Source characterisation and mid-term spatial and temporal distribution of polycyclic aromatic hydrocarbons in molluscs along the Basque coast (northern Spain)

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(Received 29 January 2015; final version received 7 May 2015)

The variability of polycyclic aromatic hydrocarbons (PAH) measured in the soft tissues of Mediterranean mussels (Mytilus galloprovincialis) and Pacific oysters (Crassostrea gigas) are investigated. Samples were collected from estuarine waters within the Basque Country (Bay of Biscay), between 2003 and 2011. PAH bioaccumulation showed some seasonality and significant differences were observed between cold (autumn–winter) and warm (spring–summer) seasons. Sites located within the ports of Bilbao and Pasaia showed the highest PAH concentrations in molluscs, and the highest percentages of samples above the established Environmental Quality Standards and Environmental Assessment Criteria. Probably due to human activities carried out in the area, no clear trends were observed, between 2003 and 2011, for the autumn data. Since the Basque coast is an area with high population density and industrial activity, the congener profiles (which reveal the predominance of tetra-aromatics) and the diagnostic ratios identified urban/industrial combustion processes as the main PAH sources. However, natural and petrogenic sources cannot be disregarded.

Keywords: mussel; oyster; polycyclic aromatic hydrocarbons (PAH); pollution sources; Basque Country

1. Introduction

Polycyclic aromatic hydrocarbons (PAH), defined as organic compounds containing two or more fused aromatic rings, occur from both natural sources (e.g. oil seeps, bitumen, coal, plant debris, forest and prairie fires) and anthropogenic sources (e.g. fossil fuels and combustion) [1]. However, incomplete combustion of organic matter at high temperatures (i.e. pyrolytic origin) is one of the major anthropogenic sources of PAH adjacent to industrial and urban areas.[2] Due to their toxicity and persistence in the marine environment, PAHs are of great environmental concern[3]; additionally, they are soluble in fatty and lipid-rich tissues and organs, where they accumulate.[4] Therefore, the use of organisms to monitor environmental contamination allows integration of the bioavailable fraction of contaminants.[5]

Within the earlier-mentioned context, the use of bivalves as indicators of chemical contamination is accepted and recommended widely (e.g. by International Conventions, such as the Oslo and Paris Convention (OSPAR), the Barcelona Convention and the Helsinki Commission);
these molluscs have limited mobility and capacity to metabolise lipophilic substances, such as PAH.[6,7] In fact, some filter-feeding biomonitors (e.g. mussels and oysters) have been used successfully as indicator organisms in environmental monitoring programmes, throughout the world (e.g. [8–12]).

In addition, the Directive 2013/39/EU, amending the Water Framework Directive (Directive 2000/60/EC) and the Environmental Quality Standards (EQS) Directive (Directive 2008/105/EC), recommend the inclusion of biota, such as molluscs, for monitoring some very hydrophobic substances that they accumulate but are hardly detectable in water. Moreover, EQS for some PAH (e.g. fluoranthene and benzo[a]pyrene) in biota have been established. Likewise, OSPAR Commission has developed Environmental Assessment Criteria (EAC) for individual PAH, for the interpretation of chemical pollution.[13] EAC indicate pollutant concentrations below which no chronic effects are expected to occur in marine species [13] (Table 1).

Along the Basque coast (Bay of Biscay: Figure 1), several studies have been undertaken on the spatial distribution of PAH in sediments (e.g. [14–16]) and molluscs (e.g. [4,17–21]), but in few (1–4) estuaries. However, very few incorporate temporal trend research in the use of biomonitors,[22,23] at the scale of the whole region. In comparison, the Basque Water Agency has monitored contaminants in molluscs within 12 estuaries for many years, within the ‘Monitoring Network for the Ecological Status Assessment of Transitional and Coastal Waters within the Basque Coast’. [24] In addition, following the Prestige oil spill, in November 2002, an intensification of the sampling programme was established by the Basque Government, in order to assess and monitor the extent of the impacts caused by the fuel oil along the Basque coast. Therefore, there presently is an extensive set of PAH measurements of biomonitors, together with information on human pressures[25]; this permits the inter-relationship between these data sets to be established, on a temporal basis. Hence, the objectives of this contribution are: (i) to study the yearly variability in concentrations of PAH, for both mussels and oysters from the Basque estuaries, for the period 2003–2007; (ii) to describe the status and trends of PAH concentrations in

