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Research Article

Assessment of Water Pollution Signs in the Brazilian Pampa Biome Using Stress Biomarkers in Fish (*Astyanax* sp.)

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Located in southern Brazil, the Pampa biome has been under constant threat due to improper management of human effluents and use of pesticides. These contaminants accumulate mainly in water resources resulting in chronic poisoning of aquatic biota. Up to date, no studies on the assessment of environmental quality in the Brazilian portion of Pampa biome have been undertaken. Thereby, our main goal in this study was to investigate the ecotoxicological risks caused by human activity in the Santa Maria River, a major water course in the Brazilian Pampa biome. Brain and muscle tissues were used for determining oxidative stress and cholinesterase biomarkers in fish (*Astyanax* sp.) exposed to urban and agricultural effluents. A substantial decrease in fish muscle acetylcholinesterase activity was observed in exposed animals, compared to controls (kept under laboratory conditions). In parallel, increased lipid peroxidation and significant changes in stress-responsive antioxidant enzymes (GST, CAT, GPx, and TrxR) were detected. In the fish brain, a significant increase in GST activity is reported. In conclusion, our results showed significant changes in biomarkers of water contamination in *Astyanax* captured in Santa Maria River, pointing to important levels of water pollution in the region and validating the use of *Astyanax* in biomonitoring programs within the Pampa biome borders.

1. Introduction

Pampa biome, located in southern Brazil, covering approximately 2% of the national territory and 63% of the Rio Grande do Sul State comprehend a large grasslands area containing a vast number of endemic species [1]. Despite its large biodiversity, the Brazilian Pampa biome is poorly studied, comparing to other five Brazilian biomes [2]. Recently, this biome has been constantly threatened due to urban and agroindustrial growth [2] which releases a large amount of pollutants in its water sources. Despite such situation, no studies concerning the impacts of the human activities on the Pampa ecosystem were undertaken so far.

Inadequate management of effluents and increasing use of agrochemicals in monocultures, especially soybeans and rice, are the main causes of environmental contamination in Pampa biome [3]. Toxicological tests are important tools to evaluate the quality of waters sources through the determination of toxic effects of chemicals on biological systems [4]. Responses to contaminants may not show obvious effects on aquatic organisms but can yield in responses at the cellular and molecular level and interfere in the life cycle of these organisms. Thereby, biochemical changes are considered as early responses to contaminants, occurring before death or manifestation of diseases and can be used as biomarkers for the detection and monitoring of such compounds [5].

Reactive oxygen species (ROS) are overproduced when organisms face environmental exposures to heavy metals, agrochemicals, and other contaminants and have been pointed as major inducers of cellular damage [6]. The implications of ROS in different tissues are related to the unbalance in cellular redox homeostasis, promoting oxidative stress and resulting in damage to biomolecules [7]. To counteract oxidative stress, antioxidant defense systems are modulated under prooxidative conditions and determinations of such changes are frequently used as biomarkers of environmental contaminations [7]. Among antioxidant defenses in cells, glutathione levels, lipid peroxidation markers, and the activity of antioxidant enzymes can be highlighted [8]. In addition to oxidative stress parameters, the activity of cholinesterases is largely

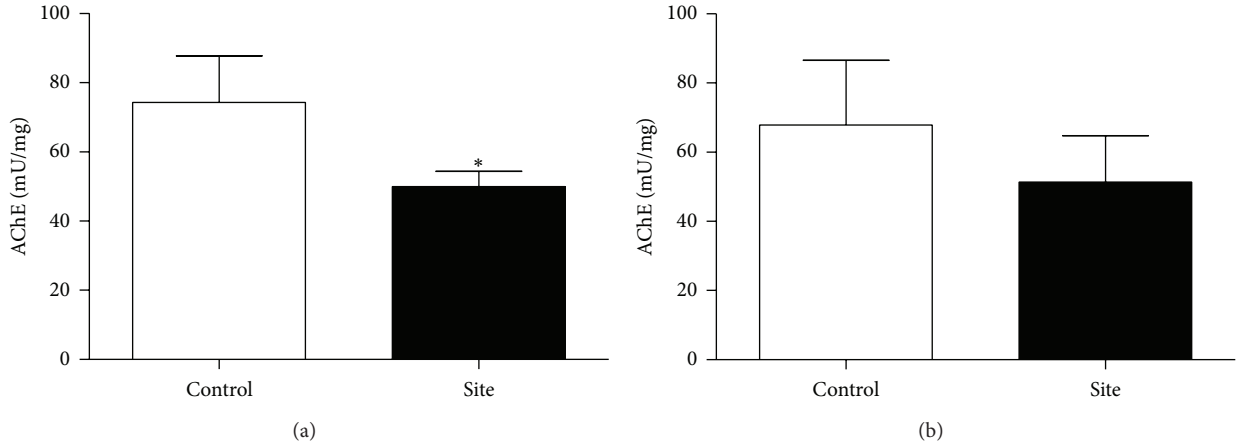

Figure 1: AChE enzyme activity in fish. Enzyme activity was determined in muscle (a) and brain (b). Data are presented as mean ± standard deviation of enzymatic activity expressed in mU/mg total protein. $^{*}P < 0.05$.

used as biomarker for the presence of organophosphate and carbamate agrochemicals in aquatic environments [9].

