Universidade do Minho
Escola de Ciências

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Université de La Rochelle
LIENSS

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sob a orientação de:
Professor Doutor José Vítor Vingada
Doutora Florence Caurant
Professor Doutor Paco Bustamante
Professor Doutor Graham J. Pierce
Doutor Alfredo López

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DECLARAÇÃO

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chemical tracers and contamination
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Northwest Iberian Peninsula

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É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTA TESE/TRABALHO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.

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Assinatura: ____

No me resigno a dar la despedida a tal altivo
y firme sentimiento que tanto impulso
y luz diera a mi vida

Manuel Altolaguirre

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ABSTRACT

The first objective of this PhD was to determine the degree of ecological segregation between five sympatric species of toothed whales (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*) inhabiting a restricted and highly productive area, the North West Iberian Peninsula (NWIP). To this end, chemical parameters analyses were used as an "alternative method" of assessing diet and habitat of these animals over different periods of integration, complementing the information given by traditional methods of investigation such as stomach contents, faeces analyses and field observations. The second objective was to evaluate the contamination status of these species in order to estimate the toxicological risk these populations face and to complete the existing database. In this context, trace elements, carbon and nitrogen stable isotopes, as well as persistent organic pollutants (POPs) were selected as chemical parameters for our investigations.

Firstly, the chemical parameters were used as ecological tracers of populations (or parts of populations) by drawing an image of the diet and habitat of the species at different time scales. We estimated that the food web has five trophic levels, with toothed whales occupying the top positions, with no significant difference in trophic positions among them, ranging from 4.3 to 5.3. While the analysis of a single chemical parameter did not completely segregate between the five species, the information gained by combining the analyses of several long-term ecological tracers (i.e. nitrogen and carbon stable isotopes in teeth and renal cadmium concentrations) revealed ecological niche segregation in two dimensions of this niche (the trophic and the spatial dimensions). However, year-to-year or seasonal variations could not be investigated, except for in common dolphin, and thus temporary overlap cannot be excluded. Additionally, we showed that polychlorinated biphenyls (PCBs) profiles could be a relevant tracer of the foraging ecology of taxonomically close species, allowing a fine separation of the species as result of their different patterns. This was particularly clear for the harbour porpoise and striped dolphin.

Secondly, the contamination status of the species in this area was evaluated, with concentrations interpreted in the light of biological and ecological factors. But the bioaccumulation of trace elements appeared to be less predictable than that of POPs. Bottlenose dolphins and harbour porpoises were found to have higher PCBs concentrations than the rest of the analysed NWIP toothed whales, as well as those of adjacent NE Atlantic waters.

The main results of this PhD highlight the relevance of exploiting these chemical parameters as ecological tracers, as well as their usefulness in the comprehensive study of structure and function of ecosystems through the role of top predators. Combined with the information on anthropogenic activities, this work can also contribute to the development and implementation of management plans and mitigation measures for these five species of toothed whales in the NWIP.

RESUMO

O primeiro objetivo desta tese foi determinar qual grau de segregação ecológica existente entre cinco espécies simpráticas de cetáceos odontocetes (i.e. golfinho-comum *Delphinus delphis*, bôto *Phocoena phocoena*, roaz-corvineiro *Tursiops truncatus*, golfinho-riscado *Stenella coeruleoalba* e baleia-piloto *Globicephala melas*) que habitam numa área restrita e altamente produtiva, o Noroeste da Península Ibérica (NOPI). Para este fim, foram analisados parâmetros químicos como um "método alternativo" que pode refletir a dieta e habitat em diferentes períodos de integração destes animais, complementando a informação obtida através de métodos tradicionais tais como as análises de conteúdos estomacais, as análises de fezes e a observação de campo levadas a cabo no NOPI. O segundo objetivo foi avaliar o estado de contaminação destas espécies, obtendo dados de base de contaminantes nestas populações e estimar o risco toxicológico. Os elementos traço, os isótopos estáveis de carbono e nitrogênio e os poluentes orgânicos persistentes (POPs) foram seleccionados como parâmetros químicos para responder aos nossos objectivos.

Em primeiro lugar, os parâmetros químicos foram utilizados como traçadores ecológicos de populações (ou partes das populações) pondo em evidência a dieta e habitat das espécies em estudo ao longo de diferentes escalas temporais. Estimou-se que a cadeia trófica possui um total de cinco niveis tróficos onde os cetáceos odontocetes estudados ocupam as posições mais elevadas sem apresentar diferenças significativas entre elas, variando desde 4.3 para 5.3. Embora a análise de um único parâmetro químico não tenha permitido segregar completamente as cinco espécies, a informação obtida combinando as análises de vários traçadores ecológicos a longo prazo (i.e. isótopos estáveis de carbono e nitrogênio em dentes e concentrações de cádmio nos rins) revela uma segregação do nicho ecológico entre as cinco espécies em duas dimensões do nicho (a dimensão trófica e a dimensão espacial). Porém, não pode ser excluída uma sobreposição temporal, uma vez que não foi possível investigar variações sazonais ou inter-anuais, excepto no caso do golfinho-comum. Adicionalmente, mostramos que perfis de policloro bifenis (PCBs) podem ser un traçador pertinente da ecologia alimentar de espécies taxonomicamente próximas permitindo uma boa separação das espécies bôto e golfinho-riscado de acordo com os seus diferentes padrões.

Em segundo lugar, foi avaliado o estado de contaminação das espécies nesta área. As concentrações foram interpretadas tendo em conta factores biológicos e ecológicos. No entanto, a bio-acumulação dos elementos traço mostrou-se menos previsível que a bio-acumulação dos POPs. O roaz-corvineiro e o bôto exibiram as concentrações mais elevadas de PCBs com respeito dos outros cetáceos odontocetes do NOPI e com respeito aos outros indivíduos de águas vizinhas do Oceano Atlântico Nordeste.

Os principais resultados desta tese mostram a relevância destes parâmetros químicos como os traçadores ecológicos e a sua utilidade no estudo da estrutura e funcionamento dos ecossistemas através do papel dos predadores de topo. Combinando estes dados com a informação sobre as atividades antropogénicas, este trabalho pode contribuir ao desenvolvimento e implementação de planos de gestão e mitigação para estas cinco espécies de cetáceos odontocetes no NOPI.

RESUMÉ

Le premier objectif de ce travail de thèse était de déterminer quel est le degré de ségrégation écologique entre cinq espèces sympatriques de cétacés odontocètes (i.e. le dauphin commun *Delphinus delphis*, le marsouin commun *Phocoena phocoena*, le grand dauphin *tursiops truncatus*, le dauphin bleu et blanc *Stenella coeruleoalba* et le globicéphale noir *Globicephala melas*) fréquentant l'aire marine restreinte et très productive du Nord-Ouest de la Péninsule Ibérique (NOPI). Pour cela, des paramètres chimiques ont été analysés dans les tissus biologiques, en tant que " méthode alternative " pouvant refléter de façon précise le régime alimentaire et l'habitat de ces animaux à différents échelles de temps d'intégration, et ainsi compléter les informations apportées par des méthodes plus traditionnelles tels que l'analyse des contenus stomacaux ou des fèces, ou encore les données d'observations en mer. Le second objectif de ce travail était d'évaluer l'état de contamination de ces espèces, pour acquérir une base de données de référence complète les concernant et estimer le risque toxicologique auquel elles peuvent faire face. Dans ce contexte, les éléments traces, les isotopes stables du carbone et de l'azote, et les polluants organiques persistants (POPs) sont les paramètres chimiques qui ont été sélectionnés pour répondre aux objectifs fixés.

Dans un premier temps, les paramètres chimiques ont été utilisés comme traceurs écologiques des populations (ou de segments de population), procurant une image du régime alimentaire et de l'habitat des espèces à différentes échelles de temps. Nous avons calculé que le réseau trophique du NOPI avait cinq niveaux trophiques, au sein duquel les espèces étudiées occupaient les plus hautes positions, comprises entre 4,3 à 5,3, et sans qu'il y ait de différence significative de position trophiques entre les espèces. Alors que l'analyse d'un seul paramètre chimique ne permettait pas ségréger les cinq espèces, les informations apportées par l'analyse de plusieurs traceurs de long terme (i.e. rapport des isotopes stables du carbone et de l'azote dans les dents et concentrations de cadmium dans les reins) ont révélé une ségrégation de leur niche écologique dans deux des dimensions possibles (les dimensions trophique et spatiale). Cependant, les variations pouvant survenir d'une année sur l'autre ou saisonnières n'ont pas pu être étudiées, excepté pour le dauphin commun, et un chevauchement temporaire des niches ne peut donc pas être totalement exclu. De plus, nous avons montré que les profils de polychlorobiphényles (PCBs) pouvaient être un traceur pertinent de l'écologie trophique d'espèces taxinomiquement proches, en permettant une séparation fine des espèces telles que le marsouin commun et le dauphin bleu et blanc, d'après les différents profiles d'accumulation de ces deux espèces.

Dans un second temps, l'état de contamination des espèces dans la zone d'étude a été évalué. Les concentrations ont été interprétées à la lumière des facteurs biologiques et écologiques pouvant influencer ces concentrations, et la bioaccumulation des éléments traces s'est révélée moins prévisible que celle des POPs. Le grand dauphin et le marsouin commun présentaient les concentrations de PCBs les plus élevées par rapport aux autres cétacés odontocètes du NOPI et par rapport à d'autres individus des eaux adjacentes de l'Atlantique Nord-Est.

Les résultats principaux de ce travail de thèse ont mis en évidence la pertinence d'utiliser ces paramètres chimiques comme traceurs écologiques, et leur utilité dans la compréhension de la structure et le fonctionnement des écosystèmes à travers le rôle des prédateurs supérieurs. En combinant ces résultats à des informations concernant les activités anthropiques, ce travail peut contribuer au développement et à l'implémentation de mesures de gestion pour ces cinq espèces de cétacés odontocètes du NOPI.

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PREFACE

In November 2002, 59 000 tons of oil from the Prestige tanker spilled off the coasts of Galicia (North-West Spain) and contaminated a vast coastal area extending from northern Portugal to Brittany (France). The oil spill had a far-reaching impact on all coastal habitats, including intertidal and beach zones, affecting top predators such as marine mammals and seabirds. Marine mammal strandings in Galicia are attended by the CEMMA (*Coordinadora para o Estudio dos Mamiferos Mariños*), which also conducted a monitoring survey of the marine mammals populations. When a marine mammal strands, the CEMMA conducts a necropsy on it to try to determine why the animal has died. As for nearly all NGOs, most of the CEMMA work is carried out thanks to the hundreds of volunteers who participate in its activities. When this oil accident occurred, I was one of those volunteers attending and the assigned candidate to conduct the study of the oil spill impact on the cetacean populations. This study was carried out by analyzing the trace elements concentrations in their tissues in collaboration with the University of La Rochelle, where most of the study was conducted. Here, in La Rochelle is located the CRMM (*Centre de Recherche sur les Mammifères Marins*) which is one of the observatories of the Federation of Research and sustainable development and also the responsible of the strandings network, marine mammal surveys and monitoring in France. The study was carried out within the "*atelier*" of ecological tracers, created in 2006 by researchers from the CRMM and from LIENSs (*Littoral, Environnement et Sociétés*) one of the biology laboratories of the University of La Rochelle. In parallel, a PhD project (conducted by Virginie Lahaye) took place to determine the use of potentially toxic trace elements, mercury (Hg) and cadmium (Cd), as tracers of the foraging ecology of the small cetaceans inhabiting the waters of the Bay of Biscay, these researches ended in 2006. Within this context and following the good results obtained from these two projects the idea to continue with the study of the Iberian cetacean populations through a “multi-tracer approach” emerged. Based on the analysis of several chemical parameters, the aim of this project was to examine ecological segregation on one hand and in another hand to assess the status of contamination of the population, with the north of Portugal included through the collaboration of the SPVS (Sociedade Portuguesa de Vida Salvagem), the association attending strandings and conducting monitoring surveys in Portugal. This idea has finally materialized into a Cotutelle PhD project carried out between two universities: The University of Minho in Braga, Portugal, and the University of La Rochelle in La Rochelle, France. The project did also include collaboration with the University of Aberdeen in Scotland where I did spend three months. The founding came from the Portuguese FCT (Fundaçao para a Ciência e a Tecnologia) for a duration of 4 years, and the biological material from the work carried out by the two stranding networks mentioned above, i.e. CEMMA and SPVS. Prey species were also sampled during two on-board surveys (PELACUS1008 and PELACUS0409) in 2008 and 2009, conducted by the Ifremer (Institut français de recherche pour l’exploitation de la mer) and the IEO (Instituto Español de Oceanografia). Moreover, all the analytical part of the project was undertaken with and thanks to the technical support of several laboratories and its qualified personal:

- *Centre Commun d’Analyses* (CCA) for trace element analyses, which is part of the LIENSs laboratory in France.
- *Plateforme de spectrométrie isotopique* for stable isotope analyses, also part of LIENSs laboratory in France.
- Marine Laboratory of Scotland, for organochlorine analyses, in Scotland.
- *Plateforme de chromatographie* for total lipid content, at the University of La Rochelle in France.
- And the *Instituto de investigaciones Marinas de Vigo*, for age determination, in Spain.

During this PhD project several collaborations were established with scientists working in parallel on the use of ecological tracers as multi-approach to study the trophic webs, especially with a former PhD student Tiphaine Chouvelon. This project began at the same time and focused on the application of this “tracer’s multi-approach” to better understand the structure and functioning of the Bay of Biscay marine ecosystem. This collaboration resulted in several publications in common in scientific journals as well as numerous communications and posters in national and international conferences.

Finally, this 4 years PhD was not only a training in research to me since I also supervised three students in “Marine Biology” and taught a total of 78 hours in “Water Quality” and 20 hours in “IT” (Information Technology).

CHAPTER I

INTRODUCTION

Pra os golfiños
mais golfos das nosas costas
Pra os cabaleiros do mar

ROLE OF MARINE MAMMALS IN MARINE ECOSYSTEMS AND THE IMPORTANCE OF STUDYING THEIR ECOLOGICAL NICHE

The studies on marine mammal ecology often deal with their role in the marine environment, but what does the term “role” mean? In an ecological sense, “role” implies something about the functional significance of a species or other taxon (Bowen 1997). Marine mammals are generally major consumers at top trophic levels in the way that most of them are apex predators. In addition with their large body size and abundance, all marine mammals must influence in some way the ecosystems of which they are a part, for example having a trophic function as top-down regulators of ecosystem functioning (Figure 1.1).

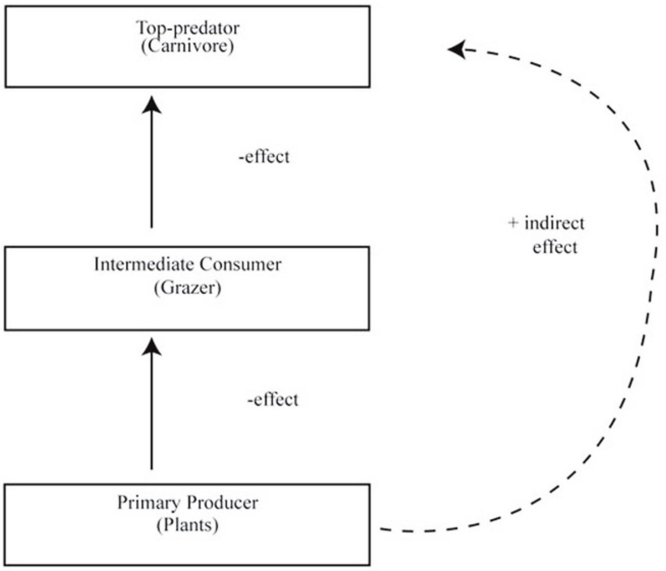

Figure 1.1 Arrows point from prey toward predator. Consumers have a negative effect on the trophic level immediately below them, but a positive effect two trophic levels away by alleviating consumer pressure. Thus, an increase of top predators causes a decrease of intermediate consumers and benefits primary producers. The indirect positive effect on primary producers may also occur if the intermediate consumers reduce their foraging in the presence of top predators (Smee 2010).

Large cetaceans may continue to play an important ecological function even after death through the downward transfer of nutrients to benthic communities (Katona & Whitehead 1988, Kemp et al. 2006), since carcasses may be an important vehicle for the dispersal of deep-sea chemosynthetic communities over large areas (Smith et al. 1989). Therefore, changes in their abundance and/or distribution can directly and/or indirectly have an influence on other species, (i.e. prey species, competitors) and on their interactions, on nutrient storage and recycling and on the communities' structure as in the case of kelp forest food web (Figure 1.2).

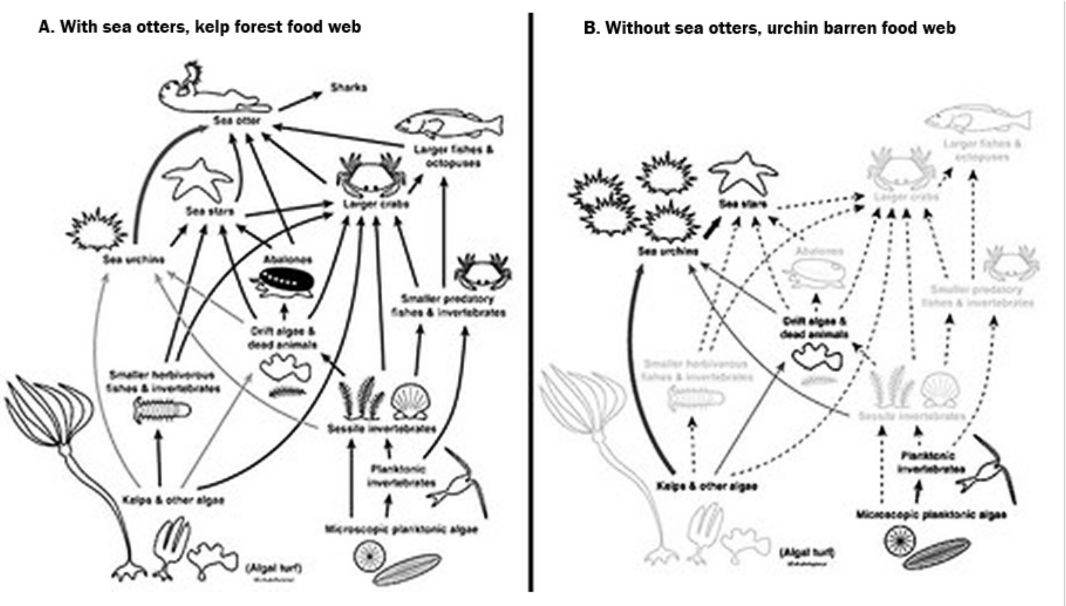

Figure 1.2 Kelp Forest food web.Sea otters can suppress sea urchin populations to such an extent that urchins are unlikely to overgraze the kelp and other members of the community. Such multi-level effects are called "top-down regulation" (Weis & Duffy 2008).

The main threats to marine mammals that can result in the decline of populations and consequently changes in their abundance and/or distribution are the interactions with human activities, which can be largely summarized in four factors: 1) incidental mortality in fishing gear, especially driftnets, 2) reduced availability of prey caused by overfishing and habitat degradation 3) contamination by xenobiotic chemicals resulting in immunosuppression and reproductive impairment and 4) environmental changes such as increased water temperatures affecting ecosystem dynamics (Bowen 1997, Bearzi et al. 2003, 2006). Several other factors affecting marine mammals can be evoked such as ship strikes, underwater noise or hunting, however their importance largely differs between species and areas.

At the same time, marine mammals, as top predators in the food webs, will integrate all the changes that occur in the ecosystems as consequence of the interactions 1) within communities and 2) between the organisms and their environment. They may depend on the productivity of the lower trophic levels and thus on the primary and detritus production. This is especially the case when bottom-up regulations occur as it has been shown in the Bay of Biscay continental shelf food web where bottom-up processes play a significant role in the population dynamics of upper-trophic-levels (Lassale et al. 2011). Additionally, levels of contaminants in certain marine mammals that die and wash ashore might provide a useful indicator of in the contamination status of marine ecosystems (Mangel & Hofman 1999). It is particularly the case of lipophilic pollutants which are bio-magnified in marine food webs. However, robust or declining marine mammal stocks are not necessarily a reliable indicator of healthy or unhealthy ecosystems and they must be considered with a variety of other indicators (Mangel & Hofman 1999). Because the importance of the marine mammals in the ecosystems is not any doubt, several recent ecosystem models, such as Ecopath, have included them as upper-trophic level species (Estes et al. 1998, Springer et al. 2003, De Master et al. 2006, Lassale et al. 2011). Many factors besides trophodynamics can be the source of ecosystem regulation and structuring (e.g. climate shifts). In consequence the relative importance of top-down and bottom-up mechanisms may be scale-dependent and vary by location (Hunt & McKinnell 2006). For that reason and in order to develop efficient marine mammal conservation strategies the ecological role of marine mammals in ecosystems still needs further studies, despite of the difficulties. One of the major reason for this is that in attempting to determine the role of marine mammals three vast disciplinary fields interact: marine ecology, marine mammal biology and fisheries ecology (Katona & Whitehead 1988). In each of these complex fields, research is expensive, manipulative experiments are rarely possible, interactions occur at quite different spatial and temporal scales making measurement of system properties difficult, and there is an inherent indeterminacy in the behaviour of these complex systems which makes simplifying deterministic explanations problematic.

Almost fifty years ago, George Evelyn Hutchinson (1957) formalized the ecological niche as an abstract n-dimensional set of points in a space whose axes represent environmental variables. In 1959 Odum defined the ecological niche as the role of the species (i.e. prey and predators) in the functioning of the ecosystems, where the ecological niche is the profession of species and the habitat their address. In subsequent elaborations, Hutchinson (1978) established a useful distinction between scenopoetic and bionomic niche axes. The bionomic axes are those that define the resources that animals use, whereas the scenopoetic axes are those that set the bioclimatic stage in which a species performs (Hutchinson 1978). After Hutchinson's original formulation, the niche has undergone many changes, but all alternative contemporary definitions retain the formalization of the niche as a multidimensional space.

One important subset of this ecological niche is the foraging niche, which can be described through the detailed study of the prey being part of a predator diet. The foraging niche allows the description of the resources used by predators through three dimensions: 1) the trophic dimension, which includes dietary preferences, trophic position and prey quality 2) spatial dimension composed by feeding habitats where resources are available and 3) temporal dimension of the availability of different resources at different times (from dial activity or different seasons of the year until yearly migratory cycles; Huisman & Weissing 2001).

The study of foraging niche was largely used to help on the understanding of species' ecological role into the ecosystem functioning, showing how these species sharing its resources (Pianka

1974). On the one hand, studying species' ecological niche can help to understand inter-species competition. When two or more species compete for a resource (limited or not) changes in the ecology of one or all the species are expected to occur to reduce competition, following three possible scenarios: 1) the species can co-exist (niche partitioning) 2) one species is excluded from a specific area or habitat (competitive exclusion or specialization; Hardin 1960) and 3) one of the species becomes extinct (Figure 1.3).

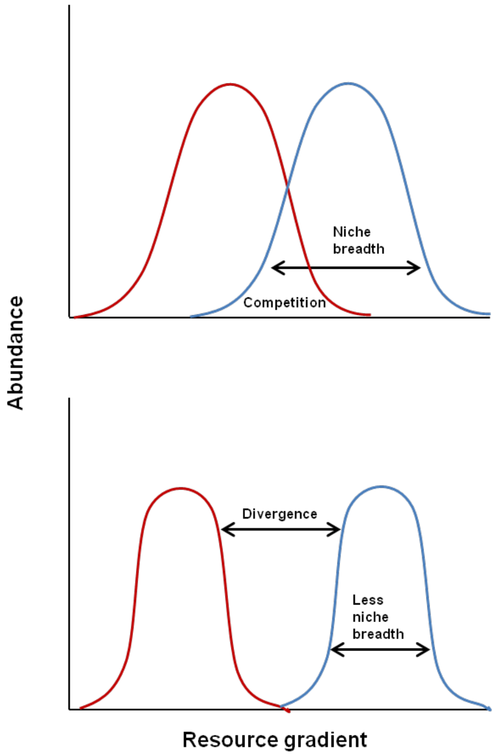

Figure 1.3 Resource partitioning (a) and competitive exclusion or niche specialization (b) caused by inter-species competition (Cunningham & Saigo 1998).

On the other hand, knowing the extent of the niche occupied by a species we can identify its diet plasticity. Thus, a large niche describes a generalist predator while a short niche corresponds to a specialist predator. However, the major difficulty here is the identification of the foraging niche dimensions to allow its fine evaluation. For example, we can consider a species as generalist since it feed on numerous different prey species, but if this species feed on a narrow range of prey sizes distribution and/or if the prey all share a similar composition (in terms of energetic contents for example) showed a more selectiveness; in that case prey size or body composition can be more important descriptors of the diet than the taxonomic composition.

In summary and in this context since scientist used the niche concept to explain the individual or population relations with their environment and consequently their ecological role into the communities, the study of the foraging niche as part of the ecological niche can help in the understanding of the role that marine mammals has into the ecosystems.

THE NORTH WEST OF THE IBERIAN PENINSULA AND ITS TOOTHED WHALES

The North West Iberian Peninsula (NWIP), comprising Galicia (north-west Spain) and northern Portugal is mainly characterized by its relatively narrow shelf, being 20-35 km wide and 100-200 m depth (Figure 1.4), and on the other hand to be under the influence of an upwelling-downwelling seasonal system.

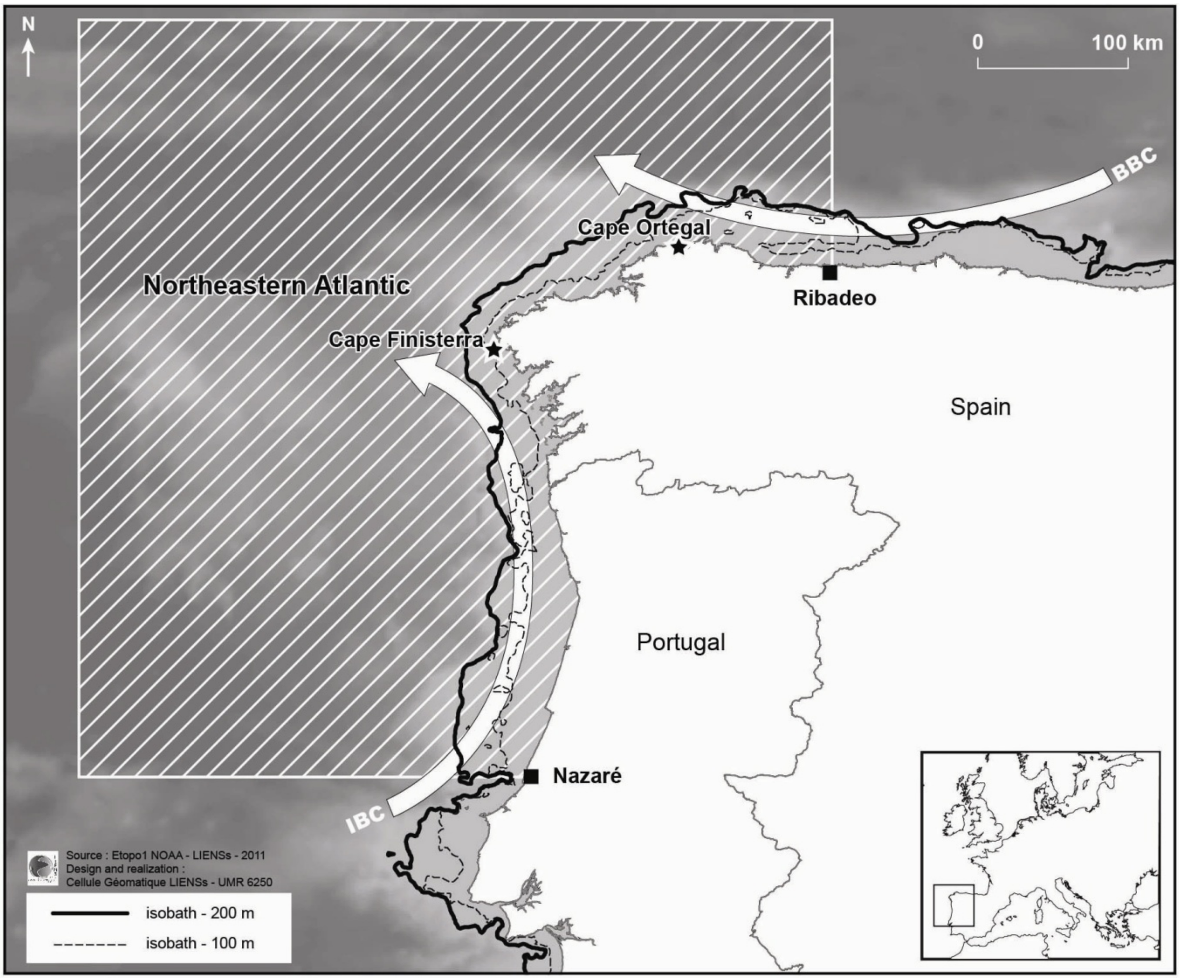

Figure 1.4 Map of the North West Iberian Peninsula (NWIP), with bathymetry in meters (black and dotted lines). The study area is framed in white and full arrows represent the main currents influencing the NW Iberian Peninsula coast. IPC: Iberian Poleward Current and BBC: Bay of Biscay Current.