| Compound                  | Abbreviation | No. of rings | Molecular mass | Quantification ions (m/z) | EQS \( (\mu g \text{ kg}^{-1}) \) | EAC \( (\mu g \text{ kg}^{-1}) \) |
|---------------------------|--------------|--------------|----------------|---------------------------|---------------------------------|---------------------------------|
| Naphthalene               | N            | 2            | 128            | 128 (127, 129)            | –                               | 340                             |
| Acenaphthylene            | Acy          | 3            | 152            | 152 (151, 153)            | –                               | –                               |
| Acenaphthene              | Ace          | 3            | 154            | 154 (151, 153)            | –                               | –                               |
| Fluorene                  | Fl           | 3            | 166            | 166 (165)                 | –                               | –                               |
| Anthracene                | A            | 3            | 178            | 178 (176)                 | –                               | 290                             |
| Phenanthrene              | Ph           | 3            | 178            | 178 (176)                 | –                               | 1700                            |
| Fluoranthene              | F            | 4            | 202            | 202 (200)                 | 150                             | 110                             |
| Pyrene                    | P            | 4            | 202            | 202 (200)                 | –                               | 100                             |
| Benzo[a]anthracene        | BaA          | 4            | 228            | 228 (226)                 | –                               | 80                              |
| Chrysene                  | C            | 4            | 228            | 228 (226)                 | –                               | –                               |
| Benzo[a]pyrene            | BaP          | 5            | 252            | 252 (250, 253)            | 25                              | 600                             |
| Benzo[c]pyrene            | BeP          | 5            | 252            | 252 (250, 253)            | –                               | –                               |
| Benzo[b]fluoranthene\(^b\) | BbF         | 5            | 252            | 252 (250, 253)            | –                               | –                               |
| Benzo[k]fluoranthene\(^b\)| BkF          | 5            | 252            | 252 (250, 253)            | –                               | 260                             |
| Perylene                  | Pe           | 5            | 252            | 252 (250, 253)            | –                               | –                               |
| Benzo[ghi]perylene        | BPe          | 6            | 276            | 276 (274, 277)            | –                               | 110                             |
| Indeno[1,2,3-cd]pyrene    | IP           | 6            | 276            | 276 (274, 277)            | –                               | –                               |
| Dibenzo[a,h]anthracene    | DBA          | 6            | 278            | 278 (276)                 | –                               | –                               |

Note: Ions used in the compound confirmation, and the established EQS (Directive 2013/39/EU), and the EAC ([13]), in \( \mu g \text{ kg}^{-1} \) dry weight, are included.

\(^{a}\)Values converted from wet weight assuming 20% of dry weight.

\(^{b}\)These two PAH are considered as a sum, and represented as Benzo[b + k]fluoranthene (BbKF).
molluscs collected in autumn from these estuaries, for the period 2003–2011; and, (iii) to identify possible sources of PAH in the study area.

2. Materials and methods

2.1. Study area

The Basque Country, located in the south eastern Bay of Biscay (Figure 1), is a mountainous coastal region, dominated by rocky shores and estuaries of limited length. Located at the mouths of the main river systems, there are 12 major estuaries with lengths of between 1 and 25 km.[26] Most of these estuaries and their adjacent coastal areas have been affected historically, to some degree, by urban and industrial wastes and/or the presence of mineral ores.[27]

In addition, the impact of the Prestige oil spill on the Basque coast, although lower than in the Galician coast, was considerable. In fact, from December 2002 to February 2003, driven by the winds and the superficial currents, part of the fuel spilled offshore of the west coast of Galicia (northwest Spain) in November 2002 arrived at the Basque coast.[23,28]

2.2. Sampling strategy

Mediterranean mussel (Mytilus galloprovincialis) and Pacific oyster (Crassostrea gigas) data were obtained, between 2003 and 2011, within the framework of the above-mentioned monitoring network; there were 13 sites sampled within the estuaries along the Basque coast (Figure 1). From winter 2003 to spring 2007, samples were collected every three months in order to monitor the impact of the Prestige oil spill; additionally, between 2007 and 2011, samples were collected only in autumn. Therefore, one sample per species, site and period was analysed.

Each sample represented a pool of around 50 wild mussels (ranging between 35 and 45 mm in length) or 30 oysters (ranging between 55 and 85 mm in length); these were collected from the intertidal zone of each sampling site and transported wet to the laboratory. There, they were retained for 24 h, in seawater from the collection site, for depuration; this procedure was adopted since tissue analysis can be influenced by sediments and detritus in the digestive tract, at the time of collection.[29] The soft tissues of each pool were separated from the shells, ground and then freeze-dried. An aliquot of the homogenised sample was withdrawn, to calculate its water percentage, by drying at 105°C for 24 h, until reaching a constant weight.

Figure 1. Location of mollusc sampling sites within the estuaries of the Basque Country: Pobeña (1), port of Zierbena (2), port of Getxo (3), Plentzia (4), Mundaka (5), Lekeitio (6), Ondarroa (7), Mutriku (8), Zumaia (9), Orio (10), San Sebastian (11), port of Pasaia (12), Hondarribia (13).
2.3. Sample analysis

For previously lyophilised molluscs, an accelerated solvent extraction technique was used. From bottom to top, the extraction cell was packed by 1 g of Florisil topped by a cellulose filter, 1 g of Na₂SO₄ and 5–10 g of lyophilised sample. PAH were eluted using a pentane/dichloromethane mixture (50:50 v/v, 15 min, 100°C, 1750 psi). A preconcentration step was carried out using a TurboVap evaporator (25°C, 6 psi). The extract was reconstituted to 15 mL with dichloromethane and filtered with a 45 µm filter. The extract clean-up procedure was performed by gel permeation chromatography. The obtained elutriate was evaporated (TurboVap rotary evaporator; 25°C, 6 psi). After reconstitution with 1 mL of iso-octane, 8 mL of sulphuric acid was added: the mixed solution was centrifuged, to obtain the organic phase.

