SETAC – Brazil

J. Braz. Soc. Ecotoxicol., v. 2, n. 2, 2007, 93-100

JBSE

Evaluation of Sub-lethal Toxicity of Cyanobacteria on the Swimming Activity of Aquatic Organisms by Image Analysis

A. S. Ferrão-Filho,[1*] R. Cunha,[1] V. F. Magalhães,[2] M. C. S. Soares[3] & D. F. Baptista[1]

[1]Laboratório de Avaliação e Promoção da Saúde Ambiental, Departamento de Biologia, Instituto Oswaldo Cruz, FIOCRUZ, Av. Brasil 4365, Manguinhos, Rio de Janeiro, RJ, Brasil

[2]Laboratório de Ecofisiologia e Toxicologia de Cianobactérias, Instituto de Biofísica Carlos Chagas Filho – CCS, Bloco G, Universidade Federal do Rio de Janeiro, Cidade Universitária, Ilha do Fundão, Rio de Janeiro, RJ

[3]Laboratório de Ficologia, Departamento de Botânica, Museu Nacional, Universidade Federal do Rio de Janeiro, São Cristovão, Rio de Janeiro, RJ

(Received July 6, 2006; Accepted April 10, 2007)

ABSTRACT

This study evaluated the sub-lethal toxicity of cyanobacteria, especially *Cylindrospermosis raciborskii*, on *Daphnia pulex* (water-flea) and *Danio rerio* (fish), through automated image processing, with the aim to develop a Real-Time Biomonitoring System (RTBS) for detection of toxic cyanobacteria in water supplies. A system composed of a video camera coupled to an image analyzer (Videomex-V®) and to a computer was used to evaluate the swimming activities of *D. rerio* and *D. pulex*. The parameters 'mean distance performed' and 'mean velocity' were used as endpoints to evaluate the effect of samples of cyanobacteria cultures or to raw water from the eutrophic Funil Reservoir. Results showed that both cyanobacteria cultures and water samples from the reservoir altered the swimming activities of *D. rerio*, elevating the values of mean distance performed and mean velocity. For *D. pulex*, the cyanobacteria cells caused the opposite effect, decreasing the swimming activity parameters, which can be related to the mechanism of action of saxitoxins. It was concluded that this system can be efficiently used in the detection of toxic blooms of saxitoxin producing cyanobacteria in water supplies.

Key words: Real-Time Biomoinitoring, toxic cyanobacteria, sub-lethal toxicity, saxitoxins, aquatic organisms

RESUMO

Avaliação da toxicidade subletal de cianobactérias na atividade natatória de organismos aquáticos através de processamento de imagem

Neste estudo avaliou-se a toxicidade subletal de cianobactérias, especialmente *Cylindrospermosis raciborskii*, em *Daphnia pulex* (microcrustáceo) e *Danio rerio* (peixe), através de processamento automático de imagem, visando ao aprimoramento de um sistema de Biomonitoramento em Tempo-Real (SBTR) para a detecção de cianobactérias tóxicas em mananciais de abastecimento. Um sistema composto de câmera de vídeo acoplada a um analisador de imagem (Videomex-V®) e a um microcomputador foi utilizado para a avaliação da atividade natatória de *D. rerio* (peixe) e *D. pulex* (microcrustáceo). Os parâmetros distância média percorrida e velocidade média foram utilizados como "endpoints" para avaliar o efeito das amostras de cianobactérias cultivadas em laboratório e água bruta do reservatório do Funil (eutrófico). Os resultados mostraram que tanto as cianobactérias de culturas laboratoriais quanto amostras de água do reservatório alteraram a atividade natatória de *D. rerio*, elevando os valores de distância percorrida e velocidade média. Para *D. pulex*, as células de cianobactérias tiveram o efeito contrário, diminuindo significativamente ambos os parâmetros da atividade natatória, o que pode estar relacionado ao mecanismo de ação das saxitoxinas. Conclui-se que este sistema pode ser utilizado com eficiência na detecção de florações tóxicas de cianobactérias produtoras de saxitoxinas em mananciais de abastecimento.

Palavras-chave: Biomonitoramento em Tempo-Real, cianobactérias tóxicas, toxicidade subletal, saxitoxinas, organismos aquáticos.

*Corresponding author: Aloysio da S. Ferrão Filho, e-mail: aloysio@ioc.fiocruz.br.

INTRODUCTION

Cyanobacterial blooms have been a concern for authorities worldwide, and have caused poisoning of wild and domestic animals (Carmichael, 1992; Chorus & Bartram, 1999), as well as humans, leading indeed to fatalities (Ressom *et al*., 1994, Carmichael *et al*., 2001). Several aquatic organisms are also affected by toxic cyanobacteria and their toxins (Christoffersen, 1996; Landsberg, 2002), including zooplankton (DeMott *et al*., 1991; Ferrão-Filho *et al*., 2000), microcrustaceans (Montagnolli *et al*., 2004), crayfish (Vasconcelos *et al*., 2001), and fishes (Kershavanath *et al*., 1994; Rodger *et al*., 1994; Baganz *et al*., 1998; Zimba *et al*., 2001).

