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Inter-species differences in polychlorinated biphenyls patterns from five sympatric species of odontocetes: Can PCBs be used as tracers of feeding ecology?

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ABSTRACT

Concentrations of thirty two polychlorinated biphenyls (PCBs) were determined in the blubber of five sympatric species of odontocetes stranded or by-caught along the Northwest coast of the Iberian Peninsula: common dolphin (*Delphinus delphis*), long-finned pilot whale (*Globicephala melas*), harbour porpoise (*Phocoena phocoena*), striped dolphin (*Stenella coeruleoalba*) and bottlenose dolphin (*Tursiops truncatus*). Multivariate analyses were applied to evaluate the ability of PCB patterns to discriminate these sympatric species and to determine which eco-biological factors influence these patterns, thus evaluating the relevance of PCB concentrations as biogeochemical tracers of feeding ecology. The five species could be separated according to their PCB patterns. Different exposure to these contaminants, a consequence of their different dietary preferences or habitats, together with potentially dissimilar metabolic capacities, likely explain these results; sex, age, habitat and the type of prey eaten were the most important eco-biological parameters of those tested. Although, no single congener has been specifically identified as a tracer of feeding ecology, 4 congeners from the 22 analysed seemed to be the most useful and around 12 congeners appear to be enough to achieve good discrimination of the cetaceans studied. Therefore, this study suggests that PCB patterns can be used as tracers for studying the feeding ecology, sources of contamination or even population structure of cetacean species from the Northwest Iberian Peninsula.

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1. Introduction

Marine mammals, occupying the upper trophic levels of marine food webs and possessing large lipid reserves, are susceptible to considerable bioaccumulation of lipophilic (fat-soluble) pollutants (Tanabe et al., 1988, 1994; Boon et al., 1994; Niimi, 1996), such as the polychlorinated biphenyls (PCBs). Commercial PCB mixtures have been produced for a wide range of industrial applications because of their properties, which include resistance to breakdown by other chemicals (WHO, 1976,1993). These properties have greatly contributed to the ubiquitous distribution of PCBs in the atmospheric, terrestrial and aquatic environments (Niimi, 1996). In the aquatic environment, overall PCB profiles change from those of the industrial products as a result of distinct congener behaviour and relative rate of degradation through physico-chemical processes in the environment, and/or the metabolic action of organisms which ingest the contaminants from the food chain or acquire them directly from the water column and marine sediments (Danis et al., 2003, 2005a,b).

Due to the variable number and position of the chlorine atoms on the biphenyl nucleus, individual PCB congeners follow different metabolic pathways. This results in the formation of diverse metabolites (Letcher et al., 2000) and different accumulation patterns (Boon et al., 1994). Thus, the PCB concentration patterns observed in marine mammals differ not only from the patterns seen in the technical formulations originally released to the environment (e.g. Arochlor, Clophen, Kanechlor, Pyralene) but also from those in their prey (Muir et al., 1988). Moreover, PCB patterns also differ among areas as a function of the type and of their distance from the source. This is a result of heavier congeners (i.e. those with higher degrees of chlorination) from regional sources adhering to organic particles and remaining closer to the source. The long-range signature includes a higher proportion of lighter congeners as a consequence of these being the more volatile PCBs and thus subject to atmospheric transport taking them some distance from their source (e.g. Staudinger and Roberts, 1996; Wania and Mackay, 2001). Therefore, PCBs can be used as a regional signature with coastal species being enriched in heavier congeners relative to oceanic species, which should present a greater proportion of the lighter compounds (e.g. Ross et al., 2004).

For seabirds and marine mammals, exposure to persistent organic pollutants through diet is the only relevant exposure pathway (Borgå et al., 2004). Thus, variation in prey preferences and/or in location of feeding grounds will result in varying tissue concentrations and patterns among different species, and among different individuals of the same species. In the same way, individual and species differences in the capacity to metabolize the different PCB congeners will result in intra- and inter-specific differences in PCB profiles in marine mammals (Boon et al., 1987; Tanabe et al., 1988; Wells and Mckenzie, 1994). Previous studies, which investigated the relative metabolic degradation of PCBs in marine mammals, demonstrated that pinnipeds had a higher capacity to metabolize PCBs, especially the highly chlorinated congeners, than cetaceans (Boon et al., 1992, 1997; Weijs et al., 2009). Such differences seem to be the consequence of differences in cytochrome P450-mediated mono-oxygenase activities. Indeed, differences were not only found between pinnipeds and cetaceans, but also among different species of the same taxonomic group (Goksøyr et al., 1992; Wells and Echarri, 1992).

There are sex-related differences in metabolism and transfer of PCBs during pregnancy and lactation that may also affect contaminant patterns in marine mammals. It is generally accepted that maternal transfer of lipophilic contaminants to the foetus and calves (during pregnancy and lactation) reduces the concentrations of these compounds in adult female marine mammals relative to males (e.g. Aguilar and Borrell, 1994). Indeed, several studies suggested that lesser chlorinated PCB congeners were preferentially eliminated from females to calves through lactation (e.g. Subramanian et al., 1986; Desforges et al., 2012), resulting in a different PCB profile in females compared to males.

These ecological, biological and physiological factors lead to different PCB signatures in individual marine mammal species (Aguilar, 1987; Aguilar and Borrell, 1994). This potentially allows a set of persistent organic pollutants to be used as a tool for the identification of different ecological features of marine mammal species or for identifying populations within the species (Aguilar, 1987; Litz et al., 2007; Yunke et al., 2011).

In this context, the first objective of the present study was to assess whether the variation in feeding preferences and sources of contamination among five sympatric odontocete species is reflected in their PCB profiles. The second objective was to assess whether or not there is a minimum number of PCB congeners that together efficiently discriminated between species and can thus potentially be used as ecological tracers. To meet these objectives, we used previously published data on PCB concentrations, lipid content, stable isotope ratios (δ^{13}C and δ^{15}N), cadmium (Cd) concentrations, age and reproductive status (Méndez-Fernandez et al., 2012, 2013, 2014) from 120 individuals of five odontocete species (i.e. common dolphin *Delphinus delphis*, harbour porpoise *Phocoena phocoena*, bottlenose dolphin *Tursiops truncatus*, striped dolphin *Stenella coeruleoalba* and long-finned pilot whale *Globicephala melas*) from the Northwest Iberian Peninsula. Stable isotope and Cd values were used as proxies of habitat and diet. δ^{13}C values are widely used in the literature as a proxy of feeding habitat, and more specifically neritic/benthic *vs* oceanic/pelagic (e.g. DeNiro and Epstein, 1978; Fry, 2006). δ ^{15}N values indicate trophic level (e.g. DeNiro and Epstein, 1978; Fry, 2006) and also vary with feeding habitat (e.g. Chouvelon et al., 2012; Ruiz-Cooley et al., 2012). Cadmium concentrations can be used as a tracer of the type of prey eaten, specifically fish *vs* cephalopod consumption, since cephalopods concentrate more cadmium (Honda et al., 1983; Bustamante et al., 1998; Lahaye et al., 2005).

