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Accumulation trends of petroleum hydrocarbons in commercial shellfish from the Galician coast (NW Spain) affected by the *Prestige* oil spill

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ABSTRACT

Aliphatic and aromatic hydrocarbons were determined in three species of commercial shellfish, namely razor shells (*Ensis arcuatus* and *Ensis siliqua*), goose barnacle (*Pollicipes cornucopia*) and sea urchin (*Paracentrotus lividus*), living in different habitats and exhibiting different feeding behaviors. The samples were collected monthly, from January 2003 to October 2004, in three stations of the Galicia coast (NW Spain), following the *Prestige* oil spill, with the aim of assessing their response to the spill and, therefore, their suitability for monitoring purposes.

The aliphatic fractions were mostly dominated by biogenic hydrocarbons, reflecting the diet composition of the organisms and their low metabolic capacity. The presence of oil was assessed by the determination of chemical markers. The analysis of the aromatic fractions revealed the occurrence of 3–6 ring parent and alkylated PAHs, consistent with a mixed petrogenic–pyrolytic origin, with the common feature of the predominance of chrysene in all samples collected after the spill. However, the distributions exhibited both temporal and interspecies variations.

The PAH concentrations (Σ13) increased significantly after the spill and decreased 6–7 months later close to background levels for the region. One year after the accident, the median values were: 58 μg/kg for razor shells, 26 μg/kg for barnacles, and 25 μg/kg for sea urchins. The temporal evolution of the PAH concentrations along the survey period was used to estimate loss rates for bioavailable PAHs in barnacles and sea urchins after the spill. Half-life values were in the order of 30 and 60 d, respectively. The results of the study demonstrate that barnacles can be suitable species for oil spill monitoring.

1. Introduction

On November 13, 2002, the 26-year old single-hulled oil tanker *Prestige*, transporting about 77,000 tonnes of heavy fuel-oil, while on route from Latvia to Singapore, suffered hull damage in the region of Cape Finisterre, approximately 50 km off the coast of Northwest Spain, due to a heavy sea and high winds. The tanker began to leak oil and after a denied safe refuge in Spain and Portugal the ship was towed further out to sea, in an attempt to avoid a dramatic impact on the economically and socially sensitive upper and lower estuaries (*rías*) of the Galician coast. Six days later, the tanker broke in two and both parts sank 240 km offshore at 3500 m water depth (Albaigés et al., 2006).

The spill of some 60,000 tonnes of fuel affected more than 600 km of the Spanish Atlantic shores that were heavily distressed by the stranding of emulsified oil or large quantities of tar balls. The accident had dramatic economic consequences for the Galician coastal ecosystem, one of the first producers of mussels worldwide, which should be closed during one year for 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 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 (Soriano et al., 2006). The use of bivalves as bioindicators 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 (O'Connor, 1996; RNO, 2002), and have also been found of application in past oil spills in Galicia (Porte et al., 2000). However, the present monitoring program encompassed also the sampling of a large variety of biota of commercial value, for regulatory purposes regarding public health issues. Among these species, benthic organisms, like sea urchins,

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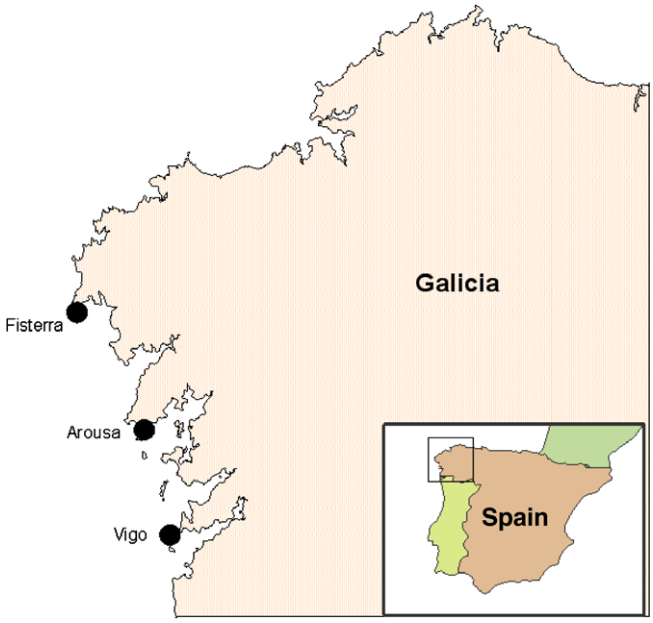

Fig. 1. Location of sampling sites along the Galician coast.

razor shells and goose barnacles were of particular importance because they were largely harvested (700, 270 and 420 tonnes/year, respectively) with an income value of around 20 M Euros/year. Moreover, they inhabited exposed areas whereas other benthic species, like clams, cockles and oysters existed in internal areas of the estuaries (*rías*) not affected by this spill.

These species were surveyed monthly, along the year following the spill, in the area more heavily affected (Fig. 1). They belong to different groups and live in different habitats, so the aim of the present study was to asses their response to the spill and, incidentally, their suitability for monitoring purposes. In fact, there is little information on the use of these species as pollution biomonitors (Shaw and Wiggs, 1980; Glegg and Rowland, 1996; Peña-Mendez et al., 1999; Al-Hassan et al., 2000).

2. Materials and methods

2.1. Commercial species

The razor shells (*Ensis arcuatus* and *Ensis siliqua*), like mussels, are bivalves but live buried in the sandy sediment. The goose barnacle (*Pollicipes cornucopia*) is a crustacean, inhabiting very exposed rocky shores. Finally, the sea urchin (*Paracentrotus lividus*) is an echinoderm that lives on rocky coasts. The three species also present differences in the feeding behavior, the razor shell being a typical filter feeder bivalve, the sea urchin feeding on plant and animal organic matter but preferentially on some types of algae and, finally, the goose barnacle collects the suspended particulate matter with their cirri, modified feathery legs used to sweep the water like sieves, collecting especially plankton.

