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Benthic fish blood as a biomarker for recent exposure to mercury

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ABSTRACT

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This study evaluated the concentration of Hg in the blood of the benthic fish species *Geophagus brasiliensis* captured from the Rio Grande and Billings Reservoir, which is located along the Upper Tietê River Basin (São Paulo, Brazil), to determine recent exposure. The relationship between blood and sediment Hg concentrations was evaluated. Sediment and fish blood samples were collected at six sampling sites during the rainy (January-March) and dry season (July-August) of 2009, and the rainy season (January) of 2010. Total Hg in blood and in sediments was determined by cold vapor atomic absorption spectrometry (CV AAS). The highest Hg concentrations in blood occurred in sampling sites located downstream from a chlor-alkali plant. Weight and length of fish were marginally associated with concentrations of Hg in blood. According to international guidelines for sediment quality, Hg concentrations were higher than Probable Effect Level (PEL) (0.49 µg/kg) in the final stretch of the Grande River and in the Billings Reservoir, while the remaining sites presented values between 0.06 and 0.35 µg/kg. Pearson correlation analysis between the concentrations of Hg in blood and sediment was positive and significant ($r=0.844$; $p<0.05$), showing that quantification of Hg in blood can be an useful tool for biomonitoring, indicating recent exposure, as well as helping as an early warning indicator of environmental contamination.

Key words: bioaccumulation, environmental quality, neotropical teleost, total mercury

RESUMO

Utilização de sangue de peixe bentônico como um biomarcador de exposição recente ao mercúrio

Neste estudo foi avaliada a concentração de Hg em sangue do peixe bentônico Geophagus brasiliensis *capturado no Rio Grande e no Reservatório Billings, da Bacia Hidrográfica do Alto Tietê (São Paulo, Brasil), para determinar exposição recente de Hg. A relação entre as concentrações de Hg no sangue e sedimento foram avaliadas. Amostras de sangue dos peixes e do sedimento foram coletadas em seis estações amostrais durante a estação chuvosa (Janeiro-Março) e seca (Julho-Agosto) de 2009, e estação chuvosa (Janeiro) de 2010. O Hg total no sangue e no sedimento foi determinado por espectrofotômetro de absorção atômica com vapor a frio (CV AAS). As maiores concentrações de Hg no sangue de peixe ocorreram nas estações de coleta localizadas à jusante de uma indústria de cloro-soda. O peso e comprimento dos peixes estiveram marginalmente* *associados com a concentração de Hg do sangue. De acordo com as normas internacionais para qualidade do sedimento, as concentrações de Hg foram maiores do que o Probable Effect Level (PEL) (0.49 µg/kg) no trecho final do Rio Grande e do Reservatório Billings, enquanto que as outras estações de coleta apresentaram valores entre 0.06 e 0.35 µg/kg. A análise de correlação de Pearson entre a concentração de Hg no sangue de peixe e no sedimento foi positiva e significativa (r=0.844;* p*<0.05), mostrando que a quantificação de Hg no sangue de peixe pode ser utilizada como ferramenta de biomonitoramento, indicando a exposição recente e auxiliando como um indicador de alerta precoce de contaminação ambiental.*

Palavras chave: *bioacumulação, qualidade ambiental, teleósteo neotropical, mercúrio total*

INTRODUCTION

Mercury (Hg) is a non-essential element that occurs naturally in the environment at low concentrations, and it originates from natural geologic emissions as well as anthropogenic activities. Worldwide, it is estimated that around 2000 to 3000 tons/year of Hg are discharged anthropically into the environment, considering global release associated with coal burning, gold mining, production of non-ferrous metals and industrial effluents (Zagatto & Bertoletti, 2006; Streets *et al.*, 2011; UNEP, 2013). Atmospheric Hg emissions since 1890 were estimated to be 200 000 tons, of which 95 % remained in soil and sediments, 3 % in surface water and 2 % in the atmosphere (Micaroni, 2000). In Brazil, industrial use of Hg and consequent release to water has been reduced in recent years owing to stricter regulations (Azevedo, 2003).

However, sediment is an active compartment of the aquatic environment, accumulating material from the water column that can become available either in solution or directly to the biota (Almeida *et al.*, 2014). Contaminants can be gradually released from the sediment, which may, therefore, act as a secondary source of pollutants, even after the primary source of pollution has been controlled (Chapman *et al.*, 1999; Burton, 2002). From an ecological perspective, sediments are habitat for the benthic community, including microbes and macrofauna, which process the organic matter (Chapman, 1990). Organisms inhabiting contaminated sediments are generally exposed to these elements and may transfer them to higher trophic levels. While concentrations of mercury in the water column are generally well below reported effects levels, the relationship between effects and diet or sediment-related exposure is unclear (Zillioux *et al.*, 1993). Inorganic mercury is transformed into methylmercury primarily in sediments (UNEP, 2013); thus, it is important to evaluate sediment samples and the possibility of the availability of this element through benthic organisms.