Emphasizing the importance of the Santa Maria River as a major water course and the environmental risks promoted by human activity in the Pampa biome, the present study was carried out in order to investigate the ecotoxicological impacts caused by urban and agricultural effluents in the region. For such a purpose, was used *Astyanax* sp., a fish broadly distributed in Brazil and of great ecological value due to its role in the trophic chain as a zooplanktivorous, insectivorous, and omnivorous species, also serving as prey for birds and other fish from higher trophic levels [10]. *Astyanax* has been used in environmental monitoring studies in other regions of Brazil [11–14]; however, the suitability of this species for biomonitoring environmental quality in the Pampa biome has not yet being addressed.

2. Materials and Methods

2.1. Animals and Sample Preparation. The animals were captured at a single site at the Santa Maria River in Rosario do Sul city, Rio Grande do Sul State, Brazil (30°15′00.04″S 54°54′42.22″O). This site was chosen because of its proximity to both agricultural and urban areas, being widely used for swimming and fishing activities. After being captured, animals were rapidly transported to the laboratory in plastic containers and transferred to aquaria with constant oxygenation, containing water of the capture site. After adaptation period, animals were anesthetized with cold water and euthanized by cervical rupture for brain and muscle tissue extraction. Tissues were homogenized in HEPES 20 mM buffer, pH 7,0, and centrifuged at 1,000 × g for 5 minutes at 4°C, a part of the supernatant removed for determination of AChE enzyme activity and levels of lipid peroxidation (TBARS) and total hydroperoxides. The remaining supernatant was centrifuged at 20,000 g for 30 minutes at 4°C to determine the activity of antioxidant enzymes, glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx), catalase (CAT), and thioredoxin reductase (TrxR). Control animals were captured in a site with apparent environmental preservation state and kept in quarantine aquaria (deionized water) with constant oxygenation and temperature (24 ± 1°C) in a 12 h light-dark cycle.

2.2. Chemicals. Glutathione reductase, glutathione reduced form (GSH), glutathione oxidized form (GSSG), t-butylhydroperoxide (t-bOOH), 5,5-dithio-bis (2-nitrobenzoic acid (DTNB)), nicotinamide adenine dinucleotide 2-phosphate reduced tetrasodium salt hydrate (NADPH), and acetylthiocholine were purchased from Sigma (São Paulo, SP, Brazil). All the other chemicals used in this study were from the highest analytical grade.

2.3. Determination of Enzyme Activity. Enzyme activity was determined in a Thermo Scientific Evolution 60S UV-Visible spectrophotometer. Antioxidant enzyme activity was determined as described previously [15] following the original methods described by Carlberg and Beng [15]; Habig and Jakoby [16]; Aebi [17]; Holmgren and Mikael [18]. Acetylcholinesterase activity was measured according to protocols described by Ellman et al. [19] using acetylthiocholine as substrate.

2.4. Glutathione Levels, Lipid Peroxidation, and Total Hydroperoxides. The end products of lipid peroxidation in fish muscle and brain were determined by the method of (Ohkawa, 1979) [20] as thiobarbituric acid reactive substances (TBARS) with minor modifications. Samples were incubated with 0.45 mM acetic acid/HCl buffer, pH 3.4, 0.28% thiobarbituric acid, and 1.2% SDS, and thereafter boiled at 95°C for 60 min to promote color reaction, measured spectrophotometrically at 532 nm. Malondialdehyde was used as a standard. The total hydroperoxide content was assessed using the xylenol orange method. This approach allows the detection of hydrogen peroxide as well as other hydroperoxides, including lipid hydroperoxides as described elsewhere [21]. Glutathione levels were measured as nonprotein thiols as described by Fernández-Vega et al. [22].

