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Using condition factor and blood variable biomarkers in fish to assess water quality

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Abstract The condition factor and blood variables, including erythrocyte lipid peroxidation (LPO) and the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), in two ecologically distinct fish species (*Astyanax fasciatus* and *Pimelodus maculatus*) were evaluated at five sites in the Furnas Hydroelectric Power Station reservoir (Brazil) to assess water quality. Aldrin/dieldrin, endosulfan, heptachlor epoxide, and metolachlor were detected at different concentrations in four of the sites. Condition factor was not directly affected by such contaminants. A negative correlation between hematocrit and heptachlor was detected in *P. maculatus*. Positive correlations between red blood cells and heptachlor as well as an interactive effect of metolachlor and aldrin/dieldrin were detected in *A. fasciatus*. The erythrocytes of both species collected from the contaminated sites showed high levels of LPO, an increase in SOD and GPx activities and a decrease in CAT activity. Although the leukocyte number and the differential percentage of leukocytes varied among the sites, the hematological variables, the LPO levels, and the antioxidant enzyme activities could be used to assess water quality, regardless of the differences in the responses of the fish species.

Keywords Organochlorine · Erythrocytes · Leukocytes · Thrombocytes · Antioxidant enzymes · Lipid peroxidation

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Introduction

Agriculture is an important activity in tropical and subtropical regions, but these production systems rely on the use of pesticides to control insects or fungal diseases and to maintain productivity. The impacts of pesticides on natural fish populations in aquatic ecosystems have been investigated, and fish biomarkers have been recognized as very useful tools for freshwater biomonitoring (Triebskorn et al. 2007; Weber et al. 2007). Laboratory and field studies have revealed numerous organic disorders in fish that have been exposed to xenobiotic molecules, with resulting changes in blood variables, growth, and reproduction (Cerqueira and Fernandes 2002).

The condition factor, a somatic biomarker, is indicative of health and reflects feeding conditions as well as energy consumption and metabolism

(Schulz and Martins-Junior 2001; Alberto et al. 2005). Toxic substances in the water may affect the growth of fish by directly changing metabolism and increasing the energy required to maintain homeostasis, or they can indirectly impact growth by reducing food availability.

In freshwater fish, most pesticides, metals, and other chemicals are taken up directly from the water by the gills, due to the large volume of water that fish need to ventilate their gills to obtain oxygen for aerobic metabolism. Toxins can also be acquired through the intestines during the transit of contaminated food. These chemicals are then distributed throughout the body via the blood. Blood cells are some of the first cells to come into contact with and be affected by xenobiotics. Blood cells also respond to changes in other tissues that have suffered some biochemical or physiological disorders due to xenobiotic exposure (Cerqueira and Fernandes 2002; Mazon et al. 2002; Ruas et al. 2008). Changes in hematology depend on the actions of xenobiotic molecules in biological systems, the concentrations of the contaminants in the water, the exposure time, and species sensitivity. Fish erythrocytes have been useful for investigations of biochemical processes at the cellular level, including oxidative stress and lipoperoxidation (Ruas et al. 2008).

Erythrocytes are essential for the transport of gases (O_2 and CO_2) from respiratory surfaces to tissues and vice versa, supplying metabolic needs and functioning in hydrogen buffering. Erythrocytes are the major site for the reactive oxygen species (ROS) production because of their role in the O_2 transport system. Leukocytes possess high phagocytic activity, participating actively in an organism's defense system and they are essential for immunological responses in fish (Serpunin and Likhatchyova 1998). Similarly, thrombocytes are involved in blood clotting and organism defense (Passantino et al. 2005). Blood plasma transports numerous nutrient molecules, ions, and metabolite residues for excretion.

The main goal of this study was to evaluate the condition factor, the blood hematological variables, and some oxidative stress biomarkers in the erythrocytes of two ecologically distinct fish species from the reservoir of the Furnas Hydroelectric Power Station (Furnas HPS), in Brazil, to assess water quality and to establish if these variables can be associated with different types of anthropogenic contamination. The oxidative stress biomarkers selected were lipid peroxidation (LPO) and the activity of specific antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). The physical and chemical water characteristics were also determined. The Furnas HPS is the largest power station in southeastern Brazil. This site was selected because it is not only located in a predominantly agricultural region but also receives untreated urban water discharges from numerous small and medium-size cities. The lambari, *Astyanax fasciatus* (benthic-pelagic species), and the mandi, *Pimelodus maculatus* (benthic species), are resident fish species that do not exhibit migration behavior and are widely distributed throughout the reservoir, allowing for comparisons between sites.

Materials and methods

Study area

Furnas HPS is located in Minas Gerais, Brazil (Fig. 1). The reservoir is the result of the damming of the Rio Grande (250 km long) and the Rio Sapucaí (170 km long), and it has a 1,440 km^2 overflow area with 21 million cubic meters and a perimeter of 3,500 km. The reservoir is bordered by 34 small- to medium-size cities, most of which engage in intense agricultural and cattle farming activities. Water and fish specimens were collected from five sites: site 1 (the reference site) at the confluence of the Grande and Sapucaí Rivers [Turvo (FU10) S20°40′835″ W 46°13′232″]; site 2, Guapé—FU20 (S20°44′331″ W 45°55′800″); site 3, Porto Fernandes—FU50 (S20°48′826″ W 45°40′567″), both in the Rio Grande axis; site 4, Barranco Alto—FU30 (S21°10′510″ W 45°57′061″), and site 5, Fama—FU40 (S21°24′074″ W 45°49′621″) in the Rio Sapucaí axis (Fig. 1).

