

Spatial and temporal trends of petroleum hydrocarbons in wild mussels from the Galician coast (NW Spain) affected by the *Prestige* oil spill

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) were determined in tissues of wild mussels (*Mytilus galloprovincialis*) from the Galicia coast (NW Spain) in order to assess the extent of the environmental impact caused by the *Prestige* oil spill (November 13, 2002). Three sampling campaigns were carried out in February, June and November 2003 at 24 stations along the Galicia coast, from La Guardia (Pontevedra) to Ribadeo (Lugo). The spatial distribution of PAHs found in the first sampling period, clearly revealed the central area (Costa da Morte) as the most affected by the oil spill. In these stations, concentrations up to 7780 µg/kg dw of the sum of 13 parent PAHs were found 2–3 months after the spill. Molecular parameters within the aliphatic and aromatic fractions confirmed the presence of the *Prestige* oil in these samples. The levels markedly decreased at most of the stations in the second sampling and recovered to levels found before the spill in November 2003, 1 year after the accident (29–279 µg/kg dw, av. 133±83 µg/kg dw). However, a certain increase was observed in some sites which could be related to the remobilization of oil residues from still unclean intertidal spots or sediments due to the winter marine weather conditions.

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1. Introduction

On November 13, 2002, the *Prestige* tanker, transporting about 77,000 tonnes of heavy fuel-oil, began to leak oil in front of Costa da Morte (Galicia, NW Spain), and 6 days later, after an erratic towing, broke in two and sank at 240 km off the Northwest coast of Spain

(Albaigés et al., 2006). The oil (No. 6 type) had a density of 0.995 kg L⁻¹ (at 15 °C) and contained 22% of aliphatic hydrocarbons, 50% of aromatic hydrocarbons and 28% of resins and asphaltenes. The spill affected more than 800 km of the Spanish Atlantic shores and had dramatic ecological and economic consequences for the Galician coastal ecosystem, one of the first producers of mussels worldwide (*Mytilus galloprovincialis*), which should be closed to commercial harvesting.

Soon after the accident a monitoring program was established by the Instituto Español de Oceanografía (IEO) in order to assess the spatial distribution and

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temporal evolution of petrogenic hydrocarbons in the affected area, which involved the sampling of water (González et al., 2006), sediments (Franco et al., 2006) and indigenous populations of mussels.

The use of bivalves as bio-indicators of chemical contamination is widely accepted and is recommended by the International Conventions, such as the OSPAR Commission, the Barcelona Convention and the Helsinki Commission. In this respect, they are widely used in coastal monitoring programmes i.e. the *Mussel Watch* in USA and the *Réseau National d'Observation* (RNO) in France (O'Connor, 1996; RNO, 2002). The interest in using these organisms relies on the fact that they are permanent inhabitants of specific sites and rather resistant to local pollution; they are also filter-feeders and have only a limited metabolic ability. Further, contaminants present in the water column, which may have not been recorded in the sediments, can accumulate in bivalve tissues to levels several orders of magnitude higher than those found in the surrounding seawater (NAS, 1980; Neff, 2002).

In the past, mussels have been successfully used to evaluate the impact of oil spills in coastal habitats, such as the *Exxon Valdez* in the Prince William sound in Alaska (Carls et al., 2001, 2004), the *Aegean Sea* in NW Spain (Porte et al., 2000) and the *Sea Prince* in Korea (Yim et al., 2002) but little information is available on their application to monitoring heavy oil spills (Tronczynski et al., 2004). In this case, it should be taken into account that besides accidental oil spills, hydrocarbons are ubiquitous in the marine environment, so that mussels are chronically exposed to these pollutants, which are linked, among others, to shipping activities (e.g. deballasting waters), urban/industrial runoff and atmospheric deposition. Therefore, the assessment of oil pollution requires the development of analytical techniques that are able to differentiate spill residues from other sources. Characteristic molecular patterns within the aliphatic and aromatic hydrocarbon fractions have been used for this purpose. These include steranes and triterpanes as well as the relative distributions of alkylated polycyclic aromatic hydrocarbons (PAHs) (Bence and Burns, 1995; Porte et al., 2000).

Steranes and triterpanes were suggested as passive tags for source recognition of oil spills by mass fragmentography (Albaigés and Albrecht, 1979). Although these compounds are not known to be toxic to marine organisms, they are useful in marine pollution monitoring as they are quite resistant to degradation and are related to the origin of the oil, so are of diagnostic value for the identification of the hydrocarbon sources. On the other hand, PAHs may be divided in two groups: those

with two and three aromatic rings, that in general are only moderately toxic, and those of high-molecular-weight, containing four or more aromatic rings, many of which are mutagenic and teratogenic (Meador et al., 1995). PAHs originated at high temperatures (combustion) are dominated by the parent species, in contrast to crude oils, that contain a wide range of alkyl-derivatives (Youngblood and Blumer, 1975). Mussels are exposed to both types of hydrocarbons, the former preferentially associated with the suspended particulate matter and the latter being mainly in the dissolved phase and more bioavailable (Porte et al., 2000).

In order to assess the extent of the impact caused by the *Prestige* oil spill on the Galician coastal ecosystem (NW Spain), using the *Mussel Watch* concept, three sampling campaigns were carried out in February, June and November 2003 at 24 stations along the coast, from La Guardia (Pontevedra) to Ribadeo (Lugo) (Fig. 1). The samples were analysed for aliphatic and aromatic hydrocarbons to determine the hydrocarbon levels and temporal trends following the accident, and identify the sources. These stations are regularly monitored for PAHs as part of the current IEO monitoring programme, the existence of previous data was important for comparing the contamination levels before and after the spill, and to agree on baseline reference values.

