Ocean and Coastal Research

Brief Communication
Special Article Collection
Souza, R. B. and Lopes, R. M. (eds), Environmental variability and hazards on the coastal and continental shelf regions of South America and the Caribbean
http://doi.org/10.1590/2675-2824071.22047vfp
ISSN 2675-2824

Bioavailability of polycyclic aromatic hydrocarbons in Santos Bay (Brazil) and its adjacent continental shelf

Vinicius Faria Patire[1,2], Ana Cecília Rizzatti de Albergaria-Barbosa[2,3,4]*, Isana Souza Barreto[4], Satie Taniguchi[2], Wellington Silva Fernandez[5], June Ferraz Dias[5], Denis A. M. da Silva[6], Marcia Caruso Bícego[2]

1. Centro Interdisciplinar de Energia e Ambiente – Universidade Federal da Bahia – Rua Barão de Jeremoabo, sn, 40170-115, Salvador, BA, Brazil
2. Laboratório de Química Orgânica Marinha – Instituto Oceanográfico – Universidade de São Paulo – Praça do Oceanográfico, 191, 05508-900, São Paulo, SP, Brazil
3. Laboratório de Geoquímica Marinha – Instituto de Geociências – Universidade Federal da Bahia – Rua Barão de Jeremoabo, sn, 40170-110, Salvador, BA, Brazil
4. LEPETRO – Instituto de Geociências – Universidade Federal da Bahia – Rua Barão de Jeremoabo, sn, 40170-110, Salvador, BA, Brazil
5. Laboratório de Ecologia Reprodutiva e do Recrutamento - Instituto Oceanográfico – Universidade de São Paulo – Praça do Oceanográfico, 191, 05508-900, São Paulo, SP, Brazil
6. Environmental and Fisheries Sciences Division - Northwest Fisheries Science Center - National Marine Fisheries Service - National Oceanographic and Atmospheric Administration – 2725 Montlake Blvd. E., Seattle, WA, 98112, USA

* Corresponding author: cecilia.albergaria@ufba.br

Abstract

This study evaluated the bioavailability of polycyclic aromatic hydrocarbons (PAHs) in Santos Bay (SB) and the adjacent Santos Continental Shelf (SCS) in Brazil. Biliary metabolites were measured in several fish species to establish a baseline for future monitoring programs. Bile samples from different species of fish were collected monthly from July to December 2005 in SB, and in August 2005 and February 2006 on SCS. Metabolite concentrations were determined using high-performance liquid chromatography with fluorescence detectors. Naphthalene, phenanthrene, and benzo[a]pyrene metabolite concentrations ranged from 24 to 810 μg g^{-1} of bile, 1.8 to 68 μg g^{-1} of bile, and below the limit of quantitation to 1.3 μg g^{-1} of bile, respectively. Despite its high concentrations, the levels of naphthalene metabolites were in regions of low-contamination, while benzo[a]pyrene metabolite were in the same range as those reported in moderately contaminated areas, which may indicate pyrolytic contamination by PAHs. No significant differences in the metabolite concentrations were found between the SB and the SCS samples or during the periods of collection. Future studies with a single biomonitoring species should be conducted, considering age, sex, and feeding condition of the individuals. The metabolite data presented in this study is an important baseline information for this urbanized region, which hosts several sources of contaminants.

Descriptors: Biomarker of exposure, PAH metabolites, HPLC/F, fish metabolites, biomonitoring.

Polycyclic aromatic hydrocarbons (PAHs) are a group of hydrophobic organic compounds widespread in the marine environment mainly due to anthropogenic activities (Bouloubassi and Saliot, 1993; Beyer et al., 2010). These compounds are particularly concerning for their mutagenic and carcinogenic effects (White, 1986; Baumann and Harshbarger, 1998) and are frequently monitored in environmental studies (Yunker et al., 2002; Beyer et al., 2010; Martins

Submitted: 04/05/2022
Approved: 03/03/2023
Editor: Rubens M. Lopes

et al., 2011; Kammann et al., 2017; Barreto et al., 2020; Snyder et al., 2020).

PAHs levels and sources of contamination in a region are often determined by analyzing abiotic matrices such as sediment (Yunker et al., 2002; Medeiros and Bícego, 2004a, 2004b; Guimarães et al., 2020), and analyzing the bioavailability of these compounds in organisms residing in contaminated areas is also essential. On the other hand, quantifying PAHs in fish tissue may underestimate exposure levels (Black, 1983; Varanasi, 1989; Beyer et al., 2010) since aquatic vertebrates have a well-developed enzyme system that efficiently metabolizes PAHs into more hydrophilic metabolites (Varanasi, 1989). In fish, PAHs are metabolized by cytochrome P4501A, by oxidation, producing short-lived compounds such as epoxides. After further metabolization, glucoronides and sulfate conjugates are excreted in the urine or secreted in the bile and rapidly eliminated (Collier et al., 2013). Determining PAH metabolites in fish bile is a useful biomarker of contamination and studies have shown that its presence correlates with recent exposure to PAHs (Collier and Varanasi, 1991; Britvić et al., 1993; Upshall et al., 1993; Anulación et al., 2020). This determination has been used in international monitoring programs (Fuchsman et al., 2001; HELCOM, 2013; Kammann et al., 2017) and environmental studies to evaluate the bioavailability of PAHs (Krahn et al., 1984, 1986, 1993; Escartín and Porte, 1999a, 1999b; da Silva et al., 2006; Pulster et al., 2020; Snyder et al., 2020; Silva et al., 2021).

