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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*

Karina C. Lopes [a, c], Aloysio da S. Ferrão-Filho [a, *], Everton G.N. dos Santos [a, b], Rodolfo A. Cunha [a], Cláudia P. Santos [a]

[a] *Laboratório de Avaliação e Promoção da Saúde Ambiental, Instituto Oswaldo Cruz, FIOCRUZ, Av. Brasil, 4365 – Manguinhos, Rio de Janeiro, RJ 21040-360, Brazil*

[b] *Fundação Instituto de Pesca do Rio de Janeiro – FIPERJ, Escritório Regional Metropolitano II, Rua Aílton da Costa, 115, sl. 606 – Vinte Cinco de Agosto, Duque de Caxias, RJ 25071-160, Brazil*

[c] *Pós-Graduação em Biodiversidade e Saúde, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil*

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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

* Corresponding author.
E-mail address: aloysio@ioc.fiocruz.br (A.S. Ferrão-Filho).

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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