2.2. Sample handling

Samples were collected manually and stored at −20 °C until analysis. At least 10 specimens were pooled as a composite sample representative of each location and sampling period. Once the samples were defrosted, the eatable part of the organism, namely, the whole soft body for razor shell and goose barnacle and the gonads for the sea urchin, was separated and homogenised.

2.3. Chemical analyses

2.3.1. Reagents

A standard solution containing the 16 EPA PAHs (10 ng/μL in cyclohexane) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Perdeuterated standards (naphthalene-d_8, anthracene-d_{10}, pyrene-d_{10} and benzo[*a*]pyrene-d_{12}, used as surrogates, were obtained from Cambridge Isotope Laboratories (Andover, USA). Suprasolv grade methanol, hexane and dichloromethane GR for analysis were obtained from Merck (Darmstadt, Germany). Silica gel (0.063–0.2 mm) and alumina 90 active neutral for column chromatography were also obtained from Merck.

2.3.2. Hydrocarbons analysis

About 8–10 g of wet tissue from each station and sampling period were mixed with sodium sulphate and Soxhlet extracted with acetone:hexane (1:3) for 12 h. and analysed as described elsewhere (Soriano et al., 2006). HPLC was used for a primary survey of parent PAHs, according to the current monitoring programme in the region, and GC–MS for full characterisation of the aliphatic and aromatic fractions.

In summary, samples to be analysed by HPLC were submitted to a clean-up step by column chromatography on deactivated alumina (10% water) and hexane elution. Thirteen PAHs were determined by HPLC (HP 1100, Agilent Technologies, Palo Alto, CA, USA) coupled with a wavelength programmable fluorescence detector (HP 1036, Agilent Technologies, Palo Alto, CA, USA) and using, respectively, the following excitation and emission wavelengths: 250 and 370 nm (phenanthrene, anthracene), 287 and 465 nm (fluoranthene), 274 and 405 (pyrene, chrysene, benz[*a*]-anthracene, benzo[*e*]pyrene), 290 and 415 nm (benzo[*b*]fluoranthene), 267 and 400 nm (benzo[*k*]fluoranthene, benzo[*a*]pyrene), 300 and 415 nm (dibenz[*ah*]anthracene, benzo[*ghi*]perylene) and 247 and 476 nm (indeno[1,2,3-*cd*]pyrene).

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 minute. 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 μg 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 0.1–0.4 μg kg^{-1} dw for phenanthrene and indeno[1,2,3-*cd*]pyrene, respectively. The reproducibility of 6 replicates was better than 70–90%. Procedural blanks were run for each set of samples.

The extracts to be analysed by GC–MS were 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 rotary evaporated and concentrated, under a gentle stream of nitrogen, to 0.5 mL.

The second fraction was solvent exchanged to dichloromethane (1 mL) and cleaned by gel permeation chromatography using a Bio-Beads 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 aliphatic and aromatic 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&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 corresponding molecular ions by the internal standard procedure using decafluorobiphenyl and recovery correction. Recoveries ranged from 50% to 60% for deuterated naphthalene and from 70% to 100% for anthracene,

