EFFECTS OF NITRITE ON HEMATOLOGICAL ASPECTS OF *ASTRONOTUS OCELLATUS* OF THE AMAZON

Maria de Nazaré Paula da Silva
INPA-Aquaculture, Alameda Cosme Ferreira, 1756
69.083-000 Manaus, AM, Brazil
Phone: +55 92 643-3191 Fax: +55 92 643-3186
e-mail: npaula@cr-am.rnp.br

Oscar Tadeu Ferreira da Costa
Dept. of Morphology, Institute of Biological Sciences
University of Amazonas

Vera Maria Fonseca de Almeida e Val
INPA-Aquaculture

Adalberto Luis Val
INPA-Aquaculture

INTRODUCTION

The Oscar (*Astronotus ocellatus*), known locally as apaiari and acará-açu, is an important commercial fish in the Amazon both as food and as ornamental fish. This fish grows up to 1.5Kg during the first year in captivity. Since 1946 when Sawaya first reported his observations on the physiological characteristics of *Astronotus*, it has been extensively studied, in special its tolerance to hypoxia.

Aquaculture has experienced a boom in the Amazon. Currently many fish farms raise fish in the Amazon. As consequence new questions have been asked. According to Val & Honczaryk (1995) water quality, fish nutrition, and reproduction in captivity are considered the main constraints currently faced by the aquaculture in the Amazon.

Nitrite (NO_2^-) is an intermediary compound that occurs naturally in both fresh and salt water bodies at concentrations lower than 0.005mg/L. Agricultural pesticides, industrial waste, and even the waste of aquaculture facilities can flow into natural water bodies and increase nitrite concentration in nature. Ammonia can reach very high levels in ponds of fish culture stations because fish is in general stocked in high density. As a consequence, nitrite that is toxic to aquatic organisms (Lewis, 1986) also reach unusual levels. Nitrite is taken up through the gills and skin, diffuses into the red blood cells and oxidizes the hemoglobin. Methemoglobin (oxidized hemoglobin) is unable to bind reversibly to oxygen (Jensen, 1990) causing an impairment of oxygen transfer.

One of the most effective defense strategies against hypoxia is metabolic depression (Hochachka and Guppy, 1987). *Astronotus ocellatus* exposed to hypoxia decreases its metabolic activity to low levels (Almeida-Val, personal observations). By decreasing the metabolism the animal increases the ability to survive natural constraints, including hypoxia. The present paper analyses the effects of nitrite on respiratory characteristics of *Astronotus ocellatus*, in particular on blood parameters and plasma lactate levels.

MATERIAL AND METHODS

Adult *Astronotus ocellatus* were obtained from a fish farm in Itacoatiara, Amazonas state. Fish were held in outdoor tanks (500L) supplied with ground water and fed daily with commercial food pellets (produced at INPA's Aquaculture Department). Feeding was suspended 48 hours prior the experiments. After acclimation period, one group of 8 fishes was exposed to nitrite (1.8 mM) during 24 hours. The control group was maintained free of nitrite. Nitrite in water was estimated as described by Jansen *et al.* (1987). After 24 hours, the animals were stunned and blood samples were collected from caudal vein using heparinized syringes.

Hematological parameters (hematocrit, hemoglobin concentration, red blood cell counts, and corpuscular constants) were estimated by classical methods. Plasma sodium and potassium levels were estimated by flame photometry (Model FC Celm 180). Plasma pH was measured using a glass micro-capillary electrode (G229A, Radiometer, Copenhagen) coupled to PHM-73 pH meter. Radiometer precision buffers were used to calibrate the electrode. Red cell pH was determined using the freeze and thaw method. Methemoglobin levels were estimated as described by Benesch *et al.* (1973). Plasma glucose and lactate levels were estimated using commercial kits (Sigma for lactate and Doles for glucose).

Gill tissue was extracted immediately after bleeding, rinsed with Ringer solution and minced in a Petri dish. Oxygen consumption was estimated in a 2 ml Gilson thermocell with a Clark-type electrode. Temperature was maintained at 30°C using a Shimadzu thermobath. Oxygen consumption is given as µmoles $O_2.min^{-1}.gwt^{-1}$. Results are expressed as mean ± SEM and the significance of the differences between treatments were estimated by ANOVA with a fiducial limit of significance of 5%.