Saxitoxins, called also Paralytic Shellfish Toxins (PST), are potent alkaloid neurotoxins produced by some marine dinoflagelates and also by some freshwater cyanobacteria such as *Anabaena circinalis* (Negri & Jones, 1995), *Aphanizomenon flos-aque* (Landsberg, 2002) and *Cylindrospermopsis raciborskii* (Lagos *et al*., 1999). Adverse effects of saxitoxins in invertebrates includes a variety of responses including reduced ingestion rates (Ives, 1985, 1987), avoidance of toxic cells by chemosensory means in copepods (Huntley *et al*., 1986; Sykes & Huntley, 1987; Teegarden & Cembella, 1996), reduced somatic growth, size at maturity, and fecundity (Dutz, 1998; Colin & Dam, 2004). Besides massive fish mortalities related to toxic blooms in marine waters documented in some studies (Landsberg, 2002), fish exposed to algal extracts containing saxitoxins can show irregular swimming behavior, lost of equilibrium and vertical orientation, diminished breading, and paralysis (White, 1977).

Behavioral or physiological responses are not always easily distinguishable by most traditional toxicity tests, and sub-lethal effects may play an important role in the alteration of the behavior of aquatic species, leading also to a decreased fitness. Also, when aquatic organisms are submitted to a toxic stress, they may show a significant alteration of their swimming activity. Thus, this parameter can be a useful tool in the evaluation of toxicity (Calow, 1993).

The detection of sub-lethal effects of toxicants by Real-Time Biomonitoring Systems (RTBS), using as endpoints the swimming activity of aquatic organisms, has an excellent potential in ecotoxicological evaluation. RTBS have been applied to the evaluation of toxicity of a wide range of toxic substances, including to the detection of harmful algal blooms (van der Schalie *et al*., 2001; Glasgow *et al*., 2004).

The aim of this work was to evaluate the potential for the use of an imaging analysis system in Real-Time Biomonitoring of harmful algal blooms, such as toxic cyanobacteria. For this purpose, an image capturing system coupled to an image analyzer (Videomex-V®) and to a computer was used to evaluate the swimming activities of *Danio rerio* (fish) and *Daphnia pulex* (water-flea), both exposed to cultures of a saxitoxin producer strain of *Cylindrospermopsis raciborskii* or to raw water from a eutrophic reservoir with occurrence of this cyanobacterium.

MATERIAL AND METHODS

Cultures of test-organisms

A strain of *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju, named CYRF, was isolated from the Funil Reservoir, located at Resende (RJ, Brazil). This strain was cultured in ASM-1 medium (Gorham *et al.*, 1964), pH 8.0, 23 ± 1°C, 40-50 µE m^{-2} s^{-1} and 12:12h light-dark cycle. The form of the strain was as straight trichomes of 100-200 µm in length.

The microcrustacean *Daphnia pulex* Leydig was obtained from Carolina Biological Supply (North Carolina, USA) and cultured in mineral commercial water (Minalba), with a hardness of 77.2 mg $CaCO_3$.L^{-1}, pH of 7.4, at 23 ± 2°C and at dim light, and fed the green algae *Ankistrodesmus falcatus* Braun at a density of 1.6×10^4 cells ml^{-1} (~0.5 mg C L^{-1}).

The cyprinid *Danio rerio* (HAMILTON, 1822) was maintained in dechlorinated tap water in aerated fish tanks, containing a maximum of 50 fish per 50 L of water, at 24 ± 1°C and fed lyophilized *Spirulina* as food every day.

Image Analysis System

For tests with *D. rerio* an static system was developed, consisting of following components: a) an image analyzer (Videomex-V®, Columbus Instruments, USA), which uses a software denominated "Multiple Object Distance Traveled (MODT); b) a video camera which send the digitalized image of animals to a monitor; c) a registering cabinet, built in acrylic (dimension 36 × 36 × 45 cm); d) an aquarium built in acrylic covered with white insulfilm (dimension: 70 × 35 × 25 cm; capacity: 20 L), with two acrylic exposing chambers inside divided in 8 boxes (9,5 × 5,0 × 2 cm), with perforated surface (wholes of 3 mm ∅) to facilitate the circulation of water; and e) a microcomputer containing a software to receive data from Videomex-V® and generate a spreadsheet in Excel format (Figure 1). For tests with *D. pulex*, a registering cabinet was built in acrylic covered with white insulfilm and EVA rubber plates of 2.0 mm thick (dimensions: 16 × 16 × 37 cm), and the exposing chamber was built in acrylic divided in three boxes (10 × 25 × 5 mm), with 1 mL capacity (Figure 2).

Tests with D. rerio

One hour prior to the experiments 8 fishes (3-4 cm length) per each trial were conditioned individually into the exposing chambers, and exposed to either strain CYRF or to raw water from Funil reservoir. Seventy two fishes exposed to clean tap water were used for controls in comparison with fishes exposed to strain CYRF, and other 40 fishes exposed to filtered Funil reservoir water (Sartorius glass fiber filters) for comparisons with animals exposed to Funil raw water. In the experiment with cultured algae eight fish were exposed to freshly harvested filaments of strain CYRF in the concentration of 10^5 cells.mL^{-1}, and in the experiments with natural samples eight fish were exposed to Funil reservoir raw water collected in two sampling dates, in 25 May 2005 and 11 February 2006. The parameters 'mean distance performed' and 'mean velocity' were used as

toxicity endpoints. After 1 hour of acclimation time, the Videomex-V® system started automatically the registering of fish activity. Five minutes before the test, the water pumps were turned on to circulate the water inside the test compartment, and samples of water from each side of the aquarium were taken for measures of dissolved oxygen, pH, conductivity and hardness. At the end of the experiment, another sample from each test compartment was taken for physical and chemical measurements. Each trial had 5 hours of registration period, with 60 registering intervals of 5 min.