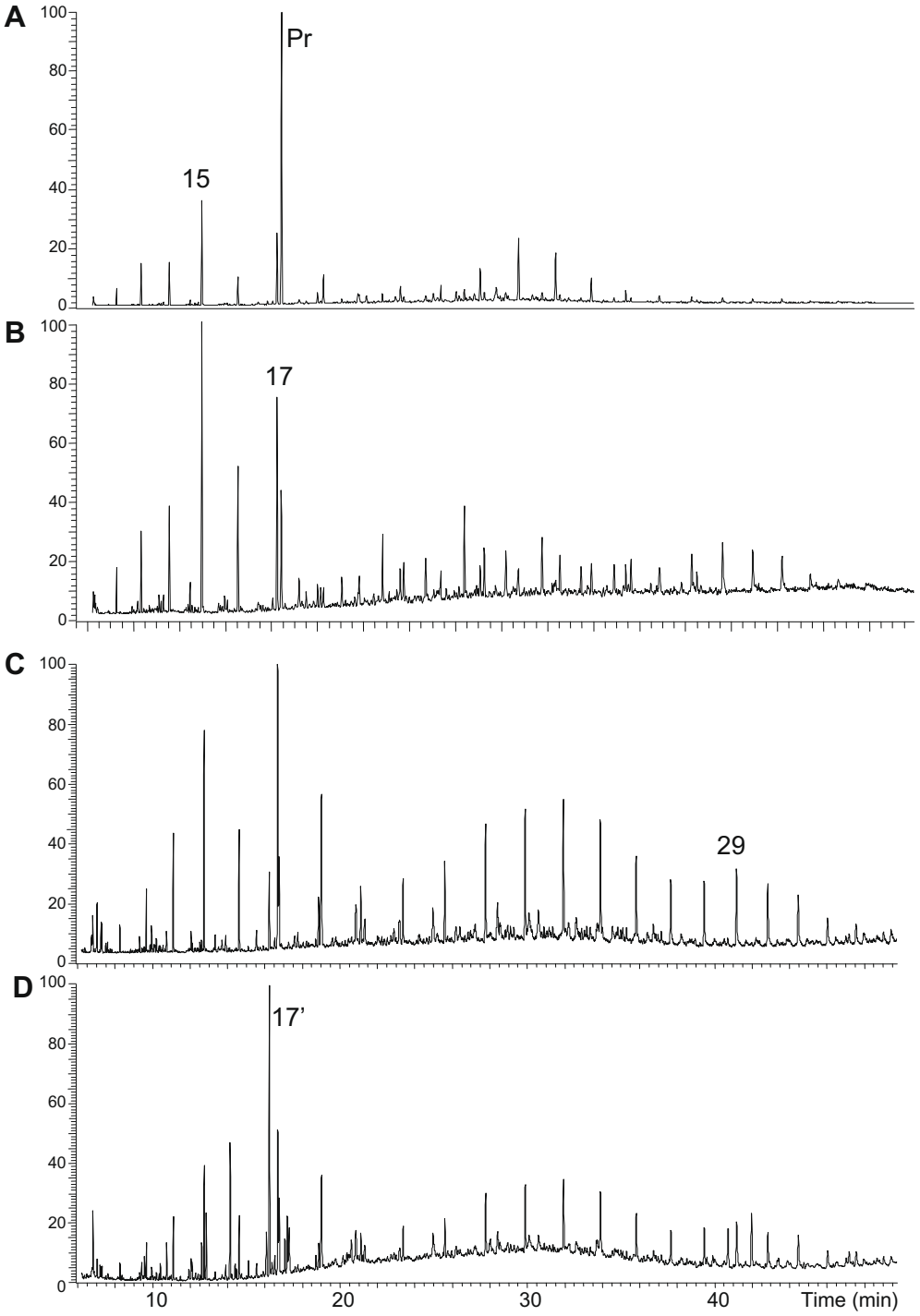

Fig. 2. Representative profiles of aliphatic hydrocarbons of barnacle (A: m/z 85), razor shell (B: m/z 85) and sea urchin (C: m/z 85, and D: TIC) samples, collected at Fisterra station in April 2003. Numbers over the peaks indicate the number of carbon atoms of *n*-alkanes. 17′: *n*-heptadecene. Pr: pristane.

pyrene and benzo[*a*]pyrene. Blanks were lower than 0.09 μg kg^{-1} dw (from naphthalene to anthracene) and 0.03 μg kg^{-1} dw (from phenanthrene to dibenzo[*ah*]anthracene). The LOD in the full scan mode ranged from 0.7 to 3.4 μg kg^{-1} dw for phenanthrene and benzo[*a*]pyrene, respectively.

2.3.3. Statistical data analysis

Statistical data treatment was carried out with the SPSS 13.0 package (SPSS Inc., Chicago, USA). Concentration ranges, means, medians and lower and upper quartiles (Box-and-Whisker plots) were analysed in order to characterise the populations. Median values were generally considered as a better representation of each data set. The normality of the datasets for the different populations was evaluated by means of the Kolmogorov–Smirnov test. Since concentration values were not normally distributed, the non-parametric Kruskall–Wallis test was used for comparing more than two groups of data. The differences among data sets were considered statistically significant when the *p*-value was lower than 0.05 (probability of 95%).

3. Results and discussion

3.1. Aliphatic fraction

Representative profiles of the aliphatic fractions of the different samples are shown in Fig. 2. In general, they are dominated by C_{15} and C_{17} *n*-alkanes and pristane, which are widespread in the marine environment (Farrington and Meyer, 1975), being characteristic of phyto and zooplankton lipids, respectively (Avigan and Blumer, 1968; Blumer et al., 1971). It is interesting to notice that pristane was largely predominant in goose barnacles, whereas the phytoplankton alkanes were more abundant in the other species. The *n*-heptadecene, often dominant in many algal species (Youngblood and Blumer, 1973), was also present in all species, particularly in the sea urchins, which preferentially feed on plant organic matter. Previous studies have also shown this type of profiles in barnacles (Morris, 1973) and sea urchin species (Mironov et al., 1981; Serrazanetti et al., 1995; Peña-Mendez et al., 1999), reflecting their diet composition and subsequent low metabolic capacity.

However, the samples collected in April 2003, three months after the oil spill, exhibited an *n*-alkane modal distribution extending up to C_{40}, which can be attributed to a petrogenic source.

In order to assess the presence of the *Prestige* oil in those samples, a detailed study of the triterpane and sterane fossil markers, currently used for oil spill fingerprinting (Daling et al., 2002), was carried out. The corresponding molecular indices shown in Fig. 3 indicate that the samples collected in April 2003 exhibited some features closer to those of the *Prestige* oil (Díez et al., 2005), which could suggest an accumulation of the spilled hydrocarbons. The different values clearly move away in the samples collected some months later (i.e. August 2003), pointing to a significant reduction of the impact of the spill until the background pollution in the region, as previously observed in areas of the Galicia coast not affected by oil spills (Soriano et al., 2006).

3.2. Aromatic fraction

The total ion chromatograms of the aromatic fractions showed a number of resolved peaks corresponding to polyunsaturated alkanes of biogenic origin, among them heneicosa-3,6,9,12,15,18-hexaene (HEH), highly branched isoprenoids (HBI) and squalene. HEH is abundant in plankton species, notably diatoms, and has been associated to spring blooms (Osterroht et al., 1983), although it was scarce in the region (Soriano et al., 2006) and consequently

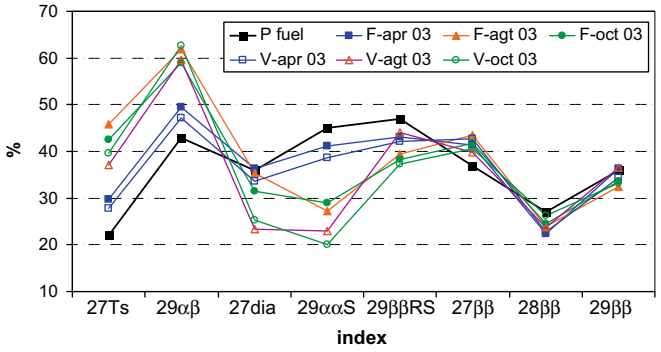

Fig. 3. Triterpane (Ts and 29αβ) and sterane (27dia, 29ααS, 29ββRS and 27–29ββ) source indicators of petrogenic hydrocarbons from sea urchins collected in Fisterra (F) and Vigo (V), compared with those of the *Prestige* fuel (P-fuel). Index definitions as in Soriano et al. (2006).

