservoir and was cultured in artificial medium (ASM-1), initial pH 8.0, at 23 °C and a 12-h/12-h light/dark cycle. This strain has been reported to produce STX and gonyautoxin [36], but also neosaxitoxin and decarbamoil neosaxitoxin [37]. The number of cells was estimated by counting samples in a Fuchs–Rosenthal hemocytometer, and biomass (dry wt) was determined by gravimetry, filtering 2 samples in glass–fiber filters (Sartorius 13400). The strain CYRF-01 presents straight filaments with variable lengths (~100 to >1000 μm), and cell biomass was estimated as 5.0768×10^{-5} μg $cell^{-1}$.

Field sampling for phytoplankton and toxin analyses

Subsurface water samples were taken from near the dam of the Funil Reservoir (surface area: 40 km^2; average depth: 22 m) in different seasons in 2007: winter (August), spring (October and November), and summer (December). For phytoplankton counting, 100-mL water samples were fixed with Lugol's solution and the methodology in Soares et al. [32] was followed. Phytoplankton biovolume (mm^3 L^{-1}) was estimated by multiplying the density of each species by the average volume of its cells, and specific biomass was expressed in mg (fresh wt) L^{-1}, assuming a specific density of phytoplankton cells of 1.0 g cm^{-3}.

For MC and STX analysis in seston, 10 L of raw water samples were taken in ice to the laboratory, from which 2 L were filtered onto glass–fiber filters (Sartorius 13400) in 2 replicates for each toxin. For the strain CYRF-01, a variable volume of culture (30–50 mL) was filtered through glass–fiber filters (Sartorius 13400) in 2 replicates. The MCs were analyzed by enzyme-linked immunosorbent assay (Plate Kit Beacon), and the STXs were analyzed by high-performance liquid chromatography as described in Ferrão-Filho et al. [27]. Toxins were expressed as MC-LR (MC with 2 amino acids, lysine [L] and arginine [R]) or STX equivalents.

Acute toxicity bioassays

The acute tests were designed to test the effect of immobilization (i.e., paralysis), and to observe the recovery, which is expected for the reversible mechanism of action of STX [16]. Experiments with raw water samples started immediately after collection or at least on the following day, with the sample kept under refrigeration. Newborns (<24 h) were placed in flat-bottomed tubes (4 replicate tubes with 10 animals each) with 30 mL of test medium. Treatments consisted of either dilutions of raw water samples performed with filtered lake water from the reservoir or concentrations of CYRF-01 strain cells established from cyanobacterial cultures diluted in *Daphnia* medium. In the former case, controls were performed with filtered lake water and in the latter case controls consisted of mineral water. To avoid nutritional effects, both controls and treatments were supplied with the green algae *A. falcatus* at a concentration of 0.5 mg C L^{-1}. Animals were left under exposure to field samples or intact cells of the strain CYRF-01 for 2 h to 3 h and checked after 0.5 h, 1 h, 2 h, and 3 h for the number of active swimming individuals. Animals were considered immobilized if they stayed for more than 10 s at the bottom of the test tube. Small movements, such as turnings and gyres along the main body axis, were not considered as active swimming. After exposure, animals were transferred to control water (mineral or filtered lake water) and checked for the number of active swimming, immobilized, and dead animals in the following interval until 24 h.

Behavioral tests

For analysis of swimming behavior, an image analysis device (Videomex-V, Columbus Instruments) was utilized, coupled to a video camera and to a computer, registering activity parameters such as time spent swimming, resting time, distance traveled, and velocity. Females 10 d old to 12 d old (1.5 mm–2.0 mm)

were placed in an acrylic chamber of 2 mL capacity, divided into 4 boxes for the exposure of 4 animals at the same time. Before the registering, animals were acclimated for 10 min to the exposure chamber and ambient conditions. After that, the Videomex-V device automatically began to register animal activity. The registering intervals were set up as 24 intervals of 5 min (300 s), and the duration of the experiment was then 120 min. Two runs with 4 animals per run and renewal of the test medium were performed for each treatment for a total of 8 replicates per treatment. Details of the system setup can be found in Ferrão-Filho et al. [14].