In order to meet safety standards for human consumption and water use, water and fish muscle samples are the matrices of choice for metals assessment (Ferreira *et al.*, 2006; Arantes *et al.*, 2009). More recently, biomarkers have been applied to determine the levels of contamination of environments and make risk assessments. In general, biomarkers can be defined as biological responses to environmental stressors that can be measured, indicating the presence, possible effects, and, in some cases, the degree of contamination.

Biomarkers can be considered as early indicators of contamination, in particular because they can be determined in tissues and biological fluids. Specifically, biomarkers indicate the exposure of an organism to xenobiotics and their metabolites and/or to metals, and they are a reflection of the intensity of such exposure (Walker *et al.*, 1996; Nordberg *et al.*, 2007). According Bernard & Lauwerys (1986), the concentration of polychlorinated biphenyl (PCB) in blood is a good indicator of concentrations accumulated in the main tissues, such as fatty tissues. Thus, measuring the internal concentrations of toxic compounds, or metabolites, in body fluids or excreta, such as blood, urine and exhaled air, may also indicate the amount of a chemical stored in one or several body compartments, or even in the whole body (Gupta, 2014).

Fish are considered excellent models for biological studies, assuming great importance in assessing environmental toxicity in impacted areas because of their presence in a variety of habitats, large geographical distribution and inclusion of species in different trophic levels (van de Oost *et al.*, 2003). Furthermore, fish have often been utilized in pollution monitoring in aquatic systems.

Studies using fish as biomarkers have most commonly quantified Hg in tissues, such as gills, liver, kidney, brain and muscles (Bastos *et al.*, 2015a,b, 2016; Carrasco *et al.*, 2011; Kütter *et al.*, 2015; Monteiro *et al.*, 2010, 2013). These studies indicate that fishes respond differently to the accumulation of Hg. Monteiro *et al.* 2010, studying the pattern of inorganic mercury accumulation in different organs of *Brycon amazonicus* (matrinxã), found higher concentrations in gills, followed by liver, heart, and, finally, white muscle. And according to their trophic level, since scavengers and herbivores have a lower concentration of mercury in muscle when compared to species of higher trophic levels (Bastos *et al.*, 2015a). For example, in the Ebro River basin (NE Spain), piscivorous fish muscle tissue showed on average 2.8 and 2.4 times more total mercury (THg) and methylmercury (MeHg), respectively, than non-piscivorous fish (Carrasco *et al.*, 2011). These studies show bioaccumulation of mercury in different fish tissues, indicating prolonged exposure to this metal.

The determination of an element in the blood of an organism reflects recent exposure to that contaminant (Mergler *et al.*, 2007), providing information for environmental quality assessment and safety standards for a given ecological guild. In the literature, most studies have used this biomarker for different animal species from temperate regions (Olson *et al.*, 1973; Ribeyre & Boudou, 1984; Wayland, 2001), while few fish studies have quantified Hg in blood matrix as a biomarker of recent exposure (van der Oost, 2003; Guilherme *et al.*, 2008).

