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Effects of crude extracts of a saxitoxin-producer strain of the cyanobacterium *Cylindrospermopsis raciborskii* on the swimming behavior of wild and laboratory reared guppy *Poecilia vivipara*

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ABSTRACT

The cyanobacterium *Cylindrospermopsis raciborskii* is an invasive species in water supply reservoirs worldwide, which can produces cylindrospermopsins and saxitoxins. In the wild, guppy (*Poecilia vivipara*) can be exposed to cyanotoxins, but those born and reared in laboratory are free of this contact. The aim of this paper was to comparatively measure the locomotor activity of 'wild' and 'lab' *P. vivipara* before and after exposure to crude extracts of two different cultures of *C. raciborskii* (CYRF-01), a saxitoxin-procucer strain. The movement of each fish was recorded using an image monitoring system (Videomex V®) before and after 48 h exposure to cyanobacterial extracts. Each experiment was performed during 4 h, with 1 h acclimation and 3 h recording period of the parameters Distance performed (DP), Swimming time (SwT), Stereotypic time (StT), Resting time (RT) and Average speed (AS). The quantification of saxitoxin in the solutions was performed by the enzyme-linked immunosorbent assay (ELISA). The weight or the total length did not influence the locomotor activity of fish in any of the experiments. The saxitoxin value was similar for both cultures (Culture 1: 7.3 μg L^{-1} and Culture 2: 8.6 μg L^{-1}). However, in experiments with Culture 1 an increased activity in most parameters was observed, while in Culture 2, a decreased activity was observed only in 'lab' fish. Wild fish was less affected, showing higher resistance to both cyanobacterial crude extracts. This study showed that different cultures of the same strain of *C. raciborskii* and with similar contents of saxitoxin are able to change the locomotor activity of *P. vivipara*, contributing to the validation of the use of behavioral parameters to the evaluation of sublethal effects of toxic cyanobacteria on fish.

1. Introduction

The cyanobacteria *Cylindrospermopsis raciborskii* (Woloszynska) Seenara and Subba Raju, 1912 is an invasive species in water supply reservoirs in Brazil, exhibiting high ecophysiologic plasticity that confers it resistance to a wide range of the temperature, pH, conductivity and light (Costa et al., 2006; Bouvy et al., 2000; Bonilla et al., 2012). They are able to produce secondary metabolites, called cyanotoxins, such as cylindrospermopsin (CYN) and saxitoxins (STXs), some of which can be extremely harmful to animals and humans (Carmichael et al., 2001; Guzmán-Guillén et al., 2015). Unlike the North American and Australian strains, that produce cylindrospermopsin, Brazilian strains of *C. raciborskii* produce saxitoxins. The saxitoxins are responsible for cases of human poisoning by consumption of seafood, such as shellfish and fish, which accumulate these toxins (Ibelings and Chorus, 2007; Etheridge, 2010).

Cyanotoxins can be both retained in cyanobacteria and released into the water during their senescence and lysis. Many aquatic organisms, especially fish, can be directly exposed to dissolved cyanotoxins and occasionally can accumulate them in several organs

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(Ferrão-Filho and Kozlowsky-Suzuki, 2011). Fish that come into contact with cyanobacteria and their toxins may be affected in their growth, development, histology, reproduction and survival. However, exposure to sublethal concentrations of cyanotoxins can also affect the life cycle of aquatic organisms in several ways (Drobac et al., 2016). For instance, some studies have showed that toxic cyanobacteria or cyanotoxins can alter the fish swimming behavior (Baganz et al., 1998, 2004; Lefebvre et al., 2005; Ferrão-Filho et al., 2007).

Behavioral changes in animals can be characterized by the attempt to adapt to the environment after a disturbance or can be a way to reduce the probability of death or the metabolic cost of the organism (Olla et al., 1980; Begout Anras and Lagardére, 2004). The swimming behavior, for example, is considered a valid and consistent index for evaluating sublethal toxicity, allowing tests that can be performed with minimal stress to the fish and enabling repeated measurements of the same individual in the experiment (Little and Finger, 1990; Siegmund and Biermann, 1993). Changes in swimming activity caused by exposure to contaminants may hamper the fish's ability to feed, avoid predation and reproduce (Little and Finger, 1990; Santos and Santos, 2013).