RESULTS AND DISCUSSION

The effects of nitrite on fish have been analysed by several authors (Colt *et al.*, 1981; Bath and Eddy, 1980; Jensen, 1990). In the bloodstream, nitrite diffuses into the red blood cell and oxidizes hemoglobin to methemoglobin impairing oxygen transport. Methemoglobin formation after nitrite exposure has been described in several fish species including *Cyprinus carpio* (Jensen *et al.*, 1987); *Semaprochilodus* and *Brycon* (Bartlett *et al.*, 1987), and *Oncorhynchus tshawytscha* (Brauner *et al.*, 1993). Nitrite exposure also results in an increase in methemoglobin levels in *Astronotus ocellatus* (Figure 1A). As a consequence of this oxygen

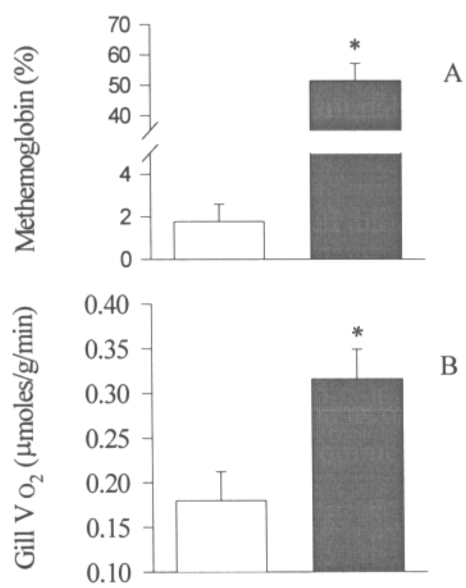

Figure 1. Methemoglobin levels (A) and gill oxygen consumption (B) of *Astronotus ocellatus* exposed to nitrite (1.8 mM) during 24 hours. * indicates significant differences ($P<0.05$) from control animals.

shortage, gill tissue itself increases oxygen consumption suggesting an increase in metabolic activity (Figure 1B). Hematological parameters did not change after nitrite exposure. Costa *et al*. (unpublished data) detected a decrease in hematocrit and red blood cell counts in *Symphysodon aequifasciata*, another Amazon cichlid, exposed to nitrite and explained the results as a consequence of hemolysis.

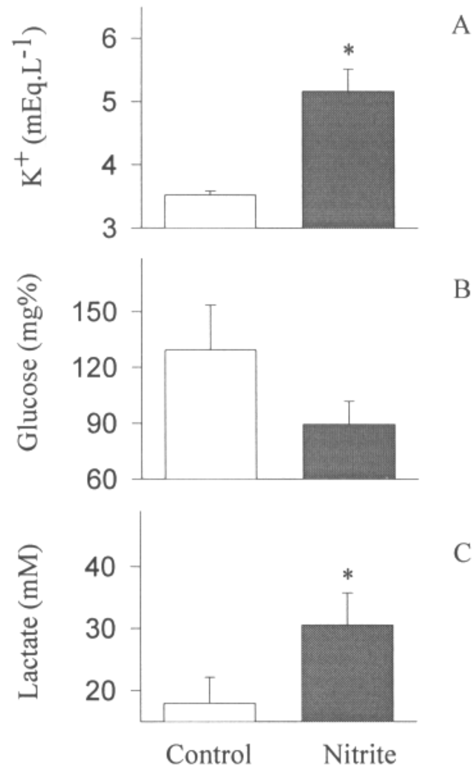

Figure 2. Plasma levels of potassium (A), glucose (B), and lactate (C) in *Astronotus ocellatus* exposed to nitrite (1.8mM). * indicates significant difference from control.

In *Cyprinus carpio* methemoglobin formation resulted in high plasma levels of potassium and lactate (Jensen *et al*., 1987). Similar results were detected in *Astronotus ocellatus* (Figure 2), indicating a rupture of cells. Interestingly, no significant change in glucose was observed after nitrite exposure while lactate levels increased suggesting activation of anaerobic glycolysis. Glycogen mobilization in liver is induced by hormones as a consequence of environmental stress. However, the increase in plasma lactate is probably due to white muscle anaerobic glycolysis which uses its own glycogen (Hochachka, 1994), explaining the non significant change in plasma glucose levels. *Astronotus ocellatus* is considered a hypoxia tolerant fish species and may accumulate high levels of glycogen in skeletal muscle.

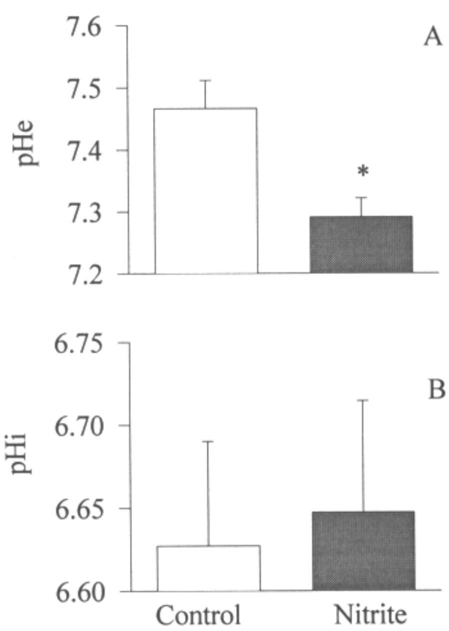

Figure 3. Blood pH and pHi of *Astronotus ocellatus* exposed to nitrite (1.8mM). * indicates significant difference from control.

There is no change in intracellular pH (Figure 3) what may be related to the effects of catecholamines. The extracellular acidosis apparently results from an impairment of H^+ excretion and/or from a nitrous acid uptake.

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