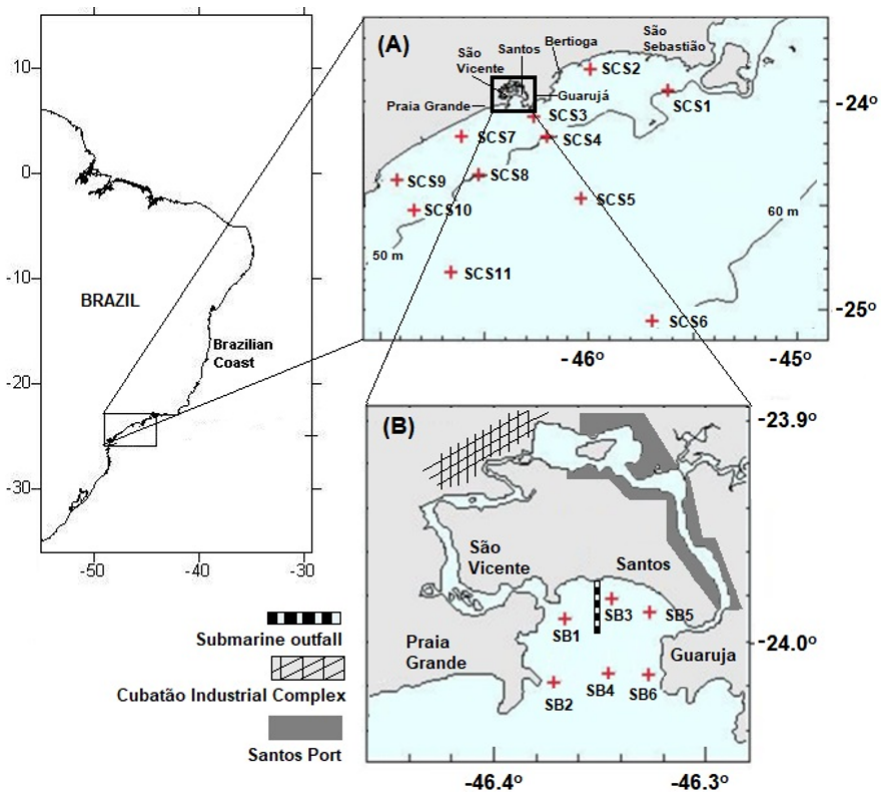

Figure 1. Geographic location of the Santos Continental Shelf (A), Santos Bay (B), main sources of contamination in the region, and areas sampled

Santos Bay (SB) and the adjacent Santos Continental Shelf (SCS) (Figure 1) are located on the southeastern coast of Brazil, in one of the most economically important areas of the country. The increase in anthropogenic activity over the last 100 years in this region contributed to the release of contaminants into the bay (Martins et al., 2011). A wide range of compounds and elements (e.g., petroleum hydrocarbons, benzothiazoles, polychlorinated biphenyls, polybrominated diphenyl ethers, cocaine, metals, etc.) have been detected in SB due to contamination sources

(Martins et al., 2007; Kim et al., 2016; Magalhães et al., 2017; Fontes et al., 2019). Petroleum exploration also contribute to the release of PAHs in the region (Azevedo et al., 2012), especially in SCS. This activity has increased substantially in the last decade, making it necessary to establish a monitoring program in the area. Some studies conducted in SB and its estuary have evaluated the bioavailability of PAHs using fish biliary metabolites (de Albergaria-Barbosa et al., 2017, 2018), but no studies have been conducted in the adjacent SCS.

This study investigated the bioavailability of PAHs in fishes in Santos Bay and Santos Continental Shelf by measuring biliary PAH metabolites in several species to establish a baseline study for future monitoring programs.

Samples were collected in SB from July to December 2005 and in August 2005 and February 2006 on the SCS. A total of six areas of the bay (Figure 1) and eleven areas of the continental shelf (Figure 1) were sampled by trawling (towing speed: 3–4 knots; 10 minutes). The species were selected based on spatial and temporal occurrence, with an emphasis on demersal fishes. The species sampled in SB were *Stellifer brasiliensis*, *Cathorops spixii*, *Larimus breviceps*, *Paralonchurus brasiliensis,* and *Orthopristis ruber*. The species sampled on the SCS were *Dactylopterus volitans*, *Paralonchurus brasiliensis*, *Paralichthys isosceles*, *Paralichthys patagonicus*, *Ctenosciaena gracilicirrhus*, *Lagocephalus laevigatus,* and *Menticirrhus martinicensis*. Tables 1 and 2 show further details on the species sampled and collection areas. After removing the gall bladder, bile was collected in a cryogenic vial until obtaining at least 20 µL of bile. Some bile samples were taken from a pool of two or more individuals of the same species collected in the same location and month (Tables 1 and 2). Samples were kept packed in dry ice until storage in an ultrafreezer at –80 °C.

