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Biomarkers of exposure to metal contamination and lipid peroxidation in the benthic fish *Cathorops spixii* from two estuaries in South America, Brazil

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Abstract Biomarkers as lipid peroxidation, metallothionein and δ-aminolevulinic acid dehydratase were determined in *Cathorops spixii* to compare the biological responses of this fish from estuaries with distinct anthropogenic influence. Three areas were selected in two estuaries in accordance with the levels of contamination for the polluted (Santos/São Vicente) and with the hydrodynamic characteristics for the non-polluted (Cananéia) estuary. Water characteristics and mercury levels in *C. spixii* confirmed a high human influence in the polluted system. In general, the biomarkers showed differences between the estuaries, suggesting disturbances in the specific cell mechanisms due to the presence of multiple xenobiotics in the contaminated system. Therefore, these biomarkers are recommended to promote more accurate information about the exposure to pollutants. Additionally, the study of the effect of the multiple xenobiotics on resident species such as the benthic fish *C. spixii* can favor a better assessment of the environmental quality of these systems.

Keywords Metallothionein · ALAD activity · Lipid peroxidation · Mercury · *Cathorops spixii* · Santos/São Vicente · Cananéia

Introduction

Metals are natural components of the environment. However, the different anthropogenic activities have increased metal concentration in the aquatic systems. Mercury occurs naturally in the environment in the forms of Hg^0, Hg^{1+} and Hg^{2+}. Human activities such as mining, sewage disposal, fossil fuel and many products like batteries, fluorescent lamps, thermometers, thermostats, paints and pesticides release this metal into the aquatic systems, causing a significant increase of Hg concentrations (Jackson 1997). Although most of the mercury in the biological system is found in the methyl-mercury form, the inorganic mercury (Hg^{2+}) can also occur and be accumulated by organisms and undergo a process of bioamplification throughout the food chain.

In fish, metal regulation and detoxification occurs mainly by the induction of metallothioneins (MT). Nevertheless, the induction of this protein differs among species and tissues (Roesijadi 1992). Generally, metals such as Zn^{2+}, Cu^{2+}, Cd^{2+} and Hg^{2+} induce the increase of MT concentrations (Fernandes et al. 2008). Moreover, δ-aminolevulinic acid dehydratase activity (ALAD) has been used as a biomarker of Pb contamination or oxidative stress in haematological systems. Despite that, few studies report ALAD activity in fish (Martin and Black 1998; Perottoni et al. 2004; Alves Costa et al. 2007).

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Santos Bay is located in Brazil on the central coast of southeastern São Paulo State (24°00′S; 46°21′W). The industrial activity is highly developed and tourism is another important economic activity. Santos has the largest commercial harbor of South America and is one of the most important petrochemical and metallurgical industrial areas in Brazil (the Cubatão industrial complex), which has around 1,100 industries. In this context, the estuary and Santos Bay is continually exposed to contamination, due mainly to the intense industrial activity in the Cubatão Industrial Complex or to old discharges and the retention of inorganic (Hg, Fe, Zn, Cu, Cd) (Hortellani et al. 2005; Cetesb 2005) and organic (Bícego et al. 2006) compounds in the sediment. All these anthropogenic sources contribute directly or indirectly to the input of contaminants to this area. In spite of the fact that the Cananéia estuary is located in the Southern coast of São Paulo State coast (25°S; 48°W), it is closed to the Paranaguá basin in the South and the Ribeira of Iguape region in the North. Although this estuary is placed among these two known polluted areas (Cetesb 2005), the Cananéia estuary has been used as a non-polluted area in biomonitoring studies along the years, as it shows low trace metal contents, nitrogen and phosphate compounds and high dissolved oxygen concentrations (Azevedo 2008). There are few studies regarding the trace metals and organic pollutants in *Cathorops spixii* for the estuary and Santos Bay. Recently, Azevedo (2008) has justified the use of this species as a bioindicador by trace metal contamination in the Santos Bay.

Therefore, considering these estuarine systems, the general objective of this work is to compare the biological responses of the benthic fish *C. spixii* from estuaries with distinct anthropogenic influence by trace metal. For this purpose, both estuaries, non-polluted and polluted, were segmented in three areas according to the levels of contamination for the polluted estuary (Santos/São Vicente) and in accordance with the hydrodynamic characteristics for the non-polluted estuary (Cananéia). The contamination process in the fish was evaluated by total mercury determination. Additionally, somatic indexes such as hepatosomatic index (HSI) and condition factor (CF), biomarkers as MT, δ-ALAD and lipid peroxidation (LPO) were also evaluated to assess the biological changes in the fish and the interdependence between these endpoints and the environmental data.

Materials and methods

Sample collection

Fish *C. spixii* were collected during Winter 2005 and Summer 2006 in three areas with distinct contamination levels within Santos/São Vicente estuarine system (San) (Fig. 1). The sites were chosen as described below:

Site 1. Santos Canal (*CS*): inner part of the system impacted by intense industrial activity. *Site 2. Santos Bay* (*BS*): less impacted by industrial activity, but with an intensive input of chemical compounds by the underwater pipeline. *Site 3. São Vicente Canal* (*CSV*): region characterized by the presence of mangrove and urban occupation.

In Cananéia estuarine-lagoon complex (Can), an environment with low anthropogenic influence, fish were collected in three sites: Cananéia Sea (MCa), Cubatão Sea (MCu) and Trapandé Bay (BT) (Fig. 1).

