



## Microcystin-LR: How it affects the cardio-respiratory responses to hypoxia in Nile tilapia, *Oreochromis niloticus*

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### ABSTRACT

The effects of microcystin on the cardio-respiratory function of Nile tilapia were analyzed 48 h after intra-peritoneal injection of microcystin-LR (MC-LR – 100  $\mu\text{g kg}^{-1}$  body weight). Exposure to MC-LR induced significant reduction in metabolic rate ( $\dot{V}\text{O}_2$ ) and increase in the critical  $\text{O}_2$  tension ( $P_{\text{cO}_2}$ ) in relation to the control group. Gill ventilation ( $\dot{V}_\text{C}$ ) and ventilatory tidal volume ( $V_\text{T}$ ) were considerably lower in fish exposed to MC-LR, probably due to an alteration in the homeostatic mechanisms, impairing the regular respiratory response of this species to environmental hypoxia. The ability to maintain the  $\text{O}_2$  extraction from the ventilatory current ( $\text{EO}_2$ ) during severe hypoxia was also significantly reduced in fish exposed to MC-LR exposure. Control fish displayed the characteristic reflex bradycardia in response to hypoxia. However, when compared to the control group, fish exposed to MC-LR presented significantly lower heart rate ( $f_\text{H}$ ) in normoxia and in all experimental hypoxic levels, probably due to a direct effect of this toxin on the cardiac tissue.

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### 1. Introduction

Under favorable conditions, cyanobacteria can form large superficial masses of green color called blooms (Reynolds, 1984). In general, 50–75% of cyanobacterial blooms produce toxins, with a possible occurrence of more than one type of toxin within the same bloom. The microcystins (MC) are the most common toxins found in freshwater blooms (Carmichael, 1994; Kondo et al., 2002), with dissolved concentrations varying from traces up to 25 000  $\mu\text{g L}^{-1}$  or higher, immediately after the collapse of a highly toxic bloom (Chorus and Bartram, 1999; Amado and Monserrat, 2010). Despite the occurrence of more than 70 variants of MC (Fastner et al., 2002), microcystin-LR (MC-LR) stands out for its high frequency in natural environments and the large volume of studies on its structure and mechanisms of action (de Figueiredo et al., 2004).

The uptake of these toxins by the cells seems to involve an active transport system, known as multi-specific bile acids transportation system (Eriksson et al., 1990) involving organic anion transporting polypeptides (OATP). The MC acts in the inhibition of protein phosphatases, especially types 1 and 2A (PP1 and PP2A) (Toivola and Eriksson, 1999; Guzman et al., 2003). The inhibition of these phosphatases leads to a state of protein hyperphosphorylation that regulate the dynamic formation of the

cytoskeleton, resulting in the disintegration of cell structure, mainly in hepatocytes (Carmichael, 1994). Besides the inhibition of phosphatases, microcystins can also affect the DNA repair systems and expression of genes (Ariza et al., 1996; Douglas et al., 2001), being able to interact with the mitochondria of animal tissues, causing oxidative stress and apoptosis (Prieto et al., 2009).

Apart from released toxins, cyanobacterial blooms can degrade water quality, causing exhaustion of dissolved oxygen (Brownlee et al., 2005) and a sharp increase in nutrients (Buryskova et al., 2006). In this context, Zhang et al. (2011) showed a strong correlation between MC and dissolved  $\text{O}_2$  in Lake Taihu, China. The authors showed that the  $\text{O}_2$  concentration of  $10.24 \pm 1.25 \text{ mg L}^{-1}$  and MCs concentration of  $0.09 \pm 0.09 \mu\text{g L}^{-1}$  found before cyanobacterial blooms changed to  $7.03 \pm 2.29 \text{ mg L}^{-1}$  and  $0.91 \pm 0.70 \mu\text{g L}^{-1}$ , respectively, during the blooms.

Several studies in teleost fish have demonstrated the effects of microcystin-LR and its variants on organs such as liver, gills, kidneys, heart, among others (Carbis et al., 1997; Best et al., 2001; Zhang et al., 2007). Damages in hepatopancreas and kidney cells (Fischer and Dietrich, 2000), cardiac myopathy (Atencio et al., 2008), gill necrosis, folded lamellar tips and mild epithelial ballooning (Carbis et al., 1997), anemia (Katharios et al., 2002; Zhang et al., 2007), and the occurrence of oxidative stress and lipoperoxidation (Prieto et al., 2006) are some of these changes.

