

Mussels as Bioindicators of PAHs Pollution within Argentinean Coastal Environments, South America

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ABSTRACT: The Bahía Blanca Estuary, located at the Atlantic coast of Argentina, includes a governmental nature reserve to protect biodiversity, lands and water; however, as the process of industrialization and urbanization is stepping rapidly at the neighbouring area, the potential increase in contaminant loads is a serious concern. After several contaminants surveys at the estuary, no single study has been yet performed in order to study the native mussels population assemblages vs. the chemical environment; then, to test the ability of *Brachidontes rodriguezii* assemblages as pollution indicators, native populations were chosen at three different sites from the Bahía Blanca Estuary. After quadrants sampling, the populations were assessed for size classification, density, sex rate, surrounding sediment and body burden of Polycyclic Aromatic Hydrocarbons (PAHs) as chemical pollution markers. Soxhlet extracted samples were measured through Capillary Gas Chromatography (Hewlett-Packard P68906C)/Mass Spectrometry (HP 5972). Mussels appeared to be better pollution indicators than sediments and could be used to classify the average pollution level at the area as "moderate" along the studied period. A positive correlational analyses was found between the population assemblage disruption vs. PAH's tissue burden, supporting the suitability of this specie to be used as biomonitor. In addition, source apportionment procedures could successfully discern between PAHs petrogenic and combustion inputs, leading to tag each site with the major contributing PAHs sources. Finally, screening values analysis concluded a certain health risk to potential consumers; thus, highlighting the urgent need of a systematic monitoring program.

Key words: Biological monitoring, Coastal environments, Persistent Organic Compounds, Hydrocarbons, Health Risk Assessment

INTRODUCTION

As it is well stated, marine and coastal areas represent especially vulnerable zones to the anthropogenic PAHs introduction via urban runoff (McCready et al., 2000), industrial processes (Simpson et al., 1996), vehicle exhausts and spillage of fossil fuels (Pettersen et al., 1997; Wang et al., 1999); therefore, the concentration of these compounds in seawater and sediments undoubtedly involve a toxicological importance to both benthic and pelagic marine organisms. As a general pathway, PAHs entering the water system can be first accumulated in fine-grained sediments and suspended particles, later remobilized in the seawater, then become bioavailable to native organisms (Wetzel and Van Vleet,

2004) and finally accumulated in biota with high rates of uptake or unable to efficiently metabolize the parent compounds (e.g. some invertebrates and mussels). Numerous literature dealing with PAHs entrance to the food web has performed research on these routes; as a consequence, mussels and other bivalves have been extensively investigated as the PAHs's access point to the pelagic food web and successively used as sentinels for biomonitoring programs (e.g., International Mussel Watch, Farrington and Tripp, 1995; European BIOMAR, Narbonne et al., 2005). Despite this, there is scarce to null studies involving both chemical and biological aspects of a native mussel community at the extensive Argentinean coast.

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The Bahía Blanca Estuary (BBE), located on the Atlantic coast of Argentina, has been recommended to be included in the RAMSAR list as the "Natural Reserve of Bahía Blanca" by The World Conservation Congress (WCC), to protect biodiversity, lands and water (2012). Despite this, the process of industrialization and urbanization is stepping rapidly at the area and the potential increase in contaminant loads is a serious concern. Then, the main aim of this work emerges as a consequence of considering this scenario and the null precedents linking the occurrence of pollutants with biological effects in sensitive species at the area. Specifically, the present research will study the presence of polycyclic aromatic hydrocarbons (PAHs) as a chemical disturbing factor and its impact over native mussel populations within the BBE, at the northern coast of the Patagonia. As a secondary objective, the selected species will be assessed as an autochthonous biological indicator to be included in future research surveys. Finally, an immediate outcome will be the generation of solid scientific basis to include the area in the RAMSAR international protection list conversation.

MATERIAL & METHODS

The BBE, at the north Patagonian coast ($38^{\circ} 40' S$ and $62^{\circ} 09' W$) has a total surface of 2300 km², of which about 410 km² of islands and 1150 km² of intertidal sector. It is a mesotidal system with very little fluvial input covered by extensive tidal flats and salt marshes (Piccolo and Perillo, 1990). This estuary has a large length/width ratio, low mean depth and a very large tidal prism in relation to a small freshwater input (Freije et al., 1981). The inner part of the estuary shows high primary production values due to a bloom of diatoms during winter and spring (Popovich et al., 2008).

On the one hand, it presents intensive anthropogenic activity at the north shoreline: oil, chemical and plastic factories, two commercial harbours, a 12-m overall length ships fishing fleet with a total catch of 600 t/yr and a big industrial city with more of 350 000 inhabitants, whose pre-filtered effluents are directly introduced into the estuarine waters. On the other hand, the inner and outermost part of the estuary presents low urbanized / rural lands, a tourist area and an artisanal fishing / recreational port (Fig. 1).

PAHs standard (mix), deuterated internal standards (naphthalene-d₈, acepnaphthene-d₁₀, phenanthene-d₁₀, crysene d-12) and benzoanthracene d-12 were provided by Sigma-Aldrich. HPLC-grade solvents (hexane, acetone, dichloromethane and methanol) were purchased from Merck. No significant amount of analytes was showed in procedural blanks. Ultrapure water (Milli-Q) was produced by a Millipore apparatus with 18.2 M Ω /

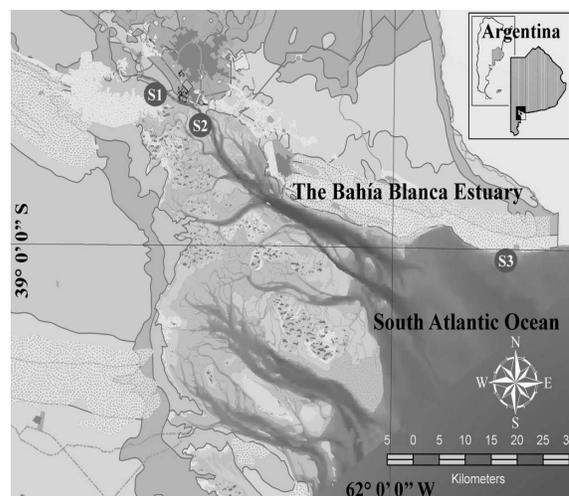


Fig. 1. Map of the area of study showing the sampling stations

cm resistivity. Merck silica gel 60 (70-230 mesh ASTM) and Aluminum oxide activated at 450 °C were heated at 120°C for 12 h prior to use. Glassware was washed with detergent (Decon), rinsed with ultrapure water and acetone and was dried at 120°C prior to use.

Indigenous mussels (*Brachidontes rodriguezii*) were collected from dock columns and rocks in surface water (0-1 m) during March 2013, on board of the IADO IV research vessel. A total of three collections of mussels were performed in the same time and in the same depth as at different sites, namely, S1, S2 and S3. In addition, at each site, 1 L of surface water (? 0,50 m depth) and a total of 500 g of surface sediment were collected and stored in clean oven-baked glass bottles before laboratory processing. "Villarino Viejo" station (S1) is located at offshore waters of a recreational/fishing port, while Puerto "Cuatros" station (S2) is in the vicinity of low urbanized/rural lands. They receive both freshwater from the Sauce Chico River, which cross a highly agriculture and cattle breeding lands. Finally, "Pehuenco" station (S3) is located at the outermost limit of the estuary and supports both a recreational beach and an artisanal fishing fleet.

