

**NITRITE CAUSES HEMATOLOGICAL DISTURBANCES IN
*COLOSSOMA MACROPOMUM***

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Abstract

The effects of acute exposure to nitrite (different concentrations during 24h) on physiological aspects of respiration of *Collossoma macropomum* (tambaqui) were estimated. The physiological stress was monitored following changes in tissue metabolic rates and hematological parameters. Increases in plasma glucose and lactate levels, and a decrease in intracellular pH indicate activation of anaerobic metabolism as the result of an impairment of oxygen unloading at tissue levels. However, the expected increase in methaemoglobin levels could not be correlated with a decrease in tissue metabolic rates, suggesting activation of cellular oxidative processes.

Introduction

Tambaqui is a native fish distributed across the Amazon and the Orinoco Hydrographic Basins. It is amongst the most important commercial fish species in Brazil. In aquacultural facilities high levels of nitrite can be found causing severe physiological disturbances. Nitrite causes, among other effects, the increase of methaemoglobin levels in fish, which is unable to bind oxygen, and so impairing tissue oxygenation. This work shows the effects of nitrite exposure on some physiological parameters of the species *Collossoma macropomum* (tambaqui).

Materials and Methods

Fish were obtained from Amazonfish Farm, near Itacoatiara City (AM). In the laboratory (Laboratory of Ecophysiology and Molecular Evolution – LEEM/COPE, INPA), the animals were acclimated over a 30 day-period before the beginning of the experiments. After this recovery period, fish were transferred to 4 experimental glass aquaria, with the following dimensions: 29 x 68 x 40 cm. Eight fishes were placed in each aquarium, which was filled with water and sodium nitrate at the following concentrations: 0 (control), 0.9, 2.7, and 3.6 mmol.l⁻¹ during 24h.

Blood was collected from the caudal vein into heparinized syringes and kept in ice. Hematological parameters (haematocrit, haemoglobin concentration, red blood cell counts, and corpuscular constants) were estimated by classical methods. Plasma sodium and potassium levels were estimated by flame photometry in a FC Celm 180 flame photometer. Plasma pH was measured using a glass micro-capillary electrode Radiometer G229A-Copenhagen, coupled to the pH meter PHM-73. Radiometer precision buffers were used to calibrate the electrode. Red cell pH was determined using freeze and thaw method. Methaemoglobin levels were estimated as described by Benesch *et al* (1973). Plasma glucose and lactate levels were estimated by enzymatic methods using commercial kits (Sigma Chem. Co. for lactate levels, and Doles® for glucose levels). Results are expressed as means ± SEM and the significance of the difference between treatments were estimated by ANOVA with a fiducially limiting of 5%.

Results and Discussion

Haematocrit values, haemoglobin concentration ([Hb]), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) of *C. macropomum* exposed to different nitrite concentrations under 24h are shown in Table 1. Haematocrit values (%) revealed a significant decrease in all tested nitrite concentrations. RBC counts were not different in any of the tested nitrite concentrations. On the other hand, MCV values decreased in the specimens exposed to nitrite at the concentration of 3.6 mmol.l⁻¹. Plasma Na⁺ levels, and extracellular pH, showed no change for all treatments. Plasma K⁺ levels were

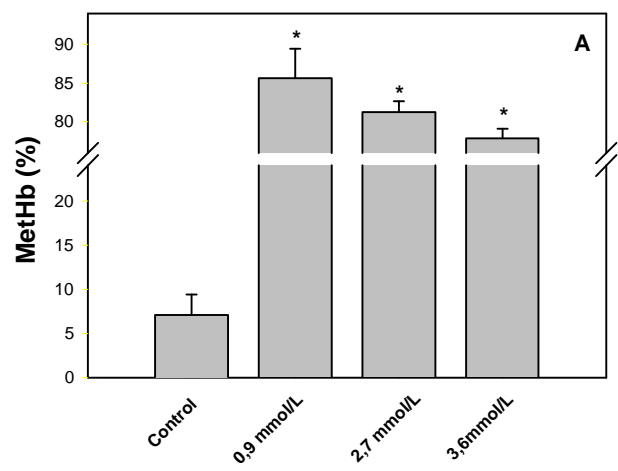
increased ($P<0.05$) in fish exposed to all treatments, which is uncommon and could only be explained as the result of cell damage. However, the analysis of *Astronotus ocellatus* (Paula-Silva *et al.*, 1996) under similar conditions showed no changes in plasma K^+ levels. Intracellular pH decreased in fish exposed to sodium nitrite at the concentration 3.6 mmol.L^{-1} , suggesting proton accumulation inside the cell.

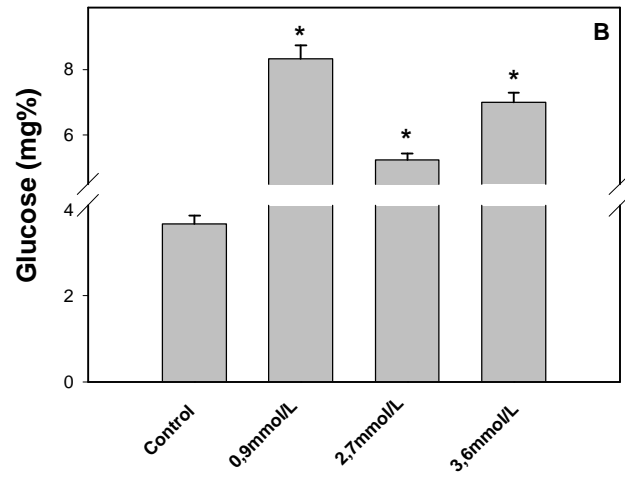
Table 1: Hematological parameters for *C. macropomum* exposed to different concentrations. Data are shown as mean \pm SEM.