Rivers and reservoirs of the Upper Tietê River

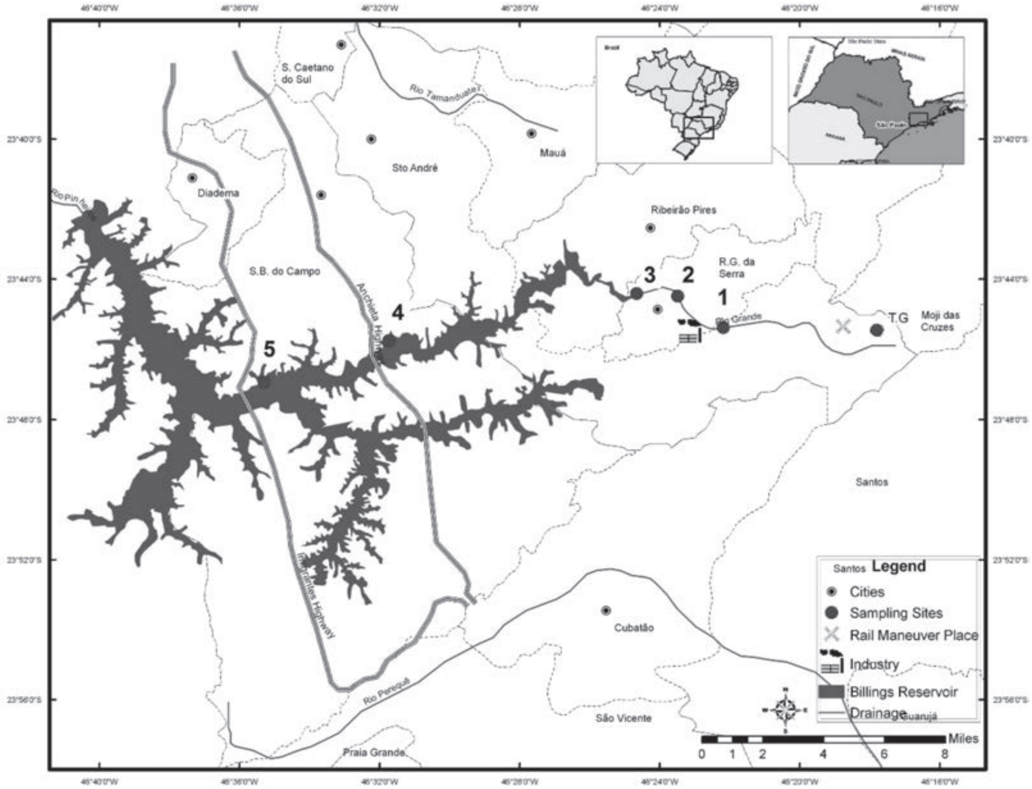

Figure 1. Map of the study area showing the Billings Reservoir and location of the Grande River, Rio Grande Reservoir, and sampling sites. In detail, location of the area in São Paulo State, Brazil. *Área de estudo mostrando o Reservatório Billings e a localização do Rio Grande, Reservatório Rio Grande, e os pontos amostrais. Em detalhe, a localização da área no Estado de São Paulo, Brasil.*

Basin are subject to strong anthropogenic influence, resulting in the exposure of the biota to several pollutants. Billings Reservoir is an important supplier of drinking water in the Metropolitan Region of São Paulo (MRSP), and it produces about 7 m^3/s of water for public supply (SABESP, 2016). For more than 50 years, this reservoir has received part of the load of industrial and domestic sewage of the Metropolitan Region of São Paulo. In 2013, about 87.6 % of the sewage produced in the MRSP was collected, but only 41.1 % was treated before it was discharged into the rivers (SNSA, 2015). Currently, Billings Reservoir is used for power generation and to increase the capacity of water supply during the dry season (April-September). Part of this water comes from the Grande River Reservoir, one of the main arms of the Billings Complex, which, however, was isolated from the main body of the reservoir at the beginning of the 1980s, in order to preserve Grande River water quality by, eliminating polluted water from the City of São Paulo (Maier *et al.*, 1997; Capobianco, 2002). Recent studies have shown that the sediment of the Grande River Reservoir presents Hg and concentrations of other metal species above Threshold Effect Level (TEL) and Probable Effect Level (PEL) values (CETESB, 2007; Hortellani *et al.*, 2013). These Hg concentrations seem to be related to a chlor-alkali plant that has been operating at the Rio Grande Reservoir since 1948 (Franklin *et al.*, 2016). However, owing to stricter Brazilian regulations (MAPA, 2002) since 2010, effluents from this chlor-alkali plant have been sent to a wastewater treatment station and are no longer discharged into the Grande River Reservoir.

Thus, while the Grande River has been subjected to industrial contamination in its lower course for more than 50 years, the presence of Hg in fish blood may reflect recent exposure to that contaminant due, mainly to its presence in the sediment, acting as a secondary source. Therefore, the purpose of this study was to measure the concentration of Hg in the blood of the benthic fish species *Geophagus brasiliensis* captured from the Grande River and Billings Reservoir to determine recent exposure in this freshwater reservoir and, hence, determine its potential use as a tool for monitoring environmental quality.

MATERIAL AND METHODS

Study Area

The Grande River is located in the Upper Tietê River Basin (Billings-Tamanduateí Sub-basin), and it is about 20 km long. Its headwaters rise within the Parque Natural Municipal Nascentes de Paranapiacaba (PNMNP), a Conservation Unit in the mountain range of Paranapiacaba (Fig. 1). This ridge is inserted within the Paulistano Plateau of the Atlantic Plateau Geomorphic Province, protecting 426 hectares of Atlantic Forest (PMSA, 2008). The climate is tropical humid, with an average annual rainfall of 3000 mm, occasionally 4000 mm. The average air temperature in the warmest months is 22 °C, and in the coldest months, it is 18 ºC (PMSA, 2008).

Near the headwaters, the river was dammed in 1900 by the British to supply water to a funicular system, forming the Gustavo Tank, a clear water pool that is currently used to supply the town of Paranapiacaba in the Municipality of Santo André (PMSA, 2008). In its medium-lower course, the Grande River crosses a heavily urbanized area, with the presence of some industries. Near the City of Rio Grande da Serra, the Grande River receives domestic and industrial effluents, forming the 112.10^6 m^3 Grande River Reservoir (SABESP, 2016). As noted above, this reservoir was isolated in 1981 from the main body of the Billings Reservoir in order to preserve water quality at appropriate levels for public supply (Maier *et al.*, 1997; Capobianco, 2002). The Grande River Reservoir now supplies water to 1.6 million people in the municipalities of Diadema, São Bernardo do Campo and part of Santo André (SABESP, 2009).