A poleward flowing slope undercurrent centred at depths of 150–300 m, which is compensating for the equatorward flows in the surface circulation, is a common feature to the coastal upwelling systems off California/Oregon, Peru/Chile, Namibia/Benguela and Canary/Iberian Peninsula (Smith 1989). Specifically, the NWIP is located in the northern limit of the NW Africa upwelling system. Therefore, during summer the Iberian Poleward Current (IPC) is 30-40 km wide and 50-100 m deep and flows southward in the vicinity of the shelf break, being driven by upwelling-favourable northerly winds. It then transports recently upwelled, cold and nutrient-rich Eastern North Atlantic Central Waters of subpolar origin (>45°N; ENACW). In contrast, between September-October until March-April the surface circulation reverses becoming the well-defined Portugal Coastal Counter Current (PCCC) that is 30 km wide and 200 m deep flowing off the NW Iberian Peninsula, and results in downwelling phenomena (Fraga 1981, Fiúza 1984, Ambar & Fiúza 1994, Figueiras et al. 2002). However, this characteristic pattern of upwelling-downwelling dynamics can vary considerably from one year to another: the spring transition (from downwelling to upwelling) can occur from February to April, while the autumn transition shows less variability and generally takes place around October (Figueiras et al. 2002). As an example the downwelling to upwelling dynamic transition in the west coast of Portugal is illustrated in Figure 1.5 through the upwelling index and temperature measured during one year. The upwelling index is considered to be an indicator of the amount of water upwelled from the base of the Ekman layer. Positive values are, in general, the result of equatorward wind stress. Negative values imply downwelling, the onshore advection of surface waters accompanied by a downward displacement of water (http://www.nwfsc.noaa.gov). Thus, according to these two factors, the upwelling period appears to be between April and September.

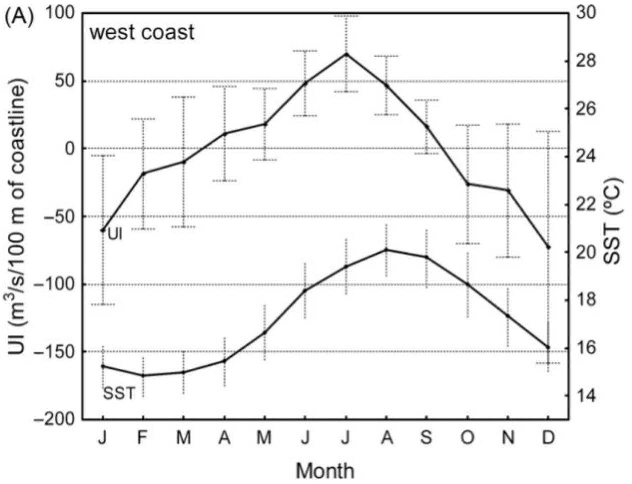

Figure 1.5 Mean monthly sea surface temperature (SST) and upwelling index (UI) over the continental shelf on the west coast of Portugal. Vertical bars denote standard deviation (Moreno et al. 2009).

The whole NW Iberian Peninsula coast is affected to a greater or lesser extent by this phenomenon that becomes strongest and more persistent in the area between Cape Ortegal and Cape Fisterra (i.e. in the north of Galicia; Blanton et al. 1984). However the northern part until the border with Asturias is especially under the influence of the Bay of Biscay Current (BBC; Figure 1.4).

The main effect of upwelling is the increase in primary production that can be multiplied by ten, reaching values up to 300g C/m^2/year (González et al. 1984), which results in considerable biodiversity in this area. More than 300 species of fish (Solórzano et al. 1988), over 75 species of cephalopods (Guerra 1992) and at least 20 marine mammal species (16 cetaceans and 4 pinnipeds) have been reported to inhabit this area (Penas-Patiño & Piñeiro-Seage 1989, Fernández de la Cigoña 1990, Pierce et al. 2010). The high biodiversity and productivity also results in intense fishing activities which are among the most important in the world. Specifically, hake (*Merluccius merluccius*), scad (*Trachurus trachurus*) and blue whiting (*Micromesistius poutassou*) are targeted by fisheries commercial value. In inshore waters, small boats target mainly molluscs and crustaceans. The offshore demersal fishery targets hake, blue whiting, scad, megrim (*Lepidorhombus whiffiagonis*), norway lobster (*Nephrops norvegicus*) and monkfish (*Lophius piscatorius*) amongst other species while purse seiners target sardine (*Sardina pilchardus*), scad and mackerel (*Scomber scombrus*). In addition, retired fishermen operate and use a variety of fishing gears, but the activity of this sector is difficult to quantify although being largely confined to the coastal zone (López et al. 2002).

The presence of small cetaceans in these waters is well known since the XVI century (Sarmiento 1757, Cabrera 1914, Penas-Patiño & Piñeiro-Seage 1989, Fernández de la Cigoña 1990) and 20 marine mammal species (16 cetaceans and 4 pinnipeds) were recorded in the area previously to 1998. Small cetaceans belong to the suborder of the toothed whales (i.e. characterized by the presence of teeth rather than the baleen of other whales) though this suborder also includes large cetaceans as for example sperm whales, beaked whales, among others. In the NWIP, the most frequently recorded species were bottlenose dolphin (*Tursiops truncatus*), common dolphin (*Delphinus delphis*), harbour porpoise (*Phocoena phocoena*), Risso's dolphin (*Grampus griseus*), long-finned pilot whale (*Globicephala melas*) and striped dolphin (*Stenella coeruleoalba*) (Cendrero 1993, López et al. 2002, 2004, Pierce et al. 2010) (Figure 1.6).

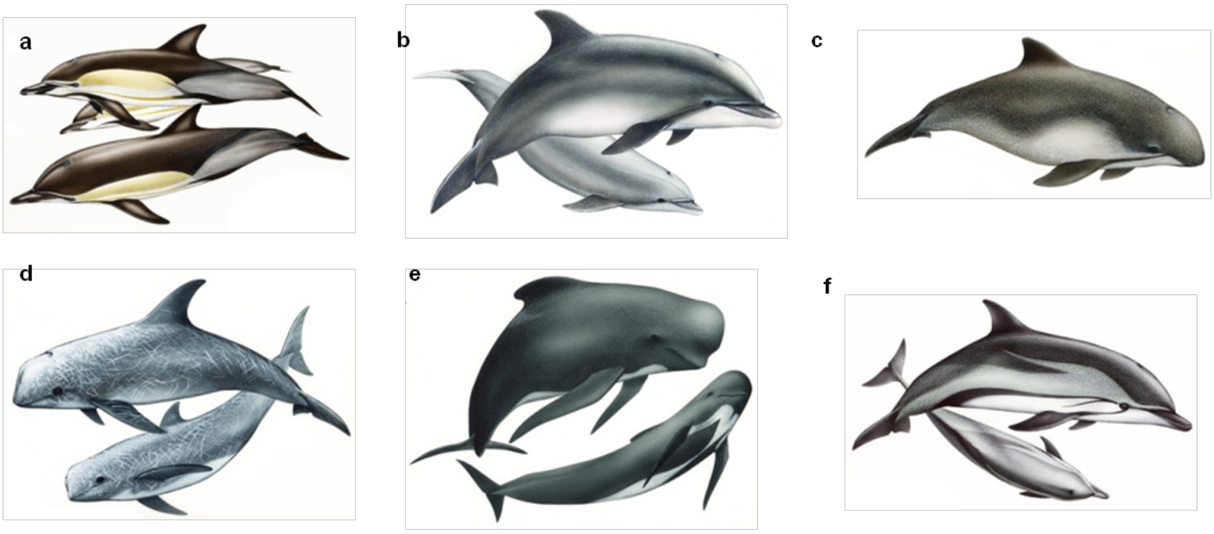

Figure 1.6 The most common toothed whale species from the NWIP. Common dolphin *Delphinus delphis* (a) bottlenose dolphin *Tursiops truncatus* (b) harbour porpoise *Phocoena phocoena* (c) Risso's dolphin *Grampus griseus* (d) long-finned pilot whale *Globicephala melas* (e) and striped dolphin *Stenella coeruleoalba* (f) (http://www.cms.int).

These species showed different distribution patterns along the NWIP coast. In terms of habitat preferences, bottlenose dolphins were seen to be associated with more productive areas (areas with higher chlorophyll-a concentrations) where the continental shelf was wider and in less extent in oceanic waters. On the contrary, common dolphins and harbour porpoises were seen most frequently in less productive areas where the continental shelf is narrowest. Common dolphin is also frequent over the 200 m depth and harbour porpoise despite its coastal preferences is also sighted where the bathymetry reaches the 1000 m depth (López et al. 2004, Cabral et al. 2005, Pierce et al. 2010, Vingada et al. 2011). Pilot whale is essentially oceanic and is known to dive up to 600 m; however it is known to forage occasionally in more coastal waters, around 50-100 me depth, since coastal prey species appear in its diet (González et al. 1994, Cabral et al. 2005, Pierce et al. 2007, 2010, Vingada et al. 2011). Risso's and striped dolphins are frequently sighted but are not abundant in the area. Striped dolphin is mainly sighted over the continental shelf, in depths of 100-1000 m (López 2003, Cabral et al. 2005, Spyrakos et al. 2011, Vingada et al. 2011), however Risso's dolphins is more commonly sighted near the coast (Pierce et al. 2010). Possible reasons for these differences in habitat use probably include different diets among the species.

Three large-scale sighting surveys recently studied the distribution and abundance of cetaceans in Europe. The first two campaigns focused on the abundance estimation of small cetaceans in European Atlantic and North Sea (Small Cetaceans Abundance in European Atlantic and North Sea, SCANS I & II) were conducted in 1994 and again in 2005 (Hammond et al. 2002, Hammond 2006). These surveys took place in European coastal waters. A third campaign was conducted in 2007 in oceanic waters to complete the SCANS campaigns by evaluating the distribution and abundance of cetaceans in offshore European Atlantic waters (Cetacean Offshore Distribution and Abundance in European Atlantic Waters, CODA) (CODA Final Report 2009). The majority of the regions of SCANS campaigns were surveyed by ship, but some coastal regions were surveyed by aircraft. However, for logistical reasons, CODA survey was only conducted by ship. The first SCANS campaign surveyed waters of the North Sea, English Channel, Celtic Sea and the Baltic Sea. SCANS-II was extended to the Gulf of Biscay and the North West of the Iberian Peninsula. These surveys were divided in several blocks, which are shown in Figure 1.7. The NWIP is included in blocks W and 3 for SCANS-II and CODA, respectively.

The interior waters of the NWIP (i.e. estuary and Rías) and continental shelf were also surveyed from continuous land and ship monitoring surveys carried out by CEMMA (Coordinadora para o Estudo dos Mamiferos Mariños) in Spain and SPVS (Sociedade Portuguesa de Vida Salvagem) in Portugal since 1990 and 2000, respectively. Both NGOs profit from European-funded national projects (Sustainable local fisheries and promotion of a safe sea for cetaceans, SAFESEA 2009-2011; Inventario y designación de la Red Natura 2000 en Áreas marinas del Estado Español, LIFE + INDEMARES 2009-2013; Conservation of Marine Protected species on Mainland Portugal, LIFE MARPRO 2012-2016) which allow to prolong these coastal surveys.

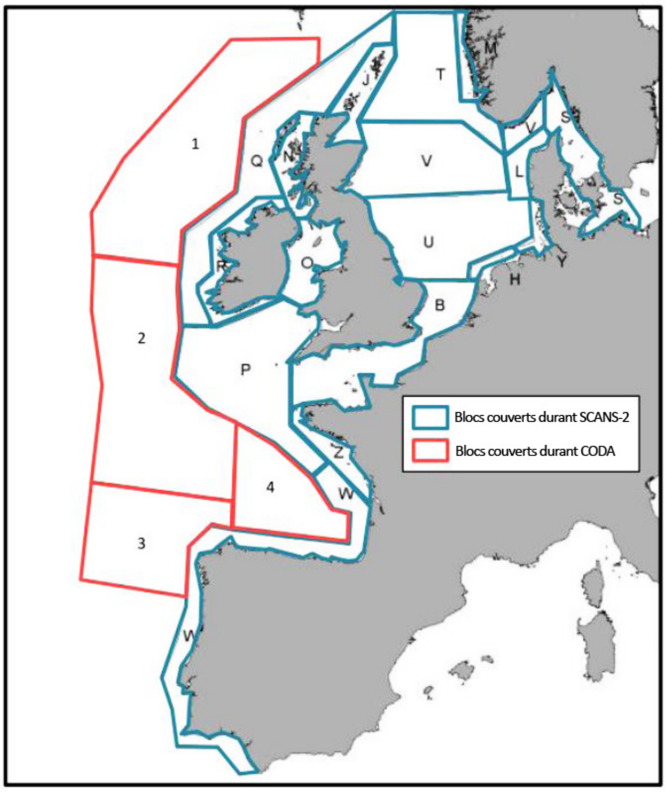

Figure 1.7 Survey region divided into the survey blocks (Hammond 2006, CODA Final Report 2009).

Data resulting from these European surveys and from local monitoring revealed that the most abundant species in the area is the common dolphin, followed by striped dolphin in oceanic waters and bottlenose dolphin in coastal waters. The other three species are less abundant, and there were not enough data to estimate the abundance of Risso's dolphin in the area despite the sightings of this species (see Table 1.1 for details).

Table 1.1 Estimates of abundance for the most common cetacean species seen during European and local shipboard surveys in the NWIP area. Coefficients of variation are given in parentheses. Figures in square brackets are 95% confidence intervals (López et al. 2004, Hammond 2006, CODA Final Report 2009, Vingada et al. 2011)

	Animal abundance			
Species	**SCANS-II**	**CODA**	**CEMMA**	**SAFESEA**
Common Dolphin *Delphinus delphis*	17 916 (0.22)	12 378 (1.23)	8 137 [4 388-13 678]	± 20 500[b]
Bottlenose dolphin *Tursiops truncatus*	3 935 (0.38)	876 (0.82)	664 [251-1 226]	nd
Harbour porpoise *Phocoena phocoena*	2 646 (0.80)	nd	< 300[a]	nd
Risso's dolphin *Grampus griseus*	nd	nd	nd	nd
Long-finned pilot whale *Globicephala melas*	nd	194 (0.88)	385 [13-1 131]	nd
Striped dolphin *Stenella coeruleoalba*	nd	7 546 (0.62)	nd	nd

nd = not determined data.
[a] Not published data from López 2003a
[b] Data recovered by ship and aircraft from coastline to 50 miles

The rate of stranding in the NWIP is one of the highest recorded in Europe, with 1.4 individuals' stranded each 10 km of coastline per year (González 1999, Sequeira et al. 1999, López et al. 2002). Common dolphin is largely the most frequently stranded species followed by bottlenose dolphin and harbour porpoise. Detailed data of stranding percentage per year are issued from published data from Galicia between 1990 and 1999 (see Figure 1.8; López et al. 2002).

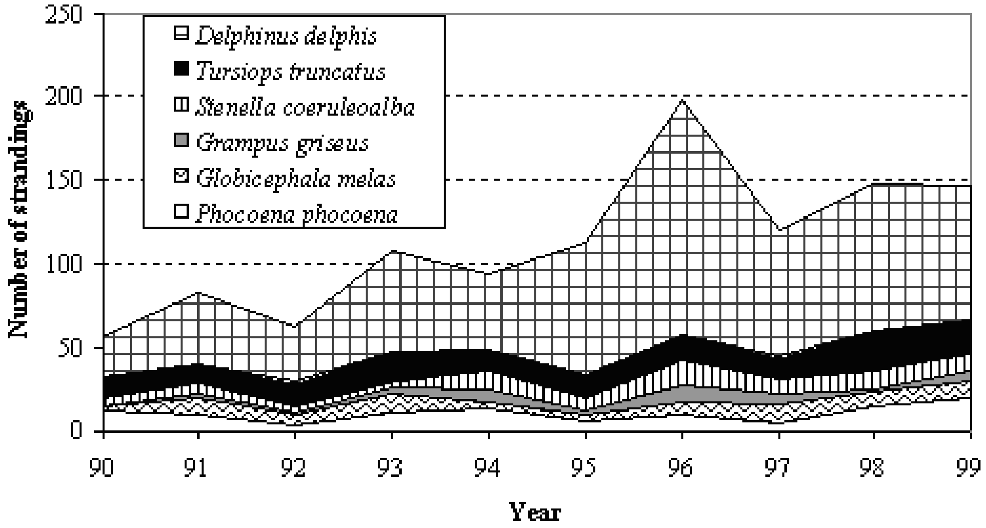

Figure 1.8 Year to year trends of the most commonly recorded species in reported strandings on the Galician coast between 1990-1999 (López et al. 2002).

The stranded peaks for almost all the species take place in winter and autumn. In winter the predominant winds from the south and southwest would drift carcasses towards the coast. Similarly, the reverse direction of winds in spring and summer, associated with upwelling, may lead to a decrease in the number of carcases reaching the shore (Figure 1.9). In autumn, as in winter, storms are more frequent and this could also influence stranding frequency, both by causing the death of animals in poor condition and by carrying dead animals towards the coast (Cendrero 1993).

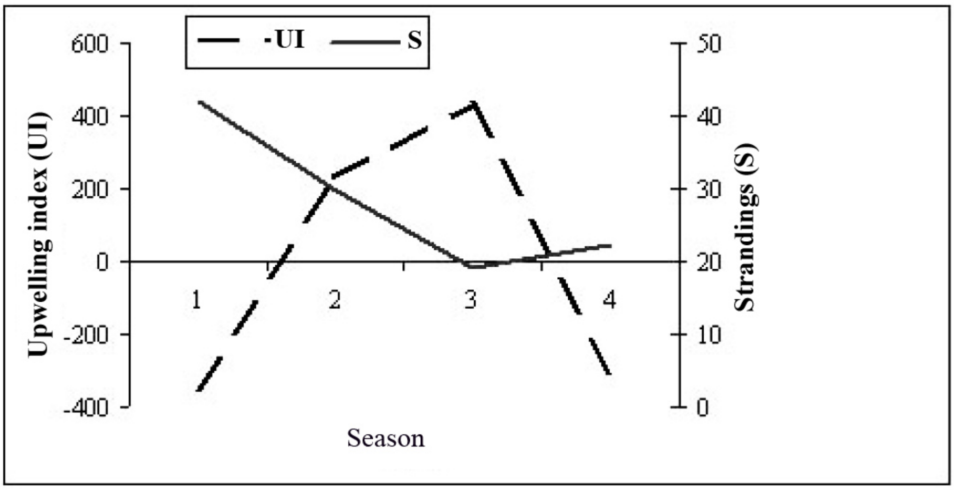

Figure 1.9 The seasonal pattern of the upwelling index, based on geostrophic winds, and annual average number of strandings of the main cetacean species, for the season, between 1990 and 1999. 1 = winter, 2 = spring, 3 = summer and 4 = autumn (López et al. 2002).

In one of the most important fishing areas in the world, it is also not surprising that by-catch records are high for cetacean species. The highest by-catch rates were estimated for gillnets, purse seine and offshore trawling (López et al. 2003, Vingada et al. 2011). Despite the species affected are related to the type of fishing nets, the majority of by-caught species are small dolphins in all the areas with common dolphins firstly but also harbour porpoise and bottlenose dolphin. In Galicia Risso's dolphins is also highly by-caught, fisheries estimated to be responsible of 29% of the population mortality. Conversely, the lowest proportion of by-catch mortality was estimated for striped dolphins (López et al. 2002, Vingada et al. 2011). It should be noted that even if the data presented Table 1.2 may seem low, the number of by-catches increases each year as a consequence of the increasing field effort and practical experienced personnel (López et al. 2002).

Table 1.2 Total numbers and percentages of proven and diagnosed by-catches recorded for the main cetacean species in Galicia (López et al. 2002).

	Proven by-catch		Diagnosed by-catch		Total by-catch	
Species	N	%	N	%	N	%
Common dolphin *Delphinus delphis*	22	3.3	133	19.8	155	23.0
Bottlenose dolphin *Tursiops truncatus*	5	3.2	17	11.0	22	14.3
Harbour porpoise *Phocoena phocoena*	4	3.9	19	18.4	23	22.3
Rissos's dolphin *Grampus griseus*	1	2.4	11	26.2	12	28.6
Long-finned pilot whale *Globicephala melas*	4	5.3	8	10.7	12	16.0
Striped dolphin *Stenella coeruleoalba*	0	0.0	4	4.9	4	4.9

As an example, the minimum estimates for population abundance of common dolphins and bottlenose dolphins in Galician waters were 8 140 and 660 respectively (see Table 1.1). Nevertheless, applying a precautionary approach it would be reasonable to specify maximum by-catch removal rates of 2% of these figures, i.e. 160 and 13 animals respectively. The data presented above suggest that by-catch mortality substantially exceeds these figures (López et al. 2003; Table 1.2).

Despite the narrowness of the continental shelf, the oceanographic features of the NWIP waters, allow supporting a highly productive ecosystem and studies carried out in the area demonstrate the importance of this region as an habitat for marine mammals. Moreover, the great pressure exerted on marine mammals through fishery activities emphasizes the need for a better knowledge of these species ecology in the area in order to plan and manage their conservation both at local and European levels as well as an extensive and long-term by-catch monitoring programme, using on-board observers to improve quantification of by-catch mortality and some kind of reduction measures.

CONSERVATIONAL STATUS OF THE NWIP TOOTHED WHALES

Conservation issues for cetaceans in the NWIP waters, and especially for toothed whales, include interactions with fisheries (as discussed above), which may be a significant cause of mortality (López et al. 2002, 2003), but also overfishing and oil spills. Vieites et al. (2004) identified the European Atlantic, especially the English Channel and Galician coast, as a major hotspot for oil spills although it should be noted that no evidence of effects of the 'Erika' oil spill have been found on marine mammals along the French coast (Ridoux et al. 2004). This is also an area with heavy boat traffic around ports, which can have an effect on the activity patterns and distribution of these species. Thus many studies pointed up the potentially harmful effects of disturbance by boats on bottlenose dolphins, common dolphins and Risso's dolphins (e.g. Nowacek et al. 2001, Hastie et al. 2003, Mattson et al. 2005, Bejder et al. 2006, Stockin et al. 2008) although some examples do exist showing that in some cases, some species as bottlenose dolphins can apparently become habituated to boat traffic (e.g. Sini et al. 2005).

Cetaceans are strictly protected by Annex IV of the Habitat directive (92/43/EEC) in the European territory and their presence can conduct to the designation of Special Areas of Conservation (SAC) (Annex II). The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the Bern convention on the conservation of European Wildlife and Natural Habitats also include cetacean species in need of specific management measures against their trade and for their conservation. In addition, international agreements were introduced for the conservation and management of these species within Bonn convention (i.e. Convention on Migratory Species, CMS, 1979; Agreement on the Conservation of Small Cetaceans of the Baltic, North East Atlantic, Irish and North Seas, ASCOBANS, 1994; Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and

Contiguous Atlantic Area, ACCOBAMS, 2001). Table 1.3 summarizes the annexes included in each of these treated.

At the national and regional levels (i.e. within the NWIP territory), some of the toothed whale species are considered endangered or threatened species and are therefore listed in the Spanish catalogue of threatened Species (Law 4/1989), as vulnerable species, in the Law of Natural Heritage and Biodiversity (Law 42/2007), which requires Spanish regional governments to design Special Areas of Conservation to preserve or restore the natural habitat of these species. Species are also listed in the Galician Catalogue of Threatened Species (Decreto 88/2007), as vulnerable species that needs a management plan, and in the Red book of Portuguese vertebrates (1993). In addition, other pieces of the Spanish and Portuguese legislation (Real Decreto n. 1727/2007 and n. 263/81, respectively) were also approved to minimize the potential negative effects of whale watching in cetacean populations and to forbid the intentional capture, transport and death of these animals. However, and despite the existing legal framework for the protection of these species, no official conservation plans exist in the NWIP yet. Table 1.4 details the conservational status of the most common toothed whales inhabiting the NWIP waters, from the international to the regional level.

Table 1.3 Summary of the annexes to the international legal framework including cetaceans as species to be protected (http://www.cms.int/; http://www.cites.org; http://ec.europa.eu/environment/nature/legislation/habitatsdirective/index_en.htm; http://www.coe.int).

	INTERNATIONAL LEGAL FRAMEWORK			
	Bonn	**CITES**	**Bern**	**Habitat Directive**
ANNEXES	**I:** Endangered migratory species	**I:** endangered species (trade only permitted in exceptional circumstances)	**I:** Strictly protected flora species **II:** Strictly protected fauna species **III:** Protected fauna species	**I:** lists the habitats of community interest (priority or not) areas of conservation whose conservation requires the designation of Special **II, IV** and **V:** establish the lists of species which must apply specific rules **III:** defines the criteria for evaluating the opportunity to join a site in Natura 2000, of conservation.by its classification as a special Area
	II: Migratory species conserved trhough agreements	**II:** vulnerable species (trade strictly regulated)	**IV:** Prohibited means and methods of killing capture and other exploitation	**VI:** establishes prohibited means and methods of killing, capture and other exploitation

Table 1.4 Summary of the key agreements and conventions on the conservation of NWIP toothed whales

				Legal framework						
Specie	**Category**			**International level**				**National and regional level**		
	Portugal	**Spain**	**IUCN**	**Bern**	**Bonn**	**CITES**	**Habitat directive**	**RBPV**	**SCTS**	**GCTS**
Common dolphin *Delphinus delphis*	LC	K	LC	II	I[1] and II	II	IV	NT	VU[2]	NC
Harbour porpoise *Phocoena phocoena*	VU	V	VU	II	–	II	IV and II	I	VU	VU
Bottlenose dolphin *Tursiops truncatus*	LC	K	LC	II	–	II	IV and II	NT	VU	VU
Striped dolphin *Stenella coeruleoalba*	LC	K	LC	II	–	II	IV	NT	NC	NC
Long-finned pilot whale *Globicephala melas*	DD	K	LC	II	II	II	IV	NT	VU[1]	NC
Risso's dolphin *Grampus griseus*	DD	NT	DD	II	II	II	IV	NT	NC	NC

[1] MEDITERRANEAN POPULATION; [2] ATLANTIC POPULATION

Category acronym: LC = Least concerned, VU = Vulnerable, DD = Data deficient, K = Insufficiently known, NT = Not threatened, NC = Not considered

RBPV = Red book of Portuguese vertebrates, SCTS = Spanish catalog of threatened species, GCTS = Galician catalog of threatened species

CHEMICAL PARAMETERS: ONE TOOL FOR TWO PERSPECTIVES IN THE STUDY OF MARINE MAMMALS' ECOLOGY

This introduction part was initiated with the important role that marine mammals can have in the marine ecosystems. For this reason, assessing the marine mammals' ecology, i.e. apex predators' ecology, is important in the understanding of marine food webs and monitoring the health status of the ecosystems. To this aim specific information on diet, habitat use, foraging habits, trophic position or contamination status of these species is required. Traditional methods or analyses have known biases and limitations when applied to marine predators. For example, in the study of diet, observational data are typically limited to predation occurring at or near the ocean surface during spring and summer months and reveal little about the foraging habits of these animals below the surface or at other times of the year. In addition, stomach content analyses and feces studies may be biased in favor of species having large masses of durable hard parts due to differential rates of digestion (e.g. cephalopod beaks, fish otoliths or crustaceans rostrum; Pierce & Boyle 1991, Tollit et al. 1997). Furthermore, these methods provide only a snapshot of the most recent meal and may therefore not be representative of the typical diet in the long-term unless analyses are carried out on numerous individuals on a long time period. Moreover they reflect the food ingested but not assimilated by the organisms.