2. Materials and methods

2.1. Sample handling

Wild mussels were collected manually and stored at $-20\text{ }^{\circ}\text{C}$ until analysis. At least 50 specimens, of all sizes were pooled as a composite sample representative of each location and sampling period. Once the samples were defrosted, mussels were homogenised and freeze-dried.

2.2. Chemical analyses

2.2.1. Reagents

A PAH mixture containing naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenzo[*ah*]anthracene and benzo[*ghi*]perylene at 10 mg/L each in cyclohexane was obtained from Dr. Ehrenstorfer (Augsburg, Germany). The following perdeuterated standards, used as surrogates, were obtained from Cambridge Isotope Laboratories (Andover, USA): naphthalene- d_8 (99%), anthracene- d_{10} (98%), pyrene- d_{10} (98%) and benzo[*a*]

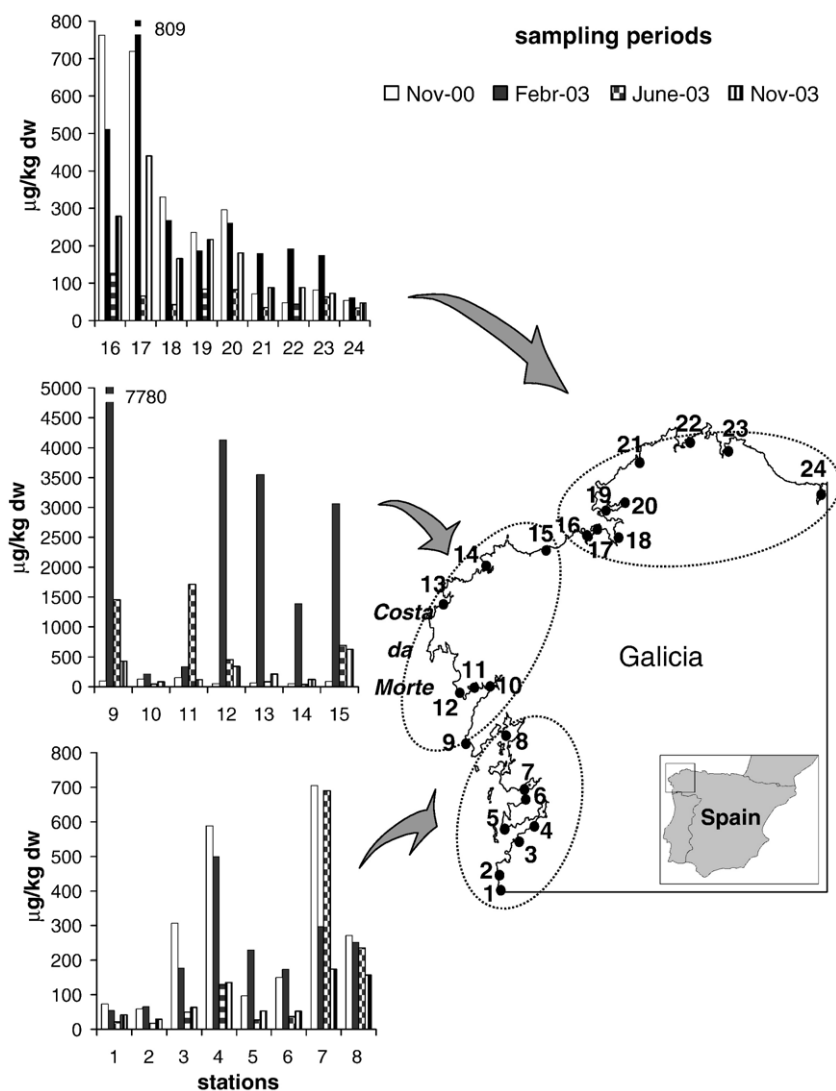


Fig. 1. Location of mussel sampling sites along the Galician coast, and PAH concentrations ($\mu\text{g/kg dw}$) in October 2000 and February, June and November 2003.

pyrene- d_{12} (98%). Decafluorobiphenyl was from Merck (Hohenbrunn, Germany). Suprasolv grade methanol, hexane and dichloromethane GR for analysis, was obtained from Merck (Darmstadt, Germany). Silica gel (0.063–0.2 mm) for analysis was also obtained from Merck and extracted with dichloromethane before use and then activated at 120 °C overnight. Anhydrous sodium sulfate for analysis and aluminium oxide 90 active neutral for column chromatography were also obtained from Merck and were activated at 400 °C and left at 120 °C overnight.

2.2.2. Analytical procedure

About 2–4 g of mussel dried tissues from each station and sampling period were Soxhlet extracted with

a 1:3 acetone:hexane mixture for 12 h. Samples to be analysed by high performance liquid chromatography (HPLC) were submitted to a clean-up step by column chromatography on deactivated alumina (10% water) and hexane elution.