PAH metabolites in the bile samples were analyzed in 2006 using high-performance liquid chromatography with fluorescence detectors (HPLC/F) (Agilent Technologies 1200 series), following the method described by Krahn et al. (1984). A more complete description of the HPLC/F conditions can be found in Albergaria-Barbosa et al. (2017). A standard solution containing naphthalene (NAP, 1.0 ng μL^{-1}), phenanthrene (PHE, 0.5 ng μL^{-1}), and benzo[a] pyrene (BaP, 1.0 ng μL^{-1}) was used as the external standard. Bile (5 µL) was injected directly into the HPLC/F system, and peaks were recorded in the chromatograms at excitation/emission wavelength pairs for each group of compounds, as follows: 290/335 nm for NAP, 249/364 nm for PHE, and 380/430 nm for BaP. Peak areas that eluted after 2 minutes were integrated, summed, and quantified as NAP, PHE, or BaP metabolite equivalents. These peaks represented all compounds present in a bile sample that fluoresce at each wavelength pair. The NAP-type metabolite (metNAP), PHE-type metabolite (metPHE), and BaP-type metabolite (metBaP) mainly comprise two-ring, three-ring, and five-ring structures, respectively. This study reports fluorescent PAHs in nonhydrolyzed bile samples at the given wavelengths, but notes the possible presence of interfering compounds (other than PAHs) that also fluoresce at these wavelengths.

Table 1. Average length (mm), weight (g) and concentrations of naphthalene (metNAP), phenanthrene (metPHE), benzo[a]pyrene (metBaP), and total biliary metabolites (TM) in µg g^{-1} of bile found in the species sampled in Santos Bay (n = number of fish used in each sample).

	Sampled Area	Species	n	Length (mm)	Weight (g)	metNAP	metPHE	metBaP	TM
July	SB3	*Cathorops spixii*	3	201 ± 41	80.6 ± 34.2	750	8.7	0.87	760
	SB5	*Stellifer brasiliensis*	1	174	71.6	78	68	0.88	150
August	SB6	*Larimus breviceps*	1	217	121	200	15	0.83	220
October	SB6	*Larimus breviceps*	1	255	237	180	15	0.45	200
November	SB3	*Paralonchurus brasiliensis*	2	200 ± 44	74.7 ± 79.9	100	7.1	0.29	110
December	SB2	*Orthopristes ruber*	1	271	226	130	12	0.77	140
	SB3	*Cathorops spixii*	1	197	60.4	30	2.5	0.39	33

Table 2. Average length (mm) and concentrations of naphthalene (metNAP), phenanthrene (metPHE), benzo[a]pyrene (metBaP), and total biliary metabolites (TM) in μg g^{-1} of bile found in the species sampled on the Santos Continental Shelf (n = number of fish used in each sample; <LOQ = concentration lower than the limit of quantitation). Individual weights not available.

	Sampled area	Species	n	Length (mm)	metNAP	metPHE	metBaP	TM
February	SCS7	*Paralonchurus brasiliensis*	3	211 ± 5	45	2.0	<LOQ	47
	SCS9	*Dactylopterus volitans*	2	189 ± 44	71	6.8	0.51	78
	SCS10	*Dactylopterus volitans*	3	230 ± 6	49	4.6	0.49	54
	SCS11	*Dactylopterus volitans*	3	206 ±28	52	3.7	0.35	56
	SCS12	*Paralichthys isosceles*	4	306 ± 6	53	2.7	<LOQ	56
	SCS16	*Dactylopterus volitans*	4	168 ± 13	99	8.8	0.60	110
	SCS17	*Menticirrhus martinicensis*	2	393 ± 2	140	11	0.41	150
	SCS17	*Dactylopterus volitans*	2	240 ± 28	59	6.0	0.53	66
	SCS18	*Lagocephalus laevigatus*	1	600	110	12	0.80	120
	SCS19	*Dactylopterus volitans*	3	196 ± 25	130	15	1.3	150
	SCS19	*Ctenosciaena gracilicirrhus*	2	186 ± 5	130	1.8	1.1	130
	SCS19	*Paralichthys patagonicus*	1	422	24	1.8	0.30	26
August	SCS2	*Dactylopterus volitans*	2	254 ± 4	240	15	0.59	260
	SCS5	*Paralonchurus brasiliensis*	3	198 ± 19	250	11	0.62	260
	SCS9	*Paralonchurus brasiliensis*	5	194 ± 10	810	22	0.39	830
	SCS11	*Dactylopterus volitans*	5	161 ± 9	93	9.1	0.44	100
	SCS17	*Dactylopterus volitans*	3	237 ± 21	100	11	0.67	110
	SCS20	*Dactylopterus volitans*	7	160 ± 9	120	8.1	0.67	130

Bile density was determined by weighing 20 aliquots of 100 μL of bile on an analytical scale. The average was 1.024 ± 0.105 mg mL^{-1}. This value was used to convert concentrations of metNAP, metPHE, and metBaP from μg mL^{-1} of bile to μg g^{-1} of bile, the most commonly used unit and the one adopted in this study.

The quality control assurance procedures of the analytical methods used in this study were adopted from Albergaria-Barbosa et al. (2018). Briefly, Atlantic salmon (*Salmo salar*) exposed to crude oil (ASMBC) was used as the control material. ASMBC was donated by the Northwest Fisheries Science Center of the United States National Oceanic and Atmospheric Administration, Seattle, USA, and it has been used as a control material since 2001. Before injecting the samples, the stability of the HPLC system was verified by analyzing at least five replicate samples of the standard solution and one sample of the control material. The performance of the system was considered stable when the standard deviations of the mean area of each analyte in the standard were less than 5% and the NAP, PHE, and BaP equivalent levels determined in the control material were within the range of control limits.