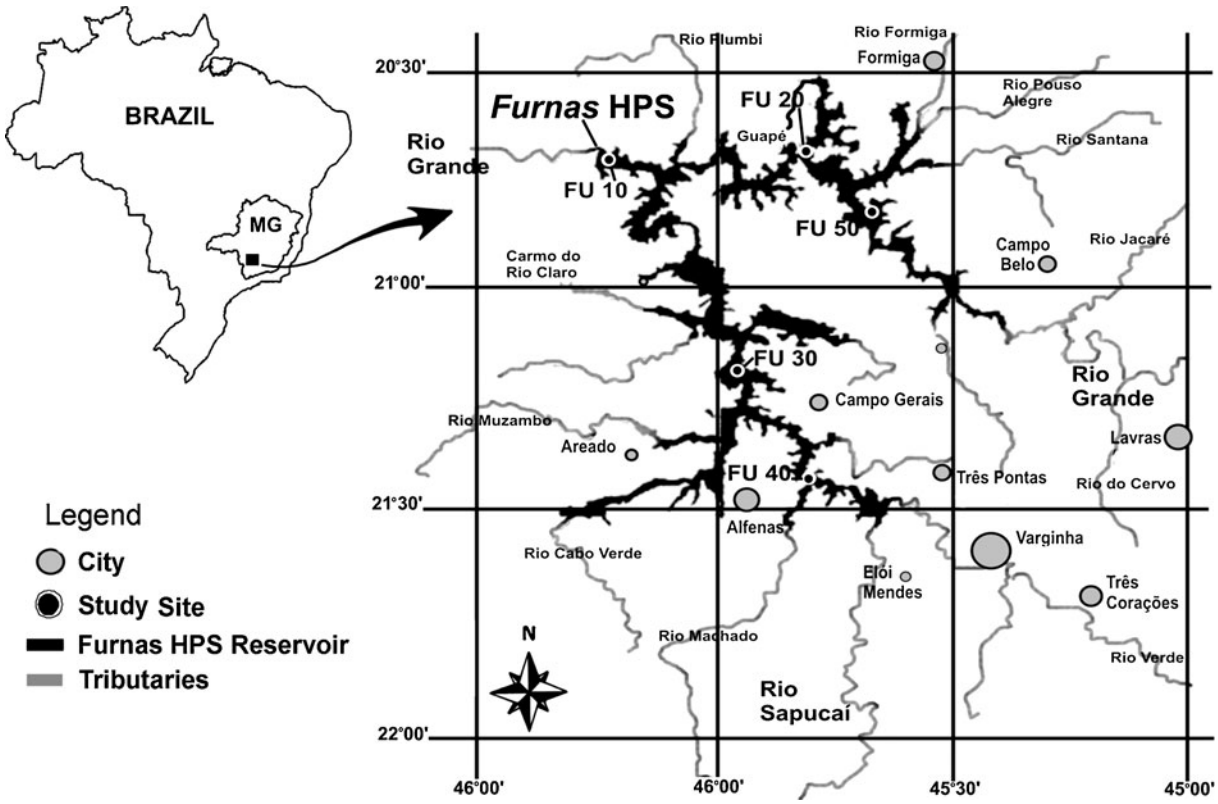

Fig. 1 Map of the Furnas Hydroelectric Power Station reservoir, Minas Gerais, Brazil, showing the sites of water and fish collection (*filled circle*): FU10 (Turvo), FU20 (Guapé), FU30 (Barranco Alto), FU40 (Fama), and FU50 (Porto Fernandes)

Fish and water collection

Lambari (*A. fasciatus*, $n = 20$/site, Wt $= 37.8 \pm 2.6$ g, Lt $= 14.3 \pm 0.3$ cm) and mandi (*P. maculatus*, $n = 15$/site, Wt $= 182.3 \pm 32.9$ g, Lt $= 25.1 \pm 1.4$ cm) specimens were collected along with water samples in June (winter season) and December (summer season) of 2006. Water samples were collected (three stations per site located 100 m apart from one another) for chemical analysis performed according to standard methods for examination of wastewater.

Water analyses

Dissolved oxygen (DO), conductivity, temperature, and pH were measured in the field using a multi-parameter water analyzer (YSI, 600XL). Alkalinity and total phosphorus were determined as described by Golterman et al. (1978). Total hardness and chloride concentration were determined following the APHA (1992) methodologies; ammoniacal nitrogen, nitrite, and nitrate were determined using the colorimetric method (Mackereth et al. 1978). Metal concentrations were determined following standards SW84603050/3051 (USEPA 1986). The aluminum concentration was determined using eriochrome cyanine R. Cadmium, copper, iron, and zinc concentrations were determined by atomic absorption spectrometry. The concentrations of pesticides in the water were determined following USEPA protocols: 2,4 dichlorophenoxyacetic acid (USEPA 8321); alachlor, atrazine,

glyphosate, hexachlorobenzene, lindane (gamma-BHC), metolachlor, methoxyclor, molinate, pendimethalin, permethrin, propanil, simazine, and trifluralin (USEPA 8270); aldrin, dieldrin, chlordane, endosulfan, endrin, heptachlor, and heptachlor epoxide (USEPA 8081); bentazon and pentachlorophenol (USEPA 8151); and dichlorodiphenyltrichloroethane (DDT isomers; USEPA, 8260) using a gas chromatograph HP 5980 and a mass spectrometer HP 5970 MSD.

Fish analyses

Fish were weighed and measured, and blood was taken through the caudal vein. The relationship between wet body mass (M_B) and total length (L_t) was calculated as $M_B = aL_t^b$, and the condition factor (K) was calculated according to the equation $K = M_B/L_t^b$, where M_B is the wet body mass (g), L_t is the total length (cm), and b is the isometry coefficient.

Blood sub-samples were used for hematological analyses immediately after sampling. The hematocrit (Hct, %) was determined using heparinized capillary tubes in a microhematocrit centrifuge. The hemoglobin concentration (Hb, g dL^{-1}) was determined using the cyanomethemoglobin method, and the red blood cell count (RBC, μL) was estimated using a modified Neubauer chamber. Mean cell volume (MCV, fL), mean cell hemoglobin (MCH, pg $cell^{-1}$), and mean cell hemoglobin concentration (MCHC, g dL^{-1}) were calculated using Hct, Hb, and RBC measurements. Blood smears were stained using polychromatic differential staining (Fast Panotic LB, Laborclin). The leukocyte and thrombocyte numbers were counted and indirectly calculated according to McKnight (1966).

The remaining blood was centrifuged, and the plasma was removed. The erythrocytes were hemolized and centrifuged at 4°C. The supernatant aliquots were stored at −70°C, and the enzyme activities and lipid peroxidation were measured spectrophotometrically (Biochrom Libra S32) at 25°C.

Lipid peroxidation was assessed by Fe^{2+} oxidation in the presence of xylenol orange (ferrous oxidation–xylenol orange) (Jiang et al. 1992). Catalase activity was determined by the decrease of the hydrogen peroxide (H_2O_2) concentration monitored at 240 nm (Aebi 1974). Superoxide dismutase activity was measured after hemoglobin precipitation and extraction in chloroform/ethanol by an indirect inhibition assay of the reduction of nitro blue tetrazolium (Crouch et al. 1981). Gluthatione peroxidase activity was measured using DTNB reagent (Hafeman et al. 1974).

Statistical analysis

Data are presented as the mean ± standard error (SEM). Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were applied to the aquatic variables to delineate geochemical groups. The correlation was applied to identify the interactions between the water variables and the biological variables. Data normality was tested using the Kolmogorov–Smirnov test. Parametric Tukey–Kramer (white and red series) and non-parametric Kruskal–Wallis (condition factor) tests were used to compare the sites. The Mann–Whitney U test was applied to compare data from June and December. The accepted significance of the data was 5% ($P < 0.05$), and analyses were conducted with XlStat 7.5 (PCA and correlations) and BioEstat v. 3.0 (ANOVA).