Statistical analyses

Median effective concentration (EC50) and median effective time (ET50) for the immobilization of the exposed animals were estimated from the acute tests by Probit analysis using the SPSS 8.0 statistical package. For analysis of behavioral parameters, a repeated measure analysis was performed using the Systat 9.0 statistical package. As we used the same dataset for 4 statistical tests (swimming, resting, distance, velocity), p values were adjusted according to Bonferroni ($p < 0.05/4 = 0.0125$). Linear regression was used to test whether the EC50 and ET50 were related to the toxin content of seston in the acute tests with water samples.

RESULTS

Phytoplankton analysis of field samples

Three main species of cyanobacteria dominated the phytoplankton, representing more than 90% of the phytoplankton biomass (Table 1). Maximum cyanobacterial biomass was observed on August (26.1 mg L^{-1}) and *Microcystis* spp. was the dominant taxa in all samples, while *C. raciborskii* ranked second in biomass, which was higher in October (17.1%; 3.4 mg L^{-1}). *Anabaena circinalis*, as well as other cyanobacteria, had a minor contribution to the phytoplankton biomass (0%–2.2%).

Acute toxicity bioassays

In the acute bioassays with raw water from the Funil Reservoir, there was an immobilization effect on animals in the 3 periods tested (Figure 1). Recovery occurred, however, only in October and November. Animals started to be immobilized in less than 1 h in December and were almost completely immobilized in 2 h, but they did not recover swimming in the following 22-h period after being placed in control water (filtered lake water). In October and November the effect was slower, with animals taking more time to be immobilized in October (3 h for complete immobilization) and fewer animals being immobilized after 2 h in November. Mortality rates were low (<20%) in all months.

The immobilization effect was higher (i.e., EC50 [2 h] and ET50 were lower) in months when STX concentration was

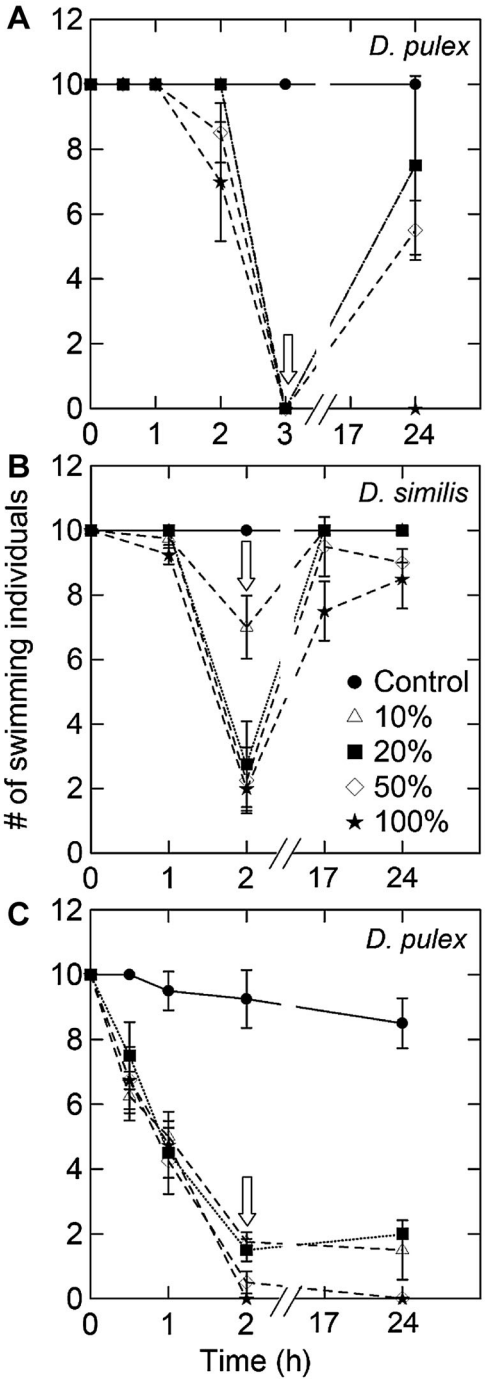

Figure 1. Acute toxicity tests with *Daphnia pulex* and *Daphnia similis* in October (**A**), November (**B**), and December (**C**) of 2007. Concentrations are in percentage water from the Funil Reservoir. Control refers to animals exposed to filtered lake water. White arrows point out the transition between the exposure and recovery period.

