

**THE EFFECTS OF HYPOXIA AND HYDROGEN SULFIDE ON THE
METABOLISM AND HEMATOLOGICAL PARAMETERS OF
TAMBAQUI (*COLOSSOMA MACROPOMUM*)**

Elizabeth Gusmão Affonso¹

1- National Institute for Amazonian Research, P.O Box 478, Manaus, AM,
69011-090, Brazil.
pgusmao@inpa.gov.br

Vera Lucia Perussi Polez²; Cristina Ferreira Corrêa²; Aurélia de Fátima Mazon¹;
Marcela Araújo³; Francisco Tadeu Rantin³

2- Department of Genetic and Evolution - Federal University of São Carlos, São
Paulo, Brazil.

3- Department of Physiological Sciences, Federal University of São Carlos.
Via Washington Luís, Km 235 – 13565-905 – São Carlos, SP – Brazil
Phone: ++55 16 260-8314/Fax: ++55 16 260-8327/e-mail:
frantin@power.ufscar.br

EXTENDED ABSTRACT ONLY - DO NOT CITE

In this study specimens of *Colossoma macropomum* were exposed to 12, 24, 48 and 96 h of sulfide and hypoxia after which samples of blood, heart, liver, and white muscle were taken for hematological, blood pH, and metabolites analysis. The acute exposures to hypoxia and sulfide were associated with elevated blood oxygen carrying capacity. The metahemoglobin concentration presented similar variation for both factors. The anaerobic metabolism of this species is an important adaptative mechanism to sulfide exposures in the natural environment. However, this mechanism is not specific to the toxic effects of this compound, since hypoxia in the tissues can be also induced by O₂ depletions in the water.

Experimental procedures

Three groups of 24 fish (control, sulfide and hypoxia) were used to in experiments. To test the effect of sulfide, fish were exposed from 10 to 13 µM. The sulfide

concentration was determined by the methylene blue method while the water was maintained in normoxic levels. Exposure to hypoxia was carried out in a flow-through system where the oxygen levels were maintained by bubbling N₂ (5mmHg). The control animals were maintained at normoxia (water PO₂ = 140 mmHg) and at the acclimation temperature.

Sampling and analysis

At 12, 24, 48 and 96 h of exposure, six fish of each treatment and control were removed. Blood samples were withdrawn for measurements of blood pH (pHe), hematological analysis (hematocrit, red blood cell count, hemoglobin concentration and metahemoglobin), glucose and lactate. After this procedure, fish were sacrificed and white muscle, liver and heart were used for analysis of glucose and lactate. The samples were deproteinized by 8% (w/v) PCA and the lactate and glucose were enzymatically determined. Glycogen was determined using 200 mg of white muscle or liver digested with KOH 6N following precipitation with ethanol and the glucosil units were analyzed by the enzymatic glucose oxydase.

Mean values of sulfide and hypoxic water groups in different times and within the groups were statistically compared to their controls using the Mann-Whitney U test. Differences were considered significant at $p < 0.05$.

Results and Discussion

Sulfide and hypoxia induced an immediate increase of blood oxygen carrying capacity in tambaqui during the 48 h of fish exposure. After 96 h of exposure to both sulfide and hypoxia, the hematological parameters returned to values similar to those of the control, but a hemolytic anemia was observed in the fish exposed to sulfide. Probably, this anemia is not a specific effect of H₂S, but a general effect due to the confinement time of 96 h. Although the increase of metahemoglobin in the present study suggests a blood sulfide oxidation, it cannot be considered a sulfide mechanism detoxification, since the results are similar to the fish exposed to hypoxia. The anaerobic metabolism is a possible physiological strategy for this species exposed to both hypoxia and high sulfide concentrations. The results obtained for liver and white muscle glycogen demonstrated that carbohydrate is an important energy source in this species during sulfide exposure. However, during exposure to hypoxia the liver and white muscle accumulated glycogen, and its

importance to the organism should be investigated. Fish submitted to high sulfide concentrations presented an increase in blood, muscle and liver glucose levels until 24 h of exposure, probably as a consequence of anaerobic metabolism. Hypoxic fish only presented higher glucose levels in the blood. Hyperglycemia could indicate that the glycolysis is an important pathway for fish under such a condition. The blood pH values obtained for tambaqui after 24 h of exposure to hypoxia and sulfide indicate that the blood pH increases again near to the control values, probably to prevent the metabolic acidity. Although some differences were observed between the effects of hypoxia and sulfide, in general, the results presented similarity. It can be suggested that tambaqui is high sulfide tolerant as a consequence of its high tolerance to hypoxia.

Acknowledgements

This study was supported by CAPES, INPA and UFSCar.

