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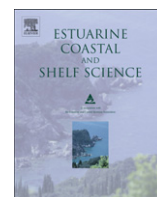
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# Polycyclic aromatic hydrocarbons in water, mussels (*Brachidontes* sp., *Tagelus* sp.) and fish (*Odontesthes* sp.) from Bahía Blanca Estuary, Argentina

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## ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs), one of the major groups of anthropogenic environmental pollutants, were firstly identified and measured in coastal waters, native mussels and fish of an industrialized South American estuary.

The aim of this study was to measure the PAHs concentrations and evaluate distribution and sources in surface seawater, native mussels (*Brachidontes* sp. and *Tagelus* sp.) and fish (*Odontesthes* sp.) from various coastal sites of the Bahía Blanca Estuary, in order to put the estuary in a global context and assess its ecotoxicological risk. For this, 40 samples of surface water were collected between August 2004 and February 2006 and analyzed by GC/MS for 17 PAHs, including the 16 EPA priority PAHs. In addition, main physicochemical parameters, nutrients and pigments were simultaneously obtained as key parameters to evaluate possible relationships. Finally, several native mussel communities and fish specimens were also analyzed for PAHs contents. The area of study is located at the Argentinean coast and presents an intensive human and industrial activity at the north shoreline.

Total PAHs ( $\Sigma 17$  PAHs) in whole surface waters ranged from undetected to more than 4  $\mu\text{g/l}$ . Seasonal PAHs levels relationship with master parameters suggested a relationship between the phytoplankton microbiological cycling and the dissolved/suspended PAHs occurrence in water. In addition, Cluster Analysis was performed to identify the homogeneous groups of individual PAHs in water and Principal Component Analysis to extract underlying common factors. The result of PCA was similar to that of the cluster analysis; both could differentiate two subsets of pyrogenic sources and a petrogenic origin.

PAHs levels in indigenous mussels (*Brachidontes* sp. and *Tagelus* sp.) ranged from 348 to 1597 ng/g (dry weight). Fish specimens (*Odontesthes* sp.) showed a mean concentration of 1095 ng/g d.w. in whole tissues. Mussel source ratios and PCA results were in good agreement with the dominant sources assessed for water samples and further enabled the linking of PAHs origins according to the sample location.

The Bahía Blanca Estuary wide mean of the PAHs burden in mussels showed that the average PAH contamination level was within the “moderate” category. Levels appeared as ecotoxicologically risk safe for the inner stations; however, they were exceeding various safety criterions at the outer. Results indicate the need of a systematic monitoring program for the area of study, in order to provide accurately assessment and management of risks for the regional population.

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## 1. Introduction

The interest in hydrophobic organic contaminants has increased rapidly during the last decades; in particular, on polycyclic aromatic hydrocarbons (PAHs), a unique class of persistent organic

pollutants (POPs) contaminating the aquatic environment. PAHs are ubiquitous pollutants which have been studied as regards to their origin, distribution and destination in the environment (Lipiatou and Salot, 1991; Kucklick et al., 1997; Yunker et al., 1999, 2002a,b; Magi et al., 2002; Ponce-Vélez et al., 2006; Arias et al., in press) and currently counted among the major groups of environmental contaminants.

For several reasons, they are of particular interest for geochemists and environmental toxicologists: first, PAHs are not

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released to the environment from a single source of origin, as are mainly industrial, petro or agricultural chemicals; in fact, they are formed in at least three ways: pirogenically, petrogenically and diagenetically. Second, when released, they are not in a relative pure chemical form like most other anthropogenic pollutants; for example, pyrogenic PAHs are frequently associated with soot carbon formed during the combustion process, thus affecting their persistence and bioavailability. Third, thousands of possible PAHs structures exist in the environment as compared to the relative limited number of other POPs. Finally, unlike other harmful organic chemicals that have been banned or regulated in discharges, PAHs continue to be released into the environment because of their widespread formation during the burning of fossil fuels and escape during petroleum recovery, transport and use. The ongoing utilization of fossil fuels on a global basis guarantees the continued release of PAHs into the environment (Burgess et al., 2003); in fact, anthropogenic activities are generally recognised to be the most important source of PAHs release into the environment (Culotta et al., 2006).

At coastal areas, anthropogenic PAHs are introduced 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). Concentrations of PAHs in seawater and sediments are of toxicological importance to both benthic and pelagic marine organisms.

Concentrations of contaminants in water are needed to obtain a better view of this part of the marine environment inhabited by plants and animals. In particular, the impacts of municipal effluents represent a major environmental issue of interest in Argentina and around the world (Chambers et al., 1997; Hinch et al., 2002). New combined municipal sewage treatment plants for road runoff and sewage are being constructed at present in the inner zone of the estuary, and no baseline data of occurrence and transport behaviour of PAHs in water are available. Therefore it is important to investigate the sources of PAHs, their relationship with the particulate phase and their seasonal behaviour in relation to several key parameters such as particle loads, nutrients and microbiological activity.

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 accumulate in biota that has high rates of uptake or is unable to efficiently metabolize the parent compounds (e.g. some invertebrates and mussels). In particular, mussels and other bivalves have been extensively used as sentinel organisms in international programs for monitoring contaminants (e.g., International Mussel Watch, Farrington and Tripp, 1995; European BIOMAR, Narbonne et al., 2005); for example, *Mytilus* sp. was the focus of many studies in the Mediterranean Sea (Storelli et al., 2001; Wetzel and Van Vleet, 2004) and *Brachidontes* sp. has also been used as monitor in Eastern Mediterranean and American surveys (Farrington and Tripp, 1995; El Nemr et al., 2004; Khaled et al., 2004; Nakhleć et al., 2006).

The process of industrialization and urbanization is stepping rapidly in South America, and in particular in Argentina – a developing country – and the potential increase in PAHs loads is a concern. Although there have been a few number of PAHs surveys at Argentinean coastal areas (Colombo et al., 1989, 2005; Cataldo et al., 2001; Arias et al., in press); there is no available information at present about PAHs in water and organisms for this strategic coastal area. Consequently, the aim of this study was to measure PAHs concentrations, evaluate distribution and seasonality and assess potential sources in surface seawater, native mussels (*Brachidontes* sp. and *Tagelus* sp.) and fish (*Odontesthes* sp.) from various coastal sites of the Bahía Blanca Estuary, in order to fulfill the

scarcity of hydrocarbons surveys at South American coasts and place them in the international context of POPs. Additionally, the generation of background information of PAHs in organisms will lead to investigate the potential suitability of indigenous mussel samples to serve as an alternative to seawater or sediments measurements used for monitoring PAHs pollution.

### 1.1. Study area

The Bahía Blanca Estuary (38° 40' S and 62° 09' W) has a total surface of 2300 km<sup>2</sup>, of which about 410 km<sup>2</sup> of islands and 1150 km<sup>2</sup> 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 harbors, a 12-m over-all 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 part of the estuary presents low urbanized/rural lands, a tourist area and an artisanal fishing/recreational port (Fig. 1).

## 2. Materials and methods

### 2.1. Water sampling and preparation for chemical analysis

Between August 2004 and February 2006, 40 seawater samples were collected in the subsurface water of the Bahía Blanca Estuary. Seventeen individual parent polycyclic aromatic hydrocarbons, including the 16 US-EPA PAHs, were analyzed at 5 different offshore locations; namely, Ing White Harbor (S1), Galván Harbor (S2), Maldonado Stream Mouth (S3), Cuatros Harbors (S4), and Villarino Viejo (S5) (Fig. 1). This group of stations is located over the Main Navigation Channel of the estuary (5–14 m depth). S1 and S2 sampling sites are both located at Ingeniero White National Harbor and Galvan National Harbor entrance respectively, two commercial harbors with high shipping activity. Industrial complexes are also settled within this area: oil, plastic polymers, by-products derivatives refineries and a small commercial fishing fleet. Next to them, S3 station receives water inputs from Maldonado Stream, running through agriculture lands and across the entire city, while S4 is located at offshore waters of a recreational/fishing port and S5 station is in the vicinity of low urbanized/rural lands. Throughout the survey a global positioning system was used to locate the sampling positions, and various sensors including salinity, temperature and depth were deployed for obtaining these master variables. Salinity was measured using the Practical Salinity Scale. A total of 1500 ml of surface water was collected from each site using pre-cleaned and oven-baked glass bottles. Samples were immediately refrigerated on board, stored on avoiding exposure to light, and then rapidly transported to the laboratory to be kept refrigerate until the extraction procedure begins. Prior to extraction, a set of perdeuterated PAHs subrogate standard was added for quantification purposes. Extraction was performed in whole water, using three consecutive hexane liquid–liquid extractions in a glass separation funnel. As stated by Witt (1995, 2002) PAHs associated with the particulate matter were also extracted, with high efficiency, comparable to the sum of separate determinations of PAHs in the water and particle phase. The extracts were combined, concentrated, dried over anhydrous sodium sulphate

(precombusted for 12 h at 450 °C), pre-concentrated by a rotary vacuum evaporator to 5.0 ml and then to 1.0 ml dryness by gently passing dry nitrogen. Just before the GC/MS injection, all the samples were spiked with the internal standard in order to evaluate the percent of recovery.