Sampling was performed using 0.25 m² quadrants for area normalization, performing up to 8 quadrants collection. Immediately after, mussels were transferred inside an ice cool box to the laboratory. The specimens at each station were treated separately, cleaned to remove sediment and particles, sorted and counted to determinate the population densities. Then, each individual was sexed in order to measure the sex ratio of each population, either directly with naked eye or microscope techniques, following the New Zealand Greenshell (2006). In addition, each individual was also assessed for length and width of the shell by means of an electronic calliper (0.01mm accuracy), selecting three

size classes of length in order to rank mussels in small [$<25\text{mm}$]; medium [$25\text{-}35\text{mm}$] and large [$>35\text{mm}$] individuals. At the end, the soft tissue was dissected, wrapped in aluminium foil and frozen at -20°C . Samples were then lyophilized during 48hs, smashed in a mortar and prepared for chemical analyses. Before extraction $100\ \mu\text{L}$ of the mixture of four predeuterated PAHs (naphthalene-d₈, acenaphthene-d₁₀, phenanthrene-d₁₀, crysene d-12) was added as surrogate standards. Then, the tissue was digested under reflux with methanol and potassium hydroxide. The non-saponifiable fraction was extracted with n-hexane; the organic phase was dried with anhydrous sodium sulphate, and evaporated down to 2mL under a gentle high purity Nitrogen stream. Extract was seeded in alumina-silica gel columns to carry out the sample clean-up and hydrocarbon isolation. Elution was performed with $70\ \text{mL}$ n-hexane-dichloromethane (9:1), and then the eluate was evaporated down to 1mL (N₂). Finally, just before the GC/MS injection, all the samples were spiked with the internal standard for recovery test.

In order to state the nutritional condition of each environment, water subsamples for determination of nitrite (NO₂-), nitrate (NO₃-), ammonium (NH₄+), dissolved inorganic phosphorous (DIP) and dissolved silicates (DSi) concentrations were treated as Grasshoff (1976); McDonald and McLaughlin (1982) and APHA-AWWA-WEF (1998) standardized methods and analyzed by the colorimetric methods described by Grasshoff et al. (1983), Treguer and Le Corre (1975a and 1975b), Eberlein and Kattner (1987) and Technicon® (1973), respectively, using a Technicon II segmented flux auto analyzer. Water subsamples were subsequently used for the determination of chlorophyll a (Chl-a) using standardized colorimetric methods (APHA, 1998). Suspended particulate matter (SPM) was determined gravimetrically in additional water subsamples, using $0.45\ \mu\text{m}$ pore diameter Whatman cellulose filters. For each sample, additional $250\ \text{ml}$ were filtered in $1.2\ \mu\text{m}$ pore diameter pre-ashed glass fiber Whatman GF/C filters and treated following Strickland and Parsons protocols (1968) for the photometric determination of Particulate Organic Matter (POM) (Beckman DU-2 Spectrophotometer).

After collection, samples were immediately refrigerated, stored on solvent cleaned glass containers avoiding exposure to light, and then rapidly transported to the laboratory to be deep frozen prior to analysis. $10\text{-}20\ \text{g}$ of wet sediment were freeze-dried and extracted. Prior to extraction, $100\ \mu\text{L}$ of perdeuterated PAHs as surrogate standards were added (Naphthalene-d₈, Acenaphthene-d₁₀, Phenanthrene-d₁₀, Chrysene-d₁₂). Soxhlet extraction apparatus was assembled and refluxed with Hexane-Acetone mixture for about two hours

for each batch of samples. After that, the dried samples were Soxhlet extracted for 12 hs, using a Hexane-Acetone 1:1 solvent mixture (EPA 3540C method). The extracts were concentrated close to $10\ \text{mL}$ in a rotary evaporator with a low temperature thermostatic bath and further concentrated up to $2\ \text{mL}$ under a gentle - high purity- Nitrogen flow. For elimination of polar components, the extracts were passed through a 5% H₂O deactivated alumina/silica gel column ($1\ \text{cm i.d.} / 15\ \text{cm}$), and PAHs were eluted with $70\ \text{mL}$ hexane/methylene chloride (9:1, v/v). Before the extract was loaded, $1\text{-}2\ \text{g}$ of Na₂SO₄ was added to the top. Eluates were evaporated down close to $10\ \text{mL}$ in a rotary evaporator and further concentration was achieved drying the extracts under Nitrogen flow at room temperature up to $1\ \text{mL}$. Then, $100\ \mu\text{L}$ of deuterated internal standard were added to extracts vials for recovery test, giving a final concentration of about $2000\ \text{ng.mL}^{-1}$.

PAHs were quantified using a gas chromatograph (Hewlett-Packard HP68906C) coupled with a quadrupole mass spectrometer (Hewlett-Packard HP5972). The GC column was an HP-5MS fused silica column ($30\ \text{m}; 0.25\ \text{mm i.d.}; 0.25\ \mu\text{m}$ film thickness) and Helium was used as a carrier gas. The mass spectrometer was operated in the electron impact mode ($70\ \text{eV}$). The samples were injected in the splitless mode at 250°C . The temperature program used was as follows: initial temperature 70°C for 2 min; heated to 150°C at $30^{\circ}\text{C}/\text{min}$ then to 310°C at $4^{\circ}\text{C}/\text{min}$; and held for 10 min. PAHs were monitored in selected ion monitoring mode (SIM). The studied parent PAHs ranged from the di-aromatics to the hexa-aromatics: naphthalene (NA), 2-methyl-naphthalene (2-M-NA), acenaphthylene (ACEPH), acenaphthene (ACE), fluorene (FL), phenanthrene (PHE), anthracene (AN), fluoranthene (FLU), pyrene (PY), benz[a]anthracene (BaA), chrysene (CHR), benz[b]fluoranthene (BbF), benz[k]fluoranthene (BkF), benz[a]pyrene (BaP), indeno[1,2,3-cd]Pyrene (IP), dibenz[ah]anthracene (DBA), benz[g,h,i]perylene (BPE). Each individual PAH compound was confirmed by the retention time and the abundance of quantification ions/confirmation ions with respect to authentic PAHs standards.

All values reported for the sediments and mussels are on a dry weight basis. Quality control for the PAHs analyses was carried out by monitoring the recovery of the internal standard (Benz-[a]-pyrene-d₁₂) spiked just before GC injection. Quantification of individual compounds was based on comparison of peak areas with internal standards and recoveries ranged from 75% to 105%. Method blanks were run with every batch of eight samples. The quantification limit for individual PAHs species was set following IOC UNESCO N° 45 (1982), and was set in average at $10\ \text{ng.mL}^{-1}$. Protocol

was validated by the use of reference material (SRM-NIST 1944).

Water content and particle size distribution were also analyzed. Water Content was determined by the sediment sub-sample weight loss at $100 \pm 2^\circ\text{C}$. For grain size analysis, sediment was dried in oven and sifted in an automatic equipment with 2 mm and 63 μm mesh sieves. As a result, three fractions were obtained: gravel (particle sizes > 2 mm), sand (particle sizes between 0.063 and 2 mm), and silt-clay (particle sizes < 0.063 mm).

Principal component analysis was carried out using a statistical package software, this allowed the summarized data to be further analyzed and plotted. Data submitted for the analysis were arranged in matrix were each column corresponded to one PAH compound and each row represented a sampling site case.

RESULTS & DISCUSSION

Mussel density at each sampled community was 710, 712 ind/m² and 10352 ind/m² for S1, S2 and S3 respectively. The Shapiro statistical test showed no difference between S1 and S2; however, S3 mussel population evidenced the highest density value, which was significantly different ($p < 5\%$) from the others (Fig. 2). The size structure was also different between each population ($p < 5\%$) (Fig. 2). Namely, for S3, a high proportion of small individuals was observed (87%) with the rest of the population characterized by medium size individuals (13%). In this case, none individual with large size was observed. On the opposite, for S1 and S2 the size distribution was a mirror image; showing 20 and 21 % for small and large individuals and 54 and 51% for large and small individuals at S1 and S2 respectively. Finally, individuals with medium sizes were not proportionately different between S1 and S2 (26% and 29%, Fig. 2).

The growth curves symbolised by the coordination of shell width vs. shell length showed two patterns: one involving S1 and S2 population and other for S3 (Fig. 2A). This last population showed a small individual's dominance up to 25 mm length with the largest mussels up to 32 mm. Apart from that, S1 and S2 showed the same growth curve shape, including individuals up to 50 mm length.

