Arch Environ Contam Toxicol
DOI 10.1007/s00244-017-0392-y

Polycyclic Aromatic Hydrocarbons in Mussels from a South American Estuary

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Received: 7 November 2016 / Accepted: 4 March 2017

Abstract Bivalves, especially mussels, have been pointed as putative species to monitor polycyclic aromatic hydrocarbons (PAHs) in marine environment. After several environmental PAHs baseline reports, the present study was conducted to assess for the first time the levels of PAHs in native mussels (*Brachidontes rodriguezii*) collected from a critical industrialized estuary of Argentina. Under this objective, after an 18-month sampling period, 34 pools of mussels were assessed for 17 PAHs, including the 16 compounds prioritized by United States Environmental Protection Agency. By means of gas chromatography–mass spectrometry analysis, results showed total PAHs concentrations in mussel's tissue ranged from under laboratory detection limits to 482.4 ng/g dry weight. Mussel body burdens were dominated by lower molecular weight PAHs, such as phenanthrene, naphthalene, and pyrene, whereas the overall PAHs profile suggested the predominance of petrogenic sources. Finally, the potential ecotoxicological impact was evaluated by applying Environmental Assessment Criteria and benzo[a]pyrene toxic equivalent factors.

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Environmental pollution by xenobiotics is a global concern, particularly for those representing persistent organic pollutants (POPs), which are able to bioaccumulate and exert biological effects on wildlife and humans. Among them, polycyclic aromatic hydrocarbons (PAHs) are ubiquitous persistent contaminants, present in complex mixtures that can be generated by different processes, including slow maturation of organic matter under geochemical gradient (petrogenic source), incomplete combustion at high temperatures of organic matter (pyrolytic source), and short-term diagenetic degradation of biogenic precursors (diagenesis) (Neff 1979). PAHs are considered priority pollutants of environmental concern representing a threat to human health due to their toxic, carcinogenic and mutagenic characteristics, reproductive impairments and endocrine disruption in lower and higher trophic levels (USEPA 2002; IARC 2010).

Several studies have reported contamination of PAHs in the air (Amador-Muñoz et al. 2013; Carreras et al. 2013; Pozo et al. 2015; Ré et al. 2015), foodstuff (Sericano et al. 1995; Barra et al. 2007; Sammarco et al. 2013; Olson et al. 2016) and marine mammals (Fossi et al. 1997; Fair et al. 2010; Flores-Serrano et al. 2014) from American coastal areas, indicating the presence of major emission sources in this region. Despite the abatement of punctual emission sources by several law restrictions, these compounds are still being released by numerous diffuse/mobile sources such as any form of combustion-engine vehicles (e.g., gasoline powered cars, diesel powered vehicles, motorcycles, aircraft, including line sources with emissions of gases and particulate matter from vehicle traffic). This

Published online: 18 March 2017

emission trend is expected to be increased in the area due to the intensive industrialization process that has taken place in South America during the past decade. Such is the case of Argentina, which increased its Gross Domestic Product 14.4% in annual average from 2003 to 2014.

Bivalves, especially mussels, have been pointed as putative species to monitor PAHs in marine environments (Sericano et al. 1995; Baumard et al. 1998a). They are widely distributed in marine waters, are easy to collect, and their low enzymatic capacity make them able to concentrate and accumulate pollutants from the environment in their tissues (Goldberg 1975; Baumard et al. 1998a, b, 1999; Francioni et al. 2007; Karacik et al. 2009; Dabrowska et al. 2013).

Bahía Blanca estuary (BBE) is the second largest estuary of Argentina. This coastal area is under constant and increasing anthropogenic pressure, including five national harbors and one of the most important industrial park in South America. Previous studies have been made to understand the sources and emissions of PAHs in water and sediments from the BBE (Arias et al. 2009, 2010, Oliva et al. 2015a, b). Despite this, precedent research that used mussels as pollutants biomonitors was performed as temporal/spatial point studies (Arias et al. 2009; Oliva et al. 2015b). The mytilid *Brachidontes rodriguezii* (d'Orbigny 1846) distributes from Uruguay to north Patagonia along the Argentinean coast (Adami et al. 2004, 2008; Torroglosa and Giménez 2015). It is the dominant organism on intertidal rocky substrata in warm-temperate shores of Argentina (Adami et al. 2004). Considering these facts, the present study was conducted to assess, for the first time, the levels of 16 USEPA priority PAHs (USEPA, 1984) in native mussels (*Brachidontes rodriguezii*), covering ~40 Km of estuarine coasts with different anthropogenic impact. Based on quarterly sampling during 18 months, the main purpose of this research was to set general tissue levels, source apportionment, and biota/sediment interaction. In addition, results were compared through worldwide reported data—particularly with those from developed countries—and the target species evaluated as a potential sentinel for future monitoring programs.

Study Area

The Bahía Blanca Estuary (BBE, Fig. 1) is located on the southeastern coast of Buenos Aires province in Argentina (38°45′–39°40′ S and 61°45′–62°30′ W). It is formed by several of northwestern–southeastern oriented tidal channels, separated by extensive intertidal flats, with patches of low salt marshes, and islands (Piccolo 2008). The Canal Principal (80-km long) is the main navigation channel and varies in width from about 3–4 km at the mouth to 200 m at the head. It is a mesotidal system with scarce fluvial input and it has a semidiurnal tidal regime.

The northern boundaries of the BBE support an intensive anthropogenic activity: two commercial harbors, cities (Bahía Blanca, Punta Alta and General Daniel Cerri, with more than 360,000 inhabitants) and one of the biggest industrial parks in South America. Therefore, the estuarine waters receive municipal wastewater and direct industrial discharges with different degrees of treatment. In addition, the main navigation channel is regularly dredged favoring the mobilization of tons of sediment.

Material and Methods

Sample Collection

Native mussels (*Brachidontes rodriguezii*) were collected from dock columns and rocks in surface water (0–1 m) trimonthly from October 2011 to February 2013. Six sampling stations with different anthropogenic impact in the surrounding area were selected for the study (Fig. 1). Physicochemical parameters (salinity, pH, and temperature) were measured in situ with a multisensor Horiba U-10. To make tissue concentrations comparable, mussels of similar size (25–40 mm shell length) were selected. A pooled sample of mussels ($n = 50$) from each site was used for measurements. Immediately after collection, mussels were transferred inside an ice-cooled box to the laboratory where mussels from each station were treated separately, extensively washed with distillated water and finally were stored at −20 °C until analytical treatment.

The different sites from the head to the mouth of the estuary were numbered from S1 to S6. Then, sampling station 1 (S1) is located in Villa del Mar, a coastal town at the middle-outer reach of the Main Navigation Chanel of the estuary. S2 is located in Ingeniero White Harbor, closed to "Luis Piedra Buena" Thermoelectric facilities. S3 is located in an abandoned dock in Galvan Harbor, the highly industrialized core of the estuary. S4 also is in the proximity of Galvan Harbor, near to a petroleum derivatives loading buoy. S5 is located in the inner part of the estuary in a small recreational/fishing harbor (Cuatreros Harbor). Finally, S6 station is close to the head of the estuary, in the vicinity of rural lands (Villarino Viejo).