The control material, standard solution, and a methanol blank were injected before, during, and after sample sequences. Concentrations in the control material and standard solution must be within the established upper and lower control limits and methanol blank levels should be less than 10% of the sample analytes. Replicate injections of selected samples were performed to ensure analytical precision and a relative standard deviation (RSD) lower than 10% was required for analyses to be considered valid. The results in all sample sets met these laboratory criteria.

The limit of quantitation (LOQ) values were determined as six times the standard deviations of six replicate injections of the standard solution, which were 0.99 μg g^{-1} for metNAP, 0.36 μg g^{-1} for metPHE, and 0.27 μg g^{-1} for metBaP.

Statistical analyses were performed using STATISTICA – version 7. The Mann–Whitney U test was used to compare spatial differences in metabolite concentrations. The level of significance for rejecting the null hypothesis (no difference between groups) was set to 0.05.

Total metabolite (TM) concentrations (sum of all analyzed metabolites) ranged from 33 to 760 μg g^{-1} of bile (230 ± 241 μg g^{-1} of bile) in SB (Table 1) and 26 to 830 μg g^{-1} of bile (152 ± 181 μg g^{-1} of bile) on the SCS (Table 2). This wide concentration range has also been reported in other studies (Pulster et al., 2020) and may have occurred due to the use of individuals of different species, sexes, sizes, and weights (Tables 1 and 2). Nevertheless, such levels agree with data described in previous studies in SB and its estuary (de Albergaria-Barbosa et al., 2017, 2018) and can be compared with other urban coastal regions (McCain et al., 1990; Escartín and Porte, 1999b; da Silva et al., 2006; Silva et al., 2021).

MetNAP was the most abundant compound, contributing from 52 to 99% of the TM in all bile samples; its concentrations ranged from 30 to 750 μg g^{-1} of bile (209 ± 245 μg g^{-1} of bile) in SB and from 24 to 810 μg g^{-1} of bile (143 ± 177 μg g^{-1} of bile) in SCS (Tables 1 and 2). MetPHE concentrations, which corresponded to 1.1 to 45% of TM levels, ranged from 2.5 to 68 μg g^{-1} of bile (18 ± 22 μg g^{-1} of bile) and from 1.8 to 22 μg g^{-1} of bile (8.5 ± 5.5 μg g^{-1} of bile) in SB and SCS, respectively. Levels of metBaP in SB and SCS ranged from 0.29 to 0.88 μg g^{-1} of bile (0.64 ± 0.25 μg g^{-1} of bile) and <LOQ to 1.3 μg g^{-1} of bile (0.57 ± 0.30 μg g^{-1} of bile), respectively, corresponding to less than 1.5% of TM in all samples. The dominance of metNAP and lower concentration of metBaP are commonly found in studies with fish bile metabolites (Krahn et al., 1986; Escartín and Porte, 1999a, 1999b; da Silva et al., 2006; de Albergaria-Barbosa et al., 2017, 2018; Pulster et al., 2020).

According to Fuchsman et al. (2001), metNAP levels above 500 μg g^{-1}, metPHE levels above 50 μg g^{-1}, and metBaP levels above 1 μg g^{-1} are indicative of bile sampled in highly contaminated environments. MetNAP levels between 300 and 500 ng g^{-1}, metPHE levels between 10 and 50 ng g^{-1}, and metBaP levels between 0.2 and 1 ng g^{-1} are indicative of bile sampled in moderately contaminated environments. Levels below the values presented above suggest areas minimally contaminated by PAHs. Only one sample in SB and one sample in SCS presented levels of metNAP in the moderated contamination range. All other samples presented low contamination by NAP. For metPHE, 43% of samples in SB and 39% in SCS were comparable to moderately contaminated areas, while 43% in SB and 61% in SCS were in the range of low-contaminated areas. For metBaP, all samples from SB and 78% of the samples from SCS presented levels in the range of moderate contamination. This indicates that the studied areas are moderately contaminated mainly by high molecular weight compounds (HMW) and slightly contaminated by low molecular weight (LMW) compounds.

The presence of LMW PAHs, such as NAP, is usually linked to petrogenic sources (Soclo et al., 2000; Colombo et al., 2006), whereas the presence of HMW compounds, such as BaP, is mainly linked to pyrolytic sources (Fernández-Tajes et al., 2011). The Santos region has historically several types of contamination. The sediment of the area is moderately contaminated by PAHs (Martins et al., 2011), especially those derived from the burning of organic matter (Medeiros and Bícego, 2004a; Martins et al., 2011). This is an important source of PAHs in the studied areas (Martins et al., 2011), mainly due to the Industrial Pole of Cubatão (IPC) activities that occur in SB (Figure 1) (Medeiros and Bícego, 2004a; de Albergaria-Barbosa et al., 2017, 2018). IPC is one of the most important metallurgical/petrochemical industrial centers in Brazil, with the presence of steel mills, oil refineries, fertilizer, cement, and chemical/petrochemical plants that sum up to 260 pollutant emission sources (Cetesb, 1999).

