

EVIDENCE OF CONTAMINATION BY OIL AND OIL PRODUCTS IN THE SANTOS-SÃO VICENTE ESTUARY, SÃO PAULO, BRAZIL

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ABSTRACT

Different components of the mixed function oxidase (MFO) system and the levels of fluorescent aromatic compounds in bile (FACs) were measured in *Cathorops spixii* in order to assess the impact of polycyclic aromatic hydrocarbons (PAHs). Fish were sampled in an estuary (Santos/São Vicente) with a history of contamination by PAHs, mainly due to the presence of the industrial complex of Cubatão city and of another of low anthropogenic influence (Cananéia) on the Brazilian coast. FACs were higher in fish from the polluted site, and the PAH 5 and 6-ring metabolites were the most frequent - with 14% and 15%, respectively. Levels of the different components of the MFO system showed the same variation profile as the FACs for both estuaries. Therefore, the values found for somatic indexes and biomarkers with data of bile PAH metabolites indicate the presence of organic contaminants, especially in the area subject to the influence of the industrial complex on the Santos/São Vicente estuary.

RESUMO

Diferentes componentes do sistema oxidase de função mista (MFO) e os níveis de compostos aromáticos fluorescentes em bile (FACS) foram determinados em *Cathorops spixii* a fim de avaliar o impacto de hidrocarbonetos policíclicos aromáticos (PAHs). Os peixes foram coletados em um estuário com histórico de contaminação por PAHs (Santos/São Vicente), devido principalmente a presença do complexo industrial na cidade de Cubatão e em outro com baixa influência antropogênica (Cananéia) na costa brasileira. FACS foram maiores nos peixes oriundos da área contaminada, sendo os metabolitos de HPAs com 5 e 6 anéis, os mais representativos com 14% e 15%, respectivamente. Os níveis dos diferentes componentes do sistema MFO mostraram o mesmo perfil de variação que os FACs em ambos os estuários. Portanto, os valores encontrados para os índices somáticos e os biomarcadores considerados, em associação com os dados de metabolitos biliares de PAHs, indicam a presença de contaminantes orgânicos, principalmente na área sob influência do complexo industrial no estuário de Santos/São Vicente.

Descriptors: Fluorescent aromatic compounds, Cytochrome P450, MFO system, *Cathorops spixii*, Brazilian coast.

Descritores: Compostos aromáticos fluorescentes, Citocromo P450, Sistema MFO, *Cathorops spixii*, Costa brasileira.

INTRODUCTION

The aquatic environment is affected by human activity and mainly by industrial discharges. Estuaries, in particular, are affected by urban, industrial, harbor and sometimes agricultural

discharges (OLIVEIRA RIBEIRO et al., 2005; VALDEZ DOMINGOS et al., 2007; AKAISHI et al., 2007). In aquatic ecosystems, contaminants bioaccumulation represent a potential risk to the biota and consequently to human health (HELLOU et al., 2006). The input of different pollutants in the

environment can affect the organism and, therefore, they are exposed to a complex mixture of contaminants (BOENING, 2000).

The liver represents one of the most important organs for bioaccumulation processes and metabolic pathways in which the monooxygenases enzymes are involved. Cytochrome P450s (CYPs) are a large superfamily of heme-proteins that play key roles in the xenobiotic biotransformation and in the synthesis and degradation of physiologically important endogenous substrates (FU et al., 2011). This family of monooxygenases includes the cytochrome *b*₅, NADPH cit. *c* P450 reductase and NADH cit. *b*₅ reductase as cofactors (SCHLENK et al. 1993; AL-ARABI; GOKSOYR, 2002; SCHENKMAN; JANSSON, 2003) and, the CYP450 activity depends on a variety of factors, such as age, sex, sexual maturity, seasonality, exposure history and diet (HARTL et al., 2007).

The analyses of PAH metabolites in fish bile provides information about its exposure to PAH compounds (VUORINEN et al., 2006). The fluorescent aromatic compounds (FACs) determination in fish bile is very important for the environmental quality evaluation to assess the organic levels of contamination and the potential risk offered for the biota. PAHs can accumulate in fish tissues inducing the activity of several enzymes, e.g. glutathione-S-transferase (VUORINEN et al., 2006), but especially the MFO system (STAGG et al., 2000; WHYTE et al., 2000; VUORINEN et al., 2006). Intracellular biotransformation of PAHs is related to mixed function oxidase (MFO) system (SCHLENK et al., 1993). Although there are considerable differences in PAH inducing capacity, the study of different components that integrate the MFO system shows a crucial role in the PAH metabolism (WHYTE et al., 2000; VUORINEN et al., 2006). MFO system also includes: i) cytochrome *b*₅, a heme-protein that acts as an intermediate in the unsaturation of fatty acids and can also act as an electron donor to cytochrome P450; ii) the NADPH cit. *c* P450 reductase, a flavoprotein responsible for the electron transfer from the NADPH to cytochromes P450 and *b*₅; and iii) the NADH cit. *b*₅ reductase, a flavoprotein responsible for the electron transfer from the NADH to cytochrome *b*₅. It is important to remark that the components of this system are also involved in electron transport of other biological processes and in apoptosis (MEHRTENS; LATURNUS; 1999).