Table 1. Percentage (% biomass) and biomass (mg wet wt L^{-1}) of the dominant cyanobacteria during the sampling period

	August		October		November		December	
Species	%	(mg L^{-1})	%	(mg L^{-1})	%	(mg L^{-1})	%	(mg L^{-1})
Anabaena circinalis	2.22	0.58	0.48	0.10	1.50	0.29	0.93	0.22
Cylindrospermopsis raciborskii	3.01	0.79	17.10	3.40	0.00	0.00	13.21	3.15
Microcystis spp.	94.55	24.67	82.42	16.34	92.16	17.50	85.84	20.45
Others	0.22	0.06	0.00	0.00	6.34	1.2	0.02	0.01

Table 2. Results of the acute tests with raw water samples from the Funil Reservoir[a]

Sample	EC50 (2 h; % raw water)[b]	ET50 (h)[c]	Microcystins		Saxitoxins	
			(ng L^{-1})	(pg × 10^3 $cell^{-1}$)	(ng L^{-1})	(pg × 10^3 $cell^{-1}$)
21 August 2007	—	—	3300	17.74	26.0	2.13
23 October 2007	112.0 (88.5–174.0)[d]	2.28 (2.02–2.89)	5420	37.57	109.6	3.35
26 November 2007	32.9 (11.5–53.7)	1.63 (1.47–1.79)	4950	31.98	140.4	8.45
19 December 2007	7.4 (9.1–16.0)	0.87 (0.74–1.02)	3500	19.44	152.4	6.96

[a] Toxins are given as equivalents of microcystin (MC)-LR and saxitoxin (STX). Cell quota for each toxin was based on cell counts for each species, considering only *M. aeruginosa* for MC and *C. raciborskii*+*A. circinalis* for STX.
[b] Median effective concentration (EC50) values are for 2-h exposure to raw water.
[c] Median effective time (ET50) and toxin values are for the 100% raw water treatments only.
[d] Values in parenthese are 95% confidence intervals. In August there was no acute test.

higher, both on a per-liter basis or as cell quota (Table 2). A significant relationship was found between EC50 and the STX content of seston ($r = –0.998$; $p = 0.025$) but not for the MC content.

An acute test with the strain CYRF-01 was conducted only with *D. similis* and showed the same effect of immobilization of animals, starting in less than 1 h and being almost complete after 2 h of exposure (Figure 2). Animals started to recover swimming 1 h to 2 h after being placed into uncontaminated water and almost all recovered within 24 h. Mortality rates were negligible in all concentrations of the strain CYRF (<10%). The EC50 (2 h) was 31.5 μg L^{-1} (13.8–48.0 μg L^{-1}), and the ET50 for each concentration is presented in Table 3.

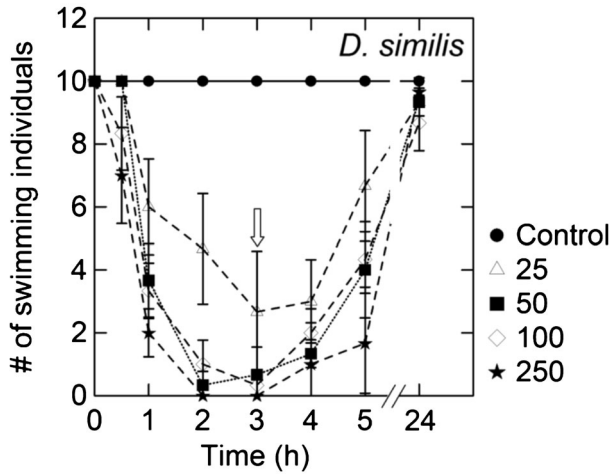

Figure 2. Acute toxicity tests with *Daphnia similis* exposed to CYRF-01 strain. Biomass concentrations are given in μg L^{-1} (corresponding cells, mL^{-1}, are given in Table 3). Control refers to animals exposed to mineral water plus green algae. The white arrow points out the transition between the exposure and recovery period.

