

# PAHs in Cultured Mussels Perna perna from a Southeastern Brazilian Bay

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A presença e a distribuição de hidrocarbonetos policíclicos aromáticos (HPAs) foram avaliadas pela primeira vez em mexilhões cultivados na Baía de Ilha Grande, o principal polo de maricultura do Estado do Rio de Janeiro, no sudeste do Brasil. As análises de HPASs no tecido dos mexilhões foram realizadas em triplicata por cromatografia em fase gasosa acoplada a espectrômetro de massas (GC-MS) em amostras compostas (cada uma com dez indivíduos), coletadas em 2011 em seis fazendas de cultivo. A concentração média para o total de HPAs (38 compostos), excluindo-se uma fazenda, foi de 14,00  $\pm$  8,53 ng g<sup>-1</sup>, enquanto que para os 16 HPAs prioritários a média foi de 9,51  $\pm$  4,78 ng g<sup>-1</sup>. Esses valores caracterizam os mexilhões cultivados como tendo baixo nível de contaminação por HPAs. A exceção foi a fazenda localizada em Mombaça, onde o total de HPAs de 584 ng g<sup>-1</sup> (16 HPAs igual a 22,4 ng g<sup>-1</sup>) e o perfil dos HPAs individuais sugerem contaminação moderada por fontes petrogênicas. Todas as concentrações medidas estão abaixo do limite estabelecido por agências internacionais de meio ambiente e de saúde que podem representar risco para a saúde humana devido ao consumo de mexilhões.

The presence and distribution of polycyclic aromatic hydrocarbons (PAHs) were evaluated for the first time in cultured mussels at Ilha Grade Bay, the largest mariculture center in the Rio de Janeiro State, Southeast Brazil. Analyses of PAHs on soft tissue were performed in triplicate by GC-MS on composite samples (10 individuals each) collected in 2011 at six mussel farms. The mean concentration of the total PAHs (38 compounds), excluding one farm, was  $14.0 \pm 8.53$  ng g<sup>-1</sup>, whereas the 16 priority PAHs was  $9.51 \pm 4.78$  ng g<sup>-1</sup>. These values characterize the farmed mussels analyzed as having low level of PAH contamination. The exception was the farm in Mombaça, where the total PAHs of 584 ng g<sup>-1</sup> (16 PAHs of 22.4 ng g<sup>-1</sup>) and the profile of individual PAHs suggest moderate contamination by petrogenic sources. All the concentrations measured were below threshold values established by environmental and health agencies as limiting to pose risk to humans by mussel consumption.

Keywords: mollusks, sentinel organism, PAHs, mariculture

# Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants in aquatic environments, derived from pyrogenic (combustion of fossil fuel and biomass) and petrogenic (crude oil and derivatives) sources.<sup>1</sup> In addition to their widespread occurrence, PAHs are contaminants of environmental concern and may pose a threat to human health because of their persistency, tendency to bioaccumulate and possible mutagenic and carcinogenic effects.<sup>2</sup> Biomonitoring (i.e., the measurement of body burden concentrations of a substance in biological samples)<sup>3</sup> is an effective approach to evaluate environmental contamination. Bivalve mollusks are usually considered as biomonitors or sentinels because these filter-feeding organisms are exposed to contaminants in dissolved and/or particulate phases, have a wide geographical distribution, sessile lifestyle and show effective bioaccumulation with little metabolic transformation when compared to animals of higher trophic levels.<sup>4</sup> Therefore, bivalve mollusks from different genera have been widely used to assess PAH contamination of aquatic systems around the world.<sup>5</sup> Although less frequent, some studies of PAHs

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contamination are also found in Brazilian coastal systems based on biomonitoring approaches.<sup>6,7,8</sup>

Another relevant aspect of the PAH body burden in mussels is related to consumption of these animals by humans. In order to supply the human demand for high-protein intake, an alternative to captured fishery is aquaculture, both in inland and marine waters. The global production of fish food from aquaculture has increased ten times in the last decades and reached 52.5 million ton in 2008.<sup>9</sup> Marine aquaculture accounted with 32.3% of this total production, represented by finfishes, crustaceans, oysters, mussels, clams, cockles and scallops.<sup>9</sup> In Brazil, the production of mussels has doubled in the last 10 years and contributed with ca. 11,700 ton of the total national production of 88,967 ton by marine aquaculture in 2004.<sup>10</sup>