Fish have a high confirmed capacity to accumulate metals and organic compounds in their tissues (FISK et al., 2001). Therefore, fish are selected as good bioindicators for environmental studies due to their capacity to accumulate pollutants in target tissues, transferring xenobiotics through trophic chain (FISK et al., 2001; BOON et al., 2002; OLIVEIRA

RIBEIRO et al. 2005). Furthermore, aquatic organisms are able to metabolize hydrocarbon compounds and to transform them into more polar, soluble by-products that are readily excreted. This metabolism is mediated via MFO system (SERAFIM et al., 2008).

Ariidae catfish, *Cathorops spixii*, commonly known as "Madamango sea catfish", has a wide geographical distribution in the Atlantic coast of South America, from Belize to the Brazilian Southeast coast, and also is the most common catfish that occurs in the Brazilian Coast (TIJARO et al., 1998). *C. spixii* spawns in late spring/early summer and has a total spawning with synchronic development of oocytes, and the fecundity is low when compared to other fish (GOMES et al., 1999; FÁVARO et al., 2005). *C. spixii* is a demersal fish species that feeds mainly the pelitic fraction (organic detritus) where the concentration of contaminants tends to be higher. Bioaccumulation and effects of organic pollutants were demonstrated in *Cathorops spixii* before (MAGALHÃES, 2005), but previous studies of our research group (AZEVEDO et al., 2009; AZEVEDO et al., 2009a) demonstrated that *C. spixii* is also an efficient sentinel species for trace metal contamination.

For a better comprehension of the biotransformation/bioaccumulation of organic compounds, it is necessary to support information regarding PAH metabolites in fish bile and evaluation of the MFO contents with biological data such as the hepatic somatic index (HSI) and condition factor (CF) to assess the toxic effects in the organism. Somatic indexes were previously used in biomonitoring studies as an efficient tool to support biomarkers responses in sentinel species (ADAMS; RYON, 1994; AZEVEDO et al., 2009a; LOMBARD et al., 2010). The CF indicates the environmental condition for living organisms such as fish and is a useful tool to compare different populations exposed to a variety of environmental stresses. According to Adams and Ryon (1994) and Karels et al. (1998), fish from polluted areas generally show lower CF values, while highest CF values are found in fish that reflect a larger adaptation to the environment. The HSI reflects the proportion between the liver and whole body (ZIMMERMAN, 1997) and HSI values tend to be lower in the period of gonadal maturation reflecting the use of hepatic reserves during the vitellogenesis processes (GURGEL et al., 2000). Fish from polluted environments usually show high HSI values (ADAMS; RYON, 1994; KARELS et al., 1998).

In the present study, responses of the MFO system in the benthic fish *C. spixii* exposed to "in situ" organic contamination were determined. Additionally, FACs in fish bile were also determined to assess the PAH bioavailability in two estuaries with different anthropogenic activities located in the Brazilian coast.

CF and HSI were also calculated in order to estimate the biological conditions of the individuals. As far as we know, this is the first study relating FACs and MFO system in a fish species in the southeastern Brazilian coast.

MATERIAL AND METHODS

Study Area

Santos' Bay is located in the southeastern coast of Brazil, as a part of São Paulo State seaboard (24°00'S; 46°21'W). The tropical rain forest climate is typical, with a heavy rainy season. The industrial activity and the tourism constitute important economic activities and recently marine petroleum exploitation activities are starting in this area. The largest commercial harbor of South America and one of the most important petrochemical and metallurgical industrial areas of Brazil (industrial complex of Cubatão city) are located in Baixada Santista region, constituted by approximately 1100 industries. The increase in the urbanization and industrialization close to the estuarine area, mainly in the last 50 years, was the principal responsible for the degradation of the mangrove vegetation and generated industrial impacts and also elevated the generation of domestic sewage effluents and solid wastes. Environmental programs of pollution control contributed to light diminution in the pollution impact in this area in the last few years, but the new petroleum exploitation activities could contribute to a new input of contaminants. For this reason, we consider very important the establishment of a monitoring program in this area.