Considering sex ratios, on the one side, the distribution of males and females was significantly different ($p < 5\%$) between S1 and the two other stations, showing a predominance of males (ratio M/F > 1). On other side, differences in the sex distribution could not be demonstrated for S3 and S4, which showed a predominance of females (ratio M/F < 1). In addition, the proportion of indeterminate individuals was significantly different between the three study stations ($p < 5\%$, Table 1). There was an increase of indeterminate individuals

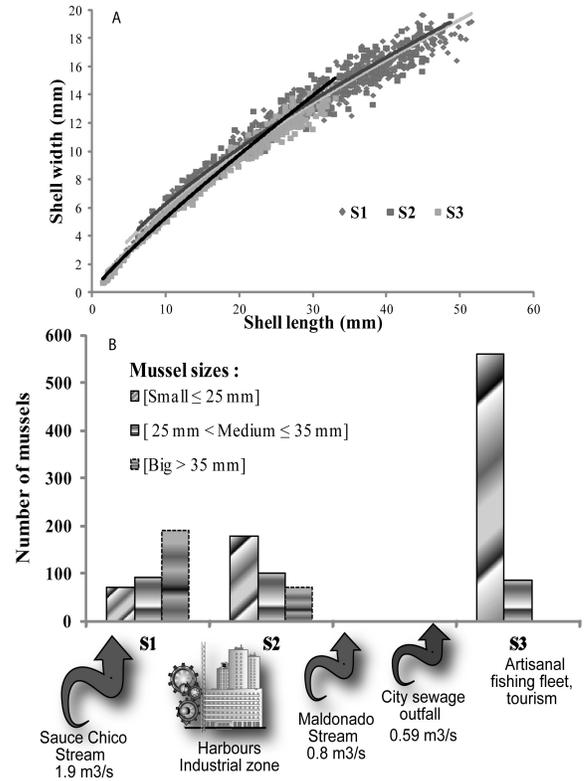


Fig. 2. (A) Mussel population's growth curves (B) Density evaluation of the three mussel populations (individual/m²) at the sampled sites in function of three size classes

from the inside (S1, 15% of the total population) to the outside of the estuary (S3, 45% of the total population).

Table 1. Sex ratio and indeterminate individuals at each mussel community

sample	S1	S2	S3
Average Sex ratio	1.48	0.79	0.82
Average indeterminate individuals/m ²	107	157	4658
Average total individuals/m ²	710	712	10352
% sex indetermination	15	22	45

Temperature, salinity and pH were in the same level at the three sampled locations (Table 2). Turbidity values defined moderately turbid waters for the three sites under study. Both inner station's turbidity is attributable to the tidal currents associated transport of sediments (silty-clay) and to the sewage network outfall proximity (Figure 1). The estuary outermost station (S3) turbidity shall be dominated by the high energy waves and sediment transport (sandy). The fact that suspended particles are commonly enriched in PAHs (Pruell et al., 1985; Budzinski et al., 1996) sets proper environmental

conditions for mussel's PAHs uptake. Opposed to that, a negative correlation between bioaccumulation factors and PAHs Log Kow is commonly found for mussels located on rocks at the air-water interface, where the water turbidity is generally low (Baumard *et al.*, 1998).

The highest POM content was found at S2 (5114 mg C/m³) and then at S1 (3861 mg C/m³). Consistently, the concentrations of nitrate, nitrite, ammonium and phosphate were higher at these stations, probably due to the inputs of the regional "Sauce Chico" and "Maldonado" freshwater streams. In agreement with the above mentioned, a decrease of Chl-a concentration was observed from the inside (10,17 mg/m³) to the outside (4,45 mg/m³) of estuary.

Sediment samples from the three sampled locations contained detectable amount of PAHs. A narrow range of PAHs concentrations was measured (Table 3); Total PAHs (summary of the 17 PAHs analyzed), ranged from 11.7 ng/g to 125.8 ng/g. The mean Total PAHs concentration recorded at the present study (68.6 ± 57.1 ng/g) indicates that the study area lies within low polluted marine environments. It is generally accepted that concentrations lower than 100 ng/g indicate a low pollution level, similar to pollution levels found in several well known unpolluted zones around the world (Table 4). The highest Total PAHs level was recorded at S2 (125.8 ng/g), an area located over the estuarine Navigation Channel (5 to 14 mts. depth, Figure 1) with high shipping activity, industrial complexes and a small commercial fishing fleet (Fig. 2). The following area in terms of concentration was S1 (68.3 ng/g) a shallow channels area located at the inner extreme point of the estuary and surrounded by agriculture lands. Finally, the lowest sediment PAHs concentration was recorded at S3 (11.7 ng/g), a sandy beach located in the proximity of a tourist village with a permanent motor-boat fishing fleet. On the other

side, Total PAHs levels found in indigenous mussels were widely above the limit of detection (LOD) of the analytical method for all the samples and ranged from 38.4 to 2048.5 ng/g d.w. (? 17 PAHs, Table 3), showing a global mean of 334.5 ± 693.5 ng/g, d.w. This high variability was mainly attributed to S3 mussels which reached a Total PAHs concentration of 2048.5 ng/g for the medium size class, showing a high percentage contribution of low molecular weight PAHs such as NA and PHE (146.9 and 1707.5 ng/g, respectively).

In terms of molecular PAHs profiles, the data firstly showed that mussels at all areas exhibited a marked dominance of PAHs up to three molecular rings, achieving an average of 73, 74 and 79% for S3, S2 and S1, respectively. This trend was in agreement with the sediment PAHs pattern which accounted 71% for S3 and S1, and 90% for S2. At first instance, it can be hypothesized that mussels are reflecting the molecular PAHs pattern of sediments; however this trend is also in agreement with the fact that higher invertebrates are more likely to accumulate low molecular weight PAHs vs. high molecular weight ones. For instance, Baumard (et al. 1998), showed a linear decrease of the PAHs bioaccumulation with increasing mass weight. Secondly, the mussel's body burden of PAHs was not correlated with the sediment PAH's content; in particular, S3 station showed the lowest sediment concentration in simultaneous with the highest PAH's tissue concentration in mussels. A rationale for this can be explored at the sediment granulometry at each site: while S3 presented a gross grain size (sandy), the sediment was silty/clay for the rest of the stations. It is well known that in general, marine organic compounds preferentially partitionate into the finest particles (Pierard et al., 1996; Baumard et al., 1999); accordingly, it can be hypothesized that the mussel body burden is in correla-

Table 2. Basic physicochemical parameters in water for the three studied stations

Variable	Units	S1	S2	S3
Turbidity	<i>N.T.U</i>	208	539	430
Temperature	°C	21.1	20.7	23.1
Salinity	<i>P.S.U.</i>	32.35	33.99	30.75
pH		8.1	8.1	8.0
Nitrite	<i>µmol/L</i>	3.65	5.06	4.97
Nitrate	<i>µmol/L</i>	10.55	44.69	0.09
Ammonium	<i>µmol/L</i>	13.89	20.68	11.37
Phosphate	<i>µmol/L</i>	1.76	2.49	0.99
Silicate	<i>µmol/L</i>	132.61	115.32	19.34
Particulate Organic Matter (POM)	<i>mg C/m³</i>	3861	5114	1544
Chlorophyll a	<i>mg/m³</i>	10.17	6.48	4.45

tion with the PAHs content at the POM phase (<0.45 μm). Assuming similar partitioning processes at each sampling site, the low POM content at S3 in comparison to S1 and S2 could have guaranteed the rapid partition and concentration of PAHs to this phase. As a consequence, a high input of PAHs at S3 would be accumulated at the POM phase and become rapidly available for surrounding mussels. In contrast, a PAHs input at S1 and S2 -with high POM values- would be diluted into the organic phase/matrix and then, become less available to the neighboring mussels.

