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Metabolites in bile of fish from São Sebastião Channel, São Paulo, Brazil as biomarkers of exposure to petrogenic polycyclic aromatic compounds

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Abstract

This study reports the analysis of polycyclic aromatic compound (PAC) metabolites, as biomarkers of exposure to PACs in marine environment. PAC metabolites were measured in bile samples from 14 species of demersal fish caught in the São Sebastião Channel (SSC), SE Brazilian coastline. Naphthalene (NPH) equivalents, phenanthrene (PHN) equivalents, and benzo[a]pyrene (BaP) equivalents were quantified using a reverse-phase high-performance liquid chromatography coupled with fluorescence detection method. For all samples, the means (±standard deviation, $n = 37$) of concentrations obtained for NPH, PHN, and BaP equivalents were, respectively, 290,000 ± 200,000 ng/g, 18,000 ± 14,000 ng/g, and 970 ± 1900 ng/g. These results indicate recent exposure of these fish to PACs in their environment. In addition, two species (*Cyclichthys spinosus* and *Prionotus nudigula*) of fish were analyzed in order to investigate local sources of PAC contamination in the SSC and the influence of the petroleum terminal in fish caught in remote areas. The results showed that these fish species potentially migrate along the channel, especially *P. nudigula*. Correlations among groups of PAC metabolites indicate the same petrogenic source for NPH and PHN equivalents and a combustion source (e.g., automobile, ships) for BaP equivalents. The ratio BaP/PHN equivalents (0.05 ± 0.07, $n = 37$) confirms the predominance of petrogenic PACs for contamination by these chemicals in this region.
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1. Introduction

Polycyclic aromatic compounds (PACs) are a class of contaminants that are found frequently in the marine environment, most commonly associated with particulates in the water column that are then deposited into the sediment. PACs are of concern due their persistence and toxicity to marine organisms. PACs with more than three aromatic rings can be carcinogenic and mutagenic (Giessing et al., 2003; IARC, 1983; Larsen, 1995). In addition, there is a great concern in investigations of PAC in environmental samples because of the transfer of these compounds from water, sediment and food to fish and other marine biota (Giessing et al., 2003; Menzie et al., 1992). For many years, analyses of PACs and hydrocarbons in sediment and surface water from coastal areas and estuaries have provided valuable information about levels and sources of these contaminants in the environment (Bouloubassi and Saliot, 1993; Law and Biscaya, 1994; Wang and Fingas, 1997; Wang et al., 1999). However, these data have not provided information about uptake of these contaminants. Furthermore, the investigation of the exposure of biota to PACs in a polluted area can be useful for environmental risk assessment.

Fish can absorb contaminants from water via the body surface or gills and also from contaminated sediment or food (Varanasi et al., 1989). Exposure of marine organisms to most organic contaminants (e.g., PCBs) has been evaluated by measuring their concentrations in tissues. However, field studies have shown that fish captured in regions highly polluted with PACs contained low levels of PACs in tissue. Because these organisms have a well-developed mixed function oxidase system, parent PACs are rapidly metabolized (Varanasi et al., 1989). The metabolites produced in the liver are secreted into the bile and stored in the gall bladder before being excreted (Au et al., 1999). Laboratory studies have shown that the presence of PAC metabolites in bile of organisms is correlated with levels of exposure (Collier and Varanasi, 1991; Britvic et al., 1993; Upshall et al., 1993; Yu et al., 1995).

The screening of bile for PAC metabolites is a convenient and relatively rapid method for monitoring fish exposure to PAC contamination in their environment (Hellou and Payne, 1987; Krahn et al., 1993a,b,c; Lin et al., 1994). The São Sebastião Channel (SSC), along SE Brazilian coast, frequently experiences oil spills or discharges as a result of the presence of a large petroleum terminal, as well as a harbor. Although studies of sediment contamination

in this area have already been reported (Medeiros and Bícego, 2004; Silva, 2003), this paper reports, for the first time in Brazilian waters, the results of a study that determined relative amounts of PAC metabolites in bile of demersal fishes from the SSC as a biomarker of PAC exposure.