Cananéia estuarine-lagoon complex, located in the southern coastal region of the São Paulo State (25°S; 48°W) was selected as reference sampling site due to their relative unpolluted waters and to their preserved estuarine ecosystem. The inner section of the estuarine-lagoon complex is subject to tidal cycles and freshwater inputs that define the regime of water circulation and water mixture in this estuarine-lagoon complex. The *C. spixii* was also present in this second system.

Collection of Animals and Sampling Sites

Fish were collected using a R/V Velliger II in winter 2005 and summer 2006 in two estuarine system at Santos/São Vicente estuarine system (*San*) affected by distinct contamination levels: Santos Bay (SB) n= 10; Santos Channel (SC) n= 10 and São Vicente Channel (SVC) n= 10. From reference area (Cananéia estuarine-lagoon complex, Can) were collected 18 animals using R/V Albacora (Fig. 1). Both sampling were performed 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. The selection of the three sites at *San* system was done according the different degrees of anthropogenic influence on the aquatic system: SC site, inner part of the system, is affected by intense industrial activity; SB site, less affected by industrial activity, at this sampling site dilution process of the chemical compounds is regulated by the marine currents; SVC site characterized by the presence of mangrove and precarious urban occupation. Can is situated into the UNESCO biosphere reserve of Iguape-Cananéia and shows an excellent conservation state (Fig. 1).

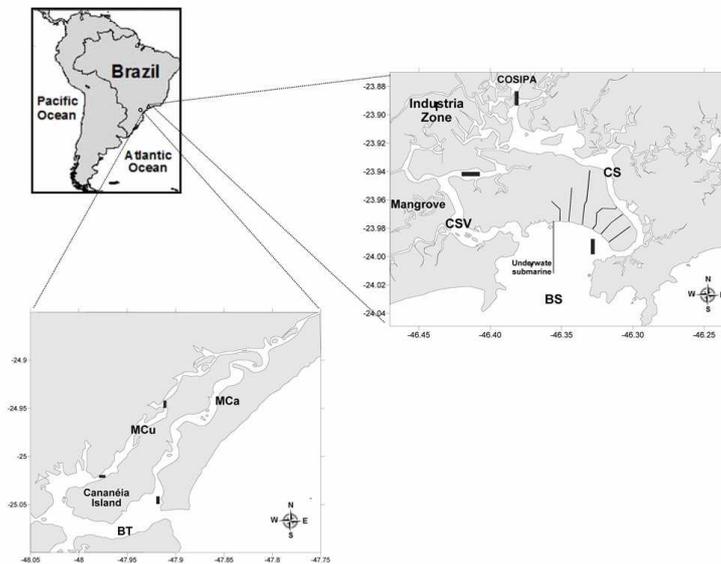


Fig. 1. Map of studied sites showing the sampling sites in Cananéia and Santos-São Vicente estuaries (Santos Bay: SB, Santos channel: SC, and São Vicente channel: SVC), São Paulo, Brazil.

C. spixii species was identified according to Figueiredo and Menezes (1978). After collection of the animals, fishes were kept in the same water of the sampling site and transported to the laboratory. The individuals were anesthetized with benzocaine (2% in water) and sacrificed through spinal section. Morphometric data (sex, total length, total body and liver weight) was annotated and the liver and bile collected. Liver samples were immediately frozen in liquid nitrogen and stored at -80°C for biochemical analyses. All biliary content was collect using a sterile syringe and stored at -20°C for subsequent analyses. Somatic indexes were calculated according with Vazoller (1996) and Lombardi et al. (2010). Hepatic somatic index (HSI) was calculated as $HSI = [\text{liver weight (g)/body weight}] \times 100$ and Condition Factor (CF) was determinate as $CF = [\text{body weight (g)/length (cm)}^3] \times 100$. The gonadal index (DK) was obtained by the expression: $(TW/TL^b) - (BW/TL^b)$, where:

TW = total weight (g)

TL = total length (mm)

BW = body weight (g)

^b = angular coefficient of the allometric expression $Y = a \cdot x^b$

Biochemical Analyses

The MFO system was measured in the microsomal fraction of the individual livers of *C. spixii* according with Livingstone (1988). Frozen livers (~1.00 g) were homogenized in buffer containing 10 mM Tris-HCl, 0.15 M KCl and 0.5 M sucrose (pH 7.6) and centrifuged three times: 500g for 15 min, 12,000g for 45 min and then at 100,000g for 90 min) Microsomal pellets were dissolved in 20 mM Tris-HCl and 20% glycerol (pH 7.6). The microsomal suspension of each sample were immediately used for the analysis. All assays were carried out in triplicate. Total cytochrome P450 and the '418-peak' (putative denatured cytochrome P450) contents were evaluated by the carbon monoxide difference spectrum of the sodium dithionite reduced sample, and absorbance values were measured in the interval 400-500 nm, using an extinction coefficient of $91 \text{ mM}^{-1} \text{ cm}^{-1}$. Cytochrome *b*₅ content was determined by the differences in spectra of microsomes reduced with 6 mM NADH versus oxidized microsomes using an extinction coefficient of $185 \text{ mM}^{-1} \text{ cm}^{-1}$ by the differences in absorbance values measured at 409 and 425 nm (ESTABROOK; WERRINGLOER, 1978).