The guppy fish *Poecilia vivipara* Bloch and Schneider, 1801 is a fish found in fresh and brackish water environments and have high tolerance to environmental variations, being considered a promising fish species in biomonitoring of aquatic pollutants (Ferreira et al., 2012; Machado et al., 2013). In the natural environment, fish can be frequently exposed to cyanobateria and their cyanotoxins, both actively through oral route or passively via direct contact of the gill epithelium (Drobac et al., 2016). On the other hand, fish born and reared in laboratory are free of this contact and are an excellent and more sensitive tool to detect such toxins.

The aim of this paper was to comparatively measure the locomotor activity in *P. vivipara*, both wild and born in the laboratory, before and after exposure to the crude extracts of *C. raciborskii*. We also tested the hypothesis that fish born in the wild would be more resistant to cyanobacterial metabolites than fish reared in the lab.

2. Material and methods

2.1. C. raciborskii culture

A saxitoxin-procucer strain of *C. raciborskii* (CYRF-01) was isolated from the Funil Reservoir (Rio de Janeiro, Brazil). Two different cultures were grown in ASM-1 medium (Gorham et al., 1964) at pH 8.0, under fluorescent light at an intensity of 40–50 μE m^{-2} s^{-1}, 12:12 h L:D cycle at 23 ± 1 °C. The cultures were lyophilized and aqueous crude extracts were used in the preparation of test solutions.

2.2. P. vivipara capture and maintenance

Wild fish were collected from Rodrigo de Freitas Lagoon (RJ, Brazil), an urban mesotrophic, coastal lagoon located in the South zone of Rio de Janeiro City. This lagoon has high inputs of organic matter from its watershed and from domestic sewage, presenting events of anoxia in the water columns and extensive fish deaths in some periods, which have been indeed correlated with harmful algal blooms (Domingos et al., 2012). These fish were maintained in dechlorinated tap water in aerated tanks, at 24 ± 1 °C and fed with commercial food. The pregnant females were separated to obtain fish free of parasitic infection or contact with cyanobacteria. A total of 26 adult *P. vivipara* from de Lagoon ('wild' fish) and 22 adult *P. vivipara* born and reared in the laboratory ('lab' fish) were used in the experiments. The fish from the lagoon was acclimated to lab conditions for 3 weeks prior to the experiments.

2.3. Image analysis biomonitoring system

A recording cabin made of acrylate with a diffuse soft lighting and an analogical video camera was used for registering fish activity. Inside, an opaque glass aquarium of 30 L capacity (35 cm × 35 cm x 25 cm) held eight holding boxes (4 cm × 4 cm x 2 cm) made of opaque acrylate with 3 mm holes, where fish were placed individually during the experiments. The movement of each fish was recorded using the image monitoring system Videomex V® (Columbus Instruments, USA) with the use of the software Multiple Objects Distance Travelled (MODT; Santos et al., 2011; Santos and Santos, 2013). Each fish was monitored before and after exposure to crude extracts of *C. raciborskii* or tap water (Control) during a period of 4 h, with 1 h for acclimation and 3 h of registering. The monitoring period was recorded in 60 intervals of 3 min and the parameters Distance performed (DP), Swimming time (SwT), Stereotypic time (StT), Resting time (RT) and Average speed (AS) were used for statistical analyses. DP is the total distance in millimeters performed by the fish during the interval. SwT is the total time in seconds during the interval in which the fish spent swimming. StT is the total time in seconds during the interval in which the fish performed some activity other than swimming. RT is the total time in seconds during the interval in which the fish spent resting. AS was calculated as the distance performed divided by the swimming time.

2.4. Experimental design

The fish were divided into three groups: (1) non-exposed to the crude extract of cyanobacteria (control), (2) exposed to 400 mg L^{-1} (as dry weight, DW) of cyanobacterial extract from Culture 1 and (3) exposed to 400 mg DW L^{-1} of cyanobacterial extract from Culture 2. In each group, both 'wild' fish and 'lab' fish were used. Among groups exposed, 30 fish were maintained individually in plastic recipients, with continuous aeration, in the concentration of 400 mg L^{-1} of the crude extracts for 48 h, being the total solution (150 mL) changed after 24 h. The 18 fish of control group were kept under the same conditions of exposed groups, but without the presence of cyanobacterial crude extract.

We performed a total of six experiments to assess the effect of crude extract of *C. raciborskii* on the swimming activity of the fish. In the first and second experiments, we used 'lab' and 'wild' *P. vivipara*, respectively, to determine if there were changes in the swimming activity without exposure to cyanobacteria ('Control group'). The third and fourth experiments were conducted using the Culture 1, and the fifth and sixth experiments were performed using Culture 2, both using 400 mg L^{-1} of cyanobacterial extract and with both, 'lab' and 'wild' fish. In all experiments, the fish were monitored 'before' and 'after' exposure to cyanobacteria. Therefore, each fish 'before' exposure consisted of their own 'control'.