Although MC-LR is the most studied variant among the microcystins, there is a dearth of studies linking fish exposure to MC-LR with changes in the in vivo cardio-respiratory function.

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Thus, the main goal of this study was to analyze the effect of MC-LR on respiratory and cardiac function of Nile tilapia, *Oreochromis niloticus*.

## 2. Materials and methods

This study was conducted in accordance with the Brazilian legislation on animal experimentation and duly approved by the Ethics in Animal Experimentation Committee/Federal University of São Carlos, Brazil.

### 2.1. Animals

Specimens of *O. niloticus* (Wt = 175 ± 37 g) were obtained from the fish farm Águas Claras, Mococa, São Paulo State, Brazil. Fish were acclimated for 30 d prior to experimentation in 1000 L holding tanks equipped with a continuous supply of well-aerated and dechlorinated water, at 25 ± 2 °C and under natural photoperiod (~12 h:12 h). The physical and chemical parameters were kept nearly constant: pH 6.7–7.3, DO 6.0–7.3 mg L<sup>-1</sup>, hardness 48–53 mg L<sup>-1</sup> (as CaCO<sub>3</sub>), alkalinity 40–43 mg L<sup>-1</sup> (as CaCO<sub>3</sub>), ammonium 0.1 mg L<sup>-1</sup>; chloride 42 mg L<sup>-1</sup>, and conductivity 110 μS cm<sup>-1</sup>. During this period, fish were fed ad libitum with commercial fish pellets.

### 2.2. Experimental design

After acclimation, specimens of *O. niloticus* were divided into two groups: Control fish (Ctrl, *n* = 8) and fish exposed to the toxin (Mcys, *n* = 8). The Mcys group received an intraperitoneal injection (0.5 mL of 0.9% saline) containing the purified form of the toxin microcystin-LR at a concentration of 100 μg kg<sup>-1</sup> body weight. The control group (Ctrl) received only saline injections (0.5 mL of 0.9% saline). Measurements of respiratory and cardiac parameters were made 48 h after injections. The purified MC-LR (*Microcystis aeruginosa*) was provided by the Cyanobacterial Research Unit of the Federal University of Rio Grande – FURG, Rio Grande do Sul State, Brazil.

Most studies on the acute lethality of MCs in fish have utilized intraperitoneal injections of extracted MCs to determine the dose that is lethal to half the test population (LD<sub>50</sub>), and the reported values range from 20 to 1500 μg kg<sup>-1</sup> body weight (Malbrouck and Kestemont, 2006). Prieto et al. (2006) and Atencio et al. (2008) submitted specimens of *Oreochromis* sp. to an intraperitoneal single dose of 500 μg kg<sup>-1</sup> MC-LR and reported acute toxic effects without mortality. Thus, the concentration of 100 μg kg<sup>-1</sup> MC-LR was selected in the present study to avoid acute toxic effects and possible death.

### 2.3. Surgical procedures

Fish were immersed in a benzocaine solution (1 g of benzocaine dissolved in 0.1 L of 99% ethanol and diluted in 10 L of water). This provided a level of anesthesia which still allowed spontaneous breathing. To monitor the respiratory frequency (*f<sub>R</sub>*) and measure the O<sub>2</sub> tensions of the inspired water (P<sub>i</sub>O<sub>2</sub>) a PE-100 catheter was inserted dorsally into the buccal cavity close to the mouth. Furthermore, the border of both opercular cleithra were cannulated (PE-50 catheter) to measure the O<sub>2</sub> tensions of the expired water (P<sub>e</sub>O<sub>2</sub>). Two ECG electrodes were placed in a ventral position, the first (positive) between the gills and the heart, and the second (negative) in a ventral position close to the pelvic fins. A reference electrode was located in the water of the experimental chamber. This preparation allowed for electrocardiographic (ECG) recordings of the first diagonal branch (D1) lead. After surgery, the fish were placed into a respirometer and, subsequently, into the experimental chamber to recover for 24 h in normoxic water (140 mm Hg) at

25 ± 1 °C (temperature for all experiments). Meanwhile, disturbances were avoided to obtain resting control values.