Laboratory Methods and Sample Processing

Soft tissues were lyophilized during 48 h and then smashed in a mortar. The analytical procedure for extraction of PAHs was performed by applying the method of UNEP/IAEA/FAO/IOC (1993). Before extraction, 100 μL of the mixture of four per deuterated PAHs (napthalene-d_8,

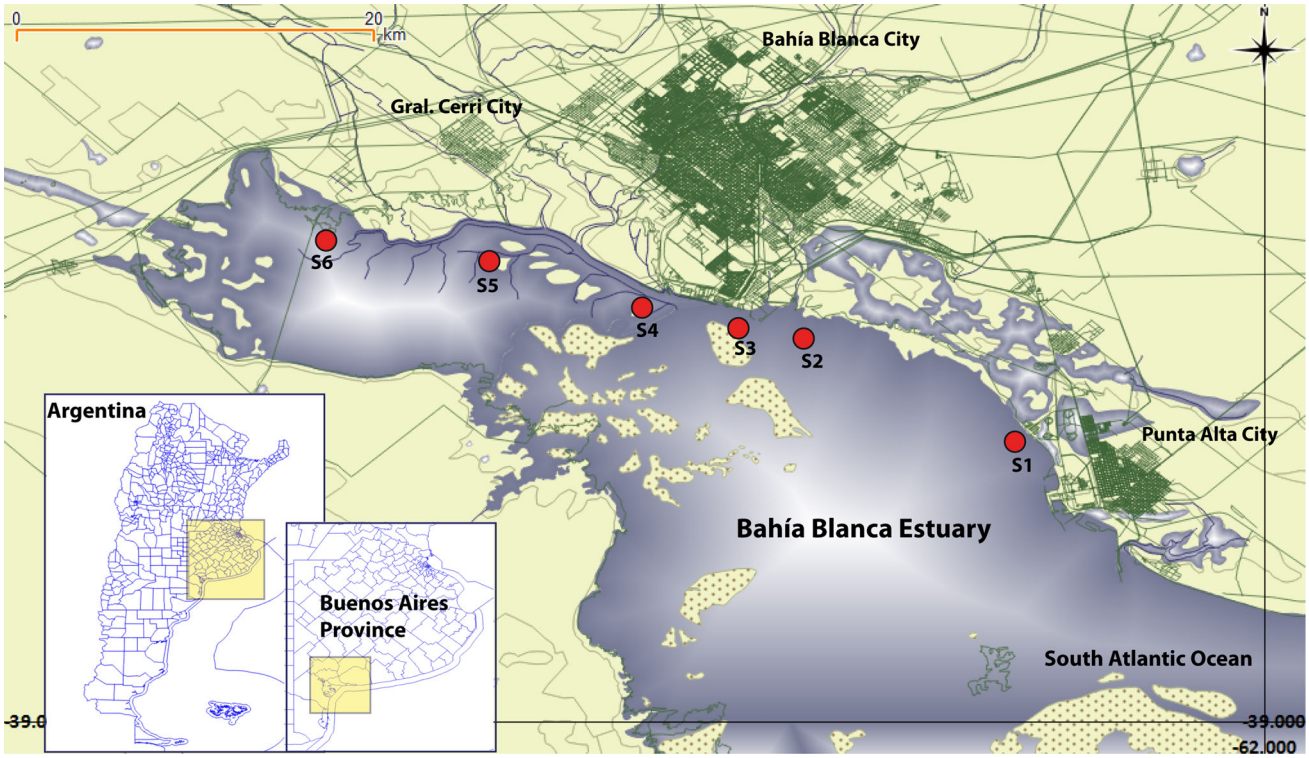

Fig. 1 Location of sampling sites in the Bahía Blanca Estuary, Argentina

acepnapthene-d_{10}, phenanthene-d_{10}, crysene-d_{12}) was added as subrogate standards. The tissue was digested with methanol using a Soxhlet-extracted (8 h) and then potassium hydroxide (0.7 M) and tri-distilled water were added and refluxed for 2 more hours. The non-saponificable fraction was extracted with n-hexane; the organic phase was dried with anhydrous sodium sulphate and concentrated close to 5 mL in a rotary evaporator with a low temperature thermostatic bath and further reduced to 1.5 mL under a gentle high purity nitrogen flow. To clean-up the extracts, a silica/alumina (2:1) gel column was used. PAHs were eluted with 70 mL of hexane-dichloromethane (9:1), and the volume of eluates was reduced to 5 mL by rotary evaporator and further to 1.5 mL under gentle nitrogen flow. Finally, just before the GC/MS injection, 100 µL of deuterated internal standard was added to extract vials for % recovery assessment.

PAHs were quantified on an Agilent 7890 B (Santa Clara, CA) gas chromatograph coupled with an Agilent 5977A mass spectrometer, using an HP-5MS fused silica column (30 m; 0.25 mm i.d.; 0.25-µm film thickness). Helium was used as a carrier gas. The mass spectrometer was operated in selected ion monitoring mode (SIM) and electron impact mode (70 eV). The samples were injected in the splitless mode at 250 °C, and the temperature program used was as follows: initial temperature 70 °C for 2 min; heated to 150 °C at 30 °C min^{-1}, then to 310 °C at 4 °C min^{-1}, and held for 10 min. Seventeen PAHs, including the 16 USEPA priority PAHs, were analyzed: naphthalene (NA), 2-methyl-naphthalene (2-M-NA), acenaphthylene (ACY), acenaphthene (ACE), fluorene (FL), phenanthrene (PHE), anthracene (AN), fluoranthene (FLU), pyrene (PY), benzo[a]anthracene (BaA), chrysene (CHR), benzo[b]- fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno[1,2,3-cd]Pyrene (IP), dibenzo[a, h]anthracene (DBA), and benzo[g, h, i]perylene (BPE). Each individual PAH compound was confirmed by the retention time and the abundance of quantification/confirmation ions with respect to authentic PAHs standards. Quantification of individual PAHs was based on the ratios analyte-peak areas/surrogate standards areas (internal standard method).

The protocol was validated by the use of certified reference material (SRM-NIST 2977).

Procedural blanks were analyzed with each batch of six samples. The detection limits (LDs) of the method were set at five times the detected amount in the procedural blank and range from 0.5 to 1.3 ng/g dry weight for individual PAH. Quality control for the PAHs analyses was performed by monitoring the recovery of the internal standard (Benzo-[a]-anthracene-d12) spiked just before GC injection; recoveries ranged from 76 to 107%.

PAH standards mixture of 17 PAHs and deuterated PAHs solutions were obtained from Supelco (Bellefonte,

PA). Solvents (hexane, methanol, and dichloromethane) were of analytical and chromatographic grade from Merck (Darmstadt, Germany). A certified reference material sample was obtained from National Institute of Standards and Technology (SRM-NIST 2977). Merck silica gel 60 (70–230 mesh ASTM) and Aluminum oxide activated at 450 °C were heated at 120 °C for 12 h before use. Glassware was washed before use with nonionic detergent, rinsed with ultrapure water and acetone/hexane, and dried at 120 °C before use.