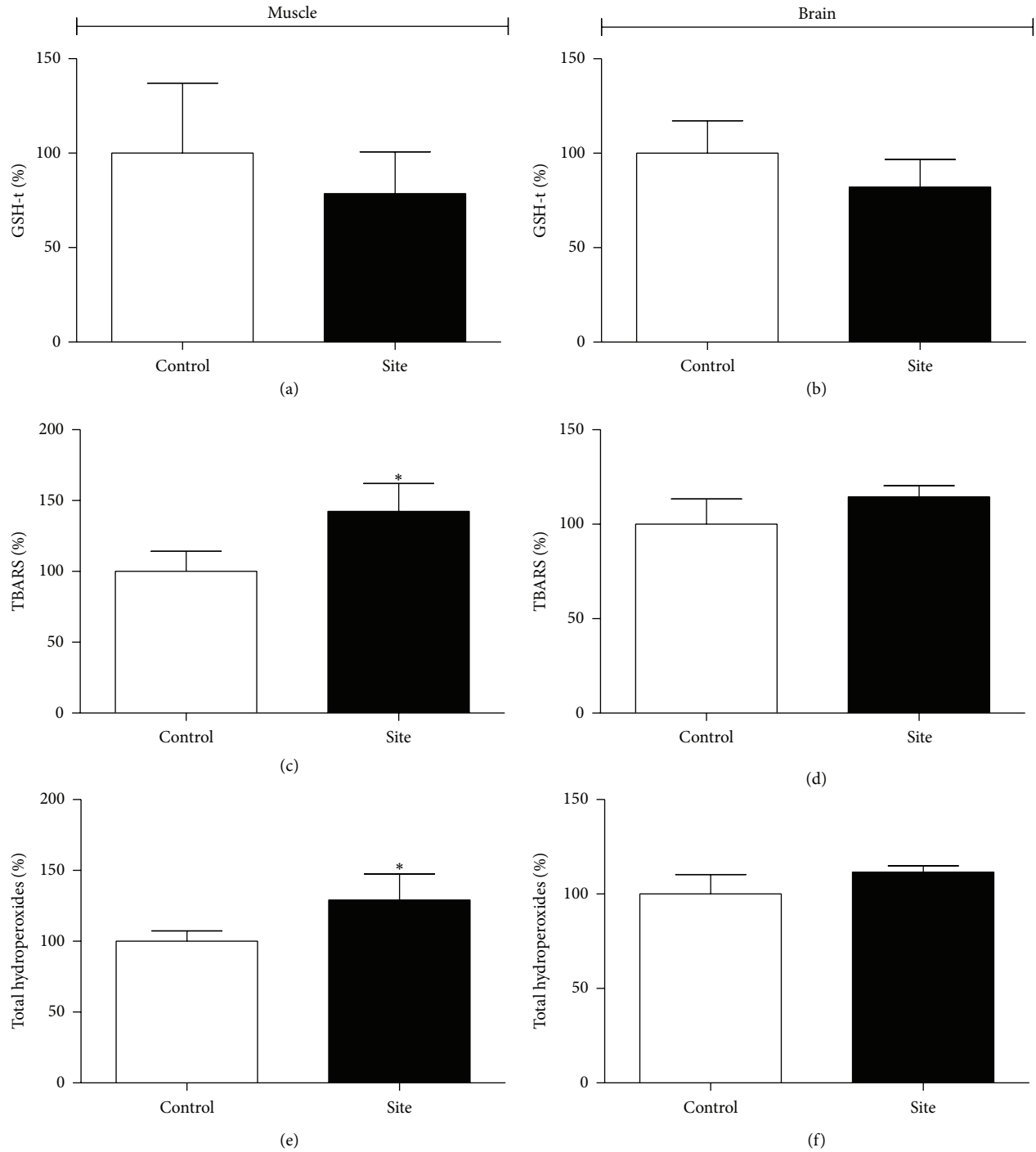

Figure 2: Oxidative stress markers in *Astyanax* sp. (a) GSH levels in muscle and (b) brain. (c) TBARS levels in muscle and (d) brain. (e) Total hydroperoxides in muscle and (f) brain. The results were expressed as percent of controls (mean ± standard deviation). $^{*}P < 0.05$.

2.5. Statistical Analysis. Statistical differences among groups were analyzed by Student's t-test. Statistically significant differences were considered when $P < 0.05$.

3. Results

3.1. Acetylcholinesterase Activity (AChE). The AChE activity was significantly ($P < 0.05$) decreased in muscle tissue (Figure 1(a)) of fish captured in the Santa Maria River in comparison to the control. In the fish brain, a slight but not significant decrease in AChE activity was observed (Figure 1(b)).