Tests with D. pulex

Ten minutes prior to the experiments, 3 individuals of *D. pulex* (1.5 to 2.0 mm length) per trial were placed individually in the exposing boxes containing 1.0 mL of suspensions of the strain CYRF in mineral water. Controls with 18 animals exposed only to green algae were performed. Animals were exposed to freshly harvested filaments of the strain CYRF in three concentrations: 5×10^3, 10^4 and 5×10^4 cells.mL^{-1}. The parameters 'mean distance performed' and 'mean velocity' were used as toxicity endpoints. After 10 min of acclimation, a 50 min registration period started automatically, with 10 registering intervals of 5 min.

Cyanobacteria countings

Cyanobacteria from Funil reservoir samples were counted according to Uthermöhl (1958). Cells of each *C. raciborkii* filament were counted in a Fuchs-Rosenthal hematocytometer and filaments were measured for estimating mean length.

Saxitoxin analysis

Saxitoxins were analyzed for total seston (2 L of raw water filtered onto Satorius glass fiber filters) and net liophylized phytoplankton (~10-20 mg).

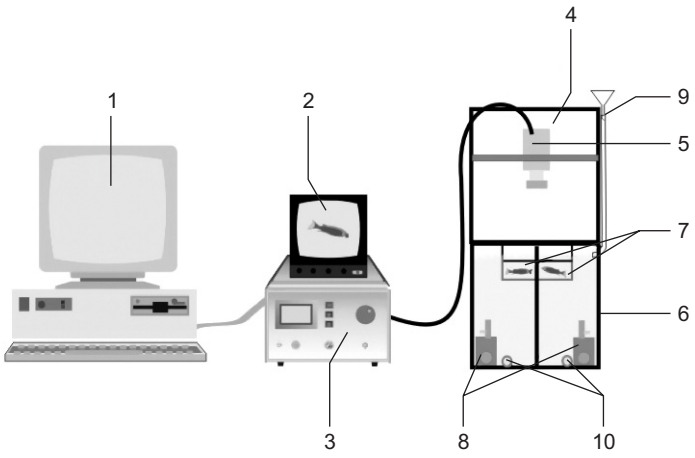

Figure 1 – Image analysis system for Real-Time Biomonitoring with *Danio rerio*. 1. microcomputer; 2. monitor; 3. Videomex-V®; 4. registering cabinet; 5. video camera; 6. glass aquarium divided in two compartments; 7. exposing chambers with 8 boxes; 8. water pumps; 9. glass funil with silicone tubes; 10. outflow of water.

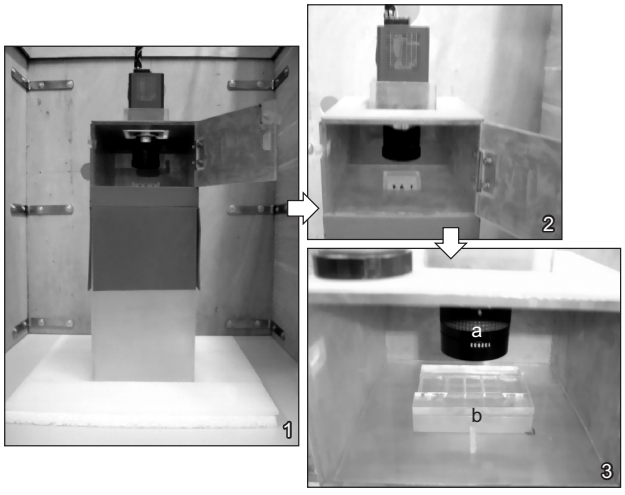

Figure 2 – Details of the registering and exposing chambers for *Daphnia pulex*. 1. Registering cabinet for *Daphnia pulex*. 2. Close of the registering cabinet. 3. Close of the video camera (a) and exposure chambers (b).

Both samples were extracted with 5 mL of 0.1 N acetic acid for 1 hour. After that samples were centrifuged at 10800 g for 10 min. and the supernatant stocked in –18ºC until the analysis. All samples were analyzed by High Performance Liquid Chromatography (HPLC) with post-column derivatization method (Oshima, 1995) in a Shimadzu/CLASS VP apparatus with fluorescence detector (RF-10A XL), adjusted to 330 nm of excitation and 390 nm of emission, using a 20 µl loop, reverse column Merck LC-18 (Lichrocart® 150 mm × 4,6 mm ∅, 5 µm). The mobile phase consisted of 2 mM hepatanosulfonate in 30 mM ammonium phosphate buffer pH 7.1:acetonitrile 100:5 for STX e neoSTX analysis and 2 mM de hepatanosulfonate in 10 mM ammonium phosphate buffer pH 7.1 for GTXs analysis with a flow rate of 0.8 mL min^{-1}. The oxidizing reagent used was 7 mM periodic acid in 10 mM sodium phosphate buffer pH 9.0 and the reaction with a flow rate of 0.4 mL min^{-1}. The oxidizing reaction was done in a 10 m coil of Teflon tubing at 80ºC. Before the detection the reaction was interrupted with a 0.5 M acetic acid. Standard solutions of saxitoxin (STX), neosaxitoxin (neoSTX), and goniautoxins (GTX 1 & 4 e GTX 2 & 3) were obtained from the National Research Council (NRC), Institute for Marine Biosciences, Canadá.