To evaluate the physiological status of mussels, the condition index (CI) and lipid content were determined. The lipid content was analyzed in the total tissue, following the method proposed by Bligh and Dyer (1959). The condition index (CI) was calculated according to Couillard et al. (1995) as follows:

$$(\text{whole soft tissue dry weight} \div \text{valve dry weight}) \times 100.$$

Data Processing and Statistical Analyses

All statistical analyses were carried out using STATISTICA 7.0 (StatSoft, Inc.). Statistical significance of compared results was determined by the one-way analysis of variance (one-way ANOVA; level of significance at $p < 0.05$). In the cases when the data did not meet the assumptions of homogeneity and normality for the parametric tests, a nonparametric test was used (Kruskal-Wallis). Used correlations (r) were calculated using Spearman correlation coefficient.

Results and Discussion

Relation of Lipid Content and Condition Index with PAHs Level

It is well known that distribution of lipophilic compounds, such as PAHs, in organisms is not only influenced by physical-chemical characteristics of the compounds but also by the organism physiological parameters (i.e., sex, maturity) and its lipid content (Livingstone 1991; Bruner et al. 1994; Baumard et al. 1998c; Ruiz et al. 2011). For example, it has been extensively demonstrated that tissues with higher lipid content accumulate PAHs to a greater extent (Livingstone 1991; Baumard et al. 1998c). In this work, lipid content varied in the range of 6.9–12.8% for all the samples and no significant differences were found between the different sampling sites (ANOVA, $p = 0.084$).

Considering the date of sampling, significant differences in lipid content values were found between summer and spring (ANOVA, $p = 0.064$). The highest lipid content was observed during spring, probably matching the highest reproductive stage period and abundance of nutrients in the environment. In fact, the EBB is characterized by a phytoplankton bloom during winter-spring (Gayoso 1999; Popovich and Marcovecchio 2008; Guinder et al. 2010, 2013). However, the lowest lipid content values were observed in summer. This trend could be attributed to natural variation though the mussel life cycle, particularly after spawning when body lipid levels may decline by 50% (Sprung 1993; Bruner et al. 1994). In addition, because no correlations were found between lipid content and total PAHs concentrations ($r = 0.17$, $p < 0.05$), the PAHs concentrations were not normalized to lipid content. This absence of correlation was in agreement with those reported elsewhere (Baumard et al. 1998c; Devier et al. 2005; Francioni et al. 2007; Ruiz et al. 2011; Yoshimine et al. 2012; Barhoumi et al. 2016) and has been attributed to the effect of endogenous and exogenous factors on the lipids levels.

The condition index (CI) is a useful indicator of bivalve growth and health (Pérez Camacho et al. 1995). It usually varies due to a complex interaction between extrinsic and intrinsic factors, such as reproductive stage, temperature, salinity, availability of food, and quality of the diet. In this study, CI varied between 4.67 and 19.90 (Table 1). Similar to that observed with the lipid content, no significant differences in the CI were found between the different sampling sites (ANOVA, $p = 0.326$), suggesting that mussels presented similar physiological conditions regardless of the sampling sites. In agreement with the lipid content, the highest values of CI were detected in spring, matching the increase in water temperature (Table 1) and food availability. CI values were significantly higher in organisms collected during spring than those collected during the rest of the year (ANOVA, $p < 0.0001$). That increment supported the hypothesis by which there is an increase in the reproductive status of *B. rodriguezii* during spring. Finally, as lipid content, there was no demonstrable correlation between total PAHs concentrations and CI ($r = 0.27$; $p < 0.05$). This suggested that despite CI is a general health indicator, it could not predict effects below a certain PAHs threshold.

Concentration and Composition of PAHs

The results of PAHs concentrations in mussels are shown in Fig. 2. From the 17 PAHs analyzed only 8 were detected and total PAHs (summary of the 8 PAHs) varied from nondetectable to 482.4 ng/g dw, showing an overall mean of 148.9 ng/g dw (Table 2). No significant seasonal variations were observed. On the one side, the highest PAHs levels were found at the S3 station (median, 208.51 ng/g dw; Fig. 2). This station is located at the core of the

Table 1 Temporal and spatial variation of condition index (CI) and total PAHs levels (ng/g dw) in mussels from the Bahía Blanca Estuary and physicochemical parameters of sea water

Season	S1	S2	S3	S4	S5	S6
Spring 2011						
pH	No data	8.20	8.11	8.13	8.27	8.17
T (°C)		15.4	15.6	15.2	15.6	15.8
Salinity		35.28	31.50	35.35	32.09	30.37
IC (%)		15.47 ± 3.13	19.90 ± 4.41	19.72 ± 4.26	17.72 ± 5.02	19.62 ± 4.08
∑PAHs		282.46	482.36	337.58	147.49	453.13
Summer 2013						
pH	8.30	8.21	8.23	8.24	8.25	8.27
T (°C)	22.2	22.8	22.8	23	23.3	22.9
Salinity	36.43	38.34	38.41	38.60	38.73	38.79
IC (%)	13.08 ± 3.65	5.26 ± 1.37	9.00 ± 3.34	9.85 ± 5.40	7.00 ± 2.28	6.73 ± 3.11
∑PAHs	11.03	387.43	<LD	229.38	275.83	11.03
Autumn 2012						
pH	8.20	8.20	No data	8.23	8.27	8.33
T (°C)	13.2	12.1		11.3	11.1	10.7
Salinity	37.39	37.32		37.32	34.71	33.37
CI (%)	8.36 ± 2.94	5.83 ± 1.45		8.94 ± 2.49	4.67 ± 1.23	5.33 ± 1.11
∑PAHs	<LD	118.89		72.90	417.38	48.13
Winter 2012						
pH	8.00	7.25	7.55	7.57	7.56	7.56
T (°C)	9.6	9.2	8.9	8.8	9.7	9.1
Salinity	35.28	33.62	33.62	33.37	31.20	31.84
CI (%)	9.15 ± 2.19	8.61 ± 1.81	8.61 ± 2.09	7.76 ± 1.70	8.24 ± 2.40	6.68 ± 1.77
∑PAHs	134.03	<LD	386.96	81.50	81.29	<LD
Spring 2012						
pH	7.98	7.96	7.82	7.99	8.02	8.09
T (°C)	16.6	18.4	19.1	18.8	18.7	20.4
Salinity	35.47	35.28	35.35	35.28	33.62	32.73
CI (%)	14.59 ± 4.26	16.02 ± 3.40	15.16 ± 4.95	12.56 ± 2.90	16.76 ± 4.61	12.45 ± 2.60
∑PAHs	128.14	135.25	204.47	118.99	30.92	<LD
Summer 2013						
pH	7.56	7.52	7.50	7.53	7.56	7.63
T (°C)	17.1	22.1	21.2	20.1	21.4	22.9
Salinity	23.89	35.47	35.79	36.43	37.64	38.54
CI (%)	9.63 ± 2.77	9.63 ± 2.12	6.66 ± 2.48	11.18 ± 2.57	8.15 ± 2.43	9.05 ± 1.96
∑PAHs	<LD	12.56	99.54	144.30	<LD	32.37

Condition index (CI): is expressed as the mean ± SD; ∑PAHs: summary of the 8 PAHs detected (ng/g dw)

industrial area from Galvan harbor and is surrounded by wastewater discharge pipelines, industrial vents, docks, shipsides, petroleum-transfer buoys, etc. On the other side, the lowest PAHs concentration was found at the area with the least human pressure, S1 station (median, 15.07 ng/g dw; Fig. 2). Furthermore, levels of PAHs tend to decrease as the distance from the heavily industrialized area of the BBE increased. This spatial trend observation was in agreement with a previous finding for sediment matrix at the area (Oliva et al. 2015a).

