



Interspecies and spatial trends in polycyclic aromatic hydrocarbons (PAHs) in Atlantic and Mediterranean pelagic seabirds

Jose L. Roscales^{a,b,*,1}, Jacob González-Solís^a, Pascual Calabuig^c, Begoña Jiménez^b

^a Research Institute of Biodiversity (IRBio) and department of Animal Biology (Vertebrates), Barcelona University, Av Diagonal 645, 08028 Barcelona, Spain

^b Department of Instrumental Analysis and Environmental Chemistry, Institute of Organic Chemistry, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain

^c Wildlife Fauna Taira Recuperation Center, Cabildo de Gran Canaria, Canary Islands, Spain

ARTICLE INFO

Article history:

Received 16 January 2011

Received in revised form

20 April 2011

Accepted 22 April 2011

Keywords:

Organic pollutants

Trophic ecology

Bioindicator

Petrel

Shearwater

ABSTRACT

PAHs were analyzed in the liver of 5 species of pelagic seabirds (Procellariiformes) from the northeast Atlantic and the Mediterranean. The main objective was to assess the trophic and geographic trends of PAHs in seabirds to evaluate their suitability as bioindicators of chronic marine pollution by these compounds. Although higher levels of PAHs have been described in the Mediterranean compared to other oceanic regions, we did not find significant spatial patterns and observed only minor effects of the geographic origin on seabird PAHs. However, we found significant higher PAH levels in petrel compared to shearwater species, which could be related to differences in their exploitation of mesopelagic and epipelagic resources, respectively, and the vertical dynamic of PAHs in the water column. Overall, although this study enhances the need of multi-species approaches to show a more comprehensive evaluation of marine pollution, seabirds emerged as poor indicators of pelagic chronic PAH levels.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous organic pollutants widely detected in both terrestrial (Edwards, 1983) and aquatic ecosystems (Hellou, 1996). PAHs have attracted a lot of scientific interest because they are considered a severe potential threat for wildlife due to their mutagenic, carcinogenic and toxic properties (World Health Organization, 1998; Laffon et al., 2006) and their capability to bioaccumulate in invertebrate species (Meador et al., 1995). Studies investigating PAHs have primarily focused on aquatic ecosystems. Once these compounds reach the aquatic medium, they become less susceptible to degradation and tend to be incorporated into sediments with long half-lives (MacRae and Hall, 1998). Therefore, PAHs can continually affect bottom-dwelling organisms and the whole ecosystem via the food web.

Hydrocarbons enter aquatic ecosystems because of human activities, mainly by incomplete combustion of organic matter with subsequent atmospheric deposition and industrial and urban runoff (Van Metre et al., 2000; Nizzetto et al., 2008). In marine environments, accidental or intentional discharges from oil

tankers, ships and fuel extraction activities are other important sources of PAHs. All of these exposure pathways increase the risk for marine biota. In fact, most PAH studies have focused on marine ecosystems affected by oil spills or coastal areas near highly industrialized regions. However, the documented long-range transport of these pollutants could also contribute to significant background levels of PAHs in remote areas of the open sea (Nizzetto et al., 2008). To our knowledge, few studies have monitored PAHs in less polluted areas, such as in pelagic ecosystems and especially in marine predators at high trophic positions (Kannan and Perrotta, 2008; Marsili et al., 1997).

As top marine predators, seabirds have been proposed as suitable biomonitors for pollutants such as PCBs or heavy metals (Furness and Camphuysen, 1997). However, in the case of PAHs, the potential for seabirds to be biomonitors has been scarcely explored and their utility remains unclear. Recently, the yellow-legged gull (*Larus michahellis*) was proposed as a good indicator of PAH contamination after comparing levels from colonies polluted by the Prestige oil spill with non-affected areas (Pérez et al., 2008). However, whereas some seabird studies have found relationships between PAH levels and trophic or geographic variables, other studies have not. Studies of PAHs in seabird eggs have documented low concentrations for certain geographic areas and species, suggesting neither biogeographical trends nor interspecific patterns for PAH levels (Shore et al., 1999; Francon et al., 2004). Moreover, some authors have reported that PAHs are not commonly found in

* Corresponding author.

E-mail address: jlroscales@iqog.csic.es (J.L. Roscales).

¹ Present address: Department of Instrumental Analysis and Environmental Chemistry, Institute of Organic Chemistry, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain.

the tissues of birds from non-contaminated sites, and when PAHs are found, they tend to be present at low levels (Hall and Coon, 1988). In addition, studies reporting interspecific differences in PAH levels in birds found significantly lower levels in bird tissues than in their prey. These studies also found greater PAH burdens in birds feeding on invertebrates (low trophic positions) compared to those feeding on vertebrates, such as fish (higher trophic positions) (Broman et al., 1990; Kayal and Connell, 1995; Lebedev et al., 1998; Custer et al., 2001). Overall, these results agree with studies on the trophodynamic of PAHs in marine food webs, which have revealed an inverse relationship between PAH burdens and trophic position and indicated that biomagnification in food webs is insignificant (Wan et al., 2007; Perugini et al., 2007; Nfon et al., 2008). This general picture concerning PAHs is probably related to the low number of studies using seabirds to monitor PAHs.

In the present study, the PAHs proposed by the U.S. EPA as priority environmental contaminants were analyzed in the livers of pelagic seabirds (*O. Procellariiformes*). We used a multi-species approach, including petrel and shearwater species with different trophic niches and breeding separately in the Atlantic and the Mediterranean (Brooke, 2004), to investigate whether PAHs in seabirds result from dietary differences or spatial differences in PAH exposure. The liver was selected as the target tissue to measure PAH exposure associated with the breeding areas of the seabirds. Birds rapidly metabolize and readily excrete PAHs (Broman et al., 1990; Troisi et al., 2006). For example, 94% of PAHs injected into chicken eggs were metabolized within 14 days (Näf et al., 1992). Therefore, the liver has been suggested to provide information

regarding short-term exposure to PAHs in vertebrates due to its large and rapid detoxification capability (Hellou, 1996). Previous studies have also successfully related liver PAH burdens to the specific wintering grounds of birds (Custer et al., 2001). To evaluate the suitability of pelagic seabirds as indicators of marine PAH contamination, we investigated the relative contribution of the species' trophic ecology and geographic origin to explain their PAH burdens. We also evaluated the sex and age of the seabirds as possible sources of intra-specific variability in PAH levels.