## 2.2. Physicochemical parameters, nutrients and pigments determination

Water subsamples for determination of nitrite ( $\text{NO}_2^-$ ), nitrate ( $\text{NO}_3^-$ ), ammonium ( $\text{NH}_4^+$ ), dissolved inorganic phosphorous (DIP) and dissolved silicates (DSi) concentrations were treated as stated by 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,b), 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 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).

## 2.3. Mussels sampling and preparation for chemical analysis

Indigenous mussels (*Brachidontes* sp. and *Tagelus* sp.) were collected from dock columns and rocks in surface water (0–1 m) in Dec 2005. A total of two collections of mussels (*Brachidontes* sp.) were performed in the same time and in the same depth as for seawater at sites S2 and S4. At S1 station, whereas no mussel communities were found, several fish specimens (*Odontesthes* sp.) were collected by artisan fishing arts at the same time as water column samples. During February 2006, additional mussel collections were performed in two outer stations, S6 and S7, namely Tres Brazas Channel (*Tagelus* sp.) and Cabeza de Buey Channel (*Brachidontes* sp.) respectively (Fig. 1). These deep water channels are commonly commercial fishing sites. An average of 50 mussels from each site was used for measurements. Mussels were selected and characterized by similar maximum length and size: 40–10 mm shell length, >8 g soft wet tissue (5–8 months old). Immediately after collection, mussels were transferred inside an ice cool box to the laboratory. ~50 individuals from each sampling were cleaned. Gills and mantle were dissected, wrapped in aluminum foil and frozen at –20 °C. Samples were lyophilized during 24–48 h and afterwards smashed in a mortar.

After that the extraction procedure followed. Briefly, the wet matrix (organism tissue) was digested under reflux with methanol and potassium hydroxide. Non-saponifiable fraction was extracted with n-hexane; the organic phase was dried with anhydrous sodium sulphate, and evaporated down to 0.2 ml 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 50 ml n-hexane-dichloromethane (9:1), and then the eluate was evaporated down to 1 ml ( $\text{N}_2$ ). Finally, just before the GC/MS injection, all the samples were spiked with the internal standard.

## 2.4. Instrumental analysis and QA/QC

PAHs were quantified using a gas chromatograph (Hewlett–Packard HP68906C) coupled with a quadrupole mass spectrometer

(Hewlett–Packard HP5972). The GC was equipped with an HP-5MS fused silica column (30 m; 0.25 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 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  $\text{min}^{-1}$  then to 310 °C at 4 °C  $\text{min}^{-1}$ ; and held for 10 min. PAHs were monitored in selected ion monitoring mode (SIM).

The studied PAHs ranged from the di-aromatics to the hexa-aromatics: naphthalene (NAP), 2-methyl-naphthalene (2-M-NAP), acenaphthylene (ACY), acenaphthene (ACE), fluorene (FLU), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLA), pyrene (PYR), benzo[a]anthracene (BaAN), chrysene (CHR), benzo[b]-fluoranthene (BbFL), benzo[k]fluoranthene (BkFL), benzo[a]pyrene (BaP), indeno[1,2,3-cd]Pyrene (InPY), dibenzo[a,h]anthracene (DiB-ah-ANT), and benzo[ghi]perylene (B-ghi-PE). Each individual PAH compound was confirmed by the retention time and the abundance of quantification ions/confirmation ions with respect to authentic PAHs standards. Quantification of individual compounds was based on the ratios analyte peak areas/surrogate standards areas (naphthalene- $\text{d}_{12}$ , acenaphthene- $\text{d}_{10}$ , phenanthrene- $\text{d}_{10}$ , chrysene- $\text{d}_{12}$ , internal standard method), using the corresponding calibration curves. Quality control for the PAHs analyses was carried out by monitoring the recovery of the internal standard (Benzo-[a]-pyrene- $\text{d}_{12}$ ) spiked just before GC injection; recoveries ranged from 75% to 105%. Method blanks for organisms were performed extracting an amount of sodium sulphate equivalent to that used with the samples were run with every batch of eight samples while for water samples, tridistilled and solvent extracted water was used as blank. Hydrocarbons were not detected in the blanks. Laboratory protocols were validated by an international intercalibration exercise (Project FMAM/BIRF 28385-AR, Global Environmental Facilities, 2005–2006) and workshops and the use of reference material for mussel samples (SRM-NIST 2977), which was extracted and analyzed in a similar fashion to the samples with good agreement with certified values (Table 1). The limit of detection (LOD) for PAHs in average was set around 1 ng/ml extract. The instrumental quantification limit for individual PAHs species was set in average at 10 ng/ml extract. The detection limits for individual PAHs in the organisms ranged from 0.10 to 0.20 ng/g tissue dry weights (d.w.). All values reported for mussels are on a dry weight basis. The chemicals used were of analytical and chromatographic grade with high purity. PAHs Standard mixtures and perdeuterated internal standards were from Ultra-Scientific Analytical Standards and Absolute Standards Inc.

**Table 1**

Concentrations ( $\text{ng g}^{-1}$ ) and confidence intervals obtained for PAHs after extraction and clean-up of the certified material SRM 2977 in comparison with the certified values.

Analyte	Certified value	Obtained value
NAP	19.0 $\pm$ 5.0	17.3 $\pm$ 5.3
2-M-NAP	18.0 $\pm$ 5.0	15.4 $\pm$ 5.3
ACE	4.2 $\pm$ 0.4	3.8 $\pm$ 0.8
FLU	10.2 $\pm$ 0.4	8.9 $\pm$ 1
PHE	35.1 $\pm$ 3.8	39.5 $\pm$ 4
ANT	8.0 $\pm$ 4.0	11.1 $\pm$ 4.1
FLA	38.7 $\pm$ 1.0	40.1 $\pm$ 2
PYR	78.9 $\pm$ 3.5	82.3 $\pm$ 4.5
BaAN	20.3 $\pm$ 0.8	20.2 $\pm$ 1.5
CHR	49.0 $\pm$ 2.0	52.3 $\pm$ 1.8
BkFL	4.0 $\pm$ 1.0	13 $\pm$ 1.1
BaP	8.4 $\pm$ 0.7	9.8 $\pm$ 1.1
InPY	4.8 $\pm$ 0.8	5.22 $\pm$ 0.9
DiB-ah-ANT	1.4 $\pm$ 0.2	2.3 $\pm$ 0.4
B-ghi-PE	9.5 $\pm$ 0.4	10.6 $\pm$ 0.9



## 2.5. Statistical analysis

Statistical significance of compared results was determined by the one-way analysis of variance (one-way ANOVA). The normality (Kolmogorov–Smirnov normality test) and homogeneity of variance (Levene's test) of data were verified and data were applied as required to fulfill ANOVA assumptions. Used correlations coefficients were calculated as Pearson *r* definition.

Multivariate analytical tools were used to reduce the number of variables. Hierarchical Cluster Analysis (HCA) was performed to identify homogeneous groups of individual variables and Principal Component Analysis (PCA) to extract underlying common factors for analysing relationships among the observed variables. Usually, as a result of an effective extraction process, PC1 accounts for the major proportion of the total data variance while the second and following PCs progressively explain smaller amounts of data variation. This tool has been applied to analyse PAHs levels variance in some study (Freeman and Catteli, 1990; Doong and Lin, 2004; Golobocanin et al., 2004; Zhang et al., 2004; Arias et al., in press; Liu et al., 2009). Prior to analysis, values under the Limit of Detection (LOD) in the data set were replaced with random values under the LOD value. First, concentrations of 17 PAHs as active variables and samples as cases were used. Before statistical analysis of data, we replaced undetectable values of the water PAHs' concentrations data set by a random number between zero and the limit of detection. Then, the data set was normalized by maximum values (McCune et al., 2002). We preferred such a way of the factors rotation to get as many positive loadings as possible in order to achieve a more meaningful and interpretable solution. All the analyses were performed using the STATISTICA statistical package, version 7.0.

## 3. Results and discussion

### 3.1. Surface water PAH distribution

Total PAHs levels (summation of 17 individual PAHs concentrations) in whole surface waters ranged from undetected to more than 4 µg/l. As whole water was processed in each sample, a wide spectrum of molecular weights was registered, including low molecular weight PAHs such as naphthalene, usually associated to the dissolved fraction, and heavy PAHs such as dibenzo[a,h]anthracene and benzo[ghi]perylene, whose occurrence in water is primarily adsorbed into the particulate fraction (Bouloubassi and Salot, 1993). The maximum concentrations were achieved at harbors entrances, namely, S1, S2 and S4. In contrast, mean levels lower than 0.7 µg/l were recorded for the remaining sampling stations (Fig. 1). This preliminary evidence associated the local harbors' anthropogenic activities with the maximum concentration of PAHs in water. For example, mean Total PAHs at S1 and S4 statistically differentiated from at least one of the rest ( $p < 0.01$  and  $p < 0.10$ , *t*-test means difference tests) and showed the highest values along the sampling period.