Nitrite levels (mmol.L^{-1})	Control	0.9	2.7	3.6
Ht %	22.62 \pm 0.76	21.56 \pm 0.49*	20.75 \pm 0.37*	20.00 \pm 0.27*
[Hb](g%)	5.37 \pm 0.18	5.97 \pm 0.22*	6.23 \pm 0.20*	5.19 \pm 0.09
RBCx10 ⁶ mm ⁻³	1.35 \pm 0.07	1.43 \pm 0.12	1.39 \pm 0.06	1.45 \pm 0.06
MCV(μm^3)	166.68 \pm 4.82	155.01 \pm 10.82	147.23 \pm 5.32	137.77 \pm 5.61*
MCH(pg)	24.07 \pm 0.42	27.16 \pm 1.53*	29.84 \pm 1.38*	26.12 \pm 0.53*
MCHC(%)	69.56 \pm 2.92	60.87 \pm 9.89*	50.43 \pm 3.45*	52.94 \pm 2.45*
Na ⁺ mEq.L ⁻¹	139.25 \pm 2.51	144.87 \pm 1.81	142.25 \pm 2.10	143.38 \pm 1.89
K ⁺ mEq.L ⁻¹	3.57 \pm 0.19	4.44 \pm 0.25*	4.20 \pm 0.27*	3.95 \pm 0.26*
pHe	7.55 \pm 0.032	7.61 \pm 0.058	7.62 \pm 0.047	7.74 \pm 0.036
pHi	6.88 \pm 0.03	6.81 \pm 0.04	6.82 \pm 0.04	6.76 \pm 0.038*

* Indicates significant difference ($P<0.05$) from control.

MetHb raised about 75% in all tested concentrations (fig.1A), indicating a depression in capacity of oxygen transport in fish blood. Plasma glucose levels (fig.1B) and lactate (fig.1C) increased in all tested concentrations.





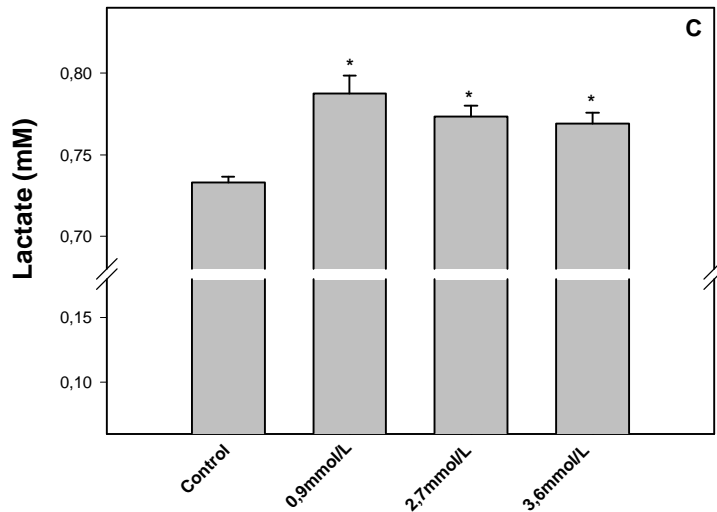


Figure 1: Plasma metHb (A), glucose (B) and lactate (C) changes of *C. macropomum* exposed to nitrite. * Indicates significant difference ($P<0.05$) from control.

Several authors reported the physiological effects of the nitrite on aquatic organisms (Williams & Eddy, 1986; Jensen, 1996; Knudsen & Jensen, 1997; Paula-Silva *et al.*, 1996; Duncan *et al.*, 1998; Costa *et al.*, 1999). Nitrite oxidizes haemoglobin to methaemoglobin (MetHb), which is unable to bind oxygen. Increased levels of MetHb cause an impairment of oxygen transfer that results in severe hypoxia and damage to several tissues. Ultimately, increased MetHb result in fish death (Arillo *et al.*, 1984). According to Hanson & Grizzle (1985), fish exposed to intermediate levels of nitrite become more vulnerable to bacterial diseases. They also suggest that nitrite may suppress fish immune system.

When exposed to nitrite, carp presents a KCl efflux from red blood cells, which is followed by an osmotic loss of water, reducing the volume of the red cells (Jensen, 1990, 1992). This fact was also observed in the present work, since animals exposed to sodium nitrite at the concentration of 3.6 mmol.l^{-1} presented a decrease in their cellular volume (MCV). Nitrite effect in the fish tissues and their immune system responses are very similar to the effects of bioaccumulation of a pollutant, not only in the plasma, but also in the gills, liver, brain, spleen, muscle, etc. (Thurston *et al.*, 1978; Margiocco *et al.*, 1983; Scarano *et al.*, 1984). Hyperventilation and shivering signals were observed in the tambaqui exposed to nitrite. Such responses could not be linked to hypoxic conditions of the environment since the aquaria were aerated during the whole experimental stage; this may be the reason why lip extension, which is common in tambaqui exposed to hypoxic situations, was not observed in the present work.

Acknowledgements

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