3.2. Glutathione Levels, Lipid Peroxidation, and Total Hydroperoxides. Despite an observed slight decrease in GSH levels, the content of this tripeptide remained statistically unaffected

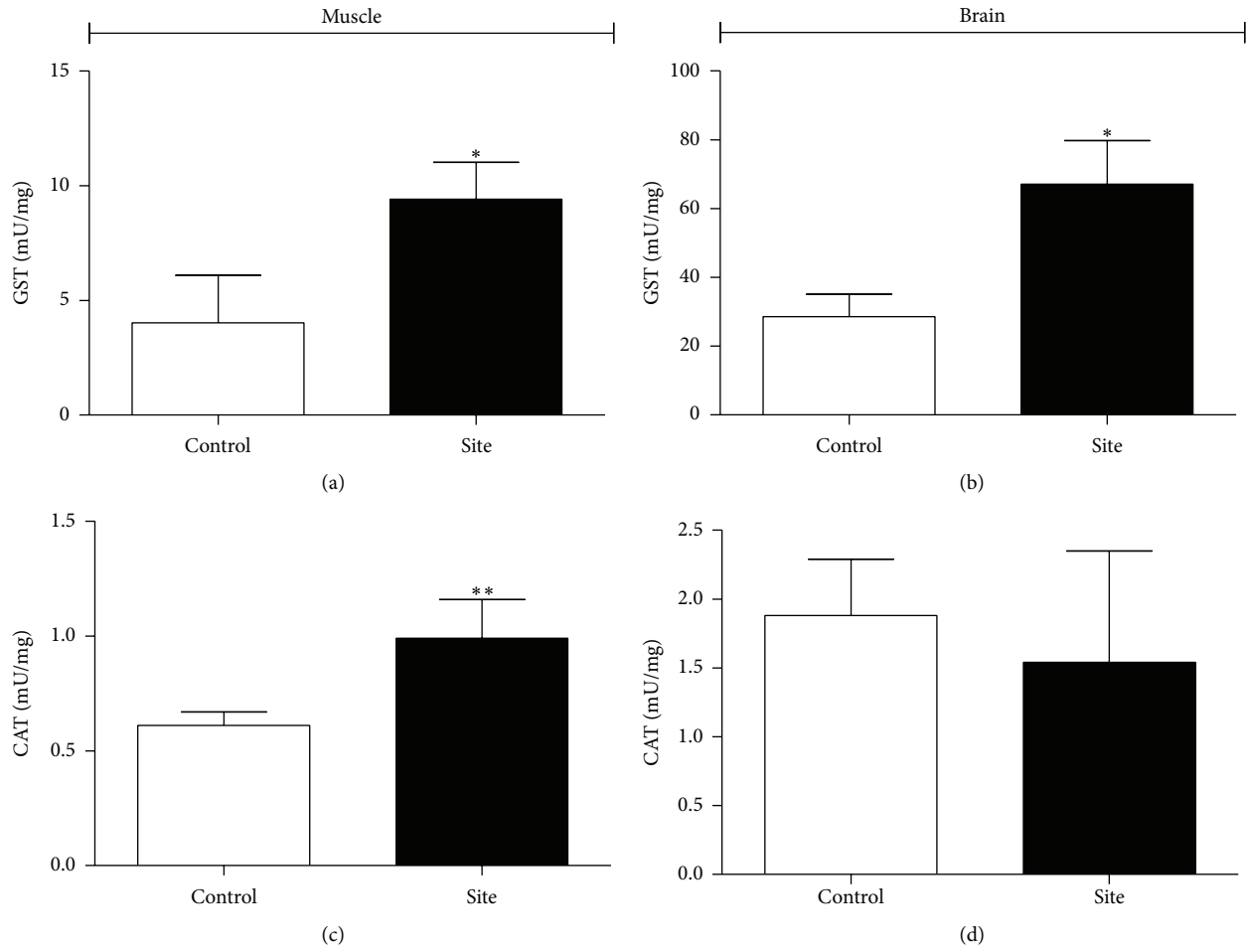

Figure 3: Activity of antioxidant enzymes in fish exposed or not to the polluted water. (a) GST activity in muscle and (b) brain tissues. (c) CAT activity in muscle and (d) brain was also determined. Data are presented as mean ± standard deviation of enzymatic activity expressed in mU/mg total protein. $^{*}P < 0.05$ and $^{**}P < 0.01$.

in muscle and brain of animals collected in the Santa Maria River (Figures 2(a) and 2(b)). However, there was a significant ($P < 0.05$) increase in TBARS content (Figure 2(c)) and total hydroperoxides (Figure 2(e)) in the muscle of exposed fish, compared to controls. There were no significant differences in TBARS and hydroperoxides in the brain of captured animals compared to control (Figure 2(d)).