Results

Water

The physical and chemical variables of the water at all sites are shown in Table 1. Temperature, dissolved oxygen, pH, and conductivity did not differ between sites. The N-ammonia, N-nitrite, and N-nitrate values in the water were higher at FU30 in June; chloride was higher at FU20 and FU50 in June, and the iron concentration was higher at FU20, FU40, and FU50 in December (Table 1). All of these variables, except for the iron concentration, were lower than the upper limits recommended by the Brazilian Environment National Council in water for biota preservation (CONAMA 357/2005). Most of the pesticides that were tested were not present at levels above the detection limits of the ana-

Table 1 Water variables from all sites of the reservoir of Furnas HPS, MG, Brazil, in June and December 2006

Variables	Site									
	FU10		FU20		FU30		FU40		FU50	
	Turvo		Guapé		Barranco Alto		Fama		Porto Fernandes	
	June	December	June	December	June	December	June	December	June	December
Dissolved oxygen (mg/L)	7.14	7.63	7.20	7.00	8.16	7.76	7.03	7.75	7.43	7.40
Temperature (°C)	22.6	24.5	21.8	25.0	20.8	25.4	20.9	24.8	21.6	24.6
pH	7.22	7.30	7.65	7.20	7.45	7.31	7.56	6.90	7.43	7.30
Conductivity (μS/cm)	33.0	36.5	30.0	36.5	32.0	43.0	33.0	38.6	30.0	35.4
Alkalinity (mg/L as $CaCO^3$)	29.3	24.1	13.8	22.5	15.4	18.0	12.7	20.0	13.0	15.0
Hardness (mg/L as CaCO3)	24.6	20	11.7	21.0	13.1	19.0	9.0	23.0	11.3	18.0
N-ammonia (mg/L N)	0.2	0.10	0.10	0.2	1.0	0.10	0.49	0.05	0.10	0.02
N-nitrite (mg/L N)	0.04	0.01	nd	0.01	0.10	0.01	0.1	0.03	0.1	0.01
N-nitrate (mg/L N)	0.25	nd	0,16	nd	0.39	nd	0.05	0.10	0.29	nd
Chloride (mg/L)	0.23	0.21	1.79	2.1	0.02	0.02	0.04	0.03	1.76	0.85
Aluminum (mg/L)	0.002	0.005	0.006	0.005	0.005	0.002	0.003	0.003	0.002	0.001
Cadmium (mg/L)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Chromium (mg/L)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Copper (mg/L)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Iron (mg/L)	0.03	0.03	0.28	0.30	nd	nd	0.64	0.71	0.25	0.20
Zinc (mg/L)	nd	nd	nd	nd	0.01	0.01	0.02	0.02	nd	nd
Aldrin and Dieldrin (μg/L)	nd	nd	1.1	0.86	0.5	nd	0.003	nd	1.03	1.0
Endosulfan (μg/L)	nd	nd	0.8	0.74	1.0	nd	1.0	nd	0.28	0.1
Heptachlor epoxide (μg/L)	nd	nd	0.45	0.4	0.8	0.31	nd	nd	0.32	0.30
Metolachlor (μg/L)	nd	nd	36	18	10	8.1	nd	nd	29	14

The standard deviation was always less than 5% of the mean value and then they were omitted

lytical methods, but different concentrations of aldrin/dieldrin, endosulfan, heptachlor epoxide, and metolachlor were detected in FU20, FU30, FU40, and FU50 (Table 1). The concentration was higher than the dissolved upper limits recommended by CONAMA 357/2005, which are 0.005, 0.056, 0.01, and 10 μg/L, respectively, for aldrin/dieldrin, endosulfan, heptachlor epoxide, and metolachlor. PCA analyses revealed an association (59%) between endosulfan and heptachlor in June at FU30 and aldrin/dieldrin, heptachlor, and metolachlor at FU20 and FU50. Strong associations (89%) between aldrin/dieldrin, endosulfan, heptachlor, and metolachlor were observed in December at FU20 and between aldrin/dieldrin, heptachlor, and metolachlor at FU50.

The HCA analysis revealed high similarity between FU20 and FU50 and between FU30 and FU40. FU10 was not similar to any of the other four sites.

Condition factor

The relationship between the wet body mass (M_B) and total length (L_t) was described by the variables $M_B = 0.001\ L_t^{3.82}$, $r^2 = 0.87$ for *A. fasciatus* and $M_B = 0.002\ L_t^{3.53}$, $r^2 = 0.92$ for *P. maculatus* (Fig. 2a, b), indicating positive allometric growth. The condition factor (K) was significantly lower in fish collected from FU30 in June (Fig. 2c, d).

Hematological variables

Figure 3 shows the values of Hct, RBC, Hb, and the hematimetric index (MVC, MHC, and MCHC) of *A. fasciatus* and *P. maculatus* collected

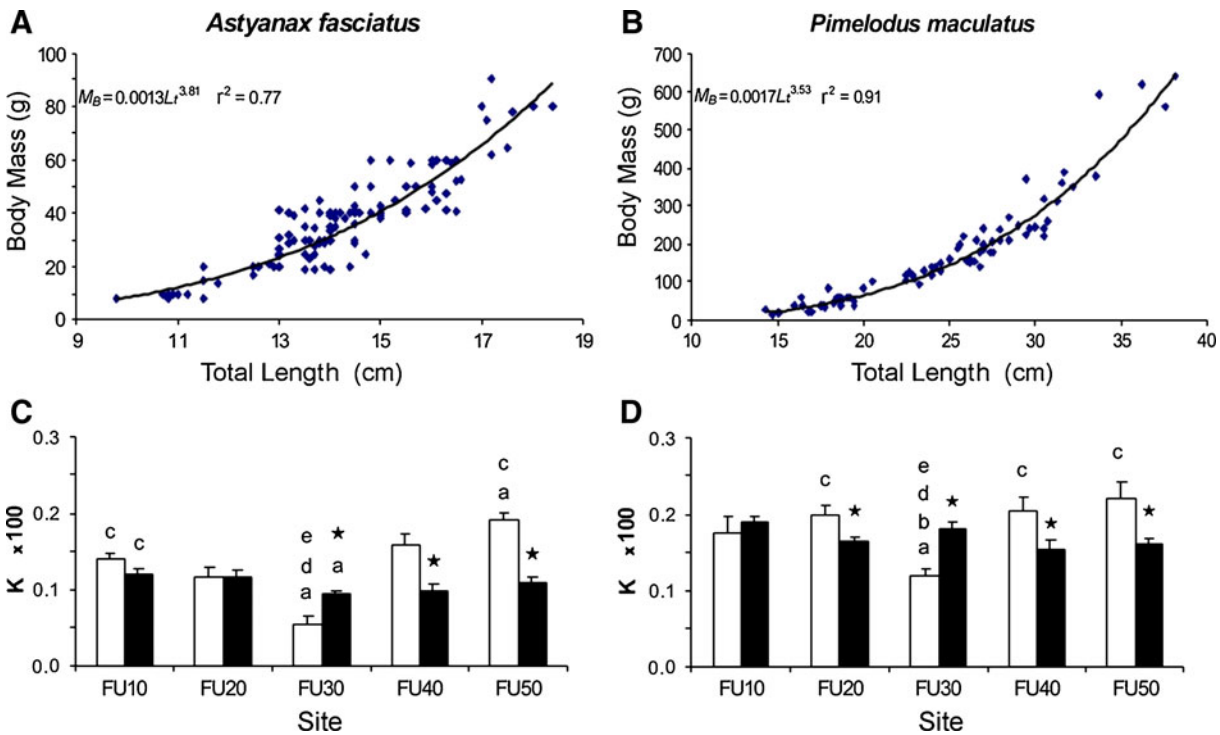

Fig. 2 Relationship between total length (L_t) and body mass (M_B) (**a**, **b**); condition factor (K) (**c**, **d**) of *A. fasciatus* and *P. maculatus* from the Furnas HPS, MG, Brazil. Labels *a*, *b*, *c*, *d*, and *e* indicate differences in relation to sites FU10, FU20, FU30, FU40, and FU50, respectively. *Asterisk* indicates a difference between June and December