2. Materials and methods

2.1. Species and sampling strategy

Five Procellariiform species were selected: the Scopoli's shearwater (*Calonectris diomedea*), the Cory's shearwater (*Calonectris borealis*), the Balearic shearwater (*Puffinus mauretanicus*), the Bulwer's petrel (*Bulweria bulwerii*) and the white-faced storm-petrel (*Pelagodroma marina*). Whereas the Scopoli's and Balearic shearwaters breed in the Mediterranean, the others nest across the Macaronesian Archipelagos in the Northeast Atlantic.

Two sampling strategies were applied to obtain fresh whole carcasses from seabird specimens. In the Mediterranean Sea, 10 Balearic and 10 Mediterranean Cory's shearwaters were accidentally captured during their breeding periods between 2003 and 2007 by longliners in the Western Mediterranean. The birds died drowned during fishery activities after predated on the baited hooks. Longliners kept the birds frozen until delivered for further processing. The sampling areas covered in this study are presented in Fig. 1. Atlantic specimens were obtained during the same period by the Wildlife Tafira Recuperation Center (Cabildo de Gran Canaria, Gran Canaria, Canary Islands), including 11 Atlantic Cory's shearwaters, 6 Bulwer's petrels and 9 white-faced storm-petrels, and were all collected within their respective breeding periods. To avoid potential selection biases from unhealthy birds, unrecoverable specimens with fractures euthanized upon arrival to the

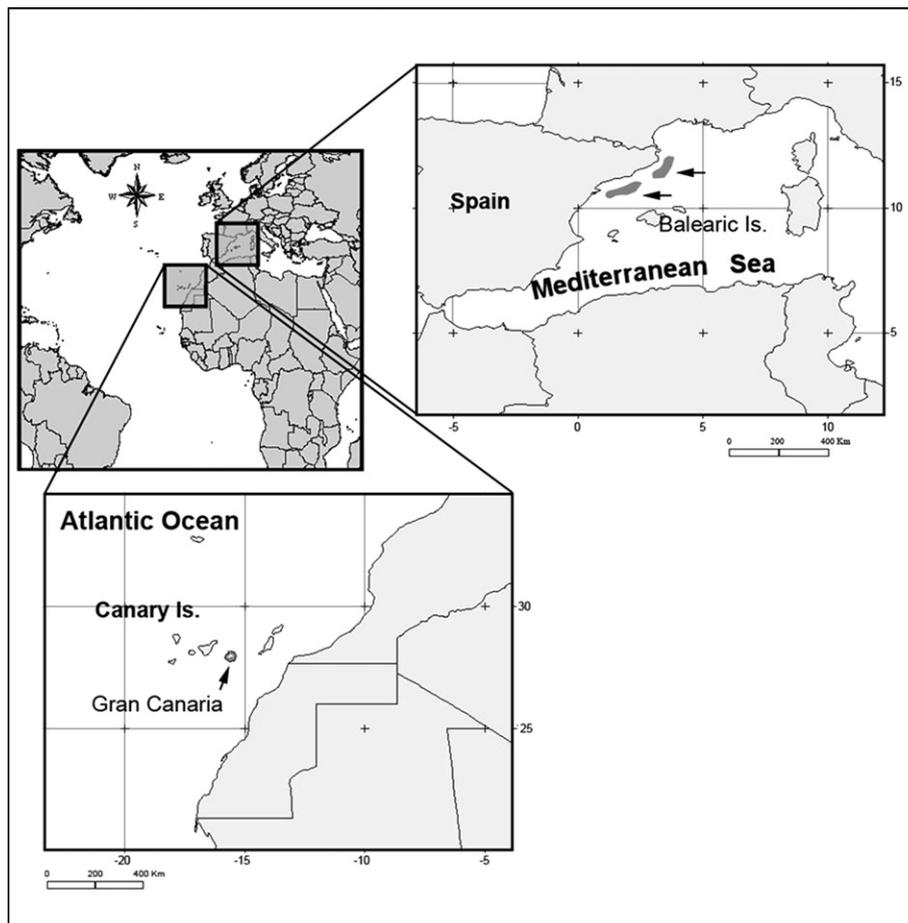


Fig. 1. Seabird sampling locations. The dark gray areas and arrows indicate the Mediterranean and Atlantic Zones zones where seabirds were collected.

recuperation center were selected. All of the specimens were frozen and preserved at -24°C . Whole livers were extracted by dissection and immediately frozen until analyses.

During the dissection procedure, all birds were sexed and aged. Sex was determined by direct observation of the sexual organs during the dissections. Age was determined by the presence of the bursa of Fabricius (Glick, 1983; Broughton, 1994) and down feathers. Only specimens with no bursa traces were considered adults. Specimens with bursa and down feathers were considered fledglings from the breeding period when they were caught, and those with bursa and no down feathers were considered juveniles. Based on these criteria, all sampled specimens were classified as adults except three Atlantic Cory's shearwaters and two white-faced storm-petrels, which had bursa and down feathers and were therefore classified as fledglings.

2.2. PAH analysis

Fifteen PAHs, among those selected by the U.S. EPA, were analyzed. PAHs ranged from di-aromatics to the hexa-aromatics: naphthalene (Naph), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phen), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), chrysene and benz[a]anthracene (Chry_BaA), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenz[a,h]anthracene (DahA), indeno[1,2,3-c,d]pyrene (IcdP) and benzo[g,h,i]perylene (BghiP).

Depending on the species, 1–4 g of fresh liver was used for PAH analysis. PAHs in the liver samples were analyzed using the procedure described by Bordajandi et al. (2004) with some modifications. Briefly, the liver samples were homogenized with anhydrous sodium sulfate. Sample homogenates were Soxhlet extracted for 8 h with dichloromethane. Further cleanup was performed using an activated silica gel column. The first fraction, eluted with 25 mL of hexane, was discarded, and a second fraction containing the selected PAHs was eluted with 25 mL of hexane/dichloromethane (3:2, v/v). The extract was concentrated to a final volume of 1 mL in acetonitrile and filtered using a 0.45 μm syringe filter (Millex HV). Five liver samples and a blank sample were included in every batch.

