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Biochemical and physiological biomarkers in *Prochilodus lineatus* submitted to in situ tests in an urban stream in southern Brazil

Marina M.P. Camargo, Cláudia B.R. Martinez *

Departamento de Ciências Fisiológicas, Universidade Estadual de Londrina, C.P. 6001, 86051-990 Londrina, Paraná, Brazil

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Abstract

The aims of the present study were to verify the suitability of short-term in situ test with the neotropical fish *Prochilodus lineatus* and to evaluate from a set of biochemical and physiological biomarkers the ones which could work as sensitivity tools for the environmental quality assessment. In situ tests were carried out for 1 week in winter and summer, at three sites along an urban stream heavily contaminated by anthropogenic activities and at a reference site. The variables analyzed were: hemoglobin content (Hb), plasma concentrations of cortisol, glucose, total protein, Na^+ and Cl^-, plasma osmolarity, liver activities of glutathione-*S*-transferase (GST) and catalase and interrenal cells area. Results showed that glycemia, interrenal cell size and GST activity, which were significantly higher in fish caged in the urban stream, were best able to distinguish between the most disturbed sites and the reference and caged *P. lineatus* showed to be a promising tool for the assessment and monitoring of tropical aquatic ecosystems.

Keywords: Biomarkers; *Prochilodus lineatus*; In situ test; Urban stream

1. Introduction

In urban aquatic environments fish may be exposed to a myriad of substances at the same time produced by different kinds of anthropogenic activities. The exposure to two or more chemicals may result in biological reponses quantitatively or qualitatively different from that expected from the action of the chemicals alone (Rand et al., 1995). In Brazil, as in other developing countries, criteria for the classification of water courses are exclusively based on physical and chemical factors (CONAMA, 1992) and chemical analyses are normally restricted to identification of a limited range of contaminants in water, mainly metals, and provide little information concerning the presence of organic contaminants such as hydrocarbons and pesticides in water and sediment. Therefore, alternative methodologies for water quality assessment and environmental monitoring other than chemical monitoring are urgent needed (Bozzetti and Schulz, 2004).

In situ tests are useful tools in ecotoxicology, mainly because they integrate ecological relevance in toxicity testing, by incorporating field fluctuations in a cost-effective way (Castro et al., 2004). In this kind of field work, focusing on environmental quality, healthy organisms are taken to the field-site and exposed directly to the potentially contaminated environment (Stien et al., 1998; Pacheco and Santos, 1999; Parrot et al., 2000; Olsen et al., 2001; Pyle et al., 2001). Here, the actual situation is identified in the field and there is no need to extrapolate laboratory results to particular local conditions (Chappie and Burton, 1997). However, it is important to have in mind that despite providing a more realistic exposure regime, outdoor field tests do not provide more realistic concentration–response information than typically generated from laboratory tests (Graney et al., 1995).

Biological changes in fish that are related to the exposure or to the effects of contaminants are called biomarkers (Peakall, 1994) and their use has led to good results in environmental risk assessment (McCarthy and Shugart, 1990; Van der Oost et al., 2003). Prominent among these biomarkers are haematological data (Soivio et al., 1973; Sampath et al., 1993) and physiological variables, such as plasma levels of metabolites (Adams et al., 1990; DiGiulio et al., 1995) and ions (Engelhardt et al., 1981; Alkindi et al., 1996; Martinez and Souza, 2002), levels of hormones like cortisol (Hontela et al., 1996; Barton et al., 1998; Hontela, 1998; Benguira and Hontela, 2000) and biochemical

* Corresponding author. Tel.: +55 43 3371 4650; fax: +55 43 3371 4467.
E-mail address: cbueno@uel.br (C.B.R. Martinez).

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variables such as detoxifying enzyme activities (Paris-Palacios et al., 2000; Teles et al., 2003; Ahmad et al., 2004). In order to make use of biomarkers its normal range of variation must be known; in other words, it is necessary to distinguish the natural variance of a given biomarker from altered values indicating stress due to contamination (Ranzani-Paiva et al., 2000; Olsen et al., 2001). In addition, the interfering effects of season on biomarkers must be elucidated, especially in tropical and subtropical regions, where interactions can be very complex (Wilhelm Filho, 1996).

In Brazil, there have been few field studies focusing on the use of native fish biomarkers as tools for water quality assessment (Wilhelm Filho et al., 2001; Winkaler et al., 2001; Martinez and Cólus, 2002; Martinez and Souza, 2002). The neotropical fish *Prochilodus lineatus* (Valenciennes, 1847) (=*P. scrofa* Steindachner, 1881), represents a well suited species to environmental monitoring as it is a bottom feeder fish which is in contact with xenobiotics in water and in sediment and also has been shown to be sensitive to variations in water quality (Mazon and Fernandes, 1999; Da Silva et al., 2004; Martinez et al., 2004).