2. Materials and methods

2.1. Area of study

The SSC, an area of coastal fisheries, is located along the SE Brazilian coast (Fig. 1). The Channel is approximately 25 km long and 2 km wide. The largest petroleum terminal in Brazil (DTCS) is located in the center of the SSC. The DTCS is responsible for 55% of the petroleum transported in Brazil, so the channel is an important area in which to study petroleum contamination. In addition to the DTCS facility, São Sebastião Harbor (SSH) is also located on the central part of this Channel. Relatively high wind-driven currents (around 1.0–4.0 knots, SW to NE; Brasil, 1993) lead to fast evaporation of the low molecular weight PACs. In addition to the strong current, the narrow channel does not allow an intense sediment deposition in the area around the harbor or DTCS (Furtado, 1995).
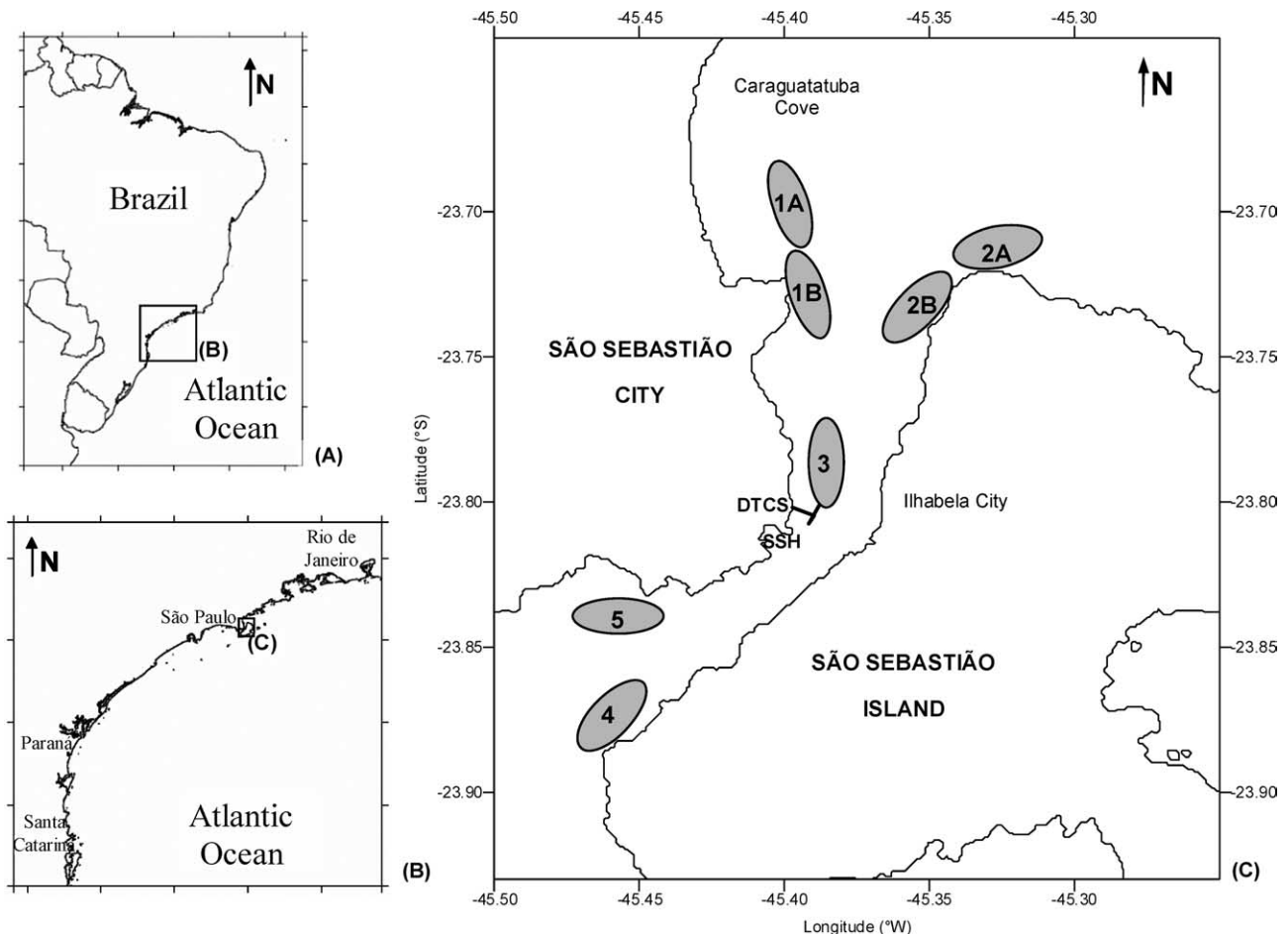

Fig. 1. Location of the São Sebastião Channel, showing the terminal of petroleum and derivatives (DTCS), São Sebastião Harbor (SSH) and the sites from which the fish were collected.

2.2. Sample collection

The sample collection sites shown in Fig. 1 were chosen according to previous investigations that measured the contamination of petroleum hydrocarbons in sediment from seven areas, using several geochemical markers, such as *n*-alkanes, isoprenoids, petroleum biomarkers (terpanes, hopanes, steranes) and PACs (Silva, 2003). In April 2004, 14 different species of fish were caught using a trawl net. Fish were transported to the laboratory on ice. The fish were identified, measured (weight and length) and gall bladders were removed from each fish. Bile was collected in sample vials by piercing the gall bladder with a solvent-rinsed scalpel and the samples were frozen immediately in liquid nitrogen and then at $-20\ ^{\circ}\text{C}$ in the laboratory. Bile samples from the same species and site were pooled when there was insufficient volume from individual samples. Table 1 shows the species of fish caught and their respective areas of collection.