PAHs were analyzed using an HPLC Jasco system consisting of a PU-1580 pump coupled to an HG-1580-31 mixer and an FP-920 fluorimetric detector with programmable excitation and emission wavelengths. Separation was achieved using a Spherisorb ODS2- C_{18} column (25 cm \times 4.6 mm and 5 μm particle size, Waters). A 20 μL loop was used to inject the samples. The initial mobile phase was acetonitrile/water (Mili-Q) (60:40, v/v), and separation was obtained using a gradient with acetonitrile increasing from 60 to 100% in 20 min at a flow rate of 1 mL/min. A calibration curve using the PAH MIX calibration mixture showed good linearity in 7 levels from 0.001 ng/ μL to 0.2 ng/ μL . The repeatability was determined at a concentration level of 0.02 ng/ μL and presented relative standard deviations (RSDs) below 7% for all the PAHs, except pyrene (13%). The reproducibility was also satisfactory, with RSD values below 6%. The instrumental limit of detection (LOD), calculated as 3 times the signal-to-noise ratio, ranged between 0.7 and 8.4 pg/ μL , except for dibenz[a,h]anthracene (13.0 pg/ μL). Recovery studies were performed at 0.5, and 10 ng/g fortification levels for each PAH, and the recoveries obtained for most of the analytes ranged from 70 to 110%.

2.3. Data analysis

SPSS 15.0 for Windows was used for statistical analysis. Concentrations below the detection limit were set to the half of this limit for statistical analysis. The distribution normality of the total PAH levels (Σ PAHs, the sum of the 15 individual PAHs analyzed) was explored by checking the Q-Q plots and the Shapiro-Wilk test (all $P < 0.05$). Σ PAHs did not show a normal distribution and thus, inter-species differences of total PAHs in adult seabird liver were evaluated by means of Kruskal-Wallis test. Mann-Whitney U -test was used for species pair comparisons. Intra-specific comparisons between Σ PAH burdens in seabirds sampled in different years (only between years with more than 2 sampled individuals) as well as between sexes were performed by means of Mann-Whitney U -test. Species fingerprints were calculated as the average percent of each PAH with respect to the Σ PAHs. Comparisons among fingerprints were performed by grouping PAHs according to

the number of aromatic rings: di- (Naph), tri- (Ace, Fl, Phen and Ant), tetra- (Flt, Pyr, Chry and BaA), penta- (BbF, BkF, BaP and DahA) and hexa-cyclic (IcdP and BghiP) PAHs. Finally, a principal component analysis (PCA) was applied to the contributions of all PAHs (except Ant, Chry, BaA and BkF which were under the limit of detection in all samples and thus cannot explain variability) to check whether seabird PAH profiles are associated with breeding locality or species. PCA was based on the analysis of the correlation matrix and the result was not rotated. PCA component matrix was used to explore the relationship between the obtained components and each PAH. Concentrations are expressed in ng/g (wet weight basis) and as mean \pm standard error, except otherwise indicated.

3. Results

PAHs were found in all of the 45 livers analyzed. We did not find any significant intra-specific relationships between sampling year and the Σ PAHs. Similarly, intra-specific comparisons (Mann-Whitney U -test) between seabirds sampled in different years did not result significant (all $P > 0.05$), indicating that the sampling year did not introduce a significant bias in our work. Intra-specific comparisons between age groups were not possible due to the low number of fledglings. Nevertheless, the Σ PAH concentrations (Table 1) and the number of PAHs detected (Table 2) were markedly lower in fledglings than in adults. In fact, the Σ PAHs in Cory's shearwater and white-faced storm-petrel fledglings were below the range of the levels obtained in adults from each species. Mann-Whitney U -test did not revealed significant sexual differences in Σ PAH (Fig. 2) for any of the studied species (all $P > 0.05$).

Among the adult specimens, Bulwer's petrels and white-faced storm-petrels presented the greatest concentrations for most of the PAHs (Table 2). Kruskal-Wallis tests showed that total PAH burdens (Table 1) differed significantly among species ($H_{40,5} = 25.94$, $P < 0.001$). Pair comparisons (Mann-Whitney U -test) indicated that the petrel species differed significantly from all the shearwater species ($P < 0.05$) but inter-species differences were not significant when comparing between two shearwater or between two petrel species. Therefore, the differences among the species were due to the higher levels found in the petrels compared to the shearwaters (Fig. 2).

Although PAH profiles in seabird livers differed among species (Fig. 3), we did not find homogeneous patterns among species or among breeding regions (i.e. Atlantic vs. Mediterranean basins). That is, Cory's and Balearic shearwaters breeding in the Atlantic and the Mediterranean, respectively, showed similar contribution of low (di- and tri-cyclic PAHs; 56.94% for Cory's shearwaters and 58.38% for Balearic shearwaters) and high (tetra-, penta- and hexa-cyclic) molecular weight PAHs in their profiles; pyrene (27.05% for Cory's shearwaters and 32.23% for Balearic shearwaters) and naphthalene (39.39% for Cory's shearwaters and 30.92% for Balearic shearwaters) were the most abundant compounds. However, whereas high-molecular-weight PAHs predominated in Scopoli's shearwaters (68.2%) breeding in the Mediterranean, low-molecular-weight-PAHs were the most abundant compounds in livers from Bulwer's petrels (89.77%) and white-faced storm-petrels

Table 1
 Σ PAH concentrations (ng/g w.w.) in the livers of pelagic seabirds breeding in the Mediterranean and the Atlantic.

Species	Age	n	Arithmetic mean	Standard deviation	Standard error	Geometric mean	Range
<i>Mediterranean</i>							
Balearic shearwater	Adults	10	6.07	3.11	0.97	5.27	1.91–10.3
Scopoli's shearwater	Adults	10	5.56	3.01	0.96	4.64	1.59–8.93
<i>Atlantic</i>							
Cory's shearwater	Adults	8	8.52	4.43	1.57	7.47	3.32–17.1
	Fledglings	3	1.60	0.82	0.47	1.48	1.02–2.54
Bulwer's petrel	Adults	6	32.5	18.2	7.43	29.1	17.2–66.2
White-face storm-petrel	Adults	6	29.8	7.37	2.99	29.0	18.5–39.8
	Fledglings	2	11.1	5.44	3.85	10.4	7.27–14.9

Table 2
Mean \pm standard error, and range (in brackets) of PAHs (ng/g w.w.) analyzed in the livers of adult and fledglings Procellariiformes. The symbol # indicates PAH levels below the detection limit or not detected. Compound abbreviations can be found in PAH analysis section.