in the present samples. HBI have also been found in diatoms and widely distributed in the marine environment, particularly in sediments (Wraige et al., 1999). Consistently, they have been found rather abundant in the benthic razor shell. The occurrence of these compounds in the region has already been reported in bivalves that may suggest a favourable habitat for these planktonic species (Porte et al., 2000). Squalene is a lipid constituent of most marine organisms and particularly of phytoplankton (Bieger et al., 1997). However, the high concentration in some species (e.g. sea urchins) may also be related to the advanced development of gonads. In fact squalene plays an important role in the biosynthesis of cholesterol, which becomes the precursor of steroid hormones and is present even in high quantities, during the development of eggs.

The corresponding GC–MS (single ion monitoring) analysis revealed the occurrence of the whole series of 3–6 ring parent and alkylated PAHs, consistent with a mixed petrogenic–pyrolytic origin. However, the distributions exhibited both temporal and interspecies variations. These are illustrated in Fig. 4, where representative profiles of the parent compounds for the three species in two sites (Fisterrra and Vigo) with one year difference (April 200 and 2004) are shown.

In general, the profiles of each organism were rather similar irrespectively of the site, thus strengthening the significance of the temporal and interspecies variations. A common feature was the predominance of chrysene in all samples collected after the spill, that was already observed in mussels collected in the same sites (Soriano et al., 2006) and also after the *Aegean Sea*, *Erika* and *Nakhodka* oil spills (Porte et al., 2000; Tronczynski et al., 2004; Koyama et al., 2004). Several authors (Varanasi et al., 1985; Meador et al., 1995a) already noticed a differential accumulation of major groups of PAHs in infaunal organisms, in which 3- and 5-ring PAHs were poorly uptaken when compared with 4-ring compounds that, apparently, offer the optimum uptake efficiency. Therefore, we have currently used this predominance as an indication of recent oil inputs. The higher abundance of alkyl-substituted over unsubstituted PAHs (not shown) also supported the predominance of petrogenic components.

On the other hand, the samples collected one year after were depleted in these components and dominated by phenanthrene and pericondensed derivatives (e.g. fluoranthene); such profiles have been widely reported in slightly polluted coastal areas (Baumard et al., 1998; Hellou et al., 2002; Soriano et al., 2006).

These features indicate that the three species, goose barnacles, razor shells and sea urchins responded to the spill, accumulating petrogenic hydrocarbons (also verified by the quantitative analysis discussed below) and, therefore, being potentially useful for oil spill biomonitoring. However, it is interesting to notice the relative

differences observed in the profiles among the species. Barnacles and sea urchins seem to preserve preferentially the lower components when the oil impact comes to an end whereas razor shells have a more conservative behaviour for all components.

Observations relating uptake to feeding strategy have explained differences in the profiles of the accumulated PAHs in marine invertebrates (Meador et al., 1995a; Law and Hellou, 1999). Physiological factors, including the rates of uptake and elimination (metabolism, diffusion, and excretion), also determine PAH body distributions. Thus, species apparently occupying the same habitat may actually be "sampling" different compartmental hydrocarbons during normal feeding and other life activities. In the present case, and based upon a limited number of studies, all these organisms seem to have reduced metabolic capacity for PAHs, except sea urchins that exhibit a cytochrome P450 monooxygenase system with certain similarities with that present in vertebrates (Den Besten, 1998). Therefore, bioavailability can be considered as the main accounting factor for the contaminant body burden.

The major route of uptake for PAHs in marine organisms has been debated for years but the present profiles seem to reflect, in the case of razor shells their association to the sedimentary habitat, and in the case of sea urchins and barnacles their feeding on water suspended plankton. The PAH profiles of these two compartments are strikingly similar with those of the inhabiting organisms. In general, the plankton profiles collected in the region after the oil spill exhibited a marked predominance of the alkylated low molecular weight components (2–3 aromatic rings), paralleling those of the seawater dissolved hydrocarbons (Salas et al., 2006). On the other hand, the sediment samples were relatively enriched in the higher PAHs (4–5 aromatic rings), which are characteristic hydrocarbons of the surface runoff (Franco et al., 2006).