Cambé stream and its tributaries constitute the main hydrological basin of Londrina, a city of 500,000 inhabitants in Paraná state, Southern Brazil; the Cambé crosses the entire city and is widely used for recreational purposes, despite being heavily impaired by anthropogenic activities. It receives diffuse and point source discharges of industrial, domestic and agricultural wastes since its headwaters. Previous chemical water analyses of the upper reaches of Cambé stream showed large amounts of metals, mainly lead and aluminum (Yabe and Oliveira, 1998). In addition, Winkaler et al. (2001) showed that feral fish from these upper areas present impaired health and more recently, Lemos et al. (2005) demonstrated that its water induces DNA damage, detected by comet assay.

Thus, the objectives of the present study were to verify the suitability of a short-term in situ test with *Prochilodus lineatus* and to evaluate from a set of biochemical and physiological biomarkers the ones which could work as sensitive tools for the assessment of the environmental quality of Cambé stream.

2. Materials and methods

2.1. In situ tests

Juveniles of *P. lineatus* (Characiformes, Prochilodontidae) weighing 28.6 ± 0.8 g (mean ± S.E., $n = 53$) were obtained from the University Hatchery Station. In situ tests were performed in the winter of 2002 (August and September) and summer of 2003 (February and March) at three sites on the upper part of Cambé stream, with strong anthropogenic influence, and at a reference site on the Apertados stream (Fig. 1). This stream is away from the urban area, present a well preserved riparian forest and is relatively free of contaminants. The climate where this study was carried out is classified as humid subtropical with warm summer and rain is distributed in all seasons, with a decline in rainfall in the winter (from June to September). In both seasons, at each site, six fish were confined for 7 days in cubic cages of 125 L, remaining in contact with the sediment. Immediately after this caging period, the fish were anaesthetized with benzocaine (0.1 g L^{-1}), to take a sample of blood from the caudal vein and then sacrificed by cervical section in order to remove the liver and anterior kidney.

2.2. Physico-chemical analysis

At each sampling-site, immediately before installing the cages and after their removal, water temperature, dissolved oxygen (DO), conductivity and pH were measured. Rainfall data for

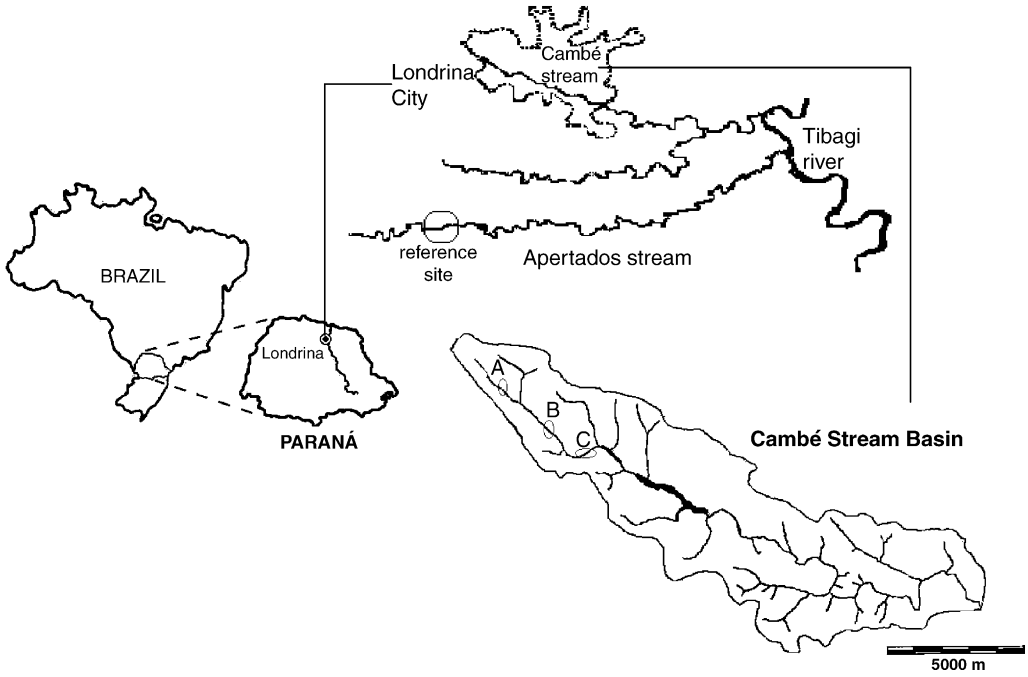

Fig. 1. Map showing the region of Londrina city where in situ tests were carried out at the reference site (Apertados stream) and the sites at Cambé stream (A, B and C).

each site in summer and winter were obtained from the Paraná State Agronomic Institute (IAPAR). Water samples collected from sampling-sites along Cambé stream were analyzed for sulphate, phosphate, nitrite and nitrate using procedures described in APHA (1998).