Notwithstanding the ongoing discussion about the sustainability of aquaculture on a global change scenario,<sup>11</sup> a key requirement of this activity is the production of safe food. For instance, it has been shown that PAH concentrations in farmed mussels are directly influenced by local environmental conditions<sup>12,13</sup> and may, in some cases, reach levels that restrict the harvesting of these animals for human consumption.<sup>14</sup>

In Brazil, data for PAHs in cultured mussels are virtually absent. Therefore, in the present work, the concentrations of up to 38 PAHs (including parental and alkylated compounds) were determined in the soft tissues of cultured brown mussels, *Perna perna* (Linneaus, 1758). The animals were collected in farms located in the Ilha Grande Bay, the main center of mariculture in the Rio de Janeiro State. The objectives were (*i*) to assess, with respect to PAH contamination, the quality of the products entering the market for human consumption and (*ii*) to evaluate the environmental health of the coastal region under investigation.

# Experimental

#### Study area and sampling procedure

The Ilha Grande Bay is a large (ca. 1,000 km<sup>2</sup>) coastal embayment located in the southern portion of the Rio de Janeiro State, which houses other smaller embayments, like Ribeira and Jacuecanga (Figure S1 in the Supplementary Information (SI) section). Human settlements around the bay are relatively few (compared to other coastal regions in the state), with a population of only ca. 170,000 inhabitants distributed in two municipalities (Angra dos Reis and Paraty). The region shows outstanding natural beauty, possesses great ecological wealth and

relevant biodiversity.<sup>15</sup> As a consequence, tourism and fisheries are among the major economic activities in the bay. With respect to mariculture activities, the Rio de Janeiro State represents 0.2% of the total national production,<sup>10</sup> being Ilha Grande Bay the most important center of mussel culture in the State.

On a regional scale, the environmental alterations caused by human activities at Ilha Grande Bay are less significant<sup>16</sup> than in other coastal bays in the state, like Sepetiba<sup>17</sup> and Guanabara<sup>18</sup> bays. However, localized environmental problems have already been identified.<sup>19</sup> These were caused by recent increase in human activities (e.g., discharge of raw sewage, commercial and recreational boating traffic) and implementation of infra-structure, including three nuclear power plants, several marinas, a large shipyard to attend the oil industry demand for rigs and vessels, a commercial port and the second national largest oil terminal.

The brown mussels (Perna perna) are cultured in Ilha Grande Bay using the floating long line system. The animals were sampled in the summer of 2011, in farms located close to the Ilha Grande Island (Matariz, Sítio Forte, Aracatiba and Dois Rios) and near the continent (Macieis and Mombaça). The farms were selected as to represent a potential gradient in PAH contamination, ranging from clean areas in the island to more contaminated ones in the continent. However, in practice, the number of farms and their locations were determined by the availability of animals during the sampling period. In each station, 30 organisms of commercial size (5-8 cm) were randomly collected by hand with gloves in the floating lines at depths < 2 m. The animals were transported in ice to the laboratory, where they were cleaned and sized before storage at −20 °C.

Sample preparation, extraction and determination of total lipid contents

In the laboratory, the 30 animals collected in each farm were separated in three replicate samples, each containing 10 animals. The pooled soft tissues of each replicate were homogenized for 20 min in a homogeneizer (Ultra Turrax, IKA, Germany) and lyophilized before extraction.

Samples (ca. 3 g dry-weight; precision  $\pm$  0.001 g) were extracted in a Soxhlet system during 24 h using 200 mL of dichloromethane (pesticide grade, Mallinckrodt, USA) and, as surrogate, *p*-terphenyl-d<sub>14</sub> (Cambridge Isotope Laboratories, USA) (100 ng). A fraction of the bulk extract was weighted (precision  $\pm$  0.0001 g) for the determination of the total lipid contents. The remainder extracts were purified in two steps: (*i*) in a glass column packed with

20 g of neutral alumina (Merck, 2 % water deactivated) and elution with 100 mL of dichloromethane and (*ii*) by gel permeation chromatography (GPC), using a Shimadzu LC10 system, a Shodex CLNpak EV2000 column and a mixture of acetone:cyclohexane (3:1, v:v).