### 2.4. Responses to hypoxia

The weight-specific oxygen uptake, or metabolic rate ( $\dot{V}O_2 - \text{mL O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ), was measured by flow-through respirometry (Kalinin et al., 1999; Thomaz et al., 2009). The fish was housed in an open flow-through respirometer while ingoing and outgoing PO<sub>2</sub> (P<sub>in</sub>O<sub>2</sub> and P<sub>out</sub>O<sub>2</sub>) were continuously recorded. Water was siphoned, via polyethylene catheters, to O<sub>2</sub> electrodes (FAC001-O<sub>2</sub>, FAC-São Carlos, SP, Brazil) housed in temperature controlled cuvettes and connected to a FAC-204AO<sub>2</sub> Analyzer. Measurements were performed for various PO<sub>2</sub> and each tension was maintained for 1 h before measurements were taken.  $\dot{V}O_2$  was calculated as:

$$\dot{V}O_2 = [(P_{in}O_2 - P_{out}O_2) \cdot \alpha O_2 \cdot \dot{V}_R] / Wt$$

where  $\dot{V}_R$  represents the constant water flow through the respirometer (L h<sup>-1</sup>),  $\alpha O_2$  denotes the solubility coefficient for O<sub>2</sub> in water (mLO<sub>2</sub> L<sup>-1</sup> mm Hg<sup>-1</sup>) and Wt the body mass (kg). The flow through the respirometer was adjusted according to Steffensen (1989) to ascertain a steady-state condition in less than 30 min.

The critical O<sub>2</sub> tension (P<sub>c</sub>O<sub>2</sub>) was determined graphically. In short, a line parallel to the abscissa axis was set in the P<sub>in</sub>O<sub>2</sub> interval in which  $\dot{V}O_2$  values were maintained constant (*p* < 0.05), and a linear regression line was fitted through the points located in lower P<sub>in</sub>O<sub>2</sub> values. The intersection of these two lines determined a set point that, projected onto the abscissa axis, allowed the estimation of P<sub>c</sub>O<sub>2</sub>.

Gill ventilation ( $\dot{V}_G - \text{mLH}_2\text{O kg}^{-1} \text{ min}^{-1}$ ) was measured according to the method of Hughes et al. (1983). Continuous measurements of the inspired (P<sub>i</sub>O<sub>2</sub>) and expired (P<sub>e</sub>O<sub>2</sub>) water O<sub>2</sub> tensions were taken by means of the implanted buccal and opercular PE catheters, respectively. The  $\dot{V}_G$  was calculated as:

$$\dot{V}_G = [(P_{in}O_2 - P_{out}O_2 / P_iO_2 - P_eO_2) \cdot \dot{V}_R] / Wt$$

Respiratory frequency (*f<sub>R</sub>* – breaths min<sup>-1</sup>) was measured from the buccal pressure variation. The buccal PE catheter was connected to a pressure transducer coupled to an amplifier of a PowerLab 8/30 data-acquisition system (ADInstruments, Australia).

The ventilatory tidal volume (*V<sub>T</sub>* – mLH<sub>2</sub>O kg<sup>-1</sup> breath<sup>-1</sup>) was calculated by dividing the gill ventilation by the respiratory frequency ( $\dot{V}_G / f_R$ ). The O<sub>2</sub> extraction from the ventilatory current by the gills (EO<sub>2</sub>%) was calculated as:

$$EO_2 = [(P_iO_2 - P_eO_2) / P_iO_2] \cdot 100$$

To obtain the heart rate (*f<sub>H</sub>* – bpm), ECG electrodes were connected to a bio-amplifier of the same data-acquisition system as described above.

After recover from surgery, the normoxic (P<sub>in</sub>O<sub>2</sub> = 140 mm Hg) control values were measured and, subsequently, the P<sub>in</sub>O<sub>2</sub> was decrementally reduced to 120, 100, 80, 60, 40, 20 and 10 mm Hg. Each O<sub>2</sub> tension was maintained for 30 min with an additional 15 min period for measurements. Normoxia was maintained by bubbling the water with compressed atmospheric air. Hypoxic levels were achieved and kept constant by dispersing pure N<sub>2</sub> in appropriate volumes into the water of the experimental tank (Thomaz et al., 2009). All of the above variables were simultaneously measured for the same individual, and each fish was always subjected to the complete experimental sequence.