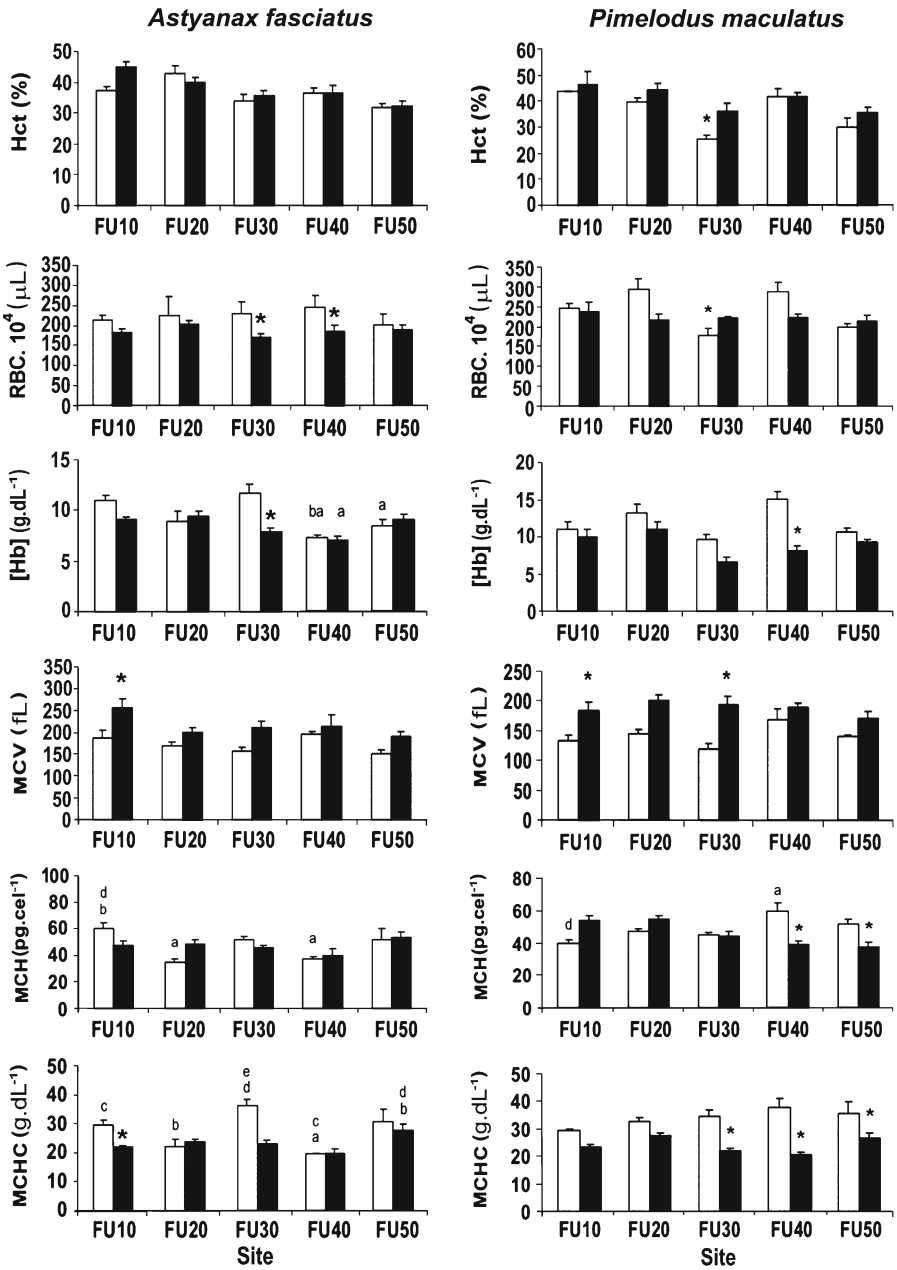

Fig. 3 Hematological variables: hematocrit (*Hct*), red blood cells (*RBC*), hemoglobin concentration ([*Hb*]), mean corpuscular volume (*MCV*), mean hemoglobin corpuscular (*MCH*), and mean corpuscular hemoglobin concentration (*MCHC*) of *A. fasciatus* and *P. maculates* from the Furnas HPS reservoir, MG, Brazil, in June (*unfilled bars*) and December (*filled bars*). Labels *a*, *b*, *c*, *d*, and *e* indicate differences from FU10, FU20, FU30, FU40, and FU50, respectively. *Asterisk* indicates a difference between June and December

in June (winter) and December (summer). No significant differences in the hematological values were found among *A. fasciatus*, with the exception of the Hb concentration and the MCHC in fish from FU40 which were lower than those of fish from FU10 (in June). A positive correlation was found between RBC and heptachlor (RBC = $199.80 + 49.1^{*}$heptachlor; $r^2 = 0.75 \mathrm{P} < 0.05$) in fish collected in June. Hematological variables in *P. maculatus* did not differ between the sites in each period, but some differences were found between June and December: the Hb concentration in FU40; MCV in FU30; MCH in FU40 and FU50; and MCHC in FU30, FU40, and FU30 were all

different from the values obtained at other sites ($P < 0.05$). A negative correlation between Hct and heptachlor (Hct = 34.73 − 16.35*heptachlor; $r^2 = 0.81$, $P < 0.03$) was found in June.

In *A. fasciatus*, the total number of leukocytes was higher in June ($P < 0.05$; Table 2). The highest and the lowest leukocyte numbers were detected in fish from FU30 and FU40, respectively. In December, the lowest value was observed in FU50. *P. maculatus* individuals from FU20, FU30, and FU50 exhibited fewer leukocytes in June (Table 2). In *A. fasciatus*, the lymphocyte percentage was higher and the percentages of monocytes and neutrophils were similar in fish from FU10 in both June and December. The monocyte frequency was lower in FU20 and FU30 ($P < 0.05$) in December. In *P. maculatus*, the lymphocytes were also the most frequent white blood cell detected in fish from FU10 in June and from FU10, FU20, and FU30 in December. The neutrophil percentage was elevated in fish from FU40 in December (Table 2). Special granulocytic cells (SGCs) or PAS-positive granular leukocytes were found in all *P. maculatus* specimens and were elevated in fish collected from FU30 in December. These cells were absent in all *A. fasciatus* collected in December.

The number of thrombocytes was lower in *A. fasciatus* than in *P. maculatus* (Table 2). There were no significant differences in the number of thrombocytes in *A. fasciatus* from all sites; however, in *P. maculatus*, the thrombocyte number was higher in fish from FU20 collected in June. Positive correlations between thrombocytes and the metolachlor concentration ($T = 4.96 + 0.19$*metolachlor, $r^2 = 0.96$, $P < 0.003$) were detected in *A. fasciatus* and *P. maculatus* collected in June.