Some master parameters, namely Turbidity, Particulate Organic Matter (POM) as well as Suspended Particulate Matter (SPM) were obtained to complement the PAH data. These parameters were measured during the same cruises at the same stations. Added to this, a number of primary production indicators (nutrients and pigments) were also measured. The recorded data are summarized in Table 2.

### 3.2. Water particulate phase

The mean concentration of SPM in the surface water ranged between 54.55 and 83.90 mg/l. SPM loads showed a spatial

variability in which the highest values were achieved at the outer zone of the estuary, defining a concentration gradient in the head to mouth direction. The highest concentrations (up to 214 mg/l) were measured at Ing. White Harbor (S1), mainly attributable to the tidal currents associated transport of sediments (Cuadrado et al., 2004) and the proximity of the local sewage network, which is continuously discharging pre-filtered solids (0.75 mm screen width, 23% solid retaining efficiency), with an estimated flow of 0.59 m<sup>3</sup>/seg and a nutrient discharge of 3976 kg N and 268 kg P per day (Tombesi et al., 2000). Others SPM contributors in the area are several domestic and industrial wastewater punctual and diffuse discharge zones at the north shoreline, including various POPs sources, like PAHs (Arias et al., in press), TBTs (Delucchi et al., 2007) and heavy metals (Marcovecchio and Ferrer, 2005).

#### 3.2.1. Particulate matter and total PAHs

The partition of PAHs between the particulate and dissolved phase should be in favor of the particulate phase, according to the octanol–water partition coefficients ( $K_{ow}$ ). For example, Readman et al. (1984) found that PAHs with mass weight (m.w.) > 200 mostly occur in the particulate phase. This mass threshold was empirically augmented by Broman et al. (1990) when studying the distribution of PAHs in the Baltic Sea. However, in this study, only anthracene (178 m.w.), pyrene (202 m.w.), chrysene (228 m.w.) and dibenzo[ah]anthracene (276 m.w.) showed correlations greater than 0.20 with the particulate phase. In the opposite, the highest correlations were achieved with the organic particulate phase (POM; chrysene,  $r = 0.94$  and dibenzo[ah]anthracene,  $r = 0.99$ ). Apart from sites S1 and S3 (S1:  $r = 0.47$ ; S3:  $r = 0.69$ ), POM showed poor linear correlations with SPM. As those sites are strongly influenced by the local sewage network discharge and the Maldonado stream's output, a possible explanation is that these discharges are functioning as PAHs sources, transporting PAHs associated with the POM.

#### 3.3. Distributions, seasonality and origin of PAHs in surface water

In order to characterize the water PAHs distribution along the coastal waters, we assessed the occurrence of mass weight groups. Usually, the scientific literature associates high molecular weight (HMW) PAHs with pyrolytic sources (Simpson et al., 1996; Dahle et al., 2003; Culotta et al., 2006). HMW includes the 226–302 molecular weight compounds group, for which the highest mutagenic activities have been described (Larsen and Larsen, 1998). These have been widely found in areas receiving direct anthropogenic PAHs inputs, and for instance, at those areas the dominance of 202 and 252 molecular weight groups proved to be characteristic (Hites et al., 1980; Bates et al., 1987; Lipiatou and Salot, 1991).

The composition pattern of PAHs in water by percent mass weight group is shown in Fig. 2. It showed a clear dominance of mass groups 202 and 252 at site S3 (46% of the Total PAHs). As seen in Section 3.1, this area receives direct inputs of the Maldonado stream outflow after crossing the urban zone. It is possible that PAHs would be entering to this area adsorbed to the organic particulate phase load, which was in good correlation with the SPM and Turbidity measures (Sections 3.2 and 3.2.1). The rest of the stations showed decreasing proportions of these anthropogenic PAHs in correlation to the decreasing levels of anthropogenic activity in the vicinity (S1: 38%, S2: 20%, S4: 26%, and S5: 14%). The wide range of compounds found at different concentrations firstly indicated that there were potentially many different sources of PAHs in the area of study, possibly including combustion followed by atmospheric fallout, oil residues, sewage outfalls and industrial wastewater. PAH sources and origins are further discussed in Sections 3.3.1 and 3.3.2.

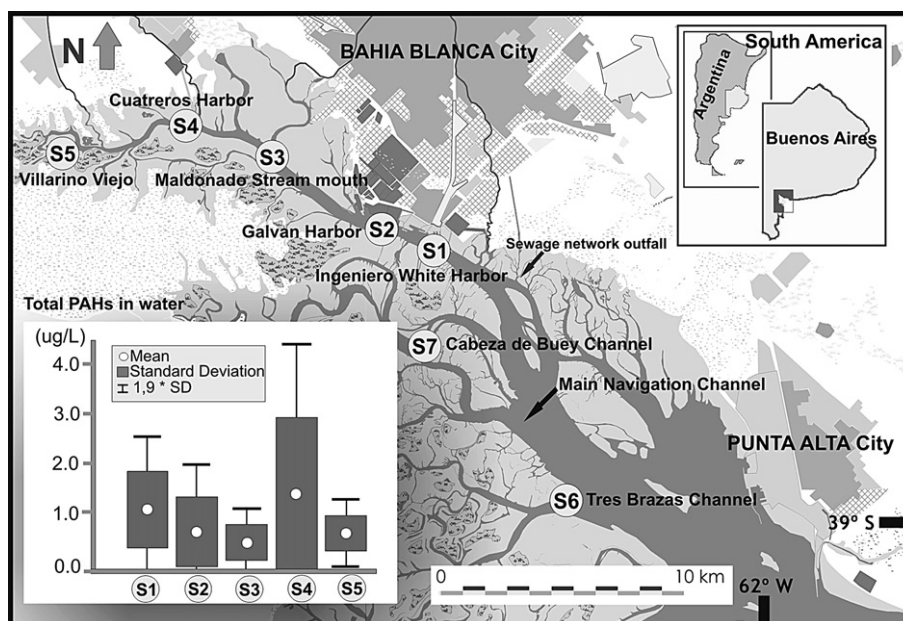


Fig. 1. Location of sampling stations 1–7 in the Bahía Blanca Estuary, Argentina, and corresponding subsurface water mean Total PAHs concentrations.

The results from different seasons have also been compared. The highest concentrations of PAHs were measured at winter ending and at the beginning of the spring season ( $4.84 \mu\text{g/L}$ , S4, September 2005). On the other hand, lower PAHs concentrations were recorded in the summer and fall, and the lowest were found in winter ( $\sim\text{LOD}$ ), just at the moment of the maximum level of algal bloom activity in the water column (Fig. 3).

The occurrence of PAHs in summer was low, a possible explanation could be found in an accelerated degradation due to the higher water temperature that affects the rate at which PAHs are degraded by microorganisms or due to a higher photooxidation process, which is also enhanced (Lee and Ryan, 1983). These processes particularly involve the lower molecular compounds with higher biodegradability ( $\text{m.w.} < 228$ ), and complain a globally observed trend (Witt 1995, 2002). From May to July, one important reason for the decreasing PAHs concentrations found could be that water presented increasing nutrients concentrations (Table 1). Annually, these nutrients lead to a rare and unique plentiful phytoplankton bloom during wintertime (Freije and Gayoso, 1988; Gayoso, 1998, 1999; Popovich and Marcovecchio, 2008), denoted by maximum concentrations of chlorophyll *a* (Table 1 and Fig. 3). Several authors have already addressed how the occurrence of high primary production leads to large vertical fluxes of particles, and consequently, to an enhanced vertical transport of particle-associated pollutants (Millard et al., 1993; Buesseler et al., 1998). Then, we suggest that during this period, the large quantity of settling particulate matter may be taking the sparingly soluble PAHs with it to the seafloor and the water itself would be cleaned relatively quickly of its pollutant contents. Furthermore, it has been well established that the growing biomass results not only in the adsorption of PAHs on freshly produced particles, but also in higher biodegradation rates (Witt 2002). Probably, all these factors were controlling the water column concentrations over this period. Although this phenomenon has been already described in several coastal zones around the world such as the Baltic Sea waters (Witt, 1995, 2002; Thorsson et al., 2008), the Swedish coasts (Gunnarsson et al., 1995) and the U.S.A. Great Lakes (Taylor et al., 1991), it is firstly observed at Argentinean coasts. Finally, at the winter end, during the period of primary production decline and algal bloom

senescence, the water PAHs concentrations achieved the maximum levels (Fig. 3). A possible explanation could be explored in the interaction between the biological cycling of phytoplankton and benthic organisms or in human seasonal anthropogenic releases. On the one hand, the settling of planktonic diatoms offered plenty of food to the benthic organisms, whose feeding activities produce an enhanced bioturbation of sediments. It has already been shown that organism's activities in sediments increase PAHs concentrations in the overlying waters (McElroy and Sisson, 1989; Ciarelli et al., 1999). In this way, we postulate that bioturbation could be responsible of the water PAHs concentrations rise at this stage. On the other hand, it is noteworthy to discuss that the water PAHs concentrations' increase correlated with the end of the winter heating period, which is globally characterized by increased use of fossil fuels for heating and subsequent higher releases of PAHs to the environment. For example, Witt (2002) observed an increment of PAHs water loads to the Baltic Sea during winter, and also Bouloubassi and Saliot (1993) observed a higher PAH flux in the dissolved and particulate phase in the Rhone River during winter because of the more intensive industrial and/or human activities.