Table 3. Results of the acute tests with the strain CYRF-01[a]

Biomass (μg L^{-1})	Cell density (cells mL^{-1})	ET50 (h)	STX (ng L^{-1})[b]
25	500	2.00 (1.52–2.73)	1.37
50	1000	1.19 (0.10–2.82)	2.74
100	2000	1.07 (0.65–1.54)	5.48
250	5000	0.71 (0.59–0.83)	10.97

[a] Median effective time (ET50) values and 95% confidence intervals (in parentheses) are given for each concentration used in the test.
[b] Nominal toxin concentrations are given as equivalents of saxitoxin (STX).

Behavioral assays

The behavioral assays with raw water samples from the Funil Reservoir revealed a consistent pattern of decreasing swimming activity of *D. similis* compared with controls with green algae (Figure 3). All parameters measured revealed significant differences relative to control ($p < 0.0125$) in the repeated measure analysis. In August, when a higher concentration of cyanobacteria was found, animals virtually stopped swimming after 45 min. In this month, as well as in the others, there was a slight recovery of swimming, and animals reached control levels by the end of the experiment.

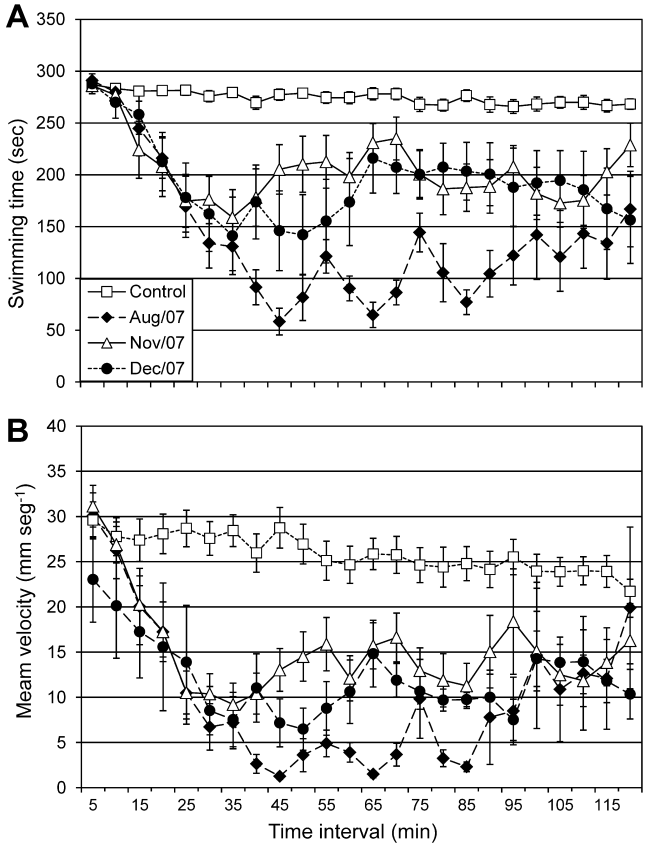

Figure 3. Parameters of the swimming activity in the behavioral tests with *Daphnia similis* exposed to raw water samples from the Funil Reservoir. Only 100% water samples were used. Control refers to animals exposed to filtered lake water plus green algae. Only swimming time (**A**) and mean velocity (**B**) are shown for each month tested. Points and bars represent the mean ± standard error for 8 replicate *Daphnia* per treatment.

The tests with the strain CYRF-01 revealed that swimming activity was affected in both *Daphnia* species. In the tests with *D. pulex*, animals showed a concentration-dependent decrease in swimming activity (Figure 4), with animals virtually stopping at the highest concentrations (500–1000 $\mu g\,L^{-1}$) at the end of the experiment. Except for the mean distance traveled and velocity at the concentration of 125 $\mu g\,L^{-1}$, all activity parameters were significantly decreased in the treatments with CYRF-01 relative to control (repeated measure analysis; $p < 0.0125$).