The 13 PAHs (phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*e*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenz[*ah*]anthracene and benzo[*ghi*]perylene) were determined by HPLC (HP 1100 apparatus, Agilent Technologies, Palo Alto, CA, USA) coupled with a wavelength programmable fluorescence detector (HP 1036, Agilent Technologies, Palo Alto, CA, USA). The column (Vydac 201 TP, Grace Vydac, Hesperia, CA, USA) was kept at 23.5 ± 0.1 °C and

eluted with a methanol:water gradient, starting with 30% methanol (0–0.5 min), then increasing the methanol content to 80% (0.5–5 min), and finally to 100% methanol (5–62 min) that was held for 1 min. After this gradient, two steps, one for cleaning with a mixture of methanol–acetone (1:1) for 11 min and another one for reconditioning the column with 30% methanol, were carried out.

Certified solutions, supplied by Dr. Ehrenstorfer were used in the quantification, using a multilevel calibration at six points between 25 and 350 $\mu\text{g kg}^{-1}$ for each of the compounds. The 2-methylchrysene was employed as an internal standard. The analytical method was subject to a continuous external quality control process by the participation in the Quality Assurance of Information for Marine Environmental Monitoring in Europe exercises (QUASIMEME II, 2003, 2004).

The limit of detection (LOD) was in the range of 0.1 to 0.4 $\mu\text{g kg}^{-1}$ dw for phenanthrene to indeno[1,2,3-*cd*]pyrene. The reproducibility of 6 replicates was better than 70–90%. Procedural blanks were run for each set of samples.

Alternatively to the previous procedure, an aliquot of the sample (2 g dw) was spiked with perdeuterated naphthalene (193 ng), anthracene (171 ng), pyrene (103 ng) and benzo[*a*]pyrene (172 ng) in ethyl acetate and Soxhlet extracted with 100 mL of dichloromethane for 24 h. The extracts were cautiously evaporated and solvent exchanged to hexane to a final volume of around 0.5 mL and fractionated by column chromatography with 6 g of neutral alumina (top) and 6 g of silica gel (bottom) both 5% deactivated with MilliQ water. Two fractions were collected; the first one containing the aliphatic hydrocarbons, eluted with 20 mL of hexane, and the second containing the PAHs, eluted with 50 mL of hexane:dichloromethane (80:20). The collected fractions were concentrated with a rotary evaporator and under a gentle nitrogen stream to 0.5 mL.

The second fraction was solvent exchanged to dichloromethane (1 mL) and cleaned by gel permeation chromatography using a BioBeads S-X12 column (45×1.0 cm) (Teknokroma, Sant Cugat, Spain) with a 0.5 mL loop and dichloromethane as mobile phase at 3 mL min^{-1} . The eluate between 5 and 8.5 min was collected and concentrated under a gentle nitrogen stream and solvent exchanged to hexane (1 mL).

The isolated fractions were analysed by gas chromatography coupled to mass spectrometry using a Trace Thermo-Electron Corporation (Austin, TX, USA) apparatus in the electron impact mode at 70 eV. Injection was performed in the splitless mode at 280 °C using hexane as a solvent. A 30 m×0.25 mm ID capillary column coated with 0.25 μm of DB-5MS stationary phase (J and W

Scientific, Folsom, CA, USA) was temperature programmed as follows: at 60 °C for 1 min, until 200 °C at 10 °C/min and finally to 320 at 4.8 °C min^{-1} , holding that temperature for 10 min. Transfer line and ion source temperatures were held at 250 and 200 °C, respectively. Acquisition was performed in the full scan mode from 50 to 350 amu at 2 scans s^{-1} and starting after 6 min.

Quantification of PAHs was conducted from the reconstructed ion chromatograms obtained from the molecular ion by the internal standard procedure using decafluorobiphenyl and recovery correction. Recoveries ranged from 50–60% for naphthalene and from 70 to 100% for anthracene, pyrene and benzo[*a*]pyrene deuterated. Blanks were lower than 0.09 $\mu\text{g kg}^{-1}$ dw (from naphthalene to anthracene) and 0.03 $\mu\text{g kg}^{-1}$ dw (from phenanthrene to dibenzo[*ah*]anthracene). The LOD in the full scan mode ranged from 0.68 to 3.4 $\mu\text{g kg}^{-1}$ dw.

3. Results and discussion

3.1. PAH concentrations in wild mussels

The total concentrations of the 13 parent PAHs determined in the collected samples along the 2003 are shown in Table 1. In February 2003, 2–3 months after the *Prestige* oil spill, the higher concentrations were observed in the area between Corrubedo and Caion (Costa da Morte), where the levels were above 1000 $\mu\text{g kg}^{-1}$ dw and up to 7780 $\mu\text{g kg}^{-1}$ dw in Corrubedo (station 9), one of the most affected sites by the spill (Fig. 1). However, the samples collected inside the Muros Ría (stations 10 and 11) showed much lower levels, possibly indicating that the fuel-oil hardly reached the inner areas, as already foreseen by the oceanographic conditions of the region (Álvarez-Salgado et al., 2006). The high values found inside the estuaries (Rías) of Vigo (st. 4) and A Coruña (st. 16–17), in the range of 500–800 $\mu\text{g kg}^{-1}$ dw, were attributed to the vicinity of urban and industrial/harbour areas. Earlier surveys already identified these sites as the major coastal “hot spots” in the region (Soler et al., 1989).

The lower values, ranging from 54 to 65 $\mu\text{g kg}^{-1}$ dw, were found near the Portuguese coast (stations 1 and 2) and at station 24 (Ribadeo) further north, where the incidence of the spill was barely discernible. The rest of stations exhibited moderate levels, between 174 and 297 $\mu\text{g kg}^{-1}$ dw.

