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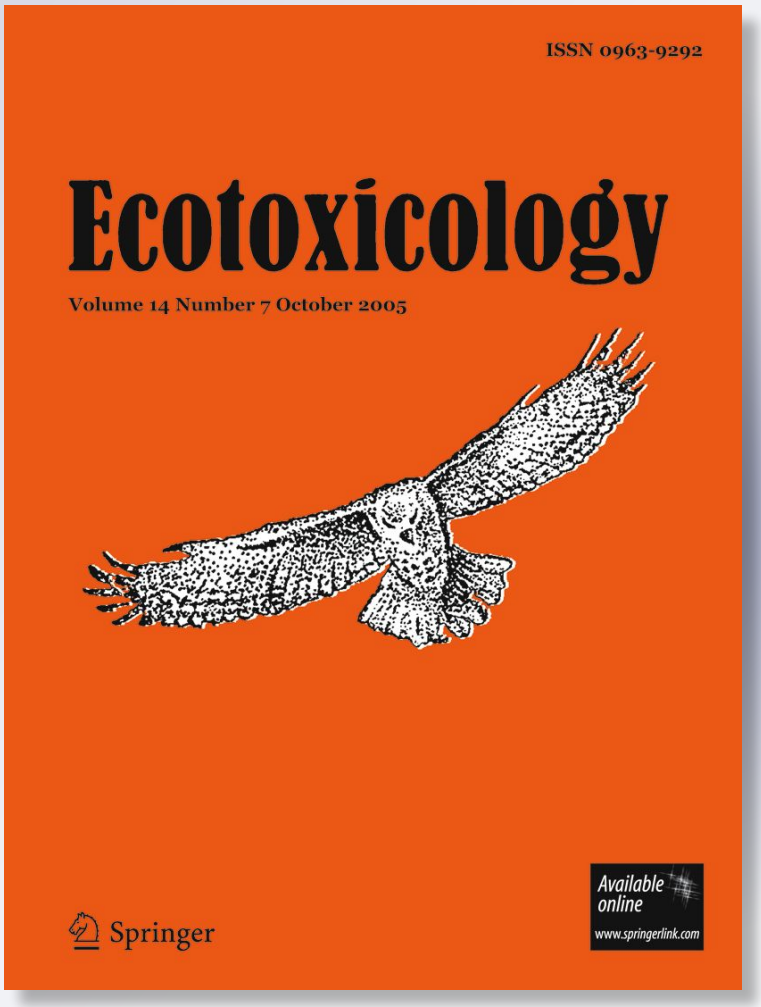

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Responses of CYP450 dependent system to aliphatic and aromatic hydrocarbons body burden in transplanted mussels from South coast of Portugal

B. Lopes · A. M. Ferreira · M. J. Bebianno

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Abstract Mussels *Mytilus galloprovincialis* were cross-transplanted at South Portugal from a reference site (site 1) to a site more contaminated with hydrocarbon compounds (site 2), and vice versa, in an active biomonitoring (ABM) concept, to assess the biotransformation capacity catalyzed by the mixed function oxygenase (MFO) system. Total alkanes (TAlk), the unresolved complex mixture (UCM), and total polycyclic aromatic hydrocarbons (TPAHs) concentration increased respectively 6, 4.4 and 4.2 fold relatively to control, in mussels transplanted from site 1 to 2. In the cross-transplant, a 48, 57 and 62% depuration of TAlk, UCM and TPAHs concentrations occurred by the end of the 3–4th week. Petrogenic and biogenic (marine and terrigenous) sources of AHs, and petrogenic and pyrolitic (biomass and oil/fuel incomplete combustion) sources of PAHs were detected at both sites. CYP450, CYT b_5 and NADPH-RED in mussels transplanted from site 1 to 2 were induced from day 0 to 28, with a total increase of 35, 32 and 35%, respectively, while biochemical equilibrium to lesser environmental contamination occurs in mussels transplanted from site 2 to 1. A significant relationship between CYP450 and NADPH-RED was found with TPAH, with distinctive behavior at the two sites. MFO system components increase with exposure time at one site and decreases in the other, reflecting an adaptation to distinct environmental hydrocarbon loads. The ABM strategy proved to be useful to understand the environment real impact on the biochemical responses in mussels' local populations. In this study, CYP450 and NADPH-RED are a useful biomarker for hydrocarbon exposure.

Keywords Active biomonitoring · Cross-transplant · Aliphatic hydrocarbons · PAHs · CYP450 · MFO system

B. Lopes · M. J. Bebianno (✉)
CIMA, Faculty of Sciences and Technologies, University of Algarve, Campus de Gambelas, 8005-130 Faro, Portugal
e-mail: mbebian@ualg.pt

A. M. Ferreira
Research Unit of Upgrading of Fishery and Farmed Products, National Institute of Biological Resources (INRB, I.P./IPIMAR), Avenida Brasília, 1449-006 Lisbon, Portugal

Introduction

Aquatic organisms are continuously exposed to organic compounds of anthropogenic origin, which can be accumulated in their tissues. The anthropogenic hydrocarbons that enter the marine environment include oil and its derivatives, synthetic combustion products and composites. The sources are complex and diverse, and include oil tanker accidents, atmospheric deposition, industrial and domestic sewage/runoff. The hydrocarbons synthesized by organisms (biogenic hydrocarbons) are also present in the environment, whose aliphatic fraction is predominant and occurs in some marine and terrestrial species (Peña-Méndez et al. 2001). Petroleum hydrocarbons include the aliphatic hydrocarbons (*n*-alkanes, cyclic and branched alkanes, isoprenoids and geochemical markers such as hopanes and steranes) and polycyclic aromatic hydrocarbons. The identification of aliphatic hydrocarbons (AHs) in organisms is based on the characteristic profiles of chemical markers that reflect the origin of the contamination: biological and/or petrogenic, as well as the degree of biodegradation. The main chemical markers used are total *n*-alkanes (TAlk) (sum of all individual *n*-alkane concentration), UCM (unresolved complex mixture); C_{17}/C_{29} ratio; CPI—carbon preference index (sum of odd *n*-alkanes C_{15}–C_{35}/sum of even *n*-alkanes C_{14}–C_{36}) and isoprenoid

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hydrocarbons (Pristane/Phytane ratio) (Carro et al. 2006). Polycyclic aromatic hydrocarbons (PAHs) are persistent organic contaminants that have been found in all biotic compartments: the atmosphere, soil and sediments, water column and in terrestrial and aquatic organisms. They are ubiquitous and part of the most studied contaminants in environmental programs due to its toxicity, persistence and accumulation along food chains (Solé 2000). PAHs are toxic, reactive and some are considered carcinogenic, as benzo(*a*)pyrene. PAHs are formed by two or more benzene rings and can be originated by organic matter decomposition during petroleum formation (petrogenic); soils or sediments processing of certain classes of organic compounds (diagenic); organisms direct synthesis (biogenic), and incomplete combustion or pyrolysis of fossil fuels and biomass (pyrolytic) (Neff 2002).

The main response of an organism to organic contamination is to detoxify and/or transform the contaminants into biologically inactive and readily excretable metabolites (Petushok et al. 2002). This metabolic detoxification employs a wide range of different enzymes that comprise phase I or functional reactions (biotransformation) and phase II or conjugation reactions with endogenous molecules. The phase I metabolism are mainly oxidative reactions catalyzed by the cytochrome P450-dependent mixed-function oxygenase (MFO) system, with the cytochrome P450 (CYP450), NADPH- cytochrome *c* P450 reductase (NADPH-RED), cytochrome b_5 (CYT b_5) and NADH-CYT b_5 reductase (NADH-RED) as main components (Di Giulio 1995; Snyder 2000).

Bivalve molluscs like mussels, have been used as sentinel species in monitoring aquatic ecosystems, as they are capable of accumulate a variety of compounds at levels much higher than those found in the surrounding waters (Solé et al. 1995a, b; Livingstone 1996; Cajaraville et al. 2000). Although MFO system is more sensitive in fish than in mussels, clear evidence of interactions between these detoxifying mechanism and hydrocarbons has been obtained in mussels from field and exposure studies (Solé et al. 1995a, b; Bebianno et al. 2007; Lopes and Bebianno 2007; Cravo et al. 2009). Moreover, mussels are true sedentary species that reflect chemical exposure, therefore are better than fish which are mobile and metabolize certain chemicals to a greater extent, so they are not found in their tissues. CYP450 and MFO system have been used as a biomarker of exposure to organic contaminants in *Mytilus galloprovincialis*, since induction on their concentrations and activities reflects the PAHs concentrations in the aquatic environment (Petushok et al. 2002; Cravo et al. 2009). Linear relationships between CYP450 and total PAHs were reported in mussels collected after an oil spill, with higher concentrations at sites near the spillage (Porte et al. 1991, 2001a, b; Solé et al. 1995b), in environmental quality assessment studies, with highest CYP450 and MFO system levels at sites with higher PAHs concentrations (Livingstone et al. 1995; Solé et al. 2000b; Bebianno et al. 2007; Lopes and Bebianno 2007), and in mussels field and laboratory exposed to PAHs (Livingstone 1998; Bebianno et al. 2007; Serafim et al. 2011).

Flavoprotein reductases are components of the microsomal electron transport system and have been characterized in fish (Williams et al. 1983) and molluscs (Livingstone et al. 1989). They play a key role in the flux regulation of the monooxygenase system and clear evidence of interaction between organic compounds and the flavoprotein NAD(P)H cytochrome *c* reductase has been obtained (Livingstone et al. 1989). CYT b_5 is primarily involved in the oxidation of various endogenous substrates and acts as an electron donor in a number of oxidative reactions canalized by CYP450. These include the anabolic metabolism of fats and steroids, as well as the catabolism of xenobiotics and compounds of endogenous metabolism (Schenkman and Jansson 2003).

In recent years, many studies highlighted the importance of using an integrated approach to assess the effects of environmental changes in coastal or estuarine zones (Peters et al. 1998, 1999; Shaw et al. 2002). The application of the active biomonitoring (ABM) strategy, in which organisms of a clean or reference site are transplanted to the site that is being monitored, gives the advantage of assuring comparable results, reducing the variability normally found in field studies (Nasci et al. 2002). The transplantation of mussels within the marine environment to evaluate the effects of contaminants and physiological (or biochemical) alterations is a useful tool to assess environmental quality, as an increasing number of studies on ABM strategy had shown, either through bioaccumulation of contaminants or biomarker (exposure and/or effect) responses (Peters et al. 1998, 1999; Solé et al. 1998; da Ros et al. 2000, 2002; Orbea et al. 2000; Nasci et al. 2000, 2002; Bodin et al. 2004; Wetzel and van Vleet 2004; Nigro et al. 2006; Bebianno et al. 2007, Serafim et al. 2011).

The South coast of Portugal is a highly important coastal zone particularly for fisheries, aquaculture (fishes and bivalves) and tourism, where the population increases markedly during the summer (to 10 fold), as well as the volume of sewage discharges and nautical activities. Although this region is characterized by median to small cities and has no major industries, some hotspots of contamination have been identified with a variety of pollutants: metals, PAHs, PCBs, and organotin compounds mainly TBT; the associated response of a specific biomarker or the use of a battery of biomarkers to assess the effects of these pollutants in the organisms were extensively studied, in some of which CYP450 and MFO system where identified as a good indicator for organic contaminants like PAHs

(Serafim and Bebianno 2001; Barroso et al. 2004; Coelho et al. 2002; Diez et al. 2005; Bebianno et al. 2007; Company et al. 2008; Cravo et al. 2009; Lopes and Bebianno 2007; Lopes 2009, Serafim et al. 2011).