Water samples from each recipient were taken for measures of toxins, dissolved oxygen, temperature, pH and conductivity.

2.5. Saxitoxin analysis

The detection and quantification of saxitoxin in the solutions of the Culture 1 and 2 were performed by the enzyme-linked immunosorbent assay (ELISA) using the kit Beacon Saxitoxin Plate (Beacon Analytical Systems) according to the manufacturer's instructions.

2.6. Statistical analysis

All statistical analyses were performed using the R program (R Development Core Team, 2014). We used the Generalized

Table 1
Results of the experiments performed with 'lab' and 'wild' *Poecilia vivipara*. Treatments were Controls (tap water), Culture 1 and Culture 2, both with 400 mg L^{-1} of the crude extract. Values are means ± standard deviations for each activity parameter. *P*-values for the statistical comparison between the monitoring periods ("before" and "after") are given. DP = distance performed, SwT = swimming time, StT = stereotypic time, RT = resting time, AS = average speed.

Exp.	Fish	n	Treat.	DP (mm)		*P*	SwT (sec)		*P*	StT (sec)		*P*	RT (sec)		*P*	AS (mm/sec)		*P*
				Before	After		Before	After		Before	After		Before	After		Before	After	
1	Lab	8	Control	363 ± 304	172 ± 337	0.100	27.8 ± 22.6	11.7 ± 17.6	0.057	88.3 ± 50.0	73.9 ± 59.5	0.440	63.8 ± 66.0	93.2 ± 68.3	0.170	10.3 ± 5.2	8.4 ± 7.3	0.360
2	Wild	10	Control	686 ± 378	674 ± 403	0.470	52.3 ± 27.2	49.7 ± 28.4	0.540	107.2 ± 23.6	92.1 ± 40.0	0.250	20.5 ± 27.2	37.5 ± 58.3	0.340	12.7 ± 1.5	11.6 ± 4.7	0.430
3	Lab	6	Cult. 1	524 ± 202	1423 ± 661	<0.001	41.1 ± 15.4	142.3 ± 36.6	<0.001	119.4 ± 14.2	21.9 ± 14.4	<0.001	19.6 ± 16.4	15.8 ± 27.3	0.150	12.8 ± 0.7	9.5 ± 2.9	<0.001
4	Wild	8	Cult. 1	407 ± 406	413 ± 185	0.031	30.0 ± 29.0	33.1 ± 14.4	0.051	98.3 ± 44.1	122.8 ± 18.3	<0.001	51.6 ± 57.8	24.1 ± 20.8	0.055	9.9 ± 5.8	12.4 ± 1.1	0.002
5	Lab	8	Cult. 2	836 ± 483	369 ± 325	0.002	57.7 ± 29.0	27.9 ± 21.2	0.001	100.8 ± 25.9	95.5 ± 47.9	0.120	21.5 ± 34.4	56.6 ± 61.7	<0.001	13.7 ± 3.4	10.7 ± 5.0	0.030
6	Wild	8	Cult. 2	555 ± 265	480 ± 327	0.053	42.7 ± 19.0	36.4 ± 24.2	0.040	115.4 ± 21.3	93.7 ± 44.4	0.080	21.9 ± 25.1	49.9 ± 61.2	0.050	12.6 ± 1.8	11.2 ± 4.8	0.230

Estimating Equation (GEE) to assess the differences in the swimming activity parameters between the measured periods ('Before' and 'After') of both 'Non-exposed' (Control) and 'Exposed' fish to toxic crude extracts of *C. raciborskii*. We also compared 'Lab' and 'Wild' fish for each measured periods ('Before' and 'After') of both 'Non-exposed' (Control) and 'Exposed' fish. This test analyzed repeat measurements of the locomotor activity data, estimating the parameters of regression and variances in the dependent timing. For each experiment, a model using each swimming activity parameter as a variable response was performed and the interaction of individual weight and total length of fish was estimated. The models were adjusted to control over-dispersion and normalization of the residuals. Finally, a goodness-of-fit statistic, the quasi-likelihood information criterion (QIC), was used for evaluating the models (Pan, 2001). The level of significance assumed for statistical tests was 5%.