### 3.3.1. Source estimates from cluster analysis

Cluster analysis was performed to identify the homogeneous groups of individual PAHs occurring in the water column of the estuary. Results are shown in the hierarchical dendrogram at Fig. 4A, which distinguished the 17 individual PAHs into two major groups. The first major group included ANT, BbFL, BkFL, BaP, InPY, DiB-ah-ANT and B-ghi-PE, which are mainly high molecular weight PAHs with 5–6 rings. The second major group included two subgroups. The first subgroup contained NAP, FLU, PYR, B-ghi-PE, BaAN, and CHR, all of them including 4 rings, with the exception of NAP. 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), although we cannot explain why ANT was classified in this group. The second subgroup included the low molecular weight PAHs with 2–3 rings or alkyl-substituted PAHs, which are abundant in petrogenic sources: 2-M-NAP, ACE, PHE, ACY, and FLU.

**Table 2**  
Main physicochemical parameters, nutrients, pigments and Total PAHs concentrations at different locations and sampling dates.

Sampling locations				Main parameters					Nutrients					Pigments	PAHs
Date	Site	Latitude	Longitude	Temperature (°C)	Salinity	Turbidity (u.n.t.)	POM (mg C/m <sup>3</sup> )	SPM (mg/l)	NO <sub>2</sub> <sup>-</sup> (μmoles/l)	NO <sub>3</sub> <sup>-</sup> (μmoles/l)	NH <sub>4</sub> <sup>+</sup> (μmoles/l)	DIP (μmoles/l)	Dsi (μmoles/l)	Chl-a (mg/m <sup>3</sup> )	Total PAHs (μg/l)
8/19/2004	S1	38° 47' 54.1"	62° 15' 48.6"	8.7	29.9	31	636	49.1	0.86	9.12	15.16	2.33	150.2	5.29	1.906
3/30/2005	S1	38° 47' 54.1"	62° 15' 48.6"	18.0	35.0	28	356	37.9	5.22	11.28	22.50	3.64	84.6	5.70	0.820
5/30/2005	S1	38° 47' 54.1"	62° 15' 48.6"	9.5	34.2	34	305	39.2	1.15	13.46	28.91	2.92	76.9	5.42	0.646
7/27/2005	S1	38° 47' 54.1"	62° 15' 48.6"	8.2	32.5	15	958	28.4	0.25	5.55	17.14	0.69	58.0	23.18	0.668
9/21/2005	S1	38° 47' 54.1"	62° 15' 48.6"	13.2	32.5	16	297	24.8	0.57	3.55	20.87	1.95	70.7	19.03	2.212
11/8/2005	S1	38° 47' 54.1"	62° 15' 48.6"	16.9	35.3	32	741	94.7	1.59	7.28	24.77	2.06	60.4	1.91	1.576
12/15/2005	S1	38° 47' 54.1"	62° 15' 48.6"	19.6	34.0	100	401	214.92	18.55	63.05	15.34	1.89	74.1	4.01	0.581
2/16/2006	S1	38° 47' 54.1"	62° 15' 48.6"	23.9	36.6	150	1177	129.4	2.94	10.72	13.45	1.75	55.6	13.16	0.014
8/19/2004	S2	38° 47' 22.7"	62° 18' 00.2"	8.3	29.7	34	1190	52.9	0.86	9.19	16.84	2.14	163.1	7.11	0.809
3/30/2005	S2	38° 47' 22.7"	62° 18' 00.2"	18.0	34.9	36	917	92.6	5.96	12.36	21.92	3.61	87.1	3.10	<LOD
5/30/2005	S2	38° 47' 22.7"	62° 18' 00.2"	9.7	34.1	41	258	49.4	1.35	14.44	30.92	3.25	79.9	5.31	0.658
7/27/2005	S2	38° 47' 22.7"	62° 18' 00.2"	8.0	32.7	17	762	27.1	0.24	4.77	9.35	0.67	56.6	24.08	<LOD
9/21/2005	S2	38° 47' 22.7"	62° 18' 00.2"	13.4	32.4	27	1233	46.8	0.58	2.63	27.18	1.77	57.7	21.52	2.104
11/8/2005	S2	38° 47' 22.7"	62° 18' 00.2"	16.7	35.5	46	453	186.2	1.89	9.41	20.86	2.41	68.9	6.20	0.659
12/15/2005	S2	38° 47' 22.7"	62° 18' 00.2"	20.2	34.0	160	1823	82.0	22.90	46.70	20.45	2.54	80.2	3.57	0.571
2/16/2006	S2	38° 47' 22.7"	62° 18' 00.2"	24.1	36.7	160	1138	150.8	2.87	10.05	20.17	1.82	55.4	8.83	<LOD
8/19/2004	S3	38° 45' 54.0"	62° 20' 25.1"	8.1	28.6	25	702	41.1	0.97	10.67	23.58	2.21	175.7	11.34	0.890
3/30/2005	S3	38° 45' 54.0"	62° 20' 25.1"	17.6	35.2	59	728	90.9	5.22	11.92	13.27	3.21	92.5	4.59	<LOD
5/30/2005	S3	38° 45' 54.0"	62° 20' 25.1"	9.4	33.6	27	130	48.2	1.56	15.50	34.29	3.48	85.0	3.91	0.645
7/27/2005	S3	38° 45' 54.0"	62° 20' 25.1"	7.8	31.8	16	777	19.9	0.10	3.83	2.63	0.45	48.6	28.86	0.291
9/21/2005	S3	38° 45' 54.0"	62° 20' 25.1"	14.8	31.6	21	374	30.3	0.17	1.36	12.62	1.23	59.8	13.36	0.637
11/8/2005	S3	38° 45' 54.0"	62° 20' 25.1"	19.3	35.5	100	1489	110.3	1.41	10.24	32.59	2.26	178.4	0.29	<LOD
12/15/2005	S3	38° 45' 54.0"	62° 20' 25.1"	21.5	34.4	85	459	72.1	21.45	21.15	22.16	2.49	91.2	4.19	0.572
2/16/2006	S3	38° 45' 54.0"	62° 20' 25.1"	23.8	37.1	171	2311	151.8	2.08	8.32	21.01	1.62	57.3	15.71	<LOD
8/19/2004	S4	38° 45' 01.7"	62° 23' 02.9"	8.0	27.5	17	856	27.5	0.91	11.10	9.26	2.03	187.4	11.89	2.413
3/30/2005	S4	38° 45' 01.7"	62° 23' 02.9"	17.4	33.9	98	931	107.6	4.32	9.08	21.35	3.04	107.0	4.17	0.907
5/30/2005	S4	38° 45' 01.7"	62° 23' 02.9"	9.1	31.9	17	221	35.0	1.37	16.05	20.84	2.83	175.2	0.47	0.642
7/27/2005	S4	38° 45' 01.7"	62° 23' 02.9"	7.6	29.5	16	1325	41.8	0.03	0.47	5.71	0.33	81.2	18.91	0.325
9/21/2005	S4	38° 45' 01.7"	62° 23' 02.9"	15.0	29.3	33	639	34.3	0.13	0.79	21.36	1.41	86.6	3.40	4.847
11/8/2005	S4	38° 45' 01.7"	62° 23' 02.9"	17.8	35.5	83	1462	28.5	1.62	10.72	26.08	1.45	90.8	0.68	0.788
12/15/2005	S4	38° 45' 01.7"	62° 23' 02.9"	22.2	34.5	107	1297	152.9	16.52	12.28	14.20	2.22	82.5	3.31	0.585
2/16/2006	S4	38° 45' 01.7"	62° 23' 02.9"	23.7	37.1	120	545	132.9	2.07	7.83	11.76	1.69	57.7	10.19	0.359
8/19/2004	S5	38° 45' 41.2"	62° 25' 22.1"	8.1	27.0	14	723	18.5	0.83	13.49	11.79	2.13	258.0	18.86	0.785
3/30/2005	S5	38° 45' 41.2"	62° 25' 22.1"	17.2	32.1	140	1181	132.4	1.94	5.63	17.31	2.54	114.9	2.04	0.801
5/30/2005	S5	38° 45' 41.2"	62° 25' 22.1"	9.1	30.0	23	130	41.3	1.23	16.65	15.46	2.71	205.1	3.37	0.649
7/27/2005	S5	38° 45' 41.2"	62° 25' 22.1"	7.9	25.7	21	1619	32.9	0.08	1.28	2.60	0.21	180.0	23.74	0.287
9/21/2005	S5	38° 45' 41.2"	62° 25' 22.1"	16.0	26.0	43	469	39.0	0.30	2.45	15.53	1.83	102.1	2.25	1.065
11/8/2005	S5	38° 45' 41.2"	62° 25' 22.1"	17.3	34.2	77	3505	22.5	1.68	11.36	20.21	2.41	80.7	0.87	<LOD
12/15/2005	S5	38° 45' 41.2"	62° 25' 22.1"	22.8	34.3	247	1214	54.7	11.30	27.10	13.07	2.19	190.0	4.85	0.742
2/16/2006	S5	38° 45' 41.2"	62° 25' 22.1"	23.5	37.0	74	1152	74.5	1.69	7.84	18.49	1.48	61.6	6.48	0.350