In June 2003, a general decrease in the total PAH concentrations occurred in all stations, although the levels were still high in some of the Costa da Morte (e.g. st. 9 and 11). This general decline may reflect a depletion of the *Prestige* oil pollution but can also be enhanced by

Table 1

Total concentrations of 13 parent PAHs* (in $\mu\text{g/kg dw}$) in wild mussels from the Galician coast at the indicated dates

Stations		Location		Sampling dates			
No.	Site	Lat. N	Long. W	Oct-2000	Febr-2003	June-2003	Nov-2003
1	La Guardia	41°52.289	08°52.487	73	54	21	42
2	Sta. Ma. Oia	41°58.212	08°53.199	59	65	17	29
3	Vigo–Samil	42°13.177	08°46.604	306	176	50	63
4	Vigo–La Guía	42°15.604	08°42.133	589	499	129	135
5	Cabo Home	42°15.007	08°52.333	97	229	27	53
6	Pontevedra–Loira	42°22.198	08°44.387	150	174	37	52
7	Pontevedra–Raxó	42°24.172	08°44.968	705	297	690	174
8	Arosa–Chazo	42°36.374	08°51.761	272	252	235	157
9	Corrubedo	42°34.684	09°04.928	101	7782	1455	430
10	Muros–Freixo	42°47.207	08°57.012	129	218	43	85
11	Muros–S. Antón	42°47.204	09°02.765	154	337	1713	121
12	Punta Insua	42°46.423	09°07.550	54	4131	456	342
13	Muxía	43°06.057	09°12.788	63	3548	82	214
14	Corme	43°14.569	08°56.642	53	1393	37	125
15	Caion	43°18.574	08°38.072	86	3059	692	625
16	Coruña–Torre	43°22.178	08°23.160	762	511	126	279
17	Coruña–Mera	43°22.946	08°20.639	720	809	65	440
18	Ares	43°22.549	08°12.842	330	268	42	166
19	Ferrol–La Palma	43°27.770	08°16.191	235	186	85	216
20	Ferrol–Pías	43°29.112	08°11.557	296	261	83	181
21	Cedeira	43°38.638	08°05.024	71	179	35	88
22	Espasante	43°43.346	07°48.302	48	192	44	88
23	Vivero	43°41.275	07°35.036	82	174	63	73
24	Ribadeo	43°31.570	07°01.032	54	61	34	47

Stations correspond to those indicated in Fig. 1.

* Sum of phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, benzo[ghi]perylene, dibenz[ah]anthracene, indeno[1,2,3-cd]pyrene.

a seasonal variability. In fact, *M. galloprovincialis* has two spawning periods in Galicia, one in autumn and another, of greater intensity, in spring, during which feeding activity and lipid concentrations are reduced (Alonso et al., 2001), and subsequently hydrophobic contaminant bioaccumulation. The decrease of PAH concentrations in late spring/early summer, matching the mussels spawning period, has also been described in other studies (Webster et al., 1997).

During this period, increasing concentrations were observed in stations located in the inner parts of certain embayments (e.g. 7 and 11), which can be the result of incidental urban and/or industrial inputs. The concentrations at the stations more distant than Costa da Morte ranged from 17 to 63 $\mu\text{g kg}^{-1}$ dw (av. 36 $\mu\text{g kg}^{-1}$ dw), probably representing the baseline levels for the region.

The situation in November 2003 at the stations of the most affected area (st. 9–15) followed a steady decreasing trend, whereas those situated northern and southern, less affected by the oil spill, exhibited concentration levels close to those found in the previous sampling campaign (29–88 $\mu\text{g kg}^{-1}$ dw). However, a certain increase was observed in many other sites (166–

440 $\mu\text{g kg}^{-1}$ dw), which could be related to the remobilization of oil residues from still unclean intertidal spots or sediments due to the winter marine weather conditions. It is interesting to note that in the survey of PAHs in coastal waters, carried out during 2003 in the same area, a similar increasing concentration trend was observed in winter, particularly in the bottom waters (González et al., 2006). This, together with the higher lipid contents of mussel tissues in autumn and winter, before the spawning period, may explain the observed increasing trend.

The range of PAH concentrations (sum of 13) found in mussels in a pre-spill survey carried out in 2000 was from 48 $\mu\text{g kg}^{-1}$ dw at station 22 (Espasante) to 762 $\mu\text{g kg}^{-1}$ dw at station 17 (A Coruña), but the majority of stations (15 out of 24) exhibited values lower than 200 $\mu\text{g kg}^{-1}$ dw. These concentrations were also comparable with those found in mussels collected 3 years after the *Aegean Sea* accident, in the area of A Coruña (Porte et al., 2000), and the *Erika*, along the Bay of Biscay (Tronczynski et al., 2004). Similar values were found as well in other European coastal areas. Besides some high levels easily recognizable as “hot

spots” (e.g. proximity to harbours or urban areas), values of 23–324 $\mu\text{g kg}^{-1}$ dw of the 14 parent PAHs (including perylene) have been reported either in the Mediterranean or in the Atlantic coasts (Baumard et al., 1998). Concentrations of 90–940 and 45–505 $\mu\text{g kg}^{-1}$ dw were found in mussels collected in the German and Danish Baltic coasts, respectively (Baumard et al., 1999; Granby and Spliid, 1995).