2. Material and methods

2.1. Study area and sampling

Fieldwork was carried out in the Northwest Iberian Peninsula (NWIP), from the Northern limit of the Galician coast in Spain (43° 31′ N, 7° 2′ W) to Nazaré on the Portuguese coast (39° 36′ N, 9° 3′ W; Fig. 1). Experienced members of the Spanish (*Coordinadora para o Estudo dos Mamiferos Mariños, CEMMA*) and Portuguese (*Sociedade Portuguesa de Vida Salvagem, SPVS*) stranding networks have been collecting stranded and by-caught cetaceans for over twenty-five years and over fifteen years, respectively. Animals were identified to species, measured, sexed and, if the decomposition state of the carcass allowed, full necropsies were performed and samples collected whenever possible. However, only animals recovered in a "fresh" state (a score of between 1 and 3 from the European Cetacean Society protocol: stranded alive, freshly dead or mildly decomposed; Kuiken and Garcia Hartmann, 1991) were selected for analyses.

The age data are from Méndez-Fernandez et al. (2013, 2014). The procedure for age determination consisted of counting Growth Layer Groups (GLGs) from tooth sections, assuming that one GLG equals 1 year (as described by Lockyer, 1993; Hohn and Lockyer, 1995).

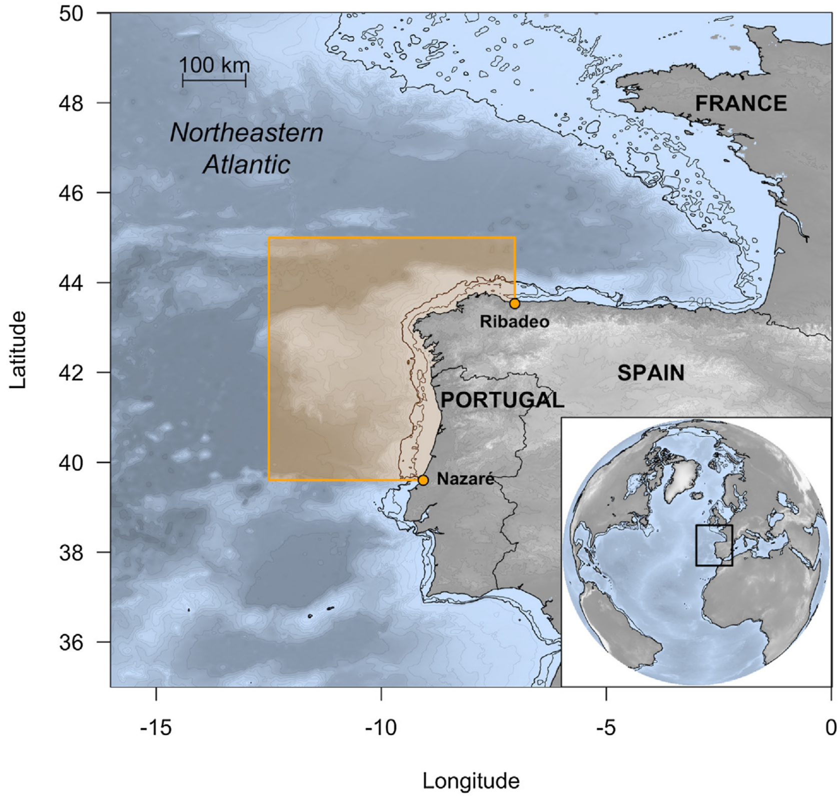

Fig. 1. Map of the study area, Northwest Iberian Peninsula. The sampling area is delimited by Nazaré and Ribadeo. Depth contours (−100 m and −200 m) are shown in grey scale.

2.2. Lipid determination and polychlorinated biphenyl (PCB) analysis

Blubber samples were previously analysed for polychlorinated biphenyls and for lipid content (Méndez-Fernandez et al., 2014). Briefly, approximately 200 mg of blubber was cut (i.e. a vertical section of the full thickness), homogenised and mixed with pre-cleaned sodium sulphate (~20 g) and then extracted by Pressurised Liquid Extraction (PLE). Following PLE, lipid content was determined by gravimetry and the rest of the extract was concentrated by Syncore (fitted with a flushback module) to ~0.5 mL and passed through silica columns, before transferring, with washings, to amber glass gas chromatography (GC) vials. Then, the concentrations of 32 PCB congeners (International Union of Pure and Applied Chemistry PCB Nos 28, 31, 52, 49, 44, 74, 70, 101, 99, 97, 110, 123, 118, 105, 114, 149, 153, 132, 137, 138, 158, 128, 156, 167, 157, 187, 183, 180, 170, 189, 194 and 209) were determined by Gas Chromatography Electron Impact Mass Spectrometry (GC–EIMS) using an HP6890 series gas chromatograph interfaced with an HP5975 MSD.

The methods employed were validated by the replicate analysis of standards and samples, regular blank controls, and through spiking experiments or analysis of certified and laboratory reference materials (LRM; cod liver oil), following the same procedure as in Méndez-Fernandez et al. (2014).

2.3. Data treatment

The similarities/differences in PCB patterns for different species and/or different groups of individuals are commonly difficult to discern in graphical output arising directly from the chemical analysis. Data reduction (e.g. dimensionality reduction) is a way of identifying patterns more readily and to provide a descriptive overview. Multivariate analyses were used to infer patterns and differences in PCB profiles. These techniques (e.g. principal component, redundancy and discriminant function analysis) have been used in previous studies for detecting differences in organochlorine compound patterns between species of marine mammals, birds and other vertebrates as well as in marine invertebrates (e.g. Schwartz and Stalling, 1991; Storr-Hansen et al., 1995; Boon et al., 1997; Borrell and Aguilar, 2005).

All the PCB congener concentrations were normalized to the lipid-based content of the blubber and thus expressed in μg/g lipid weight (lw). For statistical analyses only those congeners for which the concentrations contributed more than 10% of the total sum of the 32 congeners were selected. Thus, a total of 22 PCBs were included: CB-28, 31, 52, 49, 44, 70, 101, 99, 110, 118, 105, 149, 132, 138, 156, 187, 183, 180, 170, 189 and 194.