PAHs ratios are traditionally used to determine PAHs sources classify samples by location and estimate the importance of combustion and petroleum derived PAHs (Lipiatou and Salot 1991, Budzinski *et al.*, 1997, Yunker *et al.*, 2002). The usual index of combustion and/or anthropogenic input is an increase in the proportion of the less stable and/or kinetically produced parent PAH isomers relative to the thermodynamically stable isomers (e.g. FLU relative to PY) or to the molecular mass totals (Yunker *et al.*, 2002). Although possible sources can be inferred from PAH isomer pair ratios, the relative frequencies of PAH isomer pair ratios found in bivalves should be used cautiously since PAH can be altered to some degree during their environmental transport and bioaccumulation. Therefore, the approach of multiple PAH isomer pair ratios as diagnostic indicators rather than a single ratio alone was applied to provide a more precise estimate of the PAH contributions from different sources (Oros and Ross, 2005). We primarily used the proportions of FLU to FLU plus PY (FLU/202) and IP to IP plus BPE (IP/276). FLU/202 ratios less than about 0.40 usually indicate petroleum (oil, diesel, coal, etc), between 0.40 and 0.50 indicate liquid fossil fuel (vehicle and crude oil) combustion, while ratios over 0.50 are attributable to grass, wood or coal combustion. Similarly, IP/276 ratios less than approximately 0.20 imply petroleum, between 0.20 and 0.50 liquid fossil fuel (vehicle and crude oil) combustion while ratios over 0.50 are attributable to grass, wood or coal combustion (Yunker *et al.*, 2002). These two parent PAHs ratios are supplemented by AN to AN plus PHE (AN/178) and BaA to BaA plus CHR [BaA/228]. AN/178 ratios < 0.10 usually are taken as an indication of petroleum, while ratios > 0.10 indicate combustion (Budzinski *et al.*, 1997; Soclo *et al.*, 2000; Yunker *et al.*, 2002).

The analysis of molecular ratios indicated that the PAHs were, in most cases, of pyrolytic origin, but with inputs of petrogenic hydrocarbons close to harbours (S1) and industries (S2). The general predominance of FLU over PY consistent with elevated IP/276 and BaA/228 ratios implicated both liquid fossil fuel combustion and biomass (coal, grass, wood) as the major PAHs source (s). Biomass combustion could have both local and long range atmospheric transport sources, as the estuary is sur-

rounded by agriculture lands, in which controlled and uncontrolled fires frequently occur. Liquid fuel fossil combustion source (s) was probably a quite local source (s), in special at S3, due to the high level of motor-boat and shipping activity at the area. These results were in good agreement with the dominant sources assessed for the water column and sediments previously reported at the area (Arias *et al.*, 2009; 2010).

In order to deepen the analysis, the clustering technique was performed to identify the homogeneous groups of individual PAHs occurring in sediments and mussels. Results are shown in the hierarchical dendrogram at Figure 3, which distinguished the 17 individual PAHs into three major groups. The first major group included AN, FLU, CHR, BbF, BkF, IP, DBA and BPE, which are mainly high molecular weight PAHs with 4 to 6 rings. These PAHs are usually detected in pyrogenic sources, e.g., combustion of coal, wood, vehicle fuel and waste tire (Levendis *et al.*, 1998; Zakaria *et al.*, 2002; Wang *et al.*, 2007). The second major group contained the low molecular weight PAHs with 2-3 rings: NA, ACE, FL, ACEPH and BaA. Although there is no rationale to classify BaA in this group, these compounds are abundant in petrogenic sources. The third cluster was defined by the presence of PHE, a typical marker of diesel combustion.

After this analysis, Principal Component Analysis (PCA) was performed to identify meaningful components, which accounted for 42.91%, 20.87%, and 17.53% of the total variance, respectively. PCA loading scores higher than 0.40 were considered meaningful (Tabachnick and Fidell, 2000). First, PC1 was characterized by high loadings for PAHs such as NA, ACE, ACEPH, PHE and FLU. These PAHs belong to the Low Molecular Weight PAHs (LMW) with 2-3 rings which are abundant in petrogenic sources mainly caused by petroleum spills, e.g., fresh or used crankcase oil, crude and fuel oil (Marr *et al.*, 1999; Utvik *et al.*, 1999; Dobbins *et al.*, 2006; Gonzalez *et al.*, 2006; Ye *et al.*, 2006). Added to this, BaA appears to be the main tracer of gasoline engine (Wang *et al.*, 2009). Thus, PC1 was suggested to be indicative of volatilization or spill of petroleum-related products, e.g. from the waterway transportation industry, and interpreted as the petrogenic source of PAHs. Secondly, PC2 was characterized by significant loadings of AN, FL and BPE (three to six rings). In general the heavier PAHs are the result of combustion/pyrolytic processes and are absent in crude oil or refined products (Wang, 1999). While FL can be grouped as typical markers of pyrogenesis, the presence of a meaningful BPE loading added to the absence of PHE eigenvalue significance (as would be expected in diesel emissions) led to interpret this component as gasoline/petroleum light derivatives

Table 3. Total PAHs, molecular indices, main source contribution and screening value classification of mussels and sediments at the BBE

Sample/Site	Total PAHs (w.w.)	Total PAHs (d.w.)	FLU/202 ^{1,2}	IP276 ²	ANI/78 ³	BaA/228 ²	LMVHMW ³	Main Source	Contamination Level ⁴ /Health Risk ⁵
Sediments S1	34.4	68.2	0.51	N.D.	N.D.	0.71	3.89	Combustion	Low
Sediments S2	62.9	125.8	0.43	N.D.	N.D.	N.D.	17.50	Mixed Sources	Moderate
Sediments S3	5.3	11.7	N.D.*	N.D.	N.D.	0.64	1.80	Mixed Sources	Low
Mussels S1 small	35.1	38.4	N.D.	N.D.	N.D.	N.D.	8.44	Petrogenic	Exceedance
Mussels S1 medium	11.8	51.1	N.D.	0.86	1.00	N.D.	2.11	Combustion	Exceedance
Mussels S1 big	16.6	83.7	N.D.	0.66	1.00	N.D.	4.32	Combustion	Exceedance
Mussels S2 small	16.9	80.4	N.D.	1.00	1.00	0.79	1.47	Combustion	No Exceedance
Mussels S2 medium	28.4	142.3	N.D.	N.D.	1.00	1.00	3.66	Combustion	No Exceedance
Mussels S2 big	20.3	101.6	N.D.	N.D.	1.00	1.00	5.59	Combustion	No Exceedance
Mussels S3 small	25.9	129.8	N.D.	1.00	1.00	N.D.	1.05	Combustion	Exceedance
Mussels S3 medium	409.7	2048.5	1.00	N.D.	N.D.	1.00	48.40	Combustion	No Exceedance
Mussels S3 big	N.A. [#]	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.

¹ Budzinski et al., 1997, ²Yunker et al., 2002, ³Yuan et al., 2001; Tam et al., 2001,

⁴ Baumard et. al approach (1998), ⁵TEQ BaP screening value for fish human consumption (USEPA, 2000), * Not detected, # Not analyzed

combustion. Finally, PC3 showed significance for PY, BbF, AN and NA. BbF and BkF are components of fossil fuels and a portion of them is associated with their combustion (Rogge et al., 1993; Kavouras et al., 2001). AN and PY are general tracers of combustion and traffic emission (Fraser et al., 1997). Consequently PC3 was defined as a general traffic emission/fossil fuel combustion component. To summarize, according to HCA results, the PAHs could be subdivided into three groups with different molecular weight composition which represented three kinds of sources. Consistently and further, the results of PCA could also differentiate the three subsets sources. ~ 81% of the total variance of PAHs concentrations in mussels and sediments was explained by source contributions in PCA results, showing an equilibrium of sources: while 42.91% of the variance was explained by the contribution of petroleum/petrogenic sources, 39% (PC2, 20.87% + PC3, 17.53% of variance) was explained by the contribution of different combustion sources.