Site 1 (Tavira-T) is a relatively clean or reference site located at the eastern end of the Ria Formosa lagoon, at the mouth of River Gilão, in the proximity of bivalve aquaculture fields (oysters and clams), and with a small fishing/recreational harbor. Site 2 (Vilamoura-V) is located at the entrance of the largest recreational marina of the South coast of Portugal, with known contamination of both AH and aromatic hydrocarbon. Identified sources are related to high boat traffic, the burn of petrol fuels and the continuous lixiviation of oils in the harbor and marina areas. Previous works on environmental health assessment identified site 1 and 2 consistently with the lowest and highest hydrocarbon contamination, respectively, in which CYP450 basal concentrations also differ (Bebianno et al. 2007; Lopes and Bebianno 2007; Cravo et al. 2009; Lopes 2009).

The aim of this work is to integrate the ABM strategy using mussels *M. galloprovincialis* cross-transplanted from two sites in South coast of Portugal, to assess the response of the MFO system to distinct environmental contamination with hydrocarbons. Alterations in total hydrocarbons (aliphatic and aromatic) concentrations are evaluated at both sites, to assess the temporal variation of accumulation and elimination processes. The capacity of CYP450 to be used as a biomarker for hydrocarbons contamination depends on its ability to respond accordingly to the level of exposure, as CYP450 is induced in the presence of higher levels of these compounds; basal concentrations of CYP450 are observed in organisms exposed to lower hydrocarbon loads. This cross-transplant experiment will clarify the response of CYP450 to realistic hydrocarbon environmental contamination.

Materials and methods

Transplant strategy and sample collection

Mussels *M. galloprovincialis* (4–6 cm) were collected at site 1 ((N 37° 06,995′; W 007° 37,732′) and at site 2 (N 37° 04,406′; W 008° 07,295′) in June 2004 (Fig. 1). At the beginning of the experiment 40 mussels from each site were collected (day 0), and 200 more (that were grouped in batches of 40) were transplanted from the original site to the other, i.e. from site 1 to 2 and from site 2 to 1. After transplantation, groups of 40 mussels were collected after 7, 14, 21 and 28 days. At day 28, a group of 40 mussels was back-transplanted to their original site (that is, mussels from site 1 that had been at site 2 for 28 days, went back to site 1), and were collected 32 days later (day 60 of experiment). Simultaneously, an additional group of 40 autochthonous mussels were collected at each site, as a second control (control day 60–C60).

Fig. 1 Transplantation sites in the South coast of Portugal (site 1 Tavira; site 2 Vilamoura)

Chemical analyses

Three replicates of 3–4 mussel's whole soft tissues pools (±8 g) were homogenized with anhydrous Na_2SO_4 and Soxhlet extracted with *n*-hexane/dichloromethane (4:1) for 24 h. Samples were then purified by liquid chromatography in a 5% water-deactivated silica–alumina column. AHs were eluted in *n*-hexane and PAHs were eluted in *n*-hexane/dichloromethane.

Individual AHs were identified and quantified by capillary GC-FID, using a DB5 (J&W, Scientific, USA) fused silica column (30 m × 0.25 mm i.d.). The injector and detector temperatures were 280 and 330°C, respectively and the carrier gas was helium. Temperature was held at 70°C for 1 min, 70–300°C at 6°C/min, with a final hold-up during 15 min. Individual AHs (ng/g ww) were identified and quantified by comparison of retention times of standard compounds. A standard mixture containing the *n*-alkanes C_{15}–C_{24}, C_{28}, C_{32}, C_{36} and pristane, diluted in *iso*-octane, was used, at concentrations that varied between 6.66 and 13.33 μg/ml. The detection limit ranged between 0.1 and 0.3 ng/g. The unresolved complex mixture (UCM) of hydrocarbons was quantified using the response factor of the *n*-alkane eluting in the zone of maximum response (usually C_{22}).

Chemical markers such as UCM/TAlk ratio, CPI index, C_{17}/Pris, C_{18}/Phyt and C_{17}/C_{29} ratios have been used to identify aliphatic sources and origins. They are based on physic–chemical properties of the compounds and provide information about petrogenic (even *n*-alkanes) and biogenic sources (odd *n*-alkanes). Chemical markers are also useful in distinguish between marine biogenic sources (C_{17}) and terrigenous biogenic sources (C_{29}). The comparative persistence of compounds and the biodegradation/weathering rates are evaluated by the relative degradation of linear *n*-alkanes and branched isoprenoids, and from the proportion between unresolved and resolved alkanes.

Individual quantification of PAHs was made using a HPLC–UV, by comparison of retention times and library spectra of reference compounds. A standard mixture containing 16 individual PAHs, (EPA 610 PAH Mix, Sigma) namely naphthalene (N), acenaphthylene (Ac), acenaphthene (Ace), fluorene (Flr), phenanthrene (Phen), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benzo(*a*)anthracene (BaA), chrysene (Chry), benzo(*b*)fluoranthene (BbF), benzo(*k*) fluoranthene (BkF), benzo(*a*)pyrene (BaP), dibenzo(*a,h*) anthracene (DahP), benzo(*g,h,i*)perylene (BPer) and indeno-(1,2,3-*cd*)pyrene (IndP) was diluted in acetonitrile to reach concentrations that ranged from 0.5 and 10 μg/ml. Detection limit ranged from 0.01 to 0.24 ng/g ww, for individual PAHs. PAHs measurements were validated using a standard reference material of mussel tissue (SRM 2977; NIST, USA), that was extracted and analyzed in the same way as the samples. PAH recovery for the certified material ranged between 73 and 112%.

Several ratios among PAHs isomers, (Phen/Ant, Flu/Pyr, Ant/Ant + Phen, Flu/Flu + Pyr, BaA/BaA + Chrys and IndP/IndP + BPer) based on the temperature of formation were applied as diagnostic markers to identify the main PAHs sources found in mussels, whose limits were collected from bibliography (Baumard et al. 1998a, b; Yunker et al. 2002; Mille et al. 2007; Oros and Ross 2005; Oros et al. 2006). Oil formation requires low temperatures, in which the organic matter maturation processes originate two or three benzene ring PAHs (such as naphthalene, anthracene, and phenanthrene) which are characteristic of petrogenic sources. On the other hand, high combustion temperatures results in 4, 5 or 6 ring compound formation (as benzo(*a*)anthracene, chrysene, benzo(*a*)pyrene, benzo(*g,h,i*)perylene or indeno(1,2,3-*cd*)pyrene). The diagnostic ratios are useful in sources allocation, distinguishing between petrogenic and pyrolitic inputs, whether derived from refined products or petroleum combustion (Porte et al. 2001a, b; Solé et al. 1996a), or derived from biomass combustion (forest fires, fireplaces, incinerators, coal) (Oros and Ross 2005).

Biochemical analysis

For the analysis of CYP450, '418 peak'-putative denatured CYP450, CYT b_5 and NAD(P)H reductases, three pools of five digestive glands each, were used. The microsomal fractions were prepared at 4°C by differential centrifugation as described by Livingstone (1988). Samples were homogenized in 1:3.5 (tissue weight:buffer volume) ratio, at 4°C with 10 mM Tris–HCl pH 7.6, containing 1 mM dithiothreitol, 0.15 M KCl and 0.5 M sucrose. Following centrifugations of 500×*g* for 15 min, 10,000×*g* for 30 min and 100,000×*g* for 90 min, the resulting microsomal pellet was resuspended in 10 mM Tris–HCl pH 7.6, containing 20% (w/v) glycerol to give a protein concentration of approximately 10 mg/ml. Biochemical measurements were carried out on microsomal samples either immediately (cytochromes concentrations) or after overnight storage at 80°C (proteins concentrations and reductases activities). CYP450, '418 peak', CYT b_5 and reductases activities were assayed as described in Livingstone and Farrar (1984). CYP450 and '418 peak' concentration was measured by the carbon monoxide difference spectrum of sodium dithionite reduced samples using an extinction coefficient of 91 mM^{-1} cm^{-1} (450–490 nm). CYP450 concentration was expressed in pmol mg^{-1} proteins. Total '418 peak' was calculated in arbitrary units as described in Livingstone (1988), i.e. peak height (418–490 nm) × 1,000 mg^{-1}. Total CYT b_5 was similarly analyzed by difference spectroscopy using 50 ml of microsomal sample, 30 mM NADH and an extinction coefficient of 185 mM^{-1} cm^{-1} (409–425 nm). NADPH-dependent cytochrome *c* and NADH-dependent CYT b_5 reductases activities were measured by the increase in absorbance at 550 nm ($\varepsilon = 19.6$ mM^{-1} cm^{-1}). Microsomal protein concentration was determined by the Lowry method using bovine serum albumin as standard (Lowry et al. 1951).

Statistical analyses

Results are presented as mean ± standard deviation. The variability of hydrocarbon concentrations and MFO components were tested through the analysis of variance (ANOVA) after verification of normality and homogeneity of data. Duncan's test was used as a post hoc comparison test, to determine significant differences between variables. A significance level of 0.05 was used for all statistical analysis, i.e. a probability of $P < 0.05$ was considered significant. For each site, the relationships between hydrocarbons and MFO system with time were also evaluated by canonical correspondence analysis (CCA) using the CANOCO program (ter Braak and Smilauer 1998). The relationship between the different MFO system components and individual hydrocarbons concentrations was also assessed by regression and multivariate analysis.

Results

Aliphatic hydrocarbons

Total AH concentration (TAlk) of mussels transplanted from site 1 to 2 (Fig. 2a; Table 1) gradually increase with time, with an accumulation rate of 0.39 μg/g/day ($r = 0.98$; $P < 0.05$), reaching a concentration 6-fold higher that at the beginning (respectively 12.7 ± 2.3 and 2.1 ± 0.26 μg/g ww,

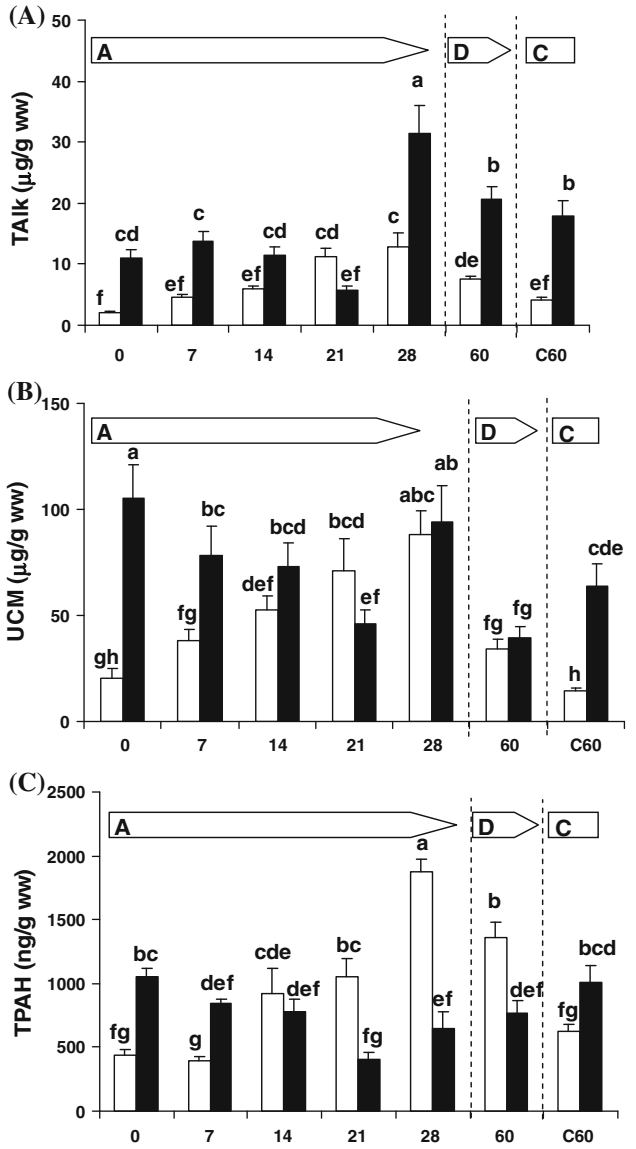

Fig. 2 TAlk (**a**), UCM (**b**) and TPAH (**c**) concentration in soft mussel *M. galloprovincialis* tissues transplanted from site 1 to 2 (*white bars*) and transplanted from site 2 to 1 (*black bars*). (*A* accumulation, *D* depuration, *C* control; mean ± standard deviation; *different letters* mean statistical differences; Duncan' s test, $P < 0.05$)

$P < 0.05$). After being back-transplanted to the original site (day T60), TAlk concentrations decreased 40% ($P < 0.05$), with mussels showing signs of recovery. However TAlk in mussels tissues after depuration were still higher than at the beginning of experiment ($P < 0.05$), but similar to those of autochthones mussels (TC60, $P > 0.05$)). TAlk concentrations in mussels transplanted from site 2 to 1 (Fig. 2a; Table 1), although similar for the first 2 weeks, afterwards decreased 48% to the 4th week, with a decreasing rate of 0.57 µg/g/day ($r = 0.97$; $P < 0.05$), and reaching lower concentrations than the initials ($P < 0.05$). A significant increase of TAlk concentrations occurred at day V28 ($P < 0.05$). When mussels were back-transplanted to site 2 for 32 days, TAlk concentrations decreased 34% (V60), attaining similar concentrations of the autochthonous mussels (VC60) ($P > 0.05$), but still significantly higher than at the beginning (V0) ($P < 0.05$).