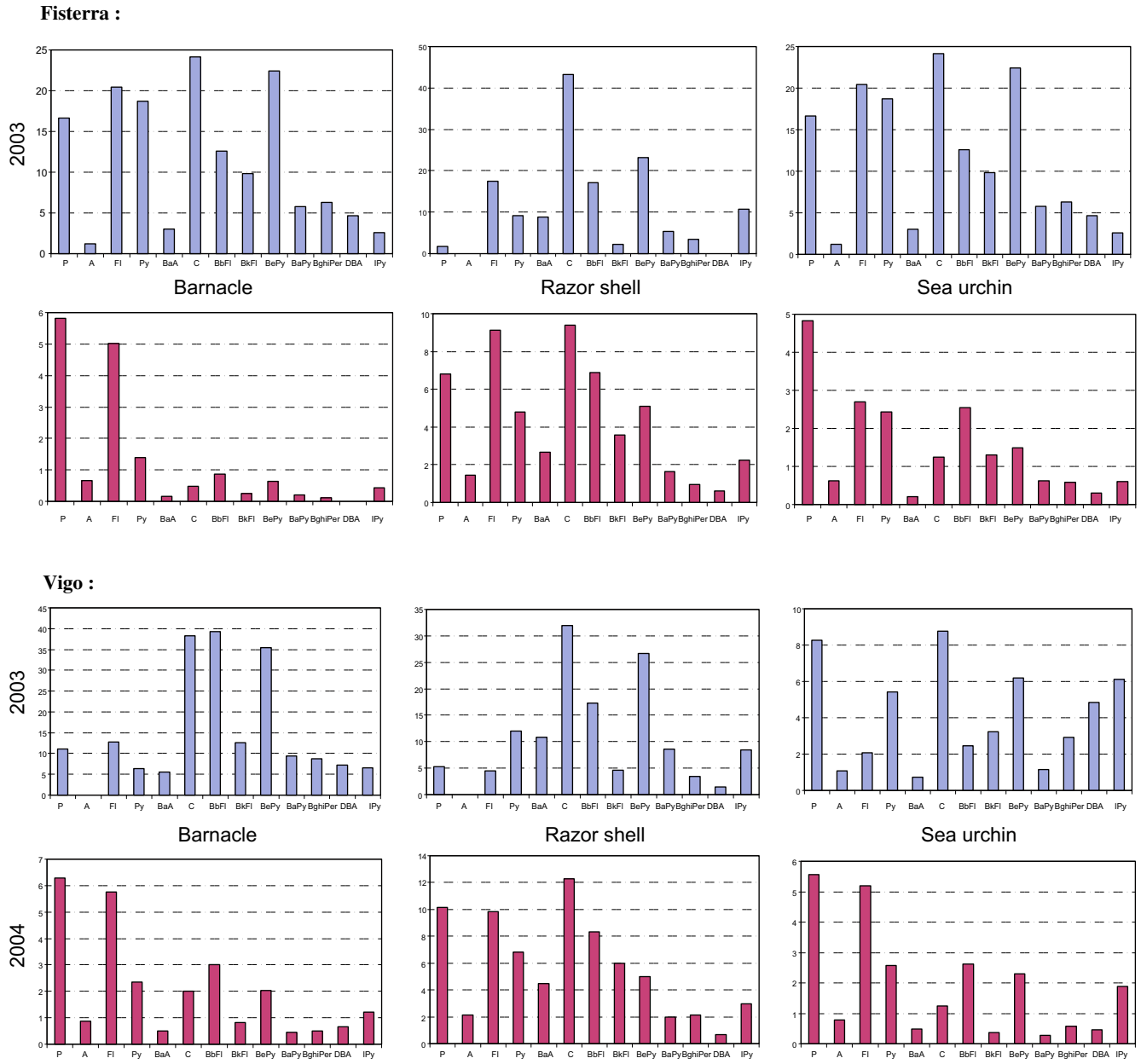

Fig. 4. Representative PAH distributions in barnacle, razor shell and sea urchin samples, collected at Fisterra and Vigo stations in April 2003 and 2004. P: Phenanthrene, A: Anthracene, Fl: Fluoranthene, Py: Pyrene, BaA: Benz[*a*]anthracene, C: Chrysene, BePy: Benzo[*e*]pyrene, BbFl: Benzo[*b*]fluoranthene, BkFl: Benzo[*k*]fluoranthene, BaPy: Benzo[*a*]pyrene, BPer: Benzo[*ghi*]perylene, DBA: Dibenz[*ah*]anthracene, IPy: Indeno[1,2,3-*cd*]pyrene.

Table 1
Total concentrations of 13 parent PAHs* (in μg/kg dw) in commercial shellfish from the Galician coast at the indicated dates. Stations correspond to those indicated in Fig. 1.

	Jan-03	Apr-03	May-03	Jun-03	Aug-03	Oct-03	Nov-03	Jan-04	Feb-04	Mar-04	Apr-04	Oct-04
Razor shell												
Fisterra		143	35	91	60	99	172		90	55	55	74
Arousa		106	38	63	80							
Vigo	335	135	58	68		54	96	102	73	48	21	27
Goose barnacle												
Fisterra		170	96	29	20	17	37	38	26	17	16	14
Arousa		659	362	154	48	16	21	43	25	21	11	10
Vigo	430	226	60	15	14	18	30	41	27	26	17	
Sea urchin												
Fisterra		152	15	25	34	16	30	26	61	22	19	23
Arousa			32	17	11		31		29	16	9	26
Vigo	2414	153	45	42	20	25	48	42	21	24	15	20
Mussel												
Fisterra		611	90		46	53	413	1330	94	201	416	200
Arousa		146	310	86	41		138	91	179	62	33	26
Vigo	1309	357	90	59	74	94	172	487	60	67	58	25

* 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.

These results confirm that after an acute exposure, organisms may eliminate almost their entire acquired burden of PAHs, whereas during chronic exposure, an appreciable fraction of the most hydrophobic may become incorporated into storage lipids and be less available for diffusive loss or metabolism (Meador et al., 1995b).

3.3. PAH concentrations and depuration rates

The total concentrations of the 13 parent PAHs determined in the collected samples along 2003 and 2004, after the *Prestige* oil spill, are shown in Table 1. Mussels are included for comparative purposes as they were extensively used in monitoring the oil spill (Soriano et al., 2006, 2007). Although the three stations were heavily affected, the concentration values in the studied organisms were generally lower than those found in mussels, even for the razor shell, which is a filter feeding bivalve, but lives buried in the intertidal sediment, that was relatively not affected in this accident (Franco et al., 2006).

The statistical treatment of the data set revealed that differences among stations for each organism were not significant along the year, irrespectively of the concentration ranges ($p = 0.920$). Conversely, differences were evident between organisms ($p < 0.005$), although not in April when the accumulation was higher ($p = 0.069$). The general accumulation trend was: mussels > razor shells > goos barnacle > sea urchin. We already showed that clams exhibit lower accumulation of hydrophobic pollutants compared to mussels (Sole et al., 1994).