Fishes were collected on board of the R/B Albacora ship, using a bottom Otter Trawl (1.6″ mesh wall and 1.2″ mesh cod end) with 11 m length, set at 8.8 m depth. Specimens of *C. spixii* were collected in Cananéia estuarine-lagoon complex ($n = 152$) and in Santos/São Vicente estuarine system ($n = 94$). These fishes were transported alive on ice to the laboratory and identified according to the morphological characteristics. In the laboratory, morphometric data were collected and muscle, blood and liver samples were dissected for chemical and biochemical analyses, respectively. The samples were frozen in liquid nitrogen and stored at −80°C for later analysis.

Water chemistry

Water temperature was determined by reversible thermometers and pH was measured using a portable potentiometer (PHM 203—Radiometer). Dissolved oxygen concentrations were determined by the Winkler method (1888). Dissolved inorganic phosphorus determination was based in method of Grasshoff et al. (1983) and dissolved inorganic nitrogen according to the method of Tréguer and Le Corre (1975), using the AutoAnalyser II—Technicon.

Mercury analysis

Mercury (Hg) determination was performed using Cold Vapour Atomic Absorption Spectrometry (CV-AAS) using a FIMS 100 from Perkin Elmer. About 200–500 mg of fish muscle and liver were digested with a mixture of concentrated HNO_3 and H_2SO_4 in Teflon vials. The analytical procedure used (wet digestion) followed the method described by Horvat (1996). The detection and quantification limit were 0.5 and 0.7 ng mL^{-1}, respectively. The validation of total Hg determination was checked with a standard reference (Dogfish liver DOLT-1, Dogfish muscle DORM-1, Mussel tissue and Oyster tissue). The analytical results showed good precision and accuracy (Table 1). Hg concentrations were reported as ng g^{-1} wet weight (w. w.).

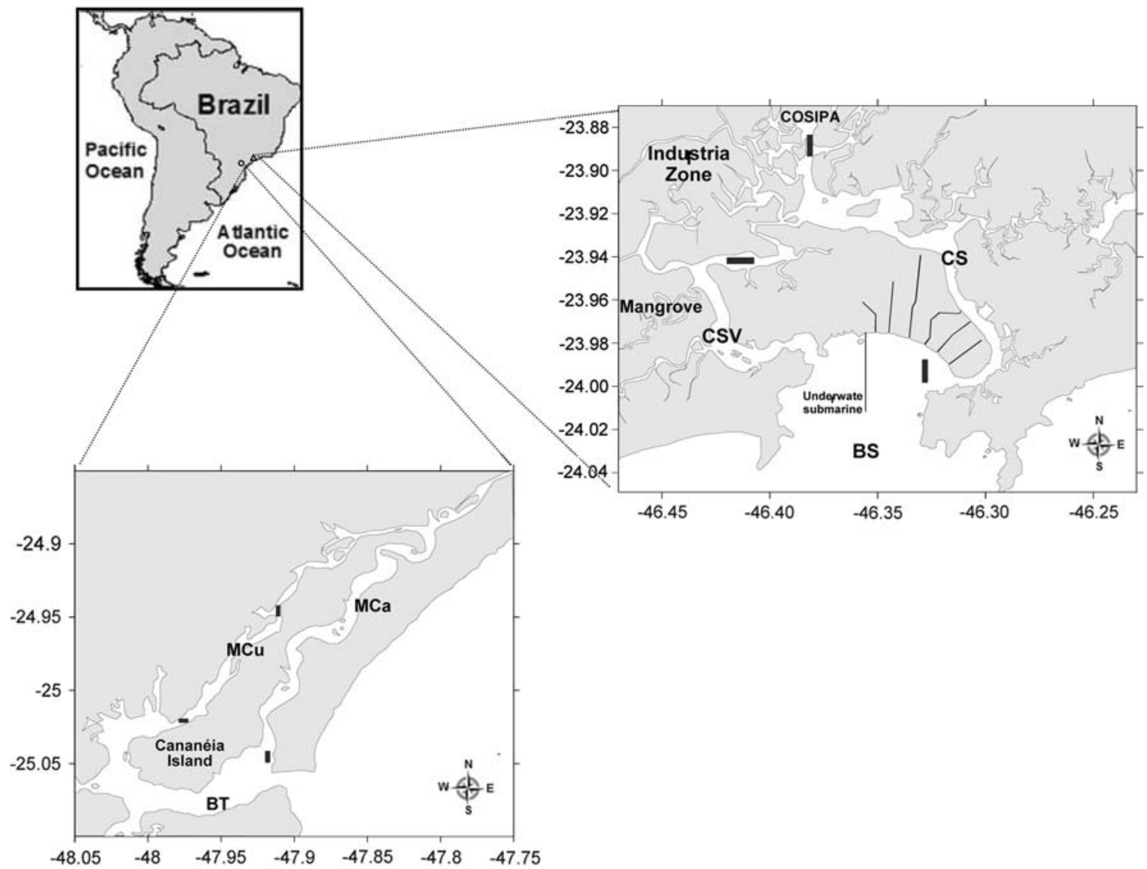

Fig. 1 Map of sampling sites showing Santos/São Vicente estuarine system and Cananéia estuarine Complex, São Paulo, Southeast coast of Brazil