3. Results

During the experiment, dissolved oxygen averaged 7.4 (±0.3) mgL^{-1}, temperature 24.0 (±1)°C, pH 6.8 (±0.6) and conductivity 233.3 (±22.3) μS/cm. The total length of the fish reared in the laboratory ranged from of 2.1–3.5 (2.8 ± 0.4) cm and the weight from 0.10 to 0.73 (0.37 ± 0.2) g. The total length of the wild fish ranged from of 3.5–4.6 (4.0 ± 0.3) cm and the weight from of 0.33–1.18 (0.77 ± 0.2) g. The GEE analysis found no influence of weight or the total length in locomotor activity of fish in any of the experiments. The saxitoxin values quantified in the water sample of Culture 1 was 7.3 μg L^{-1} and that of Culture 2 was 8.6 μg L^{-1}.

In Table 1, we present the comparisons between the measured periods ('Before' and 'After') for each swimming activity parameters of both groups of fish 'Non-exposed' (Control) and 'Exposed' to crude extracts of *C. raciborskii*, and for both Wild and Lab fish. In the first experiment, regarding the 'lab' fish without exposure to the cyanobacterial extract (Lab control), we did not find any significant changes of the swimming activity before and after exposure to tap water (Table 1). In the second experiment, with the 'wild' fish without exposure to the cyanobacterial extract (Wild control), we also did not find any significant changes of the swimming activity before and after exposure to tap water (Table 1).

In the third experiment performed with 'lab' *P. vivipara* monitored before and after exposure to 400 mg L^{-1} of the crude extract of Culture 1, there was a significant increase of about 2.7-fold in the DP and of about 3.5-fold in SwT, while a significant decrease of about 5-fold in the StT and of 1.4-fold in the AS (Table 1, Fig. 1-A).

In the fourth experiment performed with 'wild' *P. vivipara* monitored before and after exposure to 400 mg L^{-1} of the crude extract of Culture 1, there was no relevant change in the DP, a significant increase in the StT and AS, while a non-significant decrease in RT (Table 1, Fig. 1-B). Although fish did not increase swimming activity significantly (DP and SwT, $p > 0.05$), they moved faster (AS, $p = 0.002$) after exposure to the cyanobacterial extract. They also spent more time in activities other than swimming, as showed by the increase in stereotypic time after exposure to the crude extract (StT, $p < 0.001$).

In the fifth experiment performed with 'lab' *P. vivipara* monitored before and after exposure to 400 mg L^{-1} of the crude extract of Culture 2, there was a significant decrease of about 2-fold in DP ($p = 0.002$) and in SwT ($p = 0.001$) and 1.2-fold in AS ($p = 0.03$), while a significant increase of about 3-fold in RT ($p < 0.001$). Stereotypic time, however, remained unchanged (StT, $p = 0.12$) (Table 1, Fig. 2-A).