Concentrations increased significantly after the spill and decreased some months later to levels probably close to background concentrations for the region. In fact, since April–May 2003, 5–6 months after the accident, the concentrations were already below the threshold level proposed by the Food Safety Administration for commercial exploitation of these organisms (200 μg/kg dw of the sum of the 6 HAPs: benzo[*a*]anthracene, benzo[*b*] and benzo[*k*]fluorantene, benzo[*a*]pyrene, dibenzo[*a*,*h*]anthracene and indeno[1,2,3-*c*,*d*]pyrene). One year after the accident, the median values were: 58 μg/kg for razor shells, 26 μg/kg for barnacles, 25 μg/kg for sea urchins and 74 μg/kg for mussels. These can be considered as the background levels for these stations, which are rather far from direct terrestrial inputs.

Ninety days is approximately the time required under field conditions for the release of hydrocarbons after the acute exposure of mussels to oil (Farrington et al., 1980). However, in the present case, the oil was arriving in several tides to the coast until late January 2003, so probably delaying the depuration process. Moreover, in the following winter (November 03–January 04) the concentrations increased slightly, probably as a result of storms that may have reintroduced oil residues into the water column. Similar trends were observed in different intertidal species monitored in the NE Atlantic, after the *Braer*, *Sea Empress*, *Agean Sea* and *Erika* spills (Webster et al., 1997; Law et al., 2005; Porte et al., 2000; Tronczynski et al., 2004) as well as in mussels after the *Prestige* (Soriano et al., 2006).

The temporal evolution of the PAH concentrations along the survey period was used to estimate loss rates and environmental half-lives for bioavailable PAHs in the affected sites. Such processes

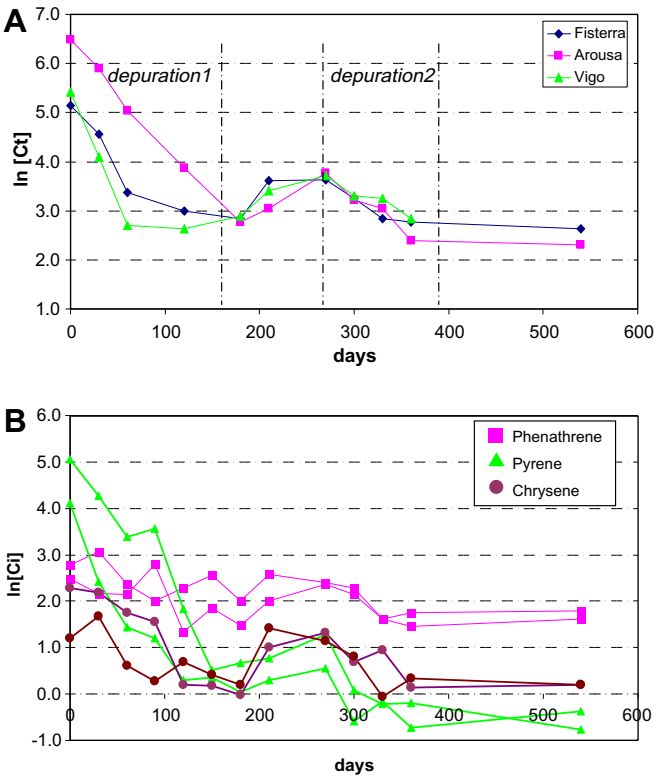

Fig. 5. Temporal trends of (A) total PAH concentrations (Σ13) in barnacles collected at the 3 sites along the studied period, and (B) of phenanthere, pyrene and chrysene in Fisterra and Arousa.

Table 2
PAH depuration and uptake rates in barnacles and sea urchins at the studied sampling stations.

Organism	Site	0–200 d (depuration1)			260–360 d (depuration2)		
		R^2	r_1	$t_{1/2}$	R^2	r_3	$t_{1/2}$
Goose barnacle	Fisterra	0.8834	0.0184	37.7	0.9310	0.0101	68.6
	Arousa	0.9945	0.0221	31.4	0.9599	0.0142	48.8
	Vigo	0.7723	0.0226	30.7	0.8117	0.0076	91.2
Sea urchin	Fisterra	0.6011	0.0091	76.2	0.8426	0.0194	35.7
	Arousa	0.9147	0.0112	61.9	–	–	–
	Vigo	0.8512	0.0152	45.6	0.9997	0.0195	35.5

are generally described using a first order (exponential decay) rate expression as follows (Fossato and Canzonier, 1976; Ernst, 1977):

$$C_t = C_0 e^{-rt}$$

where C_t is the concentration of a parent hydrocarbon detected at each sampling station at a given time t (d); C_0 is the maximum concentration of that hydrocarbon detected at this sampling station (both expressed as ng g^{-1} dry weight) and r is the mean slope of the depuration curve over the time period observed or the apparent exponential decay-rate constant. Then, the biological half-life ($t_{1/2}$), can be expressed as $\ln 2/r = 0.693/r$.

Rate constants for total PAHs loss from barnacle tissues at each sampling station were calculated from a linear regression of the data (Fig. 5A), according to the above equation. Two periods were considered, following the development of the event, the first depuration process after the spill (depuration1) and the final depuration after the second uptake during the winter period (depuration2). The corresponding constants are shown in Table 2, exhibiting small variations of half-life values between stations, thus enhancing their significance. Apparently, the primary depuration rate was slower for sea urchins. The dataset was not complete enough for a similar calculation for razor shells.