To evaluate fish blood as an efficient biomarker for recent exposure to Hg, six sites located between the coordinates 23º46'10.6"S, 46º17'38.9"W and 23º47'05.6"S, 46º35'27.8"W were selected (Fig. 1). Close to the headwaters, the Gustavo Tank (GT) was considered a "control site". Site 1 is located upstream of chloro-alkali plant, while sites 2 and 3 are within the urban area of Rio Grande da Serra City, downstream of the chloro-alkali plant. Site 4 is located in the Grande River Reservoir near a water treatment plant

(Sanitation Company of the State of São Paulo - SABESP (ETA – Grande River)), and site 5 is located in the central body of the Billings Reservoir, in an area that was cut off from the Grande River Reservoir. This site was selected because it receives domestic and industrial effluents from the rivers that reach the Grande River Reservoir.

Sampling procedure

Sediment

Sediment samples were collected during the rainy (January/March) and dry season (July/August) of 2009 at each site using van Veen and Petersen samplers, which are recommended for collecting surface samples (USEPA, 2001). Sediment grain size determination (granulometry) was performed according to CETESB L6.160 (CETESB 1995) and based on the principles of sieving and sedimentation guided by the Wentworth scale. The granulometric classification was based on the following criteria: clay (particles with diameter smaller than 0.004 mm), silt (particles from 0.004 to 0.063 mm), and sand (particles with dimensions between 0.063 and 2.0 mm). Sediment samples were placed in polyethylene bottles previously decontaminated with nitric acid solution 10 %. The samples were refrigerated until processing in the laboratory. For total Hg analysis, sediment samples were digested according to USEPA Method SW 846-3051A (USEPA, 1997), using a total sample fraction <2.00 mm. Sediment samples of 0.5 g were digested with 9.0 mL of HNO3 in a microwave oven with a closed system (ETHOS 1 Milestone) in Teflon® tubes for 10 minutes at a temperature of 175 °C (Milestone). The digest was transferred quantitatively to polyethylene tubes, and the volume was completed up to 50 mL with deionized water (18.0 M Ω). Total Hg was determined by cold vapor atomic absorption spectrometry (CV AAS) with a flow injection system (Perkin Elmer, model FIMS 100), using tin chloride (II) as the reducing agent. The analytical accuracy of the method was evaluated by using Standard Reference Material (NIST 2704 Buffalo River Sediment) with satisfactory recoveries between 80 and 120 %.

Fish

The degree to which fish from the Grande River, Grande River and Billings Reservoirs were exposed to Hg was estimated by evaluating the concentrations of Hg in blood of the cichlid *Geophagus brasiliensis* Gebr (pearl cichlid). This fish species was selected because of its wide distribution in the study sites (Furlan *et al.*, 2013), nonmigratory behavior (Froese & Pauly, 2010), omnivorous feeding habits and benthivorous feeding behavior (Nunes *et al.*, 2014). Therefore, it occupies a key position in the bioaccumulation of Hg and its transfer up the trophic food chain in this biota.

Specimens of *G. brasiliensis* collected during the rainy (January/March) and dry season (July/August) of 2009 were weighed, measured and tagged in the field. As no sample was obtained in the GT during the dry season, sampling was repeated at this site during the rainy season of 2010 (January). Monofilament gill nets (mesh size 20, 30, 35, 40 and 45 mm between adjacent nodes) of different heights and lengths were used. The nets remained for 12 hours at each site during the night and/or day to catch as many specimens as possible. When necessary, monofilament hand nets (15 and 35 mm between adjacent nodes) were also used.

The fish were kept alive until blood was sampled by caudal puncture with syringes previously treated with anticoagulant solution (EDTA-ethylenediaminetetraacetic disodium). Fish were released back into the environment after blood sampling. Blood was stored in Eppendorf microtubes treated with anticoagulant and kept under refrigeration until processing in the laboratory. Blood samples from fish with volumes ranging from 100 to 500 µL were digested with 2.7 mL of nitric acid 65 % (Carlo Erba) and 0.7 mL of hydrogen peroxide 30 % (Fluka) in a microwave oven with a closed system (ETHOS 1 Milestone) in Teflon® tubes for 15 minutes at a temperature of 230 °C (Milestone). The digest was transferred quantitatively to polyethylene tubes, and the volume was measured to 10 mL with deionized water (18.0 MΩ). Total Hg was analyzed by cold vapor atomic absorption spectrometry (CV AAS) with a flow injection system (Perkin Elmer,