Selected parent PAH (Table 1) were determined by gas chromatography coupled to a mass spectrometer. Injection was performed at 280°C in the splitless mode and a Meta X5 capillary column was used (Teknokroma, 30 m, 0.25 mm i.d., 0.25 µm thick phase film). At a constant flow of 1.6 mL min⁻¹, Helium was used as a carrier gas. The oven temperature programme was set as follows: after 1 min at an initial temperature of 80°C, it was set to 200°C at a rate of 20°C min⁻¹; subsequently, it was increased to 315°C, at a rate of 6°C min⁻¹; and with a final isothermal period of 1 min. The mass selective detector ion source temperature was fixed at 320°C. Ions were monitored in the selected ionisation monitoring mode. The certified reference material SRM 2977 Mussel Tissue (National Institute of Standards & Technology, NIST) was used to validate the analytical procedure. The mean recoveries were within the range of 80–90%; except for some 5 and 5+ ring complex PAH (indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene and benzo[ghi]perylene) that showed lower recovery rates, ranging between 60% and 70%. For these compounds, uncertainty related to the obtained results ranged between 25% and 35%.

The results have been presented as µg kg⁻¹, on a dry weight (dw) basis, while values lying below the detection limit have been reported as ‘undetected’. The congeners sum is expressed as ΣPAHs (sum of 18 individual PAH), with concentrations of congeners under the detection limit being taken as zero.

2.4. Statistical analysis

In order to describe the yearly changes in concentration of PAH along the Basque coast, the Kruskal–Wallis test and Dunn’s multiple comparison post-test were applied to the data from the period 2003 to 2007, for comparison between seasons (winter, spring, summer and autumn). Significant difference was established at α: 0.05.

In order to describe trends in the total PAH levels (ΣPAHs, sum of 18 PAH), at each of the sampling sites considered, the non-parametric Mann–Kendall test (α: 0.05) was used for autumn data sampled in the period 2003–2011. This season was selected since the longest time-series was that for autumn. In addition, molluscs in the study area are outside the normal period of spawning in autumn,[30] one of the points in which the ‘Mussel Watch’ concept is based on, in order to minimize biological changes.

2.5. PAH source characterisation

Each pollution source produces a characteristic PAH pattern.[31] Therefore, the relative abundances of PAH may provide useful signatures for recognising hydrocarbon sources in the environment.[23] In fact, the presence of lighter PAH is linked usually to a petrogenic origin,[2] while the sources of the heavier fraction are more likely to be identified as pyrolytic.[4]
In the present study, in order to discriminate PAH sources along the Basque coast, parent PAH composition profiles (as a representation of the contribution percentages) and diagnostic ratios in molluscs were obtained for all the available data (2003–2011). The PAH ratios applied as diagnostic indicators were: A/(A + Ph), F/(F + P), BaA/(BaA + C), BbkP/(BbkP + BeP) and IP/(IP + BPe) (see Table 1, for abbreviations). These ratios were usually adopted for PAH in sediments, but they are applicable to different environmental media,[32] such as bivalves (e.g. [4,33]). In all of the expressions, for ease of comparison, PAH ratios have the combustion-dominant, thermodynamically less stable isomer in the numerator. Therefore, ratios increase with an increase in the combustion input.[1]

3. Results

3.1. Yearly variability in concentrations of PAH (2003–2007)

PAH concentrations in wild molluscs analysed along the Basque coast varied considerably between species, sites and seasons, for the period 2003–2007. The pattern of PAH, by site, during the different seasonal periods and years (between 2003 and 2011) is presented in Figure S1 to Figure S13, of the Supplementary Material (hereafter SM).

The concentrations of total PAH (ΣPAHs, sum of 18 PAH) in mussels ranged from 17 µg kg\(^{-1}\) (measured at Site 11, located in the Urumea estuary, in the spring of 2004) to 10228 µg kg\(^{-1}\) (observed at Site 2, located in the port of Zierbena, in the autumn of 2006) (Table 2). Likewise, in oysters, concentrations ranged from 27 µg kg\(^{-1}\) (at Site 6, in the Lea estuary, in the winter of 2004) to 2312 µg kg\(^{-1}\) (at Site 5, in the Oka estuary, in the spring of 2007) (Table 2).

Molluscs collected from Site 1, located in the Barbadun estuary, presented the lowest ΣPAHs median concentrations for mussels (236 µg kg\(^{-1}\)) along the Basque coast, obtained throughout the study period (2003–2007). Similarly, Site 5, located in the Oka estuary, showed the lowest median concentrations for oysters (197 µg kg\(^{-1}\)). Conversely, mussels obtained from Site 2, located within the port of Zierbena (in the Ibaizabal estuary), presented the highest median concentrations for mussels (111 µg kg\(^{-1}\)) along the Basque coast, obtained throughout the study period (2003–2007).