When *D. similis* was exposed to the strain CYRF-01, it also showed decreased activity, although at concentrations much higher than those used for *D. pulex*, starting with 1000 $\mu g\,L^{-1}$ (Figure 5). Even though the animals showed both decreased swimming time and mean velocity in the first 30 min at the concentration of 1000 $\mu g\,L^{-1}$, by the end of the experiment, they showed even higher mean velocities than the control animals. At the higher concentrations of cyanobacteria, animals had virtually stopped after 25 min to 30 min of exposure and, although there was some recovery, activity parameters remained under the control values. Repeated measure analysis revealed significant differences ($p < 0.0125$) in all activity parameters between the treatments with strain CYRF-01 and control.

DISCUSSION

Using 2 different experimental designs, acute bioassays and behavioral assays, the present study showed consistently that both field water samples and an STX-producing strain of cyanobacteria isolated from the same environment altered the swimming activity of 2 *Daphnia* species. Both *Daphnia* species showed a consistent pattern of decreasing mobility and activity parameters (such as time spent swimming and resting, distance traveled, and mean velocity) after exposure to environmental samples containing a variety of cyanobacterial species and toxins (MC and STX), and to different concentrations of the STX-producing strain CYRF-01. In contrast, *D. similis* seemed to be more resistant to the strain CYRF-01 than *D. pulex*. Preliminary tests with *D. similis* at the same range of concentrations used for *D. pulex* did not result in any significant effect on activity parameters (data not shown); thus we decided to use a higher range of concentrations for *D. similis*. Also, during these tests, in which animals were continuously exposed to field samples and to the strain CYRF-01, there was some recovery of *D. similis* swimming activity, suggesting that these animals can rapidly adapt to toxins, with activation of the detoxification pathway of the toxins ingested during exposure.

Few studies have reported the effects of STX-producing strains on freshwater cladocerans. Haney et al. [13] showed that the STX-producer *Aphanizomenon floes-aquae* and pure STX reduced the thoracic appendage beating rate of *D. carinata* while increasing its post-abdomen rejection rate, which was interpreted as a behavioral response to avoid ingesting toxic cyanobacteria rather than an intoxication response as a result of STX. In this case, STX would have acted only as a chemical cue to *Daphnia* [13]. In the present study, however, *Daphnia* swimming activity was clearly affected, which suggests a true

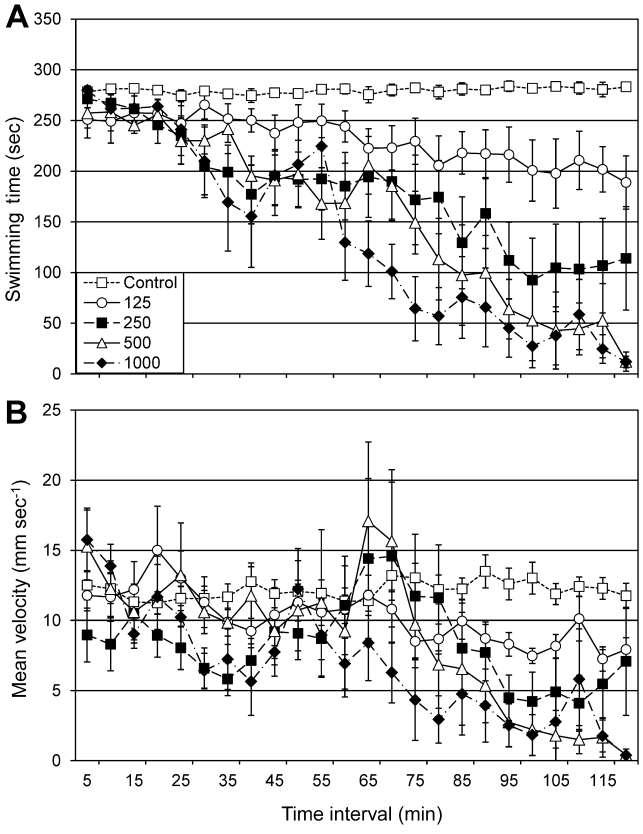

Figure 4. Parameters of the swimming activity in the behavioral tests with *Daphnia pulex* exposed to CYRF-01 strain. Control refers to animals exposed to mineral water plus green algae. Only swimming time (**A**) and mean velocity (**B**) are shown for each concentration, in biomass dry weight (μg dry wt L^{-1}). Points and bars represent the mean ± standard error for 8 replicate *Daphnia* per treatment.