NAD(P)H cytochrome *c* reductase activity was calculated by the increase in absorbance values measured at 550 nm after addition of 0.3 mM NADPH using an extension coefficient of $19.6 \text{ mM}^{-1} \text{ cm}^{-1}$. Total microsomal protein concentrations were

measured according to Lowry et al. (1951) using bovine serum albumin (BSA) as a standard.

Fish bile Fluorescent Aromatic Compounds (FACs)

Concentrations of PAH metabolites in bile content were determined according to Yang and Baumann (2006) with modifications. Bile samples were first diluted in 48% methanol (1:1500 bile/methanol) and the metabolites determined according with the sets of emission and excitation wavelengths in fluorimeter reader (spectrofluorimeter RF-5301 PC): 2-rings PAH compounds, (288 nm/330 nm, naphthalene type), 3-rings PAH compounds (267 nm/309 nm, phenanthrene type), 4-rings (334 nm/376 nm, pyrene type, 5-rings (364 nm/406 nm, benzo(a)-pyrene type) and 6-rings (380 nm/422 nm, benzo(gli)perylene type). The results were normalized to biliary concentration in $\text{ng } \mu\text{L}^{-1}$. Samples of $100 \mu\text{g mL}^{-1}$ 16 USEPA PAHs diluted in 48% methanol (1:1000, 1:1500, 1:2000, 1:3000, 1:4000, 1:5000, 1:10000) were used as standards.

Statistical Analysis

Normality of data was assessed by Kolmogorov-Smirnoff test and differences between sites in the biological parameters, variability of PAH concentrations and MFO levels were tested by ANOVA analysis. Tukey's test was performed to compare differences between groups with somatic indices and MFO compound contents. Differences among metabolites of PAHs were assessed using Post-hoc Bonferroni test. Results were represented as mean \pm standard deviation for all tests statistically significance were considered at $p \leq 0.05$.

RESULTS

Biological Aspects

All *C. spixii* sampled were mature individual, with active gonadal maturation, including spawning and post-spawning. Data about biological aspects of *C. spixii* from the non-polluted estuary (Canaanéia) and from three sites within Santos-São Vicente estuary (SC, SVC and SB) are shown in the Table 2. In order to verify the influence of the gender, all biological data and somatic indexes were first evaluated in separate male and female groups. Sex-dependent differences were not found for length, weight, HSI and CF analyses, therefore for these analyses animals were separated only by respective sampling site group. Based on weight data, fish from SB site collected in winter showed weight values significantly lower ($37 \pm 22 \text{ g}$) than fish sampled in the same period in SB ($213 \pm 78 \text{ g}$). Meanwhile, fish from

Can and SVC showed weight values significantly lower ($p < 0.05$) in the summer (Can: 45 ± 19 g; SVC: 57 ± 23 g) compared to fish collected in winter (Can: 83 ± 78 g; SVC: 75 ± 23 g). According with length values obtained, there are no significant differences between fish sampled in the both estuaries.

Based on HSI analyses, the lowest values were observed in individuals from Can (1.50 ± 0.32) and the highest values were obtained in specimens from SC (2.07 ± 0.28). However, statically differences were not observed neither between sampling sites nor seasonality. Based on CF values, only fish from SC collected in the summer showed significant differences in relation to reference site. According with the DK analysis, only males from SB sampled in the winter ($DK_M = 64.7\%$) showed significant differences in relation to reference animals. In general all females showed a displacement reservation for the reproduction of 9-13% (Table 2) while for males 10-15%, except for fishes from BS collected in winter (DK: 64.7%).

MFO system

Data concern hepatic MFO levels in *C. spixii* are shown in Figure 2. In general, significant differences were found in levels of CYP450, b_5 , NADH and NADPH. Fish from SC showed a similar pattern than fishes from SB meanwhile fishes from SVC showed similar MFO levels then individuals from reference site (Can) (Fig. 2).