Statistical analysis

A repeated measure analysis was used to detect significant differences between treatments, using Systat® 9.0 statistical package.

RESULTS

During the toxicity tests, there was not a significative alteration of the physico-chemical parameters of water. Figure 3 shows the results for *D. rerio* exposed to raw water from Funil reservoir in two dates. In May 2005, there was a significant treatment effect, elevating mean distance performed ($F_{1,46}$ = 31.65; $p < 0.001$) and mean velocity ($F_{1,46}$ = 24.59; $p < 0.001$), a significant effect of time in mean distance performed ($F_{59,2714}$ = 2.59; $p < 0.001$) and in mean velocity ($F_{59,2714}$ = 2.20; $p < 0.001$), and a significant interaction between treatment and time in mean distance performed ($F_{59,2714}$ = 3.03; $p < 0.001$). In February 2006, there was also a significant treatment effect, elevating mean distance performed ($F_{1,46}$ = 7.19; $p = 0.01$) and mean velocity ($F_{1,46}$ = 6.45; $p = 0.015$), a significant effect of time in mean distance performed ($F_{59,2714}$ = 4.36; $p < 0.001$) and in mean velocity ($F_{59,2714}$ = 2.78; $p < 0.001$), but no significant interaction between treatment and time for both parameters.

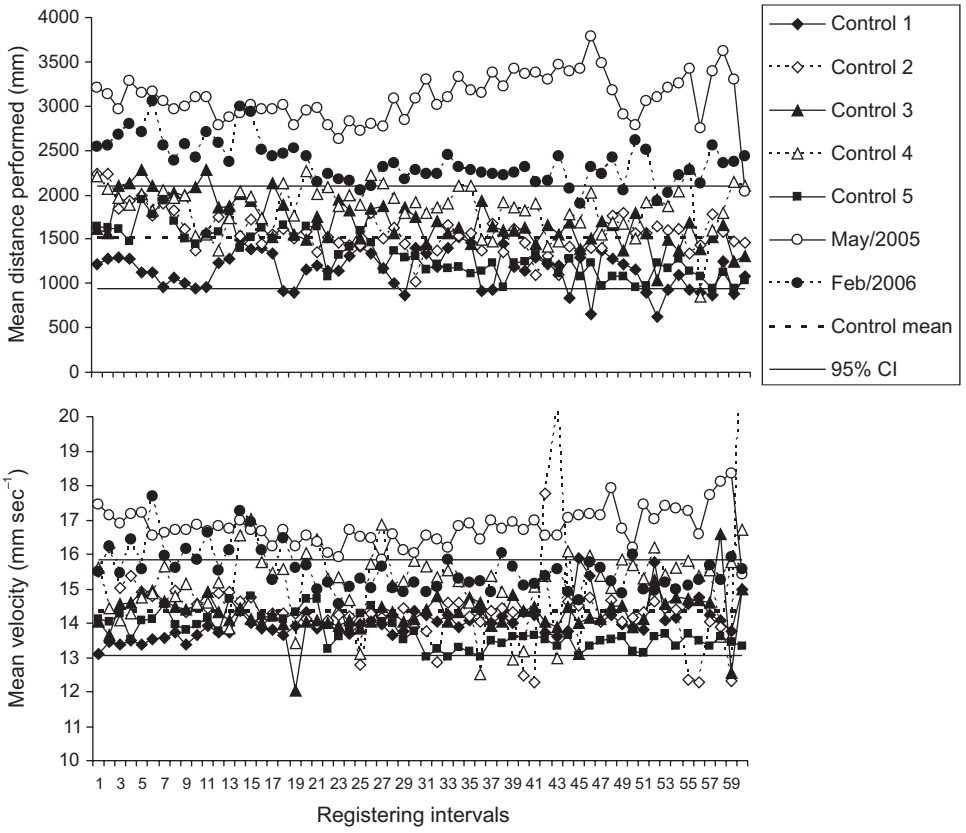

Figure 3 – Results of the tests with *Danio rerio* exposed to raw water collected from Funil reservoir in two sampling dates. The straight dark lines represent the 95% confidence intervals for data from 40 control fishes (5 control groups of 8 fishes) exposed only to filtered reservoir water, and the symbols represent mean values.