#### PAH separation and quantification

PAHs were isolated from the purified extracts by adsorption chromatography, using a glass column (1.3 cm i.d. and 30 cm height) packed with 7 g of alumina (Merck, 2 % water deactivated), 10 g of silica-gel (Merck, 5% water deactivated), 1 g of anhydrous sodium sulfate (Merck) and, in the top, 1 g of activated copper for sulfur removal. PAHs were isolated in the second fraction, which was eluted with 100 mL of a mixture of hexane:dichloromethane (1:1, v:v) pesticide grade, Mallinckrodt, USA. Volume was reduced by rotary evaporation (mod. 801, Fisaton, Brazil) and then adjusted to 1 mL in a volumetric flask.

Quantification followed a protocol based on the US-EPA 8270D (United States Environmental Protection Agency) method and was performed by gas chromatography/ mass spectrometry (GC-MS) using a Finnigan Trace-ITQ 9000 system, fitted with a J&W-MS column (5% methyl-phenyl siloxane, 30 m × 0.25 mm × 0.25 µm film). Helium was used as carrier gas adjusted to 1.2 mL min<sup>-1</sup> and the column temperature was programmed as follows: initial hold of 5 min at 50 °C, 50 °C min<sup>-1</sup> up to 80 °C, 6 °C min<sup>-1</sup> from 80 to 280 °C and a hold of 25 min at 280 °C. The injector temperature was set at 270 °C, the interface at 300 °C and ion source at 250 °C. The system operated in the electron impact mode (70 eV) and in full scan mode (*m/z* 55-450).

For instrument calibration, eight solutions (all standards > 97% pure, Sigma or Aldrich, USA) (5, 10, 20, 50, 100, 200, 400 and 1.000 ng mL<sup>-1</sup>) containing the 16 US-EPA PAHs (i.e., 16 priority PAHs), plus 2-methylnaphthalene, 1-methylnaphthalene, dibenzothiophene, 2,3-dimethylnaphthalene, perylene and the perdeuterated internal standards (naphthalene-d<sub>8</sub>, acenaphthylene- $d_{10}$ , chrysene- $d_{12}$  and perylene- $d_{12}$ ; 100 ng mL<sup>-1</sup>) were used. The following compounds were determined (i) 16 PAHs (naphthalene (N), acenaphthene (ACE), acenaphthylene (ACF), fluorene (F), phenanthrene (Ph), anthracene (A), pyrene (Py), fluoranthene (Fl), benz[a]anthracene (BaA), chrysene (Ch), benzo[b]fluoranthene (BbFl), benzo[k]fluoranthene (BkFl), benzo[e]pyrene (BePy), benzo[a]pyrene (BaPy),indeno[1,2,3-cd]pyrene (I-Py), dibenz[a,h]anthracene (DBahA) and benzo[ghi]perylene (BghiPe)) and (ii) dibenzothiophene (DBT) and its alkylated homologs

(C<sub>1</sub> to C<sub>3</sub>), 1- and 2- methylnaphthalenes; C<sub>2</sub> to C<sub>4</sub> naphthalenes, C<sub>1</sub> to C<sub>3</sub> fluorenes; C<sub>1</sub> to C<sub>4</sub> phenanthrenes; C<sub>1</sub> and C<sub>2</sub> pyrenes; C<sub>1</sub> and C<sub>2</sub> chrysenes. The determination of each alkylated series was based on the response factor of the respective non-alkylated homolog and baselines were manually adjusted, except for the 1- and 2-methylnaphtalenes for which standards (Sigma, USA) were available. Quality assurance procedures included successful analysis of a reference material (NIST 2977, National Institute of Standards and Technology), analytical blank control, recovery control of the surrogate (mean of 100.3 ± 19.7%), daily check of calibration curves. The limit of detection was 0.20 ng g<sup>-1</sup> and limit of quantification was 0.66 ng g<sup>-1</sup>. All the concentrations are expressed in a dry-weight basis.