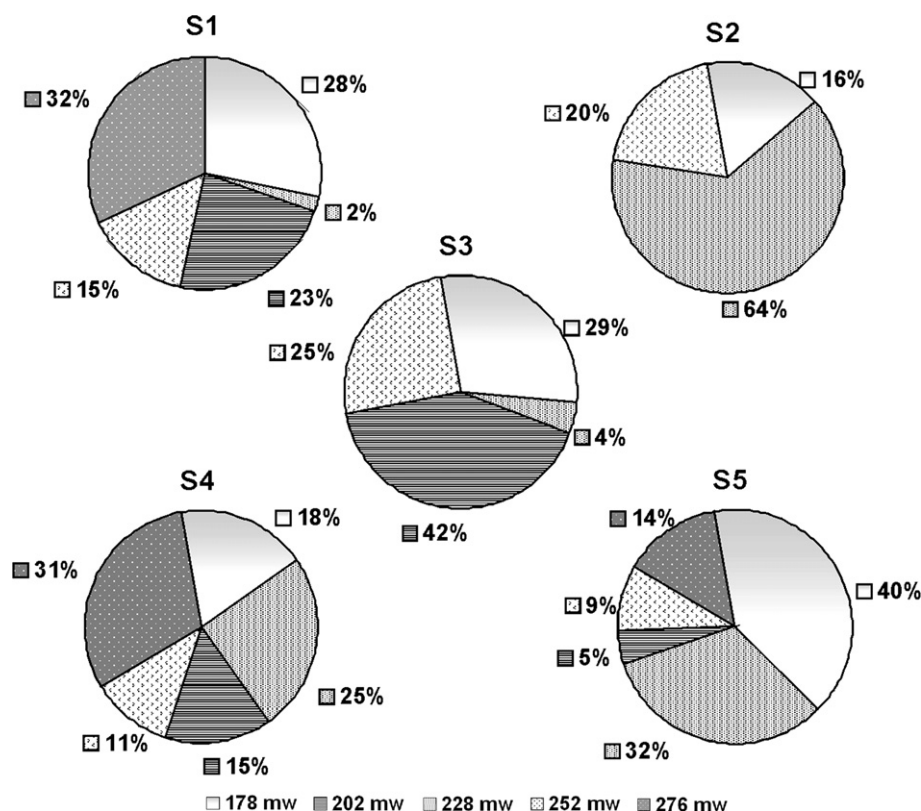


Fig. 2. Mean percent mass abundance (%) of different PAHs molecular groups in the subsurface seawater of the studied sites (S1–S5: sampling sites; m.w.: mass weight group).

### 3.3.2. Source estimates from principal component analysis

Principal Component Analysis (PCA) identified three principal components (PC1–3) accounting for 27.88%, 20.04%, and 12.02% of the total variance, respectively. PCA loading scores higher than 0.32 were considered meaningful (Tabachnick and Fidell, 2000).

The PCA results were similar to that of the cluster analysis in the previous section. PC1 was characterized by high loadings of PAHs with 5–6 rings, including benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene and dibenzo[ah]anthracene. Added to them, anthracene also scored a significant eigenvalue. In general the heavier PAHs are the result of combustion/pyrolytic processes and are absent in crude oil or refined

products (Wang et al., 1999). BbFL and BkFL are components of fossil fuels and a portion of them is associated with their combustion (Rogge et al., 1993; Kavouras et al., 2001). InPY, BaP and DiB-ah-ANT are associated with traffic emission (Fraser et al., 1997). Consequently PC1 was defined as a general traffic emission/fossil fuel combustion component. Secondly, PC2 was characterized by high negative loadings of PAHs including four rings, namely fluoranthene, pyrene, benzo[a]anthracene, chrysene and the heavier benzo[ghi]perylene. FLA and PYR are typical markers for pyrolysis or incomplete combustion. On the one hand, focusing at fuel combustion emissions, it is known that the diesel engine emission profile predominantly contains phenanthrene, fluoranthene and pyrene,

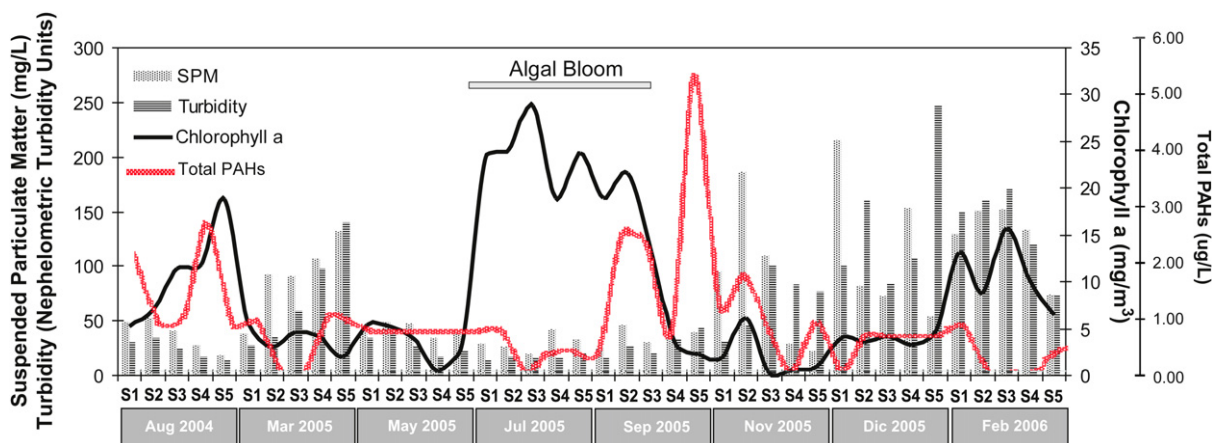


Fig. 3. Seasonal distribution of suspended particulate matter, turbidity measures, chlorophyll a and Total PAHs in the water of the Bahía Blanca Estuary, from Aug 2004 to Feb 2006.



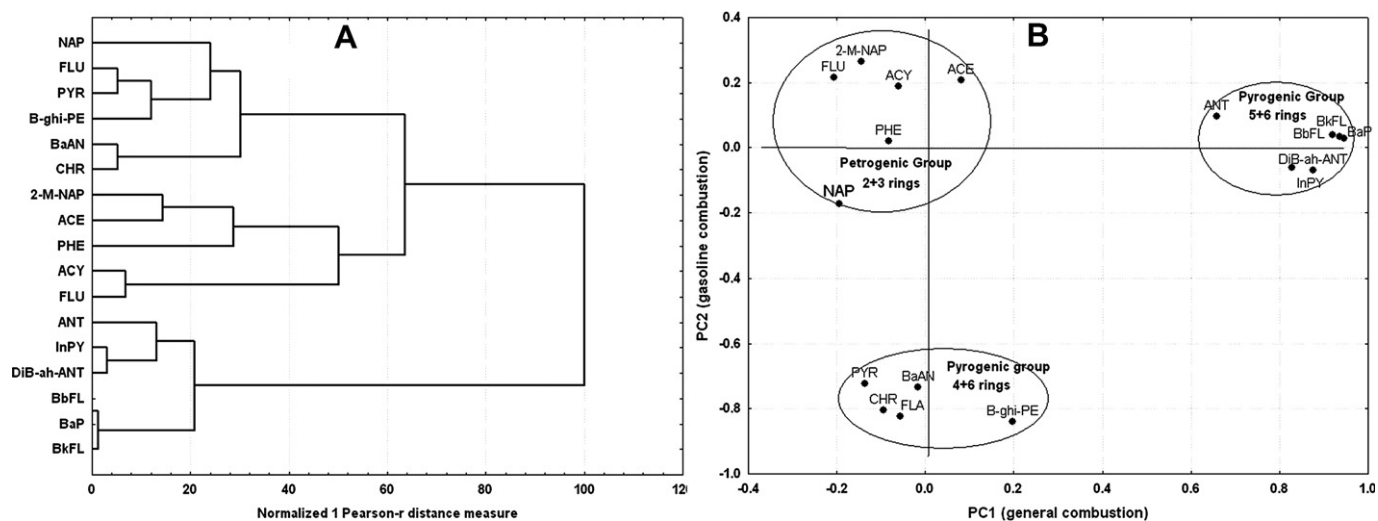


Fig. 4. A. Hierarchical dendrogram for 17 PAHs in the Bahía Blanca Estuary water column samples using Ward's method group and Pearson correlation as measure interval. B. The loadings for the individual PAHs at the principal components' plot (PC1 vs PC2).