When *D. rerio* was exposed to strain CYRF, a significant elevation of mean distance performed ($F_{1,78} = 48.92$; $p < 0.001$) and mean velocity ($F_{1,78} = 42.12$; $p < 0.001$) occurred (Figure 4). There was also a significant effect of time in mean distance performed ($F_{59,4602} = 2.27$; $p < 0.001$) and in mean velocity ($F_{59,4602} = 3.61$; $p < 0.001$), and a significant interaction between treatment and time in mean distance performed ($F_{59,4602} = 3.34$; $p < 0.001$) but not in mean velocity. *D. pulex* showed a significant alteration in swimming activity when exposed to strain CYRF (Figure 5). There was a significant effect of treatment (cell density) decreasing mean distance performed ($F_{3,47} = 4.10$; $p = 0.012$) and mean velocity only marginally ($F_{3,47} = 2.79$; $p = 0.051$). There was a significant effect of time in mean distance performed ($F_{9,270} = 12.61$; $p < 0.001$) and in mean velocity ($F_{9,423} = 24.54$; $p < 0.001$), and a significant interaction between treatment and time in mean distance performed ($F_{9,270} = 2.61$; $p = 0.006$) and in and mean velocity ($F_{27,423} = 2.98$; $p < 0.001$).

In the two sampling dates, total cyanobacteria densities in Funil reservoir ranged from 2657 cells mL^{-1} in May 2005 to 208372 cells mL^{-1} in February 2006. Two potential saxitoxin producer cyanobacteria occurred in those months, *Anabaena circinalis* with 1383 cells mL^{-1} (4% in biomass) in Feb/06, and *C. raciborskii* with 861 cells mL^{-1} (46.2% in biomass) in May/05 and 152314 cells mL^{-1} (91.2% in biomass) in Feb/06, besides potential hepatotoxin producer genera such as *Microcystis* with 1796 cells mL^{-1} (53.8% in biomass) in May/05, and 31071 cells mL^{-1} (4.4% in biomass) in Feb/06. Other cyanobacteria contributed with less than 1% biomass in those months.

The toxin analysis showed that samples from Funil reservoir contained saxitoxins (0.02 µg STX L^{-1} and 0.05 µg neoSTX L^{-1} in May/05, and 0.35 µg STX L^{-1}, 1.76 µg neoSTX L^{-1} and 1.38 µg GTX–1 L^{-1} in Feb/06). The strain CYRF contained 38.9-52.0 µg STX g^{-1} and 55.0-77.4 µg GTX–1 g^{-1}.

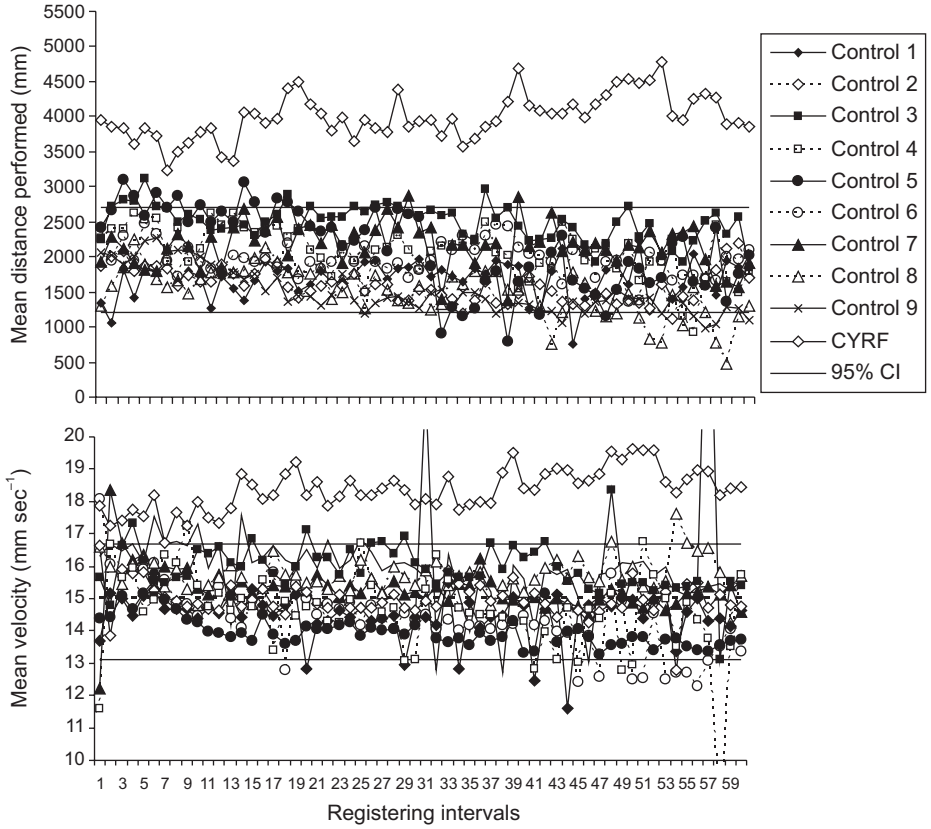

Figure 4 – Results of the tests with *Danio rerio* exposed to filaments of *C. raciborskii* (strain CYRF) in the concentration of 10^5 cells mL^{-1}. The straight dark lines represent the 95% confidence intervals for data from 72 control fishes (9 control groups of 8 fishes) exposed only to tap water, and the symbols represent mean values.