### **Results and Discussion**

#### Total contents of lipids and PAH body burden

The contents of total lipids ranged from 3.4 to 4.6% (Table 1). These values are in the range reported in the literature for mussels *Perna perna* from different coastal areas in the SE Brazilian region. For instance, in mussels collected at Guanabara Bay (Rio de Janeiro City, Rio de Janeiro State) the lipid contents ranged from 6 to 13%, with high values measured in the summer.<sup>7</sup> On the other hand, in Ubatuba City (São Paulo State) it was found values significantly lower, between 0.99 to 1.49%.<sup>20</sup> Total lipids in mussels can seasonally vary, depending on exogenous (e.g., temperature and food availability) and endogenous (reproductive stage and energetic reserve storage) factors.<sup>12,13</sup> Therefore, more samplings should be performed at Ilha Grande Bay to check for any seasonal trends in the lipid contents of the mussels *Perna perna*.

It is noteworthy that, although PAHs are lipophilic contaminants, it was not observed a correlation between lipid and total PAH contents in our samples. The lack of correlation between these two variables in the mussel tissue is frequently reported in the literature and is attributed to the endogenous and exogenous factors mentioned before.<sup>13</sup> Because of that, the lipid contents were not used to normalize the concentration of PAHs obtained in the present study.

Considering five of the mussel farms sampled (Macieis, Dois Rios, Matariz, Sítio Forte and Araçatiba), where the results were similar, the mean concentration of total PAHs (38 compounds) was  $14.0 \pm 8.53$  ng g<sup>-1</sup>, whereas for the 16 priority PAHs it was  $9.51 \pm 4.78$  ng g<sup>-1</sup> (Table 1). In these samples, the PAHs most frequently found were N and its alkylated (C1-C4) homologues as well as Ph, Ch, BePy and

Local	Macieis	Mombaça	Dois Rios	Matariz	Sítio Forte	Araçatiba
Lipids / %	4.06	3.15	3.48	4.02	4.58	4.56
PAHs <sup>b</sup>						
N <sup>a</sup>	3.65	2.60	2.45	0.94	4.81	<loq< td=""></loq<>
C1N	1.20	3.31	0.85	<loq< td=""><td>1.85</td><td>1.25</td></loq<>	1.85	1.25
C2N	1.61	7.08	nd	nd	3.98	1.21
C3N	1.07	4.99	nd	nd	2.56	1.01
C4N	nd	12.6	nd	nd	2.06	nd
ACF <sup>a</sup>	nd	nd	nd	nd	nd	nd
ACE <sup>a</sup>	nd	nd	nd	nd	nd	nd
$\mathbf{F}^{\mathrm{a}}$	nd	< LOQ	nd	nd	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>
C1F	nd	nd	nd	nd	nd	nd
C2F	nd	nd	nd	nd	nd	nd
C3F	nd	nd	nd	nd	nd	nd
DBT	< LOQ	1.30	nd	nd	< LOQ	<loq< td=""></loq<>
C1DBT	nd	12.3	nd	nd	1.26	nd
C2DBT	nd	61.5	nd	nd	nd	nd
C3DBT	nd	72.6	nd	nd	nd	nd
Ph <sup>a</sup>	2.09	6.23	1.59	1.56	3.13	2.69
C1Ph	nd	61.6	nd	nd	nd	nd
C2Ph	nd	139.	nd	nd	nd	nd
C3Ph	nd	122.	nd	nd	nd	nd
C4Ph	nd	62.3	nd	nd	nd	nd
Aª	nd	1.25	nd	nd	< LOQ	<loq< td=""></loq<>
Fl <sup>a</sup>	< LOQ	3.20	nd	nd	< LOQ	1.54
Py <sup>a</sup>	nd	nd	nd	nd	nd	2.45
C1Py	nd	nd	nd	nd	nd	nd
C2Py	nd	nd	nd	nd	nd	nd
BaA <sup>a</sup>	nd	1.27	nd	nd	< LOQ	nd
Ch <sup>a</sup>	0.97	3.04	nd	3.38	2.70	4.26
C1Ch	nd	nd	nd	nd	nd	nd
C2Ch	nd	nd	nd	nd	nd	nd
BbFl <sup>a</sup>	nd	4.01	nd	nd	1.06	1.58
BkFl <sup>a</sup>	nd	< LOQ	nd	nd	nd	<loq< td=""></loq<>
BePy	nd	nd	nd	0.84	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>
BaPy <sup>a</sup>	nd	nd	nd	1.20	nd	nd
Pe	nd	nd	nd	nd	nd	nd
I-Py <sup>a</sup>	<loq< td=""><td>nd</td><td>nd</td><td>nd</td><td>nd</td><td>0.69</td></loq<>	nd	nd	nd	nd	0.69
DBahA <sup>a</sup>	nd	nd	nd	nd	nd	nd
BghiPer <sup>a</sup>	nd	nd	nd	nd	nd	0.85
$\Sigma$ Total PAH	11.62	584.	4.88	8.26	25.6	19.9
∑ 16 PAH	7.52	22.4	4.03	7.08	13.3	15.6