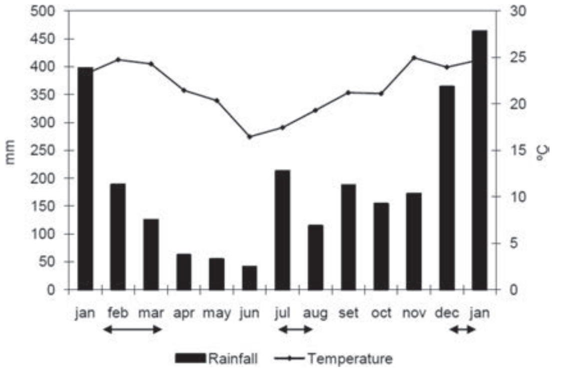

Figure 2. Total monthly rainfall (mm) and monthly mean temperature (ºC) in the Upper Tietê River Basin (January 2009 to January 2010). Arrows indicate sampling months. (Data obtained at the Centro Integrado de Informações Meteorológicas – CIIAGRO). *Precipitação mensal total (mm) e temperatura média mensal (º C) na Bacia Hidrográfica do Alto Rio Tietê (Janeiro 2009 até Janeiro de 2010). As setas indicam os meses amostrados. (Dados obtidos no Centro Integrado de Informações Meteorológicas – CIIAGRO).*

model FIMS 100), using tin chloride (II) as the reducing agent. The accuracy was assessed using certified reference material (CRM) (Bio-Rad Laboratories (Lyphochek®), Irvine, CA, USA - Level 1) in the range of 5.65 to 8.48 µg/L Hg and average value of 7.06 µg/L Hg.

Data Analysis

Statistical analyses were performed on SPSS software (version 12.0) and StatGraph Plus (version 2.1), The limit of quantification (LOQ) of the method used for blood analysis was 0.25 µg/L. Therefore, when a value was below this limit, half the limit value was used for the statistical analysis. A univariate linear model (Morrison, 1967; Breslow, 1996) was adjusted to test differences of Hg concentrations in the blood of *G. brasiliensis*. Sites and seasons were considered fixed factors, while fish weight and length were considered covariates. An ANCOVA was used to test the significance of each of these factors in the model. To verify the significance of the effect of the season and sampling sites on the mercury concentrations in the sediment, a general linear model was also adjusted. The data was previously transformed in log (x + 1). The Pearson correlation was applied between concentrations of Hg in sediment and fish blood, data was also transformed applying log (x + 1). The significance level adopted in the analyses was $p \leq 0.05$. For this last analysis, the statistical software package *Statistica* version 7.1. was also used.

RESULTS

Average monthly temperatures in the study area ranged from 16.5 ºC (dry season) to 25.0 ºC (rainy season), and monthly rainfall ranged from 40.9 mm (dry season) to 463.4 mm (rainy season 2), with abnormally high rainfalls observed during the dry season, especially in July 2009 (Fig. 2). The sampling period was considered one of the wettest years in the Upper Tietê River Basin (CETESB, 2010).

Sediment and mercury concentrations

Based on the granulometric classification of sediments, most sampling stations showed silt or sand to be predominant (Table 1). As expected, silt fraction occurred in considerable percentage

Table 1. Granulometric distribution of the sediment from the different sampling sites during the rainy (R1 and R2) and dry (D) seasons. Sampling sites according to Figure 1. *Distribuição granulométrica do sedimento em diferentes pontos amostrados durante o período chuvoso (R1 e R2) e seco (D). Pontos amostrais de acordo com a Figura 1.*

Granulometry	GT		Site 1		Site 2		Site 3		Site 4		Site 5	
	R1	R2	R1	D	R1	D	R1	D	R1	D	R1	D
Sand (%)	7.43	18.05	77.31	5.85	93.89	97.96	95.23	97.77	20.75	28.95	2.48	42.13
Silt (%)	60.05	56.08	19.16	59.13	3.96	1.06	3.23	1.36	49.73	58.79	47.31	39.60
Clay (%)	32.52	25.87	3.53	35.02	2.15	0.67	1.54	0.87	29.52	12.25	50.21	18.27

Table 2. Mercury concentrations in the sediment from the different sampling sites in the dry and rainy seasons. GT = Gustavo Tank. Sampling sites according to Figure 1. *Concentração de mercúrio no sedimento em diferentes pontos amostrados no período seco e chuvoso. GT = Tanque do Gustavo. Pontos amostrais de acordo com a Figura 1.*

Sites	Hg concentrations (mg/kg)		
	Rainy	**Dry**	**Mean**
GT	0.35	0.06*	0.21
1	0.10	0.11	0.11
2	2.30	2.94	2.62
3	1.06	0.86	0.96
4	0.27	0.10	0.19
5	0.79	0.31	0.55

* collection during the 2010 rainy season

in all seasons in lentic sites GT, 4 and 5, and sand was prevalent in river sites 2 and 3, as well as site 1 during the rainy season.