The activity of total CYP450 in fish from SC and SB was higher than those from Can site. Lower total CYP450 levels (similar to that of Can site), were observed in fish from SVC. Although the pattern of cytochrome b_5 activity and CYP450 was similar, the activity of cytochrome b_5 was high in this species. In addition, significant seasonal differences were found in total CYP450 and b_5 activities in fish from SB sampled in the summer. High levels of total CYP450 and b_5 activities were observed in fishes from SC collected in the winter. Moreover, no significant variations were detected in CYP450 and b_5 activities in fish from SVC and Can site. Significant seasonal differences were observed in the NADPH and NADH contents of specimens from SB collected in the summer, when higher values were detected compared to winter.

Fluorescent Aromatic Compounds (FACs) in Bile

Figure 3 shows the mean levels of the Σ PAH in bile extract of *C. spixii* from all studied sites. Σ PAH content was statically higher ($p < 0.05$ Bonferroni post-hoc test) in fish from SC and SVC than in *C. spixii* from Can site and SB. However, there were no differences in Σ PAH of fish from Can site (67.81 ± 4.51 ng mL⁻¹) compared to fishes from SB (62.00 ± 7.2 ng mL⁻¹) and between individuals from SC (112.8 ± 7.4 ng mL⁻¹) and SVC (99.39 ± 10.12 ng mL⁻¹).

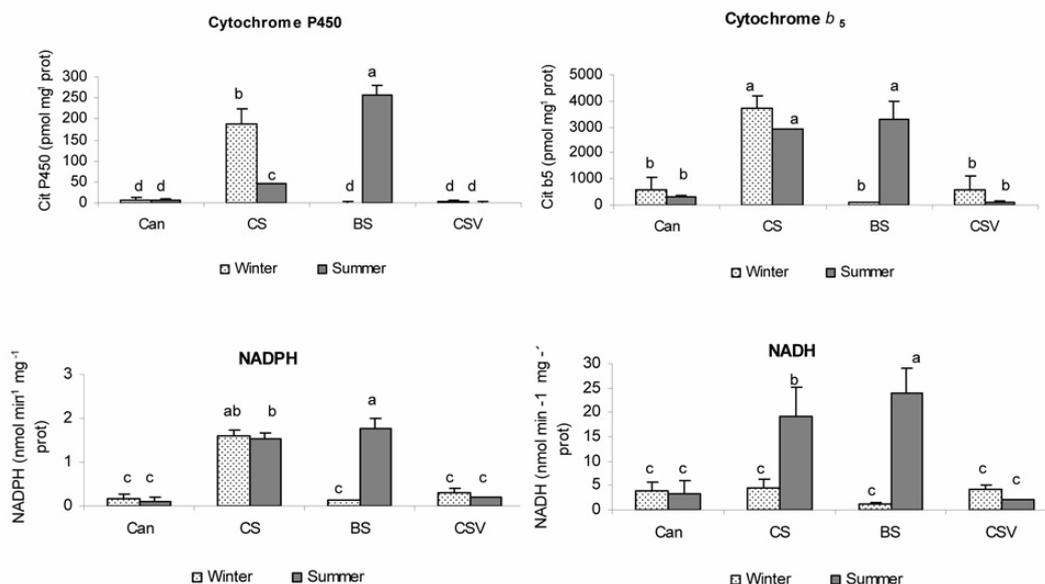


Fig. 2. Seasonal variation of monooxygenase (MFO) system for microsomal liver of *C. spixii* from Cananéia (Can) and three areas within the Santos-São Vicente estuary (SC: Santos channel; SB: Santos Bay; SVC: São Vicente channel). Data represent mean \pm sdv, and the bars followed by different letters are statistically different by Tukey post hoc test ($p < 0.05$).

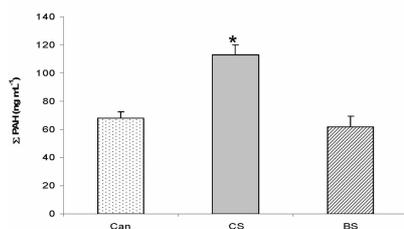


Fig. 3. Mean values \pm standard deviation of Σ PAH contents in bile of *C. spixii* from Cananéia estuary and three areas within Santos-São Vicente estuary (Santos channel: SC, Santos Bay: SB, and São Vicente channel: SVC). Distinct letters indicate significant differences by Bonferroni test ($p < 0.05$).

The concentrations of PAH metabolites, expressed according with the number of aromatic rings, are shown in the Table 3. Animals from different sites shown different profiles of aromatic metabolites. Naphthalene-like PAHs were the major PAH group in fish bile of all sites (except in SVC) and the highest contents were found in *C. spixii* from SC (40.92 ng mL^{-1}). 3-ring PAHs were also predominant in fish from SC (23.99 ng mL^{-1}). On the other hand, 4-, 5- and 6- rings-PAHs were higher in individuals from SVC (39.10 ng mL^{-1} , 22.72 ng mL^{-1} and 8.88 ng mL^{-1} , respectively). Significant differences were found in the levels of 4-, 5- and 6-rings PAH comparing individuals from the polluted areas in relation to reference site. Individuals from polluted areas showed higher concentrations of high molecular weight PAHs like benzo(a)pyrene-like (5 rings) (14% content) and benzo(ghi)perilene-like (6 rings) (15% content) then from the reference site (7% and 4% respectively).