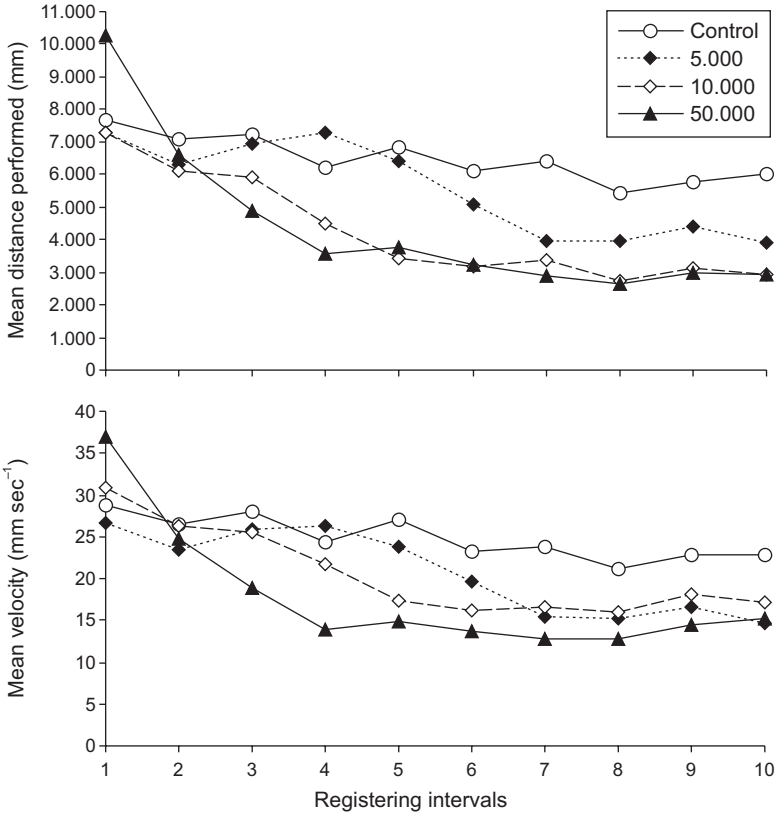

Figure 5 – Results of the tests with *Daphnia pulex* exposed to filaments of *C. raciborskii* (strain CYRF) in concentrations ranging from 5×10^3 to 5×10^4 cells mL^{-1}.

DISCUSSION

The aquatic organisms used in this study responded differently to the presence of toxic cyanobacteria in water. While *D. rerio* showed an elevation of swimming activity parameters, *D. pulex* showed the opposite trend. Although some studies have shown that cyanobacteria can alter fish swimming pattern and decrease oppercular beating rate (White, 1977), this study showed that the *C. raciborskii* altered swimming activity of *D. rerio*, elevating mean distance performed and mean velocity. Despite several reports of fish mortality in the marine environment related to red tide PST producers, few studies had found consistent evidence that saxitoxins can affect fish behavior and physiology. Lefebvre *et al*. (2005) showed that saxitoxins can alter the sensorimotor function of herring (*Clupea harengus pallasi*), decreasing its response to spontaneous and touch-activated swimming behavior. However, it was shown that fishes recovered normal motor function after 4-24 hours of continuous exposure.

Other cyanobacteria, however, can exert physiological constraints to other fish. Keshavanath *et al*. (1994) showed that tilapia (*Oreochromis niloticus*) exposed to *Microcystis aeruginosa* cells decreased opercular beating rate. Baganz *et al*. (1998) showed that spontaneous locomotor activity of *D. rerio* exposed to microcystins-LR presented a dose-response effect, but that it was dependent on the time of the day (day-time or night-time). Thus, the presence of other potential toxin producer cyanobacteria in Funil reservoir suggests that other toxins may be involved in the altered behavior of *D. rerio* and deserves further research.

Saxitoxins act by blocking sodium channels in nerve cells, leading to cessation of nerve impulse (Evans, 1965; Kao, 1965). Thus, it is likely that the decrease in swimming activity of *D. pulex* may be related to this neurotoxic effect. In another study, Ferrão-Filho *et al*. (in prep.) showed that *D. pulex* and *Moina micrura* presented progressive immobilization (stop swimming) after 24-48h when exposed to another saxitoxin producer strain of *C. raciborskii* (strain T3), and recovered swimming behavior when transferred to clean water. The same effect was observed when animals were exposed to raw water from Funil reservoir containing saxitoxins. They suggested that saxitoxins were responsible by the observed effect, likely by stopping the stimulus of nerve cells on the muscles of the second antennae, which is responsible by the movement of the animal.

Toxic effects of saxitoxin producer phytoplankton on crustacean zooplankton has been reported in many studies, mainly in the marine environment (Ives, 1985, 1987; Huntley *et al*., 1986; Sykes & Huntley, 1987; Teegarden & Cembella, 1996; Dutz, 1998; Colin & Dam, 2004). Ives (1985) has reported a 'loss of motor coordination' in copepods exposed to toxic dinoflagellates but it was not clear if this effect was related to the swimming behavior or to the feeding process of these animals. Haney *et al.* (1995) have reported a reduction in the thoracic appendages beating rate and an increase in rejection rate of particles by the post-abdomen of *Daphnia carinata* when exposed to a filtrate of *Aphanizomenon flos-aquae* and to purified saxitoxin. However, any other study has demonstrated that saxitoxins can exert inhibitory effects on the swimming movements of freshwater cladocerans.