Age	Balearic shearwater		Scopoli's shearwater		Cory's shearwater		Bulwer's petrel		White-faced storm-petrel	
	Adult	Adult	Adult	Fledgling	Adult	Adult	Adult	Fledgling		
n	10	10	8	3	6	6	6	2		
Naph	1.68 \pm 0.21 (0.87–2.79)	0.84 \pm 0.35 (#–3.28)	3.54 \pm 0.87 (0.92–8.18)	0.6 \pm 0.38 (#–1.29)	7.08 \pm 1.35 (10.00–2.45)	9.79 \pm 1.34 (4.46–12.91)	1.41 \pm 0.71 (0.7–2.13)			
Ace	0.31 \pm 0.09 (#–0.88)	0.09 \pm 0.05 (#–0.39)	0.04 \pm 0.03 (#–0.25)	#	1.7 \pm 0.41 (#–2.57)	1.14 \pm 0.43 (#–2.38)	#			
Fl	1.05 \pm 0.32 (#–6.55)	0.07 \pm 0.05 (#–0.53)	0.24 \pm 0.09 (#–0.71)	#	13.30 \pm 2.43 (8.48–22.82)	10.55 \pm 3.4 (0.84–20.43)	#			
Phen	0.49 \pm 0.18 (#–1.89)	0.62 \pm 0.17 (#–1.71)	1.03 \pm 0.26 (#–2.26)	#	5.95 \pm 2.7 (0.30–18.09)	2.23 \pm 0.44 (0.65–3.74)	0.08 \pm 0.08 (#–0.16)			
Ant	#	#	#	#	#	#	#			
Flt	0.48 \pm 0.08 (#–0.62)	0.89 \pm 0.31 (#–3.24)	0.86 \pm 0.28 (#–2.39)	#	3.34 \pm 2.05 (#–12.21)	1.73 \pm 0.50 (0.63–3.72)	#			
Pyr	2.01 \pm 0.38 (#–3.25)	2.53 \pm 0.54 (#–5.63)	2.22 \pm 0.56 (0.44–5.75)	1.00 \pm 0.15 (0.72–1.25)	0.74 \pm 0.39 (#–2.52)	4.16 \pm 2.57 (#–16.70)	9.6 \pm 3.07 (6.53–12.67)			
Chry_BaA	#	#	#	#	#	#	#			
BbF	#	0.02 \pm 0.01 (#–0.12)	0.03 \pm 0.02 (#–0.15)	#	0.32 \pm 0.32 (#–1.92)	#	#			
BkF	#	#	#	#	#	#	#			
BaP	#	0.07 \pm 0.03 (#–0.36)	0.11 \pm 0.07 (#–0.52)	#	#	#	#			
DahA	#	0.27 \pm 0.25 (#–2.56)	0.34 \pm 0.33 (#–2.67)	#	0.12 \pm 0.12 (#–0.71)	0.14 \pm 0.05 (#–0.82)	#			
IcdPyr	0.05 \pm 0.01 (#–0.46)	0.10 \pm 0.05 (#–0.47)	0.11 \pm 0.05 (#–0.42)	#	#	#	#			
BghiPer	#	0.05 \pm 0.04 (#–0.35)	#	#	#	0.11 \pm 0.11 (#–0.66)	#			

(84.43%) from the Atlantic. The abundance of tri-cyclic PAHs in livers from petrels compared to the rest of species was mainly due to the greater contributions of fluorene (46.42% and 37.36% for Bulwer's petrels and white-faced storm-petrels, respectively), which was the most abundant PAH in their profiles. In the case of Scopoli's shearwaters, the dominant presence of tetra-cyclic PAHs was due to the abundance of pyrene (47.68%) compared to the remaining PAHs.

These results were in agreement with the principal component analyses (PCA) of the relative abundance of all the analyzed PAHs (Fig. 4). We obtained two principal components, PC1 and PC2, accounting for 27.18 and 15.01% of the variance, respectively. Their graphic representation did not allow the grouping of seabirds by species or by breeding locality alone. Although most petrels were clearly segregated from *Calonectris* shearwaters, Balearic shearwaters appeared within both groups (Fig. 4). The component matrix indicated that PC1 was negatively associated with Fl, Ace

and Naph (ordered from higher to lower coefficients) and positively with Pyr, BaP and Flt. PC2 was negatively associated with Phen abundance and positively with Ace and Fl. Therefore, PCA analysis segregated between *Calonectris* shearwaters and petrels mainly due to the major presence of di- and try-cyclic PAHs in petrels.

4. Discussion

To our knowledge, this is the first study in which PAH levels have been evaluated in five pelagic seabirds breeding in two basins: the Northeast Atlantic Ocean and the Mediterranean Sea. Σ PAH levels differed among species due to the significantly greater concentrations found in Bulwer's petrel and white-faced storm-petrels related to the shearwater species (Fig. 2). Sex was a non-significant factor in explaining seabird PAH burdens but this result must be taken with caution due to the low number of males and females *per* species included in this study. Inter-species differences in PAH profiles also emerged mainly between petrels and shearwaters; PCA analysis mostly segregated petrel species from shearwaters (Fig. 4). The lack of differences in PAHs among shearwaters breeding in the Atlantic and the Mediterranean reflected a minor effect of breeding locality on PAH levels in pelagic seabirds.

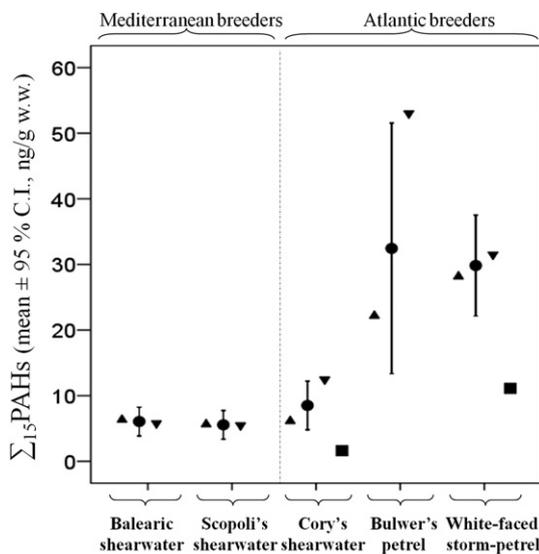


Fig. 2. Σ PAH concentrations (mean \pm 95% Confidence Interval, ng/g w.w.) in the livers of Atlantic and Mediterranean adult seabirds (circles). Triangles and inverted triangles show the mean values obtained for males and females, respectively. Squares indicate the mean levels from Cory's shearwater and white-faced storm-petrel fledglings.