Higher Hg concentrations were observed in the sediment of sites 2 and 3, and then in site 5, irrespective of the collection period. The lowest average concentrations in the dry and rainy seasons were observed at sites 1 and 4 (0.11 and 0.19 mg/kg, respectively). These average values were lower than those observed in the GT reference site (0.21 mg/kg) (Table 2). An adjusted linear model was applied between Hg concentrations in the sediment, along different seasons and sites of collection, and the results revealed marginally significant difference (F=4.18; *p*=0.067). The variance explained was 85 %. A marginally significant difference in the mean Hg concentrations was observed between sampling sites (F=4.40; *p*=0.065). However, no significant difference was noted between seasons (F=0.02; *p*=0.900).

Mercury concentrations in fish blood

A total of 105 specimens of *G. brasiliensis* was recorded, 54 collected in the rainy season and 51 in the dry season. Individuals with lower weight and length were observed in the control site (GT), and those with higher weight were observed in sites 2 and 5 (Table 3).

The determination of Hg in fish blood samples was efficient, averaging 98 % recovery. Lower average Hg concentrations in fish blood were obtained in the control site (GT) and in site 4, where all concentrations had values below the LOQ. Higher mean concentrations were recorded at sites 3 and 5 (Table 4).

The results of the general univariate linear model for Hg in fish blood indicated that the adjustment was significant for the variables, including site, weight and length (F=10.47; *p*=<0.001), and the variance explained was 60 %. The weight and length of fish were marginally associated with Hg concentrations in fish blood (F=3.88; *p*=0.052 and F=3.15, *p*=0.079, respectively). Significant differences was found

Table 3. Minimum, maximum, median, mean and standard deviation (SD) of the weight and length of *G. brasiliensis* obtained at different sampling sites in the two seasons. Sampling sites according to Figure 1. N = number of individuals; GT = Gustavo Tank. *Valor mínimo, máximo, mediano, médio e desvio padrão (SD) do peso e comprimento* G. brasiliensis *obtido em diferentes pontos amostrados em dois períodos. Pontos amostrais de acordo com a Figura 1. N = número de indivíduos; GT = Tanque do Gustavo.*

Site	N	Weight (g)				Length (cm)			
		Min - Max	**Median**	**Mean**	**SD**	**Min - Max**	**Median**	**Mean**	**SD**
GT	10	11.5 – 113.2	66.60	64.68	30.40	11.0 – 18.2	15.10	15.26	1.90
1	6	23.1 – 140.0	99.60	97.17	40.44	10.0 – 21.0	17.80	17.23	3.86
2	15	24.7 – 195.5	102.50	118.68	50.61	10.2 – 21.2	16.10	16.42	3.18
3	9	46.5 – 149.0	91.50	94.94	38.92	13.0 – 18.2	16.50	15.74	2.02
4	36	10.9 – 161.0	115.60	86.39	52.16	8.7 – 20.0	17.80	15.39	4.01
5	29	10.5 – 200.3	151.90	152.63	31.88	12.0 – 22.3	19.30	19.41	2.00
Total	105	10.9 – 200.3	119.00	108.58	52.39	8.7 – 22.3	18.00	16.77	3.48

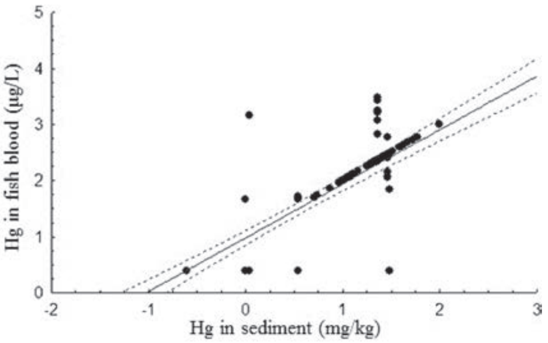

Figure 3. Linear correlation analysis between the concentrations of Hg in sediment and *G. brasiliensis* blood. (Dashed line = significance level of 95 %). *Análise da correlação linear entre a concentração de Hg no sedimento e no sangue de* G. brasiliensis*. (Linha tracejada = nível de significância de 95 %).*

between mercury concentrations in fish blood and sampled sites (F=3.76; *p*=0.002). The site-season interaction were significantly associated with Hg concentrations in fish blood (F=8.25; *p*=<0.001).

Relationship between mercury in fish blood and sediment

A correlation was found between Hg concentrations observed in *G. brasiliensis* blood and Hg concentrations found in sediment (Pearson correlation, r=0.844; *p*<0.05) (Fig. 3).

DISCUSSION

Sediment and Hg concentrations

Classification of sediment size was different among sampling stations. All sites showed a considerable amount of silt, but at the lotic sites (2 and 3) the sand fraction predominated. Grain size is a major factor that determines the distribution of metals in sediments (Zhang *et al.*, 2002), and the mean diameter of sediment particles correlates with the concentration of Hg, with lower levels being found in sandy sediments (Nelson *et al.*, 1977). Kütter *et al.* (2015) observed that Hg concentration in sediment fraction less than 63 µm was around 2 times higher than in the total fraction. However, in the Grande River, higher Hg concentrations were found in areas where sand predominated, probably because these sites (2 and 3) were located downstream of the chlor-alkali plant, a known Hg source.