Table 1 Analysis of total mercury (Hg) in reference materials

Reference material	Hg (ng g^{-1})		
	Certified values	Found values	RE (%)
Dogfish liver (DOLT-1, NRCC)	225 ± 37	249 ± 10	4.4
Dogfish muscle (DORM-1, NRCC)	798 ± 74	780 ± 0.1	0.0
Mussel tissue	61 ± 3.6	56 ± 0.8	1.7
Oyster tissue (OT, NIST)	37 ± 1.3	40 ± 2.1	5.1

Data represent mean ± SD ($n = 3$) and relative error (RE)

Somatic indexes

Individual fish were weighed, the total length measured and the liver dissected and weighed. The CF was calculated as CF = [body weight (g)/length $(mm)^3$] × 100. Hepatosomatic index was calculated using the formula HIS = [liver weight (g)/body weight] × 100.

Sample preparation

Blood were obtained by caudal vein puncture and for analysis of ALAD, individual sample was weighed (~1 mL), placed in ice-cold homogenization buffer containing NaH_2PO_4, Na_2HPO_4 0.2 M, pH 6.6 and 0.5% Triton X-100 and centrifuged at 10,000*g* for 15 min at 4°C. Samples of liver were weighed and homogenized in Tris–HCl buffer (0.02 M) at pH 8.6, 10% BHT and centrifuged at 30,000*g* for 45 min at 4°C. Two aliquots were obtained and used to malondialdehyde and MT determination. For MDA, supernatant 1-methyl-2-phenylindone solution was added, mixed and 15.4 M methanesulfonic acid added. Samples were incubated at 45°C for 60 min and centrifuged at 15,000*g* for 10 min. For MT quantification, the supernatant was heated at 80°C for 10 min to denature thermo labile proteins. The resulting preparation was re-centrifuged at 30,000*g* for 45 min at 4°C for obtaining of the low molecular weight proteins.

Biomarker assays

Aminolevulinic acid dehydratase activities were assayed using a spectrophotometric assay as described in Berlin and Schaller (1974). Hepatic lipid peroxidation was evaluated determining the concentration of malondialdehyde (MDA) and 4-hydroxyalkenals (4-HNE) produced during decomposition of polyunsaturated fatty acid peroxides of membrane lipids by spectrophotometric assay (Erdelmeier et al. 1998). Hepatic MT concentration was determined using differential pulse polarography (DPP), as described by Bebianno and Langston (1989). DPP was performed using a 646VA Processor autolab type II and an ECO Chemie IME663 Hg drop electrode. MT quantification was based on purified rabbit liver MT, MT-I (Sigma) due to the absence of fish MT standard.

Statistical analysis

The data was analyzed by one-way analysis of variance (ANOVA) and, whenever a significant effect was obtained, the Tukey's test was subsequently applied to test the interaction between the variables. P-value < 0.05 was considered for statistical significance. In order to verify the interdependence between endpoints measured as biomarkers and somatic indexes, and environmental data, the principal components analysis (PCA) was used.

Results

Environmental conditions

Environmental conditions are presented in Table 2. Temperature was significantly higher during the summer. In the winter, the highest temperature was found in the inner areas of the estuaries. In general, higher pH values were observed during the summer and a decreasing gradient from the Bay towards the inner regions of the estuaries were also verified. Dissolved oxygen (DO) was significantly higher during the winter. For the Cananéia estuary, all regions showed DO concentrations above 4.00 mL L^{-1} in this period. However, in summer, DO concentrations were more heterogeneous, with levels below 4.00 mL L^{-1}. In the Santos/São Vicente estuarine system, the highest DO values were observed in BS, while the lowest concentrations occurred in CSV. In this estuary, a drastic reduction in DO levels was observed during the summer. In general, nutrient concentrations were significantly higher in the summer. In BS lower values of nitrogen and dissolved inorganic phosphorus were observed. On the other hand, the nutrient contents in the Santos/São Vicente estuarine system were, in general, higher than in the Cananéia estuary. The highest P-PO_4^{3} concentrations were observed during the summer in both estuaries.

Mercury concentration

Mean concentrations of total Hg in the muscle of *C. spixii* are in Table 3. In the Cananéia estuary, significant seasonal variations ($P < 0.05$) were observed in fish collected in MCa and MCu. On the other hand, in the Santos/São Vicente estuary significant seasonal variations ($P < 0.05$) occurred for fish sampled in BS and CS. Fish from BT, MCu in the Cananéia estuary and CS in the Santos/São Vicente estuarine system showed higher total Hg concentrations during the winter. On the other hand, BS and CSV showed higher total Hg concentrations in the summer. In general, the highest Hg values were found in *C. spixii* from in the inner area of the Santos/São Vicente estuary (CS), near the industrial pole ($P < 0.05$). Additionally, fish collected in CSV, near the mangrove area, had lower total Hg concentrations than in Cananéia estuary. Table 4 shows total Hg concentration in different species. Hg concentrations for the Santos/São Vicente estuary were significantly lower than in the region strongly impacted by human activities. However, it is important to consider the differences between species and regions because this can modify the bioaccumulation process of Hg in the fish.