UCM in mussels transplanted from site 1 to 2 (Fig. 2b; Table 1), follows the same pattern of Talk, with a gradual increase for 4 weeks, with an accumulation rate of 2.4 µg/g/day ($r = 0.98$; $P < 0.05$), until a maximum at day T28 ($P < 0.05$) which represents an increase of 4.4 fold compared to T0. When mussels were back transplanted, UCM concentrations decreased 60% (T60), to concentrations similar to the initial ones (T0) ($P > 0.05$), but still higher than autochthonous ones (TC60) ($P < 0.05$). Conversely, UCM in mussels transplanted from site 2 to 1, decreased about 56% of initial content from day V0 to V21, with a decreasing rate of 2.6 µg/g/day ($r = 0.97$; $P < 0.05$), increasing afterwards at day 28, as occurred with *n*-alkanes. After 32 days at the original site, UCM decreased 57% (V60) ($P < 0.05$) reaching lower levels than in the autochthonous mussels (VC60) ($P < 0.05$).

The proportion between the unresolved alkanes and the resolved alkanes (UCM/TAlk) reflects the environmental weathering and degradation the hydrocarbons are subjected to. This ratio indicates if the contamination is recent (=1) or old (>1) as linear chain alkanes are more easily degraded than the branched and cyclic alkanes, whose proportional concentrations builds up with time due to the relative slower rate of degradation. In this cross-transplant experiment, the UCM/TAlk ratio ranges from 10 (T0) and 3 (TC60) for mussels transplanted from site 1 to 2, and from 9 (V0) and 2 (V60) in the cross transplant (Table 1), reflecting an introduction of recent compounds in an older hydrocarbon contamination, at both transplants.

The individual *n*-alkane distribution pattern in mussel tissues transplanted from site 1 to 2 (Table 1) shows a gradual increase of even *n*-alkanes as mussels spend more time at site 2, mainly C_{20}, C_{22}, C_{24}, C_{26} and C_{28}, reaching their maximum in day T28, after 4 weeks. These high molecular even *n*-alkanes are indicators of petrogenic sources, which were also confirmed by the CPI ≤ 1 (ratio between odd and even *n*-alkanes). On the contrary, at site 2 (Table 1) the individual profile of *n*-alkanes remain constant until day V7, while at day V14 and V28 higher molecular weight odd *n*-alkanes C_{27}, C_{29} and C_{31} showed a relatively predominance, characteristic of waxes from higher plants. This pattern is different from that of the autochthonous mussels (VC60), where even *n*-alkanes were predominant over odd *n*-alkanes (CPI = 0.43), characteristic of oil and petroleum products. Despite these alterations in individual *n*-alkane profiles at both transplants, CPI

Table 1 Individual concentration of n-alkanes and isoprenoids (ng/g ww), UCM and TAlk (μg/g ww) and chemical markers in mussels transplanted from site 1 to 2, and vice versa

	Site 1 to site 2						
	T0	T7	T14	T21	T28	T60	TC60
C_{14}	68 ± 11	149 ± 23	82 ± 16	564 ± 51	536 ± 114	642 ± 147	88 ± 21
C_{15}	18 ± 3	71 ± 10	89 ± 16	128 ± 19	266 ± 54	150 ± 6	55 ± 13
C_{16}	31 ± 7	61 ± 10	86 ± 15	180 ± 36	449 ± 106	166 ± 11	62 ± 14
C_{17}	61 ± 10	158 ± 26	176 ± 17	352 ± 68	492 ± 100	239 ± 19	87 ± 15
Pristane	71 ± 14	231 ± 22	235 ± 36	273 ± 55	917 ± 98	218 ± 9	64 ± 12
C_{18}	69 ± 13	415 ± 102	387 ± 66	420 ± 76	432 ± 106	376 ± 88	126 ± 25
Phytane	79 ± 15	395 ± 75	391 ± 70	460 ± 104	814 ± 199	439 ± 91	163 ± 13
C_{19}	132 ± 15	437 ± 87	775 ± 81	687 ± 108	580 ± 99	661 ± 105	252 ± 17
C_{20}	169 ± 17	653 ± 89	867 ± 89	1,055 ± 247	1,146 ± 239	847 ± 136	402 ± 80
C_{21}	155 ± 28	447 ± 94	703 ± 103	832 ± 95	740 ± 51	705 ± 110	426 ± 41
C_{22}	175 ± 18	458 ± 107	777 ± 94	1,205 ± 216	1,635 ± 210	906 ± 98	401 ± 87
C_{23}	161 ± 35	323 ± 62	595 ± 75	806 ± 80	848 ± 21	703 ± 91	312 ± 32
C_{24}	116 ± 24	219 ± 28	392 ± 46	663 ± 141	1416 ± 170	527 ± 103	261 ± 61
C_{25}	120 ± 22	156 ± 20	244 ± 34	405 ± 81	327 ± 14	337 ± 36	156 ± 38
C_{26}	115 ± 21	127 ± 29	184 ± 44	531 ± 122	1,008 ± 188	329 ± 55	180 ± 30
C_{27}	102 ± 19	145 ± 24	132 ± 25	368 ± 71	283 ± 28	242 ± 55	215 ± 40
C_{28}	97 ± 15	127 ± 13	108 ± 19	435 ± 101	878 ± 136	247 ± 46	228 ± 24
C_{29}	99 ± 18	142 ± 26	117 ± 24	377 ± 40	665 ± 86	201 ± 41	213 ± 33
C_{30}	81 ± 8	118 ± 23	90 ± 12	470[a]	309 ± 23	167 ± 4	206 ± 42
C_{31}	121 ± 18	166 ± 19	150 ± 35	501[a]	343[a]	108 ± 22	280 ± 33
C_{32}	73 ± 13	84 ± 11	81[a]	461[a]	322[a]	n.d.	141 ± 20
C_{33}	58 ± 6	79 ± 11	77[a]	245[a]	147[a]	n.d.	24 ± 5
C_{34}	39 ± 10	n.d.	n.d.	252[a]	311[a]	n.d.	n.d.
C_{35}	45 ± 6	n.d.	n.d.	129[a]	92[a]	n.d.	n.d.
C_{36}	n.d.	n.d.	n.d.	134[a]	230[a]	n.d.	n.d.
UCM	20.1 ± 4.9	37.9 ± 5.3	52.7 ± 6.6	70.8 ± 15.3	88 ± 11.5	34.4 ± 4.7	14.5 ± 1.8
TAlk	2.1 ± 0.3	4.5 ± 0.5	6.0 ± 0.4	11.2 ± 1.4	12.7 ± 2.3	7.6 ± 0.5	4.1 ± 0.5
UCM/TAlk	10	8	9	6	7	5	3
Pris/Phyt	0.89	0.59	0.60	0.59	1.13	0.50	0.39
C_{17}/Pris	0.86	0.68	0.75	1.29	0.54	1.10	1.36
C_{18}/Phyt	0.87	1.05	0.99	0.91	0.53	0.86	0.77
C_{17}/C_{29}	0.62	1.11	1.51	0.94	0.74	1.19	0.41
CPI	1.04	0.88	1.00	0.76	0.55	0.80	0.96
Main alkane[b]	C22 (8)	C20 (14)	C20 (14)	C22 (11)	C22 (13)	C22 (12)	C21 (10)
	Site 2 to site 1						
	V0	V7	V14	V21	V28	V60	VC60
C_{14}	380 ± 46	565 ± 83	239 ± 52	370 ± 55	523 ± 59	1,559 ± 266	1160 ± 208
C_{15}	202 ± 39	239 ± 51	66 ± 8	66 ± 9	132 ± 26	382 ± 93	380 ± 87
C_{16}	315 ± 35	290 ± 37	83 ± 8	160 ± 24	181 ± 42	707 ± 161	617 ± 141
C_{17}	246 ± 33	343 ± 45	111 ± 9	152 ± 18	204 ± 42	388 ± 53	586 ± 85
Pristane	618 ± 96	570 ± 110	32 6 ± 39	258 ± 57	204 ± 30	618 ± 82	1,595 ± 390
C_{18}	324 ± 39	829 ± 172	250 ± 33	429 ± 83	331 ± 75	992 ± 119	601 ± 120
Phytane	520 ± 85	1,193 ± 263	386 ± 84	497 ± 104	410 ± 45	788 ± 183	1,533 ± 163
C_{19}	771 ± 176	1,203 ± 171	325 ± 52	588 ± 79	545 ± 59	1,236 ± 167	619 ± 141
C_{20}	1,108 ± 156	889 ± 123	283 ± 65	549 ± 126	626 ± 130	1,648 ± 184	1,388 ± 232