3.3. Activity of Antioxidant Enzymes. A significant ($P < 0.05$) increase in GST activity was observed in muscle (Figure 3(a)) and brain (Figure 3(b)) of animals collected in the Santa Maria River, when compared to control. There was also a substantial increase in CAT enzyme activity, however, only in muscle ($P < 0.05$) (Figure 3(c)). Moreover, exposed animals also showed increased levels ($P < 0.05$) of GPx activity (Figure 4(a)) and TRxR (Figure 4(e)) in the muscle tissue, when compared to control. Glutathione reductase (GR) activity was not statistically altered, despite the fact that an increasing trend was observed in the muscle. In the brain tissue, minor changes in GPx, GR, and TrxR activity was observed.

4. Discussion

Currently, the contamination of water resources is a major environmental problem, being the aquatic environment more susceptible to anthropogenic effects, due to wastewater, industrial and agricultural discharges in watercourses [23]. These effluents are composed of various contaminants that, isolated or forming complex mistures, can pose high risk to aquatic organisms and ecosystems [2]. Thereby, studies on the assessment of environmental quality of these resources are important for determining the levels of damage caused by a range of contaminant mixtures. The Santa Maria River basin in the Brazilian Pampa biome is at constant risk, since it is located in a region of large agricultural and urban activities. Even though, at least in our knowledge, no studies on the assessment of environmental impacts caused by anthropogenic activities have been addressed in the Brazilian Pampa, especially in the Santa Maria River basin.

In the present study, a set of enzymatic biomarkers in fish (*Astyanax*) was applied to evaluate evidences of oxidative stress and changes in the activity of enzymes involved in the antioxidant cell defense machinery.