2.3.1. Differences in PCB patterns among species

Discriminant analysis (DA) was used to examine differences among the PCB patterns of the species using data from the 22 PCB congeners. However, in order to avoid problems due to collinearity of variables (i.e. concentrations of different congeners) we carried out a DA on principal component analysis (PCA) scores rather than on raw data. In addition, to better show the differences in the accumulation pattern of each PCB congener and to remove differences in absolute values of concentration between samples, PCBs were normalized to the concentration of CB-153. CB-153 (2,2′,4,4′,5,5′-hexachlorobiphenyl) was used to calculate these ratios (i.e. CB ratios) since it is the dominant congener in the great majority of aquatic mammals, and because it has a molecular structure that makes it highly resistant to biotransformation (Duinker et al., 1989; Wells and Echarri, 1992; Boon et al., 1992).

Thus, the CB ratio was calculated as follows (values vary from 0 to 1):

$$\text{CB ratio}_x = [\text{CB}_x]/[\text{CB-153}]$$

Where $[\text{CB}_x]$ is the concentration of an individual CB congener x and

[CB-153] is the concentration of CB-153.

2.3.2. Can we identify the most suitable PCBs to be used as tracers?

In order to identify the CB ratios that together efficiently discriminated between species and may thus be used as tracers; a re-sampling procedure was implemented based on the DA results. With this aim, first the most relevant principal components from the PCA that were used as input data in the DA were selected using the "elbow method" (i.e. the principal components on the left of the visual break or elbow were selected). Then, a threshold of 10% of absolute contribution was used to select the CB ratios that contributed most to each principal component. Thus, our initial DA on principal components indicated that 9 CBs were most influential. Subsequently every possible DA using a combination of 9 CB ratios out of the 22 available (i.e. 497,420 combinations) was computed, and for each DA the same computation of assignment errors was made. Afterwards, an assignment errors histogram was plotted to compare the original assignment error with the distribution obtained.

Finally, in order to identify if there was a minimum number of congeners needed to efficiently discriminate pairs of species based on DA results, a resampling procedure was implemented. For this, a discriminant analysis was produced with all CB ratios, and from 1 to 20 congeners were sequentially removed. Subsequently, for each random removal (i.e. from 1 to 20 congeners) the DA was repeated 1000 times. For each DA, the mean Euclidean distance was calculated over the first 4 axes of the DA between the centroids of all pairs of species. Finally, the mean Euclidean distance of the pairs of species were plotted with their corresponding 95% confidence intervals as a function of the number of CB ratios used.

2.3.3. Factors influencing PCB patterns

To examine the relationship between CB ratios and the set of potential ecological and biological explanatory factors, redundancy analysis (RDA) was used. Redundancy analysis is a principal component analysis in which the axes are restricted to be linear combinations of explanatory variables (Zuur et al., 2007). A RDA was performed using the combination of CB ratios leading to smallest numbers of assignment errors in the DA as response variables. Moreover, RDA requires the number of samples to exceed the number of explanatory variables, preferably by a factor of two. In total, for the RDA we analysed 118 samples and we had eleven explanatory variables, which is an acceptable ratio. Those variables were: species, sex, age, individual reproductive status, lipid content, habitat (i.e. neritic/benthic *vs* oceanic/pelagic; represented by δ^{13}C values), trophic level (i.e. high *vs* low trophic level; represented by δ^{15}N values) and type of prey eaten (i.e. fish *vs* cephalopods; represented by renal cadmium concentrations). Sex and reproductive status were considered as categorical variables and species identity was coded as a series of dummy variables. Data for the ecological and biological variables used to perform the RDA were obtained from published studies conducted on the same individuals (Méndez-Fernandez et al., 2012, 2013, 2014). Significance testing in RDA is based on a permutation test so no assumption of normality is required and collinearity between explanatory variables is not an important issue (Zuur et al., 2007).

All the statistical analyses were performed using R version 3.1.3 (R Development Core Team, 2014). The map (Fig. 1) was created using the MARMAP package (Pante and Simon-Bouhet, 2013) and figures were created using the packages MASS and ggplot2 (Venables and Ripley, 2002; Wickham, 2009).

3. Results

3.1. Differences in PCB patterns among species

The average lipid content of blubber in the five species analysed was higher in females than in males (Table 1) although the difference was not statistically significant ($p > 0.05$). Total PCB concentrations (i.e. ΣPCB_{32}) were higher in males than in females with the exception of harbour porpoises. Moreover, the species

Table 1
Arithmetic means ± standard deviation (SD) of age (in years), lipid content (%) and of the sum of 32 congener concentrations (ΣPCB_{32} in μg/g lipid weight (lw) with range in parenthesis) measured in the blubber of males and females of five odontocete species from the Northwest Iberian Peninsula. n = sample sizes for each species and sex (total of 67 males and 51 females).

Species	n	Age	Lipid	ΣPCB_{32}
Male				
Common dolphin *Delphinus delphis*	50	6.0 ± 4.9	59.0 ± 15.9	20.4 ± 16.1 (3.9–77.5)
Harbour porpoise *Phocoena phocoena*	4	7.2 ± 7.2	67.2 ± 11.1	19.8 ± 20.8 (6.7–50.8)
Bottlenose dolphin *Tursiops truncatus*	4	4.9 ± 1.6	66.3 ± 12.4	62.1 ± 41.8 (36.3–124.4)
Striped dolphin *Stenella coeruleoalba*	8	3.3 ± 5.1	57.2 ± 25.7	22.8 ± 23.3 (3.4–68.7)
Long-finned pilot whale *Globicephala melas*	1	2.0	56.0	38.7
Female				
Common dolphin *Delphinus Delphis*	31	7.3 ± 6.3	63.0 ± 15.6	11.6 ± 7.2 (1.4–27.6)
Harbour porpoise *Phocoena phocoena*	8	6.9 ± 6.6	83.2 ± 12.4	20.8 ± 21.6 (6.9–71.7)
Bottlenose dolphin *Tursiops truncatus*	3	2.3 ± 1.6	71.8 ± 0.8	48.9 ± 30.9 (13.9–72.1)
Striped dolphin *Stenella coeruleoalba*	7	6.7 ± 5.7	65.7 ± 5.7	7.6 ± 5.9 (1.7–15.4)
Long-finned pilot whale *Globicephala melas*	2	6.7 ± 6.7	69.8 ± 10.6	4.9 ± 4.2 (2.0–7.9)

that showed the highest ΣPCB_{32} concentrations were bottlenose dolphin, pilot whale and harbour porpoise (Table 1).