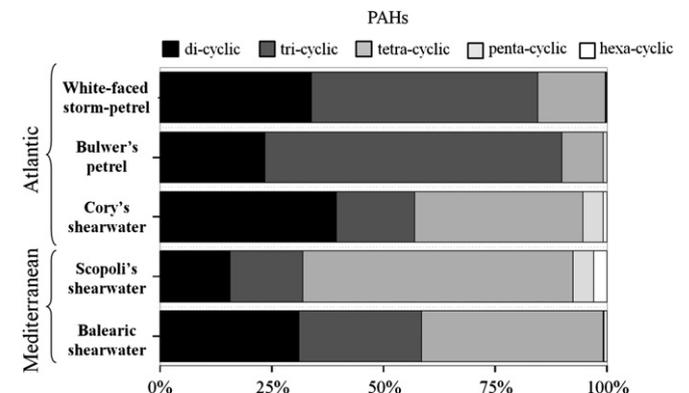


Fig. 3. PAH profiles in adult seabird livers calculated as the percent of each PAH with respect to Σ PAH.

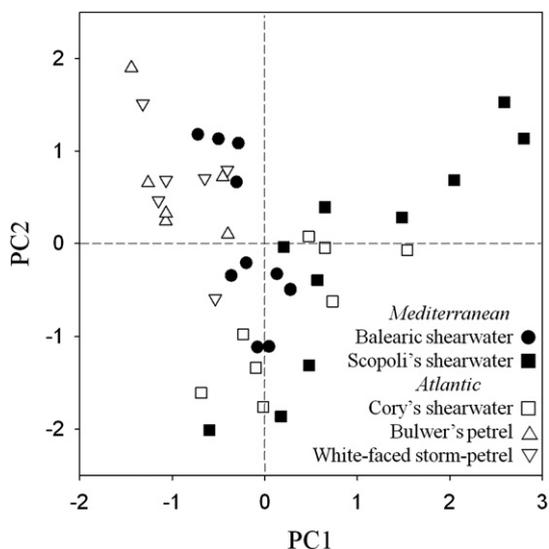


Fig. 4. Principal components (PC1–PC2) extracted from the relative contributions of the analyzed PAHs.

Temporal differences in PAH burdens have been documented in marine vertebrates mainly after local accidental discharges of petroleum into marine ecosystems (Marsili et al., 2001; Soriano et al., 2006; Pérez et al., 2008). However, similar relevant oil spills have not been documented in our sampling areas from 2003 to 2007. As far as we know, few studies have reported PAH burden differences related to the sampling year of vertebrates from areas not receiving direct pollution. In the few studies that did, temporal trends emerged in long-term temporal assessments, such as inter-decade comparisons (Kannan and Perrotta, 2008). In this study, we did not find any significant relationship between the Σ PAH levels and sampling years within the studied species. Therefore, although we cannot completely exclude some variability in PAHs due to different sampling years, it does not seem to shift our results.

Because interspecific comparisons of PAHs between regions can be confounded by trophic or detoxification efficiency differences among species, in this study, we focused on PAH differences among *Calonectris* species (Cory's and Scopoli's shearwaters) to evaluate possible spatial differences in PAHs. The Scopoli's and the Cory's shearwaters are closely related forms until recently considered subspecies of *C. diomedea* and show very similar morphology, ecology and physiology (Brooke, 2004; Gómez-Díaz and González-Solís, 2007; Gómez-Díaz et al., 2009). Therefore, because no differences in the feeding ecology or metabolic capabilities of *Calonectris* shearwaters are expected, differences in PAHs between them should be related to geographic contamination patterns. Previous studies found marked geographic patterns in POPs, such as PCBs or DDTs, and heavy metals in *Calonectris* shearwater breeding in the Atlantic and the Mediterranean due to the greater levels of these pollutants in the Mediterranean Basin (Ramos et al., 2009; Roscales et al., 2010). In fact, the Mediterranean Sea is a confined water mass with hydrogeographical conditions that have resulted in higher levels of contamination, including PAH contamination, compared with other regional seas (Martí et al., 2001; Albaigés, 2005). However, we did not find significant differences among the PAH burdens of *Calonectris* shearwaters breeding in the Atlantic and the Mediterranean. This lack of differences is likely related to the rapid elimination of PAHs by birds, masking geographic patterns of baseline marine PAH levels. Nevertheless, some dissimilarities in Scopoli's and Cory's shearwater PAH profiles could be related to their breeding grounds.

The Scopoli's shearwater PAH profiles were clearly dominated by high-molecular-weight compounds (tri-, tetra-, penta- and hexa-cyclic; Fig. 3). However, the Cory's shearwater profiles showed similar contributions of high and low-molecular-weight-PAHs. Low-molecular-weight-PAHs are the primary constituents of natural petroleum seeps, rather than urban sources of PAHs (Seruto et al., 2005; Kannan and Perrotta, 2008). Therefore, the presence of heavy PAHs in Scopoli's shearwaters could be related to the greater presence of anthropogenic sources of PAHs in the Mediterranean compared to the Atlantic. Pyrene (47.7%) was the most abundant compound in the Scopoli's shearwaters' PAH profiles, resulting in the dominance of tetra-cyclic compounds (60.48%) compared to the other PAHs. The greater abundance of pyrene is consistent with previous studies of PAHs in particulate matter in surface waters from the Western Mediterranean Sea. Particulate matter studied across the areas where the seabirds were collected and across their common feeding grounds on the Northeast Spanish coasts showed PAH profiles dominated by pyrene, likely caused by river runoffs (Dachs et al., 1997). However, the PAH profiles were not different enough between Atlantic and Mediterranean species to be segregated by the PCA analysis, indicating only a minor importance of the breeding ground locations to PAH levels. In Balearic shearwaters, also breeding and feeding in the west Mediterranean, pyrene and tetra-cyclic PAHs were also the most abundant PAHs. Nevertheless, this species showed a greater proportion of low-molecular-weight-PAHs than Scopoli's shearwaters, which could be related to different feeding habits or metabolic capabilities between the species.