In summary, concentrations below 50 $\mu\text{g kg}^{-1}$ dw can be considered as the background pollution in the region, whereas values above 200 $\mu\text{g kg}^{-1}$ dw may reflect the vicinity of urban or industrial hydrocarbon sources and above 500 $\mu\text{g kg}^{-1}$ dw be clearly assigned to “hot spots”. In this respect, the area of Costa da Morte (stations 9–15), just after the spill, and some inner parts of the estuaries (rias), chronically polluted by urban/industrial runoff, may go into the last category, whereas the northern and southern stations (e.g. 1–2 and 22–24) may show baseline values.

3.2. PAH distribution patterns

The relative abundances of the parent and alkylated PAHs may provide useful signatures for recognizing hydrocarbon sources in the environment (Bence and Burns, 1995; Boehm et al., 1997). Representative distributions of parent PAHs are shown in Fig. 2. The mussels collected at Ferrol–La Palma, an urban/industrialized site, apparently not impacted by the spill, contained the whole set of 3- to 6-ring PAHs, currently considered as combustion-derived. Nevertheless, the distributions of their alkylated derivatives (not shown) were consistent with a mixed petrogenic–pyrolytic input, similar to the average distribution found in the 2000 survey for the whole region. Conversely, the mussel distributions in the affected sites (e.g. Muxía, Cabo Home, etc.) were clearly dominated by chrysene, as was also found after the Aegean Sea and Erika oil spills (Porte et al., 2000; Tronczynski et al., 2004). However, in most of these

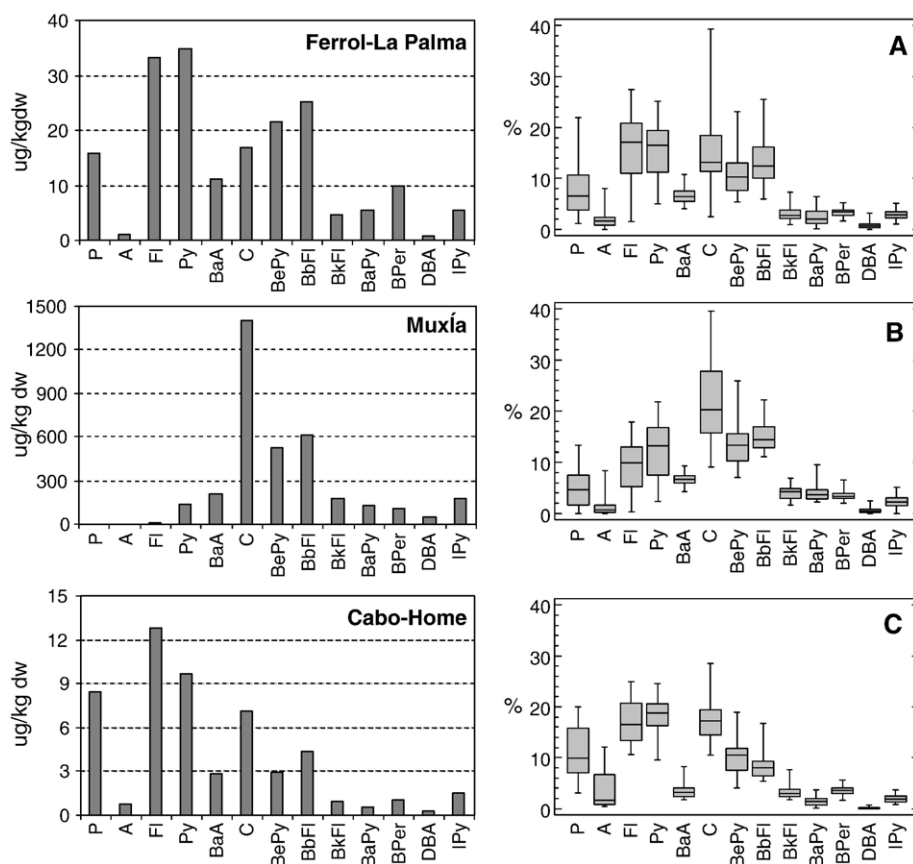


Fig. 2. Representative PAH mussel distributions (left) and average composition (Box- and Whisker plots) for all stations in (A) October 2000 and (B, C) February and November 2003. P: Phenanthrene, A: Anthracene, Fl: Fluoranthene, Py: Pyrene, BaA: Benz[a]anthracene, C: Chrysene, BePy: Benzo[e]pyrene, BbFl: Benzo[b]fluoranthene, BkFl: Benzo[k]fluoranthene, BaPy: Benzo[a]pyrene, BPer: Benzo[ghi]perylene, DBA: Dibenzo[ah]anthracene, IPy: Indeno[1,2,3-cd]pyrene.

stations (e.g. Cabo Home) the distribution patterns 1 year after the spill already resembled those found in the 2000 survey.

A radial presentation of the percentages of each PAH in different stations, along the survey period, as shown in Fig. 3, may also contribute to illustrate the effect of the spill. The distribution profile of Ferrol–La Palma

station was rather conservative from October 2000 to November 2003, besides the *Prestige* event. These profiles were also common to almost all stations monitored in 2000 and November 2003, suggesting the time span required for the recovery of the affected zone.

On the other hand, the stations initially affected by the spill displayed a totally different profile.