Somatic indexes

Table 5 shows the mean values of morphometric data of *C. spixii* captured seasonally in each sampling sites. Absence of statistically significant differences between HSI and CF for the fish from the different regions in Cananéia estuary was found. In the regions of this estuary, seasonal differences does not exist ($P < 0.05$). In general, fish from the Cananéia estuary showed lower HIS values than fish from the different areas of the Santos/São Vicente estuarine system. However, statistically significant differences were not observed between fish from Cananéia and from CS and CSV. Additionally, specimens from the Cananéia estuary showed higher value of CF than *C. spixii* from different areas in the Santos/São Vicente estuarine system, except for fish from CSV in the summer and winter periods and CS in the winter. Fish collected in CS and BS showed higher values of total length, weight and HSI ($P < 0.05$). The CF was low for the fish from BS during the winter. In addition, low values of CF were also observed in the fish collected during the summer in BS and CS.

Biomarkers

The heterogeneity observed for total Hg data is due to the larger individual variability because fish in different size classes were captured. Therefore, the verification of a larger range in relation to the total Hg levels for the evaluated area was possible. However, generally speaking, significant differences were not detected among the three regions in the Cananéia estuary. The same was obtained for the somatic data as HSI and CF because significant differences between these indexes and the regions in the Cananéia

Table 2 Water chemistry data [temperature, pH, DO, inorganic dissolved phosphorus (P-PO_4^{-3}), inorganic dissolved nitrogen (NID), nitrate (N-NO_3^-), nitrite (N-NO_2^-) and ammonia (N-NH_4^+)] for different areas in the Cananéia estuary and in Santos/São Vicente estuarine system

Region	Temp. (°C)	pH	DO (mg L^{-1})	P-PO_4^{-3}	NID (μM)	N-NO_3^- (μM)	N-NO_2^- (μM)	N-NH_4^+ (μM)
Winter								
MCa	20.93	7.84	4.76	1.30	3.86	0.19	0.93	2.73
	(19.03–21.50)	(7.54–8.10)	(4.42–4.98)	(0.77–1.94)	(3.21–4.02)	(0.14–0.29)	(0.56–1.83)	(2.25–3.67)
MCu	21.34	7.80	5.15	0.48	3.32	0.23	0.56	2.62
	(21.19–21.40)	(8.02–8.22)	(4.54–4.73)	(0.33–0.52)	(1.84–3.46)	(0.22–0.32)	(0.39–0.56)	(1.22–2.73)
BT	20.74	8.13	4.62	0.43	2.91	0.28	0.47	2.16
	(20.50–21.00)	(7.57–7.87)	(4.50–5.30)	(0.46–1.06)	(2.82–4.33)	(0.16–0.26)	(0.40–0.84)	(2.00–3.33)
CS	22.49	7.92	3.61	4.48	15.62	0.93	4.22	10.47
	(22.09–23.00)	(7.80–8.08)	(3.12–4.39)	(1.87–7.01)	(6.84–21.25)	(0.11–1.97)	(0.58–8.85)	(6.16–14.54)
BS	21.42	8.22	4.15	1.25	3.59	0.09	0.20	3.30
	(21.21–21.90)	(8.16–8.25)	(3.88–4.71)	(0.55–3.10)	(1.68–7.37)	(0.03–0.14)	(0.03–0.58)	(1.62–2.92)
CSV	22.80	7.55	3.18	6.26	24.91	0.35	1.36	23.20
	(22.00–24.50)	(7.48–7.65)	(3.01–3.36)	(5.13–7.87)	(21.82–26.70)	(0.31–0.41)	(0.96–1.48)	(20.55–24.86)
Summer								
MCa	27.71	7.83	3.82	1.77	1.67	0.17	0.11	1.38
	(25.04–29.83)	(7.50–8.09)	(3.35–4.01)	(1.10–2.70)	(0.95–2.64)	(0.07–0.35)	(0.09–0.17)	(0.79–2.12)
MCu	29.89	8.13	3.71	1.52	1.38	0.21	0.14	0.93
	(29.64–30.20)	(8.38–8.62)	(2.94–3.62)	(1.17–2.08)	(2.27–3.47)	(0.46–0.78)	(0.10–0.25)	(1.38–2.77)
BT	28.35	8.53	3.32	1.49	2.90	0.59	0.17	2.14
	(27.91–28.89)	(8.05–8.20)	(3.39–4.38)	(0.79–2.29)	(1.26–1.58)	(0.11–0.43)	(0.10–0.18)	(0.71–1.31)
CS	26.04	8.01	2.73	5.37	40.67	21.81	4.44	14.42
	(25.67–26.94)	(7.78–8.41)	(1.67–4.75)	(1.15–8.30)	(3.49–58.86)	(1.13–31.69)	(0.25–8.13)	(2.10–24.52)
BS	25.31	8.31	3.95	1.12	11.78	5.47	1.38	4.93
	(24.64–23.97)	(8.22–8.39)	(3.29–4.45)	(0.83–1.74)	(8.11–20.93)	(3.80–6.45)	(0.63–2.63)	(0.32–14.06)
CSV	27.00	7.39	1.31	5.09	49.38	7.52	3.19	38.67
	(27.00–28.00)	(7.21–7.63)	(1.09–1.62)	(4.18–5.89)	(43.33–52.71)	(5.30–9.95)	(2.16–4.25)	(39.08–44.00)

Data represent mean, maximum and minimum in parenthesis

MCa Cananéia Sea, *MCu* Cubatão Sea, *BT* Trapandé Bay, *CS* Santos canal, *BS* Santos Bay, *CSV* São Vicente canal