These known biases have created a need to develop and employ "alternative methods", as indirect chemical analysis techniques that may more accurately reflect the diet over different time-scales and that can be obtained from stranded animals but also from biopsy sampling of free-ranging animals. These alternative methods rely on the fact that predators integrate the chemical elements and/or compounds from their prey and that these chemicals may constitute signatures reflecting both the species consumed and the regions from which the prey where taken.

Among the alternative methods that assess several aspect of the marine mammals' ecology (e.g. dietary preferences, trophic position, habitat used, foraging habit) the most widely used analyses are: lipids, especially fatty acids, stable isotopes, especially carbon and nitrogen, trace elements (including essential and non-essential elements) and in some cases organochlorine contaminants. Most of the times they were used as multi-approach analyses, i.e. analyses of several chemical parameters in the same individuals, which gave a more complete and reliable information. As an example, Borrell et al. (2006) used stable isotopes together with PCBs and DDTs to investigate the population structure of bottlenose dolphins around the Iberian Peninsula. Differences in $\delta^{13}C$ and in PCB congener profiles indicated that dolphins from the Atlantic and the Mediterranean did not intermingle. However, in the Mediterranean Sea, dolphins from Catalonia and Valencia were indistinguishable, suggesting a common distribution area. In the same way, works carried out on eastern North Pacific killer whales (*Orcinus orca*) differentiated two types of killer whales, "residents" and "transients", by their genetic (Hoelzel et al. 1998), acoustic (Barret-Lennard et al. 1996), morphological (Ford et al. 2000) and feeding ecological (Ford et al. 1998) differences. A third type has been proposed as "offshore" based on their distribution in outer coastal waters between California and Alaska (Krahn et al. 2007). Therefore, in order to provide further information on this third group, their long-term diet has been investigated through the analysis of stable isotope ratios of carbon and nitrogen, persistent organic pollutants and fatty acids in biopsy samples (Herman et al. 2005, Krahn et al. 2007). The results provided evidence that the offshore individuals belonged to a third killer whale ecotype, which was again consistent with the fact that they had a different mtDNA haplotype from those of resident and transient whales (Barrett-Lennard 2000).

Fatty acids are the main constituent of most lipids, and unlike other nutrients, such as proteins that are readily broken down during digestion, fatty acids are released from ingested lipid molecules (e.g. triacylglycerols) but are not degraded (Iverson et al. 2004). Once taken up by tissues, fatty acids are either used for energy or re-esterified, primarily to triacylglycerols, and stored in adipose tissue. The essential fatty acid (but not all of them) cannot be synthesized by top predators and have to be ingested through food to satisfy the physiological needs of these organisms. In some cases and although some metabolism of fatty acids occurs within the predator, such that the composition of predator tissue will not exactly match that of their prey, fatty acids can be deposited in adipose tissue with little modification and in a predictable way (Iverson et al. 2004). Consequently they are frequently used as "proxy" of diet in marine environment, thereby providing qualitative and/or quantitative information of predator's diet (Iverson et al. 2004, Budge et al. 2006).

In the ecosystems, the natural elements present several isotopic forms. These stable (but also radioactive) isotopes of a given element differ in their atomic mass and behave differently facing to physical, chemical and biological processes. The lighter isotopes of chemical elements tend to form weaker ties and to react faster than heavy isotopes: this processes correspond to the isotopic fractionation (Peterson & Fry 1987, Fry 2006). Then biological tissues gain a self-isotopic composition. Considering the major component of the biological tissues, the predators' isotopic ratios of carbon and nitrogen basically depend: on the primary producers of the ecosystem and on the ^{13}C and ^{15}N enrichment between resource (i.e. prey) and predator, called "Trophic Enrichment Factor" (Post 2002), which is usually lower for carbon (< 1‰) than for nitrogen (3.4‰, average). The carbon isotope ratios of consumers are mostly used as "proxy" of feeding habitats reflecting the primary producers of food webs (France 1995, Hobson 1999). The nitrogen isotope ratios were mostly used as "proxy" of trophic position of species within food webs (Hobson & Welch 1992, Lesage et al. 2001). However, nitrogen isotopic ratio has recently been shown that in some contrasted ecosystem, it may be revisited also as an indicator of the feeding area (Chouvelon et al. in press).

Trace elements are released in the environment from both natural and anthropogenic sources (e.g. Zn ores for Cd, volcanism and waste incineration for Hg). They reach the ocean through river inputs and atmospheric depositions (Cossa et al. 1990*)*. Trophic transfer is then the main pathway for the intake of trace elements, but it depends on their speciation or physicochemical form in the environment that determine their availability for the organisms (Gray 2002). Trace element concentrations in an organism or in a tissue are the result of different processes of toxicodynamic and toxicocinetic including exposure, ingestion and assimilation, storage and elimination depending on the existence of detoxification processes and homeostasis regulation in the organisms (Chappuis 1991, Underwood 1977). The essential trace elements generally do not bioaccumulate to large extent in organisms since they are submitted to homeostasis regulation. The non-essential trace elements (i.e. Cd, Hg, Pb), for which no biological function has been shown in mammals at least, tend to bioaccumulate with age in the individuals (Chappuis 1991). Moreover, Hg is the only one for which biomagnification was demonstrated in the food webs, leading to greater concentrations in higher trophic level consumers (Cossa et al. 1990, Monteiro et al. 1996, Thompson et al. 1998). Since these trace elements are natural substances and have been present on the earth since its formation and since they may be released in the environment through anthropogenic activities (such as mining) or natural events (such as volcanism), trace elements and especially the non essential ones (not submitted to homeostasis) may also be potential "proxies" of the habitat or of the feeding zone of the consumers.

Persistent organic pollutants (POPs) have an exclusively anthropogenic origin*,* manufactured and spread by industry and agriculture in the environment. They are characterized by a great level of volatilization and a high physico-chemical stability leading them being transported on long distances achieving far areas from their origin source by the global distillation processes towards the Arctic and Antarctic environments (Jones & De Voogt 1999). Moreover, POPs are extremely lypophilic and tend to be efficiently accumulated in adipose tissues of the organisms.

In aquatic mammals, they enter the body almost exclusively *via* the food. However, tissue concentrations vary in relation to exposure (reflected by environmental levels, and the lipid content of prey eaten), elimination in faeces and enzymatic transformations. Since they biomagnify through food webs, the trophic level of the predator will also contribute to determine its POPs concentrations. Knowing the metabolism capacity of the individuals or species these chemical compounds may also be used as "proxy of diet preferences", but they are often coupled with other tracers (e.g. stable isotope analyses of carbon and nitrogen and/or fatty acids) in a multi-tracer approach to address an environmental issue (see previous examples).

However, the interpretation of the concentrations or ratios in predators' tissues is difficult and this is probably the main limitation to the use of these chemical forms as tracers. While all of them may give information on the habitat and environment of the individual predator trough its diet, their concentrations are highly influenced by other factors, such as their bioavailability (partly determined by the physico-chemical forms of the tracers), spatio-temporal variations in the food webs, or biological factors such as sex, species or even those linked to ontogeny (age, growth, dietary shifts) (Aguilar 1987, Vanderklift & Ponsard 2003), metabolism and physiology of the species and individuals, which must be carefully considered. In this respect, fatty acids stored in the blubber depend on the diet for some of them (having been transferred from phytoplankton through the food chain), but are synthesised by the animal itself for others. As a result it can be complex to relate the signature found in the predator to those in its prey. This is all the more complex when predators are opportunistic and exhibit numerous different prey in their diet, as it is often the case in temperate waters. Stable isotope signatures provide information on diet and also provenance of feeding (Hobson 1999). Carbon isotope ratio mainly records the signature of the primary producers and depends on the effect of environmental effect on the photosynthesis, the signature being very similar within the animals belonging to the same local food web whatever their trophic level is. On the contrary nitrogen isotope ratio is a better record of feeding area and the trophic level of the organism, but also depends on regional changes in ecosystems. Trace elements may constitute geochemical signatures or reflect dietary preferences (Caurant et al. 1994, 2009, Bustamante et al. 2004). However, their concentrations in mammals such as small cetaceans are highly influenced by the toxicodynamic (redistribution amongst tissues) and toxicokinetic (bioaccumulation *vs* elimination) of the elements. Moreover, the bioaccumulation implies the need to take into account biological factors (e.g. age and sex of the individuals) to undertake a relevant comparison among different groups or populations. Finally, POPs also reflect dietary preferences; however the biological factors, as for trace elements, have an important effect in their accumulation together with the different metabolic capacities revealed by the species or individuals, which will determine the tissue concentrations and patterns.

In this context, and considering all the advantages but also limitations offered by these "alternative" and relatively new approach, two main perspectives were proposed in the study of marine mammals' ecology through the use of trace elements and POPs analyses (in addition to the C and N stable isotopes) (Figure 1.10):

1) As **<u>ecological tracers</u>** of populations (or parts of the populations) by drawing an image of the organism's diet and habitat at different time scales into the ecosystems. Thus, according to the chemical parameter and the considered tissue, the period of integration represented by the specific signature will be different depending on the half-life of the element and the turnover of the tissue (Caurant et al. 2009). Trace elements and C and N isotopes analyses will thus allow determining the feeding habits and/or of the habitat exploited by the animals. They may also allow evaluating the degree of foraging segregation *versus* overlapping within and between populations and/or species (e.g. sympatric species) at different time scales.

2) As an evaluation of the **<u>contamination status</u>** of the species. The analyses of chemical contaminants (i.e. non-essential trace elements and POPs) allow determining baseline levels for the NWIP marine mammals and thus the identification of the most vulnerable species. Some of them can also be used as indicators of the eutrophication status of the ecosystems (e.g. through the nitrogen stable isotopes; McClelland et al. 1997). And since marine mammals are apex predators occupying the highest trophic positions into the food webs, they could be used as indicators of the health status of the ecosystems they belong to.

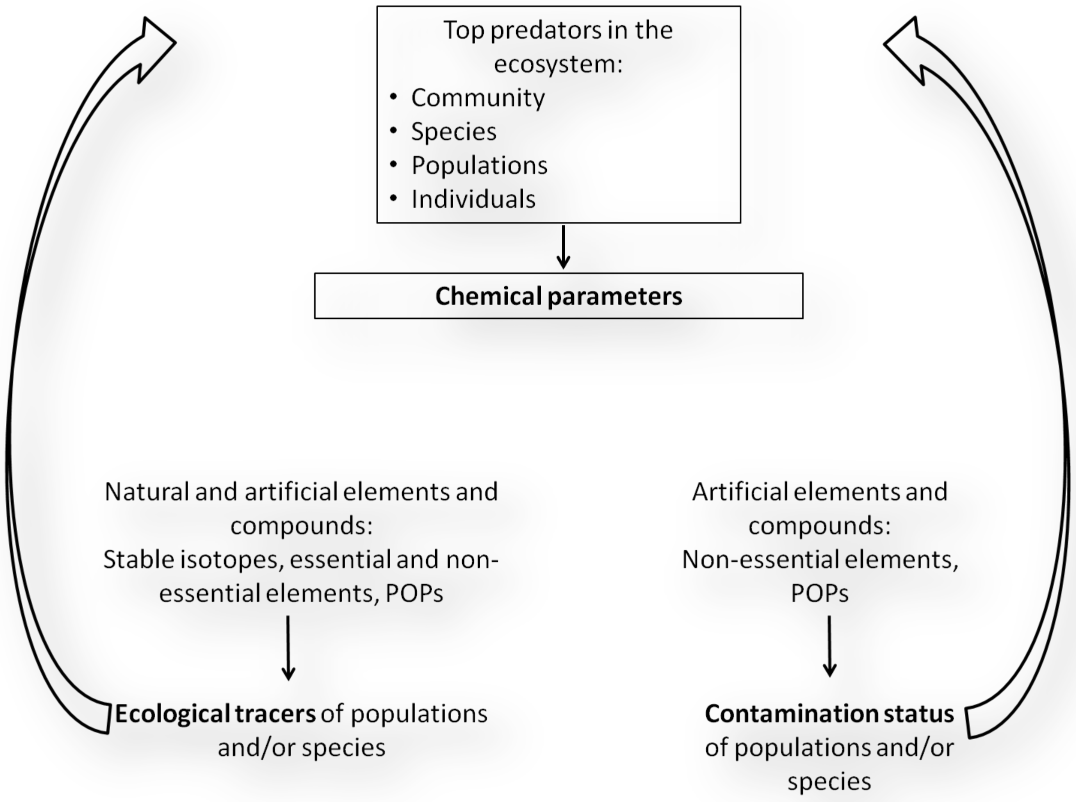

Figure 1.10 Scheme of the two perspectives applied in the marine mammals' ecology through chemical parameter analyses.

THESIS OBJECTIVES

Oceanographic features encountered along the coasts of the Iberian Peninsula appear to be favorable to support many species, leading to a very productive system. Among apex predators, sixteen species of cetaceans have been recorded. However the shelf width is only 20-35 km and depth only 100-200 m. This restricted area supporting many cetacean species raise the first question of the thesis: **Is there inter-specific ecological niche segregation or a large overlapping among the sympatric species inhabiting these waters (Objective 1)? And if segregation does exist, what is the degree of it (Objective 2)?** Moreover human activities are also important in the area; some of them (as fisheries) having a high impact on marine mammals' populations. And while almost all the European coasts display data on the levels of contamination in small cetaceans very few studies were carried out in this part of Europe despite the number of samples available. Thus the third objective was to evaluate **the contamination status of these populations and to determine the potentially most vulnerable species (Objective 3).**

To this aim, the study focused on five of the most common toothed whales in the NWIP, which were stranded and by-caught. Consequently, these species are the best represented in terms of samples available to conduct chemical analyses (López et al. 2004, Cabral et al. 2005, Pierce et al. 2010). They are: the common dolphin (*Delphinus delphis*), the harbour porpoise (*Phocoena phocoena*), the bottlenose dolphin (*Tursiops truncatus*), the striped dolphin (*Stenella coeruleoalaba*) and the long-finned pilot whale (*Globicephala melas*).

In addition to these three main objectives, several specific objectives have arised:

Objective 4: To study the trophic interactions between the toothed whales and their potential prey in the NWIP food web getting an image of the food web through the use of ecological tracers.

Objective 5: To determine which biological and ecological factors have a significant influence on the trace elements, PCBs and PBDEs concentrations in the NWIP toothed whales.

Objective 6: To determine whether PCB patterns can be used as ecological tracers to identify segregation among sympatric species.

Objective 7: To compare the contamination status of these species regarding those of adjacent waters of the Northeast Atlantic.

Objective 8: To find out about a possible temporal trend of these contaminants during the time period of the study.

THESIS OUTLINE

The thesis consists of six chapters of which four are presented as scientific articles at different stages of publication: one is published, one is under review and the two others are still under preparation for submission. The first two articles deal with the first perspective presented above (see Figure 1.10), the third article deals with both perspectives and finally the fourth one deal with the second perspective.

All co-authors of the articles are listed at the beginning of the chapters. Table 1.5 summarizes the chapters including their corresponding objectives and perspectives. Finally, chapters are followed by several Annexes including the works presented during conferences (specifically posters) as well as significant contributions carried out with others researchers in the form of articles.

Table 1.5 Summary of the chapters, objectives and perspectives included in the thesis

Chapter	Title	Objectives	Perspective
II	Foraging ecology of five toothed whale species in the Northwest Iberian Peninsula, inferred using carbon and nitrogen isotope ratios	1 and 4	Ecological tracers
III	Long-term ecological segregation among the North West Iberian Peninsula toothed whale species as evidenced using ecological tracers	1 and 2	Ecological tracers
IV	Inter-species differences for polychlorinated biphenyls patterns in five toothed whale species from the NW Iberian Peninsula	1, 5 and 6	Ecological tracers and contamination status
V	An assessment of the trace element and persistent organic pollutant (PCBs and PBDEs) levels in toothed whale species from the North West Iberian Peninsula.	3, 5, 7 and 8	Contamination status

CHAPTER II

Foraging ecology of five toothed whale species in the Northwest Iberian Peninsula, inferred using carbon and nitrogen isotope ratios

O Golfiño común (Delphinus delphis)

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Foraging ecology of five toothed whale species in the Northwest Iberian Peninsula, inferred using carbon and nitrogen isotope ratios

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ABSTRACT

The feeding ecology and habitat use of the most frequently sighted and/or regularly reported stranded or by-caught toothed whale species of the North Western Iberian Peninsula (NWIP) were examined, with a special focus on their trophic position (TP) and relationships with their prey. With this aim, the stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) of common dolphin (*Delphinus delphis*), bottlenose dolphin (*Tursiops truncatus*), harbour porpoise (*Phocoena phocoena*), striped dolphin (*Stenella coeruleolba*) and long-finned pilot whale (*Globicepahala melas*) were analyzed in muscle samples taken from stranded and by caught animals between 2004 and 2008. Stable isotopes were also measured in 17 species of fish and cephalopods previously identified as prey species, based on stomach content analyses, and in plankton. The trophic enrichment factors (TEF) were calculated for all five species and in addition, isotopic mixing models were applied to estimate the proportional contribution of each prey source to the diet of the common dolphin, which was the toothed whale species best sampled in our study. Plankton, fish and cephalopods exhibited an increasing trend in their $\delta^{13}C$ values (from -19.6‰ to -15.3‰) along the offshore-inshore axis, with a less clear spatial pattern observed for $\delta^{15}N$ values. Striped dolphins exhibited the lowest mean $\delta^{13}C$, $\delta^{15}N$ and TP values (-17.6‰, 10.8‰ and 4.3, respectively), which confirms the oceanic character of this species and its lower trophic position when compared to the other toothed whales analyzed. The common dolphin exhibited mean $\delta^{13}C$, $\delta^{15}N$ and TP values that were at an intermediate level (-17.0‰, 11.7‰ and 4.7, respectively) and results of the mixing model indicated that blue whiting (Micromesistius poutassou) was the main component of the diet. The harbour porpoise, bottlenose dolphin and pilot whale exhibited higher and very similar isotopic compositions and TPs. The mean TEF obtained between predators and their main prey were 1.4‰ for $\delta^{15}N$ and 0.8‰ for $\delta^{13}C$. These results provide information on stable isotope incorporation data for toothed whales, which are essential if conclusions are to be drawn in issues concerning trophic structures and habitat use in the NWIP.

KEY WORDS: Stable isotopes · Toothed whales · Trophic position · Trophic relationships · Isotopic mixing model · North West Iberian Peninsula

INTRODUCTION

Marine mammals are recognized as top predators in marine trophic webs but little is known about their role in the structuring of marine ecosystems (Bowen 1997). The main limitation is that the ecological needs of such large animals often exceed the temporal and spatial scales used to define community boundaries. This role is especially important in habitats with enhanced productivity such as upwelling areas, where high species richness contributes to high trophic linkage density and exceptional resource abundance (Bode et al. 2003). This is the case of the Northwest Iberian Peninsula (NWIP) waters. This area is characterized by high marine biodiversity and productivity, supported by strong nutrient enrichment during seasonal upwelling periods (Fraga 1981), and a relatively narrow (20-35 km wide) shelf. From April to September, the prevalent northerly winds favor the upwelling of the nutrient-rich Eastern North Atlantic Central Water (Fraga 1981), sustaining a high level of productivity when compared to adjacent areas. The marine fauna of the NWIP includes at least 19 marine mammal species (16 cetaceans and 3 pinnipeds; López et al. 2002). Based on strandings and sightings data, the most common cetacean species are all toothed whales: common dolphin (*Delphinus delphis*), bottlenose dolphin (*Tursiops truncatus*), harbour porpoise (*Phocoena phocoena*), long-finned pilot whale (*Globicephala melas*), striped dolphin (*Stenella coeruleoalba*) and Rissos's dolphin (*Grampus griseus*) (López et al. 2002, 2004, Pierce et al. 2010). Despite the high proportion of cetaceans inhabiting the NWIP little is known about their role into the food web and their trophic relationships. Knowledge of feeding habits, habitat use and trophic relationships within a community is needed to underpin sound management measures, in particular in areas such as the NIWP where fishery by-catch mortality (López et al. 2002, 2003), prey depletion due to overfishing, and oil spills (Ridoux et al. 2004) are the main threats to cetacean populations.

Previous studies based on stomach contents analyses, have investigated the diet of the five toothed whales in the NWIP. Striped dolphin and pilot whale are known to be mainly cephalopods eaters. However striped dolphin exhibits dietary plasticity since some crustacean species, oceanic fish such as silvery lightfish (*Maurolicus muelleri*) as well as pelagic fish species (e.g. scad *Trachurus trachurus*, sand smelt *Atherina presbyter*) were found in stomach contents of individuals stranded in the NWIP (Spitz et al. 2006, Sollmann 2011). Common dolphin mainly feeds on fish species, and the blue whiting (*Micromesistius poutassou*), sardine (*Sardina pilchardus*) and sand smelt are the most important prey species in the studied area (Santos et al. 2004c). Bottlenose dolphins have a varied diet in the NIWP, although it was strongly dominated by blue whiting and hake (*Merluccius merluccius*) (Santos et al. 2007a,b). Finally harbour porpoise from the NWIP manly feeds on pelagic and coastal fish species such as pouting (*Trisopterus luscus*) and scad (Santos et al. 2004a, Pierce et al. 2010).

These differences in diet are also supported by differences in habitat uses (Pierce et al. 2010) and could thus lead to segregation among the five toothed whale species in the area. However, it is widely recognized that stomach content analyses have a number of limitations, since information provided by stomach contents normally allows the identification of the prey consumed shortly before the stomachs were collected (Pierce & Boyle 1991) and varying time of digestion of different prey species can result in a overestimation of some species (Santos et al. 2001). Thus, insights into the trophic relationships and feeding habits of marine mammals can be obtained by measuring the ratios of naturally occurring isotopes such as those of carbon and nitrogen, in specific tissues (Hobson & Welch 1992, Ostrom et al. 1993, Muir et al. 1995). This chemical analysis constitutes a powerful complementary method to the stomach content analyses, and is based on the assumption that predator stable isotope compositions are a reflection of those of their prey (DeNiro & Epstein 1978, 1981). Ratios of the natural abundance of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) have been extensively used in studies of cetacean ecology to assess dietary variation in space and time (Abend & Smith 1995, Méndes et al. 2007, Knoff et al. 2008). Turnover rates vary among tissues depending on their metabolic activity, e.g. faster turnover rates have been measured for skin than for teeth (Walker & Macko 1999). The difference in turnover rates between tissues allows the exploration of the dietary history of individual animals over different time windows from few days to several years (Abend & Smith 1995). In addition to information on diet, $\delta^{13}C$ and $\delta^{15}N$ signatures can act as chemical tracers that reflect characteristics of the ecosystem where an animal forages, to determine the trophic position of species in marine food webs (Fry & Sherr 1984, Bode et al. 2003, 2007) but also to study migration patterns (see Hobson 1999 for review) and population substructure (Borrell et al. 2006). Stable isotopes of carbon and nitrogen show enrichment (an increase in the abundance of the heavier isotope) through food webs with increasing trophic level. The enrichment of $\delta^{13}C$ is generally small or insignificant (DeNiro & Epstein 1978, Vander Zanden & Rasmussen 2001) and, therefore, $\delta^{13}C$ is primarily used to provide information on the origin of the base of the food chain (France 1995) and can reveal information such as inshore *vs* offshore feeding preferences (Hobson & Welch 1992). On the other hand, $\delta^{15}N$ enrichment per trophic level in marine food webs is normally considerably higher, with an estimated mean trophic enrichment factor (TEF) ranging from 2 to 5‰ (DeNiro & Epstein 1978, 1981, Hobson & Welch 1992, Vander Zanden & Rasmussen 2001). Nitrogen isotope ratios are thus more useful as an indicator of trophic position although some variability of $\delta^{15}N$ has also been demonstrated, for example an inshore *vs* offshore enrichment of $\delta^{15}N$ signatures (Chouvelon et al. in press) due to the use of different nitrogen sources by phytoplankton. TEF also vary depending on the tissue analyzed (Hobson et al. 1996). For example, metabolically active tissues show less enrichment in $\delta^{13}C$ (or $\delta^{15}N$) relative to the diet than inactive or keratinous tissues (Hobson et al. 1996). This represents a potential confounding factor when using isotope ratios in different tissues to infer information on feeding ecology over different time periods.

In this context, the aim of the present study was to investigate the feeding ecology and habitat use of five toothed whales species inhabiting the NWIP waters through the use of stable isotopes analysis ($\delta^{13}C$ and $\delta^{15}N$) and based on previous stomach content studies. The carbon and nitrogen isotope ratios of known prey species (eight fish species and nine cephalopod species; see below for details) and plankton (selected as the base of the food web in the research area) were also analyzed, in order to position the predators and their potential prey in the food web and to study their trophic relationships. Following this aim, the TEF of toothed whales and prey species were calculated. For the common dolphin, which is the most abundant cetacean species in the Iberian Peninsula and also the best represented in the samples available, isotopic mixing models were also applied to estimate the proportional contribution of each prey source to its diet.

MATERIALS AND METHODS

Sample collection

The fieldwork was carried out in the North West of the Iberian Peninsula, in an area extending from the northern limit of the Galician coast (43° 31′ N, 7° 2′ W) to Nazaré on the Portuguese coast (39° 36′ N, 9° 3′ W; Figure 2.1). Stranded cetaceans were attended by experienced personnel of the Galician (*Coordinadora para o Estudo dos Mamiferos Mariños*, CEMMA) or Portuguese (*Sociedade Portuguesa de Vida Salvagem*, SPVS) stranding networks. Animals were identified to species, measured, sexed and, if the state of preservation of the carcass allowed it, full necropsies were performed and samples collected following a standard protocol defined by the European Cetacean Society (after Kuiken 1996). For this study, between 2004 and 2008, muscle tissues were sampled from stranded and by-caught individuals of five toothed whale species: common dolphin (n = 114), bottlenose dolphin (n = 9), harbour porpoise (n = 19), striped dolphin (n = 21) and long-finned pilot whale (n = 9) (see Table 2.1). After collection, muscle samples were stored at -20 °C until processed in the laboratory.

Muscle tissues of 17 species of fish and cephalopods previously identified in the literature as prey of the sampled cetacean species in the study area (see Santos et al. 2004a,c, 2007a,b) were also analyzed (see Table 2.2). All the fish samples and those of common squid (*Loligo vulgaris*) were collected with a pelagic trawl, during the PELACUS0409 survey. This survey, carried out by *Instituto Español de Oceanografía* (IEO) in spring 2009 aimed to acoustically assess pelagic marine living resources of northwestern and northern Spanish shelf waters. Plankton was also collected during the survey, at night by vertical tows (0-100 m depth) of a conical net deployed at regularly distributed stations over the shelf up to the shelf-break throughout the surveyed area (Figure 2.1).

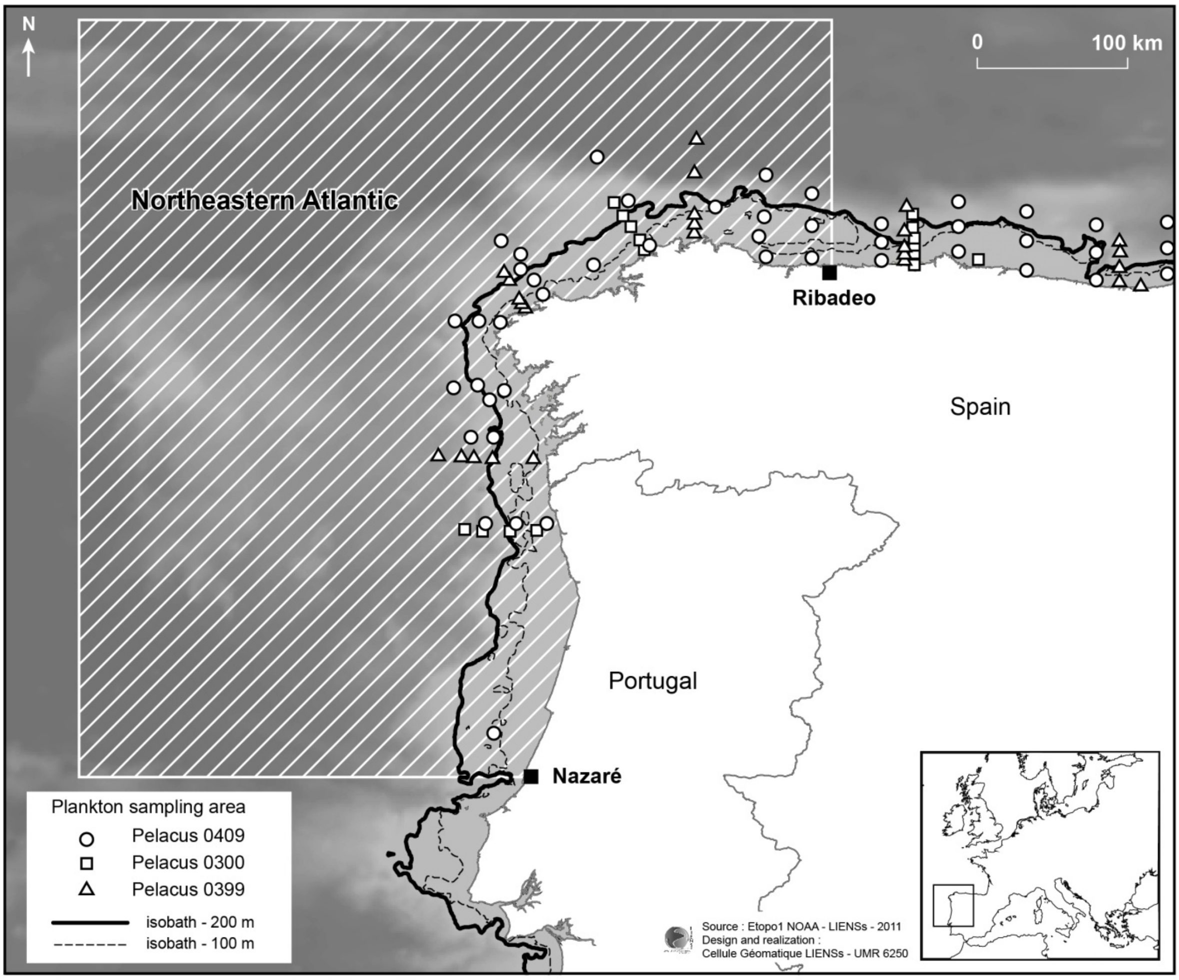

Figure 2.1 Map of the NWIP with location of plankton stations and with 100 and 200 m isobaths. The 200 m isobath was taken as the limit for the shelf-break.