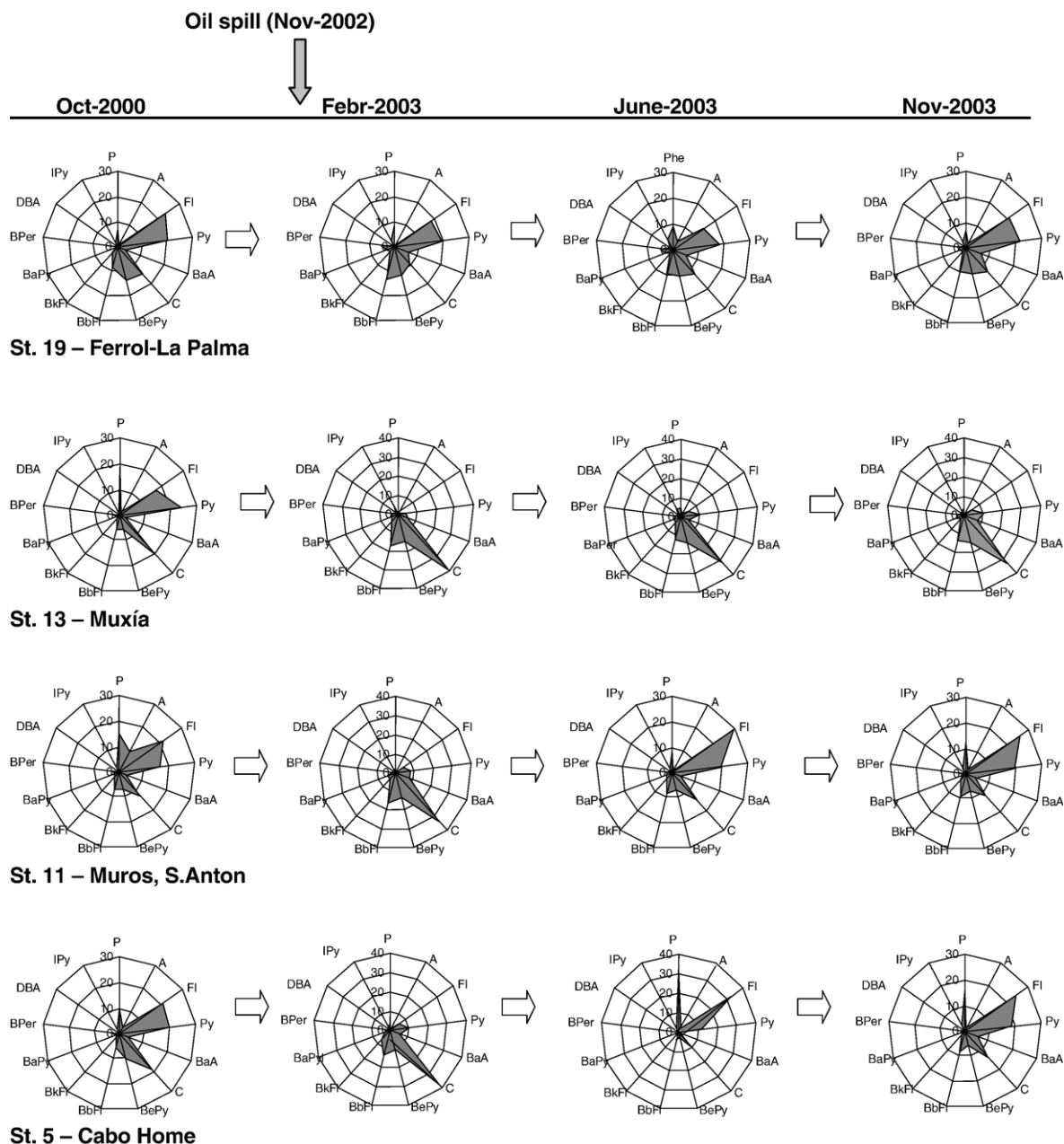


Fig. 3. Radial representation of the PAH distributions in mussels from stations 5, 11, 13 and 19 in October 2000, and February, June and November 2003.

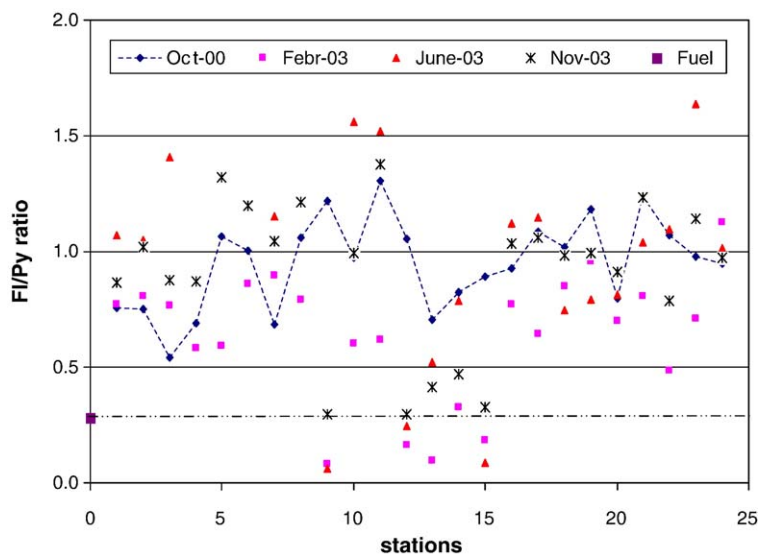


Fig. 4. Fluoranthene/pyrene ratios estimated from all collected mussel samples, and for the *Prestige* fuel oil (— · —). Pre-spill values: - - - - .