Table 1 continued

	Site 2 to site 1						
	V0	V7	V14	V21	V28	V60	VC60
C_{21}	945 ± 168	778 ± 170	221 ± 43	406 ± 66	742 ± 132	1,246 ± 307	942 ± 168
C_{22}	958 ± 150	1,087 ± 200	403 ± 70	502 ± 112	1,509 ± 226	2,071 ± 507	1,855 ± 156
C_{23}	880 ± 154	892 ± 134	344 ± 69	320 ± 36	2,610 ± 349	1,278 ± 187	1,062 ± 190
C_{24}	750 ± 112	763 ± 102	396 ± 70	266 ± 60	3,396 ± 660	1,592 ± 156	1,806 ± 111
C_{25}	720 ± 132	709 ± 157	526 ± 85	235 ± 35	1,960 ± 345	795 ± 115	474 ± 79
C_{26}	681 ± 135	877 ± 126	903 ± 106	188 ± 42	3,830 ± 665	1,088 ± 133	1,230 ± 146
C_{27}	538 ± 132	866 ± 134	1,128 ± 65	255 ± 28	3,700 ± 623	834 ± 117	384 ± 77
C_{28}	495 ± 116	85 2 ± 207	1,335 ± 110	212 ± 48	2,787 ± 683	1,112 ± 107	1,057 ± 150
C_{29}	489 ± 121	816 ± 195	1,398 ± 77	218 ± 48	2,827 ± 626	630 ± 120	308 ± 53
C_{30}	342 ± 74	661 ± 150	1,128 ± 108	204 ± 25	2,359 ± 536	689 ± 159	1,001 ± 152
C_{31}	477 ± 80	663 ± 145	1,104 ± 121	241 ± 56	2,136 ± 502	505 ± 80	379 ± 94
C_{32}	237 ± 40	343 ± 49	682 ± 101	173 ± 31	586 ± 80	994 ± 100	1,024 ± 102
C_{33}	234 ± 29	143 ± 24	341 ± 58	130 ± 26	145 ± 27	427 ± 73	171 ± 17
C_{34}	n.d.	72[a]	134 ± 23	76 ± 14	138 ± 16	478 ± 98	530 ± 112
C_{35}	n.d.	49[a]	58 ± 9	50 ± 6	48[a]	n.d.	122 ± 21
C_{36}	n.d.	n.d.	53 ± 8	40 ± 6	32[a]	n.d.	277 ± 53
UCM	105.2 ± 15.6	78.6 ± 13.4	73.1 ± 10.9	46.1 ± 6.4	94.3 ± 16.8	39.7 ± 5	63.9 ± 10,7
TAlk	11.1 ± 1.3	13.9 ± 1.6	11.5 ± 1.3	5.8 ± 0.6	31.3 ± 4.7	20.7 ± 2.1	18.0 ± 2.4
UCM/TAlk	9	6	6	8	3	2	4
Pris/Phyt	1.19	0.48	0.84	0.52	0.50	0.78	1.04
C_{17}/Pris	0.40	0.60	0.34	0.59	1.00	0.63	0.37
C_{18}/Phyt	0.62	0.69	0.65	0.86	0.81	1.26	0.39
C_{17}/C_{29}	0.50	0.42	0.08	0.70	0.07	0.62	1.90
CPI	0.98	0.93	0.95	0.84	0.92	0.60	0.43
Main alkane[b]	C20 (10)	C19 (9)	C29 (12)	C19 (10)	C26 (12)	C22 (10)	C22 (10)

TAlk: $\sum n$C14–C36; CPI (Carbon preference index): $\sum n$C15–C35)/$\sum n$C14–C36

UCM Unresolved complex mixture, *n.d* not detected

[a] only in one replicate this hydrocarbon was identified

[b] % of total

remains predominantly ≤1, indicating the presence of oil and oil products throughout this area.

The C_{17}/C_{29} ratio represents the relative contribution of marine biogenic *n*-alkanes and terrigenous biogenic *n*-alkanes. In mussels transplanted from site 1 to 2 (Table 1) this ratio changed from 0.62 (T0) to 1.11 and 1.51 (T7 and T14), indicating a substitution of terrigenous *n*-alkanes by marine *n*-alkanes. Both in the beginning (T0) and autochthonous mussels (TC60) a predominance of C_{29} in relation to C_{17} ($C_{17}/C_{29} = 0.62$ and 0.41) verifies. In mussels transplanted from site 2 to 1, the C_{17}/C_{29} ratio (Table 1) indicates a strong terrigenous influence, especially at days V14 and V28 (0.08 and 0.07, respectively). The marine biogenic influence occurred only in autochthonous mussels (VC60) with $C_{17}/C_{29} = 1.9$.

The presence of the isoprenoids pristane (Pris) and phytane (Phyt) is a marker of petrogenic products, although Pris is also considered to be produced by algae in lower proportions. Pris/Phyt ratio is ≤1 for oil and oil compounds, and ≥1 for biogenic inputs. During the cross transplant experiment the Pris/Phyt ratio ranged between 0.39 and 1.3 in mussels from site 1 transplanted to site 2, and between 0.48 and 1.1 in mussels transplanted from site 2 to 1 (Table 1), confirming that at both the sites, the main source of *n*-alkanes is petrogenic, with some biogenic inputs of pristane.

On the other hand, the ratios C_{17}/Pris and C_{18}/Phyt indicate the relative degradation between linear *n*-alkanes and branched isoprenoids, which is used to evaluate the presence of oil and the relative biodegradation of *n*-alkanes. Low values of these indices (<1) are indicative of the presence of degraded oil, whereas values between 4 and 8 indicate phytoplanktonic origin. In the transplant assays, these ratios ranged between 0.53 and 1.29 in mussels transplanted from site 1 to 2 and between 0.34 and 1.26 in those transplanted from site 2 to 1 (Table 1).

As previously, the relative slower degradation rate of branched alkanes over linear alkanes indicates the presence of degraded oil (chronic contamination). However, a recent and less degraded contamination occurred at some times, reflected by C_{17}/Pris and C_{18}/Phyt ≥ 1, as also confirmed by UCM/Talk ratio.

Polycyclic aromatic hydrocarbons

TPAH concentrations of mussels transplanted from site 1 to 2 (Fig. 2c; Table 2) remained unchanged during the first week, and increased afterwards to a maximum at day T28 ($P < 0.05$), with an accumulation rate of 66 ng/g/day ($r = 0.96$; $P < 0.05$). In this 4 week period TPAHs concentration increased 4.2 fold, relatively to control. One month after depuration at original site, TPAHs concentrations in mussel tissues decreased 27% (T60), but were still significantly higher than that of the autochthonous mussels (TC60) ($P < 0.05$), and those at the beginning (T0) ($P < 0.05$). Contrarily, in mussels transplanted from site 2 to 1 (Fig. 2c; Table 2), TPAH concentrations gradually decreased from day V0 to V21, with a loss rate of 29 ng/g/day, which corresponds to a decrease of 61.3% relatively to V0. As occurred with AHs, a significant increase at day V28 (645 ± 133 ng/g ww) ($P < 0.05$) also verifies. When mussel were back-transplanted to site 2 for 1 month, TPAH concentrations did not significantly changed ($P > 0.05$) but were lower than that of the V0 and VC60 mussels. Thus, mussels proceeding from a cleaner site (Tavira-site 1) significantly accumulate PAHs when transplanted to a more contaminated site (Vilamoura-site 2), and TPAH depuration simultaneously occurs in the opposite case, but with slower rates of elimination than of accumulation.

The distribution pattern of PAHs by ring number in both transplant assays (Fig. 3) was similar at the beginning and at the end of the experiment, at both sites, with the general predominance of aromatics with four benzene rings. At site 1, the concentration of petrogenic aromatic hydrocarbons with 2 + 3 rings was higher than the 5 + 6 ring PAHs, whereas at site 2 the predominant compounds were mainly pyrolytic aromatic hydrocarbons (4–6). In mussels transplanted from site 1 to 2 (Fig. 3a; Table 2) the proportion of 2 + 3 rings PAHs (petrogenic) gradually decreases from the beginning until day T21 with a concomitant increase of pyrolytic PAHs with 5 + 6 ring. Conversely in mussels transplanted from site 2 to 1 (Fig. 3b; Table 2), the relative proportion of 2 + 3 ringed PAHs increases at the first weeks, and remained similar between days V28 and V60. After 32 days in the original site, mussels had still the typical characteristics of site 1, that is, higher percentage of lower molecular weight PAHs.

The further analyses of individual PAHs concentrations (Table 2) indicate that BaA is the main PAH found in mussels from both sites (from 20 to 37% of the TPAH concentrations). On the other hand, Ace (3 ring), BbF (5 ring) and BPer and IndP (6 ring) are the main compounds on their ring group, and have the same behavior, that is, concentrations decreased in mussels transplanted from site 1 to 2, and increased in mussels transplanted from site 2 to 1. Thus, as for *n*-alkanes, the distribution pattern of the individual PAHs concentrations also changed, as mussels from site 1 acquires pyrolytic and loses petrogenic compounds, resembling to autochthonous mussels from site 2, while when mussels from site 2 were transplanted to a site with petrogenic characteristics, acquire these characteristics, as they proportionally accumulate more low molecular weight PAHs.

In order to evaluate the origin and sources of PAHs in transplanted mussel tissues, some diagnostic ratios were calculated and are presented in Table 2. The Phen/Ant ratio ranged between 0.88 and 3.9 indicating pyrolytic (Phen/Ant < 10) sources of PAHs, whereas the Flu/Pyr ratio indicates both petrogenic (Flu/Pyr 1<) and pyrolytic (Flu/Pyr > 1) sources of PAHs, at both sites during the transplant period. Mussels from site 1 had both sources of PAHs in the first 2 weeks and in site 2 at day 60. Ant/Ant + Phen; BaA/BaA + Chrys; IndP/IndP + BPer and Flu/Flu + Pyr (Fig. 4; Table 2) diagnostic ratios were calculated to distinguish between pyrolytic PAHs that are formed by the incomplete combustion of fossil fuels from pyrolytic PAHs with origin in biomass combustion. The Ant/Ant + Phen ratio shows that PAHs accumulated in transplanted mussels derived mainly from biomass combustion. These results are in accordance with the Phen/Ant ratio that also shows predominance of pyrolytic PAHs. At both sites, the values of Flu/Flu + Pyr ratio is consistent with PAHs emitted from the incomplete fossil fuel combustion or from a mixture of PAHs proceeding from wood combustion (or possibly from wood preservatives as creosotes) and/or coal, with only one occurrence of PAHs derived from oil (or fuels) not burnt. The Ant/Ant + Phen ratio was higher than >0.10 and in the majority of the cases where the Flu/Flu + Pyr ratio > 0.40 sustaining the conclusion that pyrolytic compounds are a strong component of PAHs accumulated by mussels, either at the low or the high contaminated site. The same trend was supported by the results of BaA/BaA + Chrys and IndP/IndP + BPer ratios, with PAHs resulting primarily from combustion and combustion of oil products. Thus, PAHs accumulated in tissues of mussels transplanted from site 1 to 2 and vice versa are mainly due to an incomplete combustion of oil and its derivatives (Ant/Ant + Phen; BaA/BaA + Chrys; IndP/IndP + BPer), presenting also petrogenic (oil not burnt) and pyrolytic PAHs derived from biomass combustion (Flu/Flu + Pyr).

Table 2 Individual and TPAHs concentration (ng/g ww) and diagnostic ratios in mussels from site 1 (T) transplanted to site 2 (V), and vice versa