Weight and length of the fish were used to calculate the feeding condition factor (FCF), which was obtained by calculating [wet weight in g/(length in cm)^3 × 100] for each fish.

2.3. HPLC/UV-F analysis of biliary metabolites of PACs

Bile samples were analyzed by high-performance liquid chromatography/ultraviolet fluorescence (HPLC/UV-F), according to Krahn et al. (1984). The reverse-phase analytical column used was a 4.6×150 mm, C18, Synergi 4 μm Hydro-RP (Phenomenex) with a 2 μm, 0.84 μL (internal volume) stainless-steel precolumn filter (Upchurch Scientific) and a 2×20 mm, C18 dry packed guard-column (Upchurch Scientific). The column temperature was 50 °C.

Table 1
Areas of collection and fish species from each area for which bile was analyzed (*n* = number of organisms in each sample)

Areas	Position		Species	*n*	Sample code
	Beginning	Ending			
1A	23°41.048′ S 045°24.306′ W	23°41.899′ S 045°23.893′ W	*Arius spixii*	4	27
			Arius spixii	6	28
			Cyclichthys spinosus	2	32
			Cyclichthys spinosus	3	33
			Lagocephalus laevigatus	1	31
			Paralonchurus brasiliensis	12	30
			Sciadeichthys luniscutis	3	29
1B	23°43.542′ S 045°23.578′ W	23°44.484′ S 045°23.783′ W	*Cyclichthys spinosus*	2	34
2A	23°43.050′ S 045°19.684′ W	23°43.140′ S 045°19.628′ W	*Cyclichthys spinosus*	2	9
			Cyclichthys spinosus	3	10
			Diplectrum formosum	3	1
			Haemulon steindachneri	1	6
			Haemulon steindachneri	1	7
			Haemulon steindachneri	3	8
			Lutjanus Synagris	1	4
			Porichthys porosissimus	1	3
			Stellifer rastrifer	2	2
			Syacium papillosum	7	5
2B	23°43.542′ S 045°23.578′ W	23°44.385′ S 045°21.060′ W	*Cyclichthys spinosus*	3	36
			Cyclichthys spinosus	3	37
			Prionotus nudigula	1	35
3	23°47.853′ S 045°22.994′ W	23°47.128′ S 045°22.706′ W	*Cyclichthys spinosus*	1	25
			Porichthys porosissimus	1	24
			Prionotus nudigula	4	23
			Symphurus tesselatus	20	26
4	23°52.479′ S 045°27.419′ W	23°52.088′ S 045°26.951′ W	*Porichthys porosissimus*	1	12, 13
			Porichthys porosissimus	2	15
			Prionotus nudigula	5	11
			Syacium papillosum	3	14
5	23°50.407′ S 045°28.096′ W	23°49.922′ S 045°27.248′ W	*Cyclichthys spinosus*	1	21
			Diapterus rhombeus	4	16
			Lagocephalus laevigatus	1	19
			Lagocephalus laevigatus	1	20
			Paralonchurus brasiliensis	9	18
			Prionotus nudigula	6	17
			Stellifer rastrifer	5	22

Acetic acid/water (5 μL/L) (solvent A) and methanol (solvent B) were used in a linear gradient as follows: with a 1.5 mL/min flow from 100% solvent A to 100% solvent B in 8 min; 13 min at 100% solvent B; 3 min to return to 100% solvent A and increase the flow to 2.7 mL/L; 6.3 min at 100% solvent A; 0.7 min to return a 1.5 mL/L flow at 100% solvent A; 6 min at 100% solvent A. The column was coupled with three UV-F detectors (Perkin–Elmer) connected in series. Bile (5 μL) was injected directly into the HPLC system and the chromatograms were recorded at excitation/emission wavelength pairs for each detector as follows: 290/335 nm for NPH; 255/380 nm for PHN; 380/430 nm for BaP. Peak areas eluting after 12 min were integrated, summed and quantified as NPH, PHN, or BaP equivalents. These peaks represent all compounds present in the bile sample that fluoresce at each wavelenght pair. A standard solution containing NPH (2.5 ng/μL), PHN (1.4 ng/μL), and BaP (0.2 ng/μL) was used as an external standard. This current investigation reports the existence of fluorescent PACs in non-hydrolyzed bile samples at the given wavelengths (above) and the possible presence of interfering compounds (other than PACs) that also fluoresce at these wavelengths cannot be disregarded. However, this is an exploratory study, which has assessed whether or not there is bioavailability of PACs and the influence of the PAC sources along the SSC.