From the distribution of sampling locations and matrix, the PCA results also showed that two population of mussels at different locations were highly characterized by petrogenic inputs (S3 and S2) while the remaining community at S1 was signed by diverse pyrogenic /combustion inputs. The petrogenic input at such stations correlated with the intense shipping activity continuously developed at those sites; as a consequence of that, motor boats residues, crankcase oil or accidental petroleum derivatives spills frequently occur. On the other side, in regards to sediments and consistently with the PAHs source prevalence of the mussels at each site, the sediment bed from S2 and S3 showed concomitant inputs from petrogenic/crude oil/diesel and combustion/traffic emission PAHs (Figure 3B). Finally and probably due to the low Total PAHs amount at the station (68.2 ng/g, d.w.) S1 sediments were unable to coordinate with positive scores at any of the three principal components.

Upon the scarcity of data explaining the physiological mechanisms underlying the diverse sexuality patterns of mussels, it has been reported an increase in birth rate and recruitment at polluted environments, probably due to the requirement of a larger density of females in order to guarantee the viability of the population. As a result, often <1 sex ratios are shown at those mussels. In this context, it can be firstly hypothesized that the higher proportion of females showed at the PAHs polluted sites S2 and S3 are a consequence of an increased birth rate triggered as a survival strategy under pollution stress. In contrast, the population located at the lowest polluted environment (S1) showed >1 sex ratios, indicating a probable natural density-dependent factor limiting mechanism.

Results also showed an increasing trend of inde-

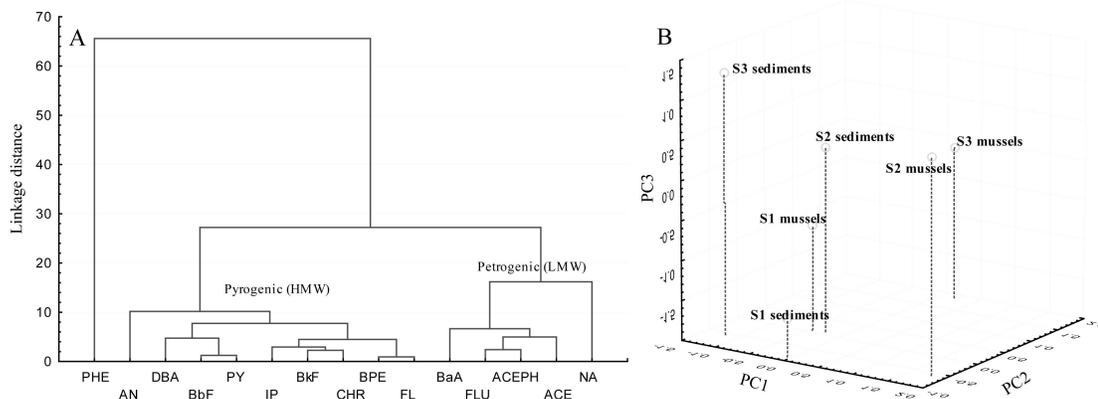


Fig.3. (A) Tree diagram for 15 PAHs as variables (single linkage, Euclidean distances). (B) Tridimensional scatterplot of factor scores coordinating with each Principal Component

terminate organisms from the inside to the outside of the estuary. As is well known, in normal conditions mussels gradually shift from somatic growth into reproduction, leading to the gametogenesis process, a high energy demanding step of the life cycle (Saucedo et al., 2008). Despite this, the physiological processes can often be altered in polluted environments (Monserrat et al., 2007) and mussels have to reallocate energy for survival and defence (in e.g.; less filtration rate, higher incidence of infections and diseases, etc.). At the light of the results, we propose that the recorded chemical stress might be indirectly inducing an elevated number of indeterminate individuals at the higher polluted communities (increasing from S1 to S3) due to the energy demands for survival and defence.

Finally, the consideration of the size structure composition at the light of the PAHs burden allowed a finer approach of the PAHs environmental contamination. An increase in the amount of large individuals was observed at the lowest polluted community (S1) and the opposite was observed at the most polluted community (S3). Similarly, a low proportion of small individuals was found at S1 in comparison to S3. As it is well stated, chemical disturbing can cause a high mortality of big individuals, which bioaccumulate PAHs from the surrounding media due to their low detoxification ability. Moreover, as stated in the above paragraphs, an increase in mortality leads to an increase of the growth rate in order to compensate, a common density-dependent factor which controls population. Density dependence occurs when the population growth rate, or constituent gain rates (e.g. birth and immigration) or loss rates (death and emigration), vary causally with population size or density (Hixon and Johnson, 2009). Then, as a result, the size structure distribution at the communities could be explained by a density dependence factor triggered by the chemical pollution at each site.

As reviewed by Barra et al. (2007), PAHs information on mussels and biota samples is scarce for South American environments. In order to place the studied PAHs concentrations in a global context, observed concentrations of sediments and marine bivalves around the world are shown in Table 4. Even though similar surveys were selected for this Table, to get better comparison parameters, the number of PAHs analysed in each study was included.

For comparative purposes, contamination level in estuarine sediments can be categorized as low (< 100 ng/g), moderate (100-1000 ng/g), high (1000-10000 ng/g) and extremely high (>10000 ng/g). Considering this, PAHs levels in the study area could be included between low to moderate polluted sediments, comparable to several coastal areas around the world, namely Gironde Estuary (France), Istanbul strait (Turkey) and Todos los Santos Bay (Mexico). According to Baumard et al. 1998 pollution in bivalves can be stated as low (0-100 ng/g d.w.), moderate (>100-1000 ng/g d.w.), high (>1000-5000 ng/g d.w.) and very high (>5000 ng/g d.w.) Then, mussels belonging at the three first levels were assessed at the area of study (from low to high) leading to a general pollution level comparable to Abu Qir Bay (Egypt), Saronikos Gulf (Greece), Galician Coasts (Spain) and San Francisco Estuary (USA). In spite of the fact that episodic PAH spikes can be measured in bivalves frequently due to oil spills and tanker accidents (Colombo et al., 2005; Soriano et al., 2006) it is generally considered that the mean Total PAHs in bivalves is highly indicative of the average contamination level of the estuary (Oros and Ross, 1995). Considering that the BBE wide mean of Total PAHs in mussels was 334.5 ng/g d.w. (17 PAHs), the average pollution level at the area was classified as "moderate" along the studied period. These results bring a solid scientific basis contributing to the BBE candidature to be included in the RAMSAR list proposed by the World

Table 4. PAHs burden at worldwide coastal and estuarine samples

Sample	Locations	N of PAHs	Range (ppb, d.w.)	References
Sediments	Kaohsiung Harbor, Taiwan	17	34- 16700	Chen et al. 2013
Sediments	Yangtze River Estuary, China	16	90-502	Li et al. 2012
Sediments	Hugli estuary, India	19	25- 1081	Guzzella et al. 2005
Sediments	Thai Coast, Thailand	17	6 -228	Boonyatumanond et al. 2006
Sediments	Estuary of the Chao Phraya River, Thailand	17	30-724	Boonyatumanond et al. 2006
Sediments	Abu Qir Bay, Egypt	16	<MDL* -2660	Khairy et al. 2009
Sediments	Istanbul strait, Turkey	16	2.1- 3152	Karadket et al. 2009
Sediments	Adriatic Sea, Italy	16	24 -501	Magi et al. 2002
Sediments	Gironde Estuary and Arcadon Bay, France	14	3- 853	Soclo et al. 2000
Sediments	Bilbao Estuary, Spain	not data	1493 -47480	Prieto et al. 2008
Sediments	Todos los Santos Bay, Mexico	16	7.6-813	Macias-Zamora et al. 2002
Sediments	Cienfuegos bay, Cuba	not data	450-10500	Tolosa et al. 2009
Sediments	Rio de la Plata Estuary, Argentina	not data	3-2010	Colombo et al. 2006
Sediments	Bahia Ushuaia, Patagonia, Argentina	16	nd - 360	Comendatore et al. 2012
Sediments	Bahia Blanca Estuary, Argentina	17	15- 10260	Arias et al. 2010
Sediments	Bahia Blanca Estuary, Argentina	17	11 - 125	<i>This study</i>
<i>Mytilus galloprovincialis</i>	Saronikos Gulf, Greece	17	1300- 1800	Valavanidis et al. 2008
<i>Mytilus galloprovincialis</i>	Galician Coasts, Spain	13	17 - 7780	Soriano et al. 2006
<i>Mytilus galloprovincialis</i>	Riade Pontevedra and Riade Vigo, Spain	16	80 - 517	Vidal-Liñan et al. 2010
<i>Mytilus galloprovincialis</i>	Bay of Biscay	14	21 - 64	Orbea et al. 2002
<i>Mytilus galloprovincialis</i>	Istanbul strait, Turkey	16	43 - 601	Karadket et al. 2009
<i>Mytilus edulis</i>	Northem Irish Sea- loughs	12	95 - 184	Guinan et al. 2001
<i>Mytilus edulis</i>	Lynæs Harbour, Denmark	19	473 - 4160	Rank et al. 2009
<i>Mactra corallina</i> and <i>Tapes decussata</i>	Abu Qir Bay, Egypt	16	242-2830	Khairy et al. 2009
<i>Mytilus californianus</i>	San Francisco Estuary, USA	25	21 - 1093	Oros and Ross 2005
<i>Perna perna</i>	Guanabara Bay, Brasil	16	69 - 239	Yoshimine et al. 2012
<i>Mytella charruana</i>	Mundaú Lagoon, Brazil	16	41 - 52	Maioli et al. 2010
<i>Brachidontes</i> sp and <i>tagelus</i> sp	Estuary of Bahiablanca, Argentina	17	349 - 1597	Arias et al. 2009
<i>Brachidontes rodriguezii</i>	Estuary of Bahiablanca, Argentina	17	38-2048	<i>This study</i>