2.3. Blood analysis

The blood collected was kept in ice until arrival at the laboratory, where an aliquot of 10 μL was used to measure the hemoglobin content by the method of cyanomethemoglobin using a spectrophotometer. The remaining blood was centrifuged for 5 min at 5000 × *g* and plasma was stored at −20 °C until the assays. Plasma glucose was measured enzymatically with a commercial kit based on glucose oxidase; total protein concentration was determined by the method of Lowry et al. (1951), using bovine serum albumin (BSA) for the calibration curve.

Plasma concentration of Na^+ was determined by flame-photometry and that of Cl^- by a colorimetric method (commercial kit), in a spectrophotometer. Osmolarity was measured by freezing the plasma in an osmometer. Cortisol was analyzed with a commercial immunoenzymatic kit (Diagnostic Systems) and the readings carried out in a microplate reader at 450 nm.

2.4. Histomorphometric analyses of interrenal cells

After removal, anterior kidney was placed in Bouin's fixative for 12 h and embedded in paraffin. Sections (5 μm) were stained with hematoxylin and eosin and examined under the light microscope. The area of interrenal cells was determined for 10 cells in representative interrenal sections from each fish. Measurements were made with an image analyzer system (Motic Cam 8 mm and Motic Images Software, Version 1.2) and an average value was determined for each fish.

2.5. Activities of glutathione-S-transferase (GST) and catalase in the liver

Fish livers were stored at −80 °C for enzymes assays. Samples were homogenized in ten volumes (w/v) of ice-cold 0.1 M K-phosphate buffer (pH 7.0) and centrifuged (14,000 × *g*) for 20 min at 4 °C, to obtain the supernatant for glutathione-*S*-transferase (GST) and catalase analyses. GST activity was determined as described by Keen and William (1976) using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. The change in absorbance was recorded at 340 nm and the enzyme activity was expressed in nmol min^{-1} mg liver protein. Catalase activity was estimated from the rate of consumption of hydrogen peroxide levels (Beutler, 1975). Change in absorbance was recorded at 240 nm and enzyme activity was expressed in μmol min^{-1} mg liver protein. Concentration of protein in the supernatant was measured by the method of Lowry et al. (1951).

2.6. Statistical analysis

For each variable studied, during a given season, differences observed among the sampling-sites on the Cambé and the reference site were tested for significance by single-factor ANOVA or the Kruskall–Wallis test, depending on whether the data followed a normal distribution and showed homogeneity of variance. To locate these differences, a multiple comparison test (Student–Newman–Keuls or Dunn's) was used. Results for winter and summer were compared by using Student's *t*-test, differences being significant when $P < 0.05$. Results are presented as mean ± S.E.

3. Results

3.1. Water analysis

Physical and chemical data on the water, obtained at the sampling-sites in each season, are given in Table 1. DO was higher than 5 mg O_2 L^{-1} in both seasons and the pH stayed close to 7. The conductivity at all the sites on the Cambé was higher than that at the reference site, being everywhere above 100 (μS cm^{-1}). Concentrations of sulphate, phosphate, nitrite and nitrate were all found to be raised at sites B and C, in comparison with site A.

3.2. Hemoglobin content (Hb)

The fish in the study had an average blood hemoglobin content of 6.41 ± 0.32 g dL^{-1}, and this did not vary significantly among the sites (Fig. 2). There was a tendency for the Hb content in summer to be smaller than that in winter, but this difference was only significant at site A.

3.3. Interrenal cell morphometry

It was noted that in the summer the areas of interrenal cells were significantly greater than in the winter (Fig. 3a). Also, fish caged at sites A and B in the summer and those at site C in both summer and winter, exhibited cells significantly larger than those of fish at the reference station.

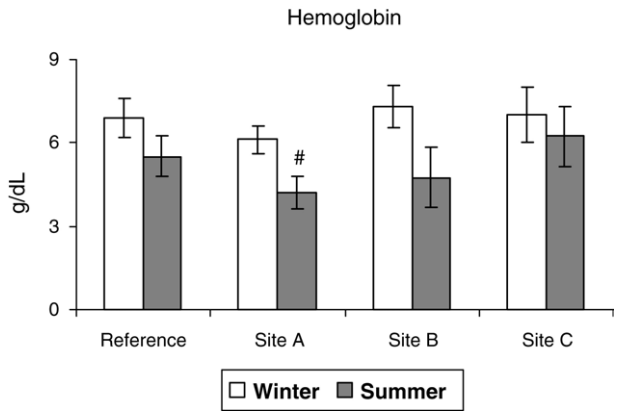

Fig. 2. Hemoglobin content of the animals caged at the three sites on the Cambé stream (A, B and C) and at the reference site, in winter and summer. The bars indicate mean and the vertical lines SE. #Indicates difference in relation to winter results ($P < 0.05$).