Table 1. Lipid content (%) and PAHs (ng g<sup>-1</sup>; dry-weight) in cultured mussels *Perna perna* collected in the summer of 2011 at Ilha Grande Bay, Rio de Janeiro State

<sup>a</sup>16 US-EPA priority PAHs marked; <sup>b</sup>N, naphthalene; C2N, C2 naphthalenes; C3N, C3 naphthalenes; C4N, C4 naphthalenes; ACF, acenaphthylene; ACE, acenaphthene; F, fluorene; C1F, C1 fluorenes; C2F, C2 fluorenes; C3F, C3 fluorenes; DBT, dibenzothiophene; C1DBT, C1 dibenzothiophenes; C2DBT, C2 dibenzothiophenes; C3DBT, C3 dibenzothiophenes; Ph, phenanthrene; C1Ph, C1 phenanthrenes; C2Ph, C2 phenanthrenes; C3Ph, C3 phenanthrenes; C4Ph, C4 phenanthrenes; A, anthracene; Fl, fluoranthene; Py, pyrene; C1Py, C1 pyrenes; C2Py, C2 pyrenes; BaA, benzo[*a*]anthracene; Ch, chrysene; C1Ch, C1 chrysenes; C2Ch, C2 chrysenes; BbFl, benzo[*b*]fluoranthene; BkFl, benzo[*k*]fluoranthene; BePy, benzo[*a*]pyrene; BaPy, benzo[*a*]pyrene; Per, perylene; I-Py, indeno[*1,2,3-cd*]pyrene; DBahA, dibenz[*a,h*]anthracene; BghiPer, benzo[*ghi]*perylene; nd: not detected; < LOQ: below limit of quantification (0.66 ng g<sup>-1</sup>).

BaPy. The exception was the farm in Mombaça, with 584 ng  $g^{-1}$  of total PAHs but only 22.4 ng  $g^{-1}$  of 16 PAHs. Alkylated PAHs, especially the C1-C3 DBT and C1-C4 Ph, were the major compounds in this sample, whereas the 5- and 6-ring PAHs were absent.

#### Evaluation of PAH contamination in cultured mussels

In the literature, there are several reports of PAH concentrations for native and cultured mussels from regions with distinct levels of contamination in Brazil and other coastal and estuarine regions in the world (Table 2). However, the comparison of PAH data from the literature must be made with caution since different methodologies are used and the number of individual PAHs quantified varies in each study. Bearing these limitations in mind, it is possible to observe that the concentrations of total PAHs (4.88 to 584 ng g<sup>-1</sup>; 38 compounds) obtained in the present study varied in the low range of values measured in mussel farms from Italy (129 to 2638 ng  $g^{-1}$ ; 11 compounds)<sup>12</sup> and from Spain (56.9 to 730 ng g<sup>-1</sup>; 13 compounds).<sup>13</sup> Since these European farms are located in places classified by their respective authors as having low to moderate contamination, and despite the constraints in data comparison mentioned before, our data suggest a low contamination level for 5 out of 6 mussel farms considered in the Ilha Grande Bay. The exception is the farm in Mombaça, with moderate level of PAH contamination.