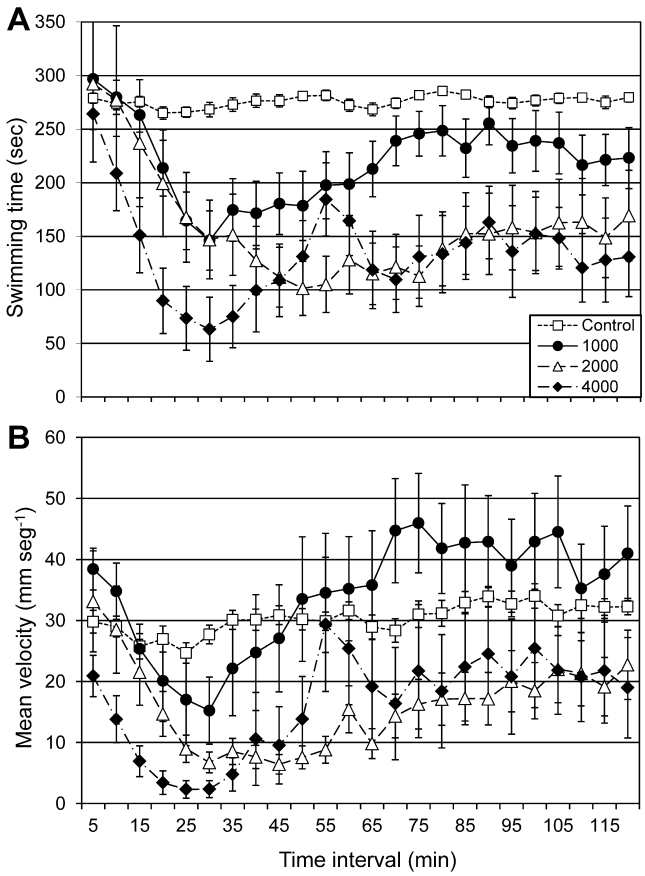

Figure 5. Parameters of the swimming activity in the behavioral tests with *Daphnia similis* exposed to CYRF-01 strain. Control refers to animals exposed to mineral water plus green algae. Only swimming time (**A**) and mean velocity (**B**) are shown for each concentration, in biomass dry weight (μg dry wt L^{-1}). Points and bars represent the mean ± standard error for 8 replicate *Daphnia* per treatment.

intoxication response. In Haney et al.'s study [13], as soon as the exposure to cyanobacteria (or toxins) ceased, animals recovered normal activity, which reinforces the hypothesis of a true behavioral response. In our acute bioassays, however, animals took some time (up to 24 h) to recover, which suggests that a detoxification response likely occurred during this period.

In another study with cladocerans, Nogueira et al. [10] showed that *D. magna* accumulated STX from *Aphanizomenon issatschenkoi* and suffered a reduction in survival and growth, in addition to a reduction in activity of cytosolic glutathione-S-transferase. Soares et al. [11] also found a reduction in survival and growth of *D. magna* fed with high proportions (75%–100%) of *C. raciborskii* (CYRF-01) in the diet, besides reduction in filtering rate and fecundity, but attributed these effects to energetic costs as a result of feeding inhibition. In both studies, however, no effects on mobility or swimming activity were reported.

Another study using the strain CYRF-01 showed a decrease in the swimming time and mean velocity of *D. pulex* [14], which was attributed to the presence of STX in field samples and in the *C. raciborskii* strain CYRF-01. In that study, however, the exposure lasted only 50 min and a higher range of concentrations was used (5000–50 000 cells mL^{-1}). In the present study, we showed that even lower concentrations of *C. raciborskii*, both from natural water samples and from cultures of a strain isolated from the same body of water, can affect the swimming activity of 2 *Daphnia* species. Acute effects on mobility were previously reported for other cladocerans, such as *Moina micrura*, when exposed to water samples from the Funil Reservoir and to another STX-producing strain of *C. raciborskii* (strain T3) [15]. Therefore, the results of different studies show that neurotoxic cyanobacteria in lakes and reservoirs can be potentially harmful to several taxa of zooplanktonic organisms.