### Table 2. Statistical parameters (min, minimum; max, maximum; mean; SD, standard deviation; median) for the sum of polycyclic aromatic hydrocarbon (ΣPAHs) concentrations (µg kg\(^{-1}\), dry weight), by location and species, for the period between 2003 and 2007.

| Site       | Location      | Estuary | Species | n  | Min. | Max. | Mean  | SD   | Median |
|------------|----------------|---------|---------|----|------|------|-------|------|--------|
| 1          | Pobeña         | Barbadun| Mussel  | 19 | 90   | 512  | 254   | 111  | 236    |
| 2          | Port of Zierbena| Ibaizabal| Mussel  | 19 | 177  | 10,228| 3589  | 2873 | 3659   |
| 3          | Port of Getxo  | Ibaizabal| Mussel  | 19 | 110  | 6855 | 1377  | 1691 | 816    |
| 4          | Plentzia       | Butroe  | Mussel  | 19 | 50   | 1777 | 730   | 452  | 617    |
| 5          | Mundaka        | Oka     | Mussel  | 8  | 185  | 3440 | 1484  | 1148 | 1348   |
| Oyster     | 11             | Lea     | Mussel  | 3  | 62   | 281  | 206   | 125  | 275    |
| Oyster     | 16             | Lea     | Mussel  | 16 | 27   | 1180 | 386   | 304  | 297    |
| 7          | Ondarroa       | Artibai | Mussel  | 19 | 81   | 2675 | 992   | 794  | 835    |
| 8          | Mutriku        | Deba    | Mussel  | 19 | 19   | 1242 | 539   | 367  | 562    |
| 9          | Zumaia         | Urola   | Mussel  | 19 | 84   | 1022 | 335   | 230  | 256    |
| 10         | Orio           | Oria    | Mussel  | 5  | 116  | 1111 | 624   | 445  | 652    |
| Oyster     | 14             | Oria    | Mussel  | 14 | 92   | 858  | 352   | 277  | 222    |
| 11         | San Sebastian  | Urumea  | Mussel  | 19 | 17   | 982  | 287   | 200  | 266    |
| 12         | Port of Pasaia | Oratzun | Mussel  | 19 | 222  | 3723 | 1163  | 951  | 840    |
| 13         | Hondarribia    | Bidaso  | Mussel  | 19 | 40   | 1663 | 438   | 447  | 300    |

Note: n, number of samples.
Table 3. Polycyclic aromatic hydrocarbon (PAH) median concentrations (µg kg\(^{-1}\), dry weight), considering data from the 13 sites sampled along the Basque coast together by season, for the study period (2003–2007).

| Seasons         | Winter (n = 65) | Spring (n = 65) | Summer (n = 52) | Autumn (n = 65) | Kruskal–Wallis test | Dunn’s test |
|-----------------|-----------------|-----------------|-----------------|-----------------|---------------------|-------------|
| Naphthalene     | 8.6             | 9.6             | 2.9             | 5.6             | ns                  | –           |
| Acenaphthylene  | 0.0             | 0.0             | 0.0             | 0.0             | ns                  | –           |
| Acenaphthene    | 0.0             | 0.0             | 0.0             | 0.0             | ns                  | –           |
| Fluorene        | 0.0             | 0.0             | 0.0             | 0.0             | ns                  | –           |
| Anthracene      | 35.0            | 27.7            | 37.0            | 47.9            | ns                  | –           |
| Phenanthrene    | 2.1             | 0.0             | 0.0             | 0.0             | \(p < .05\)         | W-S, W-Su, S-A |
| Fluoranthene    | 48.4            | 52.4            | 36.1            | 59.0            | ns                  | –           |
| Pyrene          | 58.3            | 51.5            | 44.5            | 36.3            | ns                  | –           |
| Benzo[a]anthracene | 18.1         | 17.1            | 13.5            | 28.4            | ns                  | –           |
| Chrysene        | 86.2            | 53.9            | 36.5            | 79.1            | ns                  | –           |
| Benzo[a]pyrene  | 66.0            | 46.9            | 34.9            | 49.3            | \(p < .05\)         | W-Su        |
| Benzo[e]pyrene  | 41.1            | 26.2            | 21.5            | 31.0            | \(p < .05\)         | W-Su        |
| Benzo[b + k]fluoranthene | 8.9        | 7.7             | 7.7             | 15.2            | ns                  | –           |
| Perylene        | 8.1             | 0.0             | 3.7             | 2.9             | \(p < .05\)         | W-S         |
| Benzo[ghi]perylene | 0.0          | 0.0             | 0.0             | 0.0             | ns                  | –           |
| Indeno[1,2,3-cd]pyrene | 13.0     | 9.1             | 0.0             | 0.0             | \(p < .05\)         | W-Su, W-A, S-Su, S-A |
| Dibenzo[a,h]anthracene | 9.4      | 0.0             | 0.0             | 0.0             | ns                  | –           |
| \(\Sigma\)PAHs | 491.4           | 355.3           | 308.6           | 419.0           | \(p < .05\)         | W-Su        |

Notes: \(n\), number of samples; W, winter; S, spring; Su, summer; A, autumn; ns, not significant.

The \(p\)-values obtained on the Kruskal–Wallis test, for comparison between seasons, are also included, and the seasons that significantly differed from the others (Dunn’s post-test) are indicated.

Concentrations of \(\Sigma\)PAHs (3659 µg kg\(^{-1}\)) along the Basque coast; likewise, oyster from Site 6 (297 µg kg\(^{-1}\)), located in the Lea estuary (Table 2).

Considering data from all of the sites together, the Kruskal–Wallis test showed that there were significant differences \((p < .05)\) between median values of each season, for \(\Sigma\)PAHs and for congeners acenaphthene, benzo[b + k]fluoranthene, benzo[e]pyrene, perylene and indeno[1,2,3-cd]pyrene. When Dunn’s post-test was applied for these cases, the main significant differences were observed between the median concentrations associated with cold (autumn/winter) and warm (spring/summer) seasons. However, there were also significant differences between winter and autumn, and between spring and summer for indeno[1,2,3-cd]pyrene (Table 3).