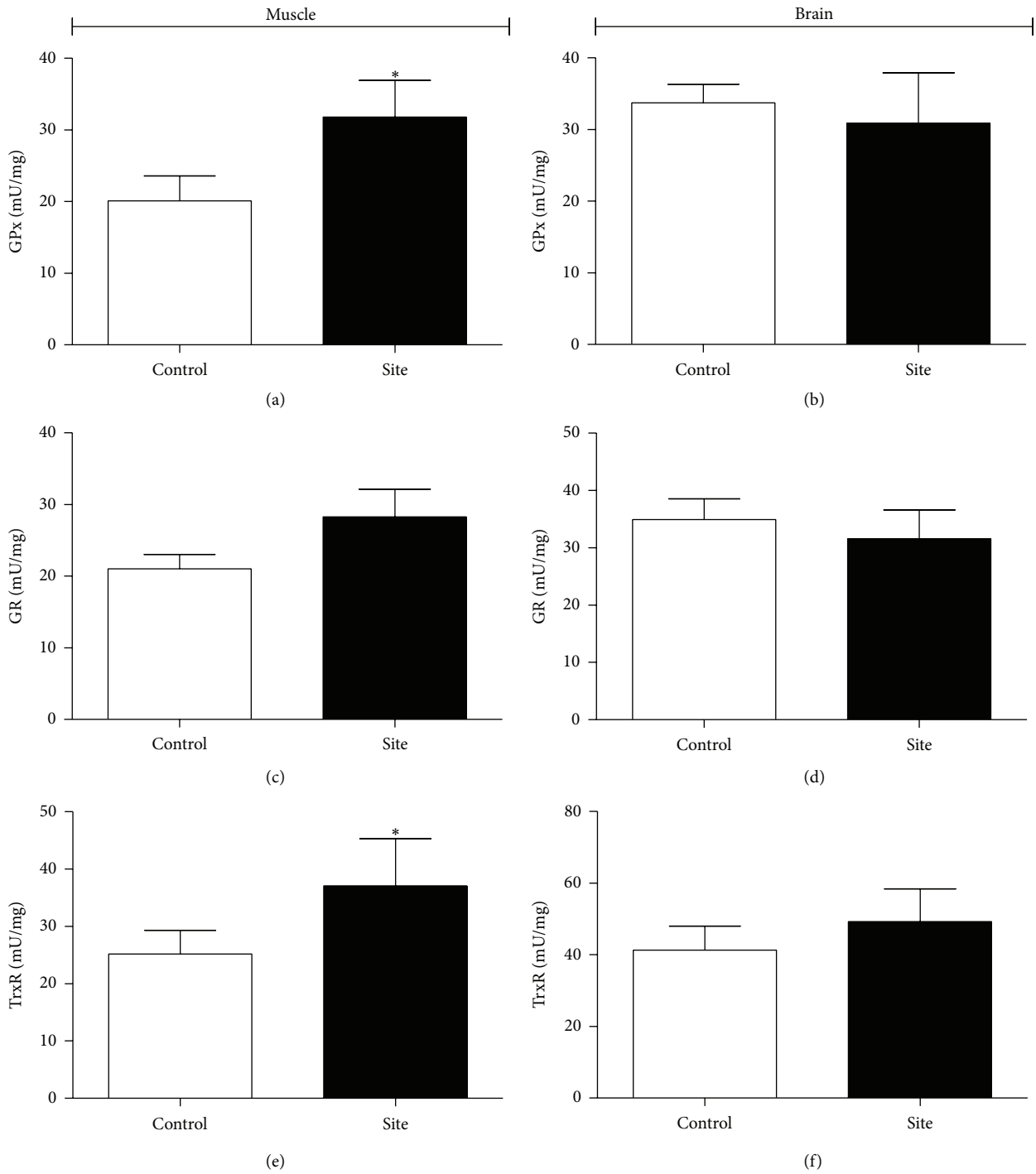

Figure 4: Activity of antioxidant enzymes in fish exposed or not to the polluted water. (a) GPx activity in muscle and (b) brain. (c) GR activity in muscle and (d) brain. (e) TrxR activity in muscle and (f) brain. Data are presented as mean ± standard deviation of enzymatic activity expressed in mU/mg total protein. $^{*}P < 0.05$.

Evidence for the presence of organophosphate and carbamate pesticides was apparent, as demonstrated by significant inhibition of fish AChE activity. Inhibition of AChE activity, as the demonstrated by our results, suggests the presence of potentially hazardous contaminants such as organophosphate (OPs) and carbamates pesticides (CARB) [24] which inactivate the enzyme by binding covalently to amino acid residues in the structure of the protein [25]. Inhibition of this enzyme is very harmful to fish, especially by compromising locomotor performance, resulting in hampered feeding and escaping behaviors [26]. Even with the prohibition of the use of some organophosphate formulations in Brazil

(Resolution: RDC n° 226, of 28 September 2004), OPs and CARB pesticides are still widely used in agriculture. Despite its apparent rapid degradation in the environment, these pesticides are not specific, presenting acute toxicity to nontarget organisms, thereby causing risk to both aquatic and terrestrial biota [27]. Even at sublethal doses, it is possible to identify environmental contamination by these pesticides for extended periods, because even after degradation in the environment, its effects on cholinesterase enzymes may last for weeks [28]. Despite the fact that CARB are considered less harmful pesticides, since they are reversible inhibitors of cholinesterases, some reports have shown the potential damage caused by this class of contaminants in aquatic organisms [29].

Enzymes involved in xenobiotic detoxification processes, including GSTs, have been widely used as biomarkers of aquatic contamination. Increases in GST activity in aquatic organism have been associated with contamination by the herbicide glyphosate [30, 31]. The observed increase in GST activity in muscle and brain of fish captured in the waters of Santa Maria River may indicate the presence of significant levels of contaminants ingested by the fish and then triggering detoxification responses. Antioxidant enzymes such as GPx, GR, TrxR, and CAT are primary defense lines against deleterious actions of reactive oxygen species (ROS) [31]. Some studies have linked the increase in antioxidant enzymes activity after exposure to organophosphate pesticides [32] and also other xenobiotics, such as heavy metals [33]. Such responses may indicate a compensatory mechanism to counteract ROS and the establishment of an oxidative stress condition in organisms exposed to a range of environmental contaminants.

The observed increase in several antioxidant enzymes in fish muscle captured in the Santa Maria River is in line with this hypothesis, pointing to the presence of prooxidative contaminants in this aquatic environment. This fact can be confirmed by the observed increase of lipid peroxidation products and hydroperoxides in exposed animals, compared to laboratory controls, which were maintained in quarantine under optimal environmental conditions. The increase in the activity of antioxidant enzymes such as CAT and TrxR in the fish captured in the studied site indicates the presence of prooxidative compounds released by human activities in the area. The TrxR is responsible for the redox cycling of thioredoxin (Trx), an electron-donor protein involved in the catalytic cycle of peroxiredoxins (Prx), among others. Like glutathione reductase (GR), TrxR is inserted into the process of cellular ROS detoxification [34]. The increased TrxR may also be acting as a compensatory mechanism increasing lipid peroxidation [35]. Regarding the increase in CAT activity, this cellular response is very common in environmentally impacted areas, as noted in the study by Bocchetti et al. (2008) [35]. In organisms exposed to heavy metals responses in terms of increases in CAT can also be observed as a response to ROS formation [36].

Overall, our results validate the use of *Astyanax* as model organism for studies on biomarkers of aquatic contamination in the Brazilian Pampa biome. In addition, the results presented here point to significant levels of water pollution in the Santa Maria River basin. The results also indicate antioxidant enzymes and acetylcholinesterase activity as well-responsive biomarkers in the fish species investigated. Additional studies are needed to determine which contaminants are present in this environment and the actual levels of biological damage to the aquatic biota are being caused by human activities in the region.