* < MDL = below the method detection limit

Conservation Congress (2012).

BaP is classified by the U.S. EPA as B2: a probable human carcinogen based on numerous adult studies in several animal species (primates, rats, mice) (WHO, 1996). In terms of human health, the PAHs carcinogenic potential may be expressed by means of the toxic equivalent of benz[a]pyrene (TEQ BaP). TEQ BaP are generated by comparing the carcinogenic effects of the measured concentrations of various representatives of polyaromatic hydrocarbons to that of BaP. Following Nisbet and Lagoy (1992) plus Larsen and Larsen equations (1998) (1), the TEQ BaP for the 17 analyzed compounds were calculated for mussels samples for comparison with the screening values of TEQ BaP of total PAHs (0.67ng/g w.w.) suggested by USEPA(2000) for human fish consumption.

$$\text{TEQ BaP} = \sum \text{TEFi} \times \text{Ci} \quad (1)$$

In equation (1), TEQ BaP is the total equivalent concentration of BaP for the 17 PAHs measured at this study; TEF is the toxic equivalent factor for individual PAHs compounds; C is the concentration of each PAH observed in target samples. The average TEQ BaP were 4.43, 0.31 and 2.17 ng TEQ BaP/g w.w. for S3, S2 and S1 mussels respectively. Consequently, two of the three sampled mussel communities were exceeding the cited USEPA acceptable guideline of fish products for human consumption. These results highlight the urgent

need of periodical monitoring surveys and government marine food advisory programs.

CONCLUSIONS

Mussels (*B. Rodriguezii*) emerged as a better average pollution indicator than sediments at the BBE and could be successfully used to classify the average pollution level at the area as "moderate" along the studied period. Source apportionment procedures could successfully discern between PAHs petrogenic and combustion inputs at each mussel community, leading to tag each site with the major contributing PAHs sources. An emerging hypothesis by which the proportion of females showed at the highest PAHs polluted sites could be a consequence of an increased birth rate triggered as a survival strategy under PAHs pollution stress was raised. The size structure distribution at each mussel communities could be explained by a density dependence factor triggered by the PAHs chemical pollution at each site. Potential mussels consumers could be in health risk; thus, highlighting the urgent need of a systematic monitoring program for the area in study, in order to provide accurately assessment and management of risks for the regional population.

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REFERENCES

- APHA-AWWA-WEF (1998). Clesceri, L.S., Greenberg, A.E., Eaton, A.D. (Eds.). Standard Methods for the Examination of Water and Wastewater, 20th ed. (Washington: American Public Health Association).
- Arias, A. H., Spetter, C.V., Freije, R. H. and Marcovecchio, J.E. (2009). Polycyclic aromatic hydrocarbons in water, mussels (*Brachidontes* sp., *Tagelus* sp.) and fish (*Odontesthes* sp.) from Bahía Blanca Estuary, Argentina. *Estuarine, Coastal and Shelf Science*, **85**(1), 67-81.
- Arias, A. H., Vazquez-Botello, A., Tombesi, N., Ponce-Velez, G., Freije, H. and Marcovecchio, J. (2010). Presence, distribution, and origins of polycyclic aromatic hydrocarbons (PAHs) in sediments from Bahia Blanca estuary, Argentina. *Environmental Monitoring and Assessment*, **160**(1), 301-314.
- AquaBio Consultants Ltd. (2006). Learning Resource for Unit Standard 16340v3 "Biology of the New Zealand Greenshell Mussel". SITO, Auckland.
- Barra, R., Castillo, C. and Machado Torres, J.P. (2007). Polycyclic aromatic hydrocarbons in the South American environment. *Reviews of Environmental Contamination and Toxicology*, 191, 1-22.
- Baumard, P., Budzinski, H., Michon, Q., Garrigues, T., Burgeot, T., and Bellocq, J. (1998). Origin and bioavailability of PAHs in the Mediterranean Sea from mussel and sediment records. *Estuarine Coastal and Shelf Science*, **47**(1), 77-90.
- Baumard, P., Budzinski, H., Garrigues, P., Narbonne, J. F., 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. *Marine Environmental Research*, **47**(5), 415-439.
- Boonyatumanond, R., Wattayakorn, G., Togo, A., Takada, H., (2006). Distribution and origins of polycyclic aromatic hydrocarbons (PAHs) in riverine, estuarine, and marine sediments in Thailand. *Marine Pollution Bulletin*, **52**(8), 942-956.
- Budzinski, H., Baumard, P., Papineau, A., Wise, S. and Garrigues, P. (1996). Focused microwave assisted extraction of polycyclic aromatic compounds from standard reference materials, sediments and biological tissues. *Polycyclic Aromatic Compounds* **9**, 225-232.
- Budzinski, H., Jones, I., Bellocq, J., Pie'rard, C., and Garrigues, P. (1997). Evaluation of sediment contamination by polycyclic aromatic hydrocarbon in the Gironde estuary. *Marine Chemistry*, **58**(1), 85-97.
- Chen, C.F, Chen, C.W., Dong, C.D. and Kao, C.M. (2013). Assessment of toxicity of polycyclic aromatic hydrocarbons in sediments of Kaohsiung Harbor, Taiwan. *Science of the Total Environment*, **463-464**, 1174-1181.
- Colombo, J. C., Barreda, A., Bilos, C., Cappelletti, N., Migoya, M. C., Demichelis, S., et al. (2005). Oil spill in the Río de la Plata estuary, Argentina: 1. Biogeochemical assessment of waters, sediments, soils and biota. *Environmental Pollution*, **134**(2), 277-289.
- Colombo, J.C., Cappelletti, N., Laschi, J., Migoya, M., Speranza, E., Skorupka, C. (2006). Sources, vertical fluxes, and equivalent toxicity of aromatic hydrocarbons in coastal sediments of the Río de la Plata Estuary, Argentina. *Environmental Science and Technology*, **40**(3), 734-740.
- Commendatore, M. G., and Esteves, J. L. (2004). Natural and anthropogenic hydrocarbons in sediments from the Chubut River (Patagonia, Argentina). *Marine Pollution Bulletin*, **48**(9), 910-918.
- Commendatore, M.G., Nievas, M.L., Amin O. and Esteves, J.L. (2012). Sources and distribution of aliphatic and polyaromatic hydrocarbons in coastal sediments from the Ushuaia Bay (Tierra del Fuego, Patagonia, Argentina). *Marine environmental Research*, **74**, 20-31.
- Dobbins, R.A., Fletcher, R.A., Benner Jr., B.A. and Hoeft, S. (2006). Polycyclic aromatic hydrocarbons in jet engines, in diesel fuels, and in diesel emissions. *Combustion and Flame* **144**(4), 773-781.
- Eberlein, K. and Kattner, G. (1987). Automatic method for determination of orthophosphate and total dissolved phosphorous in the marine environment. *Fresenius Zeitschrift fur Analytische Chemie*, **326**(4), 354-357.
- Farrington, J.W. and Tripp, B.W. (1995). International Mussel Watch Project-Final Report. (USA: NOAA Administration, pp. 55-59).
- Fraser, M.P., Cass, G.R., Simoneit, B.R.T. and Rasmussen, R.A., 1997. Air quality model evaluation data for organics C2-C36 non-aromatic hydrocarbons. *Environmental Science and Technology*, **31**(8), 2356-2367.
- Freije, R.H., Astesauain, R.O., Schmidt, A. and Zavatti, J., (1981). Relación de la salinidad y temperatura del agua con las condiciones hidrometeorológicas en la porción interna del estuario de Bahía Blanca. *Contribución Científica, IADO N° 57*, 81 pp.
- Gonzalez, J.J., Vinas, L., Franco, M.A., Fumega, J., Soriano, J.A. and Grueiro, G. (2006). Spatial and temporal distribution of dissolved/dispersed aromatic hydrocarbons in seawater in the area affected by the Prestige oil spill. *Marine Pollution Bulletin*, **53**(5), 250-259.
- Grasshoff, K. (1976). Filtration and storage. (In *Methods of Seawater Analysis* (pp. 21-24). New York: Verlag Chemie).
- Grasshoff, K., Erhardt, M. and Kremling, K. (1983). *Methods of Seawater Analysis*, second ed. (pp. 365-366). (New York: Verlag Chemie).
- Guinan, J., Charlesworth, M., Service, M. and Oliver, T. (2001). Sources and geochemical constraints of polycyclic aromatic hydrocarbons (PAHs) in sediments and mussels of two Northern Irish Sea-loughs. *Marine Pollution Bulletin* **42**(11), 1073-1081.
- Guzzella, L., Roscioli, C., Vigano, L., Saha, M., Sarkar, S.K., Bhattacharya, A. (2005). Evaluation of the concentration of HCH, DDT, HCB, PCB and PAH in the sediments along the lower stretch of Hugli estuary, West Bengal, northeast India.