Representative examples correspond to the stations of Muros, S. Anton and Cabo Home, which in February 2003 showed the oil impact (by a clear predominance of chrysene), and recovered in June and November. In turn,

the profiles of the Muxía station showed the characteristic oil accumulation from February to November 2003.

From these trends, the fluoranthene/pyrene and chrysene/benzofluoranthenes ratios were found to be

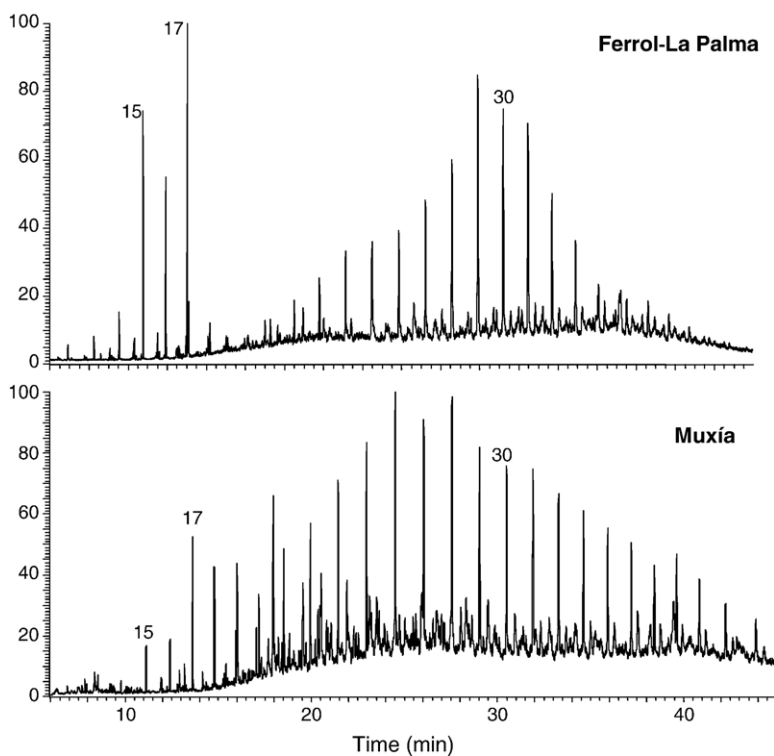


Fig. 5. Representative alkane profiles (m/z 85) in aliphatic fractions of mussels collected in February 2003. Peak numbers indicate the number of carbon atoms of n -alkanes.

adequate indicators for assessing the extent of the oil spill. The average values in the more distant stations (e.g. 1–2 and 20–25) were 0.97 and 1.16, respectively, whereas those at the stations of Costa da Morte were 0.22 and 1.92, which compared satisfactorily with those of the *Prestige* fuel oil (0.28 and 2.40). This is clearly illustrated in Fig. 4, where the individual values of the fluoranthene/pyrene ratio for all samples are shown. As it can be seen, stations 9 and 12–15 exhibit values close to the oil, whereas stations 10 and 11, located at the inner part of the Muros estuary, differed, supporting the previous statement based on total PAH concentrations (Fig. 1), that the oil did not enter the estuary. Moreover, it is interesting to notice that the values of the first survey, in February 2003, were generally in the lower range in the whole set, possibly reflecting the diffusion of the oil in the entire area.

The relative abundance of alkyl substituted over unsubstituted compounds was also used to distinguish

different sources of contamination in the case of the *Exxon Valdez* and *Erika* oil spills (Bence and Burns, 1995; Tronczynski et al., 2004). In stations 9 to 15, the alkylated derivatives were more abundant than the parent compounds, all consistent with the predominance of the petrogenic components. In this respect, the methylphenanthrene/fluoranthene and methylphenanthrene/methyldibenzothiophene ratios were found of diagnostic value. The average values in the more remote stations were 10.5 and 0.05, respectively, whereas those in the stations of Costa da Morte were 75.6 and 0.28, which compared satisfactorily with those of the *Prestige* fuel oil (72.3 and 0.30).

3.3. Molecular fingerprinting

In order to confirm the above relationship of the petrogenic hydrocarbons with the suspected source, namely the *Prestige* fuel oil, the hopane and sterane