Differences in the PAH profiles and levels in this study emerged mainly when comparing petrels with the remaining species. The Bulwer's petrels and the white-faced storm-petrels showed significantly higher levels of the Σ PAHs than shearwaters. Moreover, the PCA segregated most petrels from the rest of the seabirds due to the higher presence of di- and tri-cyclic PAHs in these species. Because the geographic origin did not explain the interspecific differences in PAH levels in seabirds, a different exposure to these contaminants through diet or different metabolic capability among species are the most probable hypotheses to explain the highest PAH burdens found in the petrels. Previous studies on Mixed-Function Oxidase (MFO) in seabirds found little enzymatic activity differences among pelagic species. A comparison between Leach's storm-petrels (*Oceanodroma leucorhoa*) and Manx shearwaters (*Puffinus puffinus*) showed similar activity between these species for most hepatic enzymes, with the exception of the aldrin epoxidase, 7-EROD and epoxide hydrolase, which showed higher activity in the case of the storm-petrel (Knight and Walker, 1982; Peakall et al., 1987). These small differences in the MFO activity between storm-petrels and shearwaters could be related to the differences we found in the PAH profiles of Bulwer's petrels and white-faced storm-petrels. The greater abundance of low-molecular-weight-PAHs in petrels than in shearwaters may be explained by the greater capability of petrels to metabolize the larger PAH compounds, resulting in a low presence of high-molecular-weight PAHs in their profiles. Therefore, the greater PAH levels in petrels compared to *Calonectris* and Balearic shearwaters do not seem to be related to a major detoxification capability of shearwaters, but to a different exposure to these compounds through their diet.

The diet of Cory's shearwaters is mainly composed of epipelagic (0–200 m depth in the water column) fish, although cephalopods, crustaceans and plankton are also included in lower proportions (Granadeiro et al., 1998). Bulwer's petrel is considered a nocturnal mesopelagic (200–1000 m) feeder with a diet composed primarily of vertically migrating mesopelagic organisms (Monteiro et al., 1996, 1998). This petrel also feeds mainly on fish (Myctophidae),

followed by cephalopods, crustaceans and plankton. To our knowledge, there are no studies investigating the diet of white-faced storm-petrels breeding in the Atlantic, but it is expected to be similar to the diet of Bulwer's petrel because this occurs with other petrels from the same archipelagos (Monteiro et al., 1996; Bolton et al., 2008). Although petrels and shearwaters feed on different prey types, both are essentially third-order consumers in their food-chains, and previous studies suggested no differences in the trophic positions of these species (Monteiro et al., 1995, 1998). Therefore, our results suggest that mesopelagic feeders are exposed to higher PAH burdens than epipelagic consumers, which could be related to specific differences in the behavior and distribution of these pollutants across the water column. However, few works have focused on PAHs in mesopelagic food-chains or deep-sea organisms (e.g. Fossi et al., 2002). Higher PAH burdens in mesopelagic, rather than epipelagic ecosystems have not been described, and previous studies showed a general surface-enrichment depth-depletion distribution for these pollutants in the water column (Dachs et al., 1997; Schulz-Bull et al., 1998; Martí et al., 2001). Nevertheless, some authors suggested that the vertical distribution of PAHs in the Mediterranean and the Atlantic seems to be governed by planktonic bioaccumulations at open sea, with a sub-maxima concentration of PAHs in zooplanktonic spots at 300 m depth. This suggests that migratory zooplankton support the flux of hydrophobic pollutants in the water column (Hobson, 1993; Dachs et al., 1997; Hodum and Hobson, 2000; Jaward et al., 2004). Migratory Myctophids feed mainly on these spots of zooplankton, and they follow them in their daily vertical migration, which could contribute to a greater exposure to PAHs. Therefore, feeding specialization of seabirds on mesopelagic prey emerges as a possible explanation for our results and implicates mesopelagic food webs as the most susceptible to accumulate and transfer PAHs to marine predators. Further investigation of PAH levels in Myctophids and in the trophic and vertical flux of PAHs is needed to evaluate this hypothesis.

In this study, we also analyzed PAH levels in Atlantic Cory's shearwater and white-faced storm-petrel fledglings. The PAH levels in fledglings were expected to reflect the local food web exposure because they are fed by their parents for several months until they fly. Differences between the white-faced storm-petrel and the Cory's shearwater fledglings were consistent with those found between adults. In addition, fledglings from both species showed lower Σ PAH levels than adults. The low PAH levels found in fledglings is probably related to the greater metabolic rate and the shorter exposure time of growing chicks. In addition, previous studies of seabirds have documented that some species tend to feed their chicks with higher trophic level preys (mainly fish) compared to the food the parents consume (Broman et al., 1990). Because PAH levels were inversely related to trophic position, dietary differences could also contribute to the differences we found in this study between PAH levels in fledglings and adults.

Comparisons of PAH levels with other marine predators are problematic. PAH levels depend on the manner in which the concentrations are expressed (wet or dry weight) and vary broadly among tissues. Accordingly, we compared our results with those reported by Troisi et al. (2006) in liver of oiled common guillemots (*Uria aalge*) from the North Atlantic. They found a mean concentration of 250 ng/g w.w. for Σ_{10} PAHs (ranging from 40 to 970 ng/g). In general, our results showed lower total PAH concentrations than those found in common guillemots, which is expected for oiled seabirds. Nevertheless, petrels showed PAH burdens around 40 ng/g and one Bulwer's petrel specimen exceeded this value. Although the proportion of carcinogenic or high-molecular-weight PAHs found in our study is significantly lower than in oiled common guillemots, the Σ PAH found in some petrels from this study are similar to the

concentrations obtained for oiled seabirds, which might be of concern.

5. Conclusions

Overall, our results indicate that PAH levels in seabirds depend mainly on the species. The lack of significant geographic patterns in seabird total PAH levels, even comparing water masses with different baseline contaminant levels such as the Mediterranean and the Atlantic, suggest that pelagic seabirds are poor indicator species of background PAH levels in the marine environment. Nevertheless, species feeding on mesopelagic preys seem to be exposed to greater PAH levels than those feeding on surface resources. This suggests that mesopelagic food webs could be more susceptible to accumulate and transfer PAHs to marine predators. Further studies are needed to verify this hypothesis. Moreover, this study proposes a multi-species approach to achieve a broader interval of risk levels due to PAH exposition and to represent a more comprehensive evaluation of marine PAH contamination.

Acknowledgments

We thank our colleagues F. J. Ramirez, R. Moreno and A. Abdennadher for reviewing earlier drafts of the manuscript and adding valuable ideas and constructive comments. We extend special thanks to L. Estevez and Y. De Vicent from the Tafira Wildlife Recovery Centre and Cabildo de Gran Canaria. We thank all the longliners and people who helped us with the sampling procedure: *Cona*, *Hermanos Galindo*, *La Maca III*, *Som i Serem*, *Palandriu*, *La Palandria*, *El Alcalde I*, *Dolores* and *Pare Joan*. We are also thankful to M. Díaz, V. Pedrocchi, M. Saez, J. Muñoz-Arnanz, T. Militão, and E. Blazquez for their professional support. J.L. Roscales was supported by a postgraduate grant from the Generalitat de Catalunya and J.González-Solís by R&C and Fondos Feder. Financial support was provided by FBBVA Biology Conservation Grants, CGL2006_01315/Bos MCEl and the Regional Government of Madrid (Project P-AMB-000352-0505).