Plankton samples were subsequently fractionated through a 200 µm mesh, carefully washed with filtered seawater, transferred to glass-fiber filters and stored frozen until subsequent isotopic analysis (Bode et al. 2007). Only nitrogen isotopes were analyzed for the PELACUS0409 samples. Carbon isotopes were also measured in samples collected during the PELACUS0399 (March 1999) and PELACUS0300 (March 2000) surveys in the same area (Bode et al. 2003). Samples from these surveys were collected as described for the nitrogen isotope samples but using a net of 20 µm mesh, thus comprising plankton in the 20-200 µm size range. The isotopic composition of this fraction is expected to be essentially the same as that of the 40-200 µm sample from 2009, as there is no significant isotopic enrichment within plankton fractions < 200 µm (see Bode et al. 2007). All isotopic values from plankton in the region were pooled to obtain representative estimates of isotopic composition taking into account spatial and temporal variability (Bode et al. 2004, 2007). Specimens of common octopus (*Octopus vulgaris*), curled octopus (*Eledone cirrhosa*) and cuttlefish (*Sepia officinalis*) were acquired in the Vigo (Galicia) fish market, while oceanic cephalopod species with no commercial value (the squids *Teuthowenia megalops*, sharpear enope *Ancistrocheirus lesueurii*, armed cranch *Galiteuthis armata*, umbrella *Histioteuthis bonnellii* and elongate jewel squid *H. reversa*) were collected during the EVHOE groundfish surveys conducted by the *Institut Français de Recherche pour l'Exploitation de la Mer* (IFREMER), from the southern part of the Bay of Biscay in autumn, 2001 to 2008.

Table 2.1 Number of individuals of the toothed whale species stranded or by-caught per year in the study area: 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).

Year	All	Dde	Pph	Ttr	Scc	Gme
2004	16	4	5	1	2	4
2005	29	21	2	2	4	0
2006	24	14	3	3	3	1
2007	60	42	5	2	9	2
2008	43	33	4	1	3	2
Total	172	114	19	9	21	9

Individuals of each species were measured, weighted and dissected to obtain portions of lateral white muscle or mantle (for fish and cephalopods, respectively), which were also stored at -20 °C prior to analysis.

Stable isotope analysis

Muscle and mantle samples were freeze-dried and then ground into a fine powder. Since lipids are highly depleted in ^{13}C relative to other tissue components (DeNiro & Epstein 1978), they were extracted from these samples using cyclohexane. To this end, an aliquot of approximately 100 mg of fine powder was agitated with 4 mL of cyclohexane for 1 h. Next, the sample was centrifuged for 5 min at 4000 g, and the supernatant containing lipids was discarded. The sample was dried in an oven at 45 °C for 48 h, and 0.35 ± 0.05 mg subsamples of lipid-free powder were then weighed in tin cups for stable isotope analyses. Plankton samples were analyzed after oven drying at 50 °C for 24 h with no lipid extraction.

These analyses were performed with an elemental analyzer coupled to an Isoprime (Micromass) continuous-flow isotope-ratio mass spectrometer (CF IR-MS). Plankton samples were analyzed using an isotope-ratio mass spectrometer (Finnigan Matt Delta Plus) coupled to an elemental analyzer (Carlo Erba CHNSO 1108).

The results are presented in the usual δ notation relative to Vienna PeeDee Belemnite Standard for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$, in parts per thousand (‰). Replicate measurements of internal laboratory standards (acetanilide) during each autorun indicate measurements errors < 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$.

Isotopic model

The trophic position (TP) of toothed whale and prey species was estimated from $\delta^{15}N$ following Vander Zanden & Rasmussen (2001):

$$TP = 2 + (\delta^{15}N_{consumer} - \delta^{15}N_{base}) / TEF$$

where δ^{15}Nconsumer is the mean $\delta^{15}N$ value of a given consumer (toothed whale, fish or cephalopod species in this case). The $\delta^{15}N$ value of < 200 μm fraction of plankton was attributed to primary consumers ($\delta^{15}N_{base}$) and thus represented the reference value at the base of the food web (TP = 2; Bode et al. 2007). In their study, Vander Zanden & Rasmussen (2001) used a mean

TEF of 3.4‰ which is a valid approximation of trophic fractionation only when averaged over multiple trophic pathways (Post 2002). However, there is increased evidence that the TEF can vary widely depending on the taxon of the consumer or depending on the type and quality of its food. To our knowledge, only two published experimental studies have determined the TEF in marine mammals. Hobson et al. (1996) fed two harp seals (*Pagophilus groenlandicus*) on a constant diet of herring (*Clupea harengus*) and obtained a TEF value of 2.4‰ for $\delta^{15}N$ in muscle tissue. The second study was carried out on three captive bottlenose dolphins and five killer whales (*Orcinus orca*) (Caut et al. 2011). A TEF value of 1.2‰ for $\delta^{15}N$ in muscle tissue was calculated for killer whales but was based on the results of a single individual that died during the experiment due to a bacterial infection. Thus we used a TEF appropriate to each major type of consumer analyzed and derived from the literature, 2.4‰ to calculate TP for marine mammals and 3.4‰ to calculate the prey TP.

Data analysis

Mean isotopic composition in muscle, and its standard deviation (SD) and 95% confidence intervals, were calculated for toothed whales, their prey and plankton. The existence of significance differences in the isotopic composition of the samples was investigated using ANOVA or Kruskal-Wallis tests among species, separately for both $\delta^{13}C$ and $\delta^{15}N$ values. The choice of test was based on the results of Shapiro (to check for normality in the sample distributions) and Bartlett (to check the homogeneity of variances) tests.

Isotopic mixing models were applied with the software package SIAR (Stable Isotope Analysis in R) to estimate the proportional contribution of sources (dietary items) within the isotopic mixture (consumer tissue) of the common dolphin. The main prey species contributing to their total diet by weight determined from stomach content analyses (Santos et al. 2004a,c) were considered as sources: blue whiting, sardine, scad, sand smelt, mackerel (*Scomber scombrus*), pouting, common squid and hake. For sand smelt, isotopic values from the literature were used

(Fernández et al. 2011a) since we did not sample this species. The isotopic values for food sources must be adjusted by appropriate enrichment factors between diet and consumer tissue (Phillips & Gregg 2003) and therefore the use of surrogate or proxy discrimination factors may not be appropriate for species or tissues for which the specific TEF are unknown (Bond & Diamond 2011). Obtaining this information is particularly challenging for marine mammals and birds because individuals must be held on an isotopically fixed diet in controlled conditions that allow for regular sampling over lengthy periods of time. We have used the two range values available in the literature (see above). Two model runs were carried out: (1) using 1.3‰ ± 0.1 for $\delta^{13}C$ and 2.4‰ ± 0.3 for $\delta^{15}N$ (Hobson et al. 1996) and (2) using 0.3‰ ± 0.2 for $\delta^{13}C$ and 1.2‰ ± 0.1 for $\delta^{15}N$ (Caut et al. 2011). Finally we ran the mixing models using default parameters (iterations = 500,000, burnin = 50,000, thinby = 15).

All models and statistical tests mentioned above were performed using the free software R, version 2.11.1 (R Development Core Team 2010).

RESULTS

Stable isotope composition of prey and plankton

Values of $\delta^{13}C$ and $\delta^{15}N$ differed significantly between prey species and plankton ($H = 105.03$ and 86.4 respectively, $P < 0.0001$). As expected, plankton showed the lowest $\delta^{13}C$ values (-19.6‰). There was wide variation in the $\delta^{13}C$ values of the different prey organisms collected from the NWIP. Plankton, fish and cephalopods exhibited $\delta^{13}C$ values (Table 2.2), ranging from -19.6‰ for the sharpear enope squid to -15.3‰ for pouting, a trend broadly consistent with an increase from the offshore system to the inshore system. Three broad groups can be identified (Figure 2.2), consisting of oceanic squids (*Teuthowenia megalops*, sharpear enope, armed cranch, umbrella and elongate jewel squid) and oceanic fish (silvery lightfish) in one group, pelagic fish (sardine, mackerel, scad), hake, blue whiting and common squid in a second group, and a third group including coastal/benthic cephalopods and fish (common octopus, cuttlefish, curled octopus and pouting).

Regarding δ^{15}N values, as for δ^{13}C, pouting exhibited the highest values (14.0‰) while the lowest mean δ^{15}N values were recorded in the oceanic squids *Teuthowenia megalops* and armed cranch squid (9.0‰ and 9.7‰, respectively), followed by sardine and the silvery lightfish (both 10.2‰). Plankton (as was the case for δ^{13}C) exhibited the lowest δ^{15}N values (5.2‰) (see Table 2.2).

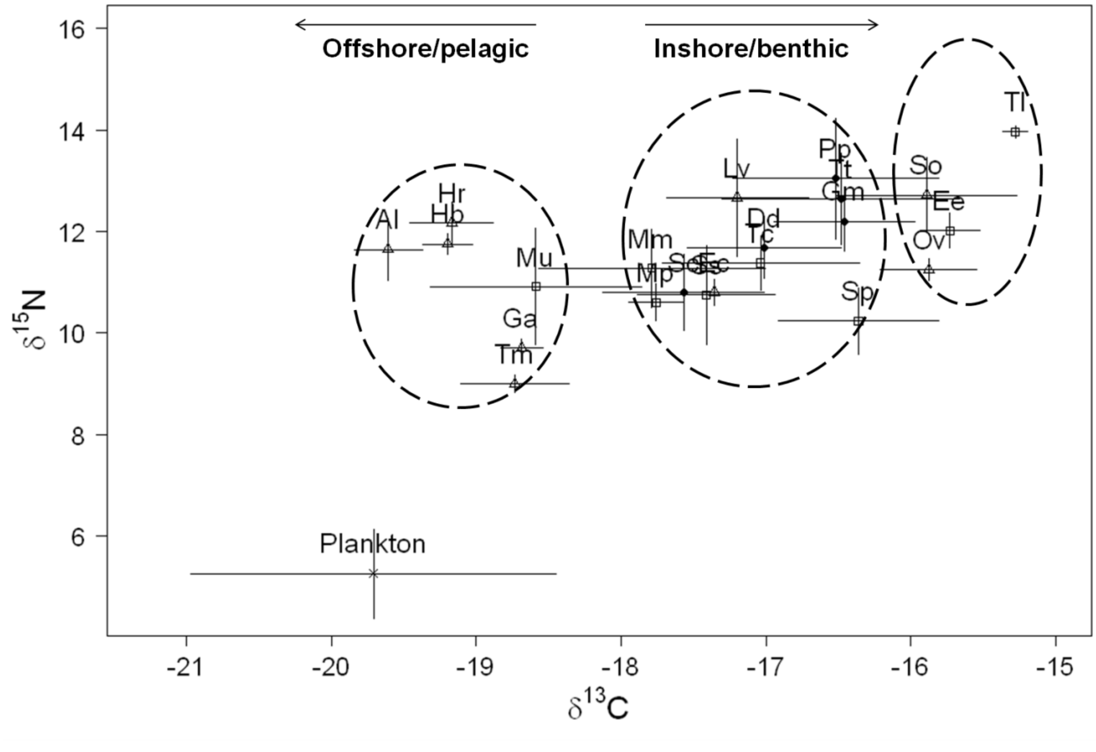

Figure 2.2 Carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope ratios (mean ± SD and ranges, ‰) in plankton and in the muscle tissue of selected fish, cephalopods, and toothed whale species of the NWIP. The discontinuous ellipses grouped the species according to their distribution on the offshore-inshore axis and δ^{13}C values: sharpear enope squid *Ancistrocheirus lesueurii* (Al), common dolphin *Delphinus delphis* (Dd), Curled octopus *Eledone cirrhosa* (Ee), armed cranch squid *Galiteuthis armata* (Ga), long-finned pilot whale *Globicephala melas* (Gm), umbrella squid *Histioteuthis bonnellii* (Hb), elongate jewel squid *H. reversa* (Hr), common squid *Loligo vulgaris* (Lv), silvery lightfish *Maurolicus muelleri* (Mu), hake *Merluccius merluccius* (Mm), blue whiting *Micromesistius poutassou* (Mp), common octopus *Octopus vulgaris* (Ov), harbour porpoise *Phocoena phocoena* (Pp), sardine *Sardina pilchardus* (Sp), mackerel *Scomber scombrus* (Ss), cuttlefish *Sepia officinalis* (So), striped dolphin *Stenella coeruleoalba* (Sc), *Teuthowenia megalops* (Tm), scad *Trachurus trachurus* (Tc), pouting *Trisopterus luscus* (Tl) and bottlenose dolphin *Tursiops truncatus* (Tt).

Stable isotope composition and trophic position of toothed whales

The muscle $\delta^{13}C$ and $\delta^{15}N$ values of the five toothed whales differed significantly between species (Kruskal-Wallis, $H = 30.39$ and 60.26, respectively, both $P < 0.0001$; Table 2.3). As revealed by the mean $\delta^{13}C$ values (Post-hoc test, $P < 0.05$) three major groups exist as delineated by their distribution along the coastal-oceanic axis (Figure 2.3). The first group was characterized by the highest $\delta^{13}C$ values (-16.5‰) and included harbour porpoise, bottlenose dolphin and long-finned pilot whale. The most depleted and highly negative mean $\delta^{13}C$ value (-17.6‰) was found in the muscle of striped dolphin whereas common dolphin exhibited a value for $\delta^{13}C$ (-17.0‰) which was intermediate between these two groups.

Comparison tests showed that $\delta^{15}N$ values in muscle of striped dolphin were significantly lower than those of the other species (Post-hoc tests, all $P < 0.05$), and $\delta^{15}N$ values in muscle of harbour porpoise were significantly higher than those in the other species with the exception of bottlenose dolphin. The $\delta^{15}N$ value of bottlenose dolphin was higher than those of common dolphin ($P < 0.01$) and long-finned pilot whale but not statistically different from the latter. Finally, $\delta^{15}N$ values were not statistically different between long-finned pilot whales and common dolphins (Table 2.3 & Figure 2.3).

Trophic positions (TP) of toothed whale species were estimated based on the $\delta^{15}N$ values of prey and primary consumers (Figure 2.4), and showed no significant differences among the five species (ANOVA, $P > 0.05$; Table 2.3).

Trophic relationship between predators and prey

The five toothed whales had higher $\delta^{15}N$ values in muscle than those obtained from the prey species, with the exception of striped dolphins which showed nitrogen stable isotope values lower than pouting. Comparing the isotopic values between diet (i.e. the main prey selected from literature for each predator species) and muscle (tissue) of the five toothed whales mean TEF values of 1.4‰ for $\delta^{15}N$ and 0.8‰ for $\delta^{13}C$ were obtained. Note that these are unweighted means which do not take into account the relative importance of the different species and included only those prey species that could be sampled.

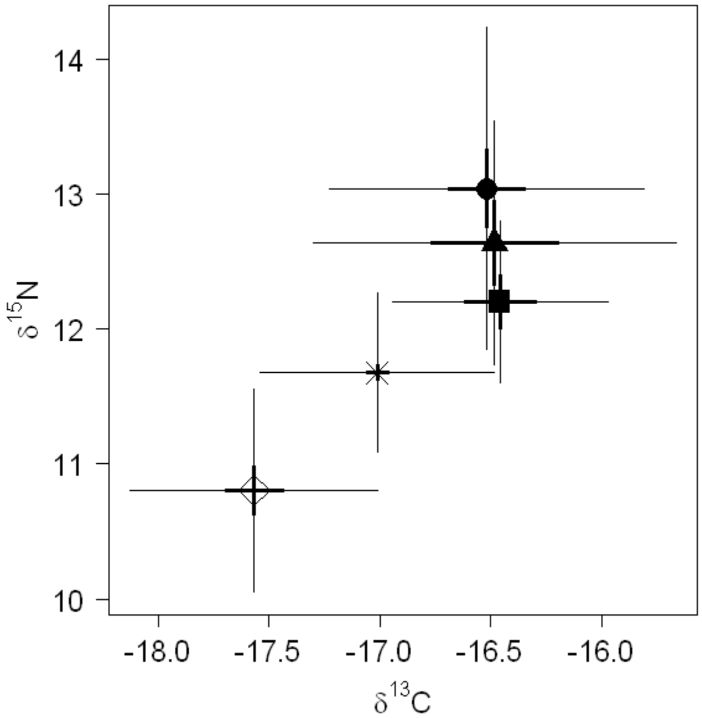

Figure 2.3 Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios in muscle tissue of striped dolphin *Stenella coeruleoalba* (◊), common dolphin *Delphinus delphis* (×), long-finned pilot whale *Globicephala melas* (■), bottlenose dolphin *Tursiops truncatus* (▲) and harbour porpoise *Phocoena phocoena* (●) from the NWIP. Data are mean (‰) ± SD and standard error (bold).

Results from the isotopic mixing model (SIAR) for common dolphin suggest a high contribution of blue whiting to the diet of this species based on muscle isotopic values. When we used the TEF from Hobson et al. (1996) (model 1, see Figure 2.5a), blue whiting showed a high mean contribution of 91%, with hake in second position but with a much lower mean contribution of 3.5%, followed by mackerel with a mean contribution of 2.9%.

In contrast, when we used the lower TEF suggested by Caut et al. (2011) (model 2, see Figure 2.5b), results indicated a higher diversity of the diet with a lower contribution of blue whiting than in model1 (50.8%) and sardine as the second most important prey species with a mean contribution of 25.5%. Mackerel and hake also exhibited a higher contribution than in model1 with 14.4% and 4.5%, respectively.

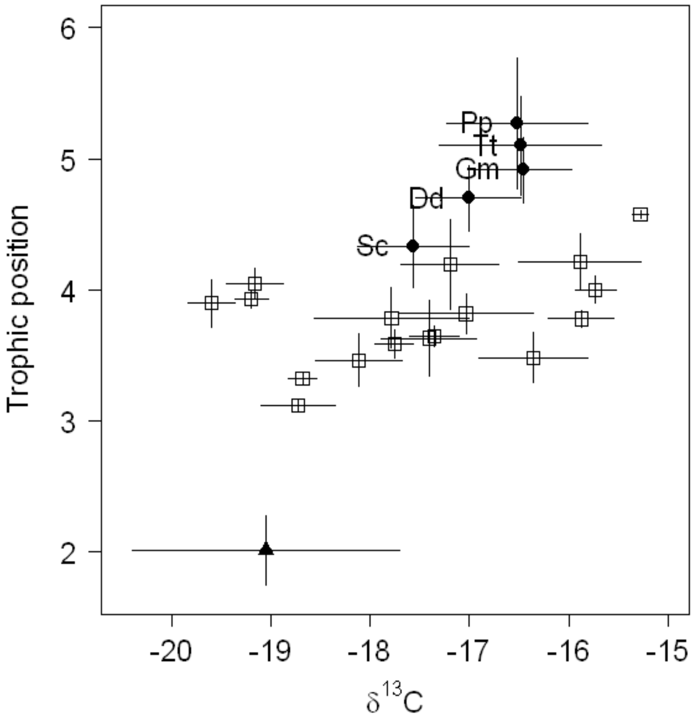

Figure 2.4 Trophic structure of the NWIP food web as determined by trophic position (TP) and carbon ($\delta^{13}C$) isotope ratios of their different components: Striped dolphin *Stenella coeruleoalba* (Sc), common dolphin *Delphinus delphis* (Dd), long-finned pilot whale *Globicephala melas* (Gm), bottlenose dolphin *Tursiops truncatus* (Tt), harbour porpoise *Phocoena phocoena* (Pp), prey species (□) and plankton (▲). Data are mean ± SD.

DISCUSSION

Spatial feeding ecology differences in the NWIP food web

δ^{13}C values are generally used as an indicator of the habitat and/or feeding zone of an organism (France 1995). Here δ^{13}C values varied from -21.0‰ to -15.1‰ with plankton being ^{13}C-depleted relative to fish, cephalopods and toothed whale species, as would be expected from its position at the base of the consumer chain. δ^{13}C isotopic values showed a decreasing trend from coastal and neritic habitats to oceanic and deep-sea habitats (Figures 2.2 & 2.3). This offshore-inshore gradient has been previously described in freshwater and marine ecosystems (Fry & Sherr 1984, Hobson 1999, Sherwood & Rose 2005). Fish and cephalopod species, which were more 13C-enriched compared to marine plankton, showed considerable segregation between species along this oceanic-coastal axis (Figure 2.2). Sharpear enope squid, umbrella squid, elongate jewel squid, *Teuthowenia megalops*, armed cranch squid and silvery lightfish have the most ^{13}C-depleted values due to their oceanic feeding habitat. Common octopus, common cuttlefish, anchovy and pouting showed the highest δ^{13}C values, consistent with their more coastal and/or benthic habitats. In the case of hake, blue whiting, mackerel, scads, common squid and sardine, the δ^{13}C values are intermediate between the previously mentioned prey species (Figure 2.2). Similar results were obtained by Fernández et al. (2011a) who analyzed stable isotope signatures of 17 prey species of bottlenose dolphins in Galicia waters. Regarding the community of toothed whales inhabiting NWIP waters, we found interspecific differences in their δ^{13}C values. These values ranged from -18.5 for striped dolphin to -15.1‰ for harbour porpoise and bottlenose dolphin and suggest that the species analyzed are spatially segregated and/or specialize on different prey in the study area. Thus structuring is present even though the continental shelf area is relatively limited, due to the narrowness of the continental shelf (< 40 km wide). The ^{13}C-depletion observed in striped dolphin and common dolphin relative to the other three toothed whale species (Figure 2.3) may result from an exploitation of oceanic, relatively ^{13}C-depleted, resources by both species. Striped dolphin shows a high level of foraging plasticity, as evidenced by several studies carried out in various part of the world (Miyazaki et al. 1973, Desportes 1985, Meotti & Podestà 1997, Ringelstein et al. 2006, Spitz et al. 2006). In the NWIP, the main fish prey of striped dolphin are blue whiting, scads and sand smelt while the main cephalopods eaten are Gonatus sp. and ommastrephids (Santos et al. 2007b). It is also known that striped dolphins in the NWIP feed on crustaceans, specifically on pelagic shrimp species (Jose Cedeira Personal communication), and on oceanic fish such as silvery lightfish. Thus, although some coastal species have been found in the stomach contents of NWIP striped dolphins, the low δ^{13}C values of the present study tend to show that this dolphin species mainly exploits the oceanic habitats of the NWIP.

Common dolphin is the most abundant cetacean in the NWIP (López et al. 2004) and the second most frequently sighted from the coast (Pierce et al. 2010). Observations from fishing boats suggest that this species is mainly found in waters of less than 200 m depth (Spyrakos et al. 2011). This apparent preferential use of shallow waters may relate to foraging preferences. The most important prey of common dolphin in Galician waters are blue whiting and sardine as shown by the analysis of stomach contents of stranded and by-caught individuals (Santos et al. 2004c) and here supported by the isotopic mixing models results, which suggested that blue whiting is the most important prey and that, depending on assumptions made about trophic fractionation, sardine may be the second most important prey (see Figure 2.5). It is clear from comparing the results of the two mixing models that the details of the results are strongly dependent on the trophic fractionation assumed and caution is thus needed in interpreting such models (see below for further discussion on this point). Common dolphin probably feeds on blue whiting on the upper part of the shelf break, while sardine is taken on the shelf and in more coastal areas. This would explain the intermediate ^{13}C value of the common dolphin, i.e. between that of the striped dolphin and those of the other toothed whales found in our study. This result is in agreement with the results of similar work carried out in the same area and in other areas of the North East Atlantic: Ireland (Das et al. 2003a), the French Channel (Das et al. 2003a) and the Bay of Biscay (Chouvelon et al. in press) (see Table 2.4).

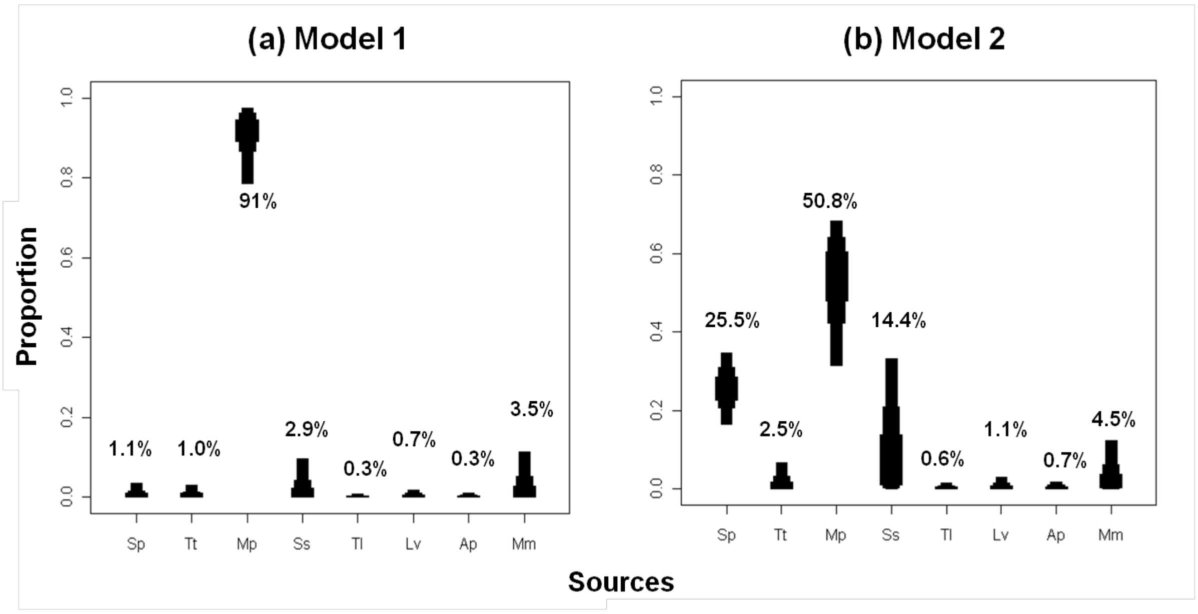

Figure 2.5 Results of SIAR mixing model (50%, 75% and 95% confidence intervals) showing the probable sources proportion in diet of *Delphinus delphis* in the NWIP. Model 1 (a) was run with a TEF of 1.3 ± 0.1 and 2.4 ± 0.3 for $\delta^{13}C$ and $\delta^{15}N$, respectively, and model 2 (b) was run with a TEF of 0.34 ± 0.2 and 1.21 ± 0.15 for $\delta^{13}C$ and $\delta^{15}N$, respectively (Caut et al. 2011, Hobson et al. 1996). The mean proportion (%) of each source is labeled: sardine *Sardina pilchardus* (Sp), scad *Trachurus trachurus* (Tt), blue whiting *Micromesistius poutassou* (Mp), mackerel *Scomber scombrus* (Ss), pouting *Trisopterus luscus* (Tl), common squid *Loligo vulgaris* (Lv), sand smelt *Atherina presbyter* (Ap) and hake *Merluccius merluccius* (Mm).