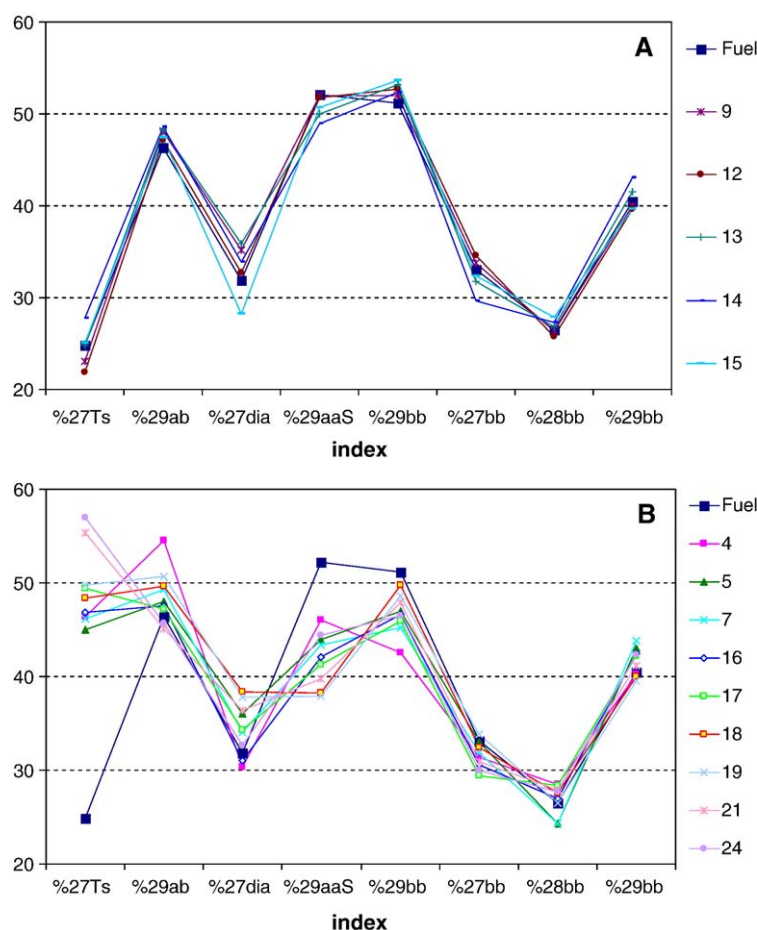


Fig. 6. Sterane and triterpane source diagnostic ratios of hydrocarbons from mussel tissues of samples collected in February 2003, compared with those of the original fuel. A: stations of Costa da Morte. B: other stations. Index definitions are indicated in Table 2.

Table 2

Diagnostic ratios used as source and weathering indicators for the *Prestige* oil samples

Diagnostic ion <i>m/z</i>	Index	Definition	Structures
191	%27Ts	$100 * Ts / (Ts + Tm)$	Ts: 18 α (H)-22,29,30-trisnorhopane Tm: 17 α (H)-22,29,30-trisnorhopane
191	%29 $\alpha\beta$	$100 * 29\alpha\beta / (29\alpha\beta + 30\alpha\beta)$	29 $\alpha\beta$: 17 α (H),21 β (H)-30-norhopane 30 $\alpha\beta$: 17 α (H),21 β (H)-hopane
217	%27dia	$100 * 27d(R+S) / [27d(R+S) + 27\beta\beta(R+S)]$	27d: 13 β (H),17 α (H)-diacholestane (20S and 20R) 27 $\beta\beta$: 14 β (H),17 β (H)-cholestane (20R and 20S)
217	%29 $\alpha\alpha$ S	$100 * 29\alpha\alpha S / (29\alpha\alpha S + 29\alpha\alpha R)$	29 $\alpha\alpha$: 24-ethyl-14 α (H),17 α (H)-cholestane (20S and 20R)
217	%29 $\beta\beta$ RS	$100 * 29\beta\beta(R+S) / [29\beta\beta(R+S) + 29\alpha\alpha(R+S)]$	29 $\beta\beta$: 24-ethyl-14 β (H),17 β (H)-cholestane (20R and 20S)
218	%27 $\beta\beta$	$100 * [27\beta\beta(R+S)] / [27\beta\beta(R+S) + 28\beta\beta(R+S) + 29\beta\beta(R+S)]$	27 $\beta\beta$: 14 β (H),17 β (H)-cholestane (20R and 20S)
218	%28 $\beta\beta$	$100 * [28\beta\beta(R+S)] / [27\beta\beta(R+S) + 28\beta\beta(R+S) + 29\beta\beta(R+S)]$	28 $\beta\beta$: 24-methyl-14 β (H),17 β (H)-cholestane (20R and 20S)
218	%29 $\beta\beta$	$100 * [29\beta\beta(R+S)] / [27\beta\beta(R+S) + 28\beta\beta(R+S) + 29\beta\beta(R+S)]$	29 $\beta\beta$: 24-ethyl-14 β (H),17 β (H)-cholestane (20R and 20S)

profiles were determined in the aliphatic fraction of the bivalve extracts. Representative alkane profiles (*m/z* 85) of a chronically polluted station (Ferrol–La Palma) and one affected by the spill (Muxía) are shown in Fig. 5. The *n*-alkane series of the former shows the planktonic *n*-C15 and *n*-C17 homologs and a range of higher alkanes, centred at *n*-C29, representative of a weathered oil residue. In turn, the affected stations exhibit a wider series, illustrative of more fresh oil.

The diagnostic molecular parameters of the fuel oil and those of representative mussel samples are displayed in Fig. 6. According to the main source markers described elsewhere for the *Prestige* oil (Díez et al., 2005), the most characteristic ones are the tetra and pentacyclic terpane (27Ts and 29 $\alpha\beta$), and the C-29 $\alpha\alpha$ S/R and $\beta\beta/\alpha\alpha$ sterane indices, below and above 50, respectively. The values displayed by the mussel samples collected in the area of Costa da Morte (stations 9, 12–14) (Fig. 6A) closely matched those of the oil, clearly confirming the fuel oil contribution, whereas the more distant stations (Fig. 6B) exhibited patterns with markedly different values.

In conclusion, even though heavy oils are barely dispersed in the water column, they can be uptaken by mussels and recognized in their tissues using the molecular marker approach. 1 year after the accident, concentrations decreased to background levels, although incidental increases may occur as a consequence of remobilization of oil residues from still unclean intertidal spots or sediments due to the winter marine weather conditions. However, even in the case that PAH values may have returned to pre-spill levels in most areas, long-term exposure to PAHs is a potential threat to marine biota and wildlife. Especially in areas where

there can be chronic and uncontrolled PAH contamination from multiple sources.

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