(Li et al., 2003; Wang et al., 2009). On the other hand, with respect to gasoline engine, benzo[a]anthracene appears to be the main tracer (Wang et al., 2009). The absence of phenanthrene eigenvalue significance (as would be expected in diesel emissions) and the presence of a meaningful benzo[ghi]perylene loading led to interpret this component as gasoline/petroleum light derivatives combustion component. Finally, PC3 presented its maximum positive loadings for PAHs such as naphthalene, 2-methyl-naphthalene, acenaphthene, phenanthrene and anthracene. These PAHs belong to the Low Molecular Weight (LMW) PAHs with 2–3 rings or alkyl-substituted PAHs, 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 and Johnsen, 1999; Dobbins et al., 2006; Gonzalez et al., 2006; Ye et al., 2006). Thus, PC3 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.

According to HCA results, the pyrogenic PAHs could be subdivided into two groups with different molecular weight composition which represented two kinds of different pyrogenic sources. Consistently and further, the results of PCA could also differentiate the two subsets of pyrogenic sources. ~60% of the total variance of PAHs concentrations in the water column was explained by source contributions in PCA results, showing that the pyrolytic origin (PC1, 27.8% + PC2, 20.04% of variance) was the prevalent explained contribution over the sampled area (Fig. 4B). Similar literature results were found in other industrialized coasts, which indicated that pyrogenic PAHs are dominant in urban aquatic environments (Budzinsky et al., 1997; Zeng et al., 1997; Pereira et al., 1999; Notar et al., 2001; Stout et al., 2004).

From the distribution of sampling locations and dates, the PCA results also showed that the highest scores of combustion factors (PC1 and PC2) were correlated to almost all the sampled sites during the cold season, from May to August. This observation was in agreement and completed the previous observation by which water PAHs concentrations' increase correlates with the end of the winter heating period. Additionally, the three highest PC3 scores (petroleum and oil inputs) corresponded to three harbor stations, namely Ing. White, Galván and Cuatros Harbors, where an intense shipping activity is continuously developed and consequently motor boats residues, crankcase oil or accidental petroleum derivatives spills are common.

### 3.4. PAHs in organisms

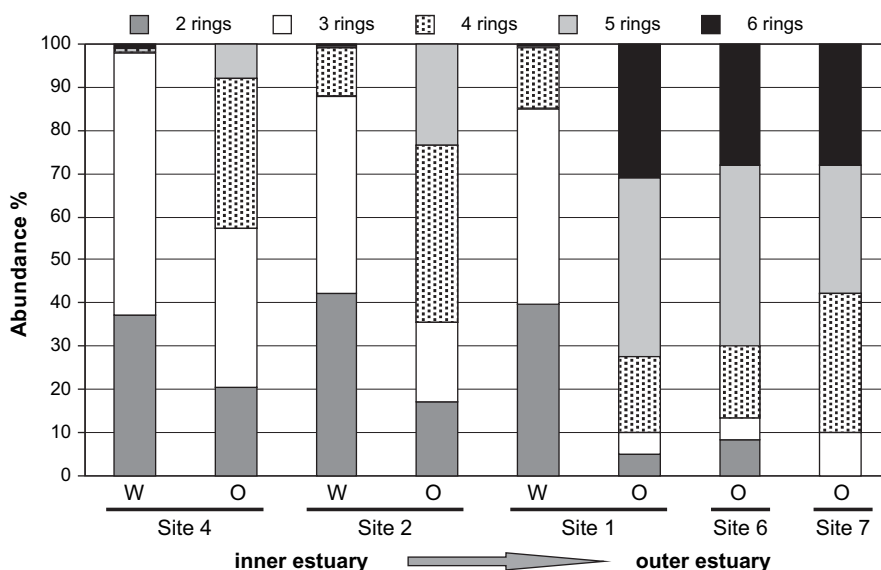
#### 3.4.1. Levels and distributions

Mussels have been addressed as bioindicators for monitoring trace toxic substances in coastal waters due to their wide geographical distribution, sessile lifestyle, easy sampling, tolerance to a considerable range of salinity, resistance to stress and high accumulation of a wide range of chemicals.

Total PAHs levels found in indigenous mussels (*Brachidontes* sp. and *Tagelus* sp.) were widely above the limit of detection (LOD) of the analytical method for all the samples and ranged from 348 to 1597 ng/g (d.w.,  $\Sigma 17$  PAHs), showing a mean of  $492 \pm 156$  for the stations located at the Main Navigation Channel (S2 and S4; Fig. 1) and a mean of  $1150 \pm 632$  for mussels at the estuary outlet stations (Tres Brazas Channel – S6 – and Cabeza de Buey Channel – S7). Fish specimens (*Odontesthes* sp.) were sampled at S1 station and reached a tissue's Total PAHs concentration of 1095 ng/g d.w., which showed a high percentage contribution of five and six molecular ring PAHs (for example, BaP: 100 ng/g, InPY: 171 ng/g, DiB-ah-ANT: 184 ng/g, and B-ghi-PE: 166 ng/g).

Generally, a mixture of PAH comprises numerous compounds of different seriousness of health effects and its 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 benz[a]pyrene, one of the most toxic and best investigated carcinogenic polyaromatic hydrocarbons. Following Nisbet and LaGoy (1992) and Larsen and Larsen (1998) equations, the calculated TEQ BaP in the samples were 339.2, 16.8, 5.3, 228.8, 580.7 ng TEQ BaP/g d.w. for S1, S2, S4, S6, S7 sites respectively.

In terms of molecular PAHs profiles, the data firstly showed that mussels from the inner zone of the estuary exhibited a marked dominance of PAHs up to four molecular rings, achieving 92% and 76% for Site 4 and Site 2 respectively. This trend followed the one observed in the water at those sites, which showed 98% and 99% respectively (Fig. 5). On the contrary, organisms (mussels and fish) located at the outer estuary locations showed higher molecular weight dominance, with proportions of 72%, 69% and 57% for the sum of 5 and 6 rings PAHs at S1, S6 and S7 sites, respectively (Fig. 5). A possible explanation for this may be found in the different dominant exposure routes of PAHs. As described in Section 3.2, the



**Fig. 5.** Polycyclic aromatic hydrocarbon (2–6 rings) distribution patterns in the water (W) and organism – mussel and fish – (O) at different locations over the Bahía Blanca Estuary Main Channel.

highest values of SPM loads were recorded in the outer zone of the estuary and were in good correlation with POM, attributable to the tidal currents associated transport of sediments and to the sewage network outfall proximity (Fig. 1). As a consequence, the organisms from the outer zone would probably be more exposed to particles and organic matter loads. The fact that suspended particles are commonly enriched in higher molecular weight PAHs (Pruell and Quinn, 1985; Budzinsky et al., 1996) could have lead to higher HMW PAHs uptake rates and so to the observed tissue profiles. On the contrary, at the inner stations, where lower SPM values were often measured, the bioaccumulation of HMW PAHs was also lower. Similar results were found by Baumard et al. (1998a) in the Mediterranean coasts, which observed negative correlations between bioaccumulation factors and PAHs Log  $K_{ow}$  for mussels located on rocks at the air–water interface, where the water turbidity was generally low.

### 3.4.2. Sources

**3.4.2.1. *Brachidontes* sp. and *Tagelus* sp.** PAHs ratios are traditionally used to determine PAHs sources to classify samples by location and estimate the importance of combustion and petroleum derived PAHs (Lipiatou and Salot, 1991; Budzinsky et al., 1997; Yunker et al., 2002a,b). 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. fluoranthene relative to pyrene) or to the molecular mass totals (Yunker et al., 2002b). 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 fluoranthene (FLA) to FLA plus pyrene (FLA/202) and indeno-[1,2,3-cd]pyrene (InPY) to InPY plus benzo[ghi]perylene (InPY/276). FLA/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