While $\delta^{13}C$ isotopic values were not significantly different between harbour porpoise, bottlenose dolphin and long-finned pilot whale, these species exhibited ^{13}C-enriched values when compared to common and striped dolphins (Figure 2.3). This could be related to a more coastal distribution of these three species or a greater use of benthic resources. Harbour porpoises tend to be seen where the shelf is narrower and where the average water depth is 90 m (Pierce et al. 2010). In this study, the harbour porpoise exhibited a mean $\delta^{13}C$ value of -16.5‰ while its main prey in Galician waters, scads and pouting (Santos et al. 2004a, Pierce et al. 2010) exhibited mean $\delta^{13}C$

values of -15.3‰ and -17.0‰, respectively. These values are consistent with the coastal distribution of both predator and prey and can explain the high $\delta^{13}C$ values obtained for harbour porpoise. These estimates are also very close to the values found for the same species in studies carried out in French Channel coastal waters (-16.1‰; Das et al. 2003a), the Irish coast (-16.5‰; Das et al. 2003a) and North Sea (-16.4‰; Das et al. 2003c) (see Table 2.4).

In the NWIP bottlenose dolphin is the species most frequently sighted from the coast (Pierce et al. 2010) but there are also sightings of this species in offshore waters (e.g. López et al. 2004). Two recent studies demonstrated the existence of ecological and genetic differences between the animals stranded in the southern part of the Galician Rías (originating from the proposed resident population in these coastal waters) and those stranded elsewhere (Fernández et al. 2011a,b). The high variability obtained in the $\delta^{13}C$ values of bottlenose dolphin in the present study could be explained by the presence in our samples of individuals from both the inshore and offshore populations, which would exploit different prey species. Santos et al. (2007a) found blue whiting and hake to be the most important prey species of stranded and by-caught bottlenose dolphins in Galicia, both numerically and in terms of biomass. Both blue whiting and hake exhibited consistent $\delta^{13}C$ isotopic values, approximately 1‰ lower than bottlenose dolphins, harbour porpoises and long-finned pilot whales (Tables 2.2 & 2.3) although the latter toothed whale species is mainly teuthophagous (Pierce et al. 2007).

The ^{13}C-enrichment (-16.5‰) measured in long-finned pilot whale tissues suggests a coastal habitat or that the individuals were mainly foraging on more coastal and/or benthic species over the sampling period (2004-2008). Although pilot whales are considered an oceanic species (Mead & Brownell 2005), foraging over the shelf is believed to take place since coastal cephalopod species such as common and curled octopus have been recorded in the stomach (González et al. 1994; Santos unpublished data) and are sometimes sighted from the coast (Pierce et al. 2010). Similar results were obtained by Spitz et al. (2011) in the northern Bay of

Biscay, who reported the diet of this species as being a unique combination of mesopelagic prey living in oceanic waters and of prey living at or close to the bottom in neritic waters.

Pattern of nitrogen isotope ratios and trophic relationships

The primary utility of nitrogen isotope ratios for animal ecology lies on their relationship with trophic level (e.g. Hobson 1999). Previous studies have indicated that pelagic foodwebs in upwelling areas are relatively short due to the prevalence of the plankton consumers (Ryther 1969) but in our case, the food web exhibited five trophic levels, with the toothed whales occupying the highest level, which is relatively long for an upwelling system. In the NWIP the TPs varied little among most fishes and cephalopods. However, a ^{15}N-enrichment occurs along the oceanic (and/or pelagic) - coastal (and/or benthic) axis, the most coastal and benthic species exhibiting the highest TP (pouting, common squid and cuttlefish as an example). Toothed whales occupy the top positions of the food web with a narrow range of TP (4.3 to 5.3), with no significant between-species differences being found in their TPs. However, an overlap in TP does not necessarily imply an overlap in diet, since animals may feed on different prey species with similar TPs.

In the present study the TEF measured in muscle of the five toothed whales with respect to muscle of their main prey was found to be 0.8‰ for δ^{13}C and 1.4‰ for δ^{15}N. These results are much lower than those obtained by previous studies but agree quite well with the results of Caut et al. (2011) who obtained TEF values from the muscle of a single captive killer whale fed on controlled diets (0.3‰ for δ^{13}C and 1.2‰ for δ^{15}N). Moreover several sources of variation, including the analytical methodology and dietary differences, have been proven to affect δ^{13}C and δ^{15}N enrichment factors (e.g. Vanderklift & Ponsard 2003). In the present study, TEF should be interpreted with caution as they were estimated based on rather few samples and only the main prey (as identified from stomach content analyses) were selected for the TEF calculations. However, due to the fact that isotope models are very sensitive to changes of TEF (Ben-David &

Schell 2001, Bond & Diamond 2011), the use of diet-dependent TEF could significantly change the results and hence the interpretation. As an example, in our mixing models, applying a lower TEF significantly changed the contribution of the main prey of common dolphin (blue whiting) from 91 to 50.8% (see Figure 2.5). Mixing models suggest a high consumption of blue whiting by common dolphins, which is in agreement with diet studies based on stomach contents (Santos et al. 2004c) and this high proportion of blue whiting would explain the low $\delta^{15}N$ values found in NWIP common dolphins.

It was not possible to investigate the extent of the temporal and/or spatial overlap in the trophic signatures between species in this study, since stable isotope values were analyzed in individuals stranded in different seasons over a period of four years over a wide area. Our sample size was insufficient to allow the investigation of these differences. Furthermore, there is also considerable variability in the isotopic composition of plankton and their consumers from year to year (Bode et al. 2007). For these reasons we used pooled values for plankton isotopic composition from several surveys to better characterize the isotopic baseline, instead of average values obtained from a single survey.

Conclusion

Despite the frequent sightings of striped dolphin and common dolphin over the continental shelf of the NWIP, their $\delta^{13}C$ and $\delta^{15}N$ values in muscle seem to indicate that they mainly feed in offshore waters although they probably target different prey species. Harbour porpoise, bottlenose dolphin and long-finned pilot whale appear to frequent the coastal (or inshore) habitats and to feed on higher trophic level prey than either striped or common dolphin. However, dietary segregation probably also occurs between these three more coastal species (the first two are mainly piscivorous but may take different sizes of prey while long-finned pilot whales feed mainly on cephalopods). The low TEF estimated in this study allows a food web with five trophic levels where the toothed whales studied occupy the top positions. In addition, the SIAR analyses confirmed that common dolphin seem to feed mostly on important economical species such as blue whiting, sardine and mackerel in our study area (as shown by previous studies on stomach contents analyses). This is also probably the case for the other four toothed whale species and highlights the need to take this information into account when designing and implementing conservation measures such as Special Areas of Conservation (SACs). By exploiting the same resources as the fisheries, toothed whales in the NWIP could be at risk from both prey depletion and incidental capture in fishing gear (by-catch) and, indeed there is abundant evidence that by-catch of cetaceans is a common occurrence (López et al. 2002, 2003, Fernández-Contreras et al. 2010). Interaction with fisheries and any other anthropogenic activity are a particular cause for concern in relation to the conservation of the recently described isolated Iberian harbour porpoise population and the resident bottlenose dolphin population inhabiting the southern Galician Rías. Our work highlights the usefulness of stable carbon and nitrogen isotope ratios together with mixing models (albeit with some caveats concerning the latter) to gain understanding on the trophic relationships and the structure of marine food webs. Because the information on diet provided by the isotopic analysis is relatively coarse but potentially covers a long time-scale, while stomach contents analysis provides snapshots of detailed diet composition, the ideal approach involves a combination of these techniques. In combination, and together with the analysis of other ecological tracers, these methods will allow information on feeding ecology to be obtained over a range of different time spans.

Table 2.2 Carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope ratios (mean ± SD, ‰), length range (cm) and mean trophic position of sampled fish, cephalopods and plankton from the NWIP. Lengths are total length for fish and dorsal mantle length for cephalopods. TP: trophic position.

Species		Length range (cm)	δ^{13}C (‰) mean ± SD	δ^{15}N (‰) mean ± SD	TP
Fish					
Sardine (*Sardina pilchardus*)	15	18.5 - 22.5	-16.4 ± 0.5	10.2 ± 0.6	3.5
Anchovy (*Engraulis encrasicolus*)	5	15.5 - 17.0	-15.7 ± 0.2	12.0 ± 0.3	4.0
Blue whiting (*Micromesistius poutassou*)	10	22.0 - 24.0	-17.8 ± 0.2	10.6 ± 0.4	3.6
Pouting (*Trisopterus luscus*)	3	20.0 - 21.0	-15.3 ± 0.1	14.0 ± 0.1	4.6
Hake (*Merluccius merluccius*)	8	25.5 - 33.0	-17.8 ± 0.8	11.3 ± 0.8	3.8
Scad (*Trachurus trachurus*)	19	12.0 - 31.0	-17.0 ± 0.7	11.4 ± 0.5	3.8
Mackerel (*Scomber scombrus*)	14	28.0 - 32.0	-17.4 ± 0.5	10.7 ± 1.0	3.6
Silvery lightfish (*Maurolicus muelleri*)	5	4.4 - 5.5	-18.1 ± 0.4	10.2 ± 0.7	3.5
Cephalopods					
Curled octopus (*Eledone cirrhosa*)	9	208.0 - 495.0	-17.3 ± 0.2	10.8 ± 0.2	3.6
Common octopus (*Octopus vulgaris*)	5	670.0 - 810.0	-15.9 ± 0.3	11.2 ± 0.2	3.8
Cuttlefish (*Sepia officinalis*)	5	150.0 - 194.0	-15.9 ± 0.6	12.7 ± 0.7	4.2
Common squid (*Loligo vulgaris*)	7	9.7 - 27.4	-17.2 ± 0.5	12.7 ± 1.2	4.2
Teuthowenia megalops	3	118.0 - 147.0	-18.7 ± 0.4	9.0 ± 0.2	-
Armed cranch squid (*Galiteuthis armata*)	2	147.0 - 308.0	-18.7 ± 0.1	9.7 ± 0.2	-
Sharpear enope squid (*Ancistrocheirus lesueurii*)	3	21.0 - 49.0	-19.6 ± 0.2	11.6 ± 0.6	-
Umbrella squid (*Histioteuthis bonnellii*)	6	27.0 - 73.0	-19.2 ± 0.2	11.7 ± 0.2	-
Elongate jewel squid (*Histioteuthis reversa*)	4	30.0 - 69.0	-19.2 ± 0.3	12.2 ± 0.4	-
Plankton					
Plankton	46	<200 (µm)	-19.06 ± 1.3	5.2 ± 0.9	2.0

Table 2.3 Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios (mean ± SD, ‰), ranges (minimum, maximum) and mean trophic position (TP) of the toothed whale species sampled along the NWIP. Groups with the same letter indicate that isotopic signatures or TP are not significantly different (pairwise multiple comparison test (MPC) in the case of Kruskal-Wallis (KW), post hoc Tukey's test in the case of ANOVA), n = number of samples.

	Species	n	Mean ± SD	Range (min, max)	Test and characteristics	Groups
$\delta^{13}C$	Common dolphin (*Delphinus delphis*)	114	-17.0 ± 0.5	-18.1, -16.3	KW + MPC tests (Holms's)	a
	Harbour porpoise (*Phocoena phocoena*)	19	-16.5 ± 0.7	-17.5, -15.1	⇳^{2}= 30.4, df = 4	b
	Bottlenose dolphin *(Tursiops truncatus*)	9	-16.5 ± 0.8	-17.2, -15,1	P < 0.0001	b
	Striped dolphin (*Stenella coeruleoalba*)	21	-17.6 ± 0.6	-18.5, -16,4		c
	Long-finned pilot whale (*Globicephala melas*)	9	-16.5 ± 0.5	-17.3, -15.7		b
$\delta^{15}N$	Common dolphin	114	11.7 ± 0.6	10.1, 13.6	KW + MPC tests (Holms's)	a,d
	Harbour porpoise	19	13.0 ± 1.2	11.5, 17.1	⇳^{2}= 60.2, df = 4	b
	Bottlenose dolphin	9	12.6 ± 0.9	11.3, 14.0	P < 0.0001	b,e
	Striped dolphin	21	10.8 ± 0.7	9.8, 12.4		c
	Long-finned pilot whale	9	12.2 ± 0.6	11.0, 13.3		d,e
TP	Common dolphin	114	4.7	3.8, 5.3	1-way ANOVA (+ Tukey's test)	a
	Harbour porpoise	19	5.3	4.4, 6.7	*F* = 0.1469, df = 1	a
	Bottlenose dolphin	9	5.1	4.3, 5.4	p = 0.4248	a
	Striped dolphin	21	4.3	3.7, 4.7		a
	Long-finned pilot whale	9	4.9	4.2, 5.1		a

Table 2.4 Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios (mean ± SD, ‰) in muscle tissue of the five toothed whale species analyzed in the present study. Values obtained for the same species by other authors are also included for comparison.

	Geographic area	n	$\delta^{13}C$ (‰) mean ± SD	$\delta^{15}N$ (‰) mean ± SD	References
Common dolphin	Bay of Biscay	26	-17.4 ± 0.5	12.1 ± 0.6	Chouvelon et al. in press
Delphinus delphis	Galicia (NW Spain)	5	-17.0 ± 0.3	13.1 ± 0.8	Bode et al. 2007
	Channel coast (NE Atlantic)	8	-16.5 ± 0.5	12.1 ± 0.4	Das et al. 2003a
	Irish coast (NE Atlantic)	14	-17.1 ± 0.4	12.2 ± 1.0	Das et al. 2003a
	NW Iberian Peninsula	114	-17.0 ± 0.5	11.7 ± 0.6	This study
Harbour porpoise	Bay of Biscay	10	-17.0 ± 0.4	13.0 ± 0.7	Chouvelon et al. in press
Phocoena phocoena	Channel coast (NE Atlantic)	4	-16.1 ± 0.6	16.5 ± 2.4	Das et al. 2003a
	Irish coast (NE Atlantic)	7	-16.5 ± 0.7	14.1 ± 1.6	Das et al. 2003a
	North Sea	46	-16.4 ± 1.6	16.2 ± 1.6	Das et al. 2003c
	NW Iberian Peninsula	19	-16.5 ± 0.7	13.0 ± 1.2	This study
Bottlenose dolphin	Bay of Biscay	7	-16.0 ± 0.7	14.5 ± 0.8	Chouvelon et al. in press
Tursiops truncatus	Galicia (NW Spain)	37	-16.5 ± 0.8	12.6 ± 0.9	Fernandez et al. 2011a
	NW Iberian Peninsula	9	-16.5 ± 0.8	12.6 ± 0.9	This study
Striped dolphin	Bay of Biscay	11	-17.5 ± 0.3	11.2 ± 0.9	Chouvelon et al. in press
Stenella coeruleoalba	Channel coast (NE Atlantic)	3	-16.7 ± 0.4	11.0 ± 1.8	Das et al. 2003a
	Irish coast (NE Atlantic)	3	-17.5 ± 0.1	10.8 ± 0.6	Das et al. 2003a
	NW Iberian Peninsula	21	-17.6 ± 0.5	10.8 ± 0.7	This study
Long-finned pilot whale	Bay of Biscay	16	-16.3 ± 0.8	13.2 ± 1.7	Chouvelon et al. in press
Globicephala melas	NW Iberian Peninsula	9	-16.4 ± 0.5	12.2 ± 0.6	This study

CHAPTER III

Long-term ecological segregation among the North West Iberian Peninsula toothed whale species as evidenced using ecological tracers

O Golfiño riscado (Stenella coeruleoalba)

Marine Ecology Progress Series submitted

Long-term ecological segregation among the North West Iberian Peninsula toothed whale species as evidenced using ecological tracers

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ABSTRACT

The waters off the Northwest Iberian Peninsula (NWIP) are characterized by high marine biodiversity and productivity, supported by the nutrients made available by seasonal upwelling, and a relatively narrow (20-35 km wide) shelf. Sixteen species of cetaceans have been recorded in this very restricted area; hence, we hypothesise that niche partitioning allows their coexistence. Thus, this study aims to assess niche segregation among the five main toothed whales inhabiting this area: the common dolphin (*Delphinus delphis*), the harbour porpoise (*Phocoena phocoena*), the bottlenose dolphin (*Tursiops truncatus*), the striped dolphin (*Stenella coeruleoalba*) and the long-finned pilot whale (*Globicephala melas*). We used cadmium (Cd) and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) as ecological tracers to assess degree of segregation in diet/trophic level and in foraging habitat, over various time-scales. Cd in the liver and kidneys and stable isotopes in teeth provide information over a time-scale of several years, while stable isotopes in muscle provide information over a time-scale of several months. Carbon isotopes values highlighted different habitat and Cd results highlighted different feeding between oceanic and neritic species. Moreover, nitrogen stable isotope values suggest different trophic levels and/or foraging habitat within each species. Thus the results reveal long-term segregation among the five toothed whales that coexist in the NWIP. In addition, our results demonstrate the ability of ecological tracers to discriminate ecological niches among closely related species in a common restricted range.

KEY WORDS: Foraging ecology · Niche segregation · Stable isotopes · Cadmium · NW Iberian Peninsula · Toothed whale species

INTRODUCTION

The ecological niche is a complex set of variables structured along three main dimensions: the trophic dimension (which includes diet composition, trophic level and prey quality), the spatial dimension (horizontally and vertically) and the temporal dimension (from dial activity patterns to yearly migratory cycles); temporal and spatial dimensions of the niche include the various environmental influences on distribution. Description of these different dimensions of the ecological niche allows estimation of the degree of inter-specific overlap *versus* segregation within a community (Pianka 1974). This is especially true in the case of sympatric species with similar ecological requirements, which would be expected to compete for resources and whose coexistence requires some degree of habitat and resource segregation (Pianka 1974). The first mechanism that allows for niche differentiation is resource partitioning (trophic dimension). In this case, different species living in the same habitat exploit the resources differently, minimizing the overlap in the size and/or species composition of the diet (Ballance 2002). The second mechanism involves the spatial and/or temporal dimensions of the availability of different resources (which become available at different times of the day or different seasons of the year; Huisman & Weissing 2001).

The study of the ecological niche dimensions as well as of the segregation processes can help to advance our understanding of ecosystem functioning. However, achieving this objective is complex in marine ecosystems due to the difficulty of measuring the ecological niche (Newsome et al. 2007). Consequently, ecologists have recently developed renewed interest in this concept due to technological advances that allow the more correct use of different chemical tracers. Among chemical tracers, the analysis of stable isotopes of C and N in marine organisms can help determine the trophic niche, in particular when combined with conventional approaches (e.g. stomach content analyses, habitat modelling) and when used together with other (bio) chemical analysis of other potential ecological tracers such as trace elements, persistent organic pollutants or fatty acids.

Several studies have investigated niche segregation in marine top predators (e.g. large teleost fish, sharks, seabirds and marine mammals) using conventional and/or chemical tracer methods. Resource partitioning among the community of deep-diving odontocetes from the Bay of Biscay (Northeast Atlantic) has been revealed by stomach contents analysis (Spitz et al. 2011). Niche partitioning in Antarctic pinnipeds has been assessed using analyses of C and N stable isotopes, showing a clear ecological segregation between species (Zhao et al. 2004). Furthermore, in tropical sympatric seabirds, stable isotopes analyses showed important overlaps of feeding niches. This result may be explained by the low productivity of tropical oligotrophic waters, leading these predators to share the same feeding resources (Cherel et al. 2008). In the Southwest Indian Ocean, stable isotopes analyses together with direct observations and behavioural budget were used to reveal resource partitioning in tropical dolphins (Kiszka et al. 2011).

Together with stable isotopes, trace elements, which are generally bioavailable compounds that marine mammals accumulate in their tissues *via* (essentially) food from their environment, offer an integrated measure of all prey assimilated over long periods of time. Depending on the combination of element and tissue used, the period of integration represented by the specific signature or concentration will differ, relating the biological half-life of the given element and its turnover rate in the tissue (Caurant et al. 2009). Thus, the principal advantage of using a combination of different ecological tracers, such as stable isotopes and trace elements, to study inter-specific niche segregation is the range of time-scales that can be covered.

Traditional dietary analysis using faeces, regurgitated food of living animals and the stomach contents of dead animals (i.e. from strandings or by-catch), provides information on the food ingested (and not assimilated) over a very short-time scale (see e.g. Santos et al. 2001) and can thus reveal trophic segregation only over the duration of the study. In contrast, ecological tracers can provide information over periods ranging from several weeks (e.g. fatty acids in blubber, stable isotopes in liver), to several months (e.g. stable isotopes in muscle) or even over the life span (e.g. tracers in hard tissues with no turnover such as teeth) (Hobson & Clark 1992, Hobson & Sease 1998, Nordstrom et al. 2008).

Ecological tracers can be viewed as proxies of habitat choice, feeding habits and trophic position. Thus the relative abundance of heavy to light carbon isotopes ($\delta^{13}C$) has been used to discriminate between habitats where phytoplankton is the only source of organic carbon (pelagic and offshore habitats) and those where macrophytes are a significant source of organic carbon (vegetated inshore and benthic habitats) (DeNiro & Epstein 1978, Fry 2006). The relative abundance of heavy to light nitrogen isotopes ($\delta^{15}N$) is commonly taken as an indicator of trophic level (Cabana & Rasmussen 1996) because its abundance increases from prey to predator due to the preferential excretion of the light isotope (Hobson 1999). Considering trace elements, cephalopods have been shown to be the major vector of Cd for predators, especially at high latitudes (Bustamante et al. 1998) and in the oceanic area (Bustamante et al. 1998, Lahaye et al. 2005). Cd has been used as a tracer of diet, e.g. in grey seals from the Faroe Islands (Bustamante et al. 2004) and the feeding zone used by consumers, e.g. common dolphins in the Bay of Biscay (Lahaye et al. 2005).

The Northwest of the Iberian Peninsula (NWIP) is at the northern limit of the East Central Atlantic upwelling system, characterized by high diversity and productive fisheries, and representing important cetacean habitat, where at least 20 species of marine mammals (16 cetaceans and 4 pinnipeds) have been recorded and where the recorded rate of stranding is one of the highest in Europe (López et al. 2002). The most abundant species of cetaceans are common dolphin (*Delphinus delphis*) and, in the coastal zones, bottlenose dolphin (*Tursiops truncatus*). Other species present in the area include harbour porpoise (*Phocoena phocoena*), striped dolphin (*Stenella coeruleoalba*), Risso's dolphin (*Grampus griseus*) and long-finned pilot whale

(*Globicephala melas*) (Cendrero 1993, López et al. 2002, 2004). The continental shelf in this area is relatively narrow, being only 20-35 km wide. The permanent presence of several toothed whale species within such a restricted area suggests the existence of mechanisms allowing the fine-scale partitioning of habitats and/or resources.

In this context, the aim of this work was to characterize habitat and resource partitioning among five of the most abundant toothed whale species living in sympatry in this area, using stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) and Cd in different tissues (muscle, tooth, liver and kidney) as ecological tracers. We hypothesized that each toothed whale species occupies its own ecological niche, defined by differences in at least one of the ecological tracers used. In relation to the trophic dimensions of the ecological niche, Cd and $\delta^{15}N$ values were used to elucidate dietary preferences and trophic position in the food web, respectively (Bustamante et al. 1998, Hobson 1999). The trophic positions of these five toothed whales and of their main prey in the NWIP food web were gathered from a recent published work (Méndez-Fernandez et al. 2012). The $\delta^{13}C$ isotope values were used to elucidate habitat use (offshore *vs* inshore, pelagic *vs* benthic), one of the spatial dimensions. The temporal dimension of this investigation was focused on common dolphin, due to its high representation in the sampling. Finally, because different animal tissues incorporate the ecological tracers in their food at different rates, they can integrate information about the ecological niche over different time periods. We therefore compare results from tissues with different turnover times in order to evaluate which degree of ecological niche segregation *versus* overlap they revealed over different time-scales.

MATERIALS AND METHODS

Study area and sampling

Fieldwork was carried out in the North West of the Iberian Peninsula (NWIP), from Northern limit of the Galician coast (43° 31' N, 7° 2' W) to Nazaré on the Portuguese coast (39° 36' N, 9° 3' W; Figure 1.4). Stranded cetaceans were attended by experienced personnel of the Galician (*Coordinadora para o Estudo dos Mamiferos Mariños*, CEMMA) or Portuguese (*Sociedade Portuguesa de Vida Salvagem*, SPVS) stranding networks. Animals were identified to species, measured, sexed and, if the state of preservation of the carcass allowed it, full necropsies were performed and samples collected following a standard protocol defined by the European Cetacean Society (after Kuiken 1991). Samples collected between 2004 and 2008 were used for this study, and muscle, teeth, liver and kidney tissues were sampled, whenever possible, from stranded and by-caught individuals of five toothed whales species: common dolphin, bottlenose dolphin, harbour porpoise, striped dolphin and long-finned pilot whale (see Table 3.1). Muscle samples are the same as previously analyzed in a recently published work (Méndez-Fernandez et al. 2012). The common dolphin was the best represented species, as a result of the high number of individuals by-caught in the Spanish and Portuguese fisheries. After the necropsies, all the tissue samples were stored at –20°C until being processed in the laboratory.

Age determination

At least 5 teeth were collected from each sampled individual, selecting the least worn/damaged and least curved teeth, to ensure sufficient material for replicate preparations. Teeth were preserved frozen or in 70% alcohol and age was determined by analysing growth layer groups (GLGs) in the dentine of teeth, following adapted methods based on Hohn and Lockyer (1995), Lockyer (1993) and Rogan et al. (2004). Teeth were decalcified and sectioned using a freezing microtome. The most central and complete sections (including the whole pulp cavity) were selected from each tooth, stained with Mayer's haematoxylin (modified by Grue) and 'blued' in a weak ammonia solution, mounted on glass slides, and allowed to dry. GLGs were counted under a binocular microscope. All readings were initially made blind (without access to individual biological data) and replicate counts were made by at least two readers.

Stable isotope analyses

Before isotopic analyses, muscle and tooth samples were freeze-dried and then ground into a fine powder. Since lipids are highly depleted in ^{13}C relative to other tissue components (DeNiro & Epstein 1978), they were extracted from muscle tissues using cyclohexane. To this end, an aliquot of approximately 100 mg of muscle powder was agitated with 4 mL of cyclohexane for 1h. Next, the sample was centrifuged for 5 min at 4000 g, and the supernatant containing the lipids was discarded. Then, the sample was dried in an oven at 45°C for 48 h, and 0.35 ± 0.05 mg subsamples of lipid-free dry powder were finally weighed in tin capsules for stable isotope analyses. Any sample for δ^{13}C analysis that may contain carbonates must be acidified and carbonates can be removed on the dry powder. Thus, carbonates were removed from tooth samples by digesting the powder with approximately 1 mL of a 4 M-hydrochloric acid solution at 45°C for 48 h. The digested contents were taken up in milli-Q ultrapure quality water, homogenized and freeze-dried at −80°C for 2 days. Finally, 1.45 ± 0.05 mg subsamples of each homogenized carbonate-free dried dental sample were weighed into tin capsules.

These analyses were performed with an elemental analyzer coupled to an Isoprime (Micromass) continuous-flow isotope-ratio mass spectrometer (CF IR-MS). The results are presented in the usual δ notation relative to Pee Dee belemnite marine fossil limestone for δ^{13}C and atmospheric N_2 for δ^{15}N, in parts per thousand (‰). Replicate measurements of internal laboratory standards (acetanilide) during each run indicate measurements errors < 0.2 ‰ for both δ^{13}C and δ^{15}N.

Trace element analyses

The equipment used in the sample processing was cleaned, and subsequently decontaminated for 24 h in a solution composed of 35 ml HNO_3 (65%) and 50 mL HCl (36%) for 1 L of Milli-Ro quality water. Fresh samples were freeze-dried, ground to powder and then each sample was treated in duplicate.

For Cd analysis, aliquots of the liver and kidney samples (0.1 - 0.3g) were digested with 6ml 67-70% HNO_3 and 2 mL 34-37% HCl. Acid digestion of the samples was carried out overnight at room temperature by using a Milestone microwave oven (30 min with constantly increasing temperature up to 120 °C, then 15 min at this maximal temperatures). Each sample was completed to 50 mL with milli-Q water. For the samples with a weight < 0.1g, the mixture was 3mL 67-70% HNO_3 / 2mL 34-37% HCl and the milli-Q water complement was 25mL. Elements were analysed using a Varian Vista-Pro ICP-OES and a Thermo Fisher Scientific XSeries 2 ICP-MS. Three control samples (two CRMs and one blank) treated and analysed in the same way as the samples were included in each analytical batch. CRMs were DOLT-4 (dogfish liver; NRCC) and TORT-2 (lobster hepatopancreas; NRCC) and the detection limit was 0.15 µg/g dry wt (weight).