### 2.5. Statistical analysis

Results are presented as means ± SD. Analysis of variance (ANOVA), complemented by Tukey–Kramer multiple comparisons

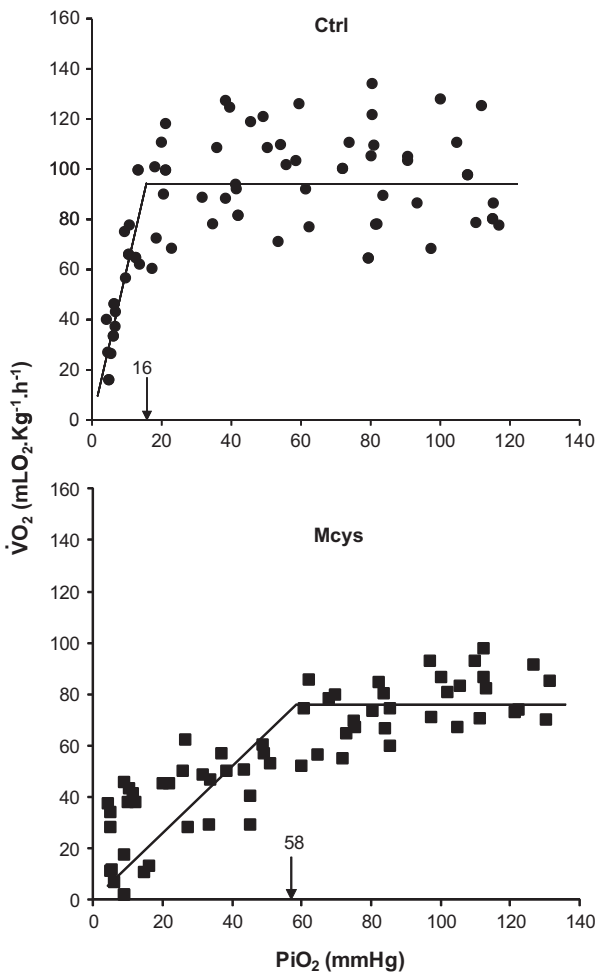
was used to check the possible existence of significant variations between the values obtained at different O<sub>2</sub> tensions for the same experimental group. The Mann–Whitney test was performed to verify the occurrence of possible differences between corresponding points in both groups. Statistical differences were considered at 5% significance (Graphpad Instat, version 3.06).

**3. Results**

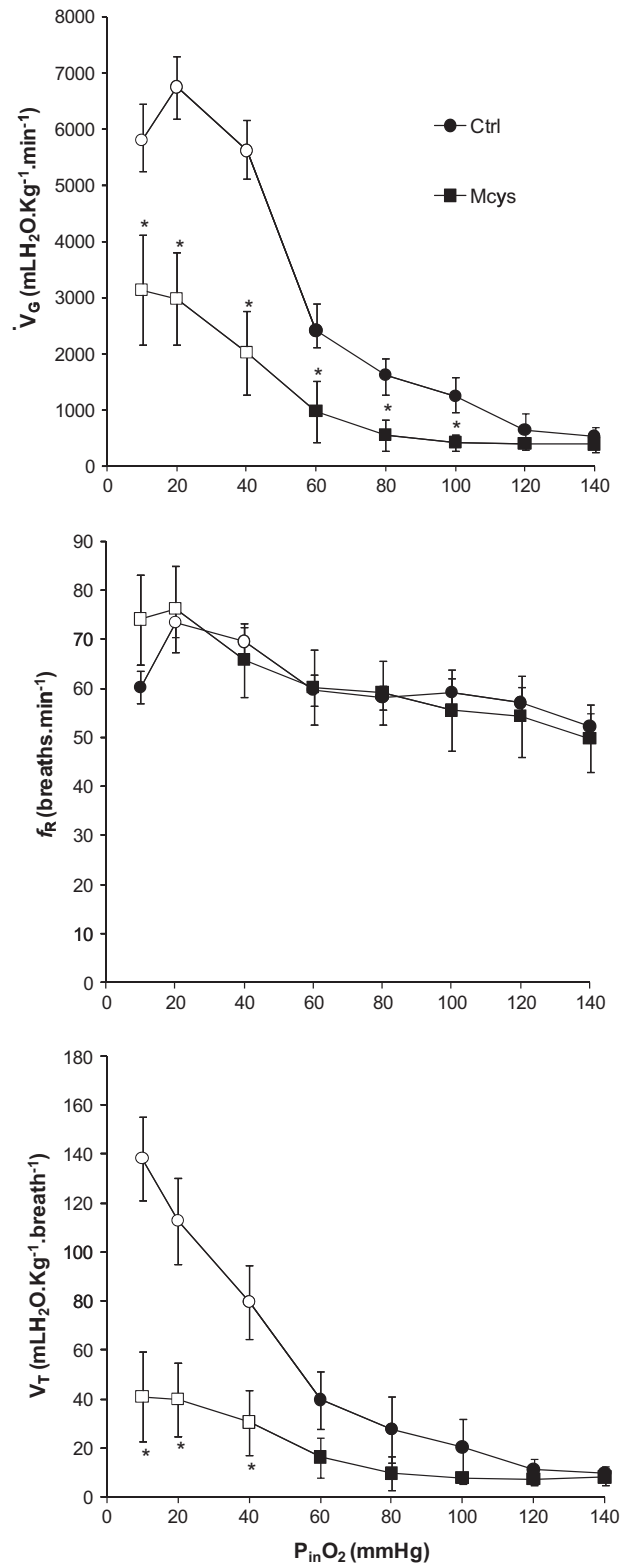
Fig. 1 shows the effects of graded hypoxia on metabolic rate ( $\dot{V}O_2$ ) and critical O<sub>2</sub> tension ( $P_{cO_2}$ ) in both experimental groups. The  $\dot{V}O_2$  of Ctrl group remained nearly constant until a  $PO_2$  of 20 mm Hg. Only during the lowest  $PO_2$  at which the fish was exposed ( $P_{inO_2}$  of 10 mm Hg) a significant reduction in  $\dot{V}O_2$  values was observed. The  $P_{cO_2}$  for this group was about 16 mm Hg. The  $\dot{V}O_2$  of the Mcys group decreased gradually and significantly below 60 mm Hg, and the  $P_{cO_2}$  was about 58 mm Hg. The  $\dot{V}O_2$  values of the Mcys group were significantly lower than that recorded for the Ctrl group in the  $P_{inO_2}$  interval between 100 and 10 mm Hg.