3.2. Status and trends of PAH (2003–2011)

In order to assess the quality status of the environment, PAH concentrations obtained in mollusc tissues in autumn (between 2003 and 2011) were compared with the available EQS established by Directive 2013/39/EU, and the EAC established by OSPAR [13] (Table 1). Most of the measured concentrations were below the EQS for fluoranthene (81%) and benzo[a]pyrene (61%), and below the EAC for fluoranthene (74%), pyrene (63%), benzo[a]anthracene (72%) and benzo[ghi]perylene (97%); while all of the measured concentrations of naphthalene, phenanthrene, anthracene and benzo[a]pyrene were below the EAC for these congeners. Mussels from Site 2 (located at the port of Zierbena) showed the highest number (up to 78%) of samples with concentrations above the established EQS and EAC, and 89% of the measured concentrations in mussels from Site 12 (located at the port of Pasaia) are above the EAC for pyrene. In fact, for the considered period (2003–2011, autumn data), these two sites showed the highest mean concentrations of \(\Sigma\)PAHs, 4340 and 1251 µg kg\(^{-1}\), respectively (Figure S14, of the SM).
Figure 2. Temporal variability of the sum of polycyclic aromatic hydrocarbons (\(\sum\)PAHs, sum of 18 PAH, in \(\mu\)g kg\(^{-1}\) dry weight) in molluscs sampled along the Basque coast, in autumn, for the period 2003–2011.

Note: O, concentrations determined in the oysters.
On the other hand, when the autumn data set was considered, for the study period 2003–2011, high temporal variability was observed for \( \Sigma \)PAHs by site. Hence, the coefficient of variation ranged between 49% (at Site 11, located in the Urumea estuary) and 116% (at Site 13, in the Bidasoa estuary), in both cases for mussels. Moreover, only mussels sampled at Site 10 (located in the Oria estuary) presented a significant decreasing trend for \( \Sigma \)PAHs (Figure 2), and the lowest concentrations along the Basque coast were measured, in general, in 2003 and 2011.

3.3. PAH congener profile

The PAH distribution profiles in molluscs collected along the Basque coast, from 2003 to 2011, were dominated mainly by tetra-aromatics, with site mean contribution to the \( \Sigma \)PAHs ranging from 47% to 66% (Figure 3). In general, mass 202 compounds (fluoranthene and pyrene, with site mean relative percentages to the \( \Sigma \)PAHs contribution of 11–25% and 13–22%, respectively) dominated the pattern; these were followed by chrysene and the sum of benzo[b]fluoranthene and benzo[k]fluoranthene (i.e. benzo[b+k]fluoranthene). In contrast, the lower molecular weight compounds (di- and tri-aromatics) were the lowest contributors to the \( \Sigma \)PAHs, with site mean values of 7–21%.

Even though there was this general predominance of tetra-aromatic compounds, the distribution profile of considered sites was not conservative throughout the study period (Figure S1 to Figure S13, of the SM). In the winter of 2003, the higher molecular weight compounds (penta- and hexa-aromatics) were predominant at all the sites sampled (57–85% of the total PAH), with benzo[e]pyrene being the most abundant PAH.

Although in the spring of 2003 the tetra-aromatics fluoranthene or pyrene were predominant along the Basque coast, in the summer and autumn of 2003, concentrations of the penta-aromatic
perylene increased considerably. In fact, this compound represented more than 30% of the total PAH at Sites 7, 9 and 11 (located in the estuaries of Artibai, Urola and Urumea, respectively), over these sampling periods.

Later, between the autumn of 2005 and the spring of 2007, naphthalene showed the highest concentrations at sites considered throughout the study period. In particular, Sites 1, 6, 9 and 13

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Figure 4. PAH congener pair ratios, by site sampled along the Basque coast, estimated from mean ratios over the period 2003–2011.
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(located in the estuaries of Barbadun, Lea, Urola and Bidasoa, respectively) showed the highest contribution of naphthalene to the total PAH (up to 26%), in some surveys.

In the more recent surveys, the accumulation of tetra-aromatics remained dominant. However, the contribution to the ΣPAHs of some other compounds, such as phenanthrene, benzo[b+k]fluoranthene and benzo[e]pyrene (especially in autumn of 2009), was also important.

3.4. **PAH ratios**

Considering the average ratio values of PAH (see Table 1, for abbreviation) at each site, for the study period, there was no common interpretation on the PAH sources (Figure 4). The A/(A + Ph) ratio showed that PAH in molluscs from the Basque coast occurred, in general, from unburned petroleum (fossil fuels) sources; although Sites 2, 3, 4 and 8 were derived from combustion. In contrast, the BaA/(BaA + C) ratio showed that PAH were derived mainly from mixed sources, with only the value at Site 8 being at the limit between combustion and mixed sources. In fact, combustion products were indicated as the predominant sources of PAH, when other ratios were considered. Most mean values of the F/(F + P) and BbkF/(BbkF + BeP) ratios suggest petroleum (liquid fuel) combustion as the main PAH source, although values of Sites 1, 2, 3 and 6 showed that PAH derived mainly from biomass and coal combustion, for the F/(F + P) ratio. In contrast, the IP/(IP + BPe) ratio showed that PAH derived mainly from biomass and coal combustion. However, at Sites 7, 8, 9, 10 and 13, petroleum (liquid fuels) combustion was indicated as the major source of PAH.