	Site 1 to site 2						
	T0	T7	T14	T21	T28	T60	TC60
Naphthalene	11.6 ± 1.6	5.4 ± 1	114.4[a]	113.6[a]	50.4 ± 7.2	11.3 ± 0.9	18.7 ± 4.3
Acenaphthylene	24.2 ± 2.7	3.1 ± 0.6	8.7[a]	30.7 ± 6.7	100.9 ± 24.9	81.1 ± 13.3	44.9 ± 9.5
Acenaphthene	105.5 ± 15.7	94.6 ± 13.3	82.7 ± 12.9	38.5 ± 6.6	241.2 ± 58.4	245.5 ± 4.4	213.1 ± 22.5
Fluorene	18.2 ± 1.9	6.9 ± 1.4	20.3 ± 4.5	73.3 ± 12.1	95.4 ± 8.5	20.6 ± 3.2	11.7 ± 2
Phenanthrene	18.8 ± 1.5	6.3 ± 1.4	16.5 ± 1.9	27 ± 3.6	17.5 ± 0.9	12.6 ± 2.6	15.3 ± 1.5
Anthracene	5.9 ± 0.3	4.2 ± 0.8	4.8 ± 1	10.4 ± 2.3	11.1 ± 1.8	3.3 ± 0.7	11.9 ± 2.5
Fluoranthene	17.9 ± 3.2	9.7 ± 1.5	34.1 ± 4	100.6 ± 13	137 ± 26.5	115.1 ± 8.9	42.1 ± 8.1
Pyrene	23.8 ± 5.9	11.0 ± 1.1	69.5 ± 10.1	66 ± 5.6	80 ± 13.4	41.5 ± 3.5	15.2 ± 0.7
Benzo(*a*)anthracene	106.0 ± 11.6	119.6 ± 4.6	257.1 ± 32.8	251.5 ± 61.4	572.5 ± 76.8	472.4 ± 69.8	168.6 ± 12.9
Chrysene	84.7 ± 3	76 ± 4.4	193.3 ± 17.6	198.1 ± 14.1	418.2 ± 3.7	338.7 ± 21.8	61.9 ± 12.1
Benzo(*b*)fluoranthene	18.7 ± 4.4	49.4 ± 8.7	147.3 ± 32.3	78.4 ± 5	69.8 ± 14.4	14 ± 2.3	15.4 ± 1
Benzo(*k*)fluoranthene	13.8[a]	0.2[a]	54.7[a]	27.9 ± 4.8	47.1 ± 5.9	1.7 ± 0.1	2.6 ± 0.6
Benzo(*a*)pyrene	0.4[a]	1.6[a]	9.1[a]	16.5 ± 3.2	19.2 ± 1.8	0.1 ± 0.01	1.2 ± 0.2
Dibenzo(*a,h*)anthracene	0.4[a]	1.2[a]	5.5[a]	48.2 ± 9	7.5 ± 1.6	0.9 ± 0.06	0.24 ± 0.04
Benzo(*g,h,i*)perylene	0.1[a]	0.5[a]	3.1[a]	18.4 ± 4.1	5.1 ± 1.1	0.2 ± 0.01	0.04 ± 0.01
Indeno(1,2,3 *cd*)pyrene	0.03[a]	0.04[a]	1.02[a]	13 ± 1.4	3.1 ± 0.7	0.1 ± 0.02	0.03 ± 0.01
TPAH	443 ± 43	390 ± 35	924 ± 192	1,055 ± 144	1,876 ± 96	1,359 ± 125	623 ± 54
Phen/Ant	3.18	1.48	3.45	2.59	1.57	3.87	1.28
Flu/Pyr	0.75	0.88	0.49	1.52	1.71	2.77	2.76
Ant/Ant + Phen	0.24	0.40	0.22	0.28	0.39	0.21	0.44
Flu/Flu + Pyr	0.43	0.47	0.33	0.60	0.63	0.74	0.73
BaA/BaA + Chrys	0.56	0.61	0.57	0.56	0.58	0.58	0.73
IndP/IndP + Bper	0.40	0.09	0.25	0.42	0.38	0.25	0.43
2 + 3 rings (%)	42	31	19	22	28	28	51
4 rings (%)	53	56	61	58	64	71	46
5 + 6 rings (%)	6	14	20	19	8	1	3
Main PAH[b]	BaA (24)	BaA (31)	BaA (28)	BaA (24)	BaA (31)	BaA (35)	Acften (34)
	Site 2 to site 1						
	V0	V7	V14	V21	V28	V60	VC60
Naphthalene	30.4 ± 6.3	17.2 ± 3.8	9.3 ± 2	n.d.	77.1[a]	73.6 ± 12	79.6 ± 13
Acenaphthylene	42.8 ± 4.1	45.9 ± 5.2	17.8 ± 4.2	19.5 ± 4.8	41.9 ± 6.4	29.9 ± 6.4	56.7 ± 2.6
Acenaphthene	78.1 ± 14.6	94.9 ± 16.8	95.4 ± 7.2	40.7 ± 7.2	69.2 ± 13.5	157.9 ± 7.4	54.3 ± 11.7
Fluorene	58.8 ± 13.4	51.3 ± 3.2	44.6 ± 10.9	15.3 ± 2.4	15.6 ± 3.2	15.2 ± 3.2	17.5 ± 3.8
Phenanthrene	24.7 ± 5.7	31.2 ± 5.6	47.8 ± 6.6	12.8 ± 2.7	33.6 ± 5.1	15.0 ± 1.9	16.9 ± 0.8
Anthracene	17.5 ± 1.1	13.2 ± 0.6	37.4 ± 6.7	14.5 ± 0.9	26.4 ± 2.8	4.7 ± 0.6	6.0 ± 0.9
Fluoranthene	135.6 ± 24.6	84.9 ± 12.8	143.9 ± 16.2	36.2 ± 8	74.9 ± 16.7	35.6 ± 7.2	85.9 ± 12.5
Pyrene	83.1 ± 9.7	43.4 ± 2.7	92.0 ± 11.1	19.0 ± 2.4	31.2 ± 5.5	46.3 ± 7.2	39.2 ± 3.4
Benzo(*a*)anthracene	319.9 ± 61.2	239.3 ± 42.4	213.2 ± 45	149.5 ± 18.7	131.4 ± 16.2	236.0 ± 30.3	262.5 ± 24
Chrysene	149.8 ± 29	180.5 ± 13.6	17.1 ± 3.7	38.8 ± 1.8	88.4 ± 3	117.5 ± 3.5	174.0 ± 18
Benzo(*b*)fluoranthene	81 ± 16	28.2 ± 4.6	33.9 ± 7.5	40.7 ± 5.7	64.9 ± 14.3	34.1 ± 5.7	94.3 ± 21
Benzo(*k*)fluoranthene	14.4 ± 2.1	11.1 ± 1.8	14.5 ± 2.6	12.0 ± 0.2	24.2 ± 2.7	0.5 ± 0.1	81.1 ± 13.2
Benzo(*a*)pyrene	8.7 ± 1.2	3.2 ± 0.7	4.7 ± 0.6	5.5 ± 1.1	0.4 ± 0.1	5.3[a]	29.9 ± 3.1
Dibenzo(*a,h*)anthracene	4.1 ± 0.5	0.60 ± 0.05	2.1 ± 0.5	2.3 ± 0.5	3.4 ± 0.4	2.7[a]	6.0 ± 0.9
Benzo(*g,h,i*)perylene	2.7 ± 0.2	0.08 ± 0.01	0.10 ± 0.01	0.07 ± 0.01	1.04[a]	1.8[a]	3.9 ± 0.8
Indeno(1,2,3 *cd*)pyrene	0.8 ± 0.1	0.05[a]	0.03[a]	0.05[a]	0.29[a]	0.8[a]	2.5 ± 0.3

Table 2 continued

	Site 2 to site 1						
	V0	V7	V14	V21	V28	V60	VC60
TPAH	1,052 ± 67	845 ± 38	774 ± 98	407 ± 51	645 ± 133	772 ± 93	1,010 ± 130
Phen/Ant	1.41	2.37	1.28	0.88	1.27	3.21	2.84
Flu/Pyr	1.63	1.96	1.56	1.91	2.40	0.77	2.19
Ant/Ant + Phen	0.42	0.30	0.44	0.53	0.44	0.24	0.26
Flu/Flu + Pyr	0.62	0.66	0.61	0.66	0.71	0.43	0.69
BaA/BaA + Chrys	0.68	0.57	0.93	0.79	0.60	0.67	0.60
IndP/IndP + Bper	0.23	0.40	0.23	0.38	0.22	0.31	0.39
2 + 3 rings (%)	24	30	33	25	35	38	23
4 rings (%)	66	65	60	60	51	56	56
5 + 6 rings (%)	11	5	7	15	15	5	21
Main PAH[b]	BaA (30)	BaA (28)	BaA (28)	BaA (37)	BaA (20)	BaA (31)	BaA (26)

TPAH Sum of 16 individual PAHs concentration

[a] only in one replicate this hydrocarbon was identified

[b] % of total

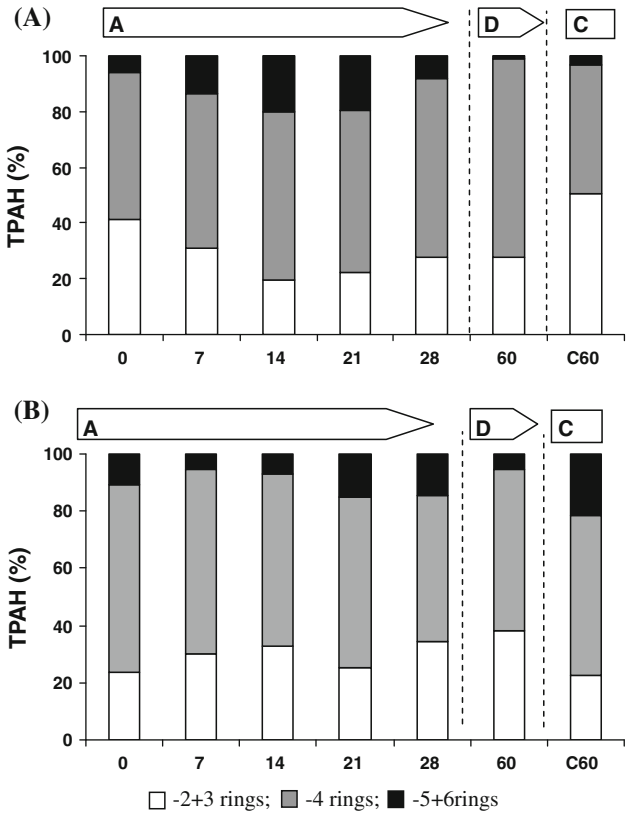

Fig. 3 TPAH grouped by rings (%), in mussels transplanted from site 1 to 2 (**a**) and transplanted from site 2 to 1 (**b**). (*A* accumulation, *D* depuration, *C* control)

MFO system

Total cytochromes concentration (CYP450, P418, and CYT b_5) and the associated reductases activities (NADPH-RED and NADH-RED) in the microsomal fraction of mussels digestive gland transplanted from site 1 to 2 and vice versa, are in Fig. 5.

The CYP450 (Fig. 5a) initial/basal concentration in both control groups (0 and C60) were significantly higher ($P < 0.05$) in site 2 (36.6 ± 5.4 and 38.3 ± 4.7 pmol/mg prot) than in site 1 (24.1 ± 3.2 and 24.2 ± 2.7 pmol/mg prot), but levels at the beginning and at the end of each transplant did not differ. CYP450 concentration decreased during the first week in mussels transplanted from site 1 to 2 and increased afterwards until a maximum at day T28 (37.2 ± 3 pmol/mg prot, $P < 0.05$), when TPAHS had also reached higher levels. CYP450 was induced with a rate of 0.74 pmol/mg prot/day ($r = 0.94$; $P < 0.05$), representing a 35% increase (1.87 fold). When mussels were back transplanted to site 1 for 60 days (T60), total CYP450 concentration decreased 4% (35.5 ± 1.8 pmol/mg prot; day T60) ($P < 0.05$), but was still significantly higher when compared to the beginning and autochthonous mussels (T0 and TC60). Contrarily, in mussels transplanted from site 2 to 1, total CYP450 concentration gradually decreased from day V0 (36.6 ± 5.4 pmol/mg prot) until day V21 (26.6 ± 2.9 pmol/mg prot) ($P < 0.05$), with a rate of 0.48 pmol/mg prot/day ($r = 0.97$; $P < 0.05$), which represents a 27% loss in these 3 weeks. At the 4th week, CYP450 showed an induction, as response to hydrocarbons increase at this week, to levels comparable to V0. After back transplanted to the site of origin, CYP450 levels at day V60 were 5% lower than at day V28, and similar to levels of V0 and of the autochthonous mussels (VC60).

"418" peak (P418) concentrations (Fig. 5b) did not changed at both sites and throughout the time, though in the 3rd and 4th week, concentration in mussels transplanted from site 1 to 2 were higher than in the inverse transplant.