- Environment International **31**(4), 523-534.
- Hixon, M. A. and Johnson, D. W. (2009). Density dependence and independence. Encyclopedia of Life Sciences.
- Karacık, B., Okay, O.S., Henkelmann, B., Bernhöft, S. and Schramm, K.-W. (2009). Polycyclic aromatic hydrocarbons and effects on marine organisms in the Istanbul Strait. Environment International, **35**(3), 599-606.
- Kavouras, I.G., Koutrakis, P., Tzapakis, M., Lagoudari, E., Stephanou, E.G., Baer, D.V. and Oyola, P. (2001). Source apportionment of urban particulate aliphatic and polynuclear aromatic hydrocarbons (PAHs) using multivariate methods. Environmental Science and Technology **35**(11), 2288-2294.
- Khairy, M., Kolba, M., Mostafa, A., EL-Fiky, A., Bahadira, M. (2009). Risk assessment of polycyclic aromatic hydrocarbons in a Mediterranean semi-enclosed basin affected by human activities (Abu Qir Bay, Egypt). Journal of Hazardous Materials, **170**(1), 389-397.
- Larsen J.C. and Larsen P. (1998). Chemical carcinogens (In: Hester, R.E. Harrison, R.M. (Eds.), Air Pollution and Health, (pp. 33-56). Cambridge: The Royal Society of Chemistry.)
- Levendis, Y.A., Atal, A. and Carlson, J.B. (1998). On the correlation of CO and PAH emissions from the combustion of pulverized coal and waste tires. Environmental Science and Technology, **32**(23), 3767-3777.
- Li, B.H., Feng, C.H., Li, X., Chen, Y.X., Niu, J.F., Shen, Z.Y. (2012). Spatial distribution and source apportionment of PAHs in surficial sediments of the Yangtze Estuary, China. Marine Pollution Bulletin, **64**(3), 636-643.
- Lipiatou E. and Salot A. (1991). Fluxes and transport of anthropogenic and natural polycyclic aromatic hydrocarbons in the western Mediterranean Sea. Marine Chemistry, **32**(1), 51-71.
- Macías-Zamora, J.V, Mendoza-Vega, E and Villaescusa-Celaya, J.A. (2002). PAHs composition of Surface marine sediments: a comparison to potential local sources in Todos Santos Bay, B.C., Mexico. Chemosphere, **46**(3), 459-468.
- Magi, E., Bianco, R., Ianni, C., and Di Carro, M. (2002). Distribution of polycyclic aromatic hydrocarbons in the sediments of the Adriatic Sea. Environmental Pollution, **119**(1), 91-98.
- Maioli, O.L.G, Rodrigues, K.C, Knoppers, B.A and Azevedo, D.A. (2010). Polycyclic aromatic and aliphatic hydrocarbons in *Mytella charruana*, a bivalve mollusk from Mundaú Lagoon, Brazil. Microchemical Journal, **96**(1), 172-179.
- Margalef, R. (1979). Ecology. (Barcelona: Omega Editorial).
- Marr, L.C., Kirchstetter, T.W., Harley, R.A., Miguel, A.H., Hering, S.V. and Hammond, S.K. (1999). Characterization of Polycyclic Aromatic Hydrocarbons in Motor Vehicle Fuels and Exhaust Emissions. Environmental Science and Technology, **33**(18), 3091-3099.
- McCready, S., Slee, D. J., Birch, G. F., and Taylor, S. E. (2000). The distribution of polycyclic aromatic hydrocarbons in surface sediments of Sidney Harbor, Australia. Marine Pollution Bulletin, **40**(11), 999-1006.
- McDonald, R.W. and McLaughlin, F.A. (1982). The effect of storage by freezing on dissolved inorganic phosphate, nitrate and reactive silicate for samples from coastal and estuarine waters. Water Research **16**(1), 95-104.
- Monserrat, J. M., Martínez, P. E., Geracitano, L. A., Lund Amado, L., Martinez Gaspar Martins, C., Lopes Leães Pinho, G. et al (2007). Pollution biomarkers in estuarine animals: critical review and new perspectives. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, **146**(1), 221-234.
- Narbonne, J.F., Aarab, N., Clè randeau, C., Daubèze, M., Narbonne, J., Champeau, O. and Garrigues, P. (2005). Scale of classification based on biochemical markers in mussels: application to pollution monitoring in Mediterranean coasts and temporal trends. Biomarkers, **10**(1), 58-71.
- Nisbet, I.C.T. and Lagoy, P.K. (1992). Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAHs). Regulatory Toxicology and Pharmacology, **16**(3), 290-300.
- Orbea, A., Ortiz-Zarragoitia, M., Solé, M., Porte, C. and Cajaraville, M.P. (2002). Antioxidant enzymes and peroxisome proliferation in relation to contaminant body burdens of PAHs and PCBs in bivalve molluscs, crabs and fish from the Urdaibai and Plentzia estuaries (Bay of Biscay). Aquatic Toxicology, **58**(1), 75-98.
- Oros, D.R. and Ross, J.R.M. (2005). Polycyclic aromatic hydrocarbons in bivalves from the San Francisco Estuary: spatial distributions, temporal trends, and sources (1993-2001). Marine Environmental Research, **60**(4), 466-488.
- Petersen, H., Naf, C., and Broman, D. (1997). Impact of PAHs outlets from an oil refinery on the receiving water area sediment trap fluxes and multivariate statistical analysis. Marine Pollution Bulletin, **34**(2), 85-95.
- Piccolo, M. C., and Perillo, G. M. E. (1990). Physical characteristics of the Bahia Blanca estuary, Argentina. Estuarine, Coastal and Shelf Science, **31**(3), 303-317.
- Pierard, C., Budzinski, H., & Garrigues, P. (1996). Grain-size distribution of polychlorobiphenyls in coastal sediments. Environmental science & technology, **30**(9), 2776-2783.
- Popovich, C., Spetter, C., Marcovecchio, J.E. and Freije, R.H., (2008). Dissolved nutrient availability during winter diatom bloom in a turbid and shallow estuary (Bahía Blanca, Argentina). Journal of Coastal Research **24**, 95-102.
- Prieto, A., Zuloaga, O., Usobiaga, A., Bartolomé L., Fernández, L.A, et al. (2008). Levels and spatial distribution of inorganic and organic contaminants in sediments along the Bilbao estuary. Marine Pollution Bulletin, **56**(12), 2082-2105.
- Pruell, R.J. and Quinn, J.G. (1985). Geochemistry of organic contaminants in Narragansett Bay sediment. Estuarine, Coastal and Shelf Science **21**, 295-312.
- Rank, J. (2009). Intersex in *Littorina littorea* and DNA damage in *Mytilus edulis* as indicators of harbour pollution. Ecotoxicology and Environmental Safety **72**(4), 1271-1277.
- Rogge, W.F., Hildemann, L.M., Mazurek, M.A., Cass, G.R. and Simoneit, B.R.T. (1993). Sources of fine organic aerosol. 2. Non-catalyst and catalyst-equipped automobiles and heavy-duty diesel trucks. Environmental Science and Tech-