Conflict of Interests

The authors declare no conflict of interests.

Acknowledgments

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References

[1] IBGE—Instituto Brasileiro de Geografia e Estatística, *Mapa de Biomas do Brasil—Primeira Aproximação*, Ministério do Meio Ambiente, Brasília, Brazil, 2004.

[2] L. F. W. Roesch, F. C. B. Vieira, V. A. Pereira et al., "The Brazilian Pampa: a fragile biome," *Diversity*, vol. 1, no. 2, pp. 182–198, 2009.

[3] M. J. Cerejeira, P. Viana, S. Batista et al., "Pesticides in Portuguese surface and ground waters," *Water Research*, vol. 37, no. 5, pp. 1055–1063, 2003.

[4] G. M. Rand, P. G. Wells, and L. S. McCarty, "Introduction to aquatic toxicology," in *Fundamentals of Aquatic Toxicology: Effects, Environmental Fate and Risk Assessment*, G. M. Rand, Ed., pp. 3–67, Taylor & Francis, Washington, DC, USA, 2nd edition, 1995.

[5] L. L. Amado, R. B. Robaldo, L. Geracitano, J. M. Monserrat, and A. Bianchini, "Biomarkers of exposure and effect in the Brazilian flounder *Paralichthys orbignyanus* (Teleostei: Paralichthyidae) from the Patos Lagoon estuary (Southern Brazil)," *Marine Pollution Bulletin*, vol. 52, no. 2, pp. 207–213, 2006.

[6] A. J. D. Cogo, A. F. Siqueira, A. C. Ramos, Z. M. A. Cruz, and A. G. Silva, "Utilização de enzimas do estresse oxidativo como biomarcadoras de impactos ambientais," *Natureza Online*, vol. 7, no. 1, pp. 37–42, 2009.

[7] M. Valko, D. Leibfritz, J. Moncol, M. T. D. Cronin, M. Mazur, and J. Telser, "Free radicals and antioxidants in normal physiological functions and human disease," *International Journal of Biochemistry and Cell Biology*, vol. 39, no. 1, pp. 44–84, 2007.

[8] P. C. Huber, W. P. Almeida, and A. Fátima, "Glutationa e enzimas relacionas: papel biológico e importância em processos patológicos," *Química Nova*, vol. 31, no. 5, pp. 1170–1179, 2008.

[9] J. T. Hamm, B. W. Wilson, and D. E. Hinton, "Increasing uptake and bioactivation with development positively modulate diazinon toxicity in early life stage medaka (*Oryzias latipes*)," *Toxicological Sciences*, vol. 61, no. 1, pp. 304–313, 2001.

[10] M. P. Godoy, *Peixes do Brasil, Subordem Characidade*, vol. 4, Franciscana, São Paulo, Brazil, 1975.

[11] U. H. Schulz and H. Martins Jr., "Astyanax fasciatus as bioindicator of water pollution of Rio dos Sinos, RS, Brazil," *Brazilian Journal of Biology*, vol. 61, no. 4, pp. 615–622, 2001.

[12] E. U. Winkaler, A. G. Silva, H. C. Galindo, and C. B. R. Martinez, "Biomarcadores histolsgicos e fisiolsgicos para o monitoramento da sazde de peixes de ribeirues de Londrina, Estado do Parana," *Acta Scientiarum*, vol. 23, pp. 507–514, 2001.

[13] A. G. Silva and C. B. R. Martinez, "Morphological changes in the kidney of a fish living in an urban stream," *Environmental Toxicology and Pharmacology*, vol. 23, no. 2, pp. 185–192, 2007.

[14] C. T. de Lemos, F. D. A. Iranço, N. C. D. de Oliveira, G. D. de Souza, and J. M. G. Fachel, "Biomonitoring of genotoxicity using micronuclei assay in native population of *Astyanax jacuhiensis* (Characiformes: Characidae) at sites under petrochemical influence," *Science of the Total Environment*, vol. 406, no. 1-2, pp. 337–343, 2008.

[15] I. Carlberg and M. Beng, "Glutathione reductase," *Methods in Enzymology*, vol. 113, pp. 484–490, 1985.

[16] W. H. Habig and W. B. Jakoby, "Assays for differentiation of glutathione *S*-transferases," in *Methods in Enzymology*, vol. 77, pp. 398–405, 1981.

[17] H. Aebi, "Catalase *in vitro*," in *Methods in Enzymology*, vol. 105, pp. 121–126, 1984.

[18] A. Holmgren and B. Mikael, "Thioredoxin and thioredoxin reductase," *Methods in Enzymology*, vol. 252, pp. 199–208, 1995.