The mean values of  $\dot{V}_G$  ( $mLH_2O\ kg^{-1}\ min^{-1}$ ),  $f_R$  (breaths  $min^{-1}$ ) and  $V_T$  ( $mLH_2O\ kg^{-1}\ breath^{-1}$ ) for both experimental groups during normoxia and graded hypoxia are presented in Fig. 2. The  $\dot{V}_G$  of the Ctrl group increased progressive and significantly above a  $P_{inO_2}$  of 60 mm Hg. The same tendency was recorded for the Mcys group, but with  $\dot{V}_G$  increases of lower magnitude. Significant

increases in the  $f_R$  values of the Ctrl group occurred only between 40 and 20 mm Hg, returning to initial values at 10 mm Hg while the  $f_R$  of the Mcys group increased significantly at 20 and



**Fig. 1.** Metabolic rate ( $\dot{V}O_2$ ) in relation to gradual reductions of  $P_{iO_2}$  (mm Hg) of control (Ctrl group,  $n = 8$ ) and MC-LR-exposed (Mcys group,  $n = 8$ ) Nile tilapia. The arrow indicates the critical oxygen tension ( $P_{cO_2}$ ) in both groups.



**Fig. 2.** The relationships between the ventilatory parameters ( $\dot{V}_G$ ,  $f_R$ , and  $V_T$ ) and the  $P_{inO_2}$  of control (Ctrl group,  $n = 8$ ) and MC-LR-exposed (Mcys group,  $n = 8$ ) Nile tilapia. Values are mean  $\pm$  SD. Open symbols indicate a significant difference in relation to the normoxic values, while asterisks indicate significant difference in relation to the control group ( $p < 0.05$ ).

10 mm Hg. The  $f_R$  values of both groups did not show any significant difference. The reductions in the water  $PO_2$  caused significant increases in the  $V_T$  of both Ctrl and Mcys groups only below 60 mm Hg. However, the  $V_T$  values of the Ctrl group were significantly higher than those of the Mcys group in the  $O_2$  tensions between 40 and 10 mm Hg.

Fig. 3 presents the mean values for  $O_2$  extraction from the ventilatory current ( $EO_2$ ) of the Ctrl and the Mcys groups in response to hypoxia. Both groups showed significant decreases of  $EO_2$  in  $O_2$  tensions below 80 mm Hg. The  $EO_2$  values of the Mcys group were significantly lower than the Ctrl ones only at 20 and 10 mm Hg.