4. **Discussion**

4.1. **Variability of PAH concentrations**

The concentration of PAH in mollusc tissues reflects the time-integrated concentrations of bioavailable PAH in the surrounding environment.[34] They include the water-soluble fraction and particles (sediment and food), as well as unassimilated PAH associated with particles on the gills or in the gut.[34] However, PAH bioaccumulation can differ between species,[35] although sometimes a clear differentiation cannot be undertaken.[10] In the present study, differences were observed between mussels and oysters, even though they could be related to the fact that sites where oysters were sampled are located, in general, within less-industrialised and urbanised areas, such as the Oka and Lea estuaries ([25]). Thus, it cannot be concluded that within the studied area, bioaccumulation of PAHs differs significantly between mussels and oysters.

In addition, PAH bioaccumulation has been observed to show some seasonality,[35] in accordance with both the biological cycle of the species [36] and the seasonal variation of anthropogenic sources. Therefore, the significant differences observed between cold (autumn–winter) and warm (spring–summer) seasons, for the period 2003–2007, in the present study could be influenced by: (i) the decrease of PAH concentrations in late spring–early summer, matching the mussel spawning period[37,38]; (ii) the increase of PAH concentrations in autumn–winter, due to the higher lipid contents in mussel tissues in that period, before the spawning[39]; (iii) the increase in the atmospheric deposition of PAH in winter, due to the greater need for heating or power generation during the colder months of the year[39]; and (iv) the increase of runoff in autumn–winter related to rainfall events, in comparison with spring–summer.[40]

Regarding the spatial variability of PAH, the zones with higher values are located usually close to urban and industrialised areas.[6] In the present study, although PAH were found in molluscs...
sampled throughout the study area, the highest $\Sigma$PAHs concentrations were observed in mussels collected from the port of Zierbena, located in the Ibaizabal estuary. Similarly, other organic compounds, such as polychlorinated biphenyls (PCB), were associated with the highest concentrations in molluscs along the Basque coast, at this site.[41] This estuary was one of the most polluted areas along the northern coast of Spain,[25,27] and has suffered from serious environmental degradation, as a result of many pollutant discharges (both industrial and domestic); these are combined with the development of the iron, steel and ship-building industries, together with mining activities.[27] In addition, between 1992 and 2011, various docks have been constructed in the western part of the Ibaizabal estuary, where the harbour infrastructures and activities are located, together with the main areas of industrial development.[42] Therefore, the high values obtained could be related also to these harbour extension works, since dredging operations could result in increased bioavailability of organic contaminants.[43]

For comparison, Site 3, located also within the Ibaizabal estuary, showed lower $\Sigma$PAHs levels in mussels. Similar values were observed in mussels collected in this estuary by Bartolomé et al. [44] (Table 4). However, Soriano et al. [23] reported even lower concentrations in Bilbao-Azcorri (Table 4), due probably to differences in the sampled locations and the prevailing hydromorphological characteristics. In fact, Sites 2 and 3 are located in the ports of Zierbena and Getxo, respectively; in contrast, Bilbao-Azcorri is located in the coastal area, where human pressures are lower.[25]

The Oiartzun estuary, with a population density of 1056 hab km$^{-2}$ in 2011, which is a harbour with shipyards, among other activities,[25] is considered also as an industrialised area and a large urban centre along the Basque coast. In fact, high concentrations of PAH were measured in mussels collected at the mouth of this estuary (Site 12); this is as observed previously for other organic compounds, such as PCB.[41]

| Location          | Species | Period          | No. PAH | Range     | Mean   | Reference |
|-------------------|---------|-----------------|---------|-----------|--------|-----------|
| **Basque Country** |         |                 |         |           |        |           |
| Bilbao-Azcorri    | Mussel  | Oct 2000–Nov 2004 | 13       | 96–1065   | 651    | [23]      |
| Ibaizabal estuary (Arriluze) | Mussel | Nov 2002–Mar 2004 | 16       | 4233–12,623 | 7677   | [17]      |
| Oka Estuary (Mundaka) | Mussel | Oct 2000–Nov 2004 | 13       | 111–155   | 131    | [23]      |
| Oka estuary       | Oyster  | Jun 2002–Sep 2004 | 10       | 289–1406  | 561    | [4]       |
| Oka estuary       | Oyster  | March-Dec 2006   | 16       | 291–1814  |        | [18]      |
| Oria estuary (Orio) | Mussel | Oct 2000–Nov 2004 | 13       | 117–495   | 230    | [23]      |
| Igueldo           | Mussel  | Oct 2000–Nov 2004 | 13       | 40–891    | 411    | [23]      |
| Bidasoa estuary (Fuenterrabia) | Mussel | Oct 2000–Nov 2004 | 13       | 86–258    | 152    | [23]      |
| **Cantabria**     |         |                 |         |           |        |           |
| S. Vicente de la Barquera | Mussel | Oct 2000–Nov 2004 | 13       | 103–245   | 174    | [23]      |
| Suances           | Mussel  | Oct 2000–Nov 2004 | 13       | 179–320   | 237    | [23]      |
| Santander-Pantalán* | Mussel | Oct 2000–Nov 2004 | 13       | 551–1805  | 1172   | [23]      |
| Santander-Pedreña | Mussel  | Oct 2000–Nov 2004 | 13       | 192–427   | 287    | [23]      |
| Laredo            | Mussel  | Oct 2000–Nov 2004 | 13       | 80–352    | 209    | [23]      |
| Castro-Urdiales   | Mussel  | Oct 2000–Nov 2004 | 13       | 73–480    | 225    | [23]      |
| **Asturias**      |         |                 |         |           |        |           |
| Navia             | Mussel  | Oct 2000–Nov 2004 | 13       | 55–226    | 112    | [23]      |
| Luarca            | Mussel  | Oct 2000–Nov 2004 | 13       | 20–146    | 74     | [23]      |
| Pravia            | Mussel  | Oct 2000–Nov 2004 | 13       | 61–536    | 269    | [23]      |
| Avilés*           | Mussel  | Oct 2000–Nov 2004 | 13       | 723–2643  | 1585   | [23]      |
| Gijón*            | Mussel  | Oct 2000–Nov 2004 | 13       | 602–5619  | 2076   | [23]      |
| Ribadesella       | Mussel  | Oct 2000–Nov 2004 | 13       | 84–291    | 141    | [23]      |