No significant differences in TM, metNAP, metPHE, or metBaP levels were found between the two areas (Mann–Whitney U test, $p > 0.05$). This means that, according to our data, the bioavailability characteristics of PAHs in the sampled species in SB and SCS were similar. As SB is closer to the sources of PAHs, the bioavailability of these compounds

in this area was expected to be greater than that of the SCS. This suggests that onshore anthropogenic activities in the SCS also serve as an important source of PAHs to fishes. However, as distinct species were found in the studied areas, it is difficult to determine the differences in contamination levels between SB and SCS. Factors such as feeding strategy, age, sex, dietary preference, habitat, and metabolic rate of each species can influence the uptake and metabolism of PAHs (Varanasi, 1989), masking the differences between the regions.

To allow for statistical analyses of metabolite concentrations during collection periods, SCS assessments were made using only the results obtained from *D. volitans*. This test was not performed when the number of species sampled in each month in SB was low. No significant differences in the levels of TM, metNAP, metPHE, or metBaP were found between the two sampling periods in the SCS (Mann–Whitney U test, $p > 0.05$). PAH metabolite levels may be associated with the season, as metabolism tends to decrease in colder months and increase in warmer months (Eggensl et al., 1996; Hylland et al., 1996; Rotchell et al., 1999). However, previous studies conducted in the Santos region found no differences in PAH metabolite concentrations in fishes collected during different periods (de Albergaria-Barbosa et al., 2018). As observed by Albergaria-Barbosa et al. (2018), studies in which such differences were found were conducted in temperate regions, where seasonal temperature variations are considerable. SCS does not have a marked temperature variation throughout the year. However, grouping organisms with different characteristics (e.g., sexes, ages, size, and maturity stages) or feeding status may mask the effects of climate and oceanographic conditions. Metabolism can also be affected by the biological characteristics of the sampled fish (Varanasi, 1989), and variations in the dataset increase by combining different organisms (Kammann, 2007). Due to the carcinogenicity of PAHs and their impacts on metabolism in the immune and reproductive systems and on ichthyoplankton (Collier et al., 2013), further studies are needed.

The levels of metabolites in the bile of demersal fishes indicated moderate contamination in SB and SCS, especially by high molecular weight PAHs. No differences in metabolite concentrations were found between the studied areas. Future studies with a single biomonitoring species should be conducted to establish differences in bioavailability between Santos Bay and Santos Continental Shelf, considering the age, sex, and feeding condition of the samples. Nonetheless, the PAH metabolite data presented in this study using multiple species are important as baseline information for this urbanized region, which harbors several sources of PAHs and other chemicals. Such data are still lacking in the literature for this area, as well as other heavily urbanized coastal areas of South America. This study can potentially support long-term monitoring studies around Santos Bay using any of the species presented here and help initiate discussions leading to regulatory decisions. Analysis of bile metabolites in fishes using high-performance liquid chromatography with fluorescence detectors may be important in present and future environmental monitoring programs.

ACKNOWLEDGMENTS

This study was developed by graduate students of the course GEOC53 “Aquatic Contamination Biomarkers” offered by the Graduate Program in Geochemistry: Petroleum and Environment (POSPETRO) of the Federal University of Bahia (UFBA) in partnership with the graduate programs in Energy and Environment (PPGEnAM) of UFBA and Oceanography of the University of São Paulo (USP). These programs are supported by *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES – Coordination for the Advancement of Higher Education Personnel), finance code 001. The fund used for the sample collection was provided by *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP – State of São Paulo Research Assistance Foundation), Grant 2003/09932-1. We are grateful to the crews of research vessels “Prof. W. Besnard” and “Velliger” for their cooperation and assistance in the sampling. The funds used for the analytical instruments

and the analyses were provided by FAPESP (Grant 2005/03801-8). Albergaria-Barbosa, Patire, and Barretos thank *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq – National Council of Scientific and Technological Development) and CAPES for their scholarships. Albergaria-Barbosa and Bícego also thank CNPq for their research grants. We thank the anonymous reviewer for the constructive comments and further suggestions, which certainly improved this updated version of the manuscript.

AUTHOR CONTRIBUTIONS

V.F.P.: Formal analysis; Investigation; Methodology; Validation; Visualization; Writing - original draft.
A.C.R.A.B.: Formal analysis; Investigation; Methodology; Supervision; Validation; Visualization; Writing - original draft; Writing - review & editing.
I.S.B.: Visualization; Writing - original draft, Writing - review & editing.
S.T.: Conceptualization; Formal analysis; Investigation; Methodology; Supervision; Writing - review & editing.
W.F., J.F.D.: Formal analysis; Methodology; Writing - review & editing
D.A.M.S.: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Validation; Visualization; Writing - review & editing.
M.C.B.: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing - review & editing.