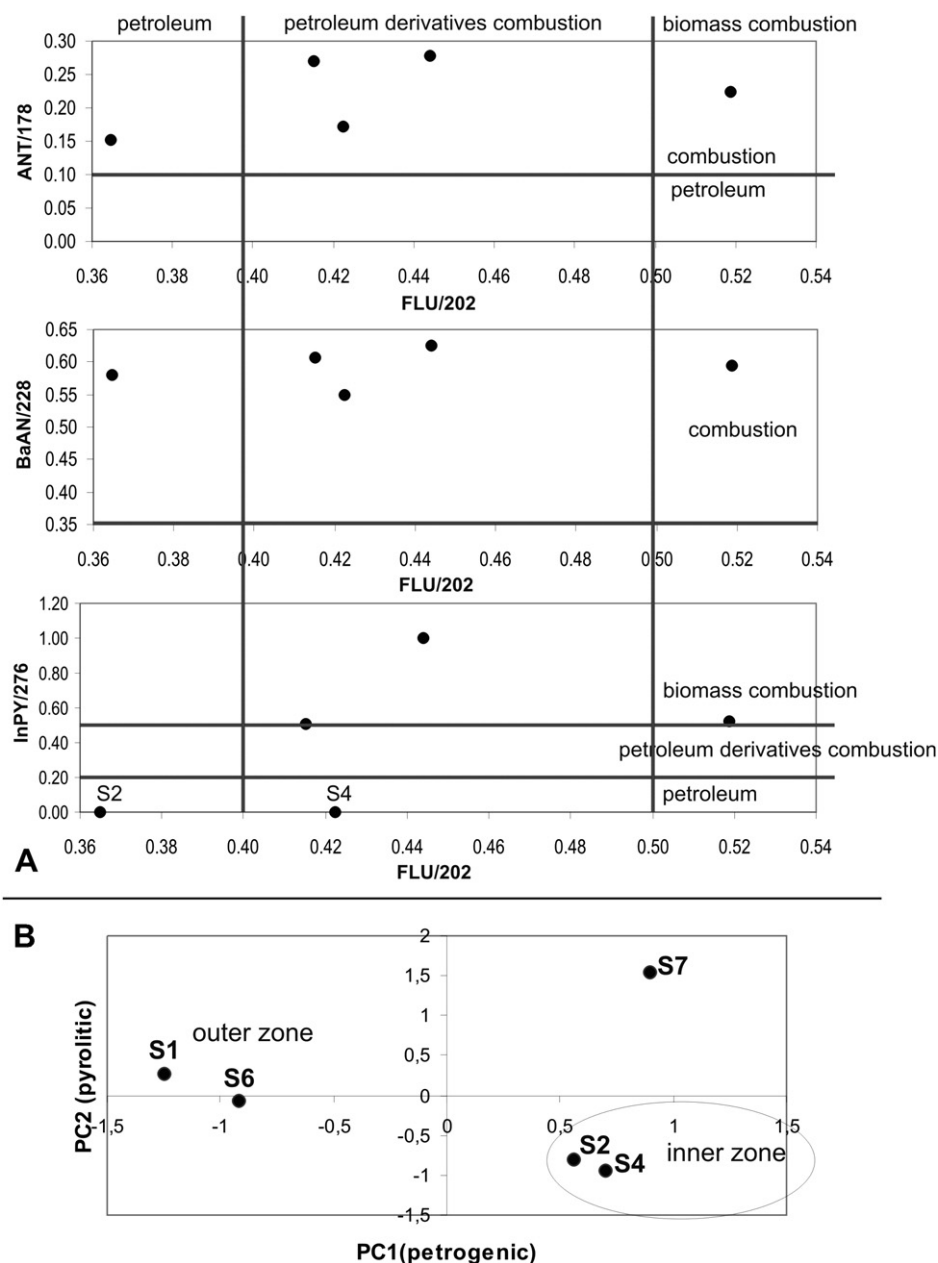
grass, wood or coal combustion. Similarly, InPY/276 ratios less than approximately 0.20 imply petroleum, between 0.20 and 0.50 liquid fossil fuel (vehicle and crude oil) combustion while ratios over 0.50 are attributable to grass, wood or coal combustion (Yunker et al., 2002b). These two parent PAHs ratios are supplemented by anthracene (ANT) to ANT plus phenanthrene (ANT/178) and benzo[*a*]anthracene (BaAN) to BaAN plus chrysene [BaAN/228]. ANT/178 ratios <0.10 usually are taken as an indication of petroleum, while ratios >0.10 indicate combustion (Budzinsky et al., 1997; Soclo et al., 2000; Yunker et al., 2002b).

The analysis of molecular ratios indicated that the PAHs were, in most cases, of pyrolytic origin, but with inputs of petrogenic hydrocarbons close to harbors (S4) and industries (S2), as seen in the mussels sampled in Galvan Harbor dock (S2) and Cuatros Harbors Harbor Dock (S4). The general predominance of fluoranthene over pyrene consistent with elevated InPY/276 and BaAN/228 ratios implicated both liquid fossil fuel combustion and biomass (coal, grass, and wood) as the major PAHs source(s) (Fig. 6A). Biomass combustion could have both local and long range atmospheric transport sources, as the estuary is surrounded by agriculture lands, in which controlled and uncontrolled fires frequently occur. Liquid fuel fossil combustion source(s) was probably a quite local source(s) due to the high level of anthropogenic activities at the estuary.

These results were in good agreement with the dominant sources assessed for the water column samples and with a previous characterization of the PAH burden in sediments (Arias et al., in press).

On the other hand, Principal Component Analysis (PCA) identified two principal components (PC1–2) accounting for 48% and 25% of the total variance, respectively.

PC1 was characterized by high positive loadings (>0.8) of low molecular weight PAHs (LMW, including PAHs of two, three and up to four molecular rings, namely, 2-M-NAP, PHE, FLA and PYR. This component was also marked with high negative loadings for heavier PAHs. Although FLA and PYR are typical markers for pyrolysis or incomplete combustion, like diesel engine emission profile (Wang et al., 2009), PAHs methyl-derivatives are abundant in petrogenic sources, e.g., fresh or used crankcase oil, crude and fuel oil (reference therein). Thus, PC1 was interpreted to be indicative of petrogenic source of PAHs.



**Fig. 6.** A. Mussel's IP/276, BaA/228 and An/178 source ratio plot vs FLU/202 ratio showing different origin zones, based on Yunker et al. (2002a,b). B. PCA score plot of biota samples.

In the other hand, PC2 was characterized by high loadings contributions ( $>0.89$ ) of HMW PAHs, namely, BaAN, CHR, InPY and DiB-ah-ANT, all of them associated with traffic emissions/gasoline engine exhausts. Therefore, this component was interpreted as a combustion/emission contribution.

As seen in Fig. 6B, the two samples with high PC1 scores and negative contribution of PC2 (S2 and S4 stations) corresponded to the previously characterized petrogenic impacted samples (source ratios, Fig. 6A). Contrarily, the rest of the samples (S1, S6 and S7) achieved either positive loadings on the PC2 axis, negatives ones for PC1 and pyrolytic/combustion source ratios punctuations. Thus, this set of samples was characterized as traffic emissions/gasoline combustion impacted samples. Further, this finding agreed and supported the above PAH transport hypothesis (Section 3.4.1) by which as outer locations are exposed to higher SPM fluxes and suspended particles are commonly enriched in higher molecular weight PAHs, they are receiving heavier PAHs loads.

**3.4.2.2. *Odontesthes* sp.** The distribution of contaminants in fish tissues is governed by complex phenomena, mainly the bioavailability of the contaminants, the diet composition and the biotransformation processes (Baumard et al., 1998b). While measurements of PAHs concentrations in fish tissue samples from urbanized coastal zones have extensively showed the presence of aromatic hydrocarbons with 1–3 benzenoid rings, indigenous *Odontesthes* sp. tissues showed a strong contribution of 4–6 rings PAHs (Fig. 5, S1). Generally the heavier PAHs in fish muscle follow a week's decreasing gradient after the uptake, and do not bioconcentrate to any substantive extent (Lemaire et al., 1990; Stein et al., 1993), however, PCA results characterized their PAHs' burden as combustion/emission origin (Fig. 6B). The occurrence of heavy PAHs could be reflecting a recent fuel/oil burning derivative uptake, which was a quite probable fact since the specimens were sampled in a shielded motorboat docking platform.