Note: No. PAH: number of PAH compounds analysed.

*Site considered as a hotspot.
Accordingly, when autumn data for the period 2003–2011 is considered, the highest percentages of samples above the established EQS and EAC are observed nearby highly urbanised and industrialised areas, such as the ports of Bilbao and Pasaia. Similar results were observed by Bellas et al. [8] in a site located close to Bilbao and other large cities along the Cantabrian coast (e.g. Ferrol, Santander, Avilés and Gijón).

On the other hand, the maximum levels in oysters along the Basque coast, for the period 2003–2007, were observed in spring and autumn of 2007 in the Oka estuary, in spite of being located in a low populated (162.7 hab km$^{-2}$, in 2011) and low industrialised area. In fact, an increase in PAH concentrations in molluscs has been observed since 2006, related probably to the population increase in the area (with an increase in traffic and heating) and the remobilisation of sediments and land, as a consequence of human activities (sanitation system, dredging and roads). Values observed in oysters sampled at the Oka estuary, in the present study (Table 2), are similar to the concentrations found in oysters sampled along this estuary by other authors [4,18] (Table 4).

Conversely, minimum mean concentrations of $\Sigma$PAHs in molluscs, for the period 2003–2007, were observed in Sites 1 and 6 (the Barbadun and Lea estuaries, respectively). These values are higher than those observed by Soriano et al. [23] in mussels, from insignificantly or moderately polluted sites located along the Cantabrian coast, such as Navia, Luarca or Ribadesella (Table 4).

Concerning temporal variability, human activities undertaken in the study area could be responsible for the absence (except for mussels in Site 10, Oria estuary) of significant trends of PAH in molluscs observed for autumn data collected between 2003 and 2011. In fact, mobilisation of sediments in some estuaries related to human activities, such as dredging and marina construction, for example, in 2005, at the mouth of the Oria estuary, to seaward of Site 10 (with an increase observed in PAH concentration since then related probably to the marina activity), or restoration of the area of Casecampo (in the Deba estuary) in 2004, could be related directly to the observed PAH concentration increases.

4.2. **PAH origin and emission sources characterisation**

The complexity of the mixture of PAH found in the environment is essentially source-dependent, with combustion sources yielding primarily parent (non-alkylated) PAH and a more complicated mixture of parent and alkylated PAH, resulting from oil inputs.[45] As mentioned previously, the Basque coast is industrialised and densely populated [27] and, as such, the motor vehicles and domestic heating are expected to constitute the most significant sources of PAH into the urban air.[46] In fact, transport is the greatest demanding sector for petroleum products in the Basque Country. However, the total demand for these products, excluding the refining sector, reduced by 10% between 2000 and 2010.[47] Coal consumption in the area is linked basically to the evolution of the industrial activity and the generation of electricity. Since reduced emission technologies are being implemented in the industrial sector and coal is being displaced gradually by natural gas for electric power generation, a decrease in the consumption of coal between 2000 and 2010 has been also observed. In fact, between 2000 and 2010, natural gas has become the major energy source in the Basque Country, displacing petroleum products to second place; these are followed by electric power, renewable energy and, finally, coal.[47]

The PAH distribution profiles observed in the present study, dominated by mass 202 and 252 compounds, are indicative of chronically polluted urban/industrial coastal areas.[31,48,49] This dominance suggests that pyrolytic PAH are arriving in larger proportion to the study area, than those of petrogenic sources.[1,49] The observed profiles are similar to those detected in mussels along the Cantabrian coast, prior to the Prestige oil spill.[23]

The PAH ratios applied in the present study, as diagnostic indicators, suggest also that PAH in the Basque coast molluscs are derived primarily from combustion; nonetheless, petrogenic sources
should not be disregarded. Similar results have been obtained for the study area, by other authors (i.e. [17,18]).