DISCUSSION

In order to characterize sampling sites studies analytical chemistry assays of water were performed (Table 1) at the same period of this study. Based on this results of analytical chemistry of water (nutrients, pH and dissolved oxygen) Can site shown characteristics of a low human influence. On the other hand, Santos-São Vicente estuary sites showed high values of nutrients, indicating anthropogenic influence. Levels of dissolved oxygen (DO) are also in accordance with this argument. Details about water chemistry to both estuaries can be obtained in Azevedo et al. (2009a).

The use of somatic indexes such as HSI, CF, and ΔK in environmental studies were considered useful tools to evaluate the influence of biotic factors in the accumulation of these types of contaminants (ADAMS; RYON, 1994; KARELS et al., 1998; COUILLARD et al., 1999; LOMBARDI et al., 2010). Azevedo et al. (2009a) observed high HSI values in *C. spixii* from polluted sites in Santos-São Vicente estuary as compared with fish from a reference unpolluted area. However, in the present study no

significant differences were observed between HSI of fish from Santos-São Vicente and those from the Cananéia estuary. This finding can be explained by sampling period, different abiotic influences and, more specifically, by the high levels of individual variations in HSI. Excepting fish from SC sampled in the summer, CF observed in *C. spixii* can be to indicating an absence of negative effects, or an adaptive response to the presence of miscellaneous chemicals in the Santos-São Vicente estuary. DK was also considered, as well as GSI index, to investigate the variation in gonadal maturation stages among the specimens studied. In general, no significant differences were observed in DK values of *C. spixii* from both estuaries. The similarity in gonad conditions between individuals could be an inner characteristic of the species. However, the highest DK values observed in males compared with females can be related to the male catfish role in the incubation of the fecundated oocytes (VAZZOLER et al., 1989; VAZZOLER 1996), so needing for it larger displacement reserves for the reproduction.

The present work integrated the levels of P450 enzymes with the PAH metabolites in bile and the ecological indexes to evaluate the impact of human activities on studied sites. Exposure to PAHs induces the total CYP450 activities especially the CYP1A family (LEE; ANDERSON, 2005). A strong induction of the total CYP450 was observed in fish exposed to 4–6-rings PAHs as benzo(a)pyrene (SAUER et al., 1993; BURNS et al., 1997; AAS et al., 2000; LEE; ANDERSON, 2005). Therefore, the family CYP450 has been largely used as a specific biomarker of PAHs exposure (SCHLENK et al., 1993; AL-ARABI; GOKSOYR, 2002) and the present results in this species support this statement.

The obtained data showed high levels of total CYP450 in individuals from SC and SB compared to individuals from SVC and Can site. These findings are in accordance with that described by other tropical fish species from south and southeast coast of Brazil (Table 4). The high levels of b_5 found in *C. spixii* are probably associated with the intra-specific endogenous processes, once this enzyme (b_5) is primarily involved in the oxidation of various endogenous substrates and acts as an electron transfer component in a number of oxidative reactions (SCHENKMAN; JANSSEN 2003). The present data is in accordance with other studies where high levels of total CYP450 and b_5 were also found in fish species from North Sea exposed to organic compounds (MEHRTENS; LATURNUS 1999). Tagliari et al. (2004) also reported high levels of total CYP450 and b_5 in the liver of *G. gymno-genys* from a polluted site in São Paulo State. These authors suggest that these increased levels are an evidence of peroxidative lesions mediated by free radicals.

Table 1. Seasonal water chemistry data from Cananéia estuary (Can) and in three sites within Santos-São Vicente estuary (SC: Santos channel; SB: Santos Bay; SVC: São Vicente channel). Data represent mean, maximum and minimum in parenthesis. Adapted from Azevedo et al (2009a).