The mean values of heart rate ( $f_H$  – bpm) of both experimental groups are presented in Fig. 4. The Ctrl group maintained a constant  $f_H$  from normoxia to the  $P_{in}O_2$  of 20 mm Hg and a significant bradycardia was recorded only at 10 mm Hg. For the Mcys group,  $f_H$  was kept constant from normoxia down to a  $P_{in}O_2$  of 40 mm Hg below which  $f_H$  decreased progressively and significantly. The  $f_H$  values of the Mcys group were significantly lower than those obtained for the Ctrl group in all the experimental  $O_2$  tensions.

#### 4. Discussion

When exposed to hypoxia, water-breathing fish usually trigger responses to maintain  $\dot{V}O_2$  either by conserving energy expenditure or reducing energy turnover, and finally by anaerobic energy metabolism. Within a certain range of ambient  $PO_2$ , fish are thus oxyregulators, but with further reductions in the  $O_2$  levels,  $\dot{V}O_2$  can no longer be maintained and they become oxyconformers. This species-specific value below which the oxygen requirements can no longer be met is often referred to as the critical oxygen tension,  $P_{c}O_2$ . It represents a key parameter which, together with the fish  $\dot{V}O_2$ , can be used to evaluate the ability of the fish to cope with oxygen limitation. This study reinforces previous findings of Fernandes and Rantin (1989) and Kalinin et al. (1999), showing that *O. niloticus* is an efficient oxyregulator, since  $\dot{V}O_2$  was maintained until the  $P_{c}O_2$  of 16 mm Hg in the control group.

Exposure to MC-LR significantly increased  $P_{c}O_2$  of *O. niloticus* to about 58 mm Hg and the species become dependent of the environmental  $PO_2$  at higher tensions, reducing their capacity to survive hypoxic conditions.

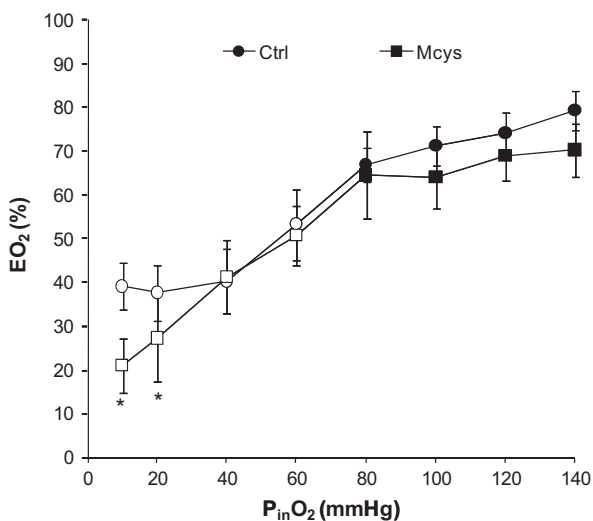


Fig. 3. The  $O_2$  extraction from the ventilatory current by the gills ( $EO_2$ ) in relation to gradual reductions of  $P_{in}O_2$  (mm Hg) of control (Ctrl group,  $n = 8$ ) and MC-LR-exposed (Mcys group,  $n = 8$ ) Nile tilapia. Values are mean  $\pm$  SD. Open symbols indicate a significant difference in relation to the normoxic values, while asterisks indicate significant difference in relation to the control group ( $p < 0.05$ ).

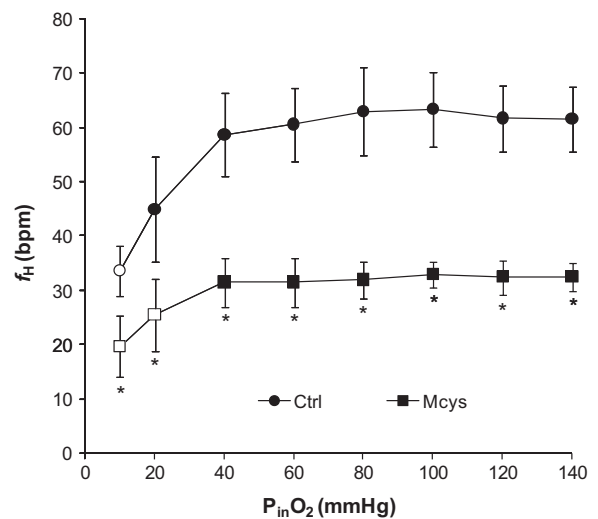


Fig. 4. The heart rate ( $f_H$ ) in relation to gradual reductions of  $P_{in}O_2$  (mm Hg) of control (Ctrl group,  $n = 8$ ) and MC-LR-exposed (Mcys group,  $n = 8$ ) Nile tilapia. Values are mean  $\pm$  SD. Open symbols indicate a significant difference in relation to the normoxic values, while asterisks indicate significant difference in relation to the control group ( $p < 0.05$ ).