The first two principal components of the PCA accounted for 84.0% of the CB ratio variability, while the first two-discriminant factors (i.e. LD1 and LD2) of the DA together explained 82.9% of the variance of the CB ratio data (Fig. 2). The DA revealed the existence of consistent differences among the five species, with harbour porpoises (shown in light blue) and striped dolphins (in orange) being clearly separated in this bi-plot. The three pilot whales were also distinctly placed relative to the striped dolphins, common dolphins and harbour porpoises, although there was overlap with one of the seven bottlenose dolphins. Between 66.7 and 100% of the individuals of each species were classified in the correct species group (Table 2). Individuals of common dolphin, bottlenose dolphin and harbour porpoise were all well classified to the correct species. However, one striped dolphin out of 15 (6.7%) was assigned to the common dolphins and one pilot whale out of three was assigned as a bottlenose dolphin. For the first discriminant axis, which clearly separated striped dolphin and, to a lesser extent harbour porpoise, from the other species (Fig. 2), the most influential principal components of the PCA were, in decreasing order: 22 and 21 (Fig. S1a). For the second discriminant axis, which clearly separated pilot whales and harbour porpoise from the bottlenose dolphin and most of the common dolphins, the most influential principal components were, in decreasing order: 22, 21, 19, 20, 18 and 17 (Fig. S1b). Finally, CB-183, 189 and 70 contributed most to principal components 22 and 21, and CB-138, 149, 70, 189, 110, 52, 118 and 99 contributed most to principal components 19, 20, 18 and 17 (Table S1). All these 9 congeners had between 4 and 7 Cl atoms per biphenyl nucleus.

Table 2
Results of the discriminant analysis (DA): classification of the individuals in each species groups. Results are presented as percentage (%) and well-classified animals are shown in bold. Each row refers to results for classification of individuals of one species (summing to 100%). Common dolphin *Delphinus delphis* (Dd), harbour porpoise *Phocoena phocoena* (Pp), bottlenose dolphin *Tursiops truncatus* (Tt), striped dolphin *Stenella coeruleoalba* (Sc) and long-finned pilot whale *Globicephala melas* (Gm).

True Species	Sample size	% classified as:				
		Dd	Pp	Tt	Sc	Gm
Dd	81	**100**	0	0	0	0
Pp	12	0	**100**	0	0	0
Tt	7	0	0	**100**	0	0
Sc	15	6.7	0	0	**93.3**	0
Gm	3	0	0	33.3	0	**66.7**

3.2. Can we identify the most suitable PCBs to be used as tracers?

The assignment errors histogram showed that the modal error rate using 9 congeners is around 12% and almost all combinations give error rates less than 20% (Fig. 3). Moreover, error rate of less than 6% was almost never achieved. However, some combinations of CB ratios led to very small numbers of assignment errors, including three combinations that led to only 4 assignment errors (3.3%). The PCBs present in these best combinations and that lead to low assignment errors were CB-105 (3 times), 110 (3), 170 (3), 180 (3), 187 (3), 194 (3), 49 (3), 189 (2), 31 (2), 138 (1) and 183 (1).

For all pairs of species, the mean Euclidean distance decreased with a decreasing number of congeners used in the DA (Fig. 4).

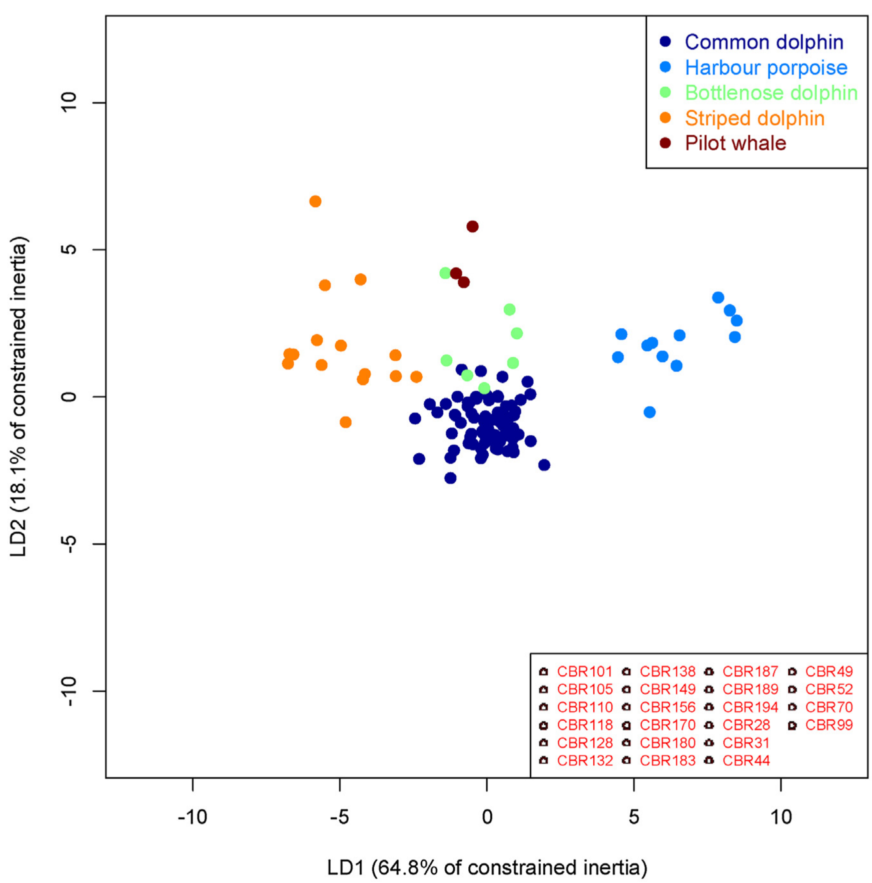

Fig. 2. Results of the discriminant analysis (DA) on principal components scores (i.e. LD1 and LD2) of individuals of common dolphin *Delphinus delphis*, harbour porpoise *Phocoena phocoena*, bottlenose dolphin *Tursiops truncatus*, striped dolphin *Stenella coeruleoalba* and long-finned pilot whale *Globicephala melas* from the Northwest Iberian Peninsula. The 22 CB ratios (CBR) included in the DA are given on the bottom right side of the bi-plot. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