Erythrocyte LPO and antioxidant enzyme activities

The LPO concentration in the erythrocytes was higher in the contaminated sites ($P < 0.05$) and significantly higher in June ($P < 0.05$), except in FU40 (Fig. 4a, b). In *A. fasciatus*, the activities of SOD and GPx were higher in FU20 and FU30 (June and December) and in FU50 (June); CAT activity in the contaminated sites

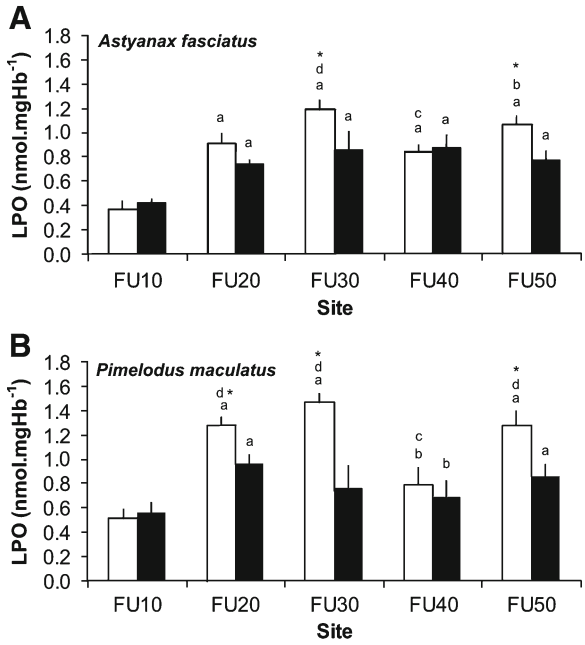

Fig. 4 Lipoperoxidation (*LPO*) in the erythrocytes of *A. fasciatus* (**a**) and *P. maculatus* (**b**) from Furnas HPS reservoir, MG, Brazil, in June (*unfilled bars*) and December (*filled bars*). Labels *a*, *b*, *c*, *d*, and *e* indicate differences from FU10, FU20, FU30, FU40, and FU50, respectively. *Asterisk* indicates a difference between June and December

was lower than that at the reference site (Fig. 5a, c, and e). A negative correlation was found between aldrin/dieldrin and SOD activity (SOD = 258.26 − 64.41*aldrin/dieldrin, $r^2 = 0.98$, $P < 0.05$) as well as GPx activity (GPx = 1.07 − 0.23*aldrin/dieldrin, $r^2 = 0.86$, $P < 0.05$). Endosulfan showed a negative correlation with CAT activity (CAT = 18.19 − 7.21*endosulfan, $r^2 = 0.74$, $P < 0.05$).

In *P. maculatus*, the SOD and GPx activities were higher in all of the contaminated sites, and significant differences were found between June and December (Fig. 5b, f). CAT activity was lower in FU20, FU30, and FU50 and significantly higher in December (Fig. 5d). Temperature and aldrin/dieldrin exhibited a positive correlation with GPx activity (GPx = 9.20 + 5.26*aldrin/dieldrin + 0.40temperature, $r^2 = 0.98$, $P < 0.05$) and an interactive effect was verified between heptachlor epoxide, alkalinity, and CAT activity (CAT = 25.63 − 11.88*heptachlor + 0.29*alkalinity, $r^2 = 0.97$, $P < 0.05$).

Table 2 Total leukocytes, differential leukocyte percentage, and thrombocyte number (mean ± SEM) of *Astyanax fasciatus* and *P. maculatus* from the reservoir of Furnas HPS, MG, Brazil, in June and December 2006

Variables	Site									
	FU10		FU20		FU30		FU40		FU50	
	Turvo		Guapé		Barranco Alto		Fama		Porto Fernandes	
	June	December	June	December	June	December	June	December	June	December
Astyanax fasciatus										
Leukocyte number 10^3 μL^{-1}	19.7 ± 2.6d	19.3 ± 1.5e	16.2 ± 1.5c	19.0 ± 2.7e	27.2 ± 3.3bde	14.4 ± 2.0*	7.8 ± 0.9ac	16.0 ± 2.1	10.5 ± 1.2c	8.9 ± 1.7ab
Lymphocytes (%)	45.2 ± 7.2e	32.6 ± 2.9	41.7 ± 6.3e	47.9 ± 6.6	40.7 ± 4,0e	26.5 ± 4.7*	42.4 ± 6.6e	31.4 ± 3.2	25.7 ± 4.2abcd	31.1 ± 4.1
Monocytes (%)	27.3 ± 7.0	43.7 ± 3.0bcde*	34.5 ± 5.3	9.7 ± 08a*	25.8 ± 4.5	29.3 ± 4.7a	34.0 ± 9.5	9.7 ± 1.7a*	44.1 ± 8.0a	25.3 ± 6.2a
Neutrophils (%)	27.4 ± 4.7	18.0 ± 3.8bcde*	23.7 ± 8.9	42.4 ± 6.9a*	33.3 ± 7.6	44.2 ± 5.6a	22.2 ± 7.9	58.2 ± 4.6a*	26.4 ± 6.3	41.8 ± 5.5a
Special granulocytic cells (%)	1.4 ± 0.9b	0	0	0	0.2 ± 0.2	0	1.4 ± 1.3	0	1.7 ± 0.8	0
Eosinophils (%)	0	5.7 ± 3.0bc*	0	0	0	0	0	0.8 ± 0.1	0	1.8 ± 1.2
Thrombocyte number 10^3 μL^{-1}	4.2 ± 0.6bc	4.8 ± 0.9	9.0 ± 1.1a	5.8 ± 1.7	7.5 ± 2.0ad	2.9 ± 1.1	5.8 ± 1.4	4.8 ± 1.1	6.8 ± 1.9	4.4 ± 0.9
Pimelodus maculatus										
Leukocyte number 10^3 μL^{-1}	39.1 ± 5.3e	23.2 ± 3.6c	14.5 ± 3.4	25.2 ± 3.5c	17.6 ± 4.0	41.3 ± 4.6abe*	28.5 ± 7.3	37.4 ± 4.0	13.6 ± 1.8a	14.2 ± 2.1c
Lymphocytes (%)	65.1 ± 13.2	60.2 ± 8.5	35.8 ± 3.3	56.0 ± 7.6	40.0 ± 7.1	50.6 ± 2.8	34.2 ± 9.1	39.4 ± 6.1	31.8 ± 5.4	40.5 ± 7.5
Monocytes (%)	22.0 ± 14.8	12.9 ± 1.9	28.4 ± 5.3	9.9 ± 1.4	29.0 ± 4.0	12.4 ± 0.6	20.9 ± 3.6	6.4 ± 1.1*	37.8 ± 7.5	18.2 ± 4.4
Neutrophils (%)	0	0	0.3 ± 0.1	0.5 ± 0.3	0	0	0	0	0	0.7 ± 0.6
Special granulocytic cells (%)	9.5 ± 4.8d	13.7 ± 3.9d	30.4 ± 2.0	25.6 ± 7.3d	14.7 ± 3.3	32.0 ± 5.7	38.3 ± 7.7a	50.0 ± 6.2ab	21.7 ± 9.2	33.7 ± 10.0
Eosinophils (%)	3.2 ± 0.5	13.2 ± 1.4	5.1 ± 0.8	8.0 ± 2.9	16.3 ± 4.6	5.0 ± 2.1	6.6 ± 3.2	4.2 ± 1.0	8.4 ± 2.3	6.9 ± 2.9
Thrombocyte number 10^3 μL^{-1}	34.5 ± 12.7	14.0 ± 12.0*	45.4 ± 17.0d	8.7 ± 4.4c*	10.4 ± 2.6	23.5 ± 20.3bd*	7.4 ± 2.3b	7.8 ± 4.8c	53 ± 1.2	14.7 ± 5.5