However, when PAHs were considered individually, different trends were observed for the lower and higher PAHs. In principle, elimination that relies solely on passive diffusion loss should be slower for the more hydrophobic PAHs, although there is conflicting information regarding persistence in tissues, even for a given PAH in one species (Meador et al., 1995b). As it can be seen in Fig. 5B, the slopes of the pyrene and chrysene curves seem to agree with this hypothesis, but phenanthrene concentrations are much more conservative. This may by due to the concurrence of acute and chronic bioaccumulation that may affect the rate of elimination and persistence of the associated PAHs in marine organisms.

These results support previous observations in the sense that the rate of elimination and persistence of PAHs in tissues of marine organisms is highly influenced by the time of exposure, so that after an acute exposure, organism's depuration is rather fast and the concentrations decline orders of magnitude in few months (Boehm and Quinn, 1977; Farrington et al., 1982). However, when the levels are close to background, both the uptake and depuration processes slow down significantly. Moreover, the time of exposure determines the persistence of those hydrocarbons constituting the chronic pollution. Finally, the present study has demonstrated that barnacles can be suitable species for oil spill monitoring.

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References

Al-Hassan, J.M., Afzal, M., Rao, C.V.N., Fayad, S., 2000. Petroleum hydrocarbon pollution in sharks in the Arabian Gulf. Bull. Environ. Contam. Toxicol. 65, 391–398.
Albaigés, J., Morales-Nin, B., Vilas, F., 2006. The *Prestige* oil spill: a scientific response. Mar. Pollut. Bull. 53, 205–207.
Avigan, J., Blumer, M., 1968. On the origin of pristane in marine organisms. J. Lipid Res. 9, 350–352.
Baumard, P., Budzinski, H., Michon, Q., Garrigues, P., Burgeot, T., Bellocq, J., 1998. Origin and bioavailability of PAHs in the Mediterranean Sea from mussel and sediment records. Estuar. Coast. Shelf Sci. 47, 77–90.
Bieger, T., Abrajano, T.A., Hellou, J., 1997. Generation of biogenic hydrocarbons during a spring bloom in Newfoundland coastal (NW Atlantic) waters. Org. Geochem. 26, 207–218.
Blumer, M., Guillard, R.R., Chase, T., 1971. Hydrocarbons of marine phytoplankton. Mar. Biol. 8, 183–189.
Boehm, P.D., Quinn, J.G., 1977. Persistence of chronically accumulated hydrocarbons in hard shell clam *Mercenaria mercenaria*. Mar. Biol. 44, 227–233.
Daling, P.S., Faksness, L.G., Hansen, A.B., Stout, S.A., 2002. Improved and standardized methodology for oil spill fingerprinting. Environ. Forensics 3, 263–278.
Den Besten, P.J., 1998. Cytochrome P450 monooxygenase system in echinoderms. Comp. Biochem. Physiol. Part C 121, 139–146.
Díez, S., Sabaté, J., Viñas, M., Bayona, J.M., Solanas, A.M., Albaigés, J., 2005. The *Prestige* oil spill. I. Enhanced biodegradation of a heavy fuel oil under simulated conditions. Environ. Toxicol. Chem. 24, 2203–2217.
Ernst, W., 1977. Determination of bioconcentration potential of marine organisms – steady-state approach. 1. Bioconcentration data for 7 chlorinated pesticides in mussels (*Mytilus edulis*) and their relation to solubility data. Chemosphere 6, 731–740.
Farrington, J.W., Meyer, P.A., 1975. Hydrocarbons in the marine environment. In: Eglinton, G. (Ed.), Environmental Chemistry, vol. 1. The Chemical Society, London, pp. 109–136.
Farrington, J.W., Albaigés, J., Burns, K.A., Dunn, B.P., Eaton, P., Laseter, J.L., Parker, P.L., Wise, S.A., 1980. Fossil fuels in bivalves. In: Goldberg, E.D. (Ed.), The International Mussel Watch. National Academy of Sciences, Washington, pp. 7–77.
Farrington, J.W., Davis, A.C., Frew, N.M., Rabin, K.S., 1982. No. 2 Fuel oil compounds in *Mytilus edulis*. *Retention and release after an oil spill*. Mar. Biol. 66, 15–26.
Fossato, V.U., Canzonier, W.J., 1976. Hydrocarbon uptake and loss by mussel *Mytilus edulis*. Mar. Biol. 36, 243–250.
Franco, M.Á., Viñas, L., Soriano, J.A., Armas, D.d., González, J.J., Beiras, R., Salas, N., Bayona, J.M., Albaigés, J., 2006. Spatial distribution and ecotoxicity of petroleum hydrocarbons in sediments from the Galicia continental shelf (NW Spain) after the *Prestige* oil spill. Mar. Pollut. Bull. 53, 260–271.
Glegg, G.A., Rowland, S.J., 1996. The *Braer* oil spill-hydrocarbon concentrations in intertidal organisms. Mar. Pollut. Bull. 32, 486–492.
González, J.J., Viñas, L., Franco, M.Á., Fumega, J., Soriano, J.A., Grueiro, G., Muniategui, S., López Mahía, P., Prada, D., Bayona, J.M., Alzaga, R., Albaigés, J., 2006. Spatial and temporal distribution of dissolved/dispersed aromatic hydrocarbons in seawater in the area affected by the *Prestige* oil spill. Mar. Pollut. Bull. 53, 250–259.
Hellou, J., Steller, S., Zitko, V., Leonard, J., King, T., Milligan, T.G., Yeats, P., 2002. Distribution of PACs in surficial sediments and bioavailability to mussels, *Mytilus edulis* of Halifax Harbour. Mar. Environ. Res. 53, 357–379.
Koyama, J., Uno, S., Kohno, K., 2004. Polycyclic aromatic hydrocarbon contamination and recovery characteristics in some organisms after the *Nakhodka* oil spill. Mar. Pollut. Bull. 49, 1054–1061.
Law, R.J., Hellou, J., 1999. Contamination of fish and shellfish following oil spill incidents. Environ. Geosci. 6, 90–98.
Law, R.J., Kelly, C.A., Graham, K.L., Woodhead, R.J., Dyrynda, P.E.J., Dyrynda, E.A., 2005. Hydrocarbons and PAH in fish and shellfish from Southwest Wales following the *Sea Empress* oil spill in 1996. 2005 International Oil Spill Conference, IOSC 2005, 3554–3560.
Meador, J.P., Casillas, E., Sloan, C.A., Varanasi, U., 1995a. Comparative bioaccumulation of polycyclic aromatic-hydrocarbons from sediment by two infaunal invertebrates. Mar. Ecol-Prog. Ser. 123, 107–124.
Meador, J.P., Stein, J.E., Reichert, W.L., Varanasi, U., 1995b. Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms. Rev. Environ. Contam. Toxicol. 143, 79–165.
Mironov, O.G., Shchekaturina, T.L., Tsimbal, I.M., 1981. Saturated hydrocarbons in marine organisms. Mar. Ecol-Prog. Ser. 5, 303–309.
Morris, R.J., 1973. Uptake and discharge of petroleum hydrocarbons by barnacles. Mar. Pollut. Bull. 4, 107–109.
O'Connor, T., 1996. Trends in chemical concentrations in mussels and oysters collected along the US coast from 1986 to 1993. Mar. Environ. Res. 41, 183–200.
Osterroht, C., Petrick, G., Wenck, A., 1983. Seasonal-variation of particulate hydrocarbons in relation to biological parameters. Mar. Chem. 14, 175–194.
Peña-Mendez, E.M., Astorga-Espana, M.S., Garcia-Montelongo, F.J., 1999. Interpretation of analytical data on *n*-alkanes and polynuclear aromatic hydrocarbons in *Arbacia lixula* from the coasts of Tenerife (Canary Islands, Spain) by multivariate data analysis. Chemosphere 39, 2259–2270.
Porte, C., Biosca, X., Pastor, D., Sole, M., Albaigés, J., 2000. The *Aegean Sea* oil spill 2. Temporal study of the hydrocarbons accumulation in bivalves. Environ. Sci. Technol. 34, 5067–5075.