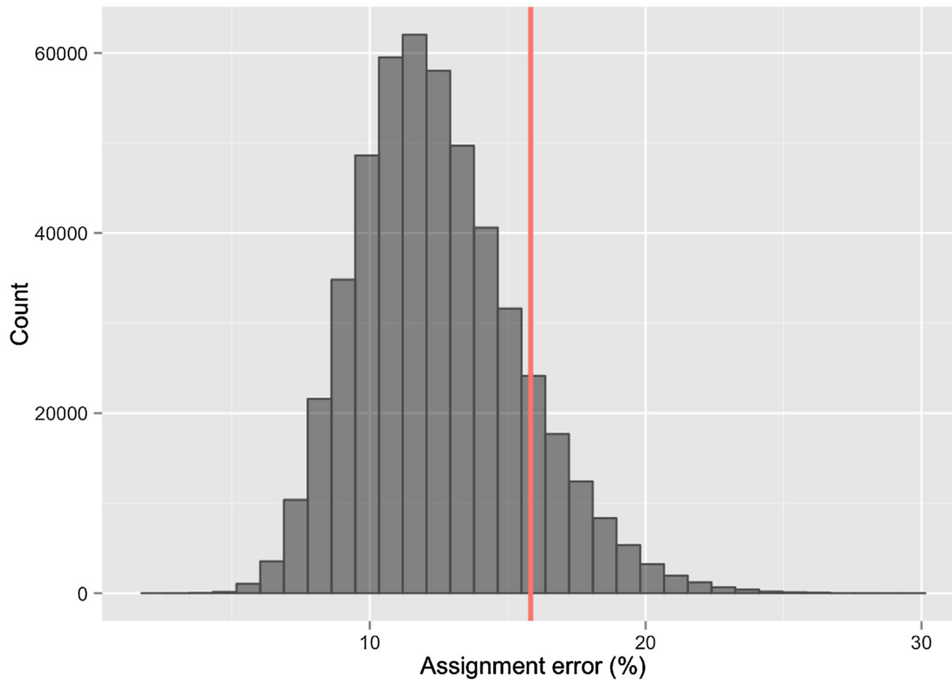

Fig. 3. Distribution of the assignment error (in%) obtained from every possible discriminant analysis (DA) using a combination of 9 CB ratios out of the 22 available. The vertical red line represents the original assignment error obtained with the 9 CB ratios selected from the first DA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

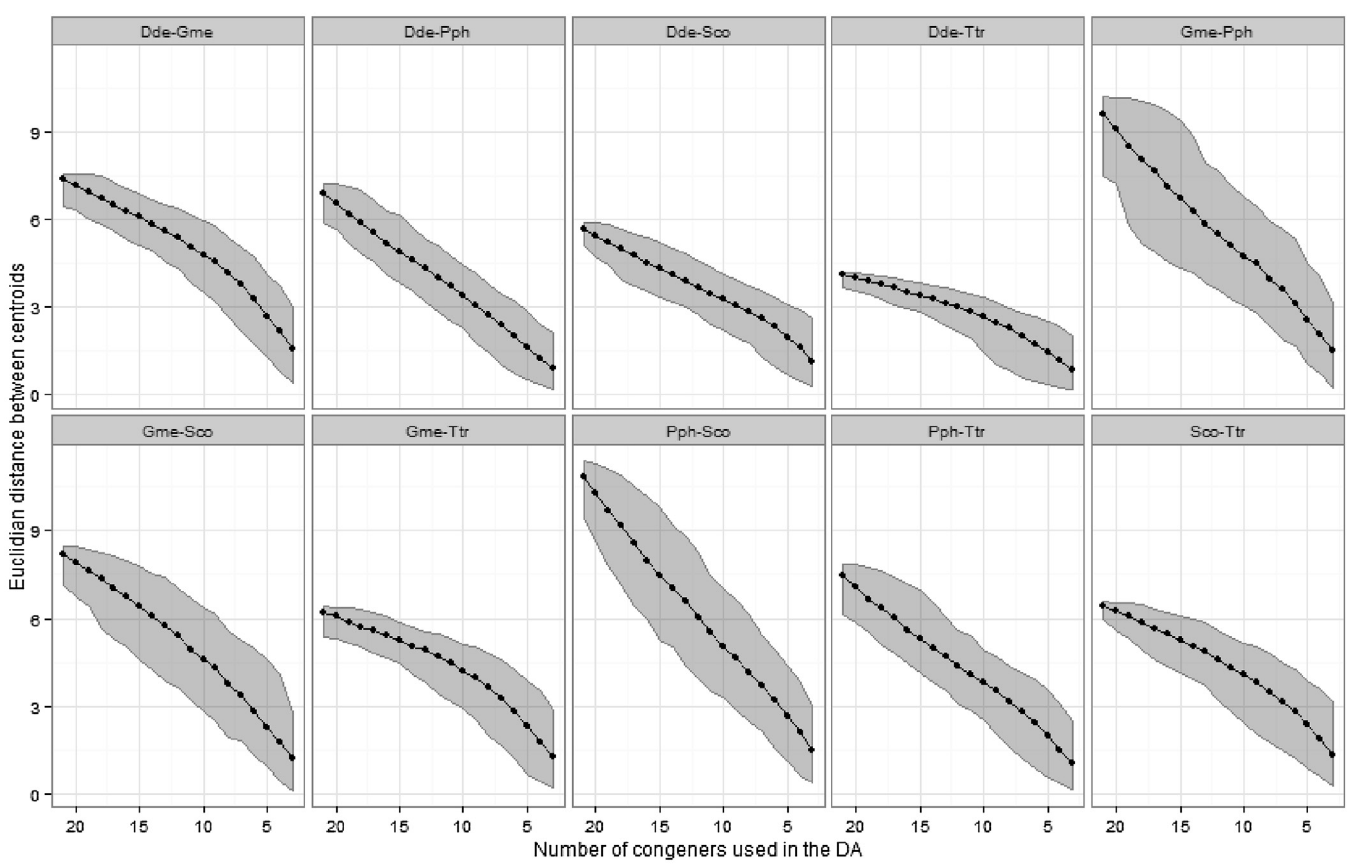

Fig. 4. Mean Euclidean distance and 95% confidence interval calculated between the centroids of all pairs of species when congeners are randomly removed. For each number of congeners removed we performed 1000 bootstraps. Common dolphin *Delphinus delphis* (Dde), harbour porpoise *Phocoena phocoena* (Pph), bottlenose dolphin *Tursiops truncatus* (Ttr), striped dolphin *Stenella coeruleoalba* (Sco) and long-finned pilot whale *Globicephala melas* (Gme).