Some studies showed the effects of STX-producing cyanobacteria on the behavior of other aquatic invertebrates. Negri and Jones [38] showed the accumulation of STX produced by the cyanobacterium *Anabaena circinalis* in *Alathyria condola* and reported an "adverse effect on feeding behavior" of the bivalve, although they have not quantified any behavioral parameter. Pereira et al. [12] estimated the accumulation of paralytic shellfish toxins from the *Aphanizomenon issatschenkoi* in the bivalve *Anodonta cygnea*, and showed that the amount of paralytic shellfish toxins provided with the diet had an inverse relationship to the clearance rate of the mussels. In spite of the fast bioaccumulation of paralytic shellfish toxins during mussel exposure, animals eliminated toxins relatively quickly, after 2 d during the depuration period, dropping to undetectable levels after 6 d to 14 d. During their experiments, mortality rates were negligible (3.5%). In our acute experiments, we also observed low mortality rates. Thus, even though STX are potent paralyzing agents and feeding inhibitors, this and the previous studies showed that they exert low lethality effects in invertebrates. Also, detoxification mechanisms may be responsible for the rapid elimination of this toxin and the recovery of swimming activity of *Daphnia* observed in our experiments.

Except for the study of Ghadouani et al. [4], no evidence was found that *Microcystis* or purified MC can cause alterations of neuromotor functions related to the feeding process of *Daphnia*. Animals exposed to microcystin-producing cells of *Microcystis* showed decreased mandibular movement and appendage beating rates, as well as an increased labral rejection rate. These effects were reversed immediately after animals were exposed back to control medium, suggesting a behavioral response rather than intoxication. When purified MC-LR was added, the same effects were observed, yet no recovery in any of these parameters was observed, indicating a toxigenic response [4]. Our results, however, showed that animals spent some time (17–24 h) to completely recover swimming movements, which suggests a toxigenic response rather than a behavioral response.

For the sake of interpretation of the results found here, the mechanism of action of the toxins present must be taken into account. The MCs act as potent inhibitors of phosphatases 1 and 2A [39] and of other important enzymes in *Daphnia* [40], also causing disruption of the midgut epithelial cells of *Daphnia* [5,40]. These damages are irreversible and more likely to be lethal to invertebrates. In contrast, STXs act as sodium channel blockers, leading to impairment of the action potential in neurons and paralysis of muscles [1]. Thus, the low lethality observed in this and other studies [12,15,16], as well as the fast immobilization and recovery, are in agreement with the mechanism of action of STX and in disagreement with the effects previously reported for MC in invertebrates [2,5].

Therefore, the effects of MC on the behavior of aquatic animals reported in the above-mentioned studies are more likely to be a consequence of the damage in important tissues, such as liver and kidney, rather than an effect on the nervous system. Therefore, although both hepatotoxins and neurotoxins were present in the field samples, taking into account the differences in the mechanism of action of MC and STX in aquatic animals explained above, the fast immobilization and recovery observed in the acute bioassays, and the reduction in swimming activity in less than 2 h during the behavioral tests, our data suggest that these specific effects result from intoxication with neurotoxins, and not from the MC present in these samples. However, we cannot rule out the possibility of a synergistic effect between hepato- and neurotoxins (including β-*N*-methylamino-L-alanine [23,24]) in the tests with field samples. In addition, no other neurotoxins or potentially neurotoxic strains of cyanobacteria were reported in the Funil Reservoir. The strain CYRF-01, however, produces only SXT, which can account for the reduction in swimming activity of *Daphnia*.

CONCLUSIONS

In conclusion, the present study showed that the behavioral parameters of the swimming activity of *Daphnia* were significantly altered by field samples and by an STX-producing strain of *C. raciborskii*, suggesting a likely involvement of these toxins in the effects observed. These effects can have consequences for sensitive zooplankton populations in the field, decreasing their fitness and allowing the establishment of cyanobacterial blooms. Therefore, neurotoxic cyanobacteria may potentially affect the behavior of aquatic animals and thus interfere in competitive and predatory relationships in the natural environment.

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