

**BLOOD CELL RESPONSES**  
**OF THE TROPICAL FISH *PROCHILODUS SCROFA***  
**TO ACUTE COPPER EXPOSURE AND SUBSEQUENT RECOVERY**

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**Abstract**

Changes in *Prochilodus scrofa* blood cells were investigated after 96-h of exposure to copper and following transference to clean water. Hematocrit, red blood cells and hemoglobin concentrations showed a significant increase after copper exposure, remaining high until the 7<sup>th</sup> day after transference to clean water. The immature blood cells (erythroblasts) also increased significantly, but did not differ from the controls on the 7<sup>th</sup> day in clean water. The changes in leukocytes occurred only in the percentage of lymphocytes, which was significantly reduced after 96-h copper exposure, remaining lower on the first and second day in copper-free water. Thrombocytes increased significantly in fish exposed to copper and remained high on the 7<sup>th</sup> day in clean water. The changes in the blood cells of *P. scrofa* reflect the animals' responses to stress caused by copper; however, after their transfer to clean water, most of the changes involved a compensatory physiological mechanism that allowed the fish to recover from copper-related damage.

**Introduction**

Blood cell responses are important indicators of changes in the internal and/or external environment of animals. In fish, exposure to chemical pollutants can induce either increases or decreases in hematological levels. The growing use of

copper in the metallurgic industry has resulted in an increase of copper ions in the natural waters of southeastern Brazil. Previous studies of the neotropical fish *Prochilodus scrofa* exposed to copper revealed drastic changes in red and white blood cells as well as in the thrombocytes (Cerqueira, 2000; Mazon et al., 2003).

Few studies, however, have focused on the process of recovery of these cells after copper was removed from water. Hence, the main purpose of this study was to evaluate the effect of acute copper exposure on *P. scrofa* blood cells and to discover how long the fish took to recover after returning to an environment of improved water quality.

### **Materials and Methods**

Three to five-month-old juvenile *Prochilodus scrofa* (W = 15-75 g) were provided by the Hydrobiology and Aquaculture Station of Furnas Hydroelectric Power Plant, Furnas, MG, Brazil, and kept in tanks at  $25 \pm 1^\circ\text{C}$  (1000 L) with a continuous flow of dechlorinated tap water (water composition: pH =  $7.3 \pm 0.2$ ; alkalinity =  $23.7 \pm 1.9 \text{ mg L}^{-1}$  as  $\text{CaCO}_3$ ; conductivity =  $8.3 \pm 0.3 \mu\text{S}$  and hardness =  $24.5 \pm 0.2 \text{ mg L}^{-1}$  as  $\text{CaCO}_3$ ) and aeration (100%  $\text{O}_2$  saturation) for at least one month prior to the experiments. The laboratory photoperiod was 12D:12L. The fish were fed with balanced fish food suitable for this species provided by the Aquaculture Research and Training Center - CEPTA/IBAMA. Water temperature, pH, hardness and alkalinity were the same as the mean values found in *P. scrofa*'s natural habitat (CETESB 1992-2000).

After acclimation to laboratorial conditions, the fish were randomly divided into two groups and each group transferred to static test aquariums not exceeding 1g fish  $\text{L}^{-1}$ . Group 1 (the control group) was kept in copper-free water while group 2 (the group exposed to copper) was exposed to  $29 \mu\text{gCu L}^{-1}$  (96-h  $\text{LC}_{50}$  for *P. scrofa*; Mazon and Fernandes, 1999). After 96 h, each group was transferred to an aquarium with clean flowing water (recovery period). The physicochemical characteristics of the water in the aquariums of both groups were maintained constant throughout the experimental period and were the same as those prevailing during the acclimation period (except for the copper concentration in the aquarium of group 2). The copper agent used was  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and the copper concentration in the water was measured using Atomic Absorption Spectrophotometry. No copper was detected in the water of the control and recovery aquariums.

To evaluate the changes in blood cells after exposure to copper and their reversibility following transference to copper-free water, random fish samples (n = 8) from each group, i.e., the control group and the one exposed to copper, were taken after the fish had been held for 96 h in a static system and 1, 2, 7, 15, 30 and 45 days after their transference to clean water. The fish were anaesthetized with 0.01% benzocaine and blood samples were withdrawn from the caudal vein into heparinized plastic tubes.

Hematocrit (Hct), red blood cell count (RBC) and hemoglobin concentration ([Hb]) were conducted immediately. Hct was determined by spinning the blood sample contained in heparinized capillary tubes in a microhematocrit centrifuge. The RBC count was carried out in a modified Neubauer chamber after saline dilution of the blood, while the [Hb] was determined by the cyanomethaemoglobin method. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from previous blood measurements.

Blood smears were fixed with methanol and stained with Leishman solution for counts of immature red blood cell, thrombocytes and leukocytes by 5000 cell count, according to the method described by McKnight (1966). To prevent errors arising from uneven cell distribution, the slides were divided into four segments and cells were counted in fields contained in parallel rows commencing from outside edge of the slide toward the inside. Differential leukocyte counts were made by identifying 200 leukocytes in each slide (Dick and Dixon, 1985). The leukocytes were classified according to their general shape and affinity to the dye (Takashima and Hibiya, 1995).