Considering a specific number of congeners (e.g. 10), the mean Euclidean distances were different according to the pair of species compared, showing that some of them were easier to discriminate than others. As an example, the pairing of harbour porpoise with striped dolphin (Pp-Sc), and pilot whale with harbour porpoise (Gm-Pp), both exhibited high mean Euclidean distances. In comparison, the lowest mean Euclidean distances were shown when bottlenose dolphins (Tt) were paired with common dolphins, pilot whales or striped dolphins (Dd-Tt, Gm-Tt and Sc-Tt, respectively) and also when common dolphins were compared with striped dolphins (Dd-Sc), showing that these species were more difficult to discriminate. Approximately half of the comparisons presented in Fig. 4 showed a clear plateau or at least a point of inflection, indicating that the analysis of more congeners would not result in a better discrimination. Where this was the case (e.g. Dd-Gm, Gm-Tr), the optimal number of congeners (showing the highest mean Euclidean distance) was between 10 and 15, which was consistent with the decreasing number of assignment errors related to the number of congeners used in the DA.

Table 3
Results of redundancy analysis (RDA) on the best CB ratios combination (i.e. CB-31, 49, 105, 110, 138, 170, 180, 183, 187, 194 and 189) in blubber of common dolphin *Delphinus delphis* (Dd), harbour porpoise *Phocoena phocoena* (Pp), bottlenose dolphin *Tursiops truncatus* (Tt), striped dolphin *Stenella coeruleoalba* (Sc) and long-finned pilot whale *Globicephala melas* (Gm). RS (reproductive status), Lipid (lipid content), CdK (cadmium concentrations in kidney), δ^{13}C (stable isotopes of carbon), δ^{15}N (stable isotopes of nitrogen). Bold values correspond to the significant variables.

Explanatory variables	*F*	*p-value*
Sex	29.5422	**0.001**
Age	22.3346	**0.001**
RS	13.7578	**0.001**
Lipid	39.5943	**0.001**
CdK	18.2575	**0.002**
Pp	12.4306	**0.002**
Dd	4.8600	**0.023**
δ^{13}C	4.7597	**0.024**
Tt	2.3000	0.123
Sc	1.0594	0.268
δ^{15}N	1.1529	0.299

3.2.1. Factors influencing PCB patterns

The CB ratios that gave the best separation of the species and the smallest number of misassignment errors (i.e. CB-31, 49, 105, 110, 138, 170, 180, 183, 187, 189 and 194) were used as response variables to compute a RDA together with the following ecological and biological parameters as explanatory variables: species, sex, age, reproductive status, lipid content, habitat, trophic level and prey type (see Material and methods for details of explanatory variables). The first two axes explained 9.8% of the CB ratio variability (Fig. 5).

All explanatory variables tested had a significant influence ($p < 0.05$) with the exception of trophic level represented by δ^{15}N values (Table 3). Additionally, either bottlenose dolphin or striped dolphin had no significant influence ($p > 0.05$) (Table 3).

The positions of individual animals in the bi-plot, represented by coloured points (Fig. 5), showed that harbour porpoises and bottlenose dolphins were positively correlated with the second axis as

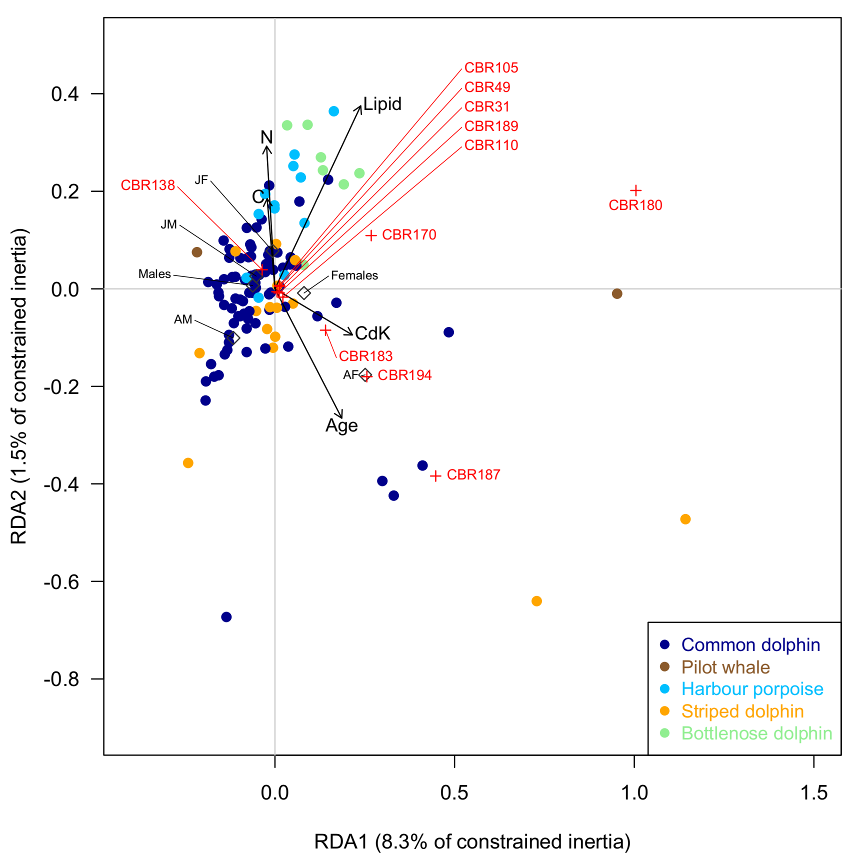

Fig. 5. Results of redundancy analysis (RDA) on the best CB ratios combination (i.e. CB-31, 49, 105, 110, 138, 170, 180, 183, 187, 194 and 189) in blubber of common dolphin *Delphinus delphis*, harbour porpoise *Phocoena phocoena*, bottlenose dolphin *Tursiops truncatus*, striped dolphin *Stenella coeruleoalba* and long-finned pilot whale *Globicephala melas* from the Northwest Iberian Peninsula. Bi-plot for axes 1–2 of significant explanatory and response variables. CdK (cadmium concentrations in kidney), C (δ^{13}C values), N (δ^{15}N values), AF (adult female), AM (adult male), JF (juvenile female), JM (juvenile male) and CBR (CB Ratios). The categorical variables (i.e. sex and reproductive status) are represented by empty black diamonds and CBR by red crosses. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

well as with lipid content and habitat. Most of the striped dolphins were associated with high renal cadmium concentrations and negatively correlated with habitat. They were also associated with tetra- to hexa- chlorinated congeners (i.e. CB-52, 99, 110, 118 and 138).