The detection limit for each group of metabolite equivalents was determined as three times the standard deviation of the mean of six replicate analyses of the lowest concentration standard solution, which were: 0.013 μg/g for NPH equivalents, 0.085 μg/g for PHN equivalents, and 0.001 μg/g for BaP equivalents. The lowest concentration solution was demonstrated to have the lowest detectable peaks ($S/N = 3/1$).

2.4. Analysis of total biliary proteins

Total biliary proteins were analyzed according to Fryer et al. (1986). Bile samples were diluted in distilled water (1:100) and $CuSO_4$ in alkaline solution and phenolic Folin-Ciocalteu reagent were added. Bovine serum albumin was used as a standard. Then, the absorbance of the final solutions was measured at 620 nm using a plate-reader spectrophotometer (Titertrek Multiskan).

2.5. Quality assurance (QA) procedures used

Bile from Atlantic salmon (Salmo salar) exposed to crude oil was utilized as a control material for quality assurance in the analyses of both biliary PAC metabolites and total biliary proteins. The Atlantic salmon were exposed for 48 h to Monterey Bay crude oil, their bile was collected, and pooled then stored at −20 °C (ASMBC sample). The ASMBC is a control material that has been used at the Northwest Fisheries Science Center (NWFSC) for bile analyses since 2001.

Before analyzing samples, the stability of the HPLC system was verified by analyzing at least 4 replicates of the standard solution and one of the control material. The HPLC performance was considered stable when the standard deviation of the mean area of each analyte in the standard was less than 5% and the control material was within the lower or upper control limits. The control material, the standard solution and a methanol blank were injected before, during and after the samples. In addition, a replicate injection of selected samples was done to insure instrument analytical precision. Concentrations of the control material were compared within the sample set and with the previous analyses of the control material. In order for the sample analyses to be considered valid the following QA criteria needed to be met: All analytes of the standards within the sample set needed to be within 15% RSD, the control material needed to be within the upper and lower control limits, the methanol blank needed to be less than 10% of the sample analytes and the replicate sample injections must be within 10% RSD of each other. If any of these criteria were not met then the samples were re-injected after corrective action to the system had been completed. For all samples sets, the analytical results met laboratory criteria.

The analyses of biliary proteins were verified by analyzing the ASMBC as a control material. A method blank, using distilled water, and replicates of one sample for each sample set were analyzed. All samples were analyzed in triplicate. All analyses needed to meet the following control criteria: (a) the results for control material within ±25% of the known concentration; (b) at least five points on the calibration curve with a correlation coefficient >0.990; (c) the difference between the concentrations of duplicate samples within ±30% and (d) the absorbance for the water blank within <0.05. If one of these criteria was not met, the plate was re-read or a new plate with other standard solution for curve (6 points), new water blank, control material, and field samples were prepared.

2.6. Statistical treatment

The values of PAC metabolites (NPH, PHN, and BaP equivalents) were presented as mean ± standard deviation for the sites with more than one sample analyzed from the same species and also for general results from each group of PAC metabolites. A correlation coefficient (r^2) was calculated for correlation analyses and concentrations were log-transformed to improve the homogeneity of the variances. Analysis of variance (σ^2) was conducted for biliary proteins and FCF.

3. Results and discussion

3.1. Normalization of the PACs metabolites using total biliary proteins and FCF

Studies on fish have verified that, during periods of fasting the amounts of fluorescent PACs increase in bile

(Collier and Varanasi, 1991; Beyer et al., 1997). In contrast, after feeding, water rapidly fills the gall bladder and the fluorescent PACs are diluted (Klaassen and Watkins, 1984). In order to normalize these effects of feeding status on equivalents of biliary PAC metabolites, the total biliary proteins were measured. Fig. 2 illustrates the inconsistent correlations between equivalents of total PAC metabolites and total biliary proteins for the three species: *Cyclichthys spinosus*, *Prionotus nudigula*, and *Porichthys porosissimus*. Although the correlation between total PAC metabolites and bile protein was good for *P. porosissimus* ($r^2 = 0.91$, $P = 0.012$), no significant correlations were found for *C. spinosus* ($r^2 = 0.038$, $P = 0.62$) or *P. nudigula* ($r^2 = 0.78$, $P = 0.12$). Concentrations of total proteins varied significantly (31 ± 40 mg/mL; $\sigma^2 = 7904.29$, $n = 4$) for *P. porosissimus*, whereas relatively low variations were observed for the other species (19 ± 10 mg/mL; $\sigma^2 = 831.48$, $n = 9$ for *C. spinosus* and 32 ± 8.7 mg/mL; $\sigma^2 = 229.01$, $n = 4$ for *P. nudigula*). Because of these inconsistent correlations between biliary proteins and PAC metabolites and also because of the poor correlations generally found for these parameters (Ariese et al., 1997; Richardson et al., 2004), the results are presented as both protein-corrected and non-corrected concentrations.