The data are presented as mean  $\pm$  SEM. The control group data are given all together, since no significant changes were found among them. After the uniformity of the groups' data was verified using the Bartlett test, the parametric analysis of variance (ANOVA) was applied to determine differences in the level of significance among the groups. Tukey's test with a 95% confidence limit was applied to compare the mean values whenever a level of significance occurred (GraphPad InStat Software, San Diego, CA).

## **Results**

No fish from the control group died during the experiment; however, 48% of the fish from the group exposed to copper died during its 96-h exposure. After its

transfer to aquariums with clean flowing water, no further mortality occurred in this group.

Figures 1 and 2 and Table 1 show the changes in the blood cells after 96 h of copper exposure and following transference to clean water. Hct, RBC and [Hb] were significantly higher in fish exposed to copper, particularly on the 1<sup>st</sup> and 2<sup>nd</sup> days following transference to clean water (Fig 1). On the 7<sup>th</sup> day in clean water, RBC and [Hb] were still significantly higher than in the control fish, but significantly lower than they had been immediately following 96 h of exposure to copper. VCM and CHCM increased after 96 h of exposure to copper but were similar to the values of the controls following transference to clean water. The percentage of immature erythrocytes (polychromatophilic and orthochromatophilic erythroblasts) increased after copper exposure, remaining high on the 1<sup>st</sup> and 2<sup>nd</sup> days after the transference to clean water (Table 1).

Table 1. Mean values  $\pm$  SEM of polychromatophilic erythroblasts (PCE) and orthochromatophilic erythroblasts (OCE) of *P. scrofa* after 96h exposure to copper and subsequent recovery

	n	PCE (%)	OCE (%)
Control	56	2.7 $\pm$ 0.05	1.3 $\pm$ 0.08
96h LC <sub>50</sub> copper exposure	8	4.5 $\pm$ 0.12*	3.8 $\pm$ 0.07*
Recovery (days)	1	8	3.8 $\pm$ 0.06* <sup>o</sup>
	2	8	3.5 $\pm$ 0.08* <sup>o</sup>
	7	8	2.6 $\pm$ 0.13 <sup>o</sup>
	15	8	2.5 $\pm$ 0.13 <sup>o</sup>
	30	8	2.6 $\pm$ 0.09 <sup>o</sup>
	45	8	2.7 $\pm$ 0.09 <sup>o</sup>

\* indicates significant difference from the controls (p<0.05) and <sup>o</sup> indicates significant difference from 96h LC<sub>50</sub> copper exposure (p<0.05)

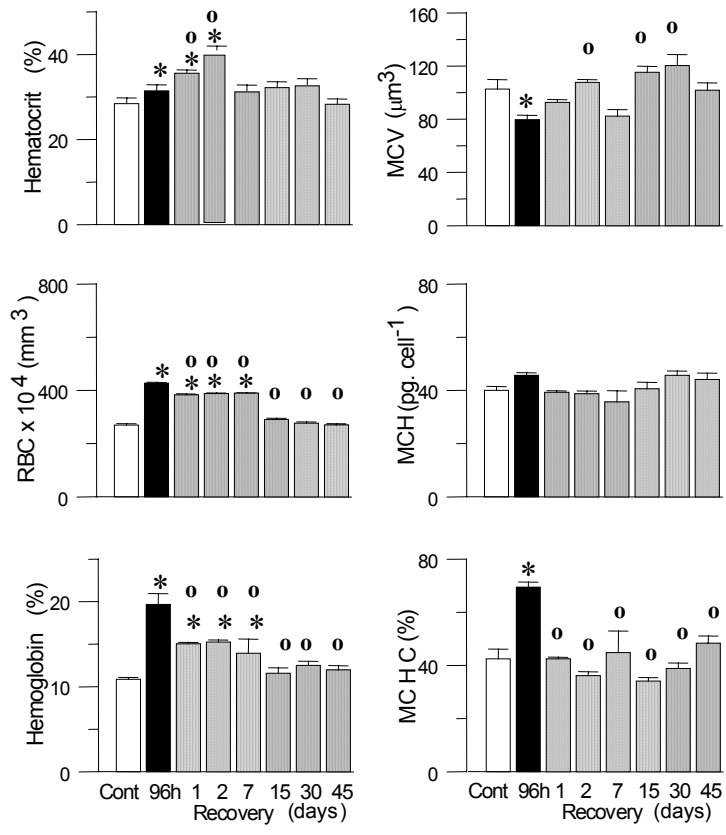


Figure 1. Changes in hematocrit (Hct), red blood cells (RBC), whole blood hemoglobin concentration ([Hb]), mean cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin content (MCHC) of *P. scrofa* after 96h of copper exposure and subsequent recovery in clean water. The bars represent the mean values (± SEM). Control fish (n = 56; open bars); 96h copper exposed fish (n = 8; black bars) and recovery (n = 8 each time; stippled bars). \* indicates significant difference from the controls (p < 0.05); ° indicates significant difference from 96h copper exposed fish (p < 0.05)