Table 1
Physical and chemical data of water measured at study sites at the beginning and end of the caging period and rainfall

Variable	Season	Sites			
		Reference	Site A	Site B	Site C
D.O. ($mg\,O_2\,L^{-1}$)	Winter	9.1 ± 1.77	5.1 ± 0.56	7.6 ± 0.28	7.0 ± 0.49
	Summer	7.8 ± 1.34	5.5 ± 0.07	7.7 ± 1.2	7.0 ± 0
Temperature (°C)	Winter	17.7 ± 0.07	22.1 ± 0.35	20.6 ± 1.13	17.4 ± 2.34
	Summer	21.4 ± 0.56	26.0 ± 0.28	22.9 ± 0.07	24.0 ± 0.07
pH	Winter	7.6 ± 0.35	7.1 ± 0.07	7.3 ± 0.07	7.5 ± 0.14
	Summer	7.6 ± 0.07	7.0 ± 0.07	7.2 ± 0.07	6.9 ± 0.63
Conductivity ($\mu S\,cm^{-1}$)	Winter	65.0 ± 10	99.0 ± 4.04	115.5 ± 0.49	223.5 ± 47.65
	Summer	83.0 ± 0	104.5 ± 4.5	138.5 ± 0.49	244.5 ± 0.49
Org. matter ($mg\,L^{-1}$)	Winter	NM	49.12 ± 0	30.32 ± 13.67	33.56 ± 29.98
	Summer		81.52 ± 32.33	71.57 ± 23.70	68.80 ± 56.87
Sulfate ($mg\,L^{-1}$)	Winter	NM	0.35 ± 0.22	4.99 ± 4.41	3.91 ± 3.63
	Summer		6.46 ± 3.85	8.93 ± 1.71	5.90 ± 2.53
Phosphate ($mg\,L^{-1}$)	Winter	NM	0.78 ± 0.69	4.05 ± 3.29	2.64 ± 2.16
	Summer		0.21 ± 0.04	0.34 ± 0.06	0.16 ± 0.06
Ammonia ($mg\,L^{-1}$)	Winter	NM	0.05 ± 0.04	0	0
	Summer		0.87 ± 0.86	0	0
Nitrite ($\mu g\,L^{-1}$)	Winter	NM	2.26 ± 0.76	9.09 ± 0.01	2.96 ± 1.74
	Summer		5.37 ± 3.82	2.0 ± 0.84	4.06 ± 0.34
Nitrate ($mg\,L^{-1}$)	Winter	NM	0.16 ± 0.04	0.87 ± 0.28	0.61 ± 0.09
	Summer		0.97 ± 0.6	1.11 ± 0.48	0.84 ± 0.73
Rainfall (mm)	Winter	0	17.2	12.4	22.0
	Summer	27.6	21.2	17.0	33.1

Values are mean ± S.E., excepting rainfall values, which correspond to the sum of the 3 days before and the 7 days of caging period. NM, not measured, quantification was not performed.

3.4. Plasma cortisol

The mean cortisol concentration in the plasma of *P. lineatus* was 163.45 ± 13.61 ng mL^{-1}. Fish caged at site C in winter reached levels as high as 200 ng mL^{-1} (Fig. 3b). This was also the only site where seasonal variation in cortisol was found, the winter level being significantly higher than the summer. There was no site on the Cambé stream where the fish presented a cortisol level significantly different from those caged at the reference site, either in summer or winter.

3.5. Plasma glucose and protein

At sites B and C, plasma glucose was higher in summer than in winter (Fig. 3c). Fish caged at site A in winter and at B and C in summer had significantly higher glucose levels than those held at the reference site, in the same period.

The total protein concentrations in the plasma are shown in Table 2. It was not observed any significant variation between winter and summer values. Significant differences among the sampling-sites on the Cambé and the reference site, in the same season, was also not found.

3.6. Osmotic and ionic variables

The osmolarity of the plasma of all fish used in tests was maintained around 260 ± 4.5 m Osm kg^{-1}. At sites A and C the summer values were significantly higher than those obtained in winter, and also than the summer values at the reference site (Fig. 4a).

Plasma sodium levels of caged fish were 148.90 ± 2.71 mM. At the reference site, the summer values were significantly lower than the winter values, while at site B, the winter level was the lowest. At site C in summer, plasma sodium was statistically higher than at the reference site (Fig. 4b). The plasma chloride levels showed no significant variation in fish caged at any of the sites, in either season. All samples presented values in the range of 108.26 ± 1.35 mM (Fig. 4c).

3.7. Catalase and GST

Activity of catalase in the liver showed no variation with respect to either different seasons or different test sites, remain-

Table 2
Plasma total protein ($mg\,mL^{-1}$) of fish caged at Cambé stream and in the reference site, in winter and summer

Site	Winter ($mg\,mL^{-1}$)	Summer ($mg\,mL^{-1}$)
Reference	18.88 ± 1.07	17.43 ± 1.22
Site A	18.85 ± 0.60	17.91 ± 1.80
Site B	21.45 ± 1.33	17.41 ± 1.50
Site C	21.54 ± 0.51	20.16 ± 2.29

Values are mean ± S.E., *N*: 4–6.