Data treatment

Cd concentration data showed a marked departure from normality which was substantially improved by log transformation. The distribution of $\delta^{13}C$ values (at least for model residuals) was approximately normal and no transformation was needed. Although $\delta^{15}N$ values were not exactly normally distributed, transformation did not improve the distribution and untransformed data were therefore used in analysis.

Differences in Cd concentrations (µg/g wet weight, w.wt) and stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) between species were tested using ANOVA (analysis of variance) with Tukey tests. To investigate the possible role of confounding factors, notably age and season but also interannual differences, additional analysis was carried out using generalized additive models (GAMs).

For common dolphin, Gaussian GAMs were fitted to stable isotopes values and Cd concentrations (log-transformed) and to identify age, seasonal and interannual trends. In these

GAMs, age was considered as a continuous explanatory variable, while the year and the season of sampling of individuals were treated as categorical explanatory variables. The age smoother was constrained to take a relatively simple form, avoiding overfitting, by setting k = 4. To test for significant interactions between age-season and age-year effects, models were fitted both with single age smoothers and with separate smoothers for each season or age and the two versions compared using an F test. If the seasonal effect was significant, we also explored using month instead of season as an explanatory variable, fitting it as a smoother, to provide greater detail on the form of the seasonal pattern. The general form of the model performed for each tracer was then:

Tracer value ~ s(age, k = 4) + s(month, k = 4) + year [+ interaction terms]

In principle this approach could be extended to species comparisons, testing species differences using models such as the following, taking into account the fact that age effects and seasonal patterns are likely to differ between species (i.e. that there are species-age and species-season interaction):

Tracer value ~ species + s(age, k = 4, by = as.factor(species))

\+ s(month, k = 4, by = as.factor(species)) + year [+ other interaction terms]

In practice, because sample sizes were small for most species, full models could not be tested. However, relationships between tracers and explanatory variables found in common dolphins were used as a guide for the construction of species comparison models, e.g. if the common dolphin model contained an age effect, this structure was used in the all species model.

For each GAM performed, the best model was found using backwards selection, removing the least important non-significant terms sequentially until all remaining effects were significant. Final selection of the “best model” was based upon the Akaike Information Criterion (AIC). Final models were checked for normality and trends in residuals and for outliers and other influential data points (Zuur et al. 2007).

Statistical analyses mentioned above were performed using R version 2.11.1 (R Development Core Team 2010) and BRODGAR 2.5.1 statistical software.

RESULTS

Age values (mean ± SD) and range together with the number of samples available by tissue and species are summarized in Table 3.1.

Cadmium concentration in kidney

Concentrations of Cd were higher in the kidney than in the liver for all species and values (mean ± SD) are summarized in Table 3.2.

ANOVA confirmed that Cd concentrations (µg/g w.wt, log-transformed) in kidney varied significantly between species (F = 5.31, $P < 0.001$), being significantly higher in striped dolphin than in common dolphin and harbour porpoise and significantly higher in pilot whale than in harbour porpoise (Tukey tests). The final GAM for Cd concentrations (µg/g w.wt, log-transformed) in the kidney of common dolphins explained 37.9% of deviance (N = 96, AIC = 133.6). There was no effect of season but the effect of age was highly significant ($P < 0.0001$, d.f. = 2.81). Cd concentrations increased with increasing age, although with a plateau in animals after around 10 years (Figure 3.1a).

A GAM model for all species which included separate age-smoothers for all species performed better than one assuming a common age smoother across all species (F test, $P < 0.0001$) and explained 47.7% of deviance. The age effect was significant for all species except for bottlenose dolphin ($P = 0.060$) showing different trends (Figure 3.2). Significant between species variation is confirmed ($P < 0.0001$) with pilot whale, striped dolphin and common dolphins all showing higher age-corrected accumulation of Cd in the kidney than harbour porpoise and striped dolphin having a higher concentration than common dolphin.

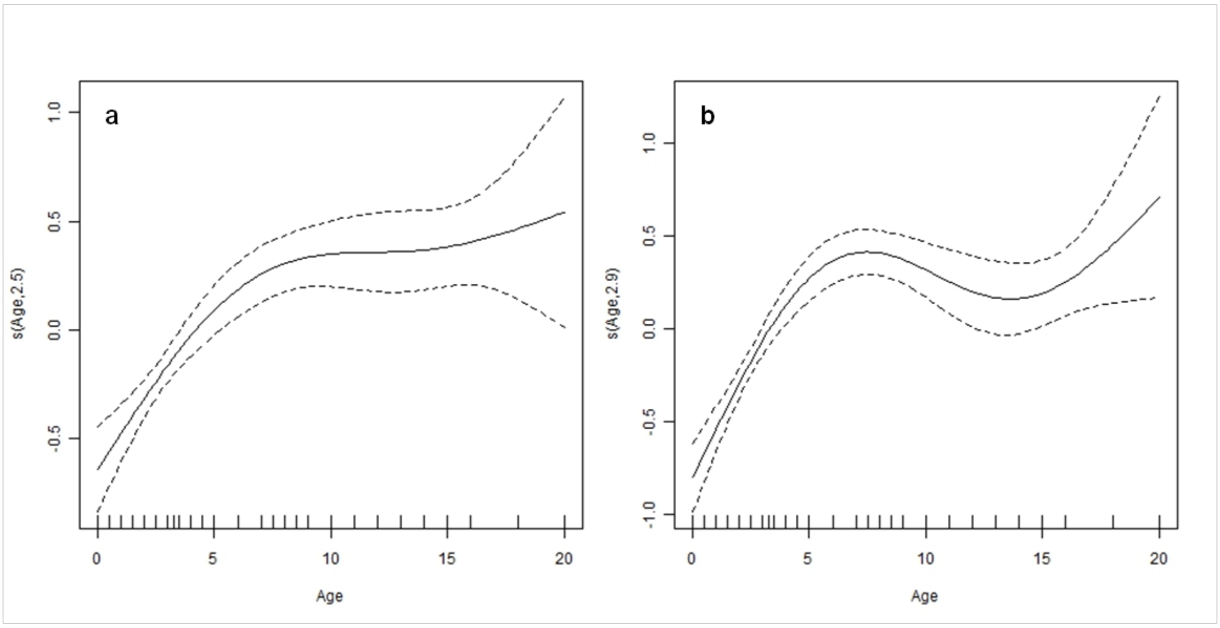

Figure 3.1 Smoothers for effects of age on cadmium concentrations (µg/g w.wt) in kidney (a) and in liver (b) of common dolphin *Delphinus delphis*. The y-axis shows the contribution of the smoother to the predictor function (in arbitrary units). Smoothers illustrate the partial effect of age, i.e. the effect of age once the effects of all other explanatory variables in the model have been taken into account. Tick marks in x-axis represent sampled toothed whales. Dashed lines represent 95% confidence bands for the smoothers.

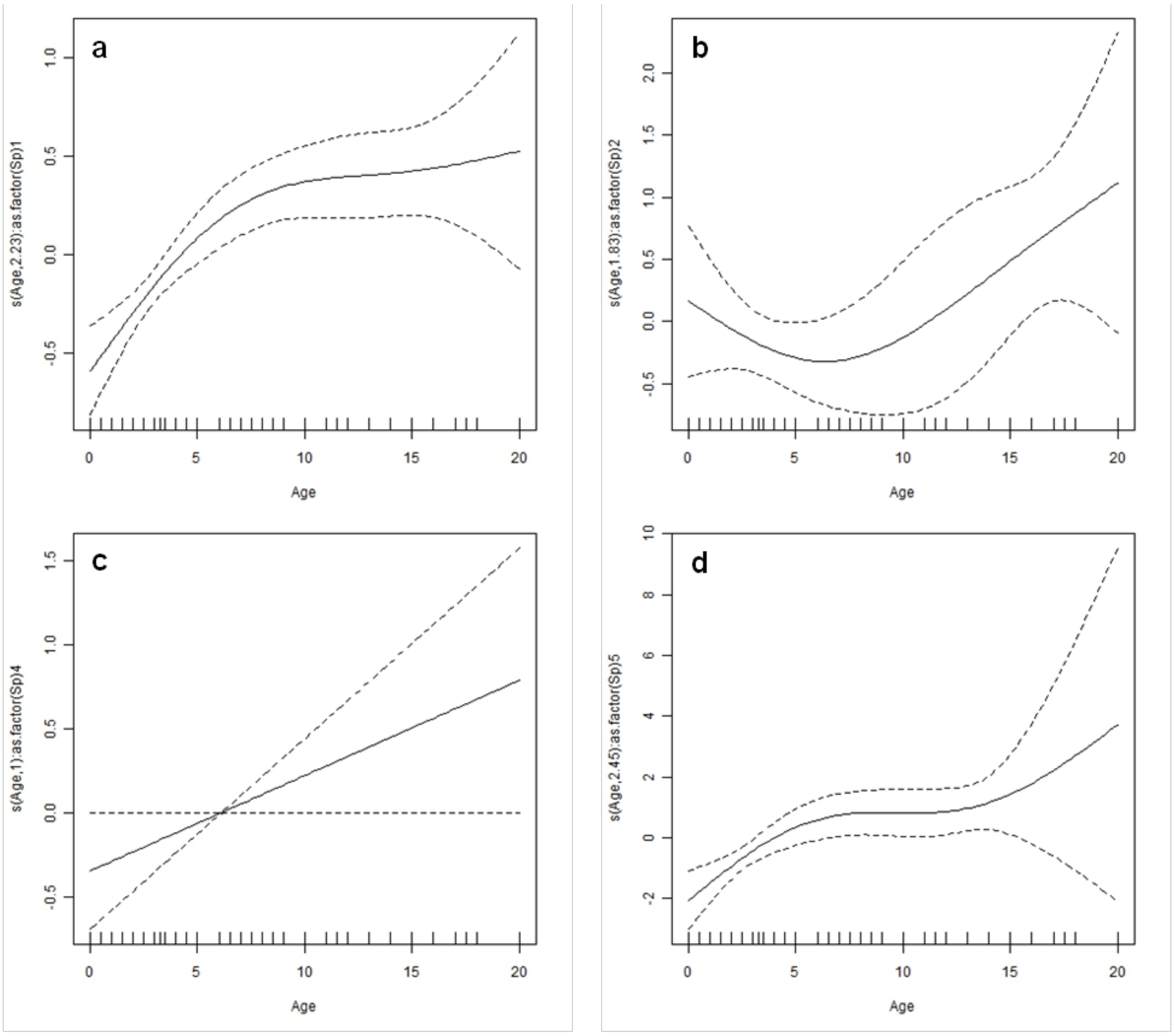

Figure 3.2 Smoothers for effects of age on cadmium concentrations in kidney (µg/g w.wt) of common dolphin *Delphinus delphis* (a) harbour porpoise *Phocoena phocoena* (b) striped dolphin *Stenella coeruleoalba* (c) and long-finned pilot whale *Globicephala melas* (d). The y-axis shows the contribution of the smoother to the predictor function (in arbitrary units). Smoothers illustrate the partial effect of age, i.e. the effect of age once the effects of all other explanatory variables in the model have been taken into account. Tick marks in x-axis represent sampled toothed whales. Dashed lines represent 95% confidence bands for the smoothers.

Cadmium concentration in liver

In liver, Cd concentrations also varied significantly between species (F = 13.85, *P* < 0.0001), being significantly higher in striped dolphin than in all the other species except pilot whale, significantly lower for harbour porpoise than in all the other species except bottlenose dolphin and significantly higher in pilot whale than in common dolphin (Tukey tests). The final model for Cd in liver of common dolphin explained 47.4% of deviance (N = 95, AIC = 110.3). Cd concentrations in liver increased with increasing age (d.f. = 2.99, *P* <0.0001) up to around age 7 (Figure 3.1b). As for Cd in kidney, seasonal and between-year differences were non-significant.

The final GAM for Cd concentrations in liver for all species included separate age smoothers for all species and explained 52.4% of deviance (N = 143, AIC = 253.9). The age effects (not illustrated) were significant for all the species except for striped dolphin and pilot whale. Again, differences between species were highly significant (*P* < 0.0001). Age-corrected concentrations were higher in pilot whale and striped dolphin than in common dolphin or harbour porpoise, and higher in common dolphin than in harbour porpoise.

Stable isotopes: $\delta^{13}C$

A summary of $\delta^{13}C$ and $\delta^{15}N$ values in the different tissues (muscle and tooth) of the five toothed whales from the NWIP is presented in Table 3.2.

$\delta^{13}C$ values (‰) in muscle and tooth varied significantly between species, as shown by ANOVA analysis (F = 11.10 and 10.91, respectively, *P* < 0.0001). Striped dolphin showed significantly lower $\delta^{13}C$ values in both muscle and teeth (Tukey tests) compared to all other species, except for common dolphin in teeth. Common dolphin also showed significantly lower $\delta^{13}C$ values than harbour porpoise in both muscle and teeth, and lower $\delta^{13}C$ values than pilot whale and bottlenose dolphin in teeth (Tukey tests) (Figure 3.3).

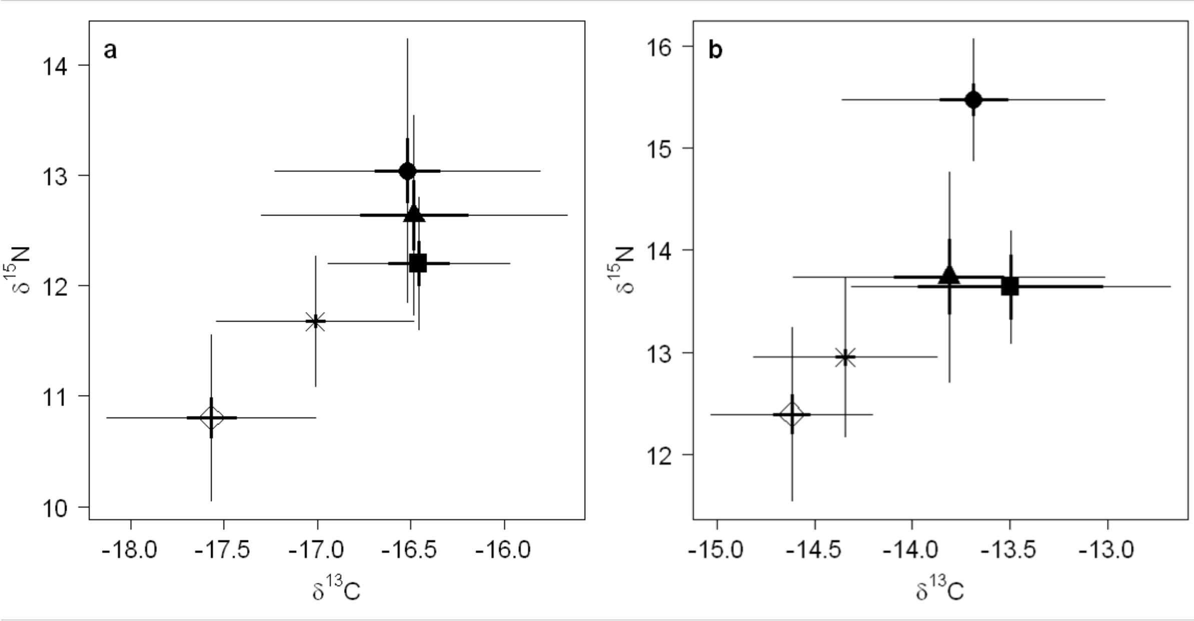

Figure 3.3 Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios isotope ratios in muscle (a) and teeth (b) of striped dolphin *Stenella coeruleoalba* (◊), common dolphin *Delphinus delphis* (×), long-finned pilot whale *Globicephala melas* (■), bottlenose dolphin *Tursiops truncatus* (▲) and harbour porpoise *Phocoena phocoena* (●) from the study area. Data are mean ± SD (‰) and standard error of the mean (SEM in bold).

The final GAM for $\delta^{13}C$ values (‰) in the muscle of common dolphin explained 26% of deviance (N = 104, AIC = 139.9). There was no effect of age but the effect of season was highly significant ($P < 0.0001$, d.f. = 2.3), with the highest values in April and the lowest in September (Figure 3.4a). For $\delta^{13}C$ in teeth of common dolphin the final GAM explained 26.5% of deviance (N = 94, AIC = 109.51). There was again no effect of age but significant effect of season, which was less prominent than in muscle ($P < 0.01$, d.f. = 2.3) but followed a similar trend (Figure 3.4b).

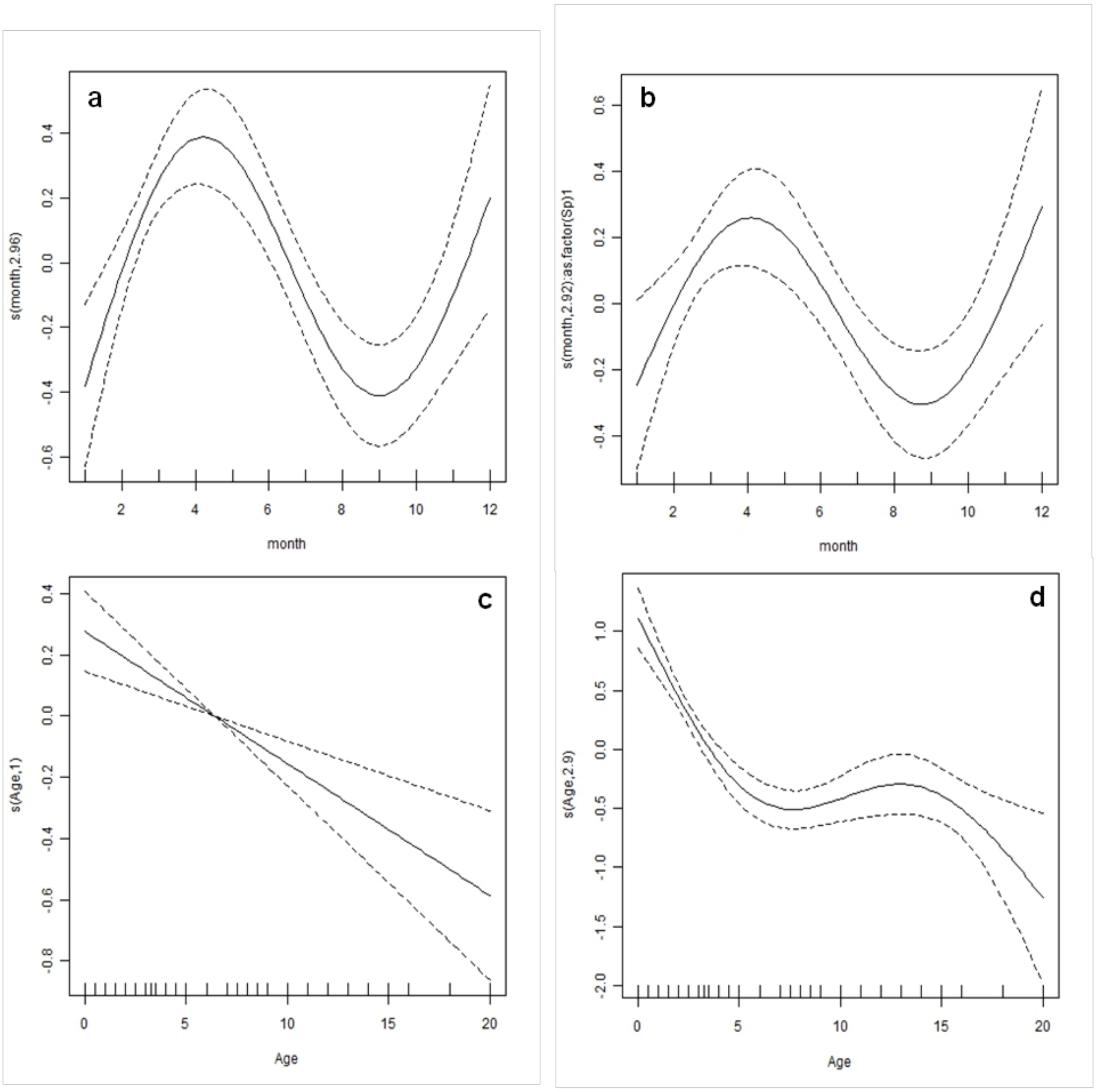

Figure 3.4 Smoothers for effects of season on δ^{13}C values in muscle (a) for effects of season on δ^{13}C values in teeth (b) for effects of age on δ^{15}N values in muscle (c) and for effects of age on δ^{15}N values in teeth (d) of common dolphin *Delphinus delphis*. The y-axis shows the contribution of the smoother to the predictor function (in arbitrary units). Smoothers illustrate the partial effect of season or age once the effects of all other explanatory variables in the model have been taken into account. Tick marks in x-axis represent sampled toothed whales. Dashed lines represent 95% confidence bands for the smoothers.

GAM results for δ^{13}C in muscle of all species confirmed clear differences between species and that the effect of season also differed between species, with the seasonal trends for common dolphin, harbour porpoise and bottlenose dolphin all being statistically significant (Figures 3.4a & 3.5a,b). The model explained 45.7% of deviance. Seasonally adjusted δ^{13}C in muscle was higher in pilot whale, porpoise and bottlenose dolphin than in common or striped dolphin, and higher in common dolphin than in striped dolphin. The GAM for δ^{13}C in teeth gave similar results, although deviance explained was only 39.2%. Between-species differences in seasonally adjusted δ^{13}C in teeth differed only in that pilot whale was statistically indistinguishable from the other species due to the very small sample size available for pilot whale teeth (N = 3) (Figure 3.5c).

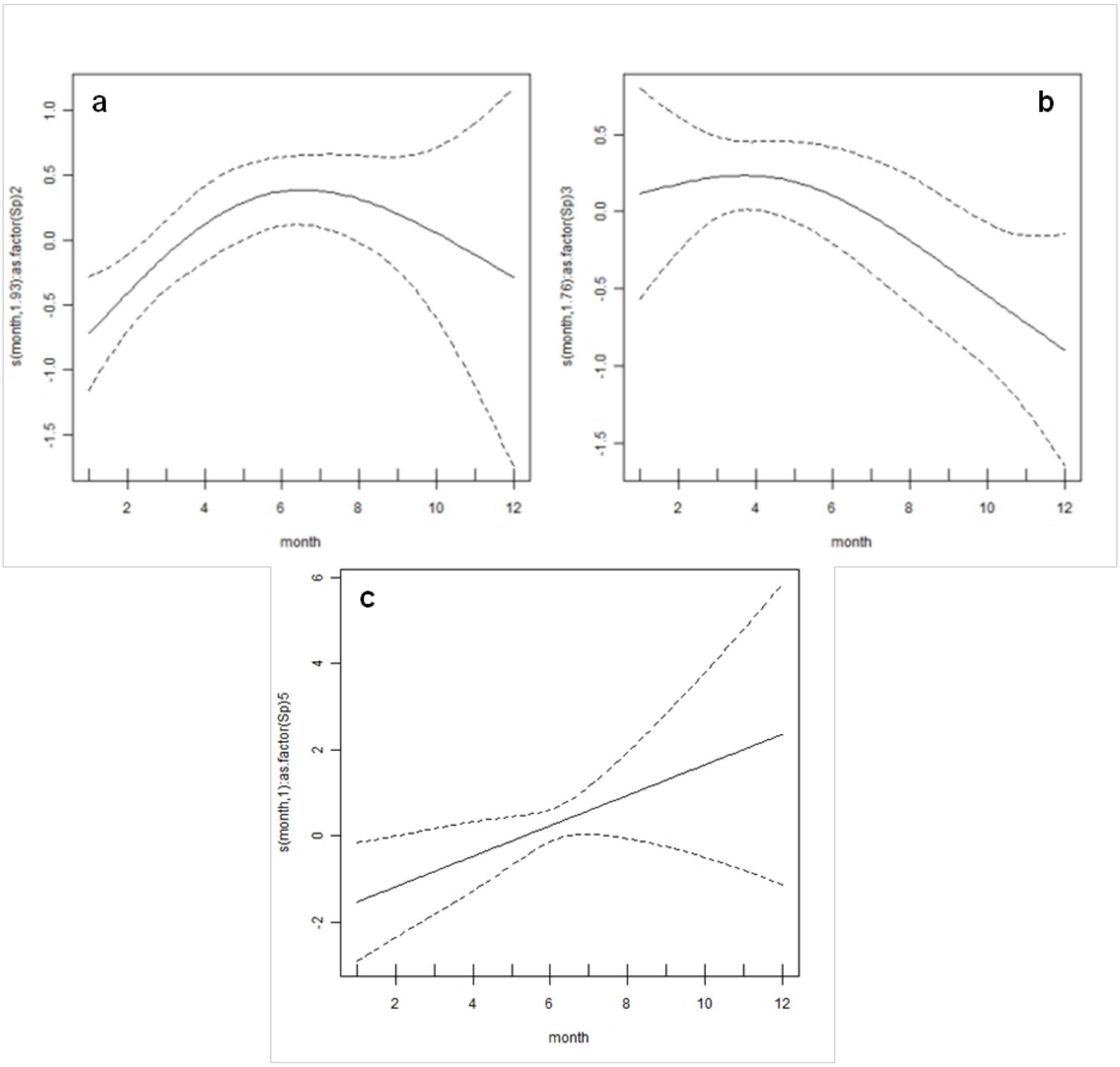

Figure 3.5 Smoothers for effects of δ^{13}C values in muscle (‰) of harbour porpoise *Phocoena phocoena* (a) bottlenose dolphin *Tursiops truncatus* (b) and in teeth of long-finned pilot whale *Globicephala melas* (c). The y-axis shows the contribution of the smoother to the predictor function (in arbitrary units). Smoothers illustrate the partial effect of month, i.e. the effect of month once the effects of all other explanatory variables in the model have been taken into account. Tick marks in x-axis represent sampled toothed whales. Dashed lines represent 95% confidence bands for the smoothers.

Stable isotopes: $\delta^{15}N$

ANOVA confirmed that $\delta^{15}N$ values (‰) in muscle and tooth also varied significantly between species (F = 25.92 and 40.61, respectively, $P < 0.0001$), being significantly lower in striped dolphin than in all the other species, except in teeth of pilot whale, and significantly higher in teeth of harbour porpoise than in all the other species (Tukey tests). Finally, $\delta^{15}N$ values in muscle of common dolphin are significantly lower than in bottlenose dolphin (Tukey tests) (Figure 3.3).

The final GAM for $\delta^{15}N$ values in muscle of common dolphin explained 19.7% of deviance (N = 97, AIC = 151.9). There was no effect of season or year but a strong negative and linear effect of age (as for Cd concentrations) ($P < 0.001$, d.f. = 1.0). $\delta^{15}N$ values decreased with increasing age (Figure 3.4c). Finally, in teeth of common dolphin the final GAM model explained 50.7% of deviance in $\delta^{15}N$ (N = 94, AIC = 161.2), with no effect of season but a strong effect of age ($P < 0.0001$, d.f. = 2.3). Here, $\delta^{15}N$ values decreased until an age of around 7 years, after which they levelled off. Although there is a suggestion of a further decrease after 12 years of age, confidence limits for older animals are too wide to confirm a trend (Figure 3.4d).

GAM results for the all-species analysis of $\delta^{15}N$ in muscle indicated that a model with separate age-smoothers for each species was not a significant improvement on a model with a common smoother for age. Although there were some influential outliers ("hat" values approaching 1.0), using separate smoothers and/or transformation of $\delta^{15}N$ produced no marked improvement in fit. The final model therefore contained a significant age effect ($P < 0.0001$, d.f. = 2.8), depicting a decline in $\delta^{15}N$, at least until age 7) and also a significant species effect ($P < 0.0001$). It explained 52.3% of deviance. Harbour porpoise, pilot whale and bottlenose dolphin all had higher $\delta^{15}N$ values than common dolphin and striped dolphin, while values for common dolphin were also higher than for striped dolphin.

Finally, GAM results for $\delta^{15}N$ in teeth of all species, the best model included separate age effects for all species (with these being significant for all species except the pilot whale) and a species effect ($P < 0.0001$), explaining 77.3% of deviance. Although there were some influential data points, the distribution of residuals appeared to be satisfactory. $\delta^{15}N$ values were higher in porpoise than in pilot whales and common dolphins, while all three of these species had higher values than striped dolphin.