FCF is another method often used to establish the nutritional status of fish and used to improve the decrease the variance in biliary PAC metabolites analyses (Escartín and Porte, 1999; Ruddock et al., 2003). No statistically significant differences ($P > 0.05$) have been observed among the FCF for fish from the same species. Fish from each species in the SSC had similar relationships (FCF) between weight and length. The mean of FCF $\pm$ SD was 5.91 ± 1.63 g/cm³ ($\sigma^2 = 36.33$, $n = 20$) for *C. spinosus*, 1.29 ± 0.07 g/cm³ ($\sigma^2 = 0.01$, $n = 16$) for *P. nudigula*, and 1.05 ± 0.12 g/cm³ ($\sigma^2 = 0.05$, $n = 5$) for *P. porosissimus*. Furthermore, no correlations were observed between the FCF and total biliary proteins. Therefore, FCF data are not used.

3.2. Level of exposure in the SSC

Concentrations of summed PACs (sum of alkyl-PACs and non-substituted PAC for each group; Table 2) were generally very low ($\sum$NPHs $\leqslant 7.7$ ng/g dry weight; $\sum$PHNs $\leqslant 26.1$ ng/g dry weight and BaP $\leqslant 11.2$ ng/g dry weight) in sediment samples collected from each of the study areas (Silva, 2003). This was likely a result of fast moving currents in the area removing sediment from the channel and transferring it to open water areas. The SSC sediments, with the exception of those from Area 4, had concentrations of PHN and/or NPH, as well as their branched chained derivatives, that were higher than those of BaP (Table 2). Area 3, close to the petroleum terminal, had the highest level of these parent PACs, especially PHN, in sediment. Area 4 was located some distance from the activities of the petroleum terminal and harbor, so pyro-
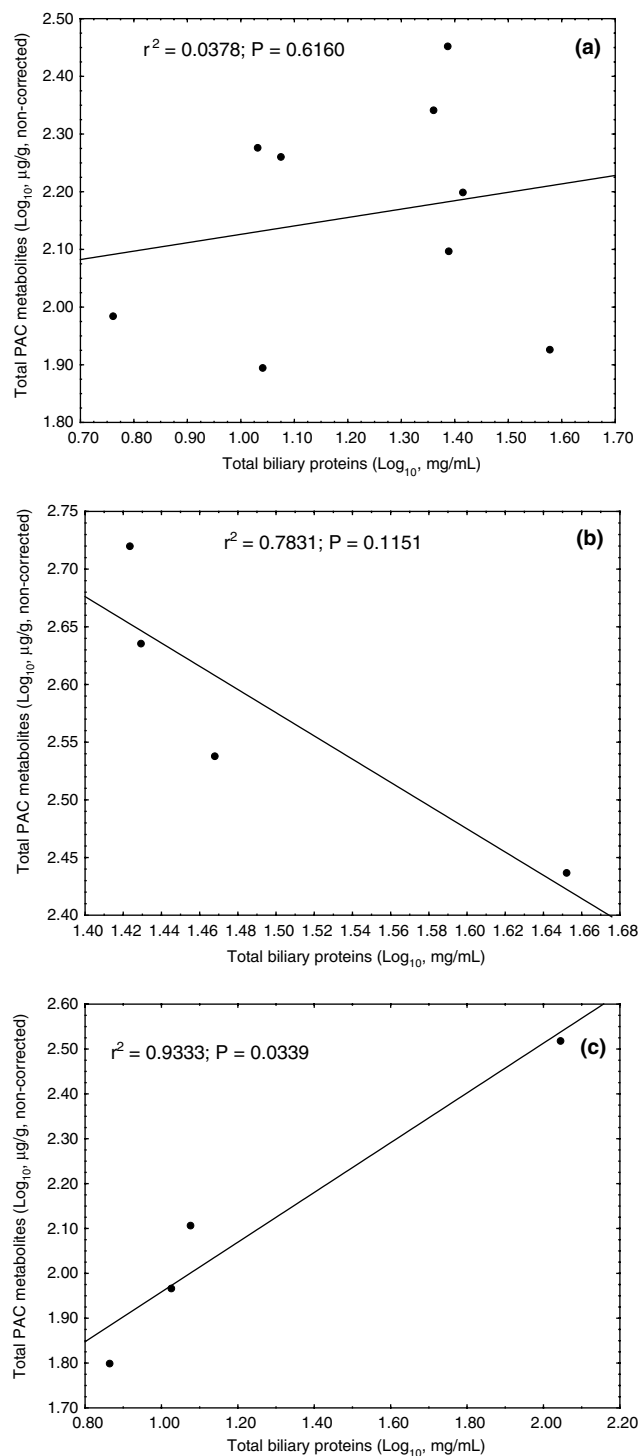

Fig. 2. Relationships between log-transformed total biliary proteins (mg/mL) and log-transformed total PAC metabolites (µg/g, non-corrected), and correlation coefficient for: (a) *C. spinosus*; (b) *P. nudigula*; (c) *P. porosissimus*.

genic compounds (e.g., BaPs) appear to be the major source of PAC pollutants in this area.