According to Baumard et al. (1998a) levels of mussels contamination by PAHs can be classified as low (0–100 ng/g dw), moderate (>100–1000 ng/g dw), high (>1000–5000 ng/g dw), and very high (>5000 ng/g dw). Following these criteria, the levels of PAH in mussels samples could be classified from low to moderate, leading to an averaged pollution level comparable to with reported values in Guanabara Bay (Brazil), Marmara Sea (Turkey) and Seine estuary and Seine bay (France; Table 3). The mean total PAHs levels observed in this study was lower than that

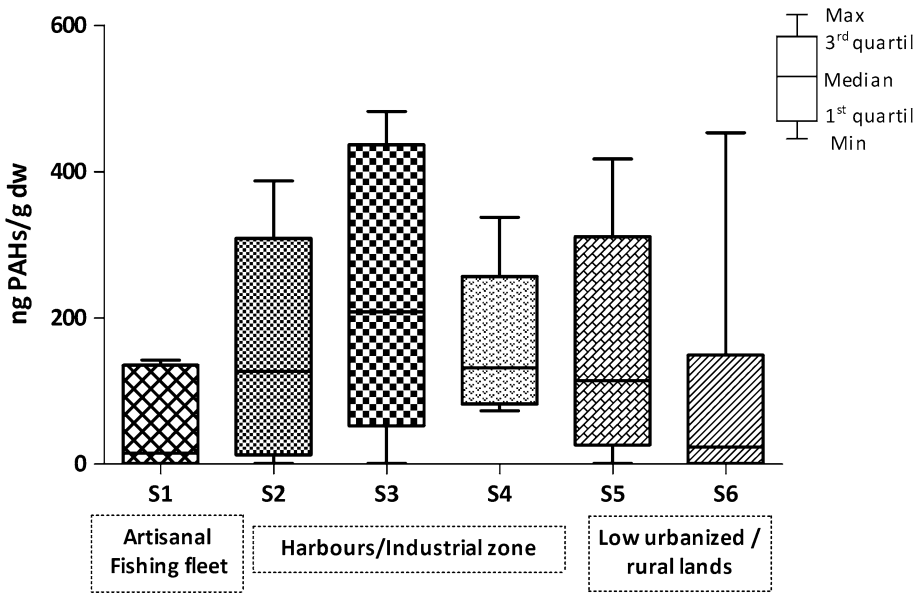

Fig. 2 Box and whisker plot for total PAHs in mussels, for each sampling site

Table 2 Range of PAHs concentration (ng/g dw) in mussels from the BBE

Sampling site	NA	2-M-NA	ACY	FL	PHE	FLU	PY	BaP	∑PAHs
S1 ($n = 5$)	<LD-1.00	<LD-1.00	<LD	<LD	<LD-10.03	<LD-12.67	<LD-31.38	<LD	<LD-134.03
S2 ($n = 6$)	<LD-170.28	<LD-30.49	<LD	<LD	<LD-136.42	<LD	<LD-40.57	<LD-148.41	<LD-387.43
S3 ($n = 5$)	<LD-70.12	<LD-38.82	<LD-33.27	<LD-1.06	<LD-279.43	<LD-53.23	<LD- 92.43	<LD	<LD-482.36
S4 ($n = 6$)	<LD-187.52	<LD-15.85	<LD-26.24	<LD-1.00	<LD-202.87	<LD-18.99	<LD-34.18	<LD	72.90-337.58
S5 ($n = 6$)	<LD-198.35	<LD-39.47	<LD-51.43	<LD	<LD-147.49	<LD-52.62	<LD-112.22	<LD	<LD-417.38
S6 ($n = 6$)	<LD-162.20	<LD-24.46	<LD	<LD	<LD-231.71	<LD	<LD-34.76	<LD	<LD-453.13

LD: lower than the detection limit

∑PAHs: sum of the 8 PAHs detected (ng/g dw)

obtained in a previous baseline for the BBE (Oliva et al. 2015b) in which a global mean of 334.5 ± 693.5 ng/g dw was reported. This difference was attributed to the different date/site of sampling. For example, the highest values of PAHs reported by Oliva et al. (2015b) were detected in Pehuenco, a site located at the outermost limit of the BBE, which was not included at the present work.

The PAH's congener distribution is usually used to discriminate PAHs sources. In general terms, petrogenic PAHs present dominance of compounds with two/three aromatic rings, whereas pyrogenic PAHs present dominance of compounds with higher molecular mass (4–6 aromatic rings). This approach should be treated with caution when studying bivalves, because PAHs can be altered to some degree during their environmental transport and bioaccumulation (Oros and Ross 2005; Arias et al. 2009).

The average composition pattern of PAHs by ring size at each sample station is shown in Fig. 3. First, data showed that the relative abundance and the composition of individual PAHs were homogeneous throughout the samples sites, suggesting a common PAHs input pattern. Mussel from the BBE presented a marked dominance of PAHs with 3 rings, achieving between 36.8 and 75% of total

Table 3 Values of PAHs concentrations (ng/g dw) in bivalves from worldwide locations and this study

Locations	Species	N° of PAHs	Total PAHs range (ng/g) dw	Contamination level[a]	Reference
America					
San Francisco estuary, USA	*Mytilus californianus*	25	21–1093	Low to high	Oros and Ross (2005)
South-central Chilean coast	*Choromytilus chorus*	13	49–3880	Low to high	Toro et al. (2004)
Guanabara Bay, Brazil	*Perna perna*	16	69–239	Low to moderate	Yoshimine et al. (2012)
Bahía Nueva, Patagonia Argentina	*Aulacomya atraatra and Tellina petitiana*	16	780–2010	Moderate to high	MassaraPaletto et al. (2008)
Bahía Blanca Estuary, Argentina	*Brachidontes sp and Tagelus sp*	17	349–1597	Moderate to high	Arias et al. (2009)
Bahía Blanca Estuary, Argentina	*Brachidontes rodriguezii*	17	38–2048	Low to high	Oliva et al. (2015b)
Bahía Blanca Estuary, Argentina	*Brachidontes rodriguezii*	17	<LD-482	Low to moderate	This study
Afric					
Dar es Salaam, Tanzania	*Saccostrea cucullata*	23	174–647	Moderate	Gaspare et al. (2009)
Bizerte lagoon, Tunisia	*Mytilus galloprovincialis*	15	107–431	Moderate	Barhoumi et al. (2016)
Algerian west coast	*Mytilus galloprovincialis*	17	68–2892	Low to high	Benali et al. (2017)
Asia					
Mokpo Bay, Korea	*Mytilus galloprovincialis*	16	<LD-96	Low	Namiesnik et al. (2008)
South and Southeast Asian coast	*Perna viridis*	19	11–1133	Low to high	Isobe et al. (2007)
Europe					
Galician Coast, Spain	*Mytilus galloprovincialis*	13	17–7780	Low to very high	Soriano et al. (2006)
N-NW Spanish Atlantic coast	*Mytilus galloprovincialis*	12	3–1015	Low to high	González-Fernández et al. (2015)
Ria de Vigo, Spain	*Mytilus galloprovincialis*	13	24–1007	Low to high	Viñas et al. (2012)
Seine estuary and Seine bay, France	*Mytilus edulis and Dreissena polymorpha*	21	15–990	Low to moderate	Rocher et al. (2006)
Guadeloupe, Caribean sea, France	*Crassostrea rhizophorae*	20	69–961	Low to moderate	Ramdine et al. (2012)
Tromsø, Northern Norway	*Mytilus edulis*	16	<5–22*	Low	Nahrgang et al. (2013)
Istanbul strait, Turkey	*Mytilus galloprovincialis*	16	43–601	Low to moderate	Karacik et al. (2009)