DISCUSSION

General comments

The ecological tracers used in this work were selected for their ability to document the main dimensions of the ecological niche along which segregation might occur in this particular restricted geographical area. Thus, the chemical elements analyzed allow discrimination of variation in foraging habits and broad-scale spatial distribution. This potentially includes both differences between species and variation within species, e.g. due to seasonal movements in common dolphin:

1) Cd concentrations indicated the main type of prey in the diet, revealing significantly differences between those cetacean species that mainly feed on fish (exhibiting the lowest Cd concentrations) and those that mainly feed on cephalopods (exhibiting higher Cd concentrations);

2) Carbon isotope values can indicate differences in the main habitats of the species, with a gradient from oceanic to coastal habitats and/or benthic to pelagic ecosystems. Values were depleted in species preying on oceanic prey;

3) Nitrogen isotope values allow determination of differences between the trophic positions of the cetacean species.

Overall, the main finding is that none of the individual ecological tracers examined reveals complete ecological segregation amongst the five species studied whereas the integration of results from all these tracers does indicate segregation of all five species (Figure 3.6).

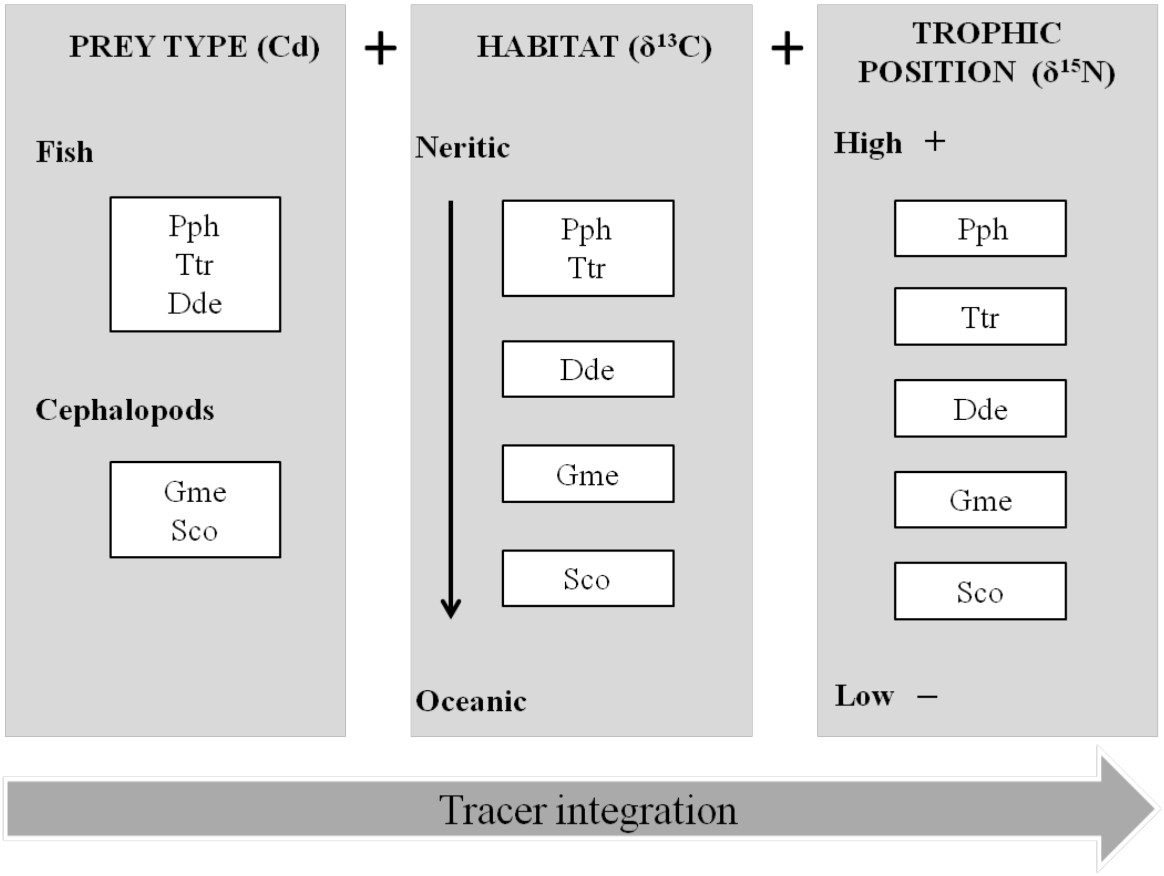

Figure 3.6 Conceptual scheme of foraging niche segregations obtained by the sum of the information given by ecological tracers' in the long-term and in the five toothed whale species. Common dolphin *Delphinus delphis* (Dde), harbour porpoise *Phocoena phocoena* (Pph), bottlenose dolphin *Tursiops truncatus*(Ttr), striped dolphin *Stenella coeruleoalba* (Sco) and long-finned pilot whale *Globicephala melas* (Gme).

Identifying the limitations of the study is necessary to delineate its range of validity. Sampling from stranded individuals does not often allow examination of inter-annual or seasonal variation since, for all but the most frequently stranded species (i.e. in the present study, for all species except common dolphin), it is very unlikely that sufficient individuals will be obtained in all seasons over several consecutive years. Therefore, the sampling in this study limited the investigation of the temporal dimension (e.g. seasonality and between-year differences) to the common dolphin. A further consideration is that all the ecological tracers are mainly transmitted *via* food and it is only differences in tracer signatures that are really informative, since similar signatures may in fact sometimes result from different combinations of different prey.

Resource partitioning inferred from Cd concentrations

Elevated concentrations of Cd in marine mammals are usually ascribed to a diet in which cephalopods and /or crustaceans predominate (Honda et al. 1983, Das et al. 2000, Bustamante et al. 2004). Cd is mainly bioaccumulated in the digestive gland of cephalopods, which contains up to 98% of the total body Cd content in some cephalopod species, and exhibits very slow Cd turnover (Bustamante et al. 2002a,b). The high bioavailability of Cd in the digestive gland indicates a high potential for the trophic transfer of this metal to upper level predators such as marine mammals (Bustamante et al. 2002a). On the other hand, it is also well-established that the cephalopods with higher Cd concentrations are those from oceanic waters (Bustamante et al. 1998, Lahaye et al. 2005). Therefore, Cd concentrations in an organism would be more closely related to the specific metal body burden in their prey (in relation to geographical variability) than to the relative position of these prey in the trophic web. Also, it must be pointed out that, when looking for inter-specific differences, other factors such as allometric effects or differential longevity among species must be considered (Sanpera et al. 1996).

Differences in Cd concentrations between different areas have already been reported in several marine mammal species. As an example, in common dolphin from the Bay of Biscay, the consumption of cephalopods appeared to be a major route of exposure to Cd and, in a previous study, the use of Cd as a tracer confirmed the existence of dietary segregation between oceanic and neritic common dolphins in the long-term scale (Lahaye et al. 2005). In the present study, Cd concentrations in both liver and kidney differentiated pilot whale and striped dolphin from the other three species, which exhibited lower Cd concentrations (Table 3.2). High Cd concentrations in pilot whale tissues are consistent with their teuthophagous feeding habits. In the NWIP this species mainly feeds on neritic and/or benthic cephalopods such as common and curled octopuses (González et al. 1994, Pierce et al. 2007). However, striped dolphin feeds on both cephalopods and fish. In the NWIP, the cephalopods eaten comprise mainly *Gonatus* sp. and ommastrephids and the fish species taken include blue whiting *Micromesistius poutassou*, scads *Trachurus trachurus* and sand smelt *Atherina presbyter* (Santos et al. 2007b). In addition, this species also takes crustaceans in the NWIP, specifically pelagic shrimp species (Jose Cedeira Personal communication), and oceanic fish such as silvery lightfish (*Maurolicus muelleri*). This fish consumption would result in lower Cd exposure of striped dolphin in comparison with pilot whales and therefore could explain their lower Cd concentrations (Table 3.2) even though no significant differences were found between them over medium and long time scales (i.e. Cd in liver and Cd in kidney, respectively). Therefore, the high Cd concentrations obtained in this study for pilot whale and striped dolphin are consistent with their important cephalopod consumption, in the medium and long-term, and the mainly oceanic distribution of striped dolphins (González et al. 1994, Fernández de la Cigoña & Oujo 1999, Forcada 2002, Spitz et al. 2006).

Conversely, bottlenose dolphin and harbour porpoise are mainly fish feeders and, in the case of harbour porpoise, mainly a coastal species (Read 1998, Fernández de la Cigoña & Oujo 1999, Santos et al. 2004b, 2007a,b). This can explain the significant differences found between harbour porpoise and the main cephalopods feeder species over a medium time-scale and between porpoise and all the other species, with the exception of bottlenose dolphin, over a long time-scale. Probably the lack of significant difference compared to bottlenose dolphin is due to their also feeding mainly on fish. Nevertheless, both species showed the lowest Cd concentrations over medium and long time-scales, there being no significant difference between them, consistent with the idea that cephalopods are the major route of exposure to Cd in marine mammals.

The higher hepatic and renal Cd concentrations recorded for common dolphin in comparison with the mainly piscivorous species strongly suggest that this species has a higher proportion of cephalopods in its diet and a more oceanic distribution (Evans 1994, Santos et al. 2004a; Table 3.2). However, significant differences were found between common dolphin and pilot whale, over a medium time-scale, and with striped dolphin, over both medium and long time-scales, meaning that segregation can exist among these species even though common dolphin also consumes some cephalopod species. At the same time, Cd concentrations of common dolphin, both in the medium and long-term, increased with age up to around 10 years old, after which concentrations reached a plateau (Figure 3.1). Similar results are well-documented in the literature for several different species of marine mammals (Caurant et al. 1994, Bustamante et al. 2004, Lahaye et al. 2006) and this pattern seems to be the result of an equilibrium between absorption and excretion in the animals and not due to a shift in diet with time.

Spatial and trophic segregation inferred from stable isotopes

For many years systematic boat- and land-based surveys have been carried out in the study area, as well as several studies on distribution and habitat preferences of cetaceans in the NWIP (López et al. 2004, Pierce et al. 2010, Spyrakos et al. 2011). These studies tried to relate coastal and at-sea sightings with environmental parameters (e.g. depth, SST and chlorophyll-a) to improve understanding of spatio-temporal trends in cetacean distribution in the NWIP. $\delta^{13}C$ values are well known to be proxy of habitat and can reflect the habitat preferences of the species (i.e. oceanic *vs* coastal or pelagic *vs* benthic). Results on $\delta^{13}C$ values obtained in the present study are generally in agreement with the habitat preferences inferred from direct observations and/or from habitat modelling.

The $\delta^{13}C$ values in the tissue analyzed (i.e. muscle and teeth) allowed a good differentiation of the most oceanic from the most coastal species. In results from both tissues, striped and common dolphins exhibited significantly lower carbon isotope ratios compared to the other species. In this area, striped dolphin is most frequently seen over depths of 100-500 m along both the western and northern coasts (López et al. 2002, Spyrakos et al. 2011). This oceanic distribution is consistent with the significantly lower $\delta^{13}C$ values (over medium and long time-scales) seen in the present study which tend to show that, despite the narrowness of the shelf and the ability to this species to shift to feeding on coastal and neritic prey, striped dolphin mainly forage in the oceanic habitats of the NWIP. Common dolphin is the most abundant cetacean in the NWIP (Aguilar 1997, López et al. 2004) and the second most frequently sighted from the coast (Pierce et al. 2010), and is mainly found in waters deeper than 200 m depth (Spyrakos et al. 2011). These characteristics would explain their low and intermediate $\delta^{13}C$ values, between those of striped dolphin and the other toothed whales (Figure 3.3). Despite the habitat overlap between common and striped dolphin on a long-term basis (as showed by the dental $\delta^{13}C$ values), $\delta^{13}C$ values in muscle tend to indicate different foraging habitats on a mid-term basis. In addition, the final GAM for common dolphin revealed marked seasonal variation in their $\delta^{13}C$ values, being more pronounced in a mid-term than in a long-term (as expected since values in muscle would relate more specifically to recent feeding) but following the same trend (Figure 3.4). These seasonal patterns in $\delta^{13}C$ values probably imply movement and might mean that common dolphin feed partly in oceanic and partly in coastal habitats. However, the significantly different Cd concentrations found between common dolphin and striped dolphin reflect that, despite this probably seasonal competition for habitat, they fed on different prey types, with less consumption of cephalopod species by common dolphin.

Based on $\delta^{13}C$ values, bottlenose dolphin, harbour porpoise and pilot whale did not show significant differences from each other over medium and long time-scales and showed the highest $\delta^{13}C$ values of the five species studied (Figure 3.3). Coastal sightings suggest that bottlenose dolphin is associated with coastal areas where the continental shelf is relatively wide and productivity relatively high (Pierce et al. 2010) but this species is also seen in offshore waters (López et al. 2004). Recent studies revealed two ecologically and genetically distinct groups of bottlenose dolphins among strandings in Galicia (northern part of the NWIP) which is consistent with the existence of both inshore and offshore forms of bottlenose dolphin (Fernández et al. 2011a,b). Thus the high variability obtained in our isotopic $\delta^{13}C$ values for bottlenose dolphin can be due to the presence in our sampling of both inshore and offshore individuals (Figure 3.3). Again based on coastal sightings, harbour porpoise tend to be seen where the shelf is narrower while boat-based sightings studies variously suggest association with water depths of 100-200m (Spyrakos et al. (2011) or an average water depth of 90m (CEMMA, unpublished data). Their coastal distribution is confirmed in this study by their high $\delta^{13}C$ values (Figure 3.3), which were significantly different from those of common and striped dolphin, on a medium- and long-term basis.

Although the pilot whale is essentially known as oceanic, it shows some degree of dietary plasticity and can apparently forage successfully in both oceanic and neritic habitats (Spitz et al. 2011), as in common dolphin, which might explain the high muscular and dental $\delta^{13}C$ values obtained for pilot whales in this study. Our results also showed a significant difference in $\delta^{13}C$ values between these two species, suggesting that they probably used different feeding areas.

Nitrogen isotopic signatures are the main source of information on resource utilization and trophic positions (TP) of the marine mammals in the food web. Here the TP inferred from $\delta^{15}N$ values are a useful tool to compare species showing overlapping in $\delta^{13}C$ values, i.e. living in the same habitat. Striped dolphins exhibited the lowest $\delta^{15}N$ values and harbour porpoise the highest (Table 3.2). The low $\delta^{15}N$ values exhibited in muscle and teeth of striped dolphin revealed the low TP of this species (Méndez-Fernandez et al. 2012). The presence of crustaceans and oceanic fish species (which usually showed low TP in the food web) may be the main reason for their low TP. Therefore, striped dolphin is well segregated from the other four species in the trophic dimension as well as in the spatial dimension (Figure 3.3). The higher $\delta^{15}N$ values in common dolphin compared to striped dolphin, over medium and long time-scales, are consistent with its more strongly piscivorous feeding habits. However, these values were still low in comparison with the other toothed whale species (Table 3.2), probably as the consequence of feeding on fish with a low TP, since the most important prey of common dolphin in Galician waters are blue whiting and sardine *Sardina pilchardus* (Santos et al. 2004a) which also showed low TP (Méndez-Fernandez et al. 2012). In addition, age has a negative effect on $\delta^{15}N$ values of common dolphin, suggesting that this species feeds lower down the food web with increasing age (Figure 3.4).

According to its $\delta^{15}N$ values, common dolphin is not significantly different from pilot whale and both species could have largely overlapping foraging ranges. However, pilot whale mainly feeds on cephalopods, as shown by Cd concentrations in this study and previous stomach content analyses (Pierce et al. 2007, Spitz et al. 2011), showed higher TP and also feeds on prey having higher TP than common dolphin (Méndez-Fernandez et al. 2012). Furthermore, pilot whales exhibit some degree of dietary plasticity allowing them to forage successfully in both oceanic and neritic habitats. This dietary plasticity can also explain their relatively high $\delta^{15}N$ values, and high variability therein, obtained in this study, and might result from a distribution which shifts seasonally between oceanic and neritic habitats. In the present study, we have insufficient samples of this species to confirm a seasonal trend in isotopic composition. Data from coastal sightings are also limited but 5 out of 6 sightings of the species during 2003-07 were during the months September to November, which would be consistent with seasonal use of coastal waters (Pierce et al. 2010, unpublished data).

Bottlenose dolphin and harbour porpoise mainly feed on fish, bottlenose dolphin being slightly more generalist than harbour porpoise (Santos et al. 2007a,b). These two species have the highest TP among the five toothed whales studied here (Méndez-Fernandez et al. 2012) which is not surprising since they have a fish-based diet although it is counter-intuitive in the sense that larger predators are expected to eat larger prey (e.g. MacLeod et al. 2006) which in turn would typically have a higher trophic level. On the other hand, the significantly higher $\delta^{15}N$ values obtained in teeth of harbour porpoise (Table 3.2) indicate that they probably feed on prey with a higher TP than those of bottlenose dolphin in the long-term. Based on the analysis of harbour porpoise stomach contents, the most important prey identified were scad (*Trachurus trachurus*), pouting (*Trisopterus luscus*) and garfish (*Belone belone*) (Santos et al. 2004b, 2007b, Pierce et al. 2010). Scad and pouting exhibit high $\delta^{15}N$ values (Méndez-Fernandez et al. 2012) which may help to explain the elevated $\delta^{15}N$ values of harbour porpoise as well as its high TP in the study area. Bottlenose dolphin also mostly feed on fish despite being slightly more generalist. The main prey, in terms of both biomass and number, are blue whiting (*Micromesistius poutassou*) and hake (*Merluccius merluccius*) (Santos et al. 2007a), which exhibit lower $\delta^{15}N$ values than harbour porpoise prey (Méndez-Fernandez et al. 2012). In consequence, consistent with previous studies on diet and the dental $\delta^{15}N$ values from the present study, over a long-time scale niche differentiation in the trophic dimension exists between both species, even though $\delta^{13}C$ isotopic values in the present study failed to show differences.

Time integration of ecological tracers

Ecological tracers were analyzed in tissues with different rates of turnover and results thus gave us access to different periods of integration. However, sampling did not give us the opportunity to study segregation over the whole year and the variations which may occur between seasons, except for the common dolphin. Foraging niche segregation probably depends on the availability of resources in the area. The study area is an upwelling area which is characterized by high biological productivity (Fraga 1981) and where primary production, biomass and phytoplankton species composition are influenced by this hydrographic perturbation and can cause large variations in nutrient availability (González et al. 1979, Tenore et al. 1995). Therefore we expected to find differences in the segregation of these species according to the time of year. Despite the seasonal movements demonstrated for common dolphin and the similar $\delta^{13}C$ values in the long-term, this species is well-separated from striped dolphin by its $\delta^{13}C$ isotopic composition over a medium time-scale, $\delta^{15}N$ isotopic composition and Cd concentrations. Thus, over a long time-scale both species mostly share the same habitat in the NWIP but feed on different resources and with different TP. The other species for which results were highly influenced by the time period of integration is the harbour porpoise. Only dental $\delta^{15}N$ isotopic composition discriminate harbour porpoise from bottlenose dolphin, the most similar species in terms of ecological niche, which means that although both species can share the same coastal habitat and type of prey, they feed on resources at different TP in the long-term. Such foraging segregation might allow their co-existence in NWIP waters.

Conclusions

To sum up, in this work we studied the main three dimensions of the ecological niche of five toothed whale species through the analyses of different ecological tracers in different soft and hard tissues, to test whether there is niche segregation to avoid competition among these five toothed whales in the NWIP waters. The sum of the information given by the long-term ecological tracers showed ecological niche segregation (although a temporal overlap could exist) of the five toothed whales studied. We hypothesize the following conceptual scheme (see Figure 3.6):

1 – Harbour porpoise has a coastal foraging niche with the highest trophic position among the five species.

2 – Bottlenose dolphin has the most similar foraging niche to harbour porpoises but its lower trophic position suggests different targeted prey and/or the use of offshore areas.

3 – Common dolphin and pilot whale can forage in both oceanic and neritic waters as shown by their $\delta^{15}N$ and $\delta^{13}C$ stable isotope ratios but the renal cadmium concentrations revealed a dietary difference (fish-based *vs* cephalopod-based, respectively).

4 – The striped dolphin showed the most oceanic ecological niche.

Consequently, the present work provides useful indications on trophic and habitat preferences in toothed whales from the NWIP and demonstrates that ecological tracers provide a relatively simple way to quantify niche differences over different time-scales, especially when looking at closely related species within a common restricted area. Future work could strengthen these conclusions through improved sampling, especially for the less well-represented species, to ensure adequate study of the temporal dimension, and through an increase in the number of the ecological tracers used.

Table 3.1 Age (mean ± SD, in years) of the toothed whales and number of samples collected per species and tissue in NWIP. Ranges (minimum, maximum) are presented in parentheses.

Species		Number of samples			
	Age	Muscle	Teeth	Liver	Kidney
Common dolphin *Delphinus delphis*	6.12 ± 5.27 (0, 20)	104	99	102	102
Harbour porpoise *Phocoena phocoena*	5.38 ± 6.11 (0, 18)	17	15	15	15
Bottlenose dolphin *Tursiops truncatus*	4.28 ± 2.00 (0.5, 7)	8	8	8	8
Striped dolphin *Stenella coeruleoalba*	4.13 ± 4.99 (0, 15)	18	20	18	18
Long-finned pilot whale *Globicephala melas*	7.39 ± 5.05 (0, 14)	9	3	8	9

Table 3.2 Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios (mean ± SD, ‰) and cadmium concentrations (mean ± SD, µg/g w.wt) in different tissues of the toothed whale species collected along the NWIP. Ranges (minimum, maximum) are presented in parentheses.

Species	$\delta^{13}C$		$\delta^{15}N$		Cd	
	Muscle	Teeth	Muscle	Teeth	Liver	Kidney
Common dolphin	-17.0 ± 0.5	-14.3 ± 0.5	11.7 ± 0.6	13.0 ± 0.8	0.4 ± 0.5	2.3 ± 2.7
Delphinus delphis	(-18.1, -16.0)	(-15.6, -13.4)	(10.1, 13.6)	(11.3, 15.2)	(< 0.01, 2.9)	(< 0.01, 16.4)
Harbour porpoise	-16.5 ± 0.7	-13.7 ± 0.7	13.0 ± 1.2	15.5 ± 0.6	0.1 ± 0.1	2.2 ± 5.3
Phocoena phocoena	(-17.6, -15.1)	(-14.7, -12.4)	(11.5, 17.1)	(14.4, 16.2)	(< 0.01, 0.4)	(0.09, 19.0)
Bottlenose dolphin	-16.5 ± 0.8	-13.8 ± 0.8	12.6 ± 0.9	13.7 ± 1.0	1.2 ± 2.8	5.7 ± 13.8
Tursiops truncatus	(-17.3, -15,2)	(-14.8, -12.3)	(11.3, 14.0)	(11.6, 15.1)	(0.01, 8.2)	(0.15, 39.8)
Striped dolphin	-17.6 ± 0.6	-14.6 ± 0.4	10.8 ± 0.7	12.4 ± 0.8	3.9 ± 3.8	10.3 ± 11.0
Stenella coeruleoalba	(-18.5, -16,5)	(-15.5, -13.8)	(9.8, 12.4)	(10.8, 14.9)	(0.04, 13.2)	(0.1, 40.7)
Long-finned pilot whale	-16.5 ± 0.5	-13.5 ± 0.8	12.2 ± 0.6	13.6 ± 0.5	8.3 ± 8.4	30.0 ± 26.9
Globicephala melas	(-17.3, -15.7)	(-14.4, -13.0)	(11.0, 13.3)	(13.3, 14.3)	(0.01, 22.2)	(< 0.01, 79.3)

CHAPTER IV

Inter-species differences of polychlorinated biphenyls patterns in five toothed whale species from the NW Iberian Peninsula: can PCBs be used as ecological tracers?

A Toniña (Phocoena phocoena)

In preparation for Environmental Pollution

Inter-species differences of polychlorinated biphenyls patterns in five toothed whale species from the NW Iberian Peninsula: can PCBs be used as ecological tracers?

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ABSTRACT

Thirty two polychlorinated biphenyls (PCBs) were determined in the blubber of five toothed whale species stranded or by-caught along the North West of the Iberian Peninsula: common dolphin (*Delphinus delphis*), harbour porpoise (*Phocoena phocoena*), bottlenose dolphin (*Tursiops truncatus*), striped dolphin (*Stenella coeruleoalba*) and long-finned pilot whale (*Globicephala melas*). Differences in the PCB patterns between individual marine mammals of the same species and between those of different species can occur as a result of the capacity to metabolize the congeners and the differences in diet, body condition, age, sex and possibly location. Multivariate analyses were applied on one hand to evaluate the ability of PCB patterns to discriminate between these five sympatric species evaluating their relevance as tracers of diet and/or habitat and on another hand to determine which biological-ecological factors have more influence in their PCB patterns. To this aim the raw data (i.e. PCB concentrations in μg/g lipid weight) were normalised to a single congener, the CB153. Both statistical approaches were greatly in agreement, allowing a well-separation of the five species according to their PCB patterns, and allowing the identification of a group of congeners (i.e. CB157, 137, 114, 149, 118, 158, 183 and 189) as possible main responsible for the discrimination among species. Differing exposures to these contaminants, resulting from different dietary preferences or feeding habitats, would explain the differences found in PCB patterns. However, the influence of different metabolic capacities among the species, as already described in the literature for some marine mammal species, could not be excluded. Contaminants could have thus the potential to be used as tracers for identifying the segregation between these species and to improve our knowledge of their relative sensitivity towards the more toxic PCB congeners.

KEY WORDS: PCBs · Patterns · Bioaccumulation · Tracers · Toothed whales · NW Iberian Peninsula

INTRODUCTION

The North Western Iberian Peninsula (NWIP) is characterised by a relative narrow shelf of 20-35 km wide and 100-200 m depth (Figure 1.4). This area is under the influence of an upwelling-downwelling system, which seems to induce a high productivity of the marine ecosystem at least during part of the year (Fraga 1981). In this area, several cetacean species coexist and are regularly observed which raises the question of the resources partitioning and trophic segregation among them. Previous studies showed a certain degree of spatial segregation with habitat preferences for the most common toothed whale species inhabiting the NWIP waters: the common dolphin (*Delphinus delphis*), the harbour porpoise (*Phocoena phocoena*), the bottlenose dolphin (*Tursiops truncatus*), the striped dolphin (*Stenella coeruleoalba*) and the long-finned pilot whale (*Globicephala melas*) (López et al. 2004, Cabral et al. 2005, Pierce et al 2010, Vingada et al. 2011). Moreover, a recent study revealed an ecological segregation among these five toothed whales in the long-term, through the analyses of several ecological tracers (carbon and nitrogen stable isotopes and cadmium) (Méndez-Fernandez et al. submitted).

Polychlorinated biphenyls (PCBs) include a group of monochloro to decachlorinated compounds with a biphenyl nucleus. There are 209 congeners of PCB because of the 10 possible substitution positions on the nucleus with a chlorine or hydrogen atom (Niimi 1996).

Commercial PCB mixtures are identified by their trade names and have been produced for a wide range of industrial applications according to their properties such as resistance to breakdown by other chemicals (WHO 1976). These properties have largely contributed to the ubiquitous distribution of PCBs in the atmospheric, terrestrial and aquatic environments (Niimi 1996).

In the marine environment PCB patterns change from those of the industrial products as a result of weathering through the metabolic action of organisms, which ingest the contaminants from the food chain or directly from the water column and marine sediments (Danis et al. 2003, 2005a,b). PCBs are hydrophobic substances (e.g. fat-soluble) and in consequence enter the food chain accumulating particularly high levels in adipose tissue of high trophic level species. Marine mammals, occupying the upper trophic levels and possessing large lipid reserves are thus susceptible to considerably bioaccumulate these lipophilic pollutants (Tanabe et al. 1988, 1994, Boon et al. 1994, Niimi 1996).

Due to the number and position of chlorine atoms, PCB congeners do not follow the same metabolic pathways which results in the formation of different metabolites (Letcher et al. 2000) and in differences in accumulation patterns and persistence of PCBs (Boon et al. 1994). This has resulted in the classification of PCBs in several groups as introduced by Boon et al. (1997, 1994), Weijs et al. (2009) and Yordy et al. (2010) and according to the presence or absence of vicinal hydrocarbons in *meta-para* and/or *ortho-meta* positions, as well as the number of *ortho*-Cls. Thus, the following groups were established: Group I, with no vicinal hydrogens (H) (e.g. CB153, 180, 183, 187, 194); Group II, with *ortho-meta* vicinal H and ≥ 2 *ortho*-Cl (e.g. CB99, 128, 138, 170, 157, 137); Group III, with *ortho-meta* vicinal H and ≤ 1 *ortho*-Cl (e.g. CB28, 31, 74, 105, 118, 156); Group IV, with *meta-para* vicinal H and ≤ 2 *ortho*-Cl (CB52, 101, 110, 114, 44, 70, 49) and Group V, with *meta-para* vicinal H and ≥ 3 *ortho*-Cl (CB149, 167).