Letters a, b, c, d, and e indicate differences in relation to FU10, FU20, FU30, FU40, and FU50, respectively

*Indicates differences between June and December

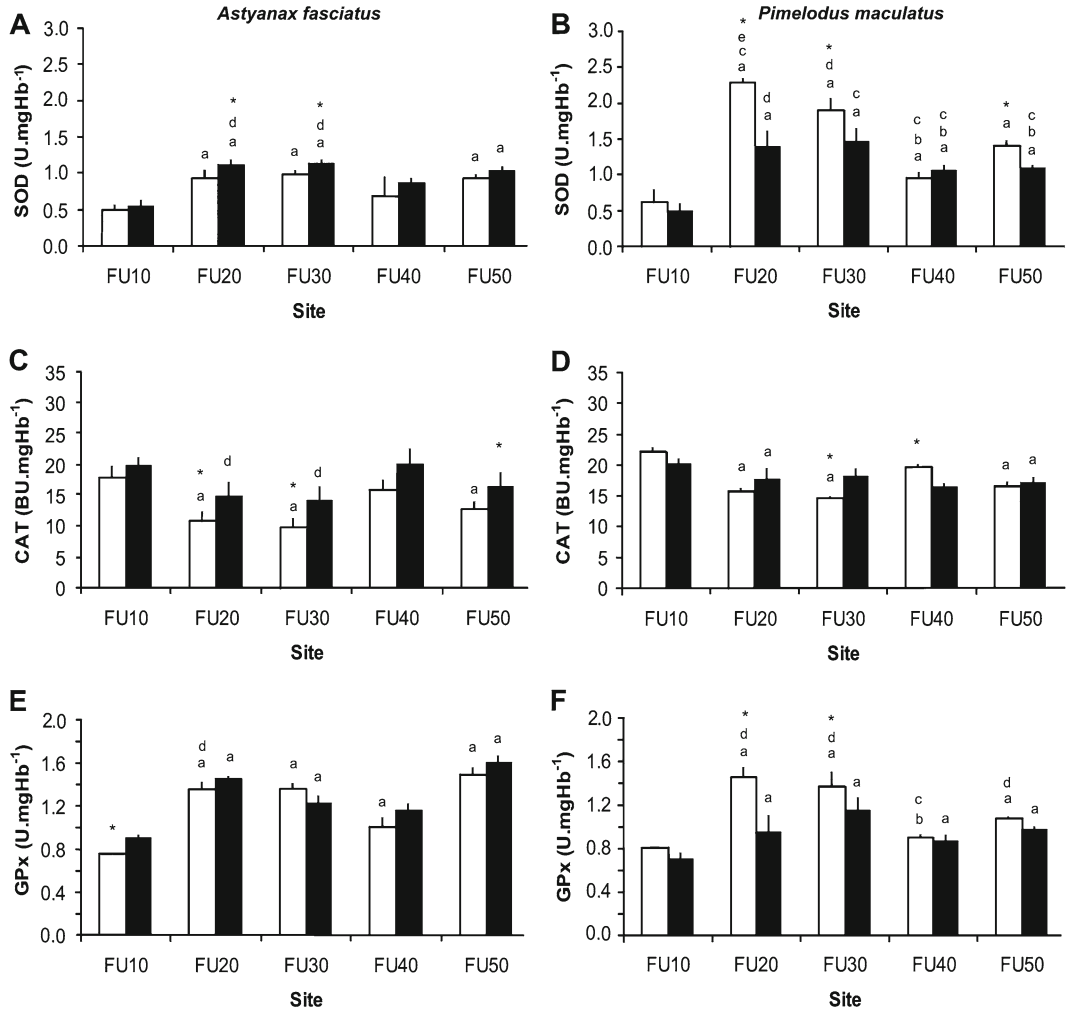

Fig. 5 Superoxide dismutase (*SOD*), catalase (*CAT*), and glutathione peroxidase (*GPx*) activity in the erythrocytes of *A. fasciatus* (**a**, **c**, and **e**) and *P. maculatus* (**b**, **d**, and **f**) from the Furnas HPS reservoir, MG, Brazil, in June (*unfilled bars*) and December (*filled bars*). Labels *a*, *b*, *c*, *d*, and *e* indicate differences from FU10, FU20, FU30, FU40, and FU50, respectively. *Asterisk* indicates a difference between June and December

Discussion

The presence of the organochlorines aldrin/dieldrin, endosulfan, heptachlor, epoxide, metolachlor (FU20, FU30, FU40, and FU50), and iron (FU40) dissolved in water characterize the Furnas HPS reservoir as contaminated. The similarity between sites located in the same river is due to the predominance of certain cultures at each riverside. The dendritic shape of the Furnas reservoir favor punctuates contamination patterns. Fish inhabiting these areas are exposed to a mixture of contaminants that differ in concentration according to the time of year, a pattern that is likely due to the periodicity of pesticide application to the agricultural landscapes, or to the dynamics of these contaminants in the reservoir (water–sediment). Organochlorines have low solubility in water and high adsorption in the organic matter, making them persistent in the environment. These compounds are retained in the sediment particulate phase and are released into

the water depending on the characteristics of the water.

The condition factor (K) and blood variables of both species demonstrated the complexity of the fishes' integrated responses to the physical and chemical characteristics of their environment, which depend on the chemical concentration, mode of action, time of exposure, and season. In general, pollutants have a negative effect on K by reducing food availability and/or by increasing the energy required to maintain homeostasis (Dethloff et al. 2001; Bervoets and Blust 2003); exceptions may be found in untreated domestic sewage pollution, which is usually related to high productivity (Schulz and Martins-Junior 2001; Alberto et al. 2005). The absence of a correlation between the contaminants and K may suggest that these two parameters are not directly affected by such contaminants, at least in these fish species. Consistent with this hypothesis, high organochlorine concentrations were also found at the FU20 and FU50 sites of Furnas HPS, and the K values were not as low as in fish from site FU30. Furthermore, organochlorine residues were detected in the gills and liver of both *A. fasciatus* and *P. maculatus* from all of the contaminated sites (unpublished data). The presence of unknown variable may explain the low K values in FU30 that were detected in the June sample.