In spite of low levels of PAC contaminants in sediments, the fish species studied show evidence of exposure to PACs. For all species of fish in this study, summed amounts of

Table 2
Concentrations (ng/g; dry w.) of parent PACs in sediment samples collected from the same sites of the fish collection

PAC	1A	1B	2A	2B	3	4	5
$\sum$ Naphthalene[a]	3.3 ± 4.2	7.0 ± 3.7	2.3 ± 4.0	1.7 ± 2.4	7.7 ± 1.2	4.4 ± 7.2	<DL
$\sum$ Phenanthrene[b]	2.4 ± 2.1	13.1 ± 0.4	1.8 ± 3.2	2.1 ± 3.0	26.1 ± 10.8	6.3 ± 8.7	<DL
Benzo[a]pyrene	1.9 ± 0.2	4.8 ± 1.2	2.0 ± 2.3	1.7 ± 2.4	11.2 ± 4.8	7.5 ± 8.4	<DL

The values are presented as a mean among the sediment samples along each area $\pm$ standard deviation (Silva, 2003).
<DL = below detection limit.
[a] $\sum$ Naphthalene = (naphthalene; 2-, 1-methylnaphthalene; 2-ethylnaphthalene; 2,6-, 1,3-, 1,7-, 2,3-, 1,5-dimethylnaphthalene; 1,6,7-, 1,4,6-, 2,3,6-, 2,3,5-, 1,4,5-trimethylnaphthalene; 1,2,3,4-tetramethylnaphthalene).
[b] $\sum$ Phenanthrene = (phenanthrene; 9-, 2-, 1-methylphenanthrene; 2,7-, 2,3-dimethylphenanthrene; 2,3,5-trimethylphenanthrene).

NPH, PHN, and BaP equivalents (mean $\pm$ SD, $n = 37$) were $290{,}000 \pm 200{,}000$ ng/g, $18{,}000 \pm 14{,}000$ ng/g, and 970 ± 1900 ng/g, respectively. Species-specific differences in the ability to metabolize PACs (Varanasi et al., 1989) and the varying amounts of exposure to PACs by the fish result in high standard deviations. The presence of PAC metabolites indicates exposure to PACs and confirms that these species of demersal fish are capable of taking up petrogenic contaminants discharged into seawater, directly from the water or from exposed food sources.

The most representative fish species (those found at a number of sites) for the region of study, *C. spinosus* and *P. nudigula*, were caught in 6 and 4 different sites, respectively. Relative amounts of NPH, PHN and BaP equivalents for *C. spinosus* in areas of the SSC are given in Table 3. Although this species, which eats small invertebrates from the bottom, does not have a major commercial importance, its biomass is relevant to the marine ecosystem (Spach et al., 2003). *C. spinosus* caught from Area 3 generally showed the highest levels of the three groups of PAC metabolites, but only one pooled bile sample was available

(Table 3). PHN and BaP equivalents from Area 3 were approximately 4- to 10-fold higher than the other areas, whereas NPH equivalents were almost 2-fold higher. This finding is in agreement with higher concentrations of PACs in sediment samples from this area where the petroleum terminal and the harbor are located, compared to the other sites sampled (Table 2).

Elevated PAC metabolites, although not as high as for Area 3, were measured in bile of *C. spinosus* from Area 5, SW of the Channel, even though all contaminant concentrations were below the detection limit in sediment samples. Furthermore, elevated PAC metabolite levels were observed in bile samples from all species of fish caught in this area (data not shown). In contrast, *C. spinosus* from Area 1B had relatively low levels of bile metabolites (Table 3) even though the sediments had elevated concentrations of PACs. These results suggest that the fish from Areas 5 and 1B may not feed primarily in the area in which they were captured.

P. nudigula feed on small crustaceans on the bottom (Teixeira and Haimovici, 1989). The highest relative

Table 3
Relative amounts of PAC metabolites (ng/g) and biliary proteins (mg/mL) in bile of *Cyclichthys spinosus*