REFERENCES

de Albergaria-Barbosa, A. C. R., Patire, V. F., Taniguchi, S., Fernandez, W. S., Dias, J. F. & Bícego, M. C. 2017. *Mugil curema* as a PAH bioavailability monitor for Atlantic west sub-tropical estuaries. *Marine Pollution Bulletin*, *114*(1), 609–614. DOI: https://doi.org/10.1016/j.marpolbul.2016.09.039

de Albergaria-Barbosa, A. C. R., da Silva, D. A. M., da Silva Rocha, A. J., Taniguchi, S., Patire, V. F., Dias, J. F., Fernandez, W. S. & Bícego, M. C. 2018. Evaluation of polycyclic aromatic hydrocarbons bioavailability on Santos Bay (Brazil) through levels of biliary metabolites. *Marine Pollution Bulletin*, *129*(2), 822–828. DOI: https://doi.org/10.1016/j.marpolbul.2017.10.006

Anulación, B. F., Ylitalo, G. M., Sol, S. Y., da Silva, D. A. M., Lomax, D. P. & Johnson, L. L. 2020. Temporal trends in aluminum smelter-derived polycyclic aromatic hydrocarbons in outmigrant juvenile *Chinook salmon* from Kitimat, British Columbia, Canada. *Marine Pollution Bulletin*, *157*, 111284. DOI: https://doi.org/10.1016/j.marpolbul.2020.111284

Azevedo, J. S., Lopes, B., Katsumiti, A., Braga, E. S., Roche, H., Ribeiro, C. A. O. & Bebianno, M. J. 2012. Evidence of contamination by oil and oil products in the Santos-São Vicente estuary, São Paulo, Brazil. *Brazilian Journal of Oceanography*, *60*(2), 117–126. DOI: https://doi.org/10.1590/s1679-87592012000200002

Barreto, I. S., de Albergaria-Barbosa, A. C. R., Patire, V. F., de Jesus Silva, M., Baldassin, P., Taniguchi, S., Montone, R. C., Gallo, H., Maranho, A. & Bícego, M. C. 2020. Bioavailability of polycyclic aromatic hydrocarbons to penguins on the coast of southeastern Brazil. *Marine Pollution Bulletin*, *157*, 111306. DOI: https://doi.org/10.1016/j.marpolbul.2020.111306

Baumann, P. & Harshbarger, J. 1998. Long term trends in liver neoplasm epizootics of brown bullhead in the Black River, Ohio. *Environmental Monitoring and Assessment*, *53*, 213–223. DOI: https://doi.org/10.1023/A:1005967631275

Beyer, J., Jonsson, G., Porte, C., Krahn, M. M. & Ariese, F. 2010. Analytical methods for determining metabolites of polycyclic aromatic hydrocarbon (PAH) pollutants in fish bile: A review. *Environmental Toxicology and Pharmacology*, *30*(3), 224–244. DOI: https://doi.org/10.1016/j.etap.2010.08.004

Black, J. J. 1983. Field and laboratory studies of environmental carcinogenesis in Niagara River fish. *Journal of Great Lakes Research*, *9*(2), 326–334. DOI: https://doi.org/10.1016/s0380-1330(83)71902-7

Bouloubassi, I. & Saliot, A. 1993. Investigation of anthropogenic and natural organic inputs in estuarine sediments using hydrocarbon markers (NAH, LAB, PAH). *Oceanologica Acta*, *16*(2), 145–161.

Britvić, S., Lucić, D. & Kurelec, B. 1993. Bile fluorescence and some early biological effects in fish as indicators of pollution by xenobiotics. *Environmental Toxicology and Chemistry*, *12*(4), 765–773. DOI: https://doi.org/10.1002/etc.5620120418

Cetesb, S. P. 1999. *Relatório anual da qualidade do ar no estado de São Paulo 1998* (Relatórios ambientais). São Paulo: Companhia de Tecnologia de Saneamento Ambiental (Cetesb).

Collier, T. K., Anulación, B. F., Arkoosh, M. R., Dietrich, J. P., Incardona, J. P., Johnson, L. L., Ylitalo, G. M. & Myers, M. S. 2013. Effects on fish of polycyclic aromatic hydrocarbons (PAHS) and naphthenic acid exposures. *Fish physiology* (Vol. 33, pp. 195–255). Elsevier. DOI: https://doi.org/10.1016/b978-0-12-398254-4.00004-2

Collier, T. K. & Varanasi, U. 1991. Hepatic activities of xenobiotic metabolizing enzymes and biliary levels of xenobiotics in English sole (*Parophrys vetulus*) exposed to environmental contaminants. *Archives of Environmental Contamination and Toxicology*, *20*(4), 462–473. DOI: https://doi.org/10.1007/bf01065834

Colombo, J. C., Cappelletti, N., Lasci, J., Migoya, M. C., Speranza, E. & Skorupka, C. N. 2006. Sources, vertical fluxes, and equivalent toxicity of aromatic hydrocarbons in coastal sediments of the Río de la Plata estuary, Argentina. *Environmental Science &amp Technology*, *40*(3), 734–740. DOI: https://doi.org/10.1021/es051672y

Eggensl, M. L., Vethaak, A. D., Leaverz, M. J., Horbach, G. J. M. J., Boon, J. P. & Seinen, W. 1996. Differences in CYP1A response between flounder (*Platichthys flesus*) and plaice (*Pleuronectes platessa*) after long-term exposure to harbour dredged spoil in a mesocosm study. *Chemosphere*, *32*(7), 1357–1380. DOI: https://doi.org/10.1016/0045-6535(96)00046-x