- nology, **27(4)**, 636-651.
- Saucedo, P.C. and Southgate, P.C. (Eds) (2008). Reproduction, Development and Growth. (In Saucedo, P.C. and Southgate, P.C. (Eds), *The pearl Oyster* (pp. 131-186) Elsevier).
- Simpson, C. D., Mosi, A. A., Cullen, W. R., and Reimer, K. J. (1996). Composition and Distribution of polycyclic aromatic hydrocarbon contamination in surficial marine sediments from Kitimat Harbor, Canada. *The Science of the Total Environment*, **181(3)**, 265-278.
- Soclo, H. H., Garrigues, P. H., and 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.
- Soriano, J.A., Viñas, L., Franco, M.A., González, J.J., Ortiz, L., Bayona, J.M. and 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. *Science of the Total Environment*, **370(1)**, 80-90.
- Strickland, J.D. and Parsons, T.R. (1968). Determination of Particulate Organic Carbon. (In Strickland, J.D. and Parsons, T.R. (Eds.) *A Practical Handbook of Seawater Analysis*. (pp.207-211). Ottawa: Fisheries Research Board of Canada).
- Tabachnick, B.G. and Fidell, L.S. (2000). *Using Multivariate Statistics*. (Boston: Pearson Education).
- Tam, N. F. Y., Ke, L., Wang, X. H., and Wong, Y. S. (2001). Contamination of polycyclic aromatic hydrocarbons in surface sediments of mangrove swamps. *Environmental Pollution*, **114(2)**, 255-263.
- Tolosa, I., Mesa-Albernas, M., Alonso-Hernandez, C., (2009). Inputs and sources of hydrocarbons in sediments from Cienfuegos bay, Cuba. *Marine Pollution Bulletin*, **58(11)**, 1624-1634.
- Treguer, P. and Le Corre, P. (1975a). Analyse des sels nutritifs sur autoanalizar II. (In Manuel D'Analyse des sels nutritifs dans L'Eau de Mer. (pp. 11-22). France: Univ Bretagne Occidentale).
- Treguer, P. and Le Corre, P. (1975b). Analyse des sels nutritifs sur autoanalizar II. Methods Richard et Kletsh (modifiée). (In: Manuel D'Analyse des sels nutritifs dans L'Eau de Mer. (pp. 50-61). France: Univ Bretagne Occidentale)
- Technicon Autoanalyzer II® (1973). *Industrial Methods* N° 186-72 W/B.
- UNESCO (1982). The determination of petroleum hydrocarbons in sediments. In: *Manuals and guides, intergovernmental oceanographic commission*. Paris, France 11, pp. 1-35.
- USEPA (United States Environmental Protection Agency) (2000). *Guidance for Assessing Chemical Contaminant, Data for use in Fish Advisories, vol. 1: Fish Sampling and Analysis*, third ed. Office of Water, Washington, DC. EPA 823-R-95-007.
- Utvik, T.I.R., Johnsen, S. (1999). Bioavailability of polycyclic aromatic hydrocarbons in the North Sea. *Environmental Science and Technology*, **33(12)**, 1963-1969.
- Valavanidis, A., Vlachogianni, Th., Triantafyllaki, S., Dassenakis, M., Androutsos, F. and Scoullou, M. (2008). Polycyclic aromatic hydrocarbons in surface seawater and in indigenous mussels (*Mytilus galloprovincialis*) from coastal areas of the Saronikos Gulf (Greece). *Estuarine, Coastal and Shelf Science*, **79(4)**, 733-739.
- Vidal-Liñán, L., Bellas, J., Campillo, J.A and Beiras R. (2010). Integrated use of antioxidant enzymes in mussels, *Mytilus galloprovincialis*, for monitoring pollution in highly productive coastal areas of Galicia (NW Spain). *Chemosphere*, **78(3)**, 265-272.
- Wang, Z., Fingas, M., Shu, Y. Y., Sigouin, L., Landriault, M., Lambert, P., et al. (1999). Quantitative characterization of PAHs in burn residue and soot samples and differentiation of pyrogenic PAHs from petrogenic PAHs-the 1994 mobile burn study. *Environmental Science and Technology*, **33(18)**, 3100-3109.
- Wang, Z., Li, K., Lambert, P., Yang, C. (2007). Identification, characterization and quantitation of pyrogenic polycyclic aromatic hydrocarbons and other organic compounds in tire wre products. *Journal of Chromatography A*, **1139(1)**, 14-26.
- Wang, D., Tian, F., Yang, M., Liu, C. and Li, Y. (2009). Application of positive matrix factorization to identify potential sources of PAHs in soil of Dalian, China. *Environmental Pollution*, **157(5)**, 1559-1564.
- Wetzel D.L and Van Vleet E.S. (2004). Accumulation and distribution of petroleum hydrocarbons found in mussels (*Mytilus galloprovincialis*) in the canals of Venice, Italy. *Marine Pollution Bulletin*, **48(9)**, 927-936.
- WHO (1996). *Drinking Water Guideline, Vol. 2: Health criteria and other supporting information*. 2nd edition. (Genova: World Health Organization).
- Ye, B., Zhang, Z. and Mao, T. (2006). Pollution sources identification of polycyclic aromatic hydrocarbons of soils in Tianjin area, China. *Chemosphere*, **64(4)**, 525-534.
- Yoshimine, R. V, Carreira, R. S., Scofield, A.L. and Wagener, A. L. R. (2012). Regional assessment of PAHs contamination in SE Brazil using brown mussels (*Perna perna* Linnaeus 1758). *Marine Pollution Bulletin*, **64(11)**, 2581-2587.
- Yuam, D. X., Yang, D. N., Terry, L., Wade, I. and Qian, Y. R. (2001). Status of persistent organic pollutants in the sediment from several estuaries in China. *Environmental Pollution*, **114(1)**, 101-111.
- Yunker, M. B., Macdonald, R. W., Goyettec, D., Paton, D. W., Fowler, B. R., Sullivan, D., et al. (1999). Natural and anthropogenic inputs of hydrocarbons to the Strait of Georgia. *The Science of the Total Environment*, **225(3)**, 181-209.
- Yunker, M. B., Macdonald, R. W., Vingarzan, R., Mitchell, R. H., Goyette, D., and 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.
- Zakaria, M.P., Takada, H., Tsutsumi, S., Ohno, K., Yamada, J. and Kouno, E. (2002). Distribution of polycyclic aromatic hydrocarbons (PAHs) in rivers and estuaries in Malaysia: a widespread input of petrogenic PAHs. *Environmental Science and Technology*, **36(9)**, 1907-1918.