Table 3 Total mercury (Hg) content in muscle of *Cathorops spixii* collected in the Cananéia (MCa, MCu and BT) and Santos/São Vicente estuaries (CS, BS and CSV)

Site	*n*		Hg (w. w.)	
	Winter	Summer	Winter	Summer
MCa	27	30	77 (39–125)	48 (<30–160)
MCu	13	37	136 (34–231)	73 (35–147)
BT	20	25	155 (65–347)	–
CS	17	21	389 (55–1,085)	199 (52–345)
BS	18	10	164 (32–163)	58 (91–340)
CSV	18	10	28 (21–104)	35 (9–80)

Data are expressed in ng g^{-1} and represent mean, minimum and maximum in parenthesis, of wet weight (w. w.)

– Not observed

estuary were not observed either. In spite of the data on biomarkers, previous statistical analyses did not reveal significant differences among fish from the three regions in the Cananéia estuary either. Therefore, the concordance of somatic indexes and total Hg levels in the regions of the Cananéia estuary between the data in the regions allowed the grouping of biomarkers to increase the sample number and thus to generate biological information of higher reliability. The absence of significant differences between males and females also allowed the grouping of the data on different biomarkers.

Hepatic lipid peroxidation in *C. spixii* is in Fig. 2. Significant seasonal differences were observed for the specimens collected in CS (Winter: 3591 nmol g^{-1} protein; Summer: 604 nmol g^{-1} protein). Higher LPO concentrations were also observed in *C. spixii* from this area, collected during the winter. On the other hand, the lowest LPO concentrations was in CSV (582 nmol g^{-1} protein) and BS (508 nmol g^{-1} protein), both during the summer period.

In specimens collected in the different areas of the Santos/São Vicente estuary ALAD activities were higher when compared to those collected in Cananéia (Fig. 3). However, ALAD activities in fish from CSV collected in the winter period (0.62 ng PBG min^{-1} mg^{-1} protein) was even lower than those in the Cananéia estuary (1.08 ng PBG min^{-1} mg^{-1} protein). Significant seasonal differences were only observed in the CSV site (Winter: 0.62 ng PBG min^{-1} mg^{-1} protein; Summer: 2.68 ng PBG min^{-1} mg^{-1} protein).

The hepatic MT levels are in Fig. 4. Fish from CSV showed lower MT levels (Winter: 0.49 mg g^{-1} protein; Summer: 0.52 mg g^{-1} protein) than those collected in the Cananéia estuary (Winter: 0.91 mg g^{-1} protein; Summer: 0.60 mg g^{-1} protein). On the other hand, the highest MT content was in *C. spixii* from CS (Winter: 2.50 mg g^{-1} protein; Summer: 3.10 mg g^{-1} protein) and BS (Winter: 2.29 mg g^{-1} protein; Summer: 2.73 mg g^{-1} protein), respectively. No significant seasonal differences in MT content were found in *C. spixii*.

Principal component analysis showed 77% of total variance, of which 31% was for PC1 and 46% for PC2 (Fig. 5). In PC1, the areas BS, CS and CVS of the Santos/São Vicente estuary were grouped with the biomarkers MT, ALAD, LPO and with the biotic and abiotic parameters HIS, TL, TW, Hg, NID, N-NO_3^-, N-NO_{2-}, N-NH_4^+ and P-PO_4^{-3}, respectively. A positive correlation was found between the different areas and parameters of this group. On the other hand, MCa, MCu and BT areas of the Cananéia estuary showed a grouping just with the parameters DO, pH, temperature and CF. Such association probably reflects the low human influence and the natural conditions of this system.

Discussion

The Santos/São Vicente estuary receives an intensive and continuous industrial and domestic effluent and some authors have identified high concentrations of different chemical compounds including the Santos Bay area

Table 4 Mean total mercury concentrations (ng g^{-1} w.w.) in muscle of fishes from different regions

Species	Location	*N*	Hg	Reference
Mugil auratus	Mediterranean Sea	46	25	Balkas et al. (1982)
Upeneus moluccensis	Mediterranean Sea	18	250	Balkas et al. (1982)
Plagioscion squamosissimus	Amazonia, Brazil	33	1,200	Porvari (1995)
Cichla temensis	Amazonia, Brazil	53	1,100	Porvari (1995)
Serrasalmus eigenmanni	Rio negro, Brazil	110	472	Dórea (2003)
Serrasalmus rhombeus	Rio Negro Brazil	22	610	Dórea (2003)
Serrasalmus nattereri	Pantanal, Brazil	1	5,270	Alho and Vieira (1997)
Cathorops spixii	Cananéia, Brazil	152	90	This work
Cathorops spixii	Santos/São Vicente, Brazil	94	164	This work