[a] Classification according to Baumard et al. (1998a); * wet weight; <LD: lower than detection limit

PAHs (Fig. 3). On the other hand, PAHs with 5 and 6 rings were absent in almost all samples. This PAHs distribution pattern is usually found in PAHs mixtures generated from petrogenic sources (Barhoumi et al. 2016; Yu et al. 2016; Wang et al. 2016). The dominance of lower molecular weight PAHs also has been reported in bivalves collected near ports and cities (León et al. 2013; Barhoumi et al. 2016: Yu et al. 2016) and was in agreement with that reported in Oliva et al. (2015b). A possible explanation for the average composition pattern could be found taking into account the hydrophobic characteristic of PAHs: because PAH's solubility decreases with increasing mass weight and as the octanol-water partition coefficient (K_{ow}) increase, the higher molecular weight PAHs tend to be absorbed onto or associated with organic particles present in the water column or sediments, and marine organisms are commonly enriched in the low molecular weight compounds (Djomo et al. 1996; Porte and Albaiges 1993; Baumard et al. 1998a, b; Thorsen et al. 2004; Tolosa et al. 2005; Barhoumi et al. 2016; Yu et al. 2016). Furthermore, mussels were obtained from dock columns and rocks, near the air-water interface without direct contact with the sediment—except for resuspension processes—and also exposed to the petroleum contamination that could exist in the upper part of the water column derived from port/industrial activities. Then, the selective PAHs

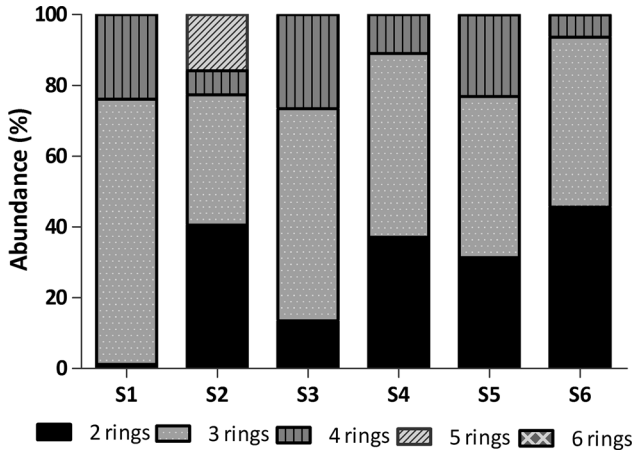

Fig. 3 Composition pattern of PAHs by ring size in mussels at the different sampling sites

bioaccumulation could be explained by the occurrence of concomitant exposure pathways, which favored the uptake of low-ringed PAHs.

In terms of abundance of individual compounds, PHE was at the top, followed by NA and PY (accounting for 47.6, 23.4, and 13.1% of total PAHs, respectively). Moreover, the frequency of detection of PHE was 100% in all the samples where PAHs were detected. On the other hand, BaP was only detected in mussels from S2, whereas FL was detected with low values just for Galvan Harbor (S3 and S4).

To deepen the analysis, the PAHs molecular-ratios approach was applied to assess putative sources. In the literature, these ratios have been traditionally used to identify sources based on the differential thermodynamic stability of congeners (Budzinski et al. 1997; Readman et al. 1987; Yunker et al. 1999, 2002). Primarily, FLU/PY + FLU and FLU/PY ratios were calculated. FLU/FLU + PY ratio <0.40 imply petroleum (coal, diesel, oil) between 0.40 and 0.50 indicate liquid fossil fuel (vehicle and crude oil) combustion, whereas ratio >0.50 is an indicator to biomass (grass, wood, or coal) combustion (Yunker et al. 2002). The global mean for this ratio in mussel samples was 0.14; this indicates that PAHs were of petrogenic origin. Moreover, a FLU/PY ratio >1 imply pyrolytic source while ratios <1 are attributable to petroleum (Baumard et al. 1998a). In all mussels samples this ratio was <1, suggesting petroleum as the main source of PAHs.

At last, a ratio of low ($\sum$ of 2–3 rings) to high ($\sum$ of 4–6 rings) PAHs (LMW/HMW) was used to identify pyrogenic (<1) and petrogenic (>1) sources of PAHs (Budzinski et al. 1997). LMW/HMW ratios were >1 for most of the mussels samples (88%) supporting the petrogenic input hypothesis. Petrogenic PAHs could be attributed to the intense shipping activity in the study area, petroleum buoys, tanker traffic, spills of fuel and lubricating oils.

Summarizing, the mussel PAH body burdens were dominated by petrogenic inputs. These results were similar in terms of concentration to those previously reported in sediments from the area of study; however, low pyrolytic inputs were found in comparison to the previous results (Arias et al. 2009, 2010; Oliva et al. 2015a), Furthermore, the decrement of heavier PAHs led to the hypothesis by which mussels were predominantly exposed to dissolved PAHs fractions rather than to sediment and suspended matter associated compounds.

Bioaccumulation

To estimate mussel PAH accumulation from sediment, bioaccumulation factors (BAFs) were calculated for the eight PAHs detected in the mussels (Baumard et al. 1998a, b). BAF is defined as the ratio between the concentration found in the mussel and the concentration found in the sediment.

In cases where the total concentration of a pollutant in sediment is potentially bioavailable, the value of BAF is close to unity (OSPAR 2009). Data of PAHs levels in sediments were obtained from a previous publication (Oliva et al. 2015a). As shown in Table 4, BAFs for NA, 2-M-NA, and PHE were higher than 1 in 23.5, 32.4, and 50.0% of mussels respectively; and thus reflected the contribution of sediments in the bioaccumulation of those PAHs. On the other hand, for the heavier PAHs with 4 rings like PY and FLU, lower values of BAF were found, suggesting a low contribution of the sediment in the bioaccumulation of these compounds (Table 4). These results are in agreement and support the settled hypothesis in the above section regarding PAH levels, sources, and composition.

Assessing PAHs Ecotoxicological Potential

BaP is the only PAH for which toxicological data are sufficient to derive a carcinogenic potency factor (Peters

Table 4 Median BAF values for mussels of the BBE

Compound	Median BAF	% of samples BAF > 1
NA	0.04	23.5
2-M-NA	0.84	32.4
ACY	1.20	8.8
FL	0.00	0.0
PHE	1.83	50.0
FLU	0.00	8.8
PY	0.22	20.6

Table 5 Risk assessment of PAHs according to values for environmental assessment criteria (EAC)

Compound	EAC	Maximum (ng/g dw)
NA	340	198.35
PHE	1700	<279.43
AN	290	<LD
FLU	110	53.23
PY	100	111.12
BkF	260	<LD
BaA	80	<LD
BaP	600	148.41
IP	110	<LD

et al. 1999). The PAHs carcinogenic potential can be assessed by calculating the toxic equivalent of benzo[a]-pyrene (TEQ BaP). TEQ BaP were calculated as follows:$TEQBaP = \sum ci \times TEF$, in which ci is the concentration of individual PAHs (ng/g) and TEF is the toxic factor of PAHs relative to BaP (USEPA 1993; Nisbet and Lagoy 1992).