Sites 1 and 4 showed lower mean values of Hg, which were even lower than the control site (GT). According to Shanker *et al.* (1996), rivers that receive large loads of pollutants by their proximity to major urban centers and areas with agricultural and industrial activities, can be considered as potential sources of mercury. Although Hg concentration in the sediment of GT was low, this site may have received some contamination in the past as a result of its location in an area that was used for mineral storage.

Analyzing only the fine sediment fraction (<63 µ) of the Billings Reservoir, Hortellani *et al.* (2013) obtained Hg concentrations between <0.30 and 2.50 µg/g. Thus, the Hg concentrations found in the sediment (total fraction) of the final stretch of the Grande River, sites 2 and 3, with mean concentrations of 2.62 and 0.96 mg/kg, are proportionally much higher, considering that the clay fraction was below 3 % in all samples from these sites. These results are also higher than those obtained from mining areas as reported by Ramos & Filho (1996) in the Preto River from the Paraíba do Sul River Basin, Brazil (0.65 mg/kg) and by Windmöller *et al.* (2007), in the Monsenhor Horta River, located in Mariana City – MG, Brazil (0.06 to 0.47 mg/kg).

The results were compared with values of sediment quality guidelines established by the Canadian Council of Ministry of Environment (CCME, 1999) for total sediment, which were adopted as Federal (CONAMA) and State (CETESB) guidelines for sediment quality monitoring to protect aquatic life. In this comparison, only site 1 presented Hg values below TEL (0.17 mg/kg), indicating that the concentration of Hg had reached threshold values which could affect the biota in both seasons and GT and site 4 in the dry season. In both seasons, Hg sediment values in sites 2 and 3 were above PEL (0.49 mg/kg) and thus had reached concentration levels likely to cause adverse effects to biota. The same was true for site 5, but only in the rainy season.

Table 4. Median, mean and standard deviation (SD) of the total mean Hg (T) concentrations in the blood of *G. brasiliensis* sampled during the rainy (R) and dry season (D) at the different sites. Sampling sites according to Figure 1. LOQ = 0.25 µg/L. GT = Gustavo Tank. *Valor mediano, médio e desvio padrão (SD) das concentrações médias do Hg total (T) em sangue de* G. brasiliensis *durante o período chuvoso (R) e seco (D) em diferentes pontos. Pontos amostrais de acordo com a Figura 1. LOQ = 0.25 µg/L. GT = Tanque do Gustavo.*

Site	Season	Median Hg (µg/L)	Mean Hg (µg/L)	SD	N
GT	**R**	10.5	10.8	5.77	10
	T	10.5	10.8	5.77	10
1	**R**	10.2	30.1	47.34	4
	D	23.2	23.2	25.10	2
	T	12.8	27.8	38.52	6
2	**R**	29.9	31.5	12.71	8
	D	11.4	14.6	12.56	7
	T	23.5	23.6	14.99	15
3	**R**	30.3	33.0	11.22	5
	D	24.8	31.9	18.16	4
	T	27.9	32.5	13.67	9
4	**R**	0.3	0.3	0.00	15
	D	0.3	0.3	0.00	21
	T	0.3	0.3	0.00	36
5	**R**	0.3	2.3	2.65	12
	D	66.4	104.1	93.76	17
	T	14.1	62.0	87.32	29
Total	**R**	4.8	12.5	18.67	54
	D	5.5	40.2	70.92	51
	T	5.2	26.0	52.81	105

Mercury concentrations in fish blood

Biomarkers have been considered as promising tools to supplement usual methods for environmental monitoring. Consequently, fish blood analysis is an alternative environmental quality early warning indicator for locations exposed to various forms of pollution (Rocha *et al.*, 1985; Sadauskas-Henrique *et al.*, 2011; Seriani *et al.*, 2012, 2015a). Fish Hg accumulation is strongly driven by ecological attributes and life history of the fish (Wiener & Spry, 1996), often explaining differences in Hg concentrations between species but also between populations or sub-populations of a given species. By accumulating pollutants, organisms can be good bioindicators of mercury pollution, revealing spatiotemporal variations in the quality of the environment in which they thrive. Although concentrations of Hg in fish indicate contamination levels of the ecosystem (Malm, 1990), variations may occur, depending on the tissue studied and on the trophic level of the species (Cizdziel *et al.*, 2003).

Some studies have demonstrated the linear correlations between fish length and Hg concentrations in fish muscle (Kehrig *et al.*, 2001; Carrasco *et al.*, 2011; Bastos *et al.*, 2015a), these works show that the muscle of older and larger fish generally presented higher levels of Hg than in younger specimens, because of exposure time. Our results showed that mercury concentrations in fish blood were marginally associated with the weight and length of fish. These results indicate that weight and length of fish may also influence the absorption of Hg from the environment, once the blood of larger fish presented the highest concentrations of total mercury.