Table 5 Morphometric data of *Cathorops spixii* in each sampling site

	n	TL (mm)	TW (g)	HSI	CF
Winter					
MCa	27	171 ± 39^{a}	55 ± 45^{a}	1.76 ± 0.25^{a}	0.32 ± 0.07^{c}
MCu	13	192 ± 48^{ab}	83 ± 78^{b}	1.75 ± 0.23^{a}	0.20 ± 0.04^{c}
BT	20	203 ± 53^{ab}	83 ± 62^{b}	1.68 ± 0.17^{a}	0.41 ± 0.10^{c}
CS	17	239 ± 35^{ab}	138 ± 97^{c}	2.07 ± 0.28^{b}	0.21 ± 0.12^{c}
BS	18	149 ± 28^{a}	37 ± 22^{a}	2.03 ± 0.52^{b}	0.004 ± 0.0003^{a}
CSV	18	195 ± 20^{a}	75 ± 23^{b}	1.66 ± 0.16^{a}	0.40 ± 0.21^{c}
Summer					
MCa	30	178 ± 11^{a}	51 ± 10^{a}	1.40 ± 0.21^{a}	0.35 ± 0.09^{c}
MCu	37	156 ± 22^{a}	44 ± 19^{a}	1.50 ± 0.31^{a}	0.45 ± 0.17^{c}
BT	25	159 ± 11^{a}	39 ± 7^{a}	1.55 ± 0.24^{a}	0.46 ± 0.07^{c}
CS	21	226 ± 34^{b}	109 ± 54^{c}	1.93 ± 0.41^{b}	0.06 ± 0.01^{b}
BS	10	284 ± 29^{b}	213 ± 78^{c}	1.83 ± 0.50^{b}	0.03 ± 0.01^{b}
CSV	10	192 ± 28^{a}	57 ± 23^{a}	1.69 ± 0.38^{a}	0.44 ± 0.07^{c}

Values are mean ± SD

Distinct letters indicate significant differences between sites ($P < 0.05$)

TL total length, *TW* total weight, *HSI* hepatossomatic index, *CF* condition factor, *n* number of individuals analyzed

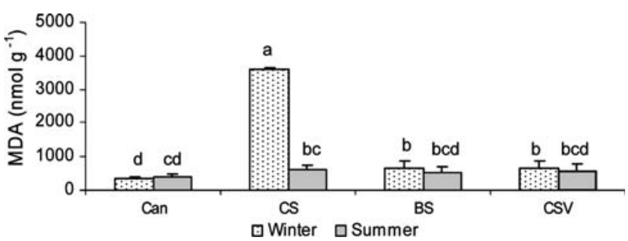

Fig. 2 Hepatic LPO levels (nmol g^{-1} protein) in *C. spixii* collected in the Cananéia estuary and in different regions in the Santos/São Vicente estuarine system. Values are expressed as mean (±SD). *Distinct letters* indicate significant differences between sites ($P < 0.05$)

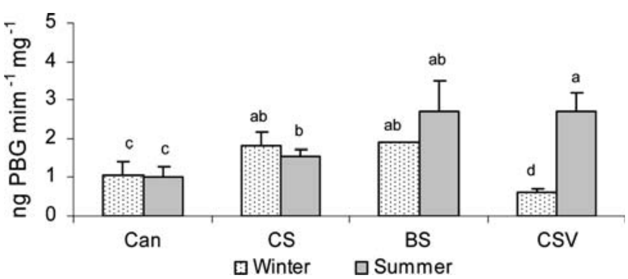

Fig. 3 ALAD activity in blood (ng PBG min^{-1} mg^{-1} protein) of *C. spixii* collected in the Cananéia estuary and in different regions in the Santos/São Vicente estuarine system. Values are expressed as mean (±SD). *Distinct letters* indicate significant differences between sites ($P < 0.05$)

(Hortellani et al. 2005; Bícego et al. 2006). In this paper, some environmental data such as pH, DO, nutrients, total Hg, and biomarkers as MT, ALAD and LPO in association with the somatic indexes as HSI and CF were evaluated in fish from a polluted and a non-polluted estuary in the Southwestern area of the Brazilian coast, in order to compare the biological responses of the *C. spixii* from the two estuaries with distinct anthropogenic influence.

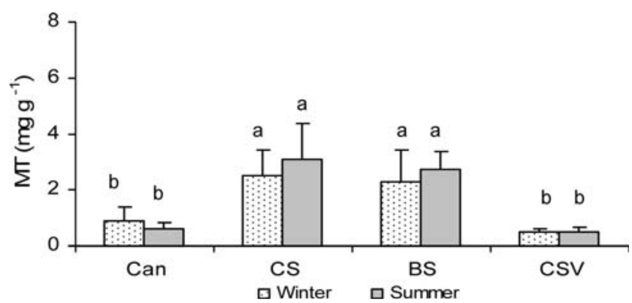

Fig. 4 Hepatic MT concentrations (mg g^{-1} w. w.) in *C. spixii* collected in the Cananéia estuary and in different regions in the Santos/São Vicente estuarine system. Values are expressed as mean (±SD). *Distinct letters* indicate significant differences between sites ($P < 0.05$)

The low DO observed in CSV during the summer period can be associated with the larger contribution and decomposition of the organic matter. The enrichment of organic matter tends to acidify the aquatics systems and reduce the DO content. The high $N\text{-}NO_2^-$ concentrations observed in CS, mainly in the summer period, in association with the lowest DO levels in this area can reflect denitrification processes or $N\text{-}NH_4^+$ oxidation. In CS, high $N\text{-}NH_4$ levels were also found. On the other hand, the high $N\text{-}NH_4^+$ concentrations in CSV can be related to organic matter oxidation. $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ found in the Santos/São Vicente estuarine system indicate a strong anthropogenic influence. In addition, the values of dissolved inorganic phosphorus were also high mainly in the inner area of this estuary, probably associated to the industrial activities. On the other hand, high OD in conjunction with low $P\text{-}PO_4^{-3}$ and $N\text{-}NO_3^-$, $N\text{-}NO_2^-$ and $N\text{-}NH_4^+$ concentrations in Cananéia reveal the low anthropogenic influence in this estuary. The environmental data such as nutrients, pH and OD in association with data of total Hg in muscular tissue