The calculated Total TEQ BaP in the samples varied from 0.01 to 0.5 ng/g dw TEQ BaP. These values were much lower than the limit of 10 ng/g proposed by the European Commission Regulation 208/2005 for edible mussels. Furthermore, these values were below the precedent baseline settled for the area (0.31–4.43 ng/g dw TEQ BaP; Oliva et al. 2015b). At first sight, these results suggest that the consumption of mussels from the BBE should not pose a problem for human health; however, additional studies with concomitant toxic substances, such as heavy metals, polychlorinated biphenyls (PCBs), pesticides must be assessed to evaluate real human health risks.

Finally, levels of PAHs in mussel of the BBE were compared with the Environmental Assessment Criteria (EAC) established by OSPAR (2009) (Table 5). The EAC represents the contaminant concentration in the environment below which no chronic effects are expected to occur in marine species, including the most sensitive species. Levels of PAHs detected in mussel from the BBE were below of the EAC in all the samples.

Conclusions

The present study allowed the following conclusions:

- The occurrence of PAHs in mussel tissues from the BBE tissues was mainly associated with anthropogenically impacted areas with a dominance of petrogenic sources.
- PAHs congeners were selectively bioaccumulated in mussels probably due to the occurrence of concomitant exposure pathways which favored the uptake of low-ringed PAHs.
- PAHs levels in mussels were comparable to other coastal sites around the world and did not raise any human consumption warning. No chronic effects were expected to occur within these organisms.
- Further studies are needed in order to further evaluate the role of biological parameters (i.e., biomarkers, reproductive cycles) in PAHs distribution and accumulation.
- As a concluding remark, the *Brachidontes rodriguezii* mussel showed to be a suitable candidate for sentinel monitoring purposes at the Bahía Blanca coastal environment.

Acknowledgments The authors thank the staff of IADO IV research vessel for the logistic and cooperation on board. This research was supported by a doctoral grant funded by the National Council of Scientific and Technological Research (CONICET-Argentina) and was part of the Ph.D. thesis of Ana Laura Oliva. This study was funded through research grants by CONICET (PIP D-738 2011) and National Agency for Promotion of Science and Technology-ANCPCyT (PICT 2010-1302).

References

Adami ML, Tablado A, López Gappa J (2004) Spatial and temporal variability in intertidal assemblages dominated by the mussel *Brachidontes rodriguezii* (d'Orbigny, 1846). Hydrobiologia 52:49–59

Adami ML, Tablado A, Sodor MA (2008) Population dynamics of the intertidal mytilid *Brachidontes rodriguezii* (Bivalvia) on a rockyshore. Thalassas 24:21–27

Amador-Muñoz O, Bazán-Torija S, Villa-Ferreira SA, Villalobos-Pietrini R, Bravo-Cabrera JL, Munive-Colín Z, Hernández-Mena L, Saldarriaga-Noreña H, Murillo-Tovar MA (2013) Opposing seasonal trends for polycyclic aromatic hydrocarbons and PM 10: health risk and sources in southwest Mexico City. Atmos Res 122:199–212

Arias AH, Spetter CV, Freije RH, Marcovecchio JE (2009) Polycyclic aromatic hydrocarbons in water, mussels (*Brachidontes* sp., *Tagelus* sp.) and fish (*Odontesthes* sp.) from Bahía Blanca Estuary Argentina. Estuar Coast Shelf Sci 85(1):67–81

Arias AH, Vazquez-Botello A, Tombesi N, Ponce-Velez G, Freije H, Marcovecchio J (2010) Presence, distribution, and origins of polycyclic aromatic hydrocarbons (PAHs) in sediments from Bahía Blanca estuary, Argentina. Environ Monit Assess 160(1):301–314

Barhoumi B, El Megdiche Y, Clérandeau C, Ameur WB, Mekni S, Bouabdallah S, Driss MR (2016) Occurrence of polycyclic aromatic hydrocarbons (PAHs) in mussel (*Mytilus galloprovincialis*) and eel (*Anguilla anguilla*) from Bizerte lagoon, Tunisia, and associated human health risk assessment. Cont Shelf Res 124:104–116

Barra R, Castillo C, Torres JPM (2007) Polycyclic aromatic hydrocarbons in the South American environment. In: Reviews of Environmental Contamination and Toxicology (pp. 1–22). Springer, New York

Baumard P, Budzinski H, Michon Q, Garrigues P, Burgeot T, Bellocq J (1998a) Origin and bioavailability of PAHs in the

Mediterranean Sea from mussel and sediment records. Estuar Coast Shelf Sci 47(1):77–90

Baumard P, Budzinski H, Garrigues P, Sorbe JC, Burgeot T, Bellocq J (1998b) Concentrations of PAHs (polycyclic aromatic hydrocarbons) in various marine organisms in relation to those in sediments and to trophic level. Mar Pollut Bull 36(12):951–960

Baumard P, Budzinski H, Garrigues P (1998c) Polycyclic aromatic hydrocarbons in sediments and mussels of the western Mediterranean Sea. Environ Toxicol Chem 17(5):765–776

Baumard P, Budzinski H, Garrigues P, Narbonne JF, Burgeot T, Michel X, Bellocq J (1999) Polycyclic aromatic hydrocarbon (PAH) burden of mussels (*Mytilus sp*.) in different marine environments in relation with sediment PAH contamination, and bioavailability. Mar Environ Res 47(5):415–439

Benali I, Boutiba Z, Grandjean D, De Alencastro LF, Rouane-Hacene O, Chèvre N (2017) Spatial distribution and biological effects of trace metals (Cu, Zn, Pb, Cd) and organic micropollutants (PCBs, PAHs) in mussels *Mytilus galloprovincialis* along the Algerian west coast. Mar Pollut Bull 115:539–550

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37(8):911–917

Bruner KA, Fisher SW, Landrum PF (1994) The role of the zebra mussel, *Dreissena polymorpha*, in contaminant cycling: I. The effect of body size and lipid content on the bioconcentration of PCBs and PAHs. J Gt Lakes Res 20(4):725–734

Budzinski H, Jones I, Bellocq J, Pie'rard C, Garrigues P (1997) Evaluation of sediment contamination by polycyclic aromatic hydrocarbon in the Gironde estuary. Mar Chem 58(1):85–97

Carreras HA, Calderón-Segura ME, Gómez-Arroyo S, Murillo-Tovar MA, Amador-Muñoz O (2013) Composition and mutagenicity of PAHs associated with urban airborne particles in Córdoba, Argentina. Environ Pollut 178:403–410

Couillard Y, Campbell PGC, Auclair JG, Pellerin-Massicotte J (1995) Field transplantation of a freshwater bivalve, *Pyganodon grandis*, across a metal contamination gradient. II. Metallothionein response to Cd and Zn exposure, evidence for cytotoxicity, and links to effects at higher levels of biological organization. Can J Fish Aquat Sci 52(4):703–715