QUASIMEME_II, 2003. QUASIMEME laboratory performance studies. Round 34. BT-4 Exercise 588. Polyaromatic hydrocarbons in biota.
QUASIMEME_II, 2004. QUASIMEME laboratory performance studies. Round 36. BT-4. Exercise 619. Polyaromatic hydrocarbons in biota.
RNO (Ed.), 2002. Surveillance du milieu marin. Travaux du RNO. Ifremer et Ministère de l'Ecologie et du Développment Durable. ISSB 1620-1124.
Salas, N., Ortiz, L., Gilcoto, M., Varela, M., Bayona, J.M., Groom, S., Alvarez-Salgado, X.A., Albaigés, J., 2006. Fingerprinting petroleum hydrocarbons in plankton and surface sediments during the spring and early summer blooms in the Galician coast (NW Spain) after the *Prestige* oil spill. Mar. Environ. Res. 62, 388–413.
Serrazanetti, G.P., Pagnucco, C., Conte, L.S., Cattani, O., 1995. Hydrocarbons, sterols and fatty-acids in sea-urchin (*Paracentrotus lividus*) of the Adriatic Sea. Chemosphere 30, 1453–1461.
Shaw, D.G., Wiggs, J.N., 1980. Hydrocarbons in the inter-tidal environment of Kachemak Bay, Alaska. Mar. Pollut. Bull. 11, 297–300.
Sole, M., Porte, C., Albaigés, J., 1994. Mixed-function oxygenase system components and antioxidant enzymes in different marine bivalves: its relation with contaminant body burdens. Aquat. Toxicol. 30, 271–283.
Soriano, J.A., Viñas, L., Franco, M.Á., González, J.J., Ortiz, L., Bayona, J.M., Albaigés, J., 2006. Spatial and temporal trends of petroleum hydrocarbons in wild mussels from the Galician coast (NW Spain) affected by the *Prestige* oil spill. Sci. Total Environ. 370, 80–90.
Soriano, J.A., Viñas, L., Franco, M.A., González, J.J., Nguyen, M.H., Bayona, J.M., Albaigés, J., 2007. Spatial and temporal trends of polycyclic aromatic hydrocarbons in wild mussels from the Cantabrian coast (N Spain) after the *Prestige* oil spill. J. Environ. Monitor. 9, 1018–1023.
Tronczynski, J., Munschy, C., Héas-Moisan, K., Guiot, N., Truquet, I., Olivier, N., Men, S., Furaut, A., 2004. Contamination of the Bay of Biscay by polycyclic aromatic hydrocarbons (PAHs) following the T/V "*Erika*" oil spill. Aquat. Living Resour. 17, 243–259.
Varanasi, U., Reichert, W.L., Stein, J.E., 1985. Bioavailability and biotransformation of aromatic hydrocarbons in benthic organisms exposed to sediment from an urban estuary. Environ. Sci. Technol. 19, 836–841.
Webster, L., Angus, L., Topping, G., Dalgarno, E.J., Moffat, C.F., 1997. Long-term monitoring of polycyclic aromatic hydrocarbons in mussels (*Mytilus edulis*) following the *Braer* oil spill. Analyst 122, 1491–1495.
Wraige, E.J., Johns, L., Belt, S.T., Massé, G., Robert, J.-M., Rowland, S., 1999. Highly branched C25 isoprenoids in axenic cultures of *Haslea ostrearia*. Phytochemistry 51, 69–73.
Youngblood, W.W., Blumer, M., 1973. Alkanes and alkenes in marine benthic algae. Mar. Biol. 21, 163–172.