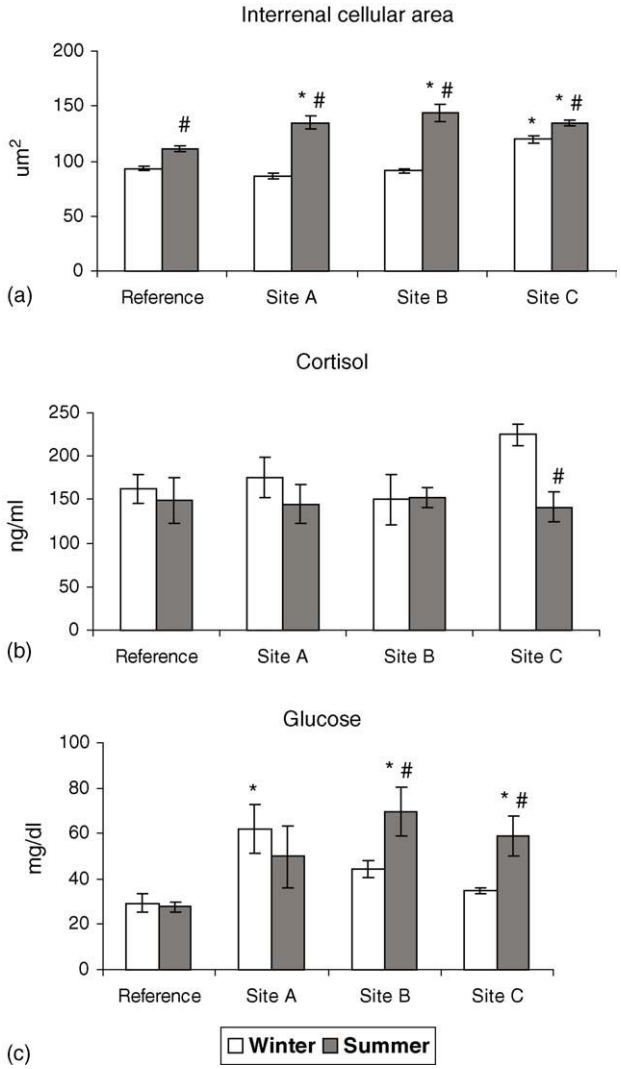

Fig. 3. Cellular area of the interrenal cells (a) and plasmatic concentration of cortisol (b) and glucose (c) of the animals caged at the three sites on the Cambe stream (A, B and C) and at the reference site, in winter and summer. The bars indicate mean and the vertical lines SE. *Indicates difference from reference site at the same season, #indicates difference in relation to winter results ($P < 0.05$).

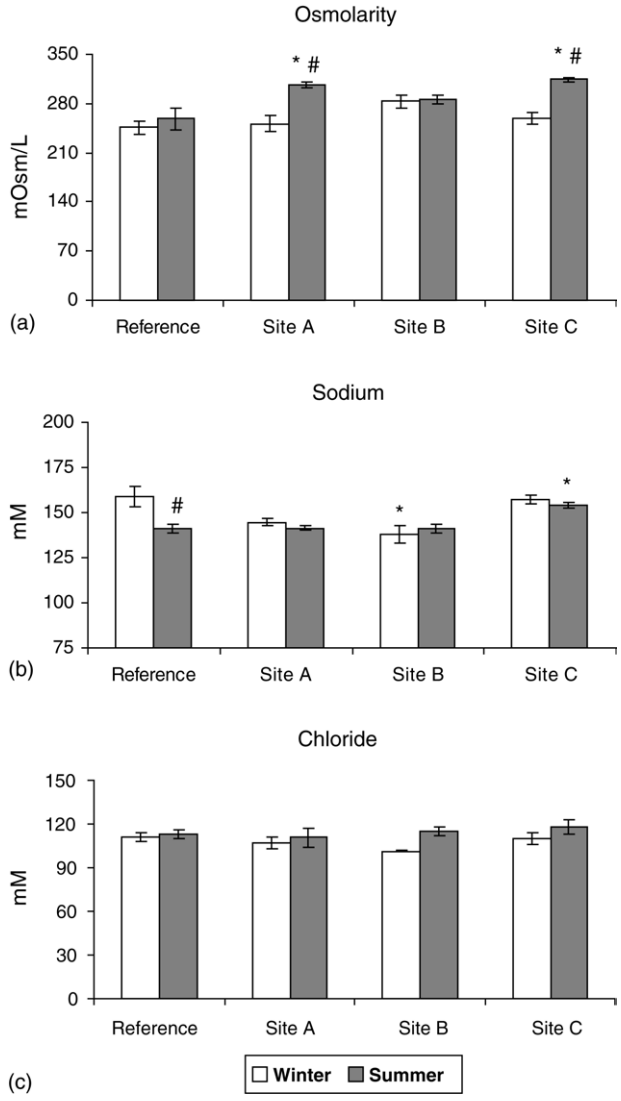

Fig. 4. Plasmatic osmolarity (a) and plasmatic concentrations of sodium (b) and chloride (c) of the animals caged at the three sites on the Cambe stream (A, B and C) and at the reference site, in winter and summer. The bars indicate mean and the vertical lines the SE. *Indicates difference from reference site at the same season, #indicates difference in relation to winter results ($P < 0.05$).