Dabrowska H, Kopko O, Turja R, Lehtonen KK, Góra A, Polak-Juszczak L, Warzocha J, Kholodkevich S (2013) Sediment contaminants and contaminant levels and biomarkers in caged mussels (*Mytilus trossulus)* in the southern Baltic Sea. Mar Environ Res 84:1–9

Devier MH, Augagneur S, Budzinski H, Le Menach K, Mora P, Narbonne JF, Garrigues P (2005) One-year monitoring survey of organic compounds (PAHs, PCBs, TBT), heavy metals and biomarkers in blue mussels from the Arcachon Bay, France. J Environ Monit 7(3):224–240

Djomo JE, Garrigues P, Narbonne JF (1996) Uptake and depuration of polycyclic aromatic hydrocarbons from sediment by the zebrafish (*Brachydanio rerio*). Environ Toxicol Chem 15(7):1177–1181

Fair PA, Adams J, Mitchum G, Hulsey TC, Reif JS, Houde M, Muire D, Wirtha E, Wetzelf D, Zolmana E, McFee W (2010) Contaminant blubber burdens in Atlantic bottlenose dolphins (*Tursiops truncatus*) from two southeastern US estuarine areas: concentrations and patterns of PCBs, pesticides, PBDEs, PFCs, and PAHs. Sci Total Environ 408(7):1577–1597

Flores-Serrano RM, Iturbe-Argüelles R, Pérez-Casimiro G, Ramírez-González A, Flores-Guido JS, Kantún-Balam JM (2014) Ecological risk assessment for small omnivorous mammals exposed to polycyclic aromatic hydrocarbons: a case study in northeastern Mexico. Sci Total Environ 476:218–227

Fossi MC, Marsili L, Junin M, Castello H, Lorenzani JA, Casini S, Leonzio C (1997) Use of nondestructive biomarkers and residue analysis to assess the health status of endangered species of pinnipeds in the south-west Atlantic. Mar Pollut Bull 34(3):157–162

Francioni E, Wagener A, Scofield ADL, Depledge MH, Cavalier B, Sette CB, Carvaose L, Lozinsky C, Mariath R (2007) Polycyclic aromatic hydrocarbon in inter-tidal mussel *Perna perna*: Space–time observations, source investigation and genotoxicity. Sci Total Environ 372(2):515–531

Gaspare L, Machiwa JF, Mdachi SJM, Streck G, Brack W (2009) Polycyclic aromatic hydrocarbon (PAH) contamination of surface sediments and oysters from the inter-tidal areas of Dar es Salaam, Tanzania. Environ Pollut 157(1):24–34

Gayoso AM (1999) Seasonal succession patterns of phytoplankton in the Bahía Blanca Estuary (Argentina). Botanica Marina 42(4):367–375

Goldberg ED (1975) The mussel watch—a first step in global marine monitoring. Mar Pollut Bull 6(7):111

González-Fernández C, Albentosa M, Campillo JA, Viñas L, Fumega J, Franco A, Besada V, González-Quijano A, Bellas J (2015) Influence of mussel biological variability on pollution biomarkers. Environ Res 137:14–31

Guinder VA, Popovich CA, Molinero JC, Perillo GM (2010) Long-term changes in phytoplankton phenology and community structure in the Bahía Blanca Estuary, Argentina. Mar Biol 157(12):2703–2716

Guinder VA, Popovich CA, Molinero JC, Marcovecchio JE (2013) Phytoplankton summer bloom dynamics in the Bahía Blanca Estuary in relation to changing environmental conditions. Cont Shelf Res 52:150–158

IARC (2010) International Agency for Research on Cancer.Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures, Vol 92. http://monographs.iarc.fr/ENG/Monographs/vol92/mono92.pdf

Isobe T, Takada H, Kanai M, Tsutsumi S, Isobe KO, Boonyatumanond R, Zakaria MP (2007) Distribution of polycyclic aromatic hydrocarbons (PAHs) and phenolic endocrine disrupting chemicals in South and Southeast Asian mussels. Environ Monit Assess 135(1–3):423–440

Karacik B, Okay OS, Henkelmann B, Bernhöft S, Schramm KW (2009) Polycyclic aromatic hydrocarbons and effects on marine organisms in the Istanbul Strait. Environ Int 35(3):599–606

León VM, Martínez-Gómez C, García I, Campillo JA, Benedicto J (2013) Spatial distribution and temporal trends of polycyclic aromatic hydrocarbons in *Mytilus galloprovincialis* from the Iberian Mediterranean coast. Environ Monit Assess 185(2):1055–1070

Livingstone DR (1991) Towards a specific index of impact by organic pollution for marine invertebrates. Comp Biochem Physiol C Comp Pharmacol 100(1):151–155

MassaraPaletto V, Commendatore MG, Esteves JL (2008) Hydrocarbon levels in sediments and bivalve mollusks from Bahía Nueva (Patagonia, Argentina): An assessment of probable originand bioaccumulation factors. Mar Pollut Bull 56(12):2082–2105

Nahrgang J, Brooks SJ, Evenset A, Camus L, Jonsson M, Smith TJ, Lukina J, Frantzen M, Giarratano E, Renaul PE (2013) Seasonal variation in biomarkers in blue mussel (*Mytilusmedulis*), Icelandic scallop (*Chlamys islandica*) and Atlantic cod (*Gadus morhua*)—Implications for environmental monitoring in the Barents Sea. Aquat Toxicol 127:21–35

Namiesnik J, Moncheva S, Park YS, Ham KS, Heo BG, Tashma Z, Katrich E, Gorinstein S (2008) Concentration of bioactive compounds in mussels *Mytilusgalloprovincialis* as an indicator of pollution. Chemosphere 73(6):938–944

Neff JM (1979) Polycyclic aromatic hydrocarbons in the aquatic environment. Applied Science Publishers LTD, London

Nisbet IC, LaGoy PK (1992) Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAHs). Regul Toxicol Pharmacol 16(3):290–300

Oliva AL, Quintas PY, La Colla NS, Arias AH, Marcovecchio JE (2015a) Distribution, sources, and potential ecotoxicological risk of polycyclic aromatic hydrocarbons in surface sediments from Bahía Blanca estuary, Argentina. Arch Environ Contam Toxicol 69(2):163–172

Oliva AL, Ovaert J, Arias AH, Souissi S, Marcovecchio JE (2015b) Mussels as bioindicators of PAHs pollution within argentinean coastal environments, South America. Int J Environ Res 9(4):1293–1304

Olson GM, Meyer BM, Portier RJ (2016) Assessment of the toxic potential of polycyclic aromatic hydrocarbons (PAHs) affecting Gulf menhaden (Brevoortia patronus) harvested from waters impacted by the BP Deepwater Horizon Spill. Chemosphere 145:322–328

Oros DR, Ross JR (2005) Polycyclic aromatic hydrocarbons in bivalves from the San Francisco estuary: spatial distributions, temporal trends, and sources (1993–2001). Mar Environ Res 60(4):466–488

OSPAR (2009) Background Document on CEMP Assessment Criteria for QSR 2010.OSPAR Publication Number 461/2009