Examination of the bi-plot also highlighted a cluster of individuals in the lower part of the bi-plot corresponded to old common and striped dolphin females. These individuals were also associated in this part of the bi-plot with higher concentrations of the hepta- and octa- chlorinated congeners (i.e. CB-183, 187 and 194), as well as with high cadmium concentrations and high age values. Finally, common and striped dolphin individuals showed the lowest segregation within the bi-plot (Fig. 5).

4. Discussion

4.1. Differences in PCB patterns and identification of most suitable PCBs to be used as tracers

The PCB profiles of the five odontocete species differed substantially and could be successfully distinguished from each other (Fig. 2), with all the common dolphins, bottlenose dolphins and harbour porpoises being correctly assigned to species (Table 2). Pilot whales exhibited the lowest percentage of successful classification. However, the sample size was very small for this latter species. As such, one out of three incorrectly classified animals resulted in only a 66.7% of well classification (Table 2). Harbour porpoise and striped dolphin were the species with the greatest separation on the DA plot and thus presented the most significant difference with respect to PCB profiles (Fig. 2). The habitat where these animals feed is likely to be a factor that strongly influences the PCB profiles. Higher pollutant burdens are generally found in species inhabiting coastal regions due to the close proximity of these animals to possible emissions, discharges and losses of persistent organic pollutants in temperate areas (Storr-Hansen and Spliid, 1993). Moreover, the pattern of the PCBs may differ according to the distance from the source; the lighter congeners are more volatile, and thus are capable of being transported over longer distances (Aguilar and Borrell, 2005). Therefore, the proportion of the highly chlorinated congeners decreases with distance from the source. Additionally, the relative abundance of heavy to light carbon isotopes has also been used to discriminate between habitats, pelagic/offshore habitats where phytoplankton is the only source of organic carbon and vegetated inshore/benthic habitats where macrophytes may be an additional source of organic carbon (DeNiro and Epstein, 1978; Fry, 2006). Likewise, $\delta^{15}N$ values also vary strongly with habitat (between inshore and offshore systems, with latitudes and between oceanic basins) and are used as an indicator of feeding habitat (e.g. Chouvelon et al., 2012; Ruiz-Cooley et al., 2012). Based on the stable isotope composition and direct observations at sea of the five species studied, we can affirm that in the waters off the NWIP the harbour porpoise is a coastal species, while the striped dolphin mainly inhabits offshore waters (Table 4). The discrimination between both species on the basis of their PCB profiles was consistent with the information available on features of their ecology. The other three species can be observed in both coastal and offshore waters, and are therefore likely to feed in both, as indeed may be inferred from their diets in this region (see Table 4 and references therein) and, based on this research, their PCB profiles.

Among the three combinations of PCBs that generated only four assignment errors, eleven of the selected PCBs (i.e. CB-105, 110, 170, 180, 187, 194, 49, 189, 31, 138 and 183) presumably reflect interspecific differences in habitat but also in feeding preferences. In fact, a subsequent DA performed with this combination of PCBs confirm this statement giving a better separation of the species than if we consider the PCBs selected from the first DA (Tables S2 and S3).

Moreover, whatever the pair of species is considered, the mean Euclidean distance separating them decreases with the number of congeners randomly removed (Fig. 4). However, different pairs of species had different mean Euclidean distances between them as might be expected based on the eco-biological differences presented (see Table 4) and PCB profiles. The highest mean Euclidean distances between species correspond to harbour porpoise in comparison with striped dolphin and pilot whale, the species that are the most different from each other in terms of eco-biological traits. The lowest mean Euclidean distances correspond to the ecologically closest species, the common dolphin when compared with striped and bottlenose dolphins.

The assignment errors together with the mean Euclidean distances show that around 12 congeners is enough to well separate the species. This is a good result since PCB analysis requires considerable effort in terms of data reduction, quality assurance, and processing. Potential quality control and comparability issues when applying this approach widely include variability in co-elution patterns, different sets of congeners analysed by different laboratories (although the ICES 7 CB-28, 52, 101, 118, 153, 138, 180 is an internationally recognised set of PCBs), and normal inter-laboratory variation. Hence, the minimum number of congeners needed to allow discrimination of the species is an important issue since the cost of analyses can be greatly reduced by lowering the number of congeners that are analysed.

Although it was not possible to identify specific congeners as representing the most useful tracers, it is clear that among the set of 12 congeners selected, those that were more frequently repeated in the discriminant analyses performed (i.e. CB-110, 138, 183, 189) should be included.

4.2. Factors influencing specific PCB patterns

The variability of PCB patterns in marine mammals basically depends on differences in their pollutant intakes, which is a direct consequence of the specific feeding preferences and associated habitats (e.g. Tanabe et al., 1988; Borrell and Aguilar, 2005), as well as capacities to metabolize and/or eliminate (e.g. maternal transfer) some congeners. However, identifying whether the separation is due to differences in PCB metabolism or differences in ecological and/or biological traits of the species is a difficult issue.

This research revealed a good separation among species based on PCB profiles, that is in agreement with recent studies, using stable isotopes and other ecological tracers, showing that these species occupy different foraging niches within the NWIP (Fernández et al., 2013; Méndez-Fernandez et al., 2013). Among the set of explanatory variables used to elucidate this issue (Fig. 5), only trophic level, represented by $\delta^{15}N$ values, had no significant influence on CB ratios. More specifically, in the NWIP food web, these species have the same range of trophic level with no significant differences among them (Table 4). However, occupying the same trophic level does not necessarily mean that these species exploit the same food resource. This is reflected in the RDA since the prey type variable, which is represented by the renal cadmium concentrations of the specimens analysed, has a significant effect on the CB ratio data and is, together with habitat, the most important ecological explanatory variable (see Table 3).

Cadmium is a trace element used as an indicator of the type of prey eaten by the various odontocetes. More specifically, it helps distinguish fish *vs* cephalopod consumption, cephalopods being animals that concentrate more cadmium (Honda et al., 1983; Bustamante et al., 1998; Lahaye et al., 2005). This would explain the positive correlation found between higher cadmium concentrations and the majority of the striped dolphins, some common

Table 4
Background on the feeding ecology of the odontocetes studied in the Northwest Iberian Peninsula, obtained from previous studies on: stomach contents analysis, land-based and sea surveys and chemical analyses (stable isotopes of carbon and nitrogen and renal cadmium concentrations). Trophic levels were calculated following Vander Zanden and Rasmussen (2001) in Méndez-Fernandez et al. (2012, 2013).