In the sixth experiment conducted with 'wild' *P. vivipara*

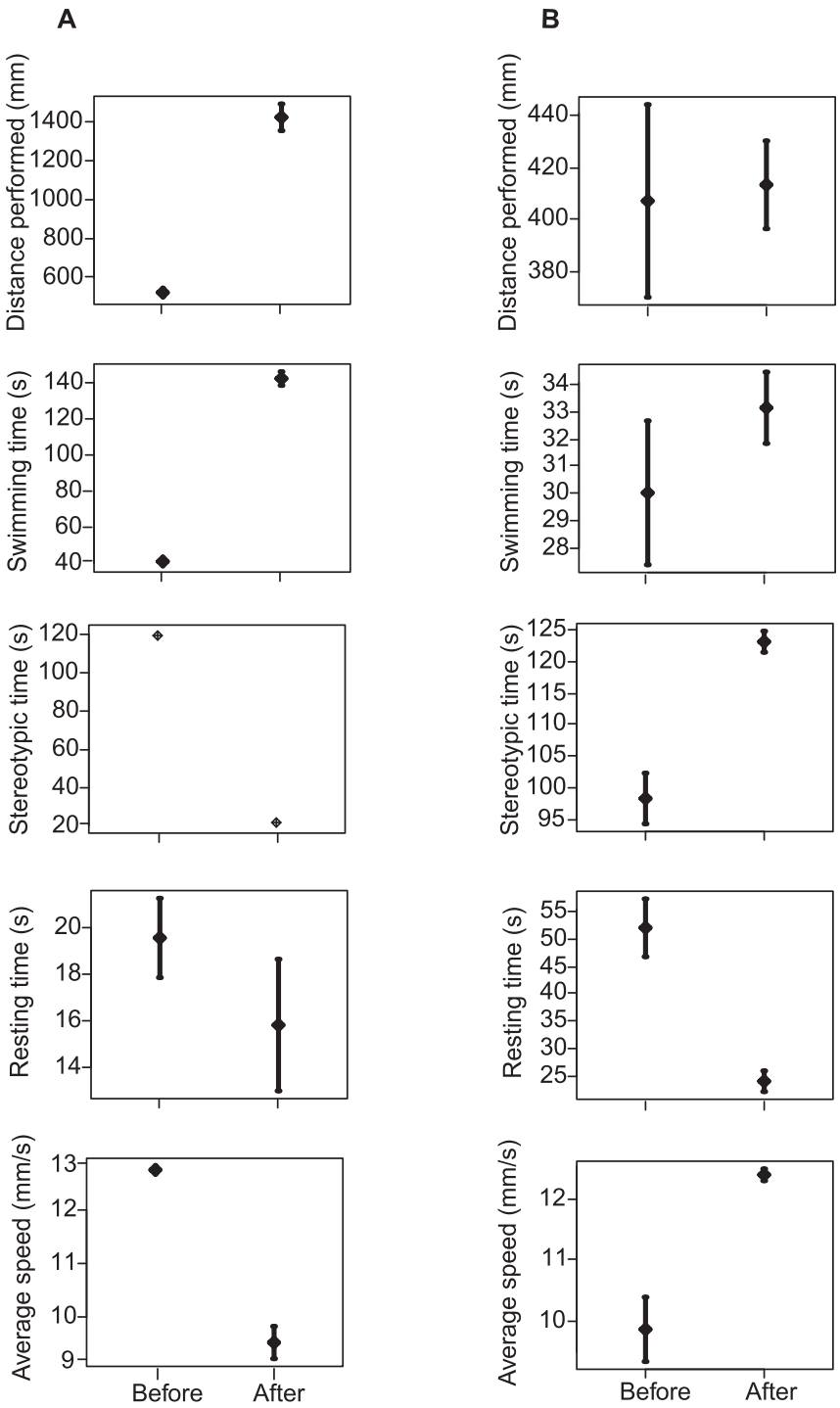

Fig. 1. Behavioral parameters of 'lab' (A) and 'wild' (B) *Poecilia vivipara* before and after exposure to 400 mg L^{-1} of the crude extract of *Cylindrospermopsis raciborskii* ('Culture 1'). Bars show the mean ± standard error.

monitored before and after exposure to 400 mg L^{-1} of the crude extract of Culture 2, there was a slight but significant decrease in SwT ($p = 0.04$). All other parameters remained unchanged (Table 1, Fig. 2-B).

Comparing the Lab and Wild fish activities in each moment of exposure ('Before' and 'After') for each experiment (Table 2), we observed no significant differences between Lab and Wild fish in the control groups, either before or after exposure to tap water. The same was observed for Lab and Wild fish exposed to Culture 2. However, significant differences in all activity parameters were observed between Lab and Wild fish, both before and after exposure to Culture 1.

4. Discussion

The toxic effects of cyanotoxins in fish can be evaluated by invasive or non-invasive methods. Among the invasive methods, the intraperitoneal injections and oral applications have been used