Region	pH	DO	P-PO ₄ ³⁻	N-NO ₃ ⁻	N-NO ₂ ⁻	N-NH ₄ ⁺
Winter						
Can	7.84 (7.54-8.10)	4.76 (4.42-4.98)	1.30 (0.77-1.94)	0.19 (0.14-0.29)	0.93 (0.56-1.83)	2.73 (2.25-3.67)
SC	7.92 (7.80-8.08)	3.61 (3.12-4.39)	4.48 (1.87-7.01)	0.93 (0.11-1.97)	4.22 (0.58-8.85)	10.47 (6.16-14.54)
SB	8.22 (8.16-8.25)	4.15 (3.88-4.71)	1.25 (0.55-3.10)	0.09 (0.03-0.14)	0.20 (0.03-0.58)	3.30 (1.62-2.92)
SVC	7.55 (7.48-7.65)	3.18 (3.01-3.36)	6.26 (5.13-7.87)	0.35 (0.31-0.41)	1.36 (0.96-1.48)	23.20 (20.55-24.86)
Summer						
Can	7.83 (7.50-8.09)	3.82 (3.35-4.01)	1.77 (1.10-2.70)	0.17 (0.07-0.35)	0.11 (0.09-0.17)	1.38 (0.79-2.12)
SC	8.01 (7.78-8.41)	2.73 (1.67-4.75)	5.37 (1.15-8.30)	21.81 (1.13-31.69)	4.44 (0.25-8.13)	14.42 (2.10-24.52)
SB	8.31 (8.22-8.39)	3.95 (3.29-4.45)	1.12 (0.83-1.74)	5.47 (3.80-6.45)	1.38 (0.63-2.63)	4.93 (0.32-14.06)
SVC	7.39 (7.21-7.63)	1.31 (1.09-1.62)	5.09 (4.18-5.89)	7.52 (5.30-9.95)	3.19 (2.16-4.25)	38.67 (39.08-44.00)

DO: dissolved oxygen (mg L⁻¹), P-PO₄³⁻:inorganic dissolved phosphorus, N-NO₃⁻: nitrate, N-NO₂⁻: nitrite, and N-NH₄⁺: ammonium. Nutrients in µM.

Table 2. Total length (TL), total weight (TW), hepatic somatic index (HSI) and condition factor (CF) of *Cathorops spixii* from Cananéia estuary (Can) and three areas within the Santos-São Vicente estuary (SB: Santos Bay, SC: Santos channel and SVC: São Vicente channel). Data represent mean ± standard deviation. n = 18 (Can), 10 (SB), 10 (SC) and 10 (SVC) fish for sampling period.

	TL (mm)	TW (g)	HSI	CF	%DK _F	%DK _M
Winter						
Can	192±48 ^a	83±78 ^b	1.76±0.23 ^d	0.96±0.09 ^d	11.9	11.3
SC	239±35 ^a	138±97 ^{ab}	2.07±0.28 ^d	0.91±0.12 ^d	11.6	64.7
SB	149±28 ^a	37±22 ^c	2.03±0.52 ^d	1.04±0.13 ^d	12.4	10.9
SVC	195±20 ^a	75±23 ^b	1.66±0.16 ^d	0.99±0.06 ^d	12.3	11.4
Summer						
Can	166±22 ^a	45±19 ^c	1.50±0.32 ^d	0.92±0.05 ^d	13.2	15.0
SC	226±34 ^a	109±54 ^{ab}	2.02±0.38 ^d	0.83±0.08 ^c	12.6	12.3
SB	284±29 ^a	213±78 ^{ab}	1.83±0.50 ^d	0.91±0.09 ^d	9.7	12.5
SVC	182±28 ^a	57±23 ^c	1.69±0.38 ^d	0.91±0.08 ^d	11.4	10.2

Distinct letters indicate significant differences by Tukey test ($p < 0.05$). %DK_F=gonadal index of females; %DK_M= gonadal index of males

Table 3. Total amounts of PAH metabolites (ng mL⁻¹) expressed as aromatic ring numbers in *C. spixii* from Cananéia estuary (Can) and within three areas in the Santos-São Vicente estuary (SC: Santos channel; SB: Santos Bay; and SVC: São Vicente channel).Data are expressed as mean and range (max-mix).

Site	2 rings	3 rings	4 rings	5 rings	6 rings	ΣPAH
Can	33.62 (19.25-57.57)	19.69 (15.58-34.77)	6.79 (5.82-9.52)	4.90 (3.53-8.80)	2.81 (1.85-4.40)	67.80 (42.95-98.28)
SB	26.23 (17.39-41.22)	12.15 (8.11-17.72)	12.38 (8.54-14.89)	8.52 (4.85-10.79)	2.86 (1.75-3.91)	62.14 (56.93-74.92)
SC	40.92 (33.29-46.34)	23.99 (18.44-30.63)	29.44 (6.29-66.16)	12.53 (6.37-25.12)	5.95 (3.37-10.47)	112.82 (93.00-169.17)
SVC	18.94 (17.85-55.78)	9.73 (8.68-10.78)	39.10 (24.95-53.26)	22.72 (11.10-34.34)	8.88 (5.07-12.69)	99.38 (71.95-126.82)

Table 4. Levels of the MFO system in fish liver microsomes from reference (*) and polluted sites. Data are mean \pm standard deviation. Cytochrome P450 and b_5 (pmol mg prot⁻¹) and NADPH cytochrome *c* reductase (nmol min⁻¹ mg prot⁻¹).