Higher concentrations of Hg were found in sites 2 and 3 in the rainy season and in sites 3 and 5 in the dry season. Lower values occurred in GT and sites 4 and 5 in the rainy season and site 4 and GT in the dry season. These results show that the sites with the higher mean concentration of Hg in fish blood were those located downstream of the chlor-alkali plant (sites 2 and 3) and at site 5, in the main body of the Billings Reservoir. Billings Reservoir for many years, the latter has been the recipient of polluted water from Pinheiros River, an urban river in São Paulo City, in order to increase power generation in the Henry Borden Hydroelectric Power Plant (CETESB, 1990). With the exception of this site, the lowest concentrations of Hg in fish blood found in the dry season could be explained by a cascade of events. Specifically, the low temperatures during the dry season might have significantly reduced the basal metabolism of fish. This could have led to a reduction in swimming activity and corresponding decrease in the search for food. Such decrease could then reduce exposure to contamination (Rankin & Jensen, 1993). Changes in fish blood properties have proven to be successful biomarkers of the presence of high concentrations of different metals in the Parque Ecológico do Tietê-SP, in which tilapia showed higher numbers of erythrocytes, leukocytes, lymphocytes, erythroblasts, and mean corpuscular volume, when compared with tilapia from a control site (Seriani *et al.*, 2015b). As an experimental standard, cytogenetic effects were observed in tilapia after seven days of exposure to different forms of Hg (Seriani *et al.*, 2015b). These authors have recommended that fish blood be employed as a biomarker of exposure in places where there is a presence of contaminants, such as metals in the aquatic environment. Results showed our Hg extraction method was efficient, and furthermore, the concentration of Hg recorded in *G. brasiliensis* blood was determined to be significantly positively correlated to Hg concentrations in sediment (r=0.844; p<0.05).

Our results showed higher concentrations of Hg at the sites with the greatest anthropogenic influence (site 3 and 5). Site 5 is located in an area of the Billings Reservoir that has received a large load of industrial pollutants owing to the Pinheiros River, and site 3 is located in a stretch with high levels of mercury in the sediment, as a result of effluent discharges of a chlor-alkali plant (CETESB, 2007). Although these effluents are no longer released into the Grande River (<0.0002 mg/L) (Furlan, 2010), high levels of Hg are still found in the sediment, indicating that it remains an active source of contamination.

In site 4, a high Hg concentration in the sediment was recorded; however, low Hg values in fish blood were observed. This fact is probably related to the high copper (Cu) concentrations recorded in the sediment of this reservoir. According to CETESB (2010), Cu concentrations up to 4596 µg/g were recorded, and it was established that the presence of copper may decrease by more than 50 % the retention of Hg by benthic organisms (Nordberg *et al.*, 2007). At this site, the main copper source to the reservoir can be related to the use of algicides, such as copper sulfate, in order to control cyanobacteria at the water treatment station intake (Moschini-Carlos *et al.*, 2010; Cardoso-Silva *et al.*, 2014).

High values of total Hg were found in the blood of *G. brasiliensis* during the dry season at site 5 (Table 4), which may be related to the resuspension of the sediment caused by thermal destratification, which is commonly documented in tropical shallow reservoirs during water column mixing when some elements, such as nutrients previously retained in the sediment, can be made available to the water column (Vaz, 1996; Ramírez & Bicudo, 2005). It could also be related to the contribution of soil eroded during the 2007 to 2010 construction of the southern part of the Rodoanel, a perimeter highway (GESP, 2011). Also with the reproductive aspects of the species could also reduce feeding activity. Under these circumstances, fish would use their body reserves, instead of effective feeding, which would, in turn, affect metal intake (Jesus & Carvalho, 2008).

Our results further showed that the pearl cichlid of the Grande River and Billings Reservoir reflected Hg concentrations in the sediment. The observed correlation between blood Hg and sediment Hg values can, to some extent, be explained by the feeding tactic employed by *G. brasiliensis*, which involves revolving the substrate, snapping it with the protrusion of the upper jaw, and expelling it through the mouth and opercular openings (Sabino & Castro, 1990). Similar relationships were observed by Zhou & Wong (2000), who found that concentrations of Hg in *Aristichthys nobilis* (bighead carp) muscle were positively correlated with sediment concentrations, as well as Ikingura & Akagi (2003) studying several species of fish in Tanzanian reservoirs.

CONCLUSIONS

Hg in blood can be used as a measurement the evidence of persistence and/or contaminant availability in the environment, and, hence, as an indicator of an environmental liability. Our results also suggest that the selection of a species with benthic habits was appropriate, indicating its potential as a biomonitoring tool. Mercury in the blood of fishes as a biomarker of recent exposure can be used as a complement to currently used monitoring tools, helping to predict future environmental contamination and, helping to prevent observed effects at higher levels of biological organization.

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