Escartín, E. & Porte, C. 1999a. Biomonitoring of PAH pollution in high-altitude mountain lakes through the analysis of

fish bile. *Environmental Science & Technology*, *33*(3), 406–409. DOI: https://doi.org/10.1021/es980798a

Escartín, E. & Porte, C. 1999b. Hydroxylated PAHs in bile of deep-sea fish. Relationship with xenobiotic metabolizing enzymes. *Environmental Science & Technology*, *33*(16), 2710–2714. DOI: https://doi.org/10.1021/es9902322

Fernández-Tajes, J., Flórez, F., Pereira, S., Rábade, T., Laffon, B. & Méndez, J. 2011. Use of three bivalve species for biomonitoring a polluted estuarine environment. *Environmental Monitoring and Assessment*, *177*(1), 289–300. DOI: https://doi.org/10.1007/s10661-010-1634-x

Fontes, M. K., de Campos, B. G., Cortez, F. S., Pusceddu, F. H., Moreno, B. B., Maranho, L. A., Lebre, D. T., Guimarães, L. L. & Pereira, C. D. S. 2019. Seasonal monitoring of cocaine and benzoylecgonine in a subtropical coastal zone (Santos Bay, Brazil). *Marine Pollution Bulletin*, *149*, 110545. DOI: https://doi.org/10.1016/j.marpolbul.2019.110545

Fuchsman, P. C., Leigh, K. B. & Barber, T. R. 2001. Ecological assessment of PAHs in fish. *Sediments guidance compendium*. Palo Alto, CA: EPRI.

Guimarães, L. M., França, E. J. D., de Arruda, G. N. & de Albergaria-Barbosa, A. C. R. 2020. Historical inputs of polycyclic aromatic hydrocarbons in the preserved tropical estuary of the Itapicuru River, Bahia, Brazil. *Marine Pollution Bulletin*, *156*, 111218. DOI: https://doi.org/10.1016/j.marpolbul.2020.111218

Helcom. 2013. HELCOM core indicators: Final report of the HELCOM CORESET project. Helsinki: Helsinki Comission.

Hylland, K., Sandvik, M., Skåre, J. U., Beyer, J., Egaas, E. & Goksøyr, A. 1996. Biomarkers in flounder (*Platichthys flesus*): an evaluation of their use in pollution monitoring. *Marine Environmental Research*, *42*(1), 223–227. DOI: https://doi.org/10.1016/0141-1136(95)00034-8

Kammann, U. 2007. PAH metabolites in bile fluids of dab (*Limanda limanda*) and flounder (*Platichthys flesus*): Spatial distribution and seasonal changes (7 pp). *Environmental Science and Pollution Research — International*, *14*(2), 102–108. DOI: https://doi.org/10.1065/espr2006.05.308

Kammann, U., Akcha, F., Budzinski, H., Burgeot, T., Gubbins, M. J., Lang, T., Menach, K. L., Vethaak, A. D. & Hylland, K. 2017. PAH metabolites in fish bile: From the Seine estuary to Iceland. *Marine Environmental Research*, *124*, 41–45. DOI: https://doi.org/10.1016/j.marenvres.2016.02.014

Kim, B. S. M., Salaroli, A. B., de Lima Ferreira, P. A., Sartoretto, J. R., de Mahiques, M. M. & Figueira, R. C. L. 2016. Spatial distribution and enrichment assessment of heavy metals in surface sediments from Baixada Santista, Southeastern Brazil. *Marine Pollution Bulletin*, *103*(1), 333–338. DOI: https://doi.org/10.1016/j.marpolbul.2015.12.041

Krahn, M. M., Myers, M. S., Burrows, D. G. & Malins, D. C. 1984. Determination of metabolites of xenobiotics in the bile of fish from polluted waterways. *Xenobiotica; the Fate of Foreign Compounds in Biological Systems*, *14*(8), 633–646. DOI: https://doi.org/10.3109/00498258409151461

Krahn, M. M., Rhodes, L. D., Myers, M. S., Moore, L. K., MacLeod, W. D. & Malins, D. C. 1986. Associations between metabolites of aromatic compounds in bile and the occurrence of hepatic lesions in English sole (*Parophrys vetulus*) from Puget Sound, Washington. *Archives of Environmental Contamination and Toxicology*, *15*(1), 61–67. DOI: https://doi.org/10.1007/bf01055249

Krahn, M. M., Ylitalo, G. M., Buzitis, J., Bolton, J. L., Wigren, C. A., Chan, S.-L. & Varanasi, U. 1993. Analyses for petroleum-related contaminants in marine fish and sediments following the Gulf oil spill. *Marine Pollution Bulletin*, *27*, 285–292. DOI: https://doi.org/10.1016/0025-326x(93)90035-i

Magalhães, C. A., Taniguchi, S., Lourenço, R. A. & Montone, R. C. 2017. Organochlorine pesticides, PCBs, and PBDEs in liver and muscle tissues of *Paralonchurus brasiliensis*, *Trichiurus lepturus* and *Cathorops spixii* in Santos Bay and surrounding area, São Paulo, Brazil. *Regional Studies in Marine Science*, *16*, 42–48. DOI: https://doi.org/10.1016/j.rsma.2017.08.010