Area	Sample code (n)	Proteins (mg/mL)	PAC metabolites[a] (ng/g)			PAC metabolites[a] (ng/g, protein-corrected)		
			NPH equiv.	PHN equiv.	BaP equiv.	NPH equiv.	PHN equiv.	BaP equiv.
1A	32 (2), 33 (3)	22.9; 24.5	$160{,}000 \pm 45{,}000$	7200 ± 2200	150 ± 70	7000 ± 2100	310 ± 100	6.5 ± 3.1
1B	34 (2)	5.8	93,000	3300	60	16,000	570	10
2A	9 (2), 10 (3)	37.8; 26.0	$120{,}000 \pm 35{,}000$	5500 ± 2300	110 ± 20	4000 ± 1800	190 ± 110	3.8 ± 1.2
2B	36 (3), 37 (3)	11.9; 11.0	$120{,}000 \pm 47{,}000$	7200 ± 4900	160 ± 60	$11{,}000 \pm 3700$	610 ± 400	14 ± 4.7
3	25 (1)	24.3	250,000	32,000	1100	10,000	1300	50
5[b]	21 (1)	10.7	$180{,}000 \pm 5000$	8700 ± 400	190 ± 10	$16{,}000 \pm 470$	810 ± 40	18 ± 0.9

n = number of organisms in each sample.
NPH: naphthalene; PHN: phenanthrene; BaP: benzo[a]pyrene; equiv. = equivalents.
[a] Mean of samples $\pm$ standard deviation. Value without SD means only one sample from the area.
[b] Only one sample was analyzed, but it was done in duplicate.

Table 4
Relative amounts of PAC metabolites (ng/g) and biliary proteins (mg/mL) in bile of *Prionotus nudigula*

Area	Sample code (n)	Proteins (mg/mL)	PAC metabolites (ng/g)			PAC metabolites (ng/g, protein-corrected)		
			NPH equiv.	PHN equiv.	BaP equiv.	NPH equiv.	PHN equiv.	BaP equiv.
2B	35 (1)	29.4	330,000	15,000	290	11,000	510	10
3	23 (4)	44.9	260,000	13,000	290	5800	290	6.5
4	11 (5)	26.8	410,000	22,000	150	15,000	820	5.6
5	17 (6)	26.5	500,000	25,000	280	19,000	940	11

n = number of organisms in each sample.
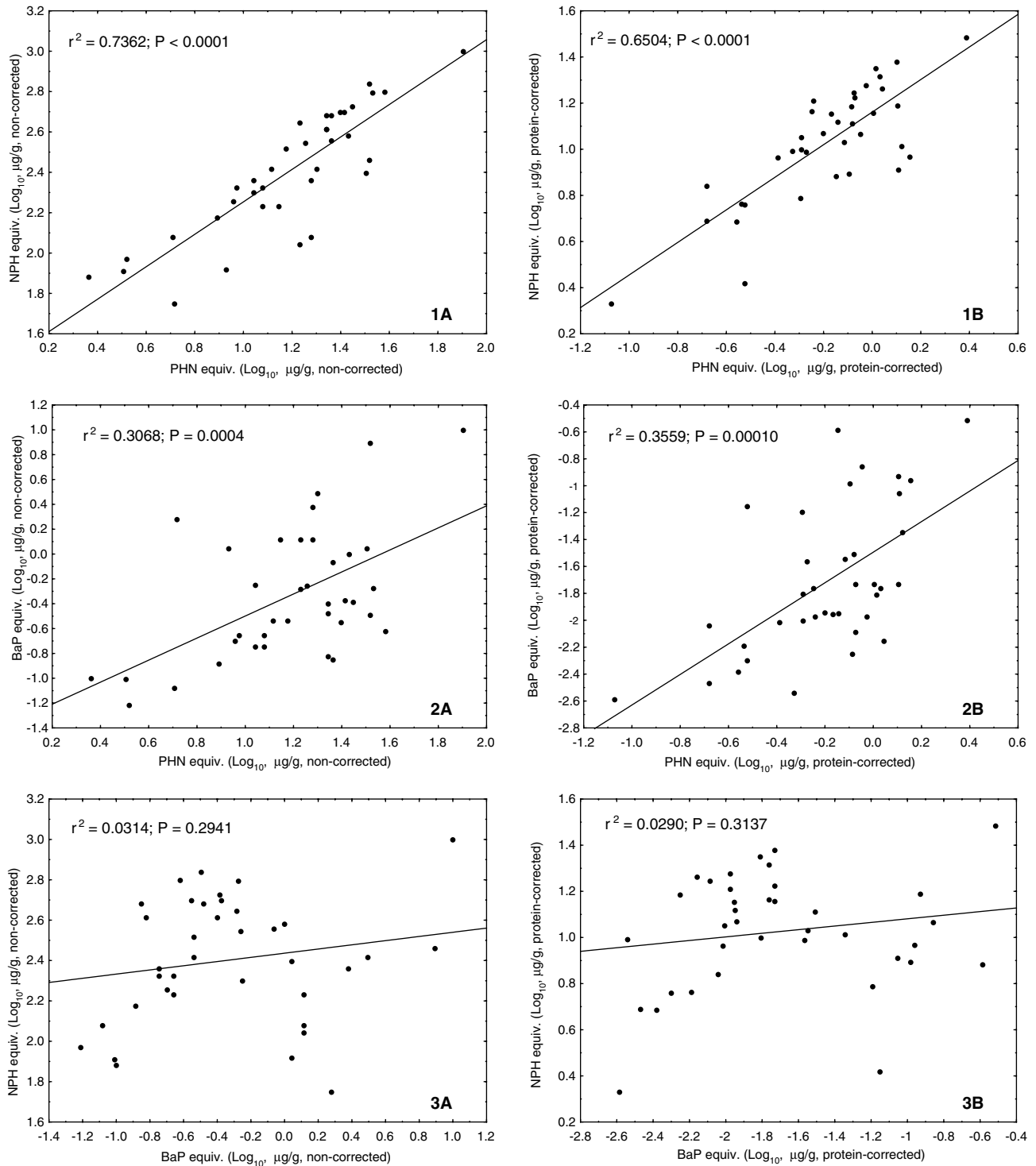NPH: naphthalene; PHN: phenanthrene; BaP: benzo[a]pyrene; equiv. = equivalents.