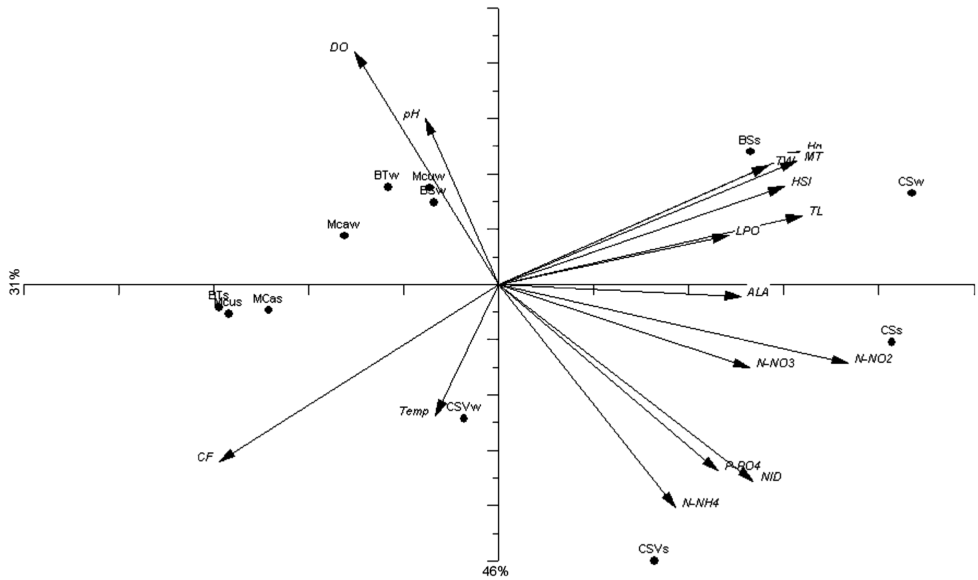

Fig. 5 Principal components analyze of biomarkers, somatic indexes, morphometric and environmental data in *C. spixii* collected seasonally in each sampling site

of *C. spixii* obtained for the different regions in the Cananéia estuary reinforce the use of this system as a reference area due to the low anthropogenic influence. Moreover, these results indicate natural characteristics of one estuary of the Southwest of the Brazilian coast.

The seasonality of most of the biomarkers evaluated in this study reinforce the influence of the abiotic parameters, because pH, salinity and temperature variations can modify the bioavailability of the contaminants in aquatic system. The summer period is characterized as a rainy season. Thus, the larger rainfall in this period favors the input of contaminants or can also dilute these compounds due to the larger contribution of less saline water in the system.

The total Hg content in the muscular tissue of *C. spixii* obtained in this study show higher concentrations in the fish from CS, followed by BS, in relation to the specimens from the Cananéia estuary. This variation observed for both winter and summer periods can be associated to the input of total Hg in these areas, by industrial activities, and by urban discharges (i.e., underwater emissary). On the other hand, the low total Hg values obtained for *C. spixii* from CSV suggest a smaller bioavailability of this element because the low hydrodynamics in association with the characteristics of the sediment in this area are favorable conditions for the retention of the chemicals in the sediments. Thus, the chemical pollutants are less available for the biota. Finally, although the total Hg determination in *C. spixii* was done in muscle and not in liver, the contents of this metal in the muscular tissue show that these individuals are exposed to total Hg, especially in CS.

Detection of modified abiotic and biotic processes constitutes an important tool to predict the best managing strategy for the coastal ecosystems. Therefore, one of the most important purposes of biomonitoring is to assess environmental risk and, in this context, the integration of the biotic and abiotic components is very important. More recently, some authors proposed the use of ecological indexes like hepatosomatic and gonadosomatic indexes and CF in biomonitoring studies to evaluate the influence of biotic processes or as an additional tool in biomonitoring approaches (Adams and Ryon 1994). Fish from polluted environments usually show an increase in the HSI (Karels et al. 1998). This pattern was also observed in *C. spixii* from the Santos/São Vicente estuary. In the present study, the data suggest the presence of different xenobiotic compounds in the environment due to human activities. On the other hand, the low HSI in fish from the reference site reflect a lower hepatic stress in those individuals. The CF indicates that a living organism such as fish is in good physiological conditions and is useful in the comparison among populations exposed to different environmental stress conditions. In the present study, the smaller CF values were in fish sampled in the different sites within the Santos/São Vicente estuarine system, reflecting the different environmental conditions.

In most of the aquatic organisms, increases in the MT concentration or decrease of the ALAD activity are associated to trace metals contamination. Some authors relate changes in the levels of these enzymes with the exposure to Cd, Cu, Hg, Ag and Pb (Amiard et al. 2006; Monserrat et al. 2007). As a consequence, MT and ALAD are used as biomarkers in biomonitoring of aquatic environments. On the other hand, evaluation of the oxidative stress by LPO is a non-specific biomarker and therefore should be analyzed in association with other biomarkers (Monserrat et al. 2007). In general, organisms from polluted environments show an increase in the LPO process (Ferreira et al. 2005).