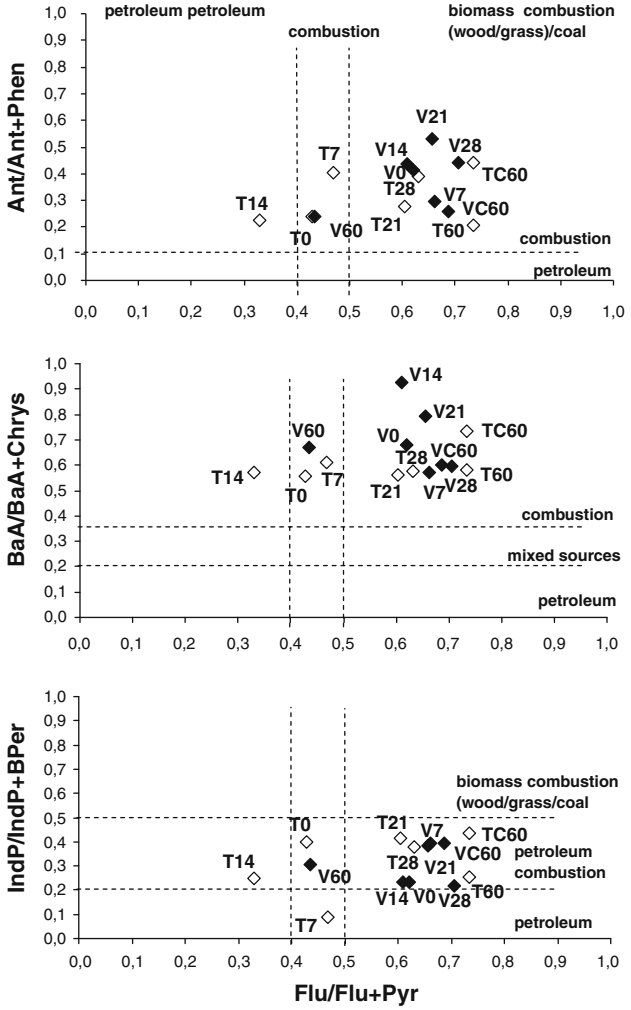

Fig. 4 Ant/Ant + Phen, BaA/BaA + Chris and IndP/IndP + BPer versus Flu/Flu + Pyr ratio in mussels transplanted from site 1 to 2 (*white diamonds*) and transplanted from site 2 to 1 (*black diamonds*)

NADPH-RED activity (Fig. 5c) in both control groups of both transplants did not significantly differ, although site 2 presents higher activities. NADPH-RED activity in mussels transplanted from site 1 to 2 followed the same pattern of CYP450, that is, after a week with no significant changes, reductase activity increased to a maximum at 4th week (21.7 ± 2.8 nmol/min/mg prot; $P < 0.05$), with a rate of 0.29 nmol/min/mg prot/day ($r = 0.91$; $P < 0.05$), which represents a 32% induction (1.48 fold) relatively to T0. After spending 60 days at original site, NADPH-RED activity did not significantly changed (TC60—19.3 ± 1.8 nmol/min/mg prot). Though NADPH-RED activity was comparable at both sites at the beginning of transplants, it was significantly higher in mussels transplanted from site 1 to 2 between the day 14 and 28. When mussels were transplanted from site 2 to 1, the NADPH-RED activity did not significantly changed with time, nonetheless activities in this period of 4 weeks are significantly lower than in the autochthonous organisms (day C60).

Cytochrome b_5 (Fig. 5d) concentration is not significantly different in mussels from both control groups of both transplants, although basal concentrations were higher at site 1. Mussels transplanted from site 1 to 2 showed an induction of CYT b_5 from T0 (20.4 ± 1.2 pmol/mg prot) to T28 (27.4 ± 1.5 pmol/mg prot), augmenting 35% (1.34 fold) from the initial base level, with an increase rate of 0.22 pmol/mg prot/day ($r = 0.91$; $P < 0.05$). However, these increments were not significant with time. CYT b_5 concentrations at time T60 was comparable to levels found in both controls (T0 and TC60). Mussels transplanted from site 2 to 1 showed no significant changes in CYT b_5 concentration with time (which ranged from 14.4 ± 1.6 to 19.8 ± 2.5 pmol/mg prot) but levels were always lower than those of mussels transplanted from site 1 to 2.

The NADH-RED activities (Fig. 5e) from both transplant experiments remained unchanged with time and were similar in mussels from both the sites. NADH-RED activity varied from 70.5 ± 13.8 to 78.6 ± 1.1 pmol/mg prot and from 65.9 ± 7.9 and 70.7 ± 6.5 pmol/mg prot, respectively from days 0 and C60 and to mussels transplanted from site 1 to 2 and transplanted from site 2 to 1.

Relation between CYP450 and hydrocarbons

The relationship between cytochrome P450 (and remaining MFO system components), analyzed in the digestive gland and the total hydrocarbons concentration (AHs and PAHs) accumulated mussels tissues, were evaluated by regression analysis, in both transplants together and separately. The results show a significant relationship between CYP450 and TPAH when both transplants were used together (CYP450 = 0.010 TPAH + 20.8; $r = 0.75$, $P < 0.05$), or separately (site 1 to 2 CYP450 = 0.011 TPAH + 17.2; $r = 0.95$, $P < 0.05$; site 2 to 1 CYP450 = 0.017 TPAH + 18.7; $r = 0.86$, $P < 0.05$) but not with TAlk concentration in any case. CYP450 also have a direct relationship with UCM, but only when data from both transplants were used simultaneously ($r = 0.59$; $P < 0.05$). In the remaining MFO system components, only NADPH-RED activity was positively related with TPAH concentrations, when data from both transplants were analyzed simultaneously ($r = 0.78$; $P < 0.05$) and in mussels transplanted from site 1 to 2 ($r = 0.95$; $P < 0.05$). NADPH-RED activity was also positively related with TAlk concentrations in mussels transplanted from site 1 to 2 ($r = 0.86$; $P < 0.05$). CYT b_5 and NADH-RED were not related with AHs and PAHs, in any of the situations.

In order to evaluate which of the individual hydrocarbon had greater influence in the CYP450 concentration, CCA

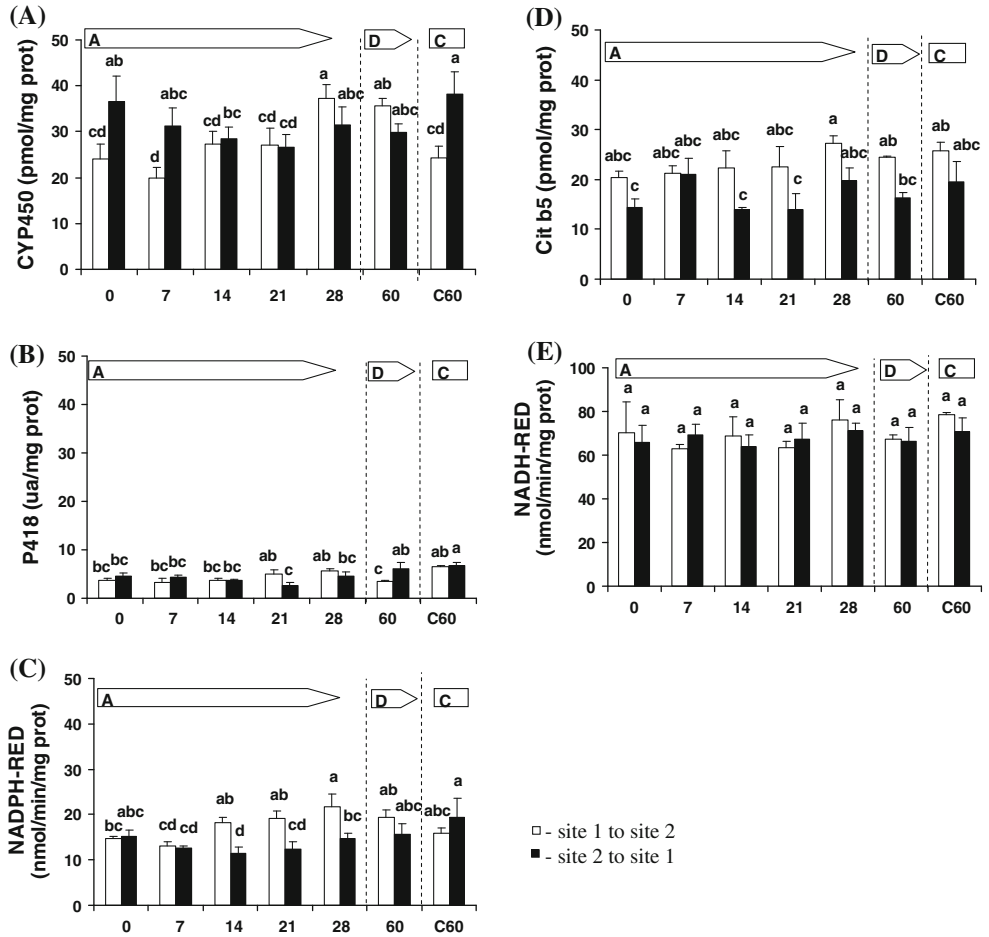

Fig. 5 Total cytochrome P450 (**a**), P418 (**b**) concentrations and NADPH-RED activity (**c**), cytochrome b_5 concentration (**d**), and NADH-RED activity(**e**), in the digestive gland microsomal fraction of *M. galloprovincialis* transplanted from site 1 to 2 and transplanted from site 2 to 1. (*A* accumulation, *D* depuration, *C* control; mean ± standard deviation; *different letters* mean statistical differences; Duncan' s test, $P < 0.05$)

analysis was also used. The individual AH and aromatic hydrocarbons data from both transplants were transformed as proportion of the total concentration, and were used separately (Fig. 6a, b) and together (Fig. 6c). In the first case (Fig. 6a), axis 1 and 2 explains 81% of the data variance, and clearly separates mussels from the site 1 from those of site 2 in the first axis, with those from site 1 associated with isoprenoids and low molecular weight *n*-alkanes (C_{14}–C_{23}). The second axis discriminates the time of exposure to *n*-alkanes, showing that MFO system components increase with exposure time at one site and decreases in the other, towards to a biochemical equilibrium to lesser environmental contamination.

When individual PAH concentrations were analyzed (Fig. 6b), axis 1 and 2 account for 70% of the data variance, and the two transplants are separated by the second axis, where in mussels from site 1 the individual concentrations of 4–6 ringed aromatics builds up with time. The first axis discriminates MFO system components and individual PAHs by molecular weight, showing a decreasing importance of MFO with lower molecular weight PAHs accumulation with time, in mussels from site 2. On the contrary, MFO acquires more importance as mussels from site 1 accumulates more high molecular weight PAHs.

When the hydrocarbons were analyzed simultaneously (Fig. 6c) (axis 1 and 2 explain 67% of the data variance), the same trend is observed, i.e. the two transplant experiments are projected apart by the second axis, confirming the distinctive behavior at the two sites. Axis 1 separates the MFO system components and low weight AHs + high molecular weight PAHs as time goes by in mussels from site 1, and MFO system components decrease with high weight AHs + low weight PAHs, at mussels from site 2.