Species	Habitat	Trophic level	Food source
Common dolphin *Delphinus delphis*	Oceanic/neritic	4.7	Mixed feeder (blue whiting, sardine, hake and sand smelt, but also sepiolids, common and curled octopus)
Harbour porpoise *Phocoena phocoena*	Neritic	5.3	Piscivorous (pouting, scad and blue whiting)
Bottlenose dolphin *Tursiops truncatus*	Neritic (and offshore ecotype)	5.1	Mainly piscivorous (blue whiting and hake)
Striped dolphin *Stenella coeruleoalba*	Oceanic	4.3	Mainly teuthophagous (cephalopods and crustaceans, but also blue whiting, sand smelt and scad)
Long-finned pilot whale *Globicephala melas*	Oceanic/neritic	4.9	Teuthophagous (common and curled octopus)

Adapted from Santos et al. (2007, 2013, 2014), Spitz et al. (2006, 2011), Pierce et al. (2010), Fernández et al. (2011), Spyrakos et al. (2011), and Méndez-Fernandez et al. (2012, 2013).

dolphins and one of the three pilot whales (Fig. 5), which feed on cephalopods in the NWIP (Table 4). Moreover, common dolphins showed the highest dispersion, which is consistent with their mixed diet (Table 4), and the fact that the proportion of the different prey species in their diet can vary according to the oceanic or neritic origin of the individuals (Pusineri et al., 2007).

In the same way, Barone et al. (2014) compared PCB profiles of fishery products (i.e. fish, cephalopods and crustaceans) from Southern Italy, and highlighted a species-specific bioaccumulation of contaminants and differences in PCB profiles among the three different groups of seafood. Specifically, invertebrates (i.e. cephalopods and crustaceans) had a high percentage contribution of lower-chlorinated PCBs such as CB-28 and 52 with a slight predominance of CB-138, while in fish samples high-chlorinated congeners such as CB-180 were more prominent. Similarly, in the RDA, low chlorinated congeners such as CB-105, 49 31, 110 and 138 seems to be more associated with most of the striped and common dolphins (Fig. 5), and therefore with the mainly cephalopod feeders in the area. On the contrary, the high-chlorinated congeners CB-170 and 180 showed a positive relationship with most of the harbour porpoises and bottlenose dolphins, which are largely piscivorous in the NWIP (Fig. 5). Boon et al. (1997) reviewed the types of metabolic behaviour of several PCB congeners in five species of mammals (seals, otters and cetaceans) and revealed that CB170 and 180 are highly resistant to biotransformation and consequently difficult to metabolise. These congeners are also among those exhibiting a high octanol-water partition coefficient (log Kow $\approx$ 7) indicating their high liposolubility (Jäntschi and Bolboacà, 2006; Walters et al., 2011). The high lipid content exhibited by harbour porpoise and bottlenose dolphin (67.2 and 66.3% for males and 83.2 and 71.8% for females, respectively, Table 1) compared to the other species may thus partly explain the high ratio of these both congeners and the positive relationship with lipid content in these individuals (Fig. 5).

With respect to the various biological factors investigated, sex had an important effect on PCB accumulation and patterns, primarily due to the maternal transfer during gestation and lactation. The physicochemical characteristics of the congeners govern this transfer, with the less lipophilic and lower molecular weight congeners being those primarily transferred to the foetus (e.g. Greig et al., 2007; Desforges et al., 2012). Thus, the RDA bi-plot revealed a cluster of several individuals positively correlated with age and with kidney cadmium concentration (linked to diet), as well as with hepta- and octa- chlorinated CBs (CB-183, 187 and 194) (Fig. 5). Of the individuals belonging to this cluster, there are individuals of common and striped dolphin species, all of them are females and 60% were found to be mature. Thus, CB-183, 187 and 194, which are high-chlorinated congeners, probably accumulates with age in females and may be not well transferred to offspring. Consequently, the older females, which have probably been pregnant and hence eliminated some less-chlorinated PCB congeners, may have high concentrations of these congeners as was previously reported in several marine mammal species (e.g. Desforges et al., 2012; Peterson et al., 2014).

Despite the evident effects of eco-biological factors, the effect of metabolism on PCB patterns cannot be overlooked. Differences in metabolic capacities have been demonstrated between pinnipeds and cetaceans (Tanabe et al., 1988; Goksøyr et al., 1992; Wells et al., 1996), in which the iso-enzymes of the cytochrome P450 1A and 2B subfamilies (CYP1A and CYP2B) seem to play a key role in the biotransformation of PCBs. However, there are few comparable studies on odontocete species, and taxonomic proximity should not be taken as an indication of identical metabolic capacities. Indeed, substantial interspecific variation in the capacity to degrade persistent organic pollutants has been observed between common and striped dolphins (Marsili and Fossi, 1996; Borrell and Aguilar, 2005), and between other taxonomically close vertebrates (Fossi et al., 1995). The set of congeners used in the RDA, which resulted in a high segregation among species and low intra-species dispersion, could be involved in the species' metabolic capacities. Nevertheless, with the information currently available, it is impossible to distinguish the contribution of metabolism to the observed PCB patterns.

5. Conclusion

The results presented in this paper show clear differences in PCB patterns between different sympatric species of odontocetes. These differences are a presumably consequence of the effect of a mixture of metabolism and eco-biological parameters, with sex, age, habitat and the type of prey eaten probably being the most important among all that were tested. No single congener has been identified as a tracer of feeding ecology. However, 4 congeners (i.e. CB-110, 138, 183 and 189) from the 22 analysed seemed to be the prime ones and around 12 congeners appear to be enough to efficiently discriminate the species. Further studies are necessary to evaluate the potential generalisation of these results. Thus the same data treatment will have to be applied to other data sets of different species and/or different geographical areas in order to confirm the relevancy and the universality of these results. Moreover, the effectiveness of this data treatment for addressing issues associated with the conservation and management of wildlife could be significant. The importance of defining ecological diversity and populations has been the subject of much debate (ICES, 2014), and genetic together with ecological segregation is the fundamental basis for identifying true populations. This is all the more important considering that the impact of localized anthropogenic threats, such as contamination, differs among populations. The profile of persistent organic pollutants, such as PCBs, was previously proposed as an additional tool to identify segregation of marine mammal populations (as in Borrell et al., 2006; Herman et al., 2005; Krahn et al., 2007; Pierce et al., 2008). In light of these results, the proposed data treatment can be used as a complementary tool in the identification of marine mammal populations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind.2016.11.013.

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