The occurrence of cyanobacterial blooms is usually related to increased input of nutrients in the water (eutrophication) (Havens, 2007). In this context, one could expect that the combined eutrophication and cyanobacterial blooms would decrease water  $PO_2$ , and thus, fish would be exposed to combined effects of microcystins and environmental hypoxia. Therefore, the fish ability to keep a constant metabolic rate and/or  $P_{c}O_2$  would be impaired, decreasing their chances of surviving in this environmental condition.

Prieto et al. (2006) reported significant increases in the activity of the antioxidant system of *O. niloticus* after exposure to MC-LR at a dose of  $500 \mu\text{g kg}^{-1}$  body weight injected intraperitoneally. The authors observed lipid peroxidation in gills, liver and kidney. According to Davis (1975) and Steffensen (2006), when exposed to  $O_2$  tensions below its  $P_{c}O_2$ , oxyregulators suffer reduction in their metabolic rate according to the decrease in environmental  $PO_2$ . According to these authors, this is probably due to a gradual reduction in the efficiency of homeostatic mechanisms involved in  $O_2$  extraction by the gills and in the gas diffusion to tissues. The higher  $P_{c}O_2$  of the Mcys group indicates that the respiratory homeostasis was modified by this cyanotoxin, either in the  $O_2$  extraction or diffusion to the tissues, even at lower MC doses.

Changes in the respiratory homeostasis may be related to changes in blood oxygen capacity, probably due to a possible decrease in the hematocrit and hemoglobin concentration. Studies have demonstrated that microcystin can damage hematopoietic tissues of teleost fish such as spleen, liver and especially kidney (Quentel and Obach, 1992; Matushima, 1995), interfering with blood cell formation (Katharios et al., 2002; Zhang et al., 2007) and, consequently with  $O_2$  transport. As the most important hemogenic organs in fish (Ozaki, 1982), it is possible that intraperitoneal injection of MC-LR may have resulted in hypofunction of this organ which, allied to the damage occurring in the liver, caused anemia, which influenced the increase in  $P_{c}O_2$  recorded in the Mcys group. A similar scenario was described by Zhang et al. (2007) for the crucian carp, *Carassius carassius*, submitted to intraperitoneal injections of microcystin of 50 and  $200 \mu\text{g kg}^{-1}$  body weight.

Increases in gill ventilation ( $\dot{V}_G$ ) as the primary response to reduced environmental  $O_2$  tension has been observed in several fish species such as pacu, *Piaractus mesopotamicus* (Kalinin et al., 2000),

trahira, *Hoplias malabaricus*, giant trahira, *Hoplias lacerdae* (Rantin et al., 1992), and common carp, *Cyprinus carpio* (Saunders, 1962; Lomholt and Johansen, 1979), among others. In this study, both experimental groups presented significant increases in  $\dot{V}_G$  during exposure to graded hypoxia. This hyperventilation was characterized by a larger increase in  $V_T$  than in  $f_R$ . The predominance of this strategy has been documented for a number of teleost species (Smith and Jones, 1982; Fernandes and Rantin, 1989). According to Rantin et al. (1992), this mode of response may seem surprising, because fish possess a large potential for increase of respiratory frequency due to an often intermittent breathing pattern (Lomholt and Johansen, 1979). The motivation for this strategy could be a lower energy cost of the response, assuming that conservation of a constant velocity of muscular contraction is energy saving, whereas a higher frequency of contraction is limited by work against high internal viscosity of the muscle and a high viscosity of water (Rantin et al., 1992).

According to the “fundamental equation of respiratory physiology” proposed by Dejours (1981),  $(\dot{V}_G/\dot{V}O_2) \cdot EO_2 \cdot P_{in}O_2 = 1$ , the maintenance of a constant metabolic rate when the environment  $O_2$  concentration declines is possible by means of an increase in  $\dot{V}_G$  and/or extraction in  $EO_2$ . Due to the limited capacity of  $EO_2$  increases in fish, enhancements in  $\dot{V}_G$  are required to maintain a constant  $\dot{V}O_2$  (Kalinin et al., 1996). This statement implies that fish survival in hypoxic environments can be compromised if the ability to increase  $\dot{V}_G$  is affected. Therefore, although exhibiting a similar respiratory strategy during hypoxia, the Mcys group had significantly lower  $\dot{V}_G$  values when compared to the Ctrl, showing that exposures to MC-LR somehow impaired the normal respiratory responses in this group.