Martins, C. C., Bícego, M. C., Mahiques, M. M., Figueira, R. C. L., Tessler, M. G. & Montone, R. C. 2011. Polycyclic aromatic hydrocarbons (PAHs) in a large South American industrial coastal area (Santos Estuary, Southeastern Brazil). Sources and depositional history. *Marine Pollution Bulletin*, *63*(5), 452–458. DOI: https://doi.org/10.1016/j.marpolbul.2011.03.017

Martins, C. C., Mahiques, M. M., Bícego, M. C., Fukumoto, M. M. & Montone, R. C. 2007. Comparison between anthropogenic hydrocarbons and magnetic susceptibility in sediment cores from the Santos Estuary, Brazil. *Marine Pollution Bulletin*, *54*(2), 240–246. DOI: https://doi.org/10.1016/j.marpolbul.2006.11.006

McCain, B. B., Malins, D. C., Krahn, M. M., Brown, D. W., Gronlund, W. D., Moore, L. K. & Chan, S.-L. 1990. Uptake of aromatic and chlorinated hydrocarbons by juvenile chinook salmon (*Oncorhynchus tshawytscha*) in an urban estuary. *Archives of Environmental Contamination and Toxicology*, *19*(1), 10–16. DOI: https://doi.org/10.1007/bf01059807

Medeiros, P. M. & Bícego, M. C. 2004a. Investigation of natural and anthropogenic hydrocarbon inputs in sediments using geochemical markers. I. Santos, SP – Brazil. *Marine Pollution Bulletin*, *49*(9), 761–769. DOI: https://doi.org/10.1016/j.marpolbul.2004.06.001

Medeiros, P. M. & Bícego, M. C. 2004b. Investigation of natural and anthropogenic hydrocarbon inputs in sediments using geochemical markers. II. São Sebastião, SP – Brazil. *Marine Pollution Bulletin*, *49*(11), 892–899. DOI: https://doi.org/10.1016/j.marpolbul.2004.06.002

Pulster, E. L., Gracia, A., Armenteros, M., Toro-Farmer, G., Snyder, S. M., Carr, B. E., Schwaab, M. R., Nicholson, T. J., Mrowicki, J. & Murawski, S. A. 2020. A first comprehensive baseline of hydrocarbon pollution in Gulf of Mexico fishes. *Scientific Reports*, *10*(1), 1–14. DOI: https://doi.org/10.1038/s41598-020-62944-6

Rotchell, J., Bird, D. & Newton, L. 1999. Seasonal variation in ethoxyresorufin O-deethylase (EROD) activity in European eels *Anguilla anguilla* and flounders *Pleuronectes flesus* from the Severn estuary and Bristol channel. *Marine Ecology Progress Series*, *190*, 263–270. DOI: https://doi.org/10.3354/meps190263

da Silva, D. A. M., Buzitis, J., Krahn, M. M., Bícego, M. C. & Pires-Vanin, A. M. S. 2006. Metabolites in bile of fish from São Sebastião Channel, São Paulo, Brazil as biomarkers of exposure to petrogenic polycyclic aromatic

compounds. *Marine Pollution Bulletin*, *52*(2), 175–183. DOI: https://doi.org/10.1016/j.marpolbul.2005.08.016

Silva, J. S., Alves, R. N., de Paulo, D. V., Jr., C. F. M., de Melo Alves, M. K. & Carvalho, P. S. M. 2021. Biliary polycyclic aromatic hydrocarbons and enzymatic biomarkers in *Eugerres brasilianus* along four tropical estuaries. *Marine Pollution Bulletin*, *163*, 111919. DOI: https://doi.org/10.1016/j.marpolbul.2020.111919

Snyder, S. M., Olin, J. A., Pulster, E. L. & Murawski, S. A. 2020. Spatial contrasts in hepatic and biliary PAHs in Tilefish (*Lopholatilus chamaeleonticeps*) throughout the Gulf of Mexico, with comparison to the Northwest Atlantic. *Environmental Pollution*, *258*, 113775. DOI: https://doi.org/10.1016/j.envpol.2019.113775

Soclo, H. H., Garrigues, P. & Ewald, M. 2000. Origin of polycyclic aromatic hydrocarbons (PAHs) in coastal marine sediments: Case studies in Cotonou (Benin) and Aquitaine (France) areas. *Marine Pollution Bulletin*, *40*(5), 387–396. DOI: https://doi.org/10.1016/s0025-326x(99)00200-3

Upshall, C., Payne, J. F. & Hellou, J. 1993. Induction of MFO enzymes and production of bile metabolites in rainbow trout (*Oncorhynchus mykiss*) exposed to waste crankcase oil. *Environmental Toxicology and Chemistry*, *12*(11), 2105–2112. DOI: https://doi.org/10.1002/etc.5620121118

Varanasi, U. 1989. *Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment* (1st ed.). Boca Raton: CRC Press.

White, K. L. 1986. An overview of immunotoxicology and carcinogenic polycyclic aromatic hydrocarbons. *Environmental Carcinogenesis Reviews*, *4*(2), 163–202. DOI: https://doi.org/10.1080/10590508609373342

Yunker, M. B., Macdonald, R. W., Vingarzan, R., Mitchell, R. H., Goyette, D. & Sylvestre, S. 2002. PAHs in the Fraser River basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. *Organic Geochemistry*, *33*(4), 489–515. DOI: https://doi.org/10.1016/s0146-6380(02)00002-5