Pérez Camacho A, Labarta U, Beiras R (1995) Growth of mussels (*Mytilus edulis galloprovincialis*) on cultivation rafts: influence of seed source, cultivation site and phytoplankton availability. Aquaculture 138(1):349–362

Peters CA, Knightes CD, Brown DG (1999) Long-term composition dynamics of PAH-containing NAPLs and implications for risk assessment. Environ Sci Technol 33:4499–4507

Piccolo MC (2008) Climatological features of the Bahía Blanca Estuary. In: Neves R, Baretta J, Mateus M (eds) Perspectives on integrated coastal zone management in South America. IST Press, Lisbon, pp 231–239

Popovich CA, Marcovecchio JE (2008) Spatial and temporal variability of phytoplankton and environmental factors in a temperate estuary of South America (Atlantic coast, Argentina). Cont Shelf Res 28(2):236–244

Porte C, Albaiges J (1993) Bioaccumulation patterns of hydrocarbons and polychlorinated biphenyls in bivalves, crustaceans, and fishes. Arch Environ Contam Toxicol 26(3):273–281

Pozo K, Estellano VH, Harner T, Diaz-Robles L, Cereceda-Balic F, Etcharren P, Pozo K, Vidal V, Guerrero F, Vergara-Fernández A (2015) Assessing Polycyclic Aromatic Hydrocarbons (PAHs) using passive air sampling in the atmosphere of one of the most wood-smoke-polluted cities in Chile: the case study of Temuco. Chemosphere 134:475–481

Ramdine G, Fichet D, Louis M, Lemoine S (2012) Polycyclic aromatic hydrocarbons (PAHs) in surface sediment and oysters (*Crassostrea rhizophorae*) from mangrove of Guadeloupe: levels, bioavailability, and effects. Ecotox Environ Safe 79:80–89

Ré N, Kataoka VMF, Cardoso CAL, Alcantara GB, Gomes de Souza JB (2015) Polycyclic aromatic hydrocarbon concentrations in gas and particle phases and source determination in atmospheric samples from a semiurban area of Dourados, Brazil. Arch Environ Contam Toxicol 69(1):69–80

Readman JW, Mantoura RFC, Rhead MM (1987) A record of polycyclic aromatic hydrocarbon (PAH) pollution obtained from accreting sediments of the Tamar estuary, UK: evidence for non-equilibrium behaviour of PAH. Sci Total Environ 66:73–94

Rocher B, Le Goff J, Peluhet L, Briand M, Manduzio H, Gallois J, Devier MH, Geffard O, Gricourt L, Augagneur S, Budzinski H, Pottier D, André V, Lebailly P, Cachot J (2006) Genotoxicant accumulation and cellular defence activation in bivalves chronically exposed to waterborne contaminants from the Seine River. Aquat Toxicol 79(1):65–77

Ruiz Y, Suarez P, Alonso A, Longo E, Villaverde A, San Juan F (2011) Environmental quality of mussel farms in the Vigo estuary: pollution by PAHs, origin and effects on reproduction. Environ Pollut 159(1):250–265

Sammarco PW, Kolian SR, Warby RA, Bouldin JL, Subra WA, Porter SA (2013) Distribution and concentrations of petroleum hydrocarbons associated with the BP/Deepwater Horizon Oil Spill, Gulf of Mexico. Mar Pollut Bull 73(1):129–143

Sericano JL, Wade TL, Jackson TJ, Brooks JM, Tripp BW, Farrington JW, Mee LD, Readmann JW, Villeneuve JP, Goldberg ED (1995) Trace organic contamination in the Americas: an overview of the US National Status and Trends and the International 'Mussel Watch' programmes. Mar Pollut Bull 31(4):214–225

Soriano JA, Viñas L, Franco MA, González JJ, Ortiz L, Bayona JM, Albaigés J (2006) Spatial and temporal trends of petroleum hydrocarbons in wild mussels from the Galician coast (NW Spain) affected by the Prestige oil spill. Sci Total Environ 370(1):80–90

Sprung M (1993) The other life: an account of present knowledge of the larval phase of *Dreissena polymorpha*. In: Nalepa TF, Schloesser DW (eds) Zebra mussels: biology, impacts and control. Lewis Publishers, New York, pp 39–54

Thorsen WA, Cope WG, Shea D (2004) Bioavailability of PAHs: effects of soot carbon and PAH source. Environ Sci Technol 38(7):2029–2037

Tolosa I, De Mora SJ, Fowler SW, Villeneuve JP, Bartocci J, Cattini C (2005) Aliphatic and aromatic hydrocarbons in marine biota and coastal sediments from the Gulf and the Gulf of Oman. Mar Pollut Bull 50(12):1619–1633

Toro B, Palma-Fleming H, Navarro JM (2004) Organic pollutant burden of the giant mussels *Choromytilus chorus* from the south-central Chilean coast. Chemosphere 55(2):267–275

Torroglosa ME, Giménez J (2015) Sperm ultrastructure in two species of *Brachidontes* (Bivalvia, Mytilidae) from the south-western Atlantic Ocean. J Mar Biol Assoc 95(05):991–998

UNEP (1993) United Nations Environment Programme. UNEP/FAO/IOC/IAEA. Guidelines for monitoring chemical contaminants in the sea using marine organisms. Reference Methods for Marine Pollution Studies No. 6

US EPA (1984) Health effects assessments for polycyclic aromatic hydrocarbons (PAHs). US Environmental Protection Agency, Environmental Criteria and Assessment Office, EPA 549/1-86-013, Cincinnati. 1984

USEPA (1993) US Environmental Protection Agency. Provisional Guidance for Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons. EPA/600/R-93/089, July 1993

USEPA (2002) Polycyclic Organic Matter. US Environmental Protection Agency. Available at: https://www.epa.gov/sites/production/files/2016-09/documents/polycyclic-organic-matter.pdf

Viñas L, Franco A, Blanco X, Bargiela J, Soriano JA, Perez-Fernandez B, Gonzalez JJ (2012) Temporal and spatial changes of PAH concentrations in *Mytilus galloprovincialis* from Ria de Vigo (NW Spain). Environ Sci Pollut Res 19(2):529–539

Wang J, Bi Y, Henkelmann B, Pfister G, Zhang L, Schramm KW (2016) PAHs and PCBs accumulated by SPMD-based virtual organisms and feral fish in Three Gorges Reservoir, China. Sci Total Environ 542:899–907

Yoshimine RV, Carreira RS, Scofield AL, Wagener ALR (2012) Regional assessment of PAHs contamination in SE Brazil using brown mussels (*Perna perna* Linnaeus 1758). Mar Pollut Bull 64(11):2581–2587

Yu ZL, Lin Q, Gu YG, Ke CL, Sun RX (2016) Spatial–temporal trend and health implications of polycyclic aromatic hydrocarbons (PAHs) in resident oysters, South China Sea: a case study of Eastern Guangdong coast. Mar Pollut Bull 110(1):203–211

Yunker MB, Macdonald RW, Goyettec D, Paton DW, Fowler BR, Sullivan D, Boyd J (1999) Natural and anthropogenic inputs of hydrocarbons to the Strait of Georgia. Sci Total Environ 225(3):181–209

Yunker MB, Macdonald RW, Vingarzan R, Mitchell RH, Goyette D, Sylvestre S (2002) PAHs in the Fraser River basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. Org Geochem 33(4):489–515