[19] G. L. Ellman, K. D. Courtney, V. Andres Jr., and R. M. Featherstone, "A new and rapid colorimetric determination of acetylcholinesterase activity," *Biochemical Pharmacology*, vol. 7, no. 2, pp. 88–95, 1961.

[20] G. L. Ellman, "Tissue sulfhydryl groups," *Archives of Biochemistry and Biophysics*, vol. 82, no. 1, pp. 70–77, 1959.

[21] R. Y. Tomita and Z. Beyruth, "Toxicologia de agrotóxicos em ambiente aquático," *Biológico*, vol. 64, no. 2, pp. 135–142, 2002.

[22] C. Fernández-Vega, E. Sancho, M. D. Ferrando, and E. Andreu, "Thiobencarb-induced changes in acetylcholinesterase activity of the fish *Anguilla anguilla*," *Pesticide Biochemistry and Physiology*, vol. 72, no. 1, pp. 55–63, 2002.

[23] J.-H. Jung, R. F. Addison, and W. J. Shim, "Characterization of cholinesterases in marbled sole, *Limanda yokohamae*, and their inhibition in vitro by the fungicide iprobenfos," *Marine Environmental Research*, vol. 63, no. 5, pp. 471–478, 2007.

[24] T. Bálint, T. Szegletes, Z. S. Szegletes, K. Halasy, and J. Nemcsók, "Biochemical and subcellular changes in carp exposed to the organophosphorus methidathion and the pyrethroid deltamethrin," *Aquatic Toxicology*, vol. 33, no. 3-4, pp. 279–295, 1995.

[25] M. H. Fulton and P. B. Key, "Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects," *Environmental Toxicology and Chemistry*, vol. 20, no. 1, pp. 37–45, 2001.

[26] G. M. Benke and S. D. Murphy, "Anticholinesterase action of methyl parathion, parathion and azinphosmethyl in mice and fish: onset and recovery of inhibition," *Bulletin of Environmental Contamination and Toxicology*, vol. 12, no. 1, pp. 117–122, 1974.

[27] M. Crestani, C. Menezes, L. Glusczak et al., "Effect of clomazone herbicide on biochemical and histological aspects of silver catfish (*Rhamdia quelen*) and recovery pattern," *Chemosphere*, vol. 67, no. 11, pp. 2305–2311, 2007.

[28] L. Glusczak, D. dos Santos Miron, M. Crestani et al., "Effect of glyphosate herbicide on acetylcholinesterase activity and metabolic and hematological parameters in piava (*Leporinus obtusidens*)," *Ecotoxicology and Environmental Safety*, vol. 65, no. 2, pp. 237–241, 2006.

[29] R. Cattaneo, B. Clasen, V. L. Loro et al., "Toxicological responses of *Cyprinus carpio* exposed to a commercial formulation containing glyphosate," *Bulletin of Environmental Contamination and Toxicology*, vol. 87, no. 6, pp. 597–602, 2011.

[30] B. Halliwell and J. Gutteridge, *Free Radicals in Biology and Medicine*, vol. 1, Oxford University Press, New York, NY, USA, 2007.

[31] S. Peña-Llopis, M. D. Ferrando, and J. B. Peña, "Impaired glutathione redox status is associated with decreased survival in two organophosphate-poisoned marine bivalves," *Chemosphere*, vol. 47, no. 5, pp. 485–497, 2002.

[32] A. Rodriguez-Ariza, N. Abril, J. I. Navas, G. Dorado, J. López-Barea, and C. Pueyo, "Metal, mutagenicity, and biochemical studies on bivalve molluscs from Spanish coasts," *Environmental and Molecular Mutagenesis*, vol. 19, no. 2, pp. 112–124, 1992.

[33] L. Flohé and J. R. Harris, *Peroxiredoxin Systems: Structures and Functions*, vol. 44, Springer, 2007.

[34] L. Zhong and A. Holmgren, "Essential role of selenium in the catalytic activities of mammalian thioredoxin reductase revealed by characterization of recombinant enzymes with selenocysteine mutations," *The Journal of Biological Chemistry*, vol. 275, no. 24, pp. 18121–18128, 2000.

[35] R. Bocchetti, D. Fattorini, B. Pisanelli et al., "Contaminant accumulation and biomarker responses in caged mussels, *Mytilus galloprovincialis*, to evaluate bioavailability and toxicological effects of remobilized chemicals during dredging and disposal operations in harbour areas," *Aquatic Toxicology*, vol. 89, no. 4, pp. 257–266, 2008.

[36] G. Atli and M. Canli, "Enzymatic responses to metal exposures in a freshwater fish *Oreochromis niloticus*," *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, vol. 145, no. 2, pp. 282–287, 2007.