ing in all samples at 41 ± 1.91 μmol min^{-1} per mg liver protein (Fig. 5a). In fish caged at site A, GST activity was higher in summer than in winter, while at site C, the summer level was higher than at the reference site (Fig. 5b).

4. Discussion

Physical and chemical variables are essential in the assessment of water quality and they provide important information on the alterations that may occur as the season changes. The temperatures and rainfall presented here varied as expected in subtropical climate: all sites were hotter and wetter in summer than in winter, on average. The reference site, in relation to the ones along Cambé stream, showed lower value of conductivity, higher value of DO and a constant value of pH, which indicate the most favorable condition of its waters and validate it as a reference site. None of the sites in the urban stream showed a DO below 4 mg O_2 L^{-1}, the critical oxygen content according to Esteves (1988). The conductivity at site C was higher than at other sites; values in excess of 100 μS cm^{-1} have been reported at polluted sites (CETESB, 2001) and could reflect poor quality water (Olsen et al., 2001). At sites B and C an increased loading of nutrients was indicated by phosphate, nitrite and nitrate concentrations, suggesting a higher discharge of effluents into the stream at these sites.

Hematological variables have been used to indicate physiological state, as well as in the control of diseases and stress in fish (Martinez et al., 1994). However, owing to the wide variety of factors that affect these variables (Winkaler et al., 2001; Guijarro et al., 2003), such as the season, diseases, age, sexual maturity and environmental changes (Heath, 1987), it is often hard to interpret correctly the hematological status of fish. The

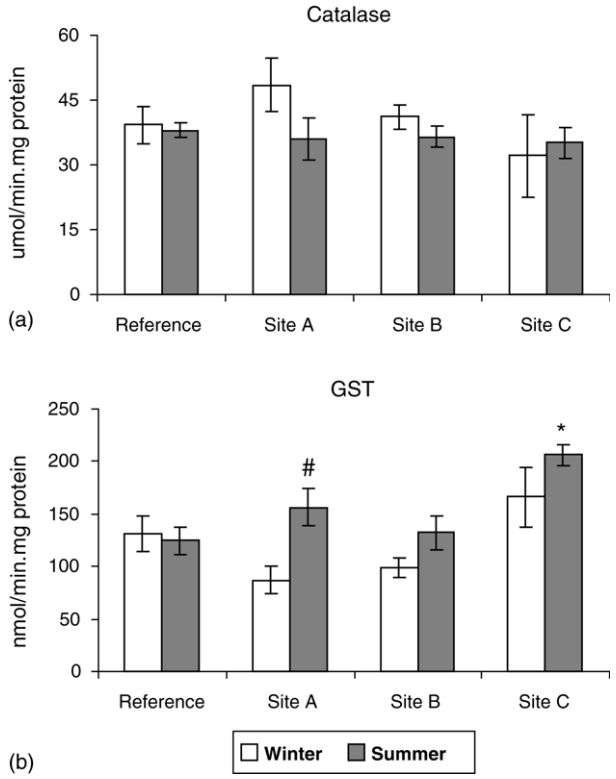

Fig. 5. Hepatic activity of catalase (a) and GST (b) of the animals caged at the three sites on the Cambé stream (A, B and C) and at the reference site, in winter and summer. The bars indicate mean and the vertical lines SE. *Indicates difference from reference site at the same season, #indicates difference in relation to winter results ($P < 0.05$).

fish used in this study showed a general tendency to have lower hemoglobin contents in summer than in winter. However, the common situation described in the literature is that Hb levels increase at higher temperatures (Martinez et al., 1994; Guijarro et al., 2003). Thus, in the present study, other parameters rather than temperature must have led to the reduced Hb in summer and it becomes clear that Hb content is not very suitable for use as a biomarker in the field, in view of the number of factors that can influence this hematological variable.

In fish, the corticosteroid hormones, including cortisol, are synthesized by the interrenal cells, structures that are homologous to the adrenal cortex in mammals (Donaldson, 1981; Wendelaar-Bonga, 1997; Hontela, 1998). Morphometry of interrenal cells can reveal alterations such as hypertrophy, which may provide information on the working state of these cells and also on the stress level the fish is experiencing (Norris et al., 1997).