In this sense, variations observed in the overall PAH composition pattern in the present study could be indicative also of secondary PAH sources, such as natural or petrogenic. [50] Regarding natural sources, perylene percentages above 30% of the total PAH observed in the estuaries of Artibai, Urola and Urumea, in summer and autumn 2003, could be indicative of a natural perylene source. [31]

Concerning petrogenic sources, the generalised larger presence of penta-aromatics (e.g. benzo[e]pyrene) observed along the Basque coast, in the winter of 2003, could be related to the arrival of fuel originating from the Prestige, over that period. Similarly, along the Cantabrian coast, in April 2003, Soriano et al. [23,37] observed distribution profiles dominated by the tetra-aromatic chrysene and an increase in the contribution of the penta-aromatics, benzo[b]fluoranthene and benzo[e]pyrene. On the other hand, higher concentrations of lighter PAH (e.g. naphthalene), observed between 2005 and 2007, could indicate that molluscs were exposed to fresh petroleum hydrocarbons. [51]

In relation to diagnostic ratios, they exhibit less variation and provide a more incisive tool to discriminate PAH sources, where only one or two sources dominate. [1] In that sense, ratios for the smaller parent PAH (e.g. those of molecular mass 178 and 202) can distinguish between combustion and petroleum sources. Ratios for larger parent PAH (e.g. those of molecular mass 228, 252 and 276) can distinguish fossil fuel, liquid fuel combustion and solid (biomass/coal) combustion PAH sources. [1,52] However, the co-existence of multiple sources could make difficult the source apportionment process. [2] Since the molecular mass 202, 228 and 252 parent PAH were major constituents in the parent profiles of all molluscs samples in the present study, ratios for mass 178 (A/A + P) and 276 (IP/IP + BPe) could be less definite for the PAH source characterisation in the area than ratios for mass 202 (F/F + P), 228 (BaA/BaA + C) and 252 (BbkF/BbkF + BeP).

4.3. PAH in molluscs and sediments

Molluscs, as filter-feeders, are exposed to pollutants that enter either dissolved in water, or absorbed on the filtered particles. Since PAH are hydrophobic compounds with very low water solubility, their concentrations in water are usually very low. In fact, they tend to rapidly absorb onto suspended material and sediments. [53] Therefore, the relative importance of water or sediment in the accumulation of pollutants in molluscs depends upon factors such as their habitat and feeding strategy. [54] When filter-feeding molluscs, such as mussels and oysters, are considered, the major uptake route is from water (either dissolved or particulate). However, pollutants in sediments are also important in areas of sediment resuspension, such as external estuarine areas, where samples had been collected in the present study.

In order to characterise the bioaccumulation of PAH in molluscs from the Basque coast, in relation to their levels in sediment, data obtained in the present study (\(\sum PAHs\), sum of 18 PAH) were compared with available information on PAH concentrations in sediment from the same monitoring network. Since data in sediment were not obtained exactly at the same locations and on the same dates when molluscs were sampled, this information should be considered only as an approximation. However, this kind of integration could be useful as a line of evidence in ecological risk assessment, as has been recently studied by Walker et al. [55]

The \(\sum PAHs\) concentrations in molluscs from the Basque coast, obtained in the present study (17–10228 µg kg\(^{-1}\)), for the period 2003–2011, are relatively low in comparison to the concentrations of PAH in the surface sediments of the Basque estuaries (11–152755 µg kg\(^{-1}\)), obtained by Legorburu et al. [16] for the period 2009–2012. However, the composition profiles for both
molluscs (the present study) and sediments [16] followed a similar distribution, with the mass 202 and 252 compounds being dominant. In fact, mussels located close to sediment or in a high turbidity water column could be enriched in higher molecular weight and less-water-soluble PAH. [36] On the other hand, for both matrices, the PAH ratios showed combustion as the main source of PAH.

5. Conclusions

The highest levels of parent PAH along the Basque coast were found in molluscs located close to the major cities and industrialised areas, such as the Ibaizabal and Oiartzun estuaries; the lowest were observed in the mouth of the Lea and Barbadun estuaries. PAH bioaccumulation, for the period 2003–2007, showed some seasonality throughout this period; further, significant differences were observed between cold and warm seasons.

When autumn data for the period 2003–2011 is considered, for status and trend assessment, the highest percentages of samples above the established EQS and EAC are observed nearby the major cities. However, no clear temporal trends were detected throughout the study area, probably related to human activities undertaken.

Concerning the PAH sources within the Basque coast, an industrialised and densely populated area, it is likely that the main PAH source is input from urban/industrial combustion processes. However, the PAH composition profiles and diagnostic ratios have identified natural and petrogenic sources, which cannot be disregarded.

Acknowledgements

Data used in this contribution were obtained from the projects ‘Monitoring Network for the Ecological Status Assessment of Transitional and Coastal Waters within the Basque Coast’ and ‘IMPRES’ undertaken by AZTI-Tecnalia for the Basque Water Agency and the Basque Government, respectively. We wish to thank AZTI-Tecnalia staff for assistance in the collection of data and Professor Michael Collins (School of Ocean and Earth Science, University of Southampton (UK) and AZTI-Tecnalia, Marine Research Division), for advising us on some details of this paper. The suggestions from two anonymous reviewers and the editor of the Journal have considerably improved the first version of this manuscript. This is contribution number 708 from AZTI-Tecnalia Marine Research Division.

Disclosure statement

No potential conflict of interest was reported by the authors.

Supplemental data

Supplemental data for this article can be accessed at 10.1080/02757540.2015.1051041.

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