The mechanism of action must be different in different taxa. While in *D. pulex* the saxitoxins appear to be acting by inhibition of the nerve impulse, in fish a stimulatory effect appears to have occurred. It is not possible, however, to affirm if the effects observed in fish are either physiologically or behaviorally mediated. It is not likely that fish nerve system was overstimulated by saxitoxins containing filaments of *C. raciborskii* in the water, since the opposite trend was predicted taking into account the mechanism of action of these toxins. The hyperactivity observed in fish could be a result of gill irritation by contact with toxic filaments, or even the alteration of physico-chemical parameters, if they could "smell" some different compound in the water. However, the hypothesis that other unidentified toxins would be present can not be discarded, both in Funil water and in strain CYRF.

Concluding, *D. pulex* showed a sensitive response to *C. raciborskii* filaments in the water, decreasing swimming activity with increasing filament and saxitoxins concentrations, which is compatible with the mechanism of action of these toxins. The fish *D. rerio* showed an opposite trend, increasing swimming activity, and the mechanism of action of these cyanobacteria on these fish remains unclear and deserves more investigation. Nevertheless, our image analysis system seem to be efficient in detecting effects of toxic cyanobacteria and can be used in Real-Time Biomonitoring of harmful algal blooms in water supplies.

Acknowledgements — We thank Jobson and Ricardo by the field sampling and toxin analysis. We thank FAPERJ by the first author fellowship (Proc. # 151.218/2005) and Minalba Alimentos e Bebidas Ltda by the grant to participate of ECOTOX 2006.

REFERENCES

BAGANZ, D., STAAKS, G. & STEINBERG, C., 1998, Impact of the toxin, microcystin-LR on behaviour of zebrafish, *Danio rerio*. *Wat. Res., 32*: 948-952.

CALOW, P., 1993., *Handbook of Ecotoxiclogy*. Volume I. Blackwell Scientific Publications, Oxford, 478 p.

CARMICHAEL, W. W., 1992, Cyanobacteria Secondary metabolites: the cyanotoxins. *J. App. Bacteriol., 72*: 445-459.

CARMICHAEL, W. W., AZEVEDO, S. M. F. O., NA, J. S., MOLICA, R. J. R., JOCHIMSEN, E. M., LAU, S., RINEHART, K. I., SHAW, G. R. & EAGLESHAM, G. K., 2001, Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins. *Envir. H. Per., 109*: 663-668.

CHORUS, I. & BARTRAM, J., 1999, *Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management*. E & FN Spon, London, 416 p.

CHRISTOFFERSEN, K., 1996, Ecological implications of cyanobacterial toxins in aquatic food webs. *Phycologia, 35*: 42-50.

COLIN, S. P. & DAM, H. G., 2004, Testing for resistance of pelagic marine copepods to a toxic dinoflagellate. *Evol. Ecol.*, *18*: 355-377.

DEMOTT, W. R., ZHANG, Q. & CARMICHAEL, W. W., 1991, Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and three species of *Daphnia*. *Limnol. Oceanogr., 36*: 1346-1357.

DUTZ, J., 1998, Repression of fecundity in the neritic copepod *Acartia clausi* exposed to the toxic dinoflagellate *Alexandrium lusitanicum*: relationship between feeding and egg production. *Mar. Ecol. Prog. Ser., 175*: 97-107.

EVANS, M. H., 1965, Cause of death in experimental paralytic shellfish poisoning (PSP). *Br. J. Exp. Path., 46*: 245-253.

FERRÃO-FILHO, A. S., AZEVEDO, S. M. F. O. & DEMOTT, W. R., 2000, Effects of toxic and non-toxic cyanobacteria on the life history of tropical and temperate cladocerans. *Freshw. Biol., 45*: 1-19.

FERRÃO-FILHO, A. S., COSTA, S. M., RIBEIRO, M. G. L. & AZEVEDO, S. M. F. O. (in prep.) Effects of a saxitoxin producer strain of *Cylindrospermopsis raciborskii* (cyanobacteria) on the swimming movements of cladocerans. (submitted to Environ. Toxicol.).

GLASGOW, B. H., BURKHOLDER, J. M., REED, R. E., LEWITUS, A. J. & KLEINMAN, J. E., 2004, Real-time remote monitoring of water quality: a review of current applications, and advancements in sensor, telemetry, and computing technologies. *J. Exp. Mar. Biol. Ecol., 300*: 409-448.

GORHAM, P. R., MCLACHLAV, J. R., HAMMER, V. T. & KIM, W. K., 1964, Isolation and culture of toxic strains of *Anabaena flos-aquae* (Lyngb.) de Bréb. *Verein Theor. Angew. Limnol., 15*: 796-804.

HANEY, J. F., SASNER, J. J. & IKAWA, M., 1995, Effects of products released by *Aphanizomenon flos-aquae* and purified saxitoxin on the movements of *Daphnia carinata* feeding appendages. *Limnol. Oceanogr., 40*: 263-272.

HUNTLEY, M. E., SYKES, P., ROHAN, S. & MARIN, M., 1986, Chemically mediated rejection of dinoflagelate prey by the copepods *Calanus pacificus* and *Paracalanus parvus*: mechanism, occurrence and significance. *Mar. Ecol. Prog. Ser., 28*: 105-120.