Differential leukocyte counts (Fig. 2A) showed that lymphocytes were the most frequent white blood cells in the control *P. scrofa* ( $63 \pm 1\%$ ) and the proportion of these cells was reduced to 50% in fish exposed to  $29 \mu\text{gCu L}^{-1}$ . A further significant reduction was found during the first two days of the recovery period and, on the 7<sup>th</sup> day, the percentage of lymphocytes was similar to that of the controls. The percentage of neutrophils was low compared to monocytes in the control fish. Neither of the cell types showed significant changes after exposure to copper. Basophils were not found in the prepared smears and eosinophils were very rare (less than 0,20 %).

The percentage of thrombocytes increased in fish exposed to copper and was found to be similar to the control fish on the 15<sup>th</sup> day in clean water (Fig. 2B).

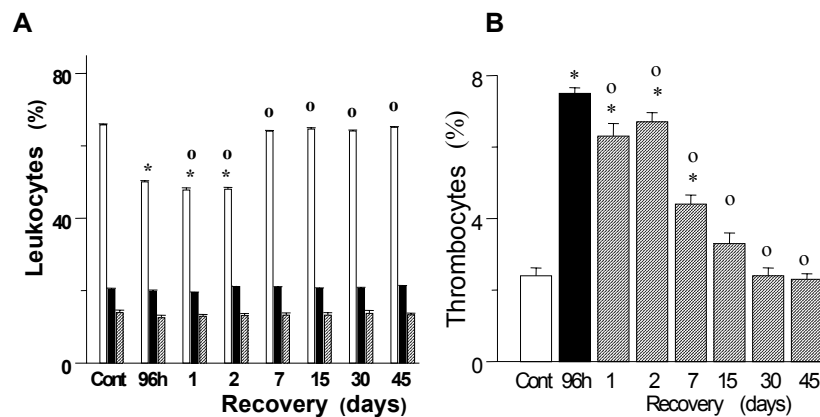


Figure 2. **A.** Lymphocyte (open bars), monocyte (black bars) and neutrophil (stripped bars) percentages of *P. scrofa* leukocytes after 96h copper exposure and subsequent recovery in clean water. **B.** Thrombocyte percentage of *P. scrofa* leukocytes after 96h copper exposure (black bar) and subsequent recovery in clean water (stripped bars); control fish (open bar). The bars represent the mean values ( $\pm$  SEM). Control fish (Cont,  $n = 56$ ); 96h copper exposed fish ( $n = 8$ ) and recovery ( $n = 8$  each time). \* indicates significant difference from the controls ( $p < 0.05$ ); <sup>o</sup> indicates significant difference from 96h copper exposed fish ( $p < 0.05$ )

## Discussion

The direct effects of copper on circulating blood cells were usually associated with an increased disintegration of erythrocytes or, in the case of more sensitive species, to damage of the hemopoietic system (Svobodová et al., 1994). However, some contradictory responses have been found (Wilson and Taylor, 1993; Heath, 1995; Nussey et al., 1995a,b) even in the same species. In *P. scrofa*, the increase of Hct, RBC and [Hb] with significant changes in the MVC and MCHC blood indices suggests a possible hemoconcentration after copper exposure for 96h. Similar increases of Hct, RBC and [Hb] were also reported by Mazon et al. (2003) in *P. scrofa* exposed to copper, but the changes were not coupled to changes in the blood indices. During the 7 days of the recovery period, the changes in the red blood cells (increase in the RBC and Hb concentration with no significant changes in the MCH and MCHC blood indices and cell size) suggest a compensatory response of this species to heighten the blood's O<sub>2</sub> carrying capacity.

The reduction of the lymphocyte percentage seems to be a general response to metal exposure (Mishra and Srivastava, 1980; Dick and Dixon, 1985; Svobodová et al., 1994) caused by increasing corticosteroid levels in the blood. Neutrophil and monocyte percentages in blood are expected to decrease during acute copper exposure (Svobodová et al., 1994), since these blood cells are vital to protect the body against bacterial infection in damaged tissue. However, no changes were found in *P. scrofa* either following acute copper exposure in which cell degeneration, rupture and peeling of lamellar epithelial are known to be intense (Mazon et al., 2002), nor during the recovery period.

Because thrombocytes are the blood cells involved with blood clotting, their increased percentage in *P. scrofa* exposed to copper may evidence a compensatory response to reduce bleeding from the damaged branchial vascular tissue. The return of thrombocyte percentages to the levels of the control fish in clean water coincided with the main changes observed in the restoration of gill tissue (Cerqueira et al., 2002).

In conclusion, the changes in the blood cells reflect the responses to the effects of stress caused by copper and, after transference to clean water, most of the changes are evidence of compensatory responses that enable fish to recover from copper-related damage.

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