Specie	Localization	n	P450	b_5	NADPH	Reference
<i>Gymnogeophagus gymnogenys</i>	Rio Grande do Sul State, Brazil *	41	220 \pm 56	320 \pm 27	----	Tagliari et al., 2004
<i>Gymnogeophagus gymnogenys</i>	Rio Grande do Sul State, Brazil	60	n.d.	170 \pm 80	----	Tagliari et al., 2004
<i>Oreochromis niloticus</i>	São Paulo State, Brazil *	34	170 \pm 140	60 \pm 33	120 \pm 50	Thomaz et al., 2004
<i>Brycon cephalus</i>	São Paulo State, Brazil *	06	170 \pm 70	120 \pm 70	120 \pm 80	Thomaz et al., 2004
<i>Brycon cephalus</i>	São Paulo State, Brazil	05	230 \pm 130	100 \pm 10	380 \pm 150	Leitão et al., 2000
<i>Colossoma macropomom</i>	São Paulo State, Brazil	09	310 \pm 190	250 \pm 75	420 \pm 190	Leitão et al., 2000
<i>Oreochromis niloticus</i>	São Paulo State, Brazil	06	160 \pm 90	60 \pm 25	290 \pm 130	Leitão et al., 2000
<i>Oreochromis niloticus</i>	Billings, São Paulo State, Brazil	07	810 \pm 220	200 \pm 60	----	Bainy et al., 1999
<i>Pleuronectes platessa</i>	North Sea, German	02	520 \pm 170	100 \pm 20	----	Mehrtens and Laturnus 1999
<i>Limanda limanda</i>	North Sea, German	25	340 \pm 300	160 \pm 110	----	Mehrtens and Laturnus 1999
<i>Gadus morhua</i>	North Sea, German	03	220 \pm 30	60 \pm 40	----	Mehrtens and Laturnus 1999
<i>Cathorops spixii</i>	São Paulo State, Brazil *	18	7 \pm 4	488 \pm 337	0.2 \pm 0.08	This work
<i>Cathorops spixii</i>	São Paulo State, Brazil	27	80 \pm 46	2208 \pm 1650	0.90 \pm 0.70	This work

In addition, the variation of NADH cit. b_5 reductase in general followed the same pattern of Cytochrome b_5 in fish from SC and SB (high levels), and in fish from SVC and Can site (low levels) following the profile of the total CYP450. These findings suggest the influence of the flavoprotein NADH cit. b_5 reductase in the cytochrome b_5 activities. NADPH cytochrome *c* reductase also showed the same pattern of total CYP450. This co-factor is an electron donor to CYP450 and changes is probably affecting the function of the monooxygenase system. The lower levels of CYP450 in individuals from SVC are explained in part by the presence of a large mangrove area. This type of ecosystem plays an important role in the immobilization of the contaminants in the sediments or in the biotransformation of organic pollutants due to the intense biological activity (BIANCHI, 2007).

Seasonality effects found in total CYP450, b_5 , NADPH and/or NADH of fish from SC and/or SB reinforces the influence of organic contaminants in this system. In special in the summer period, fish from SB showed high levels of all CYP450 analyzed components. The summer period in this region is characterized by a heavy rainy season. Thus, the large rainfall in this period influences the input of contaminants or can also act by diluting these compounds. Finally, the present data supports the influence of organic compounds in Santos-São Vicente estuarine system as a potential risk to the biota.

FACs analysis in fish bile content confirmed a high anthropogenic influence in Santos-São Vicente estuary in comparison with Cananéia. The data

presented in this work showed the effect of contaminants on the expression of CYP450. The present work reinforce the use of these enzymatic systems as an efficient tool to assess environmental impacts in field studies. Finally, the use of biomarkers associated with somatic indexes and chemical analyses are essential to understand anthropogenic impacts and generate a more effective diagnosis of environmental health effects.

ACKNOWLEDGMENTS

This work was supported by CAPES (Brazilian Agency for Science and Technology), Oceanographic Institute of São Paulo University and the Laboratory of Ecotoxicology and Environmental Chemistry of the University of Algarve. Azevedo J. S. was a recipient of fellowships from CAPES-PDEE (BEX 2176/07-6) and this study make a part of the FAPESP Project - MOBIO, Process number 2005/50769-2.

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(Manuscript received 11 January 2011; revised 13 July 2011; accepted 20 November 2011)