The same observation, i.e., low level of PAH contamination in most of the mussel farms sampled, is valid when data for native mussels are considered (Table 2).

Excluding Mombaça, the PAH concentrations in the present study are comparable, for example, to unpolluted sites in the Mediterranean coast  $(25-80 \text{ ng g}^{-1})^{21}$  and are below the value of 100 ng g<sup>-1</sup> for total PAHs suggested as background concentration for mussels from Guanabara Bay.<sup>7</sup> Similarly, our data are well below concentrations of total PAHs measured in highly contaminated regions, like Guanabara Bay (60-6271 ng g<sup>-1</sup>)<sup>7</sup> in Brazil, and Victoria Harbor (600-22,858 ng g<sup>-1</sup>)<sup>22</sup> in Hong Kong (Table 2).

#### Source apportionment of PAHs

A variety of diagnostic ratios (or proxies) based on selected PAH compounds are traditionally used as bi-plot diagrams [e.g., Fl/(Fl+Py), BFl/(BFl+BePy) or I-Py/(I-Py+BghiPer] by several authors to infer the relative contributions of petrogenic and/or pyrolytic PAHs to environmental compartments, as well as to indicate the type of material combusted (oil, diesel, coal, biomass, etc.).<sup>30-32</sup> However, PAH diagnostic ratios could not be calculated in the present study because many compounds were below the limit of quantification in most samples.

In the cases when diagnostic ratios cannot be calculated, PAH profiles are an alternative to source assignments of these compounds. It is well known that combustion processes produce mainly high molecular weight compounds (HMW; 4-6 rings), whereas in petroleum it is found a higher proportion of low molecular weight compounds (LMW; 2-3 rings).<sup>31,32</sup> The relative contributions of LMW and HMW PAHs, considering only the 16 priority compounds and excluding the alkylated homologues,

Table 2. PAH concentrations (ng g<sup>-1</sup>; dry-weight) in cultured and native mussels from coastal regions in Brazil and worldwide

Local	Animal	Туре	Number of PAHs	Total PAHs range / (ng g <sup>-1</sup> )	Reference
Brazil					
Ilha Grande Bay-RJ	Perna perna	cultured	38	4.88-584	present study
Guanabara Bay-RJ	Perna perna	native	35	60-6271	7
São Sebastião Channel-SP	Perna perna	native	16	180-1630	23
Mundau-Manguaba Lagoons-PB	Mytella charruana	native	17	41.4-525	8
Wordwide					
Ligurian Coast/Italy	M.galloprovincialis	cultured	11	129-2638	12
Ria de Vigo/Spain	M.galloprovincialis	cultured	13	59.6-739	13
Mediterranean Coast	M.galloprovincialis	native	18	25-80	21
Venice Lagoon/Italy	M.galloprovincialis	native	17	67-2434	24
Saronikus Gulf/Greece	M.galloprovincialis	native	17	184-2454	25
Boston Harbor - Massachusetts Bay/USA	M.edulis	native	24	44-3333	26
South and Southeast Asia	Perna viridis	native	19	11-1133	27
Victoria Harbor/Hong Kong	Perna viridis	native	15	600-22,858	22
Bahia Blanca Estuary/Argentina	Brachyodontes sp and Tagelus sp	native	17	349-1597	28
San Francisco Bay/USA	mussels (non specified)	native	25	21-1093	29



Figure 1. Percentages of LMW (2-3 ring) and HMW (4-6 ring) PAHs, considering only the 16 priority PAHs.

suggested accumulation of petrogenic PAHs in Macieis, Sítio Forte and Dois Rios (LMW > HMW). On the other hand, in Araçatiba and Matariz pyrogenic PAHs are more important (LMW < HMW) (Figure 1). In Mombaça, it was found similar proportions of LMW and HMW PAHs, indicating mixture of the two sources (petrogenic and pyrogenic).