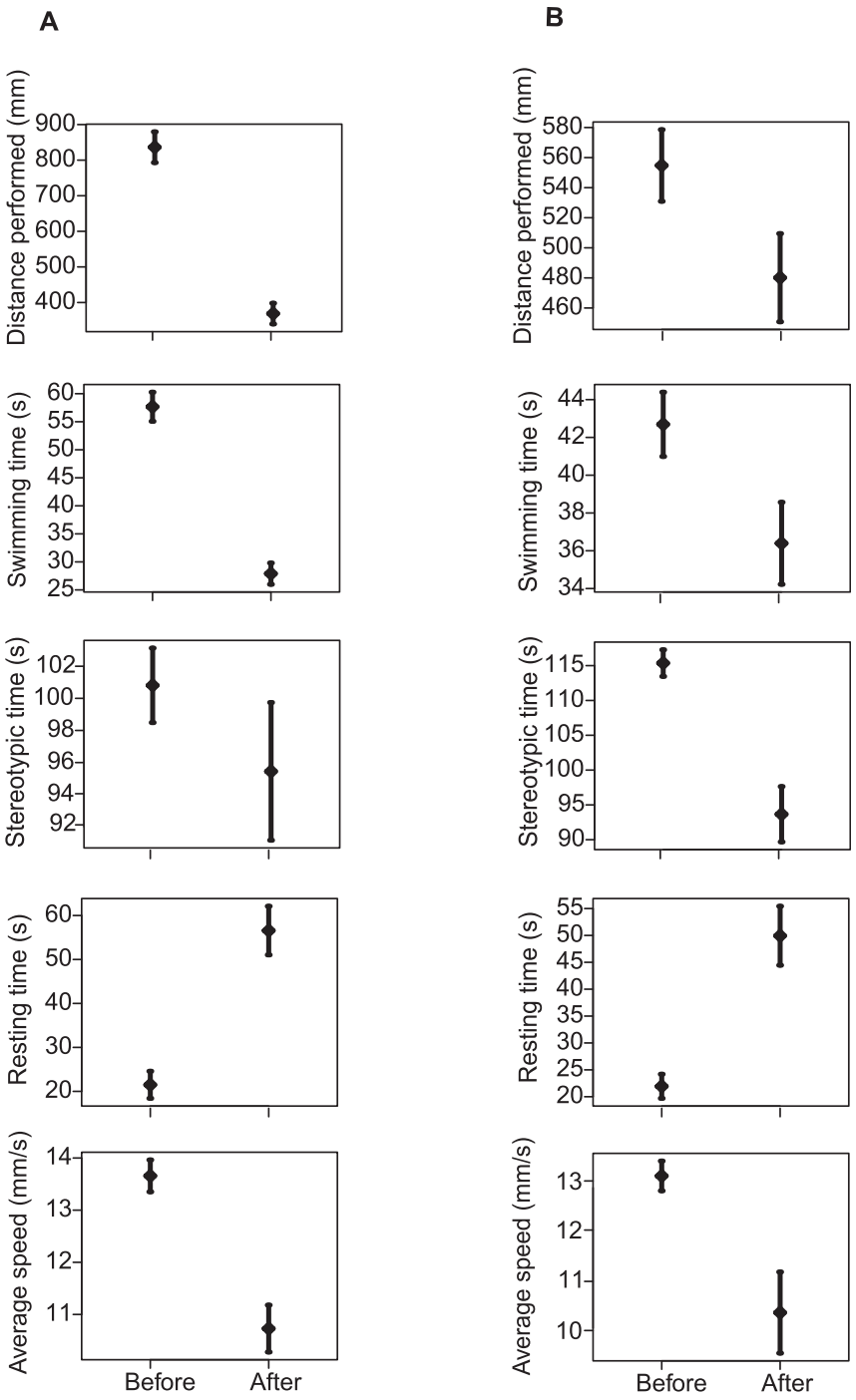

Fig. 2. Behavioral parameters of 'lab' (A) and 'wild' (B) *Poecilia vivipara* before and after exposure to 400 mg L^{-1} of the crude extract of *Cylindrospermopsis raciborskii* ('Culture 2'). Bars show the mean ± standard error.

in previous toxicological studies (Phillips et al., 1985; Tencalla et al., 1994; Cazenave et al., 2008; Bakke et al., 2010). In contrast, the use of behavioral parameters, such as those applied in this study, is considered minimally invasive to assess the sensitivity of the fish to toxins and also detect sublethal levels of cyanotoxins able to generate disturbances (Siegmund and Biermann, 1993). Considering this, besides the fact that we used native fish submitted to extracts of a cyanobacterial strain isolated from Rio de Janeiro State, in the concentration range of toxins found in Brazilian waters (Ferrão-Filho et al., 2009, 2010), this study is ecologically relevant as it was performed in a more realistic exposure scenario.

The behavioral assessment of 'lab' and 'wild' fish monitored without exposure to cyanobacteria extract (control group) did not show significant changes in any parameter evaluated, showing that the simple procedure of exposing the fish to the different recipients did not cause sufficient stress to significantly alter their swimming behavior. Also, few differences in activity parameters between 'lab' and 'wild' fish (Table 2) showed that these fish had essentially the same behavior, despite their origin. However, prominent

Table 2
Experiments performed with 'lab' and 'wild' *Poecilia vivipara*. Treatments were Controls with tap water (Ctrl), Culture 1 (Cult. 1) and Culture 2 (Cult. 2), both with 400 mg L^{-1} of the crude extract. Values are means ± standard deviations for each activity parameter. *P*-values for the statistical comparisons between the laboratory ('Lab') and wild ('Wild') fish are given. DP = distance performed, SwT = swimming time, StT = stereotypic time, RT = resting time, AS = average speed.