The interrenal cell area in *P. lineatus* in the present study was greater in summer than in winter, at the reference site and all the test sites on the Cambé stream. It is possible that the endocrine system of the caged fish was more stimulated in the summer than in the winter, leading to greater production and secretion of cortisol. With respect to the reference site, the interrenal cell areas of fish held at sites A and B in summer, and at C in either season, were significantly enlarged. This hypertrophy coincided with the hyperglycemia (relative to the reference site) observed at B and C locations in summer.

Hyperglycemia in animals is often related to the mobilization of energy needed for an increase in the metabolic rate, occasioned by external conditions such as raised temperature. In the present case, the fish caged at sites B and C showed higher plasma glucose levels in the summer, as the results obtained by Guijarro et al. (2003), who analyzed specimens of tench. A rise in glycemia may also represent the mobilization of energy in response to temporary stressors, such as handling or variations of temperature or pH, or the presence of contaminants in the water (Lohner et al., 2001).

The glycemia of the fish caged at sites A, B and C was significantly higher than that at the reference site. In other study, specimens of *P. lineatus* also exhibited raised plasma glucose levels when exposed to lead for 24 h (Martinez et al., 2004), and the control level observed in that experiment was quite similar to the glycemia in the fish at the reference site described here. Tavares-Dias et al. (2002) also found that the glycemia of pacus *Piaractus mesopotamicus* was raised after exposure for 8 days to copper sulphate, while Barcarolli and Martinez (2004) identified hyperglycemia in piavuçus (*Leporinus macrocephalus*) exposed to aluminium for 24 h. Furthermore, fish confined to lakes contaminated with oil refinery effluents showed hyperglycemia (Martin and Black, 1996). A rise in blood glucose is very common in animals confronting a stressful situation and it is one of the major effects of the secretion of catecholamine and corticosteroid hormones that occurs in such situations (Brown, 1993).

Cortisol is the main corticosteroid hormone in fish, synthesized and secreted by the interrenal cells located in anterior kidney (Hontela, 1998). A raised cortisol level in the plasma has generally been used as a sign of stress response in fish (Wendelaar-Bonga, 1997).

In situations of acute stress, the cortisol level rises just a few minutes after exposure to the stressor, and returns to normal within a few hours. The levels of cortisol found in *P. lineatus* were very high in comparison with basal values recorded for teleosts, which range from 5 to 50 ng mL^{-1} (Pickering, 1981). It is likely that this resulted from the stress caused by cage confinement and also by handling at the time of sampling (Wendelaar-Bonga, 1997). Rotland et al. (2001) found that cortisol increased significantly in gilthead sea-bream (*Sparus aurata*) submitted to an hour of stress from handling and confinement, and the level remained high, although somewhat lower, in fish exposed to the same type of stress for 7 days.

Seasonal variation in the cortisol response has been described in several species of fish, the winter level normally being higher, and the same tendency was observed in the current study. Rising levels of cortisol have also been reported in the blood of fish exposed to several types of chemical stressors (Norris et al., 1999). Ruyet et al. (2003) showed that this hormone increased in the Atlantic turbot (*Scopthalmus maximus*) exposed to high concentrations of ammonia for 7 and 14 days; Ytrestoyl et al. (2001) found the same in salmon (*Salmo salar*) exposed to aluminium and Donaldson (1981) in salmon exposed to copper for up to 48 h, while Hontela et al. (1996) found that the level of cortisol was high in rainbow trout (*Oncorhynchus mykiss*) exposed

to cadmium from 2 to 96 h and that it stayed high at the end of 7 days exposure.

In the fish caged at the various sites on the Cambé stream, no difference was observed between the plasma cortisol at any of the test sites and that at the reference site. However, the hyperglycemia seen in fish at site A, in winter, and at sites B and C, in summer, could be interpreted as a secondary stress response, triggered by the release of cortisol in these fish. In other words, since a cortisol peak occurs moments after the exposure to an acute stress and the hormone rapidly recovers its normal level, the blood sample taken at the end of 7 days in the cage might not show such a peak, even though one of its side-effects, a raised level of glucose, was observed. This difficulty in establishing a consistent correlation pattern between the stress responses that are primary (i.e., plasma cortisol alterations) and those that are secondary (i.e., changes in plasma glucose) was already pointed out by Teles et al. (2004).

In this study, the cortisol results also did not correlate with the interrenal cell hypertrophy. These cells must have continued working at an augmented rate for longer than the cortisol response, owing to the persistence of the stress stimulus. McBride et al. (1979) observed a similar response in rainbow trout exposed to leachate from sanitary landfill tips: the cortisol increased just a few hours after the exposure, whereas the effects on the interrenals were noted only in fish exposed for 7 days. The present data lead to the conclusion that the fish caged in Cambé stream were exposed to stressors to which they were ready to respond, as shown by plasma glucose and interrenal cell morphometry.