Multiple contaminants in the water may result in an interactive effect on the blood variables and other tissues (Adhikari et al. 2004; Koprucu et al. 2006). The Hct, the RBc, the Hb concentration, and the hematimetric indexes of both studied species were similar to those previously determined for *A. fasciatus* (Alberto et al. 2005) and *P. maculatus* (Ranzani-Paiva et al. 2000). However, the variability in these measurements between the sites demonstrates the influence of environmental characteristics and differences in the physiological adjustments between these two species. The positive correlation between RBC and heptachlor in *A. fasciatus* suggests an adjustment to maintain the O_2 cascade from the gills to the tissue, while in *P. maculatus*, the negative correlation between Hct and heptachlor implies a reduction of the efficiency of O_2transport and the absence of any hematological compensatory adjustment to overcome this effect. Changes in RBC, Hb concentration, and MCV are directly related to the need for O_2 in oxidative metabolism and the efficiency of O_2 uptake from the environment and its transport to tissues.

The higher LPO concentration in the erythrocytes of *A. fasciatus* and *P. maculatus* are evidence of oxidative stress in fish collected at the FU20, FU30, and FU50 sites in June, as these sites have a higher concentration of organochlorine. Oxidative stress occurs when the balance between oxidants and antioxidants is disrupted and the excessive generation of reactive oxygen species produces LPO (Scandalios 2005).

Changes in the activity of the antioxidant enzymes SOD, CAT, and GPx to maintain the function and integrity of cells in most animals, as well as their activation in response to exposure to pollutants, have been reported in erythrocytes and various tissues (Bainy et al. 1996; Chebab et al. 2009; Kaminski et al. 2009). The higher activity of the antioxidant enzymes SOD, CAT, and GPx in the erythrocytes of *A. fasciatus* and *P. maculatus* collected in the contaminated sites were not sufficient to remove ROS and neutralize their effects, resulting in an increase of LPO. The inhibition of CAT activity in the erythrocytes of both species collected in the contaminated sites contributed to higher levels of LPO. The differences of SOD, CAT, and GPx activities between the *A. fasciatus* and *P. maculatus* clearly showed the different susceptibilities of these enzymes to a mixture of toxic compounds. This phenomenon has been previously emphasized by Ruas et al. (2008), who measured the activities of SOD, CAT, and GPx in the erythrocytes of three cichlid species living in the same contaminated environment.

In vitro and in vivo studies have shown that individual pesticides/herbicides increase the production of ROS and enhance SOD, CAT, and GPx activities in a time- and dose-dependent manner, while a mixture of these toxicants may have different effects. CAT is inhibited by endosulfan in chicken erythrocytes (Aggarwal et al. 2009). SOD and CAT activity increased in the liver of rats treated with endosulfan or chlorpyriphos and decreased in those treated with both chemicals in combination (Chebab et al. 2009). CAT activity increased in the muscles of fish living in a

river that was contaminated heavily with pesticides/herbicides (Michelo et al. 2006).

Although specific immunity in fish is less developed than that in birds and mammals, fish have a non-specific resistance system that protects them against pathogenic and environmental factors (Passantino et al. 2005). Leukocytes are involved in specific and non-specific defense mechanisms, but most pollutants affect the defense system in fish. In general, lymphocytes are the most numerous white blood cells (70–80%), as they are involved in antibody production and inflammatory process. These cells were relatively scarce in both of the studied species (20–48% in *A. fasciatus* and 21–66% in *P. maculatus*), while previous studies found 70% lymphocytes in the same species (Silva-Souza et al. 2000). These findings suggest a possible immunosuppression in fish from the Furnas HPS reservoir. Furthermore, the number of monocytes and neutrophils observed indicates the mobilization of these cells from lymphoid tissues in response to bacterial infection. Monocytes are the precursors of tissue macrophages, which ingest foreign materials and are also involved in the immune response as antigen-presenting cells that transmit information about the ingested material to lymphocytes. Neutrophils are involved in non-specific immunity, migrating to the infection site and recognizing, ingesting, and destroying bacteria and other pathogens (Tavares-Dias et al. 2007). The role of SGCs is not well established, but as they are considered as a type of neutrophil, they may be involved in inflammatory and phagocytic processes. Our results support the hypothesis that the percentage of SGCs increases in fish stressed by low water quality, such as during chronic exposure to contaminants (Martins et al. 2002), as these cells were always present in fish collected in the Furnas HPS reservoir (except in *A. fasciatus* in December).

Thrombocyte function is related to blood coagulation and phagocytosis as a link between innate and adaptive immunity (Passantino et al. 2005). Increases in the number of thrombocytes have been reported in *Cyprinus carpiovar* exposed to sublethal concentrations of endosulfan (1 μgL^{-1}) under laboratory conditions (Shafiq-ur-Rehman 2006). In the present study, there was no correlation of thrombocytes with the endosulfan present in water. The low concentrations in most sites, excluding FU30 and FU40 in June, or the low sensitivity of *A. fasciatus* and *P. maculatus* species may have influenced the responses of these fish. However, the effect of metolachlor on the thrombocyte number was evident in the positive correlation between these variables. This response may be due to a direct effect on thrombocyte production or to an indirect effect due to the increased gill tissue damage (Paulino, personal communication).

Conclusion

Fish exposed to multiple contaminants in their natural environments present substantial variability in most physiological and biochemical variables. Such responses are expected, as numerous factors influence organic responses. Considering the complexity of the aquatic environment and the coexistence of inducing and inhibiting chemicals, this study provides evidence for the importance of using several biomarkers to estimate risks in complex situations. The use of a set of biomarkers can enhance the likelihood of identifying areas/species that are threatened by chemicals, especially in cases where differences in sensitivity may be found among the species. Among the biomarkers used in this study, the hematological variables, the erythrocyte LPO levels, and the activity of the antioxidant enzymes can be used to assess water quality, regardless of the difference in responses between fish species. Furthermore, the higher energy demand of fish needed to repair the damage caused by the environment may affect reproductive activity, thereby reducing these specimens in the reservoir and fish diversity over an extended period.

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References

Adhikari, S., Sarkar, B., Chatterjee, A., Mahapatra, C. T., & Ayyappan, S. (2004). Effects of cypermethrin and carbofuran on certain hematological parameters and predictions of their recovery in a freshwater teleost, *Labeo rohita* (Hamilton). *Ecotoxicology and Environmental Safety, 58*, 220–226.

Aebi, H. (1974). Catalase. In: H. U. Bergmayer (Ed.), *Methods of enzymatic analysis* (pp. 671–684). London: Academic Press.

Aggarwal, M., Naraharisetti, S. B., Sarkar, S. N., Rao, G. S., Degen, G. H., & Malik, J. K. (2009). Effects of subchronic coexposure to arsenic and endosulfan on the erythrocytes of broiler chickens: A biochemical study. *Archives of Environmental Contamination and Toxicology, 56*, 139–148.

Alberto, A., Camargo, F. M., Verani, J. R., & Costa, O. F. T. (2005). Health variables and gill morphology in the tropical fish *Astyanax fasciatus* from a sewage-contaminated river. *Ecotoxicology and Environmental Safety, 61*, 247–255.

APHA (1992). *Standard Methods for the Examination of Water and Wastewater* 18th (ed.). Washington, DC: American Public Health Association.