Exp.	Treat.	Moment	DP (mm)		*P*	SwT (sec)		*P*	StT (sec)		*P*	RT (sec)		*P*	AS (mm/sec)		*P*
			Lab	Wild		Lab	Wild		Lab	Wild		Lab	Wild		Lab	Wild	
1 vs 2	Ctrl	Before	363 ± 304	686 ± 378	0.100	27.8 ± 22.6	52.3 ± 27.2	0.090	88.3 ± 50.0	107.2 ± 23.6	0.100	63.8 ± 66.0	20.5 ± 27.2	0.043	10.3 ± 5.2	12.7 ± 1.5	0.170
1 vs 2	Ctrl	After	172 ± 337	674 ± 403	0.520	11.7 ± 17.6	49.7 ± 28.4	0.430	73.9 ± 59.5	92.1 ± 40.0	0.640	93.2 ± 68.3	37.5 ± 58.3	0.350	8.4 ± 7.3	11.6 ± 4.7	0.740
3 vs 4	Cult. 1	Before	524 ± 202	407 ± 406	0.002	41.1 ± 15.4	30.0 ± 29.0	0.002	119.4 ± 14.2	98.3 ± 44.1	0.005	19.6 ± 16.4	51.6 ± 57.8	<0.001	12.8 ± 0.7	9.9 ± 5.8	0.003
3 vs 4	Cult. 1	After	1423 ± 661	413 ± 185	<0.001	142.3 ± 36.6	33.1 ± 14.4	<0.001	21.9 ± 14.4	122.8 ± 18.3	<0.001	15.8 ± 27.3	24.1 ± 20.8	0.046	9.5 ± 2.9	12.4 ± 1.1	0.002
5 vs 6	Cult. 2	Before	836 ± 483	555 ± 265	0.962	57.7 ± 29.0	42.7 ± 19.0	0.920	100.8 ± 25.9	115.4 ± 21.3	0.024	21.5 ± 34.4	21.9 ± 25.1	0.440	13.7 ± 3.4	12.6 ± 1.8	0.740
5 vs 6	Cult. 2	After	369 ± 325	480 ± 327	0.260	27.9 ± 21.2	36.4 ± 24.2	0.240	95.5 ± 47.9	93.7 ± 44.4	0.510	56.6 ± 61.7	49.9 ± 61.2	0.450	10.7 ± 5.0	11.2 ± 4.8	0.450

differences were found between 'lab' and 'wild' fish when they were exposed to Culture 1, showing that while 'lab' fish had its activity highly altered by Culture 1 extract, 'wild ' fish showed little or no response.

In experiments conducted with the cyanobacterial extract of Culture 1 we clearly observed an increase in the activity parameters DP and SwT of the 'lab' fish, while these parameters remained unchanged in the 'wild fish'. Regarding stereotypic time (StT), 'lab' and 'wild' fish showed an opposite trend: while 'lab' fish decreased StT, 'wild' fish increased this activity. Stereotypic behavior in fish could be considered as activities other than swimming, such as breeding, feeding or fight (Hughes and Blight, 1999). As in our experiments animals were caged in separate arenas, it is difficult to interpret such results. However, as 'lab' fish had a decrease of about 5-fold in the stereotypic time while an increase of about 3.5-fold in swimming time, it is reasonable to suppose that a stress response caused fish to reallocate time (and energy) from stereotypic to a more active swimming behavior. This could be interpreted as a scape response from the contaminated site due to stress caused by cyanobacterial metabolites.

In the experiment using the cyanobacterial extract of Culture 2, an opposite response was observed in 'lab' fish: the parameters DP and SwT decreased after exposure to the cyanobacterial extract. However, the saxitoxin values obtained in the extract samples from each culture were similar (7.3 and 8.6 μg L^{-1} for cultures 1 and 2, respectively), indicating that this toxin was not related to the behavioral changes observed. Therefore, different cultures of the same strain of *C. raciboorkii* caused different effects on locomotor activity of *P. vivipara*, possibly induced by action of other secondary metabolites not evaluated in this study. In fact, cyanobacteria are able to produce a variety of bioactive metabolites, such as oligopeptides with protease inhibiting activity, cyclic peptides and alkaloids (Leflaive and Ten-Hage, 2007; Schwarzenberger et al., 2012; Silva-Stenico et al., 2012; Švercel, 2013). Also, the production of metabolites in cyanobacteria may vary with culture conditions, such as age, medium, temperature and light (Carneiro et al., 2009). Although culture conditions in our experiments were about the same, it is likely that different compounds may have acted in the different tests with different cultures of *C. raciborskii*.