IVES, J. D., 1985, The relationship between *Gonyaulax tamarensis* cell toxin levels and copepod ingestion rate, pp. 413-418. *In*: D. M. Anderson, A. W. White & D. G. Baden (ed.), *Toxic dinoflagellates*. Elsevier, New York.

IVES, J. D., 1987, Possible mechanisms underlying copepod grazing responses to levels of toxicity in red tide dinoflagellates. *J. Exp. Mar. Biol. Ecol., 112*: 131-145.

KAO, C. Y., SUZUKI, C. Y., KLEINAHUS, T. & SIEGMAN, M. J., 1967, Vasomotor and respiratory depressant actions of tetrodotoxin and saxitoxin. *Arch. Inter. Pharm., 165*: 438-50.

KESHAVANATH, P., BEVERIDGE, M. C. M., BAIRD, D. J. & LAWTON, L. A., 1994, The functional grazing response of a phytoplanktivorous fish *Oreochromis niloticus* to mixtures of toxic and non-toxic strains of the cyanobacterium *Microcystis aeruginosa*. *J. Fish. Biol., 45*: 123-129.

LAGOS, N., ONODERA, H., ZAGATTO, P. A., ANDRINOLO, D., AZEVEDO S. M. F. O. & OSHIMA, Y., 1999, The first evidence of paralytic shellfish toxins in the freshwater cyanobacterium *Cylindrospermopsis raciborskii*, isolated from Brazil. *Toxicon., 37*: 1359-1373.

LANDSBERG, J. H., 2002, The effects of harmful algal blooms on aquatic organisms. *Rev. Fish. Sci., 10*: 191-193.

LEFEBVRE, K. A., ELDER, N. E., HERSHBERGER, P. K., TRAINER, V. L., STEHR, C. M. & SCHOLZ, N. L., 2005, Dissolved saxitoxin causes transient inhibition of sensorimotor function in larval Pacific herring (*Clupea harengus pallasi*). *Ma. Biol., 147*: 1393-1402.

MONTAGNOLLI, W., ZAMBONI, A., LUVIZOTTO-SANTOS, R., J. & YUNES, J. S., 2004, Acute Effects of *Microcystis aeruginosa* from the Patos Lagoon Estuary, Southern Brazil, on the Microcrustacean *Kalliapseudes schubartii* (Crustacea: Tanaidacea). *Arch. Environ. Contam. Toxicol., 46*, 463-469.

NEGRI, A. P. & JONES, G. J., 1995, Bioaccumulation of paralytic shellfish poisoning (PSP) toxins from the cyanobacterium *Anabaena circinalis* by the freshwater mussels *Alathrya condola*. *Toxicon., 33*: 667-678.

OSHIMA, Y., 1995, Manual on harmful marine microalgae, pp. 81-94. *In*: G. M. Hallegraeff, D. M. Anderson & A. D. Cembella (eds.), *IOC manuals and guides no. 33*. UNESCO, Paris.

RESSOM, R., SOONG, F. S., FITZGERALD, J., TURCCZYNOWICZ, L., EL SAADI, O., RODER, D., MAYNARD, T. & FALCONER, I. (eds.), 1994, *Health effects of toxic Cyanobacteria (Blue-green Alage)*. National Health and Medical Research Council, Australian Government Public Service.

RODGER, H. D., TURNBULL, T., EDWARDS, C. & CODD, G. A., 1994, Cyanobacterial (blue-green algal) bloom associated pathology in brown trout, *Salmo truta* L., in Loch Leven, scothland. *J. Fish Dis., 17*: 177-181.

SYKES, P. F. & HUNTLEY, M. E., 1987, Acute physiological reactions of *Calanus pacificus* to selected dinoflagellates: direct observations. *Mar. Biol., 94*: 19-24.

TEEGARDEN, G. J. & CEMBELLA, A. D., 1996, Grazing of toxic dinoflagellates, *Alexandrium* spp., by adult copepods of coastal Maine: Implications for the fate of paralytic shellfish toxins in marine food webs. *J. Exp. Mar. Biol. Ecol., 196*: 145-176.

UTERMÖHL, H., 1958, Zur Vervollkommung der quantitativen Phytoplankton-Methodik. *Verh. Int. Ver. Limnol., 9*: 1-38.

VAN DER SCHALIE, W. H., SHEDD, T. R., KNECHTGES, P. L. & WIDDER, M. W., 2001, Using higher organisms in biological early warning systems for a real-time toxicity detection. *Biosens. Bio., 16*: 457-465.

VASCONCELOS, V., OLIVEIRA, S. & TELES, F. O., 2001, Impact of a toxic and a non-toxic strain of *Microcystis aeruginosa* on the crayfish *Procambarus clarkii*. *Toxicon., 39*: 1461-1470.

WHITE, A. W., 1977, Dinoflagellate toxins as probable cause of an Atlantic herring (*Clupea harengus hnrengus*) kill, and pteropods as apparent vector. *J. Fish. Res. Bd. Can., 34*: 2421-2424.

ZIMBA, P. V., KHOO, L., GAUNT, P. S., BRITTAIN, S. & CARMICHAEL, W. W., 2001, Confirmation of catfish, *Ictalurus punctatus* (Rafinesque), mortality from *Microcystis* toxins. *J. Fish Dis., 24*: 41.