**Table 3**  
Occurrence of PAHs in marine bivalves and invertebrates from different worldwide field collections.

Mussels and oysters	Feeding mode	Location	Total PAHs range (ppb)	N° PAHs	N° sites	d.w./w.w.	Contamination level	Reference
<b>Europe</b>								
<i>Mytilus edulis</i>	Filter feeder	Norway	500–12,845	32	11	d.w.	Moderate to very high	Knutzen and Sortland, 1982
<i>Mytilus</i> sp., <i>C. gigas</i>	Filter feeder	France	nd–300,000 <sup>a</sup>	n.e.	110	d.w.	Low to very high	Claissie, 1989
<i>Mytilus edulis</i>	Filter feeder	Northern Baltic Sea	440	19	3	d.w.	Moderate	Broman et al., 1990
<i>Mytilus galloprovincialis</i>	Filter feeder	Mediterranean	25–390	14	23	d.w.	Low to moderate	Baumard et al., 1998a,b
<i>Mytilus edulis</i>	Filter feeder	Western Baltic Sea	90–3900	31	25	d.w.	Low to high	Baumard et al., 1999
<i>Mytilus edulis</i>	Filter feeder	Northern Irish Sea-loughs	95–184	12	8	d.w.	Low to moderate	Guinan et al., 2001
<i>Mytilus galloprovincialis</i>	Filter feeder	Ligurian Coast, Italy	129–2638	12	5	d.w.	Moderate to high	Piccardo et al., 2001
<i>Crassostrea virginica</i>	Filter feeder	Mobile Bay, Alabama, USA	32–1189	11	3	d.w.	Low to very high	Peachey, 2003
<i>Mytilus galloprovincialis</i>	Filter feeder	Spain, Galician Coasts	17–7780	13	24	d.w.	Low to very high	Soriano et al., 2006
<i>Mytilus</i> spp.	Filter feeder	Iceland, Norway and Sweden	40–11,670	32	22	d.w.	Low to very high	Skarphedinsdottir et al., 2007
<i>Mytilus galloprovincialis</i>	Filter feeder	Saronikos Gulf, Greece	1300–1800				Moderate to high	Valavanidis et al., 2008
<b>North America</b>								
<i>Mytilidae</i>	Filter feeder	Gulf of Mexico and USA	76–7530	17	4	d.w.	Low to very high	Wade et al., 1989
<i>Mytilus edulis</i>	Filter feeder	Puget Sound, WA	40–63,600	24	9	d.w.	Low to very high	Krishnakumar et al., 1994
Mussels and oysters	Filter feeder	USA Coasts	192–503 <sup>b</sup>	44	191	d.w.	Moderate	NOAA, 1998
<i>Mytilus</i> spp.	Filter feeder	San Francisco Bay	180–4100	34	6	d.w.	Moderate to high	Miles and Roster, 1999
<i>Mytilus galloprovincialis</i>	Filter feeder	San Diego Bay, USA	1831–23,985	19	6	d.w.	High to very high	Anderson et al., 1999
<i>Crassostrea virginica</i>	Filter feeder	Florida, USA	361–11,026		14	d.w.	Moderate to very high	Fisher et al., 2000
<i>Mytilus edulis</i>	Filter feeder	Gulf of Maine, USA	92 (median)		56	d.w.	Low	Chase et al., 2001
Mussels and oysters	Filter feeder	USA Coasts	77–1100	24	214	d.w.	Low to high	O'Connor, 2002
<i>Mytilus californianus</i>	Filter feeder	San Francisco Estuary	21–1093	25	10	d.w.	Low to high	Oros and Ross, 2005
<i>Crassostrea gigas</i>	Filter feeder	San Francisco Estuary	184–6899	25	8	d.w.	Moderate to very high	Oros and Ross, 2005
<b>Asia</b>								
<i>Perna viridis</i> and <i>Mytilus edulis</i>	Filter feeder	East Coast of China	456–3495	24	7	d.w.	Moderate to high	Fung et al., 2004
<i>Perna viridis</i>	Filter feeder	Hong Kong Coastal Waters	402–1849	15	5	d.w.	Moderate to high	Siu et al., 2008
<i>Mytilus galloprovincialis</i>	Filter feeder	Mokpo Bay, Korea	96	16	1	d.w.	Low	Namiesnik et al., 2008
<b>South America</b>								
Several Mussel and Oyster species	Filter feeder	Central and South America coastal locations	28–13,800	18	56	d.w.	Low to very high	NOAA Technical Memorandum, 1995
<i>Ostrea iridescent</i>	Filter feeder	Acajutla, El Salvador	37–18,000	–	5	d.w.	Low to very high	Michel and Zengel, 1998
<i>Crassostrea virginica</i>	Filter feeder	Terminos Lagoon, Mexico	2470–42,500	42	5	d.w.	High to very high	Noreña-Barroso et al., 1999
<i>Curculia fluminea</i>	Filter feeder	Rio de la Plata Estuary, Argentina	1000–15,800	n.s.	11	d.w.	High to very high	Colombo et al., 2005
<i>Perna perna</i>	Filter feeder	Guanabara Bay, Brazil	60–6000	35	10	d.w.	Low to very high	Francioni et al., 2007
<i>Aulacomya atra atra</i> , <i>Tellina petitiata</i>	Filter feeder/detritus feeder	Bahía Nueva, Patagonia, Argentina	9–273	16	10	d.w.	Low to moderate	
<i>Brachydontes</i> sp. and <i>Tagelus</i> sp.	Filter feeder	Bahía Blanca Estuary, Argentina	810–2010	8–15	8	d.w.	Moderate to high	Massara Paletto et al., 2009
			349–1597	17	4	d.w.	Moderate to high	This study

Values represent all sites and PAHs measured. Sites are the number of sites sampled and PAHs the number of individual PAHs analyzed. FF: filter feeder, DF: detritus feeder, n.s.: not specified, d.w.: dry weight, w.w.: wet weight.

<sup>a</sup> Mean for most stations 3000–5000 ng/g.

<sup>b</sup> Annual mean values.



### 3.5. Biota (fish and mussels) PAHs burden in comparison to worldwide previously reported

As reviewed by Barra et al. (2007), PAHs information on mussel and biota samples is scarce for South American environments. In order to place the studied organisms PAHs concentrations in a global context, observed concentrations of marine bivalves around the world are shown in Table 3. Additionally, for comparative purposes, we used the classification approach generated by Baumard et al. (1998a). For them the contamination level in an estuary can be categorized in bivalves 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.). Finally, in order to get better comparison parameters, the number of PAHs analyzed in each study was included.

Results listed in Table 3 firstly showed that the levels found in several high polluted areas around the world were widely above 1000 ng/g (d.w.) (Porte and Albaiges, 1993; Anderson et al., 1999; Colombo et al., 2005) achieving high to very high levels of contamination at the scale described by Baumard et al. When comparing sites, it could be observed that the number of PAHs analyzed had a strong influence in the final classification of a site. For instance, the 16 EPA PAHs analyzed by Francioni et al. (2007) in Guanabara Bay, Brazil, led to a “moderate” level of pollution following Baumard’s classification scale, however, when considering the totality of PAHs analyzed in that work (35), the strong occurrence of dibenzothiophene and PAHs methyl-derivatives led to a “very high” level of classification.

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, 2005). The Bahía Blanca Estuary wide mean of Total PAHs in mussels was 857 ng/g d.w. (17 PAHs), which suggested that the average PAHs contamination level was within the “moderate” category along the studied period.

### 3.6. Assessing PAHs ecotoxicological potential

Studies from the 1960s onward began to report PAHs pollutants sites and since then, PAHs concentrations that may cause adverse effects in marine organisms are incipiently reported around the world.

Concerning PAHs in fish, although their exposure cannot be reliably determined by measuring fish tissue levels, the showed PAHs’ tissue burden results represent a new baseline data that can be interpreted using several toxicity criteria. For example, anthracene (found in ~20 ppb) is known for damaging the gills of fish upon exposure to quite low levels of the compound (McCloskey and Oris, 1993). In addition, Total PAHs values found in this study (around 1 ppm) are in the level of “abnormalities triggering” for some fish species embryos (Crals et al., 2002). Moreover, concentrations of PAHs in sediments up to 10 ppm have been found in local sediments (Arias et al., in press), while worldwide guidelines indicate potential for effects in fish in the 1 ppm range or lower. Finally, concentrations of PAHs in water as low as 1 ppb (local range is from not detected to 4 ppb) has been reported to cause serious effects on fish larvae and sublethal effects in fish (Payne et al., 1988). Taking all into account, it is important to start addressing the potential health problems for fish in the area of study in order to provide better insight into tissue’s level–response relationship and actual ecotoxicological risk.

In the other hand, results point mussels as a raising concern for the area in study. While PAH levels as low as 300 ng/g (d.w.) are known to trigger genotoxic response in some species (Francioni

et al., 2007), concentrations above this threshold were found at all samples (Section 3.4.1). Finally, the risks associated to organism’s consumption by humans are difficult to estimate, since for most PAHs there are no general regulations. For example, USA regulations differ according to the state, e.g., California calculated 44 ppb as a level of benzo[a]pyrene equivalent PAHs in fish or shellfish tissue that, when consumed in certain frequencies, will not pose a significant human health risk (OEHHA, USA, 2007) while the Maine state fish tissue action levels for PAHs were set in 3 ppb (Maine Center for Disease Control and Prevention, 2001). Besides, the European Commission Regulation 208/2005 proposed the limit of 10 ppb for benzo[a]pyrene in edible mussels.

In this work indigenous mussels exhibited increasing BaPEq values when moving to the outer estuary, from 5.3 (S4) to 580.7 (S7) ng/g d.w. (ppb) (Section 3.4.1). While these levels appeared as risk safe for the inner stations (S4 and S2), they were exceeding various safety criteria at S6 and S7. Results indicated the need of a systematic monitoring program for the area in study, in order to provide accurate assessment and management of risks for the regional population.

## 4. Conclusions

Levels of Total PAHs in the surface water at various coastal locations of the Bahía Blanca Estuary were found from moderate to low concentrations, registering a wide spectrum of molecular weights.

Seasonal PAHs levels relationship with master parameters suggested a relationship between the phytoplankton microbiological cycling and the dissolved/suspended PAHs occurrence in water.

About PAHs sources, both HCA and PCA could differentiate two subsets of pyrogenic sources and a petrogenic one for local waters. While pyrogenic sources appeared to be dominant over the local waters, the highest petrogenic sites corresponded to three harbor stations, namely Ing. White Harbor, Galván Harbor and Cuatros Harbors, where an intense shipping activity was continuously developed.

Molecular PAHs profiles found in mussels and fish were different at the inner and outer estuary sampling sites. Evidence suggested that these differences could be owed to different dominant exposure routes of PAHs.

Mussel source ratios and PCA results were in good agreement with the dominant sources assessed for the water column samples and with previous PAHs burden characterization in sediments. PCA also enabled the linking of PAHs origins according to the sample location.

The Bahía Blanca Estuary wide mean of the PAHs burden in mussels suggested that the average PAH contamination level was within the “moderate” category over the study period. While levels appeared as ecotoxicologically risk safe for the inner stations, they were exceeding various safety criteria at the outer. Results indicate the 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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