Wild fish had previous contact with various substances and microorganisms, including cyanobacteria, in the natural environment while laboratory fish were kept under controlled conditions. Contrary to 'lab' fish, 'wild' fish had small and less significant changes in most parameters with the exception of StT and AS when exposed to the extract of Culture 1, while no significant changes were detected when exposed to Culture 2. Therefore, it is likely that there is a greater resistance to effects of the cyanobacterial metabolites in the 'wild' fish than in fish reared in the lab.

Most of the research about the effects of toxic cyanobacteria was carried out with hepatotoxins such as microcystins (Ibelings and Havens, 2008; Ferrão-Filho and Kozlowsky-Suzuki, 2011). Few studies, however, dealt with effects of toxic cyanobacteria or cyanotoxins on fish behavior. Baganz et al. (2004), for example, found that fish exposed to MC–LR presented a dose-dependent response, but that it was dependent on the time of the day (daytime or night-time). During the day, the activity of fish increased in the lower concentration (0.5 μg L^{-1}), while the higher concentration (50 μg L^{-1}) led to a significant reduction in activity. Studies about the effects of saxitoxins in fish behavior are even scarcer. Lefebvre et al. (2005) showed that STXs can alter the sensorimotor function of herring (*Clupea harengus pallasi*), decreasing its response to spontaneous and touch-activated swimming behavior. However, the normal motor function

recovered after 4–24 h of continuous exposure. This fact may explain some of our results of increased activity after exposure.

The effect of saxitoxin in aquatic organisms is referred to as paralytic, by blocking of sodium channel and impairment of electrical impulses in nerve cells (Evans, 1965). Ferrão-Filho et al. (2007) exposed *D. rerio* to the living cells of the same strain of *C. raciborskii* (CYRF-01) used in this study and also to raw water from Funil Reservoir (RJ), from which this strain was isolated. Similarly to our experiment using Culture 1, the authors reported an increase in locomotor activity in fish after exposure. In both studies, fish paralysis was not observed. The authors attributed the increased activity possibly to irritant compounds, such as lipopolysaccharids, present in all gram-negative bacteria, including cyanobacteria (Wiegand and Pflugmacher, 2005). In a complex matrix like a crude cyanobacterial extract, other compounds with biological activity in fish could be also responsible for the effects observed (Ferrão-Filho and Kozlowsky-Suzuki, 2011; Osswald et al., 2008). On the other hand, the results with the Culture 2 showed a decreased activity in 'lab' fish, which might suggest the mechanism of action of saxitoxins, with realocation of energy to survive likely related to costs of detoxification process (Wiegand and Pflugmacher, 2005).

In our experiments deaths were not observed in fish exposed to 400 mg L^{-1} of the cyanobacterial crude extract for 48 h. Studies by Zagatto et al. (2012) also showed no effect on survival of adult fish *D. rerio* after 96 h of exposure to live cells of *C. raciborskii*, but high lethality was observed in the same fish larvae in 7 days of exposure. Although no lethality was observed in the present study, extracts of *C. raciborskii* were able to alter the swimming behavior of *P. vivipara*. Therefore, the presence of toxic cyanobacteria in the aquatic environment can influence several aspects of the fish life-cycle, such as scape from predators, foraging capacity and reproduction. These results contribute to the validation of the use of behavioral parameters to the evaluation of sublethal effects of toxic cyanobacteria on fish.

5. Conclusion

The crude extract of *C. raciborskii* was clearly able to change the locomotor activity of fish born in the laboratory, but had less effect on the fish collected from Rodrigo de Freitas Lagoon, suggesting a higher resistance of the wild fish to metabolites produced by this cyanobacterium. Also, different cultures of the same strain of *C. raciborskii* affected the locomotor activity of 'lab' *P. vivipara* in opposite directions, suggesting that saxitoxin was not the toxin responsible for those changes. Nevertheless, these alterations in the locomotor activity of *P. vivipara* can cause biological and ecological constraints in naïve fish, not previously exposed to cyanobacteria.

Ethical statement

This study was authorized by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA, license no. 15898-1) and approved by the Animal Ethics Committee of the Oswaldo Cruz Foundation (CEUA- Fiocruz no. L-020/2015) in accordance with the guidelines of the Brazilian College for Animal Experiments (COBEA).

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