The levels of MDA and 4-HNE, products of LPO were significantly higher in CS during the winter. This area is characterized by intense industrial activities and therefore, LPO reflect the effect of toxic compounds from the industrial activities. On the other hand, the increased levels of malonaldehyde in this area reflect a natural deputative condition to maintain the cell balance. Finally, the LPO data obtained for *C. spixii* should be analyzed in association with other antioxidant enzymes because the endogenous metabolism can also promote the LPO.

The induction of MT in fish exposed to metal contamination, especially Hg and Cd in the environment, is documented in literature (Schmitt et al. 2007; Fernandes et al. 2008). However, a wide range of factors such as age, sex, size, and reproductive status can also affect the induction of MT (Lacorn et al. 2001). The PCA found a positive correlation among MT levels and length of *C. spixii*. Bebianno et al. (2007) show a direct relationship between these variables and total Hg for the fish *Aphanopus carbo* from Madeira Island. Increases in the MT hepatic levels in *C. spixii* from CS and BS suggest exposure to trace metals such as Hg. In the benthic *Trisopterus luscus* higher MT concentrations indicate that the sediments is the principal reservoir of pollutants in the aquatic environment (Fernandes et al. 2008). The smaller MT hepatic levels in *C. spixii* from CSV are in accordance with the same justification for *T. luscus*—since, besides the fact that *C. spixii* is also a benthic fish, the CSV region is characterized by low hydrodynamic processes and mangroves areas, suggesting that the sediments this area are, in fact, a reservoir of pollutants as Hg. The significant increase of MT levels in *C. spixii* from CS and BS suggests an induction of this biomarker. In fish from CSV, MT levels were significantly lower which is related to less bioavailable forms of metals in this area, since mangroves are well known for acting as a sink for many contaminants. Thus, the chemical from the industrial activities are not bioavailable for the organisms.

Aminolevulinic acid dehydratase activity is a specific biomarker of lead exposure in several fish species (Martin and Black 1998). However, when organisms are exposed to a mixture of contaminants, like in the natural environment, interactions occur masking a possible ALAD inhibition due to Pb exposure (Berglind 1986). In fact, the induction of ALAD activity as a consequence of Pb exposure showed in this work is questionable due to the absence of specific data on Pb concentrations in *C. spixii*. Nevertheless, the large amount of historic data of trace metal contamination in the Santos/São Vicente estuarine system (Hortellani et al. 2005) suggests a strong influence of Pb. Additionally, it is also known that ALAD functions in the metabolic synthesis of the group heme (Alves Costa et al. 2007). Moreover, Schmitt et al. (2007) pointed out that ALAD inhibition is not uniformly sensitive to Pb in all species. ALAD data obtained in *C. spixii* were not conclusive because a decrease in the ALAD activities in fish from areas with historical contamination, such as CS and BS, was not observed. However, the multiple xenobiotics in the environment can "mask" ALAD responses by antagonic processes. On the other hand, decreases in the ALAD activities were found for *C. spixii* from CSV in the winter period. The reduction in the ALAD activities in fish from CSV is associated with the higher bioavailability of trace metals in the sediments in this area.

Additionally, although the PCA grouped ALAD with different impacted areas of the Santos/São Vicente estuarine system, decreases in the ALAD activities also occur as a consequence of a hematological modulation. Therefore, the authors strongly recommended an extensive hematological evaluation and other trace metal determination such as Pb in *C. spixii*, in order to verify the efficiency of ALAD as an exposure biomarker to different areas in the Santos/São Vicente estuarine system. Therefore, it is suggested that the determination of haemoglobin content as an additional tool to verify if ALAD induction is a consequence of altered haematological status.

In general, the biomarkers evaluated in this study show differences between the areas exposed to anthropogenic influence in the Santos/São Vicente estuarine system in comparison to the Cananéia estuary, suggesting disturbances in the specific cell mechanisms due to the presence of multiple xenobiotics in the Santos/São Vicente estuarine system. Cellular responses were obtained by modifications in LPO, ALAD, and MT contents. Despite this, variations in the concentrations of secondary products of the LPO were not necessarily in accordance with the negative effects and indicate protecting responses. On the other hand, decreases in the MT levels are probably associated to negative effects because they can change specific biological functions and promote higher additional stress susceptibility.

The PCA showed the influence of the biological parameters as length and weight in the biomarkers such as MT and ALAD. Therefore, the biological aspect is recommended to promote more accurate information about the exposure to pollutants.

The knowledge about the sources of contamination and distribution of chemicals in the Santos/São Vicente estuarine system is very important so the best way to manage and to control the impact caused by the anthropogenic activities is adopted, because this region is one of the most important industrial zones in Brazil. Moreover, the study of the effect of the multiple xenobiotics on resident species, such as the benthic fish *C. spixii*, can favor a better management of this system. In this context, the integration between some biomarkers, somatic indexes and the environmental data is the best way to understand and to generate a more effective environmental diagnosis. There are few studies about the use of biomarkers for biomonitoring of the Cananéia and Santos/São Vicente estuaries. Finally, this study reinforces the strong anthropogenic influence on the Santos/São Vicente estuarine system, mainly in the inner area of this estuary where industrial activities are intense and shows the suitability of *C. spixii* as sentinel species for biomonitoring studies.

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