The limitation of considering the ratios of LMW and HMW is that these groups consider only the 16 priority PAHs and other compounds present in petroleum, like DBT, as well as the alkylated PAHs are excluded. In this sense, the individual PAH profile obtained in the sample from Mombaça (Figure 2) shows elevated concentrations of alkylated PAHs, particularly for DBT and Ph, which comprised more than 95% of the total PAHs. Moreover, the profile of the alkylated PAHs exhibited the classical "bell shape" format, suggesting the presence of non-degraded petrogenic PAHs.<sup>32</sup> Therefore, these results are clear evidences of the presence of petrogenic PAHs in Mombaça, rather than a mixture of sources as indicated by the ratio LMW/HMW.



Figure 2. Profile of individual PAHs (ng  $g^{-1}$ ) obtained in the sample collected at Mombaça.

The presence of petrogenic PAHs in the mussels collected in Mombaça, where the contamination by PAHs may be considered moderate (as previous discussion), could be explained by the traffic of recreational boats and/or commercial ships in the Jacuecanga Embayment. Probably the second source is more relevant, because a large shipyard is located nearby the farm in Mombaça (Figure S1 in the SI section). The farm at Macieis is located near to an oil terminal, which might explain the predominance of petrogenic PAHs in the mussels of this farm. However, the relatively low concentration of total PAHs at Macieis (11.62 ng g<sup>-1</sup>; Table 1) does not characterize, at least in the sample analyzed, environmental contamination derived from the activities of the oil terminal.

The mussels from farms located at the western side of the Ilha Grande Island accumulated PAHs that can be assigned to petrogenic (Sítio Forte) or pyrolytic (Araçatiba and Matariz) sources, as discussed before. The traffic of small boats from fishermen or tourists might be the sources of PAHs to these sites, but in all cases the contamination level is low. Finally, the farm in Dois Rios is located at the eastern side of the island and far from human activities, and accordingly very low total PAH concentration (4.88 ng g<sup>-1</sup>) was measured.

#### Potential human health risk

There are no specific regulations in Brazil about concentrations of PAHs in shellfishes that are safe for human consumption. In other countries, however, several guidelines for PAHs are proposed for food safety. For instance, the commission for the protection of the marine environment of the North-East Atlantic named OSPAR (Oslo and Paris Convention) set up quite variable concentrations of individual PAHs in edible mussels (all values in a dry-weight basis): 5-50 ng g<sup>-1</sup> for anthracene, 1000-10,000 for fluoranthene or pyrene and 500-50,000 for phenantrene or benzo(a)pyrene.<sup>33</sup> More recently, the European Commission Regulation 208/2005 proposed the limit of 10 ng g<sup>-1</sup> (wet weight) for benzo(a)pyrene in soft tissue of mussels.<sup>34</sup> Considering a median moisture content of 14%,35 this threshold is equivalent to 71.4 ng g-1 dry-weight. Moreover, the US EPA considers the concentration of 6000 ng g<sup>-1</sup> (wet weight), which is equivalent to 44,400 ng g<sup>-1</sup> dry-weight, as the limit of total PAHs that may present risks to human consumers of fish and shellfish.36

In all samples of mussel tissues analyzed in 2011 the PAH concentrations were below the threshold values in the guidelines cited above. Therefore, our data suggest that the consumption of mussels cultured at Ilha Grande Bay does not represent a vector of human exposure to PAHs. However, it was pointed out that to attest the overall good quality of the mussels, the contents of metals and sanitary parameters should also be considered.

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## Conclusion

The concentrations of PAHs measured in the present study revealed a general low level of contamination of the cultured mussels from Ilha Grande Bay, Rio de Janeiro State. Despite our limited data set, based on a single sampling, the mean concentration of  $14.0 \pm 8.53$  ng g<sup>-1</sup> for total PAHs (including alkylated compounds) and of  $9.51 \pm 4.78$  ng g<sup>-1</sup> for the 16 priority PAHs may be considered as background values for the region. Moreover, based on international criteria, the mussels do not represent risk for human consumption, at least with respect to PAH contamination. On the other hand, since the sample at Mombaça exhibited moderate level of PAH contamination by petrogenic sources, it is important to implement a monitoring program to assess the quality of the mussels for human consumption and the local environmental health.

## Supplementary Information

Supplementary material (map with Ilha Grande Bay location showing the mussel farms) is available free of charge at http://jbcs.sbq.org.br as a PDF file.

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