Discussion

Bivalve molluscs are considered good bioindicators of contaminants in aquatic systems. These organisms are capable of concentrating certain compounds to levels much

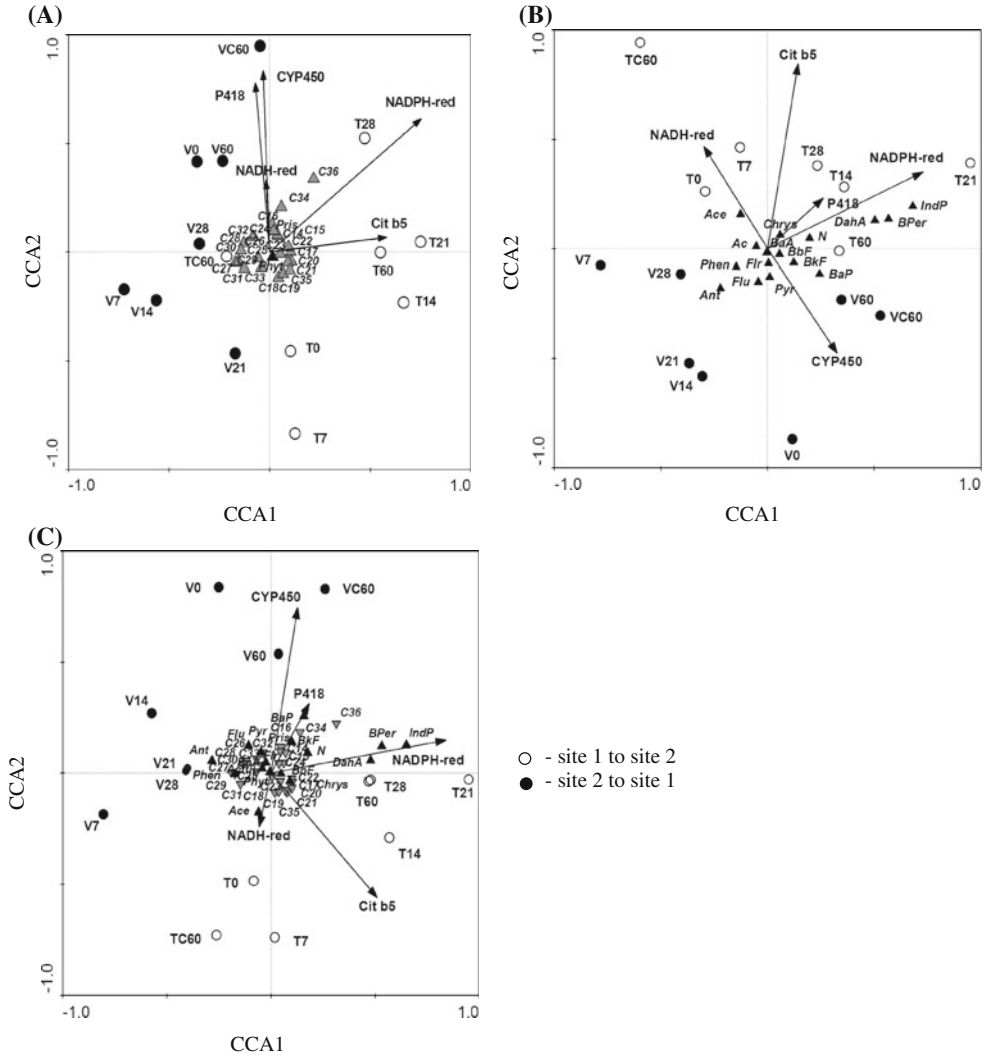

Fig. 6 Canonical correspondence analysis (CCA) between MFO system components and the individual *n*-alkanes concentrations (**a**), individual PAHs concentration (**b**) and the two types of hydrocarbons together (**c**), considering both the transplants (hydrocarbons as proportion of the total)

higher than those found in seawater (Cajaraville et al. 2000), providing information on contamination trends. Biochemical responses associated to particular contaminants can be used to assess how organisms cope with pollution, integrating information on health status of the organisms and the environment.

Hydrocarbons levels (TAlk, UCM and TPAH) found in mussels from site 1 and 2 are of the same order of magnitude of those previously found in this same area (where site 1 consistently showed lower levels of contamination relatively to site 2) (Bebianno et al. 2007; Serafim et al. 2008; Cravo et al. 2009) (Table 3), but lower than those found in the NW Portuguese coast (Lima et al. 2007), and lower than in mussels from areas affected by oil spills or tanker accidents, such as the Prestige (Soriano et al. 2006) and the Aegean Sea (Porte et al. 2000a, b). Nevertheless, the highest PAH concentrations found in mussels from site 2 are similar to those in some polluted areas of Mediterranean like the Ebro delta and Barcelona area (Porte et al. 1991; Solé et al. 1995a, b; Porte et al. 2001a, b), Venice and the Adriatic Sea (Wetzel and van Vleet 2004) (Table 3). Levels of MFO system components in mussels from site 1 and 2 are of the same order of magnitude as those found in the same area in previous works, in which CYP450 basal concentrations at site 1 are lower than at site 2, where elevated CYP450 levels are associated to highest hydrocarbon contamination. (Bebianno et al. 2007; Lopes and Bebianno 2007; Cravo et al. 2009; Lopes 2009) (Table 3). On the other hand, MFO system components at site 2 are at the same degree of those found in sites with chronic hydrocarbon contamination like Venice (Livingstone et al. 1995; Solé et al. 2000a; Solé 2000), and the Adriatic Sea (Livingstone et al. 1995), the Ebro delta and Barcelona area (Porte et al. 1991, 2001a, b; Solé et al.

1994, 1995a, b), or in sites near to an oil spill (Porte et al. 2001a, b; Solé 2000) (Table 3).

TAlk and UCM

In mussels transplanted from site 1 to 2 (Fig. 2a) TAlk and UCM increased 6 and 4.4 fold higher than the initial concentrations, with increasing rates of 0.39 and 2.4 μg/g/day, respectively. After a 60 days period in the original site, mussels showed signs of recovery, by the depuration of 40 and 60% of accumulated hydrocarbons, indicating a fast elimination to levels similar to those of autochthones mussels. The changes in *n*-alkanes profile indicate a petrogenic source due to an increase of isoprenoids and even *n*-alkanes, characteristic of oil/petroleum sources. Nevertheless, an increase of odd *n*-alkanes (C_{17}, C_{27} and C_{29}) also occurred. These *n*-alkanes are biogenic, since C_{17} is typical of *n*-alkanes synthesized by phytoplankton, while C_{27} and C_{29} are synthesized by land higher plants. Marine and terrigenous *n*-alkanes are accumulated through microalgae and particles that mussels filter from the water column (Wetzel and van Vleet 2004). When mussels are transplanted from the site more contaminated (2) to the site with background levels of contamination (1) (Fig. 2), a decrease of 48 and 56% of original TAlk and UCM occurred by the 3rd week with a elimination rate of 0.57 and 2.6 μg/g/day respectively, reaching at day 21 half of the initial concentration ($P < 0.05$). A change in *n*-alkane individual profile also occurred, but both petrogenic and biogenic sources occur simultaneously.

These results are in agreement with data obtained on an ABM strategy carried out in the Adriatic Sea, where mussels *M. galloprovincialis* were transplanted to the channels of Venice for a period of 3 weeks. In this case total AHs concentration increase 7-fold than the baseline levels, mainly due to even *n*-alkanes of high molecular weight, CPI was next to one (petroleum and oil products) and individual profile in mussels tissues was similar to the sediments which had petrogenic characteristics (Wetzel and van Vleet 2004). Signs of depuration and recovery were described in mussels transplanted from contaminated sites to cleaner sites, in the Bay of Biscay (Orbea et al. 2000) and the Venice Lagoon (Da Ros et al. 2000, 2002). Several cross-transplantation works to study metallic or organic contamination refers to a loss of the accumulated contaminants within a 3–6 weeks period, in mussels transplanted from a polluted site to a cleaner one, and a significant accumulation in the inverse situation (Da Ros et al. 2000; Orbea et al. 2000; Nasci et al. 2002; Nigro et al. 2006).

Polycyclic aromatic hydrocarbons

A 4-fold TPAHs increase, mainly due to the increase of high molecular weight PAHs from 4 to 6 rings (fluoranthene, anthracene, chrysene, benzo(*b*)fluoranthene, benzo(*g,h,i*)perylene and indeno(1,2,3 *cd*)pyrene) typical of incomplete combustion biomass and of fossil fuel products (Fig. 4), were accumulated in mussels transplanted from site 1 to 2. Low weight PAHs concentration, like acenaphthylene, acenaphthene and fluorene decreased during the first 3 weeks, whereas the concentrations of phenanthrene and anthracene remained unchanged (Figs. 2, 3). An elimination of 61.3% of initial TPAHs levels was observed in mussels transplanted from site 2 to 1, with simultaneous changes in individual profiles. In fact, low molecular weight PAHs like acenaphthene, phenanthrene and anthracene, present in oil products and characteristic of petrogenic sources, increased over time with the gradual reduction of pyrolytic high molecular weight PAHs (4–6 rings) (Fig. 2, 3). The alterations between the proportion of low molecular weight PAHs (petrogenic) and high molecular weight PAHs (pyrolitic) indicate that both types of sources are present, but with different predominant proportion at each site (Fig. 4). However, in transplanted mussels the predominance of petrogenic or pyrolitic signature is gradually replaced by the compound signature of the destination site, confirming that accumulated compounds found in mussels tissues reflect the different environmental predominant source of contamination.

Similar results of accumulation and depuration were obtained in other studies where mussels are transplanted to a more contaminated site and cross-transplanted to a less contaminated site. After a 4 week period, Serafim et al. (2011) in the South coast of Portugal found a three fold TPAH increase in mussels transplanted to a more contaminated site, and a decrease of 3.4 folds-less of the initial TPAHs body burden (Table 3). This type of changes was also observed in mussel *M. galloprovincialis* transplanted from the Adriatic Sea to the inner channels of Venice lagoon where a background petrogenic signature was overlapped by a greater accumulation of pyrolytic PAHs, due to intensive maritime traffic; TPAHs in mussels tissues increased from <20 to 5320 ng/g ww (Table 3) (Wetzel and van Vleet 2004). Francioni et al. (2007) reported a 3-fold accumulation of pyrolitic PAHs in mussels *Perna perna* transplanted from a clean site at Guanabara bay to a more contaminated one, in which the individual profile of PAHs changed from mainly petrogenic to pyrolytic with enhanced 4–6 ring PAHs. Contrarily, in the cross-transplant 75% of the TPAHs concentrations decreased. In mussels cross-transplanted between two sites in the Bay of Biscay for 3–6 weeks, TPAHs concentrations in the mussel tissues increased significantly in mussels transplanted to a high contaminated site while mussels in the inverse transplant presented a significant loss of the initial contaminant body burden (Orbea et al. 2000). Related results were also detected in mussels transplanted from a clean site of the

Table 3 Hydrocarbons concentration and MFO system components concentration and activities, in mussels from Europe