*O. niloticus* presents high values of  $O_2$  extraction ( $EO_2$ ), in the range of 75–80% (Fernandes and Rantin, 1989; Kalinin et al., 1996). The increased  $\dot{V}_G$  while  $EO_2$  remains relatively high and constant is one of the most important respiratory compensations for reductions in environmental  $O_2$  (Fernandes and Rantin, 1989). The  $EO_2$  values of the Mcys group were significantly lower than the Ctrl ones in the more hypoxic tensions (20 and 10 mm Hg). Carbis et al. (1997) observed necrosis in gill cells of carp, *C. carpio*, exposed to blooms of cyanobacteria producing microcystin. Prieto et al. (2006, 2007) showed that exposure of *O. niloticus* to microcystin at sublethal doses ( $500 \mu\text{g kg}^{-1}$  body weight) caused oxidative stress and subsequent cellular destruction (lipid peroxidation) in various organs, including gills. An impairment of gill cells caused by lipid peroxidation could explain the reduced  $EO_2$  presented by the Mcys group during exposure to severe hypoxia in the present study.

Steffensen et al. (1982) argued that hypoxia-tolerant species exhibit a great potential to increase  $\dot{V}_G$  with concomitant maintenance of  $EO_2$ . In our study, both the ability to increase  $\dot{V}_G$  and maintain  $EO_2$  were impaired in the Mcys group, which reinforces the reduced survival chances in hypoxic environments.

Heart rate ( $f_H$ ) have been used as an indicator of toxic effects of environmental contaminants in fish, and reductions in their values with concomitant increases in gill ventilation are common responses in fish exposed to a variety of xenobiotics (Heath, 1995; Teuschler et al., 2005). According to Farrell (1984), the reflex bradycardia produced by hypoxia can be valuable to ensure the performance of the heart during myocardial hypoxia. Therefore, the reduction in  $f_H$  of *O. niloticus* at  $O_2$  tensions near the  $P_{C}O_2$  probably preserved the energy expenditure of the heart when the  $O_2$  availability became reduced. Furthermore, bradycardia increases the residence time of blood within the ventricle, which could allow an increased extraction of  $O_2$  by the spongy myocardium. In the present study, the  $f_H$  values of the Mcys group were significantly lower than the Ctrl in all  $O_2$  tensions analyzed. In the Mcys group, a significant bradycardia appeared earlier, at 20 mm Hg, while in

the Ctrl group a significant decrease in  $f_H$  occurred only at 10 mm Hg. Although both groups showed a similar bradycardic response to hypoxia, fish of the Mcys group presented significantly lower  $f_H$  values than those of the Ctrl group in all  $O_2$  tensions analyzed, indicating that MC-LR exposure affected the normal heart function of *O. niloticus*. Atencio et al. (2008), submitted specimens of *O. niloticus* to intraperitoneal injections of microcystin-LR ( $500 \mu\text{g kg}^{-1}$  body weight) and, besides the injuries in organs such as liver and kidneys, they verified the occurrence of histopathology in the cardiac myocytes. Among these changes, myopathies with fibrolysis processes and generalized edema were observed in the cardiomyocytes due to a possible direct effect of MC-LR in the heart tissue. Accordingly, in the present study, a possible injury of heart tissue caused by MC-LR exposure could explain the reduced  $f_H$  and earlier bradycardia observed in the Mcys group, prejudicing the normal heart function.

## 5. Conclusions

Intraperitoneal injection of microcystin-LR (MC-LR –  $100 \mu\text{g kg}^{-1}$  body weight) reduced the ability to increase  $\dot{V}_G$  and to maintain a constant  $\dot{V}O_2$  and  $EO_2$ , impairing the maintenance of an adequate respiratory response to hypoxia. Moreover, MC-LR decreased  $f_H$  and anticipated the occurrence of hypoxic bradycardia, presumably due to damages in cardiac myocytes. Thus, exposure to MC-LR may reduce the survival chances of *O. niloticus* in hypoxic environments.

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