Concentrations of individual ions and total osmolarity in blood plasma are physiological variables that have been used as indicators of the effects of pollution on fish (Abel, 1989). Shifts in the hydromineral balance may be a consequence of the action of pollutants on organs involved in osmoregulation, on the endocrine system, on metabolism or on active transport processes (Martinez and Cólus, 2002). In this study, sodium levels were significantly lower in fish caged at site B in winter, with respect to the reference site. This lowering of plasma sodium may arise from the general response of the organism to stress (Martinez and Cólus, 2002), since it may represent water inwards movement due to enhanced branchial permeability, or even the inhibition of the Na^+/K^+—ATPases in the gills triggered by many different pollutants (Cerqueira and Fernandes, 2002; Martinez et al., 2004). However, the chloride ion content remained unchanged and was similar to that already described for *P. lineatus* (Cerqueira and Fernandes, 2002; Mazon et al., 2002). Fish at site C exhibited increased $[Na^+]$ and osmolarity. This probably reflects the high conductivity of the water found at this site, which is usually associated with the discharge of large quantities of salt, such as could arise from domestic and industrial waste (Winkaler et al., 2001).

Glutathione-*S*-transferases (GST) are a family of detoxifying enzymes, which participate in phase II of the detoxification of foreign compounds (Pesonen et al., 1999; Paris-Palacios et al., 2000; Van der Oost et al., 2003). They catalyze conjugation of various electrophilic compounds with reduced glutathione (Olsen et al., 2001). Raised GST activity has been associated with defensive adaptation of the organism to the presence of a variety of organic compounds in the environment (Gallagher et al., 2001).

In the *P. lineatus* caged in the Cambé, the liver activity of GST was consistently higher in summer than in winter, significantly so at site A. Ronisz et al. (1999) found a positive correlation between the activity of GST in female blenny (*Zoarces viviparus*) and the water temperature. High GST levels were also found by Wilhelm Filho et al. (2001) in the cichlid *Geophagus brasiliensis* caught in unpolluted water in the summer. The authors affirmed that the high temperatures promoted greater oxygen consumption and hence a rising of the antioxidant defenses of the fish, including superoxide dismutase and GST activities.

The fish caged at site C in the summer exhibited GST activities (200 nmol min^{-1} mg liver protein) significantly higher than at the reference site. Vigano et al. (2001) encountered similar values in European flounder (*Platichtys flesus*) exposed for 6 days to sediment contaminated with domestic and industrial effluents. Increased liver GST activity has been reported in various other publications after the exposure of fish to polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and pesticides (Van der Oost et al., 2003) and to water contaminated with domestic waste, heavy metals and organic compounds (Ahmad et al., 2004) and in fish inhabiting sites with strong anthropogenic influence (Vigano et al., 1998). The higher GST activity in the fish caged at site C on the Cambé stream indicates that these animals had their detoxifying activity stimulated, possibly by the presence of contaminants in the water.

Catalase acts exclusively on H_2O_2, eliminating it from the cell by converting it to O_2 and water. The hepatic catalase activity in the fish subjected to in situ tests exhibited no seasonal variation, nor any difference between sampling sites. Wilhelm Filho et al. (2001) also detected no seasonal variation in catalase activity in fish sampled in months of the year that differed considerably in temperature. Ronisz et al. (1999) found no correlation between catalase activity in female viviparous blennies and the water temperature.

Van der Oost et al. (2003), reviewing the results of several investigations into catalase activity in fish exposed to PAHs, PCBs and pulp mill effluents, found that around 60% of laboratory studies and 40% of those in the field did not reveal alterations of the activity in fish exposed to toxic agents, in comparison with controls.

Nevertheless, Paris-Palacios et al. (2000) observed a linear rise in catalase activity with both exposure time and copper concentration in the zebra fish (*Brachydanio rerio*) and Ahmad et al. (2004) reported increased catalase activity in eels subjected to in situ tests in a harbour contaminated with mixed effluents in Portugal. Thus, it may be that the short exposure time influenced the catalase activity in the present study in such a way that it did not vary significantly between fish caged either at different sites or in different seasons. Therefore, among the biochemical variables analyzed here, the GST activity in the liver appears to be a more suitable biomarker than catalase activity in the circumstances of this research.

5. Conclusions

- Amongst the variables analyzed here, glycemia, interrenal cell size and GST activity were best able to distinguish between the most pollution-affected sites and the reference.
- Considering the number of variables that exhibited alterations with respect to reference-site values and its location, downstream of the other sites, site C showed to be the most impaired site on the Cambé stream.
- Considerable refinement of some handling techniques used during sampling will be necessary if the resulting stress is to be diminished, so that it interferes less in related variables such as the cortisol level.
- Caged *P. lineatus* proved to be suitable for the assessment of water quality in a disturbed urban stream and could be employed in biomonitoring programmes in subtropical regions.

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