Fig. 3. Correlation among log-transformed concentrations ((A) non-corretcted and (B) protein-corrected) of PAC metabolites in bile of all samples from São Sebastião Channel: (1) PHN and NPH equivalents, (2) PHN and BaP equivalents, and (3) BaP and NPH equivalents.

amounts of total PAC metabolites in bile of this species were observed from samples collected in Area 5 (Table 4). For *P. nudigula*, the rank order, in decreasing concentrations: Area 5 > Area 4 > Area 2B > Area 3 (Table 4). Similar to the results for *C. spinosus*, concentrations of PAC metabolites in bile of *P. nudigula* did not agree well

with sediment results in terms of spacial distribution, suggesting that these fish may move along the Channel and feed in other areas. Furthermore, studies of benthic macrofauna in the SSC found that the moderately low total biomass in Area 5 (6.69 g/0.195 m^2; Pires-Vanin, Oceangraphic Institute, University of São Paulo, 2004, unpublished results).

Muniz and Pires (1999) also obtained relatively low biomass especially for surface deposit-feeders trophic group. Thus, the results from these benthic community studies suggest that this area is not a preferential feeding ground for demersal fish.

Good correlations were obtained between PHN equivalents and NPH equivalents (Fig. 3). Correlations were not as strong between PHN equivalents and BaP equivalents and no significant correlations were observed between BaP and NPH equivalents (Fig. 3). These results show that petrogenic PACs, such as PHN and NPH, are likely obtained from the same source, such as petroleum discharged into the channel. On the other hand, BaP most likely has a source related to combustion (e.g., automobiles) that is unrelated to the source of petrogenic PACs (Wang et al., 1999).

The source of parent PACs in the marine environment can be demonstrated by the relationship between PHN (or NPH) and BaP equivalents in bile. When PHN or NPH metabolite levels are high, a petrogenic source of PACs is evident. Conversely, when BaP metabolites are high, a pyrogenic source is predominate. In some studies, levels of PAC metabolites in bile were measured to evaluate petroleum contamination in aquatic environment. For example, Krahn et al. (1992), studied fish from Prince William Sound, Alaska, following the *Exxon Valdez* 11 million-gallon oil spill. In fish species caught even 1 year postspill, high amounts of NPH and PHN equivalents were found, ranging from 270,000 to 2,600,000 ng/g for NPH equivalents and 44,000 to 380,000 ng/g for PHN equivalents. In another study, Krahn et al. (1986) investigated the 170,000-gallon oil spill in Columbia River, Oregon. In the site closest to the spill, five days postspill, fish bile was found to contain 200,000 ng/g of NPH, 210,000 ng/g of PHN, and 2,100 ng/g of BaP equivalents. In contrast, Escartín and Porte (1999) found the highest levels for BaP equivalents in bile of fish from NW Mediteranean, close to high urbanized areas like Barcelona Harbor, suggesting a pyrolytic source for the corresponding PAC.

In this current study with fish from São Sebastião Channel the mean ratio of BaP/PHN equivalents was 0.05 ± 0.07 ($n = 37$). This finding suggests that petrogenic PAC sources are the predominant contributors to summed PACs in this region.

4. Conclusions

High levels of NPH and PHN metabolites in bile of demersal fishes from São Sebastião Channel showed the presence of petrogenic sources of these contaminants, likely as a result of inputs of petroleum and derivatives to these waters. Furthermore, the analyses of bile from several species of fish, especially *C. spinosus* and *P. nudigula*, in different areas of the SSC showed that these species appear to migrate along the channel, because elevated PAC metabolites were found in fish from sites in which sediment contamination by petroleum hydrocarbons was relatively low

and, conversely, lower relative amounts of PAC metabolites in bile of fish where the sediment contamination was higher.

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