Bainy, A. C. D., Saito, E., Carvalho, P. S. M., & Junqueira, V. B. C. (1996). Oxidative stress in gill, erythrocytes, liver and kidney of Nile tilapia (*Oreochromis niloticus*) from a polluted site. *Aquatic Toxicology, 34*, 151–162.

Bervoets, L., & Blust, R. (2003). Metal concentrations in water, sediment and gudgeon (*Gobio gobio*) from a pollution gradient: Relationship with fish condition factor. *Environmental Pollution, 126*, 9–19.

Cerqueira, C. C. C., & Fernandes, M. N. (2002). Gill tissue recovery after copper exposure and blood parameter responses in the tropical fish, *Prochilodus scrofa*. *Ecotoxicology and Environmental Safety, 52*, 83–91.

Chebab, S., Belli, N., Leghouchi, E., & Lahouel, M. (2009). Oxidative stress induced by two pesticides: Endosulfan and chlorpyriphos. *Environnement, Risques & Santé, 8*(5), 425–432.

CONAMA - Conselho Nacional do Meio ambiente (2005). Res CONAMA 357/2005. Classificação dos corpos de água, diretrizes ambientais para enquadramento, condições e padrões de lançamento de efluentes. Brasília.

Crouch, R. K., Gandy, S. C., & Kimsey, G. (1981). The inhibition of islet superoxide dismutase by diabetogenic drugs. *Diabetes, 30*, 235–241.

Dethloff, G. M., Bailey, H. C., & Maier, K. J. (2001). Effects dissolved cooper on select hematological biochemical and immunological parameters of wild rainbow trout (*Oncorhynchus mukiss*). *Archives of Environmental Contamination and Toxicology, 40*, 371–380.

Golterman, H. L., Clymo, R. S., & Ohnstad, M. A. M. (1978). *Methods for Physical and Chemical Analysis of Freshwaters. IBP Handbook. 8*. Oxford: Blackwell Scientific Publications.

Hafeman, D. G., Sunde, R. A., & Hoekstra, W. G. (1974). Dietary selenium on erythrocyte and glutathione peroxidase in the rat. *Journal of Nutrition, 104*, 580–587.

Jiang, Z. Y., Hunt, J. V., & Wolff, S. P. (1992). Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. *Analytical Biochemistry, 202*, 384–389.

Kaminski, P., Kurhalyuk, N., Jerzak, L., Kasprzak, M., Tkachenko, H., Klawe, J. J., et al. (2009). Eco-physiological determinations of antioxidant enzymes and lipoperoxidation in the blood of White Stork *Ciconia ciconia* from Poland. *Environmental Research, 109*, 29–39.

Koprucu, S. S., Koprucu, K., Ural, M. S., Ispir, U., & Pala, M. (2006). Acute toxicity of organophosphorous pesticide diazinon and its effects on behavior and some hematological parameters of fingerling European catfish (*Silurus glanis* L.). *Pesticide Biochemistry and Physiology, 86*, 99–105.

Mackereth, F. J. H., Heron, J., & Talling, J. F. (1978). *Water analysis: Some revised methods for limnologists*. Cumbria: Scientific Publication.

Martins, M. L., Moraes, F. R., Fujimoto, R. Y., Nomura, D. T., & Fenerick, J. (2002). Respostas do híbrido tambacu (*Piaractus mesopotamicus* Holmberg, 1887 macho x *Colossoma macropomum* Cuvier, 1818 fêmea) a estímulos simples ou consecutivos de captura. *Boletin do Instituto de Pesca, 28*, 195–204.

Michelo, S., Kennedy, C., Roy, M., Victor, W., Masahiro, Y., & Yoshimitsu, M. (2006). Pesticide/herbicide pollutants in the Kafue river and a preliminary investigation into their biological effect through catalase levels in fish. *Japanese Journal of Veterinary Research, 54*, 119–128.

Mazon, A. F., Monteiro, E. A. S., Pinheiro, G. H. D., & Fernandes, M. N. (2002). Hematological and physiological changes induced by short-term exposure to copper in freshwater fish, Prochilodus scrofa. *Brazilian Journal of Biology, 62*, 621–631.

McKnight, I. M. (1966). A hematological study on the mountain white. *Prosopium williasonit*. *Journal of the Fisheries Research Board of Canada, 23*, 45–64.

Passantino, L., Cianciotta, A., Patruno, R., Ribaud, M. R., Jirillo, E., & Passantino, G. F. (2005). Do fish thombocytes play an immunological role? Their cytoenzimatic profiles and function during an accidental piscine candidiasis in aquarium. *Immunopharmacology and Immunotoxicology, 27*(2), 345–356.

Ranzani-Paiva, M. J. T., Silva-Souza, A. T., Pavanelli, G. C., Takemoto, R. M., & Eiras, A. C. (2000). Hematological evaluation in commercial fish species from the floodplain of the upper Paraná River, Brazil. *Acta Scientarium, 22*, 507–513.

Ruas, C. B. G., Carvalho, C. S., Araújo, H. S. S., Espíndola, E. L. G., & Fernandes, M. N. (2008). Oxidative stress biomarkers of exposure in the blood of cichlid species from a polluted river. *Ecotoxicology and Environmental Safety, 71*, 86–93.

Scandalios, J. G. (2005). Oxidative stress: Molecular perception and transduction of signals triggering

antioxidant gene defenses. *Brazilian Journal of Medical and Biological Research, 38*(7), 995–1014.

Schulz, U. H., & Martins-Junior, H. (2001). Astyanax fasciatus as bioindicator of water pollution of Rio Sinos, RS, Brazil. *Brazilian Journal of Biology, 61*(4), 615–622.

Serpunin, G. G., & Likhatchyova, O. A. (1998). Use of the ichthyohaematological studies in ecological monitoring of the reservoirs. *Acta Veterianaria Brunensis, 67*, 339–345.

Shafiq-ur-Rehman (2006). Endosulfan toxicity and its reduction by selenium: A behavioral, hematological and peroxidative stress evaluation. The *Internet Journal of Toxicology, 3*(1). www.ispub.com/.../endosulfan_toxicity.html.

Silva-Souza, A. T., Machado, P. M., & Almeida, S. C. (2000). Haematology of fish from Tibagi River. I. Differential white blood cell counts in *Pimelodus maculatus* females. *Boletin do Instituto de Pesca, 26*, 33–39.

Tavares-Dias, M., Ono, E. A., Pilarski, F., & Moraes, F. R. (2007). Can thrombocytes participate in the removal of cellular debris in the circulating blood of teleost fish? A cytochemical study and ultrastructural analysis. *Journal of Applied Ichthyology, 23*(6), 709–712.

Triebskorn, R., Telcean, I., Casper, H., Farkas, A., Sandu, C., Stan, G., et al. (2007). Monitoring pollution in River Mures, Romania, part II: Metal accumulation and histopathology in fish. *Environmental Monitoring and Assessment, 141*(1), 177–188.

USEPA (1986). Test method for evaluating solid waste Report, no. SW846, Washington, DC.

Weber, C., Peter, A., & Zanini, F. (2007). Spatio-temporal analysis of fish and their habitat: A case study on a highly degraded Swiss river system prior to extensive rehabilitation. *Aquatic Science, 69*, 162–172.