References

- Albaigés, J., 2005. Persistent organic pollutants in the Mediterranean Sea. In: Saliot, A. (Ed.), *The Mediterranean Sea*. Springer, Berlin, pp. 89–149.
- Bolton, M., Smith, A.L., Gómez-Díaz, E., Friesen, V.L., Madeiros, R., Bried, J., Roscales, J.L., Furness, R.W., 2008. Monteiro's storm-petrel *Oceanodroma monteiroi*: a new species from the Azores. *Ibis* 150, 717–727.
- Bordajandi, L.R., Gómez, G., Abad, E., Rivera, E., Fernández-Bastón, M.M., Blasco, J., González, M.J., 2004. Survey of persistent organochlorine contaminants (PCBs, PCDD/Fs, and PAHs), heavy metals (Cu, Cd, Zn, Pb, and Hg), and arsenic in food samples from Huelva (Spain): levels and health implications. *Journal of Agricultural and Food Chemistry* 52, 992–1001.
- Broman, D., Näf, C., Lundbergh, I., Zebühr, Y., 1990. An in situ study on the distribution, biotransformation and flux of polycyclic aromatic hydrocarbons (PAHs) in an aquatic food chain (seston-*Mytilus edulis* L.–*Somateria mollissima* L.) from the Baltic: an ecotoxicological perspective. *Environmental Toxicology & Chemistry* 9, 429–442.
- Brooke, M., 2004. *Albatrosses and Petrels Across the World*. Oxford University Press, Oxford.
- Broughton, J.M., 1994. Size of the bursa of fabricius in relation to gonad size and age in Laysan and black-footed albatrosses. *The Condor* 96, 203–207.
- Custer, T.W., Custer, C.M., Dickerson, K., Allen, K., Melancon, M.J., Schmidt, L.J., 2001. Polycyclic aromatic hydrocarbons, aliphatic hydrocarbons, trace elements, and monooxygenase activity in birds nesting on the North Platte River, Casper, Wyoming, USA. *Environmental Toxicology & Chemistry* 20, 624–631.
- Dachs, J., Bayona, J.M., Raoux, C., Albaigés, J., 1997. Spatial, vertical distribution and budget of polycyclic aromatic hydrocarbons in the Western Mediterranean seawater. *Environmental Science & Technology* 31, 682–688.
- Edwards, T.N., 1983. Polycyclic aromatic hydrocarbons (PAHs) in the terrestrial environment. A review. *Journal of Environmental Quality* 12, 427–441.
- Fossi, M.C., Borsani, J.F., Di Mento, R., Marsili, L., Casini, S., Neri, G., Mori, G., Ancora, S., Leonzio, C., Minutoli, R., Notarbartolo di Sciarra, G., 2002. Multi-trial biomarker approach in *Meganyctiphanes norvegica*: a potential early indicator of