	TAlk (μg/g ww)	UCM (μg/g ww)	PAHs (ng/g ww)	CYP450 (pmol/mg prot)	NADPH-red (nmol/min/mg prot)	CYT b_5 (pmol/mg prot)	References
M. edulis							
Galicia	0.33–0.65[c]	7–25,6[c]	13.2–46.3	0.52–1.83	1.25–2.80		Solé et al. (1996)
Galicia		7–717[c]					Porte et al. (2000a, b)
M. galloprovincialis							
Adriatic Sea		27.6–107.6[c]	14.2–50.4[c]	57.4 ± 9.6			Livingstone et al. (1995)
Adriatic Sea	7.8–40.6[c]	79.6–156[c]	260[c]				Wetzel and Van Vleet (2004)
Adriatic Sea	(Site O) n.d ↑ 36.9[c]		(Site O) < 20 ↑ 5,320[c]				Wetzel and Van Vleet (2004)
Arcachon			55.8–484[c]				Baumard et al. (1998a, b)
Barcelona			2,700[a]	68.6 ± 13.9	19.3 ± 2.3	74. ± 16.9	Porte et al. (1991)
Ebro delta			235[b]	56.2 ± 5.1	24.9 ± 1.6	54.2 ± 2.1	Solé et al. (1994)
Ebro delta			100[a]	47.7 ± 7.2	16.4 ± 2.5	63.1 ± 5.6	Porte et al. (1991)
Ebro delta			1,100–3,400	30–86	8–25	40–90	Solé et al. (1995a, b)
Galicia	0.13–0.83	1.5–39.7	21–203	43–67			Porte et al. (2001[a])
Galicia			22–270	43–62	14–16		Solé (2000)
Galicia			4.2–1,556[c]				Soriano et al. (2006)
Mediterranean Sea			111–4,222	40–69			Solé et al. (1995b)
Mediterranean Sea				40–80	14–27		Solé (2000)
Mediterranean Sea			19–2,675	56–86	20–25		Porte et al. (2001b)
NW Portugal	8,000–33,740[c]	70–434[c]	24,800–109,800[c]				Lima et al. (2007)
South Portugal (site 2)	37.7 ± 6.8	204.8 ± 57	874 ± 57	80 ± 5	16.3 ± 1.4	65.1 ± 6.1	Serafim et al. (2008)
South Portugal	Site 1-10.5 ± 1.6	Site 1-80 ± 8	Site 1-427 ± 62	Site 1-46.1 ± 2.8	Site 1-13.1 ± 1.7	Site 1-14.1 ± 1.6	Lopes (2009)
	Site 2-38.3 ± 6.7	Site 2-205 ± 57	Site 2-887 ± 122	Site 2-69.8 ± 8	Site 2-16.4 ± 2.1	Site 2-65.2 ± 4.9	
South Portugal			Site 1-375.4 ± 148	Site 1-46.3 ± 2.8			Bebianno et al. (2007)
			Site 2-875.8 ± 247	Site 2-69.6 ± 8			
South Portugal			Site 1-49.3 ± 0.6	Site 1-35.4 ± 6.7	Site 1-11.8 ± 2.6	Site 1-20.73 ± 10	Cravo et al. (2009)
			Site 2-511 ± 13.9	Site 2-50 ± 15	Site 2-12.9 ± 1.5	Site 2-24.8 ± 5	
South Portugal			Site A-294 ↑ 944	Site A-19.6 ↑ 25.1	Site A-13.9 ↑ 19.5	Site A-6.1 ↑ 7.7	Serafim et al. (2011)
			Site B-649 ↓ 189	Site B-24 ↓ 17.9	Site B-26.1 ↓ 16.9	Site B-6.7 ↑ 9.9	
Venice		92–311.6[c]	31.2–189.8	65.9 ± 1.7			Livingstone et al. (1995)
Venice	0.28–1.44[c]	19.6–115[c]	18.2–100.8[c]	24–57			Solé et al. (2000[a])

Table 3 continued

	TAlk (µg/g ww)	UCM (µg/g ww)	PAHs (ng/g ww)	CYP450 (pmol/mg prot)	NADPH-red (nmol/min/mg prot)	CYT b_5 (pmol/mg prot)	References
Venice	16–26.2[c]	163.4–181.4[c]	800–1800[c]				Wetzel and Van Vleet (2004)
Venice			17–139	24–66	8–33		Solé (2000)

↑ Increased to, ↓ decreased to

[a] ng/g ww chrysene equivalents

[b] Lipid µg/g chrysene equivalents

[c] Values in wet weight, calculated from the original data publish in dry weight, assuming a 5:1 wet/dry weight ratio

Bay of Cannes to a more contaminated site, where high molecular weight PAHs such as benzo(*b*)fluoranthene and benzo(*k*)fluoranthene, due to oil combustion coming from an intense maritime traffic, are present in the mussels transplanted to harbour areas (Damiens et al. 2007). Likewise, when mussels *Geukensia demissa* were transplanted from a site chronically contaminated with oil products to a site with low levels, a reduction of TPAH concentrations in mussel tissues also occurred, followed by an improvement of the filtration and growth rates, but not by the condition index (Culbertson et al. 2008). Fabbri et al. (2006) observed a two fold increase of TPAHs when mussels were transplanted from a clean site in the Adriatic Sea to the coastal lagoon of Pialassa Baiona (Ravenna, Italy) contaminated with pyrolytic PAHs, while in the Bay of Hong Kong, mussels *P. viridis* TPAHs concentrations also increased when mussels were transplanted from a remote site to sites contaminated with hydrocarbons, (Cheung et al. 2001).

In general, the accumulation rate of TPAH in mussels is faster, whereas the elimination kinetic is slower and can take weeks or months, depending on the biotic and abiotic factors (Neff 2002). This opposite behavior of accumulation versus elimination and the relative contribution of low and high molecular weight compounds in PAHs levels during different phases of the transplant experiments were also observed in CCA analyses (Fig. 6). Site 2 is directly affected by the biggest recreational marina of the South Portuguese coast, with an intensive maritime traffic. Mussels from this site had the highest proportion of pyrolytic PAHs originated by fossil fuels incomplete combustion relatively to petrogenic PAHs, diagnostic of uncombusted petroleum products (gasoline, diesel oil). Contrarily, site 1 is directly impacted by a small fisheries harbor, where low molecular weight PAHs represents a significant component of the total PAHs. This small harbor has enhanced nautical activities in the summer, with the concomitant pyrolytic PAHs increase. By transplanting mussels from site 2 (highly contaminated site) to site 1 (background contamination), a gradual adaptation of the individual PAHs distribution profile occurred, from mainly pyrolytic with petrogenic inputs, to a profile with softer pyrolytic characteristics and higher petrogenic contribution (small oil and fuels boat spills).

MFO system

CYP450 and MFO system have been used as a biomarker of exposure to organic contaminants in *M. galloprovincialis*, since their concentrations and activities reflects the PAHs concentrations in the aquatic environment (Livingstone 1996; Petushok et al. 2002; Cravo et al. 2009). Basal concentrations of CYP450 are observed in organisms

exposed to lower hydrocarbon loads while this enzymatic system is induced in the presence of higher levels of these compounds, either due to an oil spill (Solé et al. 1996; Porte et al. 2000a, b), or due to laboratory and field exposition (Livingstone 1998; Livingstone et al. 1995; Livingstone 1998; Solé et al. 2000a, b; Bebianno et al. 2007; Lopes and Bebianno 2007; Serafim et al. 2011).

CYP450 increased 64% relatively to basal concentration (0.75 pmol/day), in mussels from site 1 transplanted to site 2 (Fig. 6), showing an induction pattern similar to the accumulation pattern of PAHs and alkanes and confirming the capacity of mussels to cope with the presence of an increased organic contamination. In mussels transplanted from site 2 to 1, CYP450 decreased 27% in relation to initial concentrations, with a decreasing rate of 0.47 pmol/day, simultaneously with a decrease in hydrocarbons concentration, indicating an adaptation of the MFO system towards to biochemical equilibrium to lesser environmental contamination loads. These inverse behaviors are also present in the CCA analyses (Fig. 6) that shows that CYP450 and the MFO system components acquire more importance as mussels spent more time at site 2, and are less related to mussel transplanted to site 1. Furthermore, CYP450 presented a linear relationship with TPAHs concentrations ($P < 0.05$) in both transplant assays, which is in agreement with other works where CYP450 concentrations were related to hydrocarbons loads, either in field (Livingstone et al. 1995; Porte et al. 2001a, b; Cravo et al. 2009), after an oil spill (Solé et al. 1996; Peters et al. 1999; Porte et al. 2000a, b), or due to laboratory exposition to hydrocarbons (Livingstone 1988; Livingstone et al. 1997; Petushok et al. 2002).

Comparable results were obtained by Serafim et al. (2011) where mussel CPY450 induction occurred after 2 weeks in a transplant from a less contaminated site to a site more contaminated with PAHs, while in the cross-transplant, CPY450 significantly decreased to the 21st day, indicating an adaptation of the MFO system to environmental changes in organic contamination levels (Table 3). Bebianno et al. (2007) also reported a positive relationship between TPAHs and the induction of CYP450 found in mussels transplanted from site 1 to 2, although in the cross-transplantation CYP450 did not suffer a significant decrease (Table 3). Förlin et al. (1996) observed an induction of benzo(*a*)pyrene hydroxylase (BPH) (mediated by CYP450) of 2–3-fold in mussels *M. edulis* transplanted from a clean site (Faroe Islands) to a PAHs contaminated site (Skagerrak, North Sea). The induction of this enzyme activity occurred at the same time that total PAHs was accumulated in mussels' tissues to concentrations 2.5-fold higher. Similarly, an increase of CYP1A protein up to 1.4-fold and an increased of the DNA damage (1.2-fold) occurred in mussels *M. edulis* transplanted from Port Quinn (reference site) to New Brighton (urban/industrial site, with high PAHs and PCBs levels) in the UK, after 13 weeks (Shaw et al. 2002). Peters et al. (1998) reported a preferential elevation of CYPlA-immunopositive protein with 3 weeks transplantation from a relatively clean site to a site containing 3–6 fold higher tissue levels of PAHs and PCBs is consistent with the observations of increased digestive gland CYPlA-immunopositive protein in *Mytilus* sp. with laboratory exposure to PCBs (Livingstone et al. 1997) or field exposure to PAHs (Solé et al. 1996). In the United Kingdom (Peters et al. 1999) mussels in sites with higher levels of CYP1A-immunopositive protein showed higher levels of BPH activities and they were positively correlated with each other and tended to be highest at sites with greatest PAH body burden.

On the other hand, though NADPH cytochrome *c* reductase activity (NADPH-RED) was comparable at both sites at the beginning of transplants, it was significantly higher in mussels transplanted from site 1 to 2 between the day 14 and 28, and followed the activity of CYP450 and the TPAH accumulation pattern, presenting an increase of 32% relatively to initial activities (Fig. 6). This flavoprotein is an electron donor to cytochrome P450 and changes in this activity with TPAHs were also detected by other authors (Livingstone et al. 1985; Livingstone 1988; Porte et al. 1991, 2001b; Solé et al. 1995b; Serafim et al. 2008, 2011) (Table 3).

Similarly, the concentration of cytochrome b_5 increased 35% during the 4 weeks of the transplant from site 1 to 2, and decreased during the same period in cross-transplanted mussels (Fig. 6d), even though there is no significant relationship with the hydrocarbons content in tissues. CYT b_5, contrarily to the remaining MFO system components, is primarily involved in the oxidation of various endogenous substrates like fats and steroids (Solé et al. 1995a; Schenkman and Jansson 2003), acting as an electron transfer component. Changes in CYT b_5 content can therefore be related to differences in the metabolic or hormonal status of the mussels, as well as associated with changes in abiotic (temperature, season) and biotic (food state, reproductive stage) factors provoked by the displacement. Other studies have shown that CYT b_5 in *M. galloprovincialis* does not respond to PAH levels or other organic contaminants (Solé et al. 1994, 1995a, b; Porte et al. 1991; Cravo et al. 2009; Serafim et al. 2008, 2011) (Table 3). Furthermore, despite the fact that there was not differences between sites, the variation of NADH CYT b_5 reductase activities (NADH-RED) followed, in general, the pattern of CYT b_5 in both transplants, which is not surprising since this flavoprotein largely depends on CYT b_5 activities (Schenkman and Jansson 2003) rather than hydrocarbon contamination.

Results indicate an induction of the MFO system components in transplanted mussels to cope with the increase

of environmental organic contamination. Other authors report similar concentrations and activities of the MFO system and show that variations in this system reflect the PAHs concentrations of the environment (Porte et al. 1991; Solé et al. 1995b; Bebianno et al. 2007; Serafim et al. 2008, 2011; Cravo et al. 2009).

Conclusions

This ABM strategy using CYP450 and MFO system components revealed different biochemical adaptations to hydrocarbon environmental changes. In fact, the CYP450 induction in the presence of elevated hydrocarbons concentrations showed that mussels are capable of coping with this type of organic pollution, while when at situations where depuration can occur, CYP450 moves to an equilibrium towards basal levels found in local populations where hydrocarbon pollution are at background levels. ABM also revealed significant alterations in total and individual profiles of both AH and aromatic hydrocarbons, as mussels gradually acquire the characteristic profiles of the destination site, reflecting the different sources and origins of hydrocarbons present at each site.

CYP450 dependent system proved to be a good and suitable biomarker for hydrocarbons exposition, explaining the differences between basal biotransformation rates in local populations from sites with distinct loads of hydrocarbons, thus reflecting the environment primary role in the biochemical characteristics of mussels' populations.

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