- health status of the Mediterranean “whale sanctuary”. *Marine Environmental Research* 54, 761–767.
- Franson, J.C., Hollmén, T.E., Flint, P.L., Grand, J.B., Lancot, R.B., 2004. Contamination in molting long-tailed ducks and nesting common eiders in the Beaufort Sea. *Marine Pollution Bulletin* 48, 504–513.
- Furness, R.W., Camphuysen, C.J., 1997. Seabirds as monitors of the marine environment. *ICES Journal of Marine Science* 54, 726–737.
- Glick, B., 1983. *Bursa of fabricius*. In: Farner, D.S., King, J.R., Parkes, K.C. (Eds.), *Avian Biology*. Academic Press, New York, pp. 443–500.
- Gómez-Díaz, E., González-Solís, J., 2007. Geographic assignment of seabirds to breeding origin: combining morphology, genetics, stable isotopes and trace elements in Cory's shearwater. *Ecological Applications* 17, 1484–1498.
- Gómez-Díaz, E., González-Solís, J., Peinado, M.A., 2009. Population structure in a highly pelagic seabird, the Cory's shearwater *Calonectris diomedea*: an examination of genetics, morphology and ecology. *Marine Ecology Progress Series* 382, 197–209.
- Granadeiro, J.P., Monteiro, L.R., Furness, R.W., 1998. Diet and feeding ecology of Cory's shearwater *Calonectris diomedea* in the Azores, north-east Atlantic. *Marine Ecology Progress Series* 166, 267–276.
- Hall, R.J., Coon, N.C., 1988. *Interpreting Residues of Petroleum Hydrocarbons in Wildlife Tissues*. Biological Report 88(15). US Fish and Wildlife Service, Washington, DC, USA.
- Hellou, J., 1996. Polycyclic aromatic hydrocarbons in marine mammals, finfish, and molluscs. In: Beyer, W.N., Heinz, G.H., Redmon-Norwood, A.W. (Eds.), *Environmental Contaminants in Wildlife: Interpreting Tissues Concentrations*. Lewis Publisher, Boca Raton, pp. 229–250.
- Hobson, K.A., 1993. Trophic relationship among Arctic seabirds: insights from tissue-dependent stable-isotope models. *Marine Ecology Progress Series* 95, 7–18.
- Hodum, P.J., Hobson, K.A., 2000. Trophic relationships among Antarctic fulmarine petrels: insights into dietary overlap and chick provisioning strategies inferred from stable-isotope ($d^{15}N$ and $d^{13}C$) analyses. *Marine Ecology Progress Series* 198, 273–281.
- Jaward, F.M., Barber, J.L., Booij, K., Dachs, J., Lohmann, R., Jones, K.C., 2004. Evidence for dynamic air–water coupling and cycling of persistent organic pollutants over the open Atlantic Ocean. *Environmental Science & Technology* 38, 2617–2625.
- Kannan, K., Perrotta, E., 2008. Polycyclic aromatic hydrocarbons (PAHs) in livers of California sea otters. *Chemosphere* 71, 649–655.
- Kayal, S., Connell, D.W., 1995. Polycyclic aromatic hydrocarbons in biota from the Brisbane River Estuary, Australia. *Estuarine, Coastal and Shelf Science* 40, 475–493.
- Knight, G.C., Walker, C.H., 1982. A study of the hepatic microsomal monooxygenase of sea birds and its relationships to organochlorine pollutants. *Comparative Biochemistry and Physiology* 73, 211–221.
- Laffon, B., Fraga-Iriso, R., Pérez-Cadahía, B., Méndez, J., 2006. Genotoxicity associated to exposure to Prestige oil during autopsies and cleaning of oil-contaminated birds. *Food and Chemical Toxicology* 44, 1714–1723.
- Lebedev, A.T., Poliakova, O.V., Karakhanova, N.K., Petrosyan, V.S., Renzoni, A., 1998. The contamination of birds with organic pollutants in the Lake Baikal region. *Science of the Total Environment* 212, 153–162.
- MacRae, J.D., Hall, K.J., 1998. Biodegradation of polycyclic aromatic hydrocarbons (PAH) in marine sediment under denitrifying conditions. *Water Science and Technology* 38, 177–185.
- Marsili, L., Fossi, M.C., Casini, S., Savelli, C., Jiménez, B., Junin, M., Castello, H., 1997. Fingerprint of polycyclic aromatic hydrocarbons in two populations of southern sea lions (*Otaria flavescens*). *Chemosphere* 34 (4), 759–770.
- Marsili, L., Caruso, A., Fossi, M.C., Zanardelli, M., Poli, E., Focardi, S., 2001. Polycyclic aromatic hydrocarbons (PAHs) in subcutaneous biopsies of Mediterranean cetaceans. *Chemosphere* 44, 147–154.
- Martí, S., Bayona, J.M., Albaigés, J., 2001. A potential source of organic pollutants into the northeastern Atlantic: the outflow of the Mediterranean deep-lying waters through the Gibraltar Strait. *Environmental Science & Technology* 35, 2682–2689.
- Meador, J.P., Stein, J.E., Reichert, W.L., Varanasi, U., 1995. Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms. *Reviews of Environmental Contamination and Toxicology* 143, 79–165.
- Monteiro, L.R., Furness, R.W., Del Nevo, A.J., 1995. Mercury levels in seabirds from Azores, Mid-North Atlantic Ocean. *Archives of Environmental Contamination and Toxicology* 28, 304–309.
- Monteiro, L.R., Granadeiro, J.P., Furness, R.W., 1998. Relationship between mercury levels and diet in Azores seabirds. *Marine Ecology Progress Series* 166, 259–265.
- Monteiro, L.R., Ramos, J.A., Furness, R.W., Del Nevo, A.J., 1996. Movements, morphology, breeding, moult, diet and feeding of seabirds in the Azores. *Colonial Waterbirds* 19, 82–97.
- Náf, C., Broman, D., Brunström, B., 1992. Distribution and metabolism of polycyclic aromatic hydrocarbons (PAHs) injected into eggs of chicken (*Gallus domesticus*) and Common eider duck (*Somateria mollissima*). *Environmental Toxicology and Chemistry* 11, 1653–1660.
- Nfon, E., Cousins, I.T., Broman, D., 2008. Biomagnification of organic pollutants in benthic and pelagic marine food chains from the Baltic Sea. *Science of the Total Environment* 397, 190–204.
- Nizzetto, L., Lohmann, R., Gioia, R., Jahnke, A., Temme, C., Dachs, J., Herckes, P., Di Guardo, A., Jones, K.C., 2008. PAHs in air and seawater along a North-South Atlantic transect: trends, processes and possible sources. *Environmental Science & Technology* 42, 1580–1585.
- Peakall, D.B., Jeffrey, D.A., Boersma, D., 1987. Mixed-function oxidase activity in seabirds and its relationship to oil pollution. *Comparative Biochemistry and Physiology* 88, 151–154.
- Pérez, C., Velando, A., Munilla, I., López-Alonso, M., Oro, D., 2008. Monitoring polycyclic aromatic hydrocarbon pollution in the marine environment after Prestige oil spill by means of seabirds blood analysis. *Environmental Science & Technology* 42, 707–713.
- Perugini, M., Visciano, P., Manera, M., Turno, G., Lucisano, A., Amorena, M., 2007. Polycyclic aromatic hydrocarbons in marine organisms from the Gulf of Naples, Tyrrhenian Sea. *Journal of Agricultural and Food Chemistry* 55, 2049–2054.
- Ramos, R., González-Solís, J., Forero, M.G., Moreno, R., Gómez-Díaz, E., Ruiz, X., Hobson, K.A., 2009. The influence of breeding colony and sex on mercury, selenium and lead levels and carbon and nitrogen stable isotope signatures in summer and winter feathers of *Calonectris* shearwaters. *Oecologia* 159, 345–354.
- Roscales, J.L., Muñoz-Arnanz, J., González-Solís, J., Jiménez, B., 2010. Geographical PCB and DDT patterns in shearwaters breeding across the NE Atlantic and the Mediterranean archipelagos. *Environmental Science & Technology* 44, 2328–2334.
- Schulz-Bull, D.E., Petrick, G., Bruhn, R., Duinker, J.C., 1998. Chlorobiphenyls (PCB) and PAHs in water masses of the northern North Atlantic. *Marine Chemistry* 61, 101–114.
- Seruto, C., Sapozhnikova, Y., Schlenk, D., 2005. Evaluation of the relationship between biochemical endpoints of PAH exposure and physiological endpoints of reproduction in male California Halibut (*Paralichthys californicus*) exposed to sediments from a natural oil seep. *Marine Environmental Research* 60, 454–465.
- Shore, R.F., Wright, J., Horne, J.A., Sparks, T.H., 1999. Polycyclic aromatic hydrocarbon (PAH) residues in the eggs of coastal-nesting birds from Britain. *Marine Pollution Bulletin* 38, 509–513.
- Soriano, J.A., Viñas, L., Franco, M.A., 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. *Science of the Total Environment* 370, 80–90.
- Troisi, G.M., Bexton, S., Robinson, I., 2006. Polyaromatic hydrocarbons and PAH metabolite burdens in oiled common guillemots (*Uria aalge*) stranded on the East coast of England (2001–2002). *Environmental Science & Technology* 40, 7938–7943.
- Van Metre, P.C., Mahler, B.J., Furlong, E.T., 2000. Urban sprawl leaves its PAH signature. *Environmental Science & Technology* 34, 4064–4070.
- Wan, Y., Jin, X., Hu, J., Jin, F., 2007. Trophic dilution of polycyclic aromatic hydrocarbons (PAHs) in a marine food web from Bohai Bay, North China. *Environmental Science & Technology* 41, 3109–3114.
- World Health Organization, 1998. *Selected Non-heterocyclic Polycyclic Aromatic Hydrocarbons*. Environmental Health Criteria 202. World Health Organization/International Program on Chemical Safety.