In consequence, the PCB patterns observed in marine mammals differ from the patterns seen in the technical formulations originally released to the environment (e.g. AROCHLOR) but also from those in their prey (Muir et al. 1988). Moreover, differences in the PCB pattern between individual marine mammals of the same species (i.e. intra-species variability) and between those of different species (i.e. inter-species variability) can occur as a result of the capacity to metabolize the congeners (Boon et al. 1987, Tanabe et al. 1988, Wells & Mckenzie 1994). Specifically, previous studies, which investigated the relative metabolic degradation of PCBs in marine mammals, demonstrated that pinnipeds have higher capacity to metabolize PCBs, especially the high chlorinated ones, than cetaceans (Boon et al. 1992, 1997, Weijs et al. 2009).

These differences seem to be the consequence of difference in cytochrome P450 mediated mono-oxygenase activities. Indeed, these differences were not only found between pinnipeds and cetaceans, but also between different species of the same groups (Goksøyr et al. 1992, Wells & Echarri 1992, Boon et al. 1994). Moreover, toxicological studies have shown that several biological and ecological factors, specific of each individual and species, can affect this metabolization in mammals including marine species and therefore have to be taken into account (Aguilar 1987, Aguilar & Borrell 1994). Thereby, male marine mammals accumulate PCBs throughout their life, but females generally transfer a large part of their PCBs burden to their progeny during gestation and lactation as a result of their lipophilic nature and their biological persistence (Addison & Brodie 1987, Tanabe et al. 1994). Both these processes produce sex- and age-related variations of the patterns of PCB levels which differ 1) between species and/or 2) between populations of a single species (Wells & Echarri 1992, Wells et al. 1996, Borrell & Aguilar 2005). In consequence, these processes need special attention. Furthermore, differences in feeding habits may be especially important because food contributes over 90% of the organochlorine intake by mammals (Campbell et al. 1965). Variation in food composition or in location of feeding grounds can thus produce variations in tissue residue levels among different species due to their different prey preferences. But these variations can also be related with sex and age, since pregnant and lactating females may have different feeding habits compare to immature females and also to males of the same species (Addison & Smith 1974, Tanabe et al. 1982, Aguilar & Borrell 1994). Consequently, all these differences related with biological factors, as age and sex, and ecological factors lead to different PCB exposure of the individuals and therefore differences in the PCB patterns and concentrations in their tissues.

The dissimilarity in the patterns is commonly imperceptible when graphing the data directly from the chemical analysis and the descriptive interpretation is usually convoluted. Therefore, data reduction is essential for identifying patterns more readily and for providing a descriptive overview. Thus multivariate analysis (e.g. principal component analysis and discriminant function analysis) were used by previous studies for detecting differences in PCB patterns between species of marine mammals, birds and other vertebrates but also in marine invertebrates (Schwartz & Stalling 1991, Aguilar et al. 1993, Storr-Hansen et al. 1995, Wells et al. 1996, Boon et al. 1997, Mckenzie et al. 1997, Borrell & Aguilar 2005, Carro et al. 2006). Such statistical techniques can be used to study the relationship between biological and ecological variables, proper to a species, and POP concentrations as was carried out by Pierce et al. (2008) for common dolphin and harbour porpoise through redundancy analysis. Therefore, these techniques can provide specific information on differences in PCB patterns between closely or related species, which often is imperceptible in the raw data (Wells et al. 1996).

Thus, the objectives of this paper was firstly to describe the main sources of variation in PCB patterns between five toothed whale species inhabiting the waters of the NWIP by using multivariate analyses and taking into account age- and sex- related effects as well as information about feeding habits compiled from previous studies. Secondly the objectives were to identify the main congeners and/or PCB patterns that may characterize the species and their food habits, and thus constitute signatures and maybe ecological tracers. However, concentrations of PCBs in blubber of marine mammals are known to vary with age, although in different ways for mature males and mature females (Aguilar 1987). In this study, we restricted our analyses to the sexually immature individuals of both sexes since no bias due to transfer would have to be accounted for in this group. Therefore a total of 32 PCB congeners were analyzed in 72 blubber samples from immature individuals of the five species which were found stranded or were by-caught between 2004 and 2008.

MATERIALS AND METHODS

Study area and sampling

Fieldwork was carried out at the North West 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; Figure 1.4). Stranded cetaceans were attended by experienced personnel of the Galician (Coordinadora para o Estudo dos Mamiferos Mariños, CEMMA) or Portuguese (Sociedade Portuguesa de Vida Salvagem, SPVS) stranding networks. Animals were identified to species, measured, sexed and, if the state of preservation of the carcass allowed it, full necropsies were performed and samples collected following a standard protocol defined by the European Cetacean Society (after Kuiken 1991). Blubber tissue was sampled between 2004 and 2008 from individuals being in a good state of preservation. Among the five toothed whale species the common dolphin was the most represented species as a result of the great number of individuals by-caught in the Spanish and Portuguese fisheries. The blubber samples were taken from the left side in front of the dorsal fin. Complete vertical cross-sections of the samples were collected, to prevent any possible effects of stratification of the blubber. After the necropsies, all samples were removed, wrapped separately in aluminum foil and frozen at –20°C until required for analysis.

At least five teeth were also collected from each sampled individual, selecting the least worn/damaged and least curved teeth, to ensure sufficient material for replicate preparations. Teeth were preserved frozen or in 70% alcohol and age was determined at the IIM (*Instituto de Investigaciones Marinas*) of Vigo. The procedure consisted of counting Growth Layer Groups (GLGs) from teeth sections, assuming that one GLG equals 1 year (Lockyer 1993, Hohn & Lockyer 1995). We referred to Boyd et al. (1999) for the age of sexual maturity for each species. Then a total of 72 animals from the five species were statistically analyzed. The numbers of individuals for each species and sex are summarised in Table 4.2.

Lipid determination

The total lipid content was determined using the Folch et al. (1957) modified method. Between 100 and 150 mg of blubber samples were cut in small slices then lipids extracted three times successively with a mixture of chloroform:methanol (1:2, 2:1 and 4:1, v/v). A volume of 6.5mL of 1% NaCl solution was added and the mixture separated into 2 phases. The lower layer containing lipids was collected and water removed by addition of dry sodium sulfate. These extracts were shaken and stored at 4°C for 1h. Centrifugation at 3000g during 10 minutes was used to separate the organic extract from the particulate material and the solvent was removed under a stream of nitrogen in a water bath at 40°C. When all the solvent has evaporated the weight of residue was determined and the lipid content calculated by gravimetry.

Extraction and clean-up for polychlorinated biphenyl (PCB) analysis

Samples were extracted by Pressurised Liquid Extraction (PLE) (Walsham et al. 2006). For each extraction an approximately 200 mg of blubber was cut (in vertical section), homogenised and mixed with sodium sulphate (~ 20 g). This was spiked with appropriate internal standards (PCBs by GC-MS: ^{13}C-CB28, ^{13}C-CB52, ^{13}C-CB101, ^{13}C-CB153, ^{13}C-CB138, ^{13}C-CB156, ^{13}C-CB180, ^{13}C-CB189, ^{13}C-CB194 and ^{13}C-CB209; PBDEs: FBDE160[1]). Samples were then refrigerated overnight. Solvent washed PLE cells (100 mL) were packed as follows: solvent washed filter paper, pre-washed sodium sulphate (10 g), 5% deactivated alumina (30 g), solvent washed filter paper and the samples/sodium sulphate mixture prepared as above.

Samples were extracted by PLE using an ASE 300 (Dionex Ltd., Camberley, Surrey, UK) under elevated temperature (100°C) and pressure (1500 psi). Five minutes of heating was followed by 2 x 5 min static cycles. The cell flush was 50% total cell volume (i.e. 25% of the cell volume for each flush = 25 mL per flush) with a 120 s purge (using nitrogen) at the end of each sample extraction. The extraction solvent was *iso*-hexane.

Following PLE, the extract for PCB analyses was concentrated by Syncore (fitted with flushback module) to ~ 0.5 mL and passed through silica columns, before transferring with washing to amber glass GC vials. The concentrated extracts were analyzed for PCBs by GC-EIMS (Gas Chromatography Electron Impact Mass Spectrometry).

Determination of PCBs by Gas Chromatography-Electron Impact Mass Spectrometry (GC-EIMS)

The concentrations of 32 PCB congeners (IUPAC PCB numbers 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 GC–EIMS using an HP6890 series gas chromatograph interfaced with an HP5975 MSD, fitted with a cool on-column injector and a 50 m x 0.22 mm x 25 µm SGE HT-8 column (SGE, Milton Keynes, UK). The initial oven temperature was 80°C, which was held for 1 minute. The temperature was raised at 20°C min^{-1} up to 170°C and held at this temperature for 7.5 minutes. This was followed by a ramp of 3°C min^{-1} up to a final temperature of 290°C at which it was held for 10 minutes. The MS was set for selective ion monitoring (SIM) with a dwell time of 50 ms. Calibration standards containing all 32 PCB congeners and covering the concentration range 0.6-500 ng/mL were analyzed in triplicate, and the average response used to compute the calibration curve. Correlation coefficients of at least 0.99 were achieved for all PCBs.

Quality control

All methods were validated by the replicate analysis of standards and samples, and through spiking experiments or analysis of certified reference materials (CRMs). Limits of detection (LoDs) were determined through the repeated analysis of a low spiked sample and the LoDs calculated from 4.65 x SD (standard deviation) of the mean concentration. LoDs were dependent on the sample size. The replicate analysis of standards on separate days gave coefficient of variation (CV%) of ~ 3% for PCBs analyzed by GC–EIMS. Recoveries greater than 75% were achieved for PCB and PBDE spiked samples and CRMs.

Internal quality control procedures incorporated the use of a laboratory reference material (LRM) for all determinants, and also a CRM for PCBs, in each batch of samples. Procedural blanks were performed with each batch of samples, and the final concentration adjusted accordingly. The data obtained from the LRM were transferred onto NWA Quality Analyst and Shewhart charts were produced with warning and action limits being drawn at ± 2x and ± 3x the standard deviation of the mean, respectively. CRM data were accepted if recoveries were between 75% and 120% of the certified concentration. Quality assurance was further demonstrated through successful participation in the QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) Laboratory Performance Studies.

Data treatment

Normalization of data

All the concentrations of PCB congeners were normalized to the lipid-based content of the blubber and thus expressed in µg/g lipid weight (lw). In addition, the PCB patterns of species can be compared independently of differences in absolute concentration by normalization. This normalization consisted to divide the concentration of each PCB by the concentration of another single PCB (Boon et al. 1992). As CB153 (2,2′, 4,4′, 5,5′-hexachlorobyphenyl) is the dominant congener in a great majority of aquatic mammals, and it has a molecular structure that makes it highly recalcitrant towards biotransformation (Duinker et al. 1989, Wells & Echarri 1992, Boon et al. 1992, 1994, Storr-Hansen et al. 1995), normalized PCB patterns were expressed as values of RCB for all congeners, which can be expressed as a fraction of unity:

$$RCBx = [CBx] / [CB153]$$

where RCBx is the ratio between the concentration of individual PCB congeners (i.e. CBx) and concentration of CB153.

Redundancy Analysis (RDA)

To examine relationship between RCB congeners in the blubber and the set of potential biological and ecological explanatory factors we used Redundancy Analyses (RDA). RDA requires the number of explanatory variables to be smaller than the number of samples. We analysed 72 samples and 4 explanatory variables were selected: sex, lipid content, and habitat (oceanic, neritic or both of them) and food (piscivorous or teuthophagous feeders) which is an acceptable ratio. Sex, habitat and food were considered as nominal variables. Data from ecological and biological factors were obtained from previous studies carried out on the same species and/or in the same area (Santos et al. 2004a,b, 2007a,b, Spitz et al. 2006, 2011, Pierce et al. 2010) and on the same individuals (Méndez-Fernandez et al. submitted). RDA assumes that the underlying relationships between variables are generally linear, which was supported by initial data exploration. Significance testing in RDA is based on a permutation test and no assumption of normality is required and colinearity between explanatory variables is not an issue. Between species variation in RCB patterns as function of explanatory variables was also analyzed using RDA.

Discriminant Analysis (DA)

The discriminant analysis was used to examine differences among species in PCB patterns. As for RDA only immature individuals of the five species were analyzed and the variables included in the DA were RCB congeners. In order to identify the number of congeners needed to efficiently discriminate species based on DA results, a resampling procedure was implemented in R 2.14.1 (R Development Core Team 2011). We successively removed from 1 to 26 congeners at random (*ncr*: number of congeners removed) and recomputed discriminant analyses. For each new DA, the Euclidian distance between the 5 species centroids was calculated. One hundred replications were performed for each *ncr*. The averaged Euclidian distance between species was then plotted against *ncr*.

RESULTS

A summary of the PCB data (i.e. concentrations of the sum of the 32 congeners in µg/g lw, ΣPCB_{32}), lipid content (%) and biological and ecological data of the immature individuals of the five toothed whale species are summarized in Tables 4.2 & 4.3.

Factors influencing PCB patterns

Table 4.1 summarizes results from RDA, which related RCB data to lipid content, food (piscivorous *vs* teuthophagous) and habitat (oceanic, neritic or both). Sex variable was tested in a previous RDA and removed since no significant influence was obtained ($P > 0.05$). All investigated explanatory variables together explained 42% of the variation of RDA data, among which the first two axes respectively explained 58% and 36% of (explained) RCB variability.

Table 4.1 Results of redundancy analysis (RDA) on ratios of PCBs in blubber of immature toothed whale species. Total inertia = 1.00, constrained inertia = 0.42, sum of all canonical eigenvalues = 0.034. F statistics of conditional effects refer to the increase in explained variation due to adding extra explanatory variable.

Explanatory variable	*F*	*P*
Habitat	4.75	0.0028
Lipid content	10.85	0.0002
Food	2.59	0.468

We observed close relationships between axis 1, lipid content and neritic variables, as well as between axis 2, teuthophagous and oceanic variables (Figure 4.1). Hence, the most important explanatory variables were lipid content ($P < 0.0005$), and habitat ($P < 0.005$) (Table 4.1). The congeners CB170 and 180 which belong to structural groups I and II are well-correlated and negatively to axis 1 (i.e. lipid content and neritic variables). CB101, 110, 52, 187 and 149 were clearly linked to the axis 2 (i.e. teuthophagous and oceanic variables) (Figure 4.1).

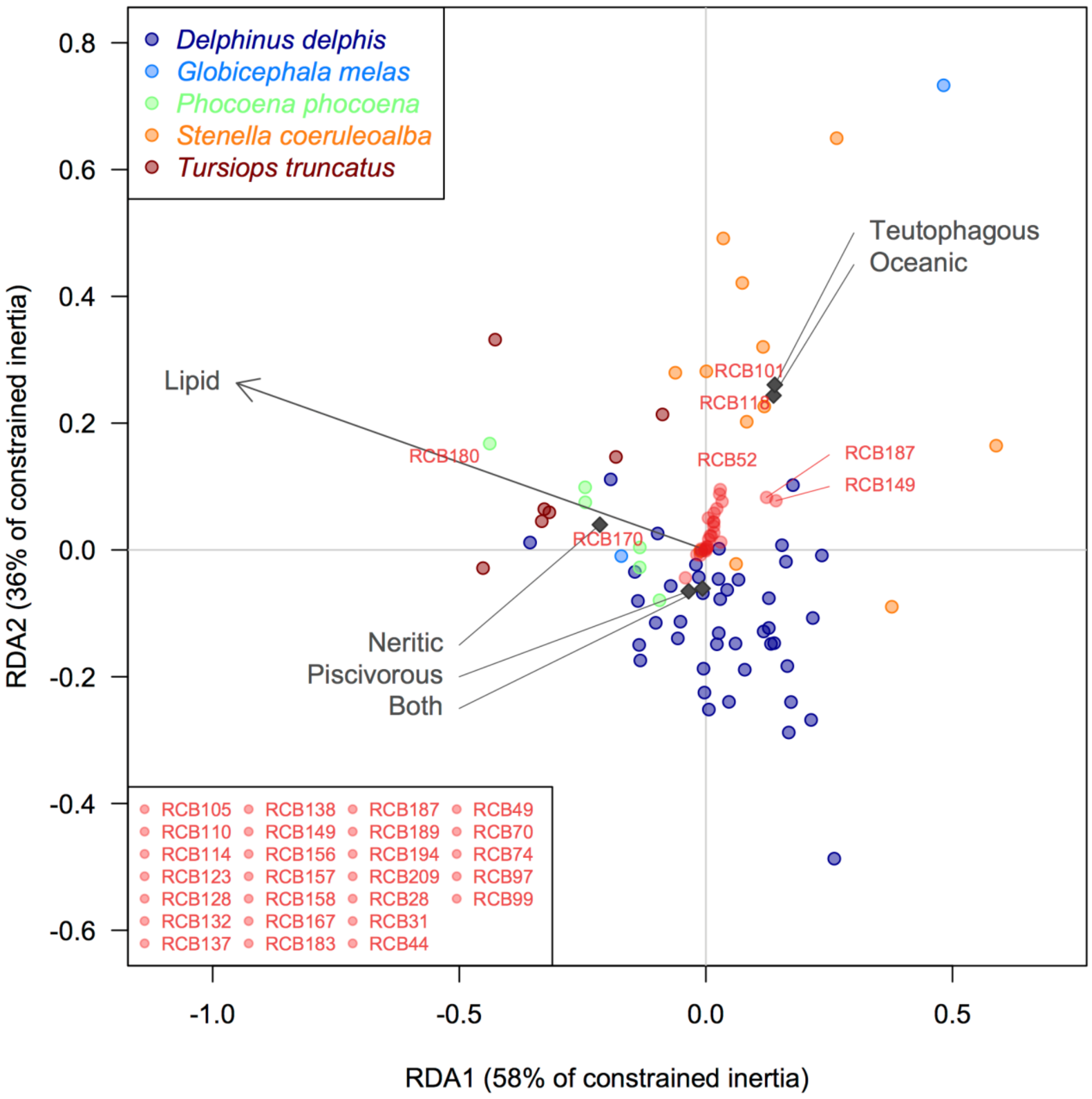

Figure 4.1 Results of redundancy analysis (RDA) on PCB ratios in blubber of immature individuals from common dolphin *Delphinus delphis*, harbour porpoise *Phocoena phocoena*, bottlenose dolphin *Tursiops Truncatus*, striped dolphin *Stenella coeruleoalba* and long-finned pilot whale *Globicephala melas* from the NWIP: bi-plot for axes 1-2 of explanatory and response variables. The bottom legend indicated the congeners which are not well-separated in the bi-plot.

Individual plots showed that most of the harbour porpoises and bottlenose dolphins were those with the highest lipid content percentage (Figure 4.1). Note that these specimens were well correlated with axes 1, and then with neritic habitat and CB170 and 180. Examination of bi-plots also highlighted that striped dolphins and one pilot whale displayed higher CB101, 110, 52, 187 and 149 values in their blubber than animals from the other species (Figure 4.1). These animals are also well correlated with axis 2, therefore with oceanic habitat and teuthophagous feeding habits.

Differences of PCB patterns among species

A first discriminant analysis (DA) was carried out in order to identify differences in RCB patterns among individuals belonging to the different species. Figure 4.2 shows the bi-plot of the first two discriminant factors (i.e. LD1 and LD2). The DA pointed out the existence of difference among the five species. Harbour porpoises and striped dolphins were clearly separated in this bi-plot. The DA also allowed a classification of each individual in the group species based upon the canonical scores and then all the individuals were classified in the correct species group. In the axis 1, which strongly separated striped dolphins from the other species, the RCBs with the highest importance were in the decreasing order: CB157 > 137 > 114 > 149 > 118 > 158 and 183. In the axis 2, which strongly separated the harbour porpoises, the RCBs having the highest importance were CB157 > 137 > 149 > 183 > 158 > 118 and 189.

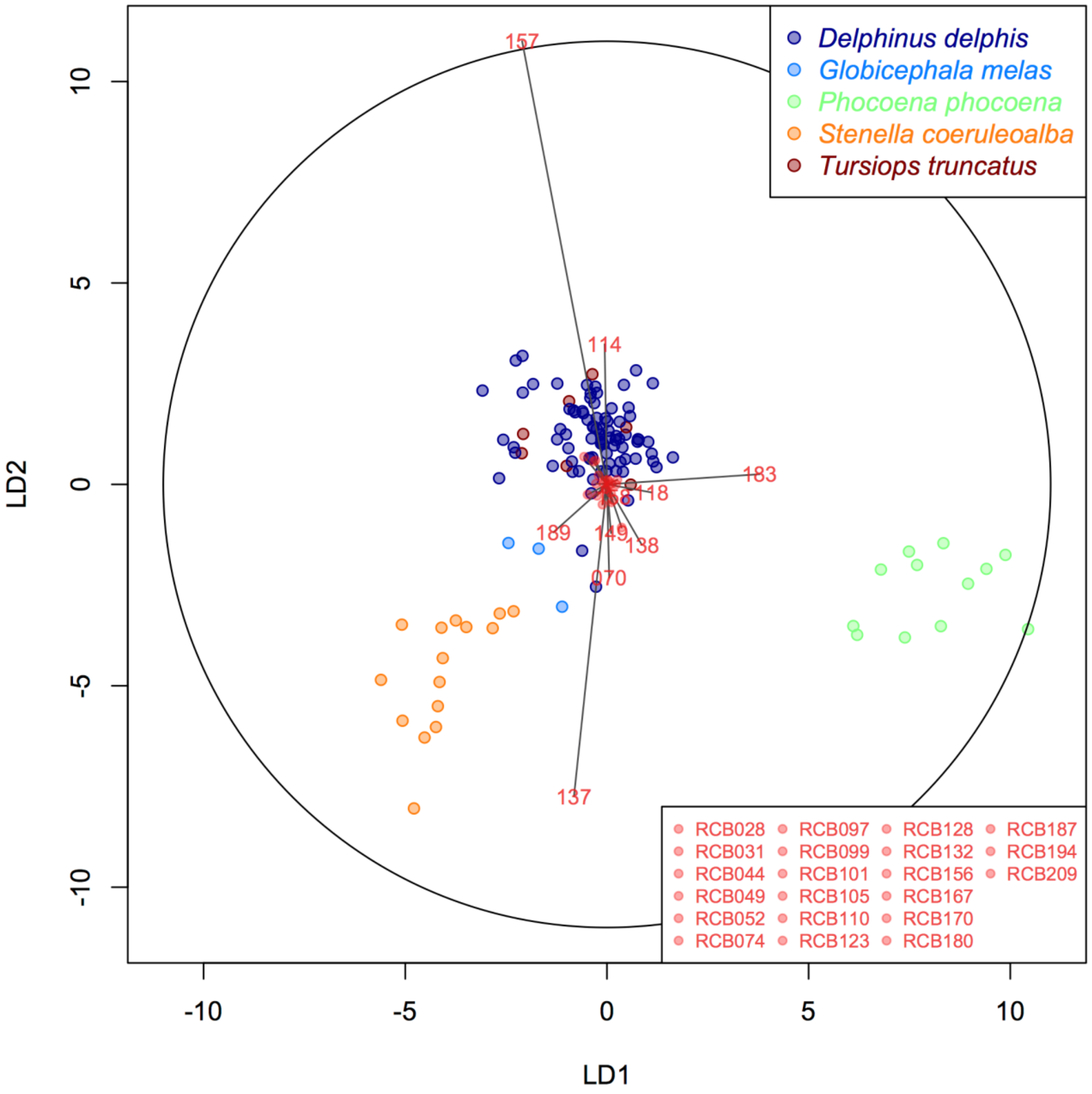

Figure 4.2 Results of discriminant analysis (DA) on PCB ratios of immature individuals from common dolphin *Delphinus delphis* (Dde), harbour porpoise *Phocoena phocoena* (Pph), bottlenose dolphin *Tursiops Truncatus* (Ttr), striped dolphin *Stenella coeruleoalba* (Sco) and long-finned pilot whale *Globicephala melas* (Gme) from the NWIP: bi-plot for axes 1-2 (i.e. LD1 and LD2). RCB = ratio of each PCB concentration divided by the concentration of CB153 (see Materials and methods).

In addition, Mean Euclidean Distances (MED) between species and in comparison with common dolphins and harbour porpoises were calculated and for each number of congeners removed (N) 100 DAs were computed (Figure 4.3). Figure 4.3a shows that the MED between common dolphins, bottlenose dolphins and pilot whales were very low and do not varied with the removing of congeners. The higher MED were found between common dolphins and harbour porpoises, and between common dolphins and striped dolphins. The good separation among the species is kept up to approximately 20 congeners removed, where the curves cross. On the other hand, considering Figure 4.3b, the MED between harbour porpoise and the rest of species indicated a good separation of all of them with a markedly decreased of these MED values after 10 congeners removed, and an approximation between curves from 20 congeners removed.

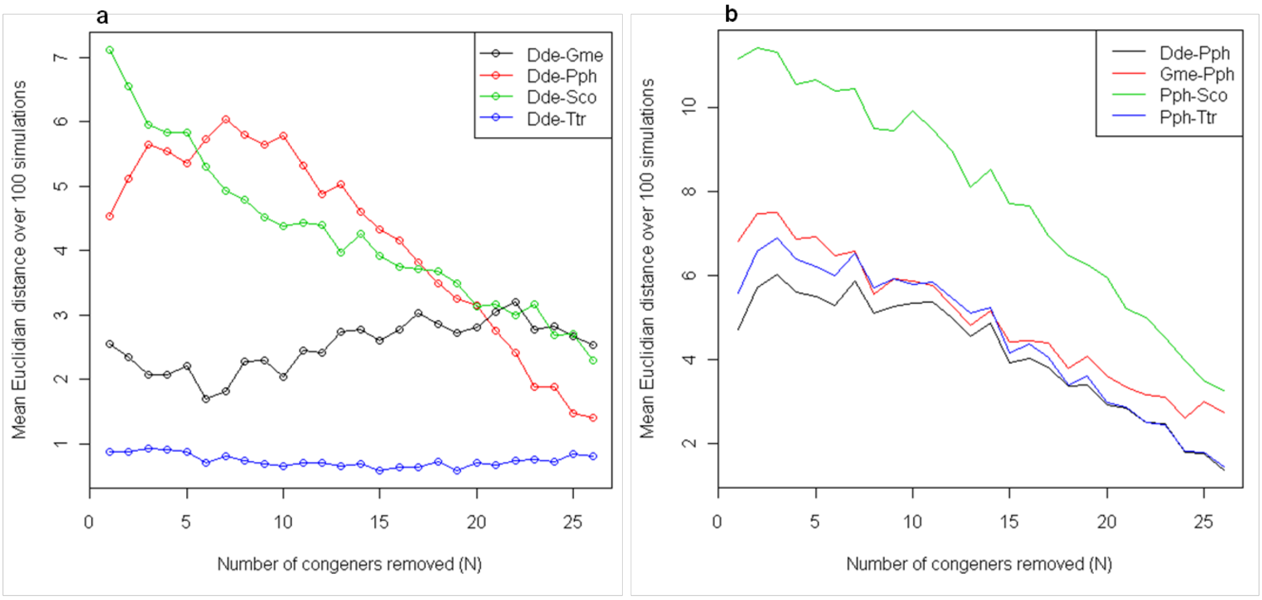

Figure 4.3 Mean Euclidean Distance (MED) between species in comparison to common dolphin *Delphinus delphis* (Dde) (a) and harbour porpoise *Phocoena phocoena* (Pph) (b). For each N (number of congeners removed), 100 discriminant analyses (DA) were computed. Bottlenose dolphin *Tursiops Truncatus* (Ttr), striped dolphin *Stenella coeruleoalba* (Sco) and long-finned pilot whale *Globicephala melas* (Gme).

A visual example of the effect of the number of congeners removed on MED is given in Figure 4.4. Here, the DA with 32 congeners is presented in the top left panel. The five remaining panels show examples of DA when 5, 10, 15, 20 and 25 randomly chosen congeners are removed. Here, the Euclidian distances between harbour porpoise, striped dolphin and common dolphin decrease regularly with the number of congeners removed. However, these 3 species remain clearly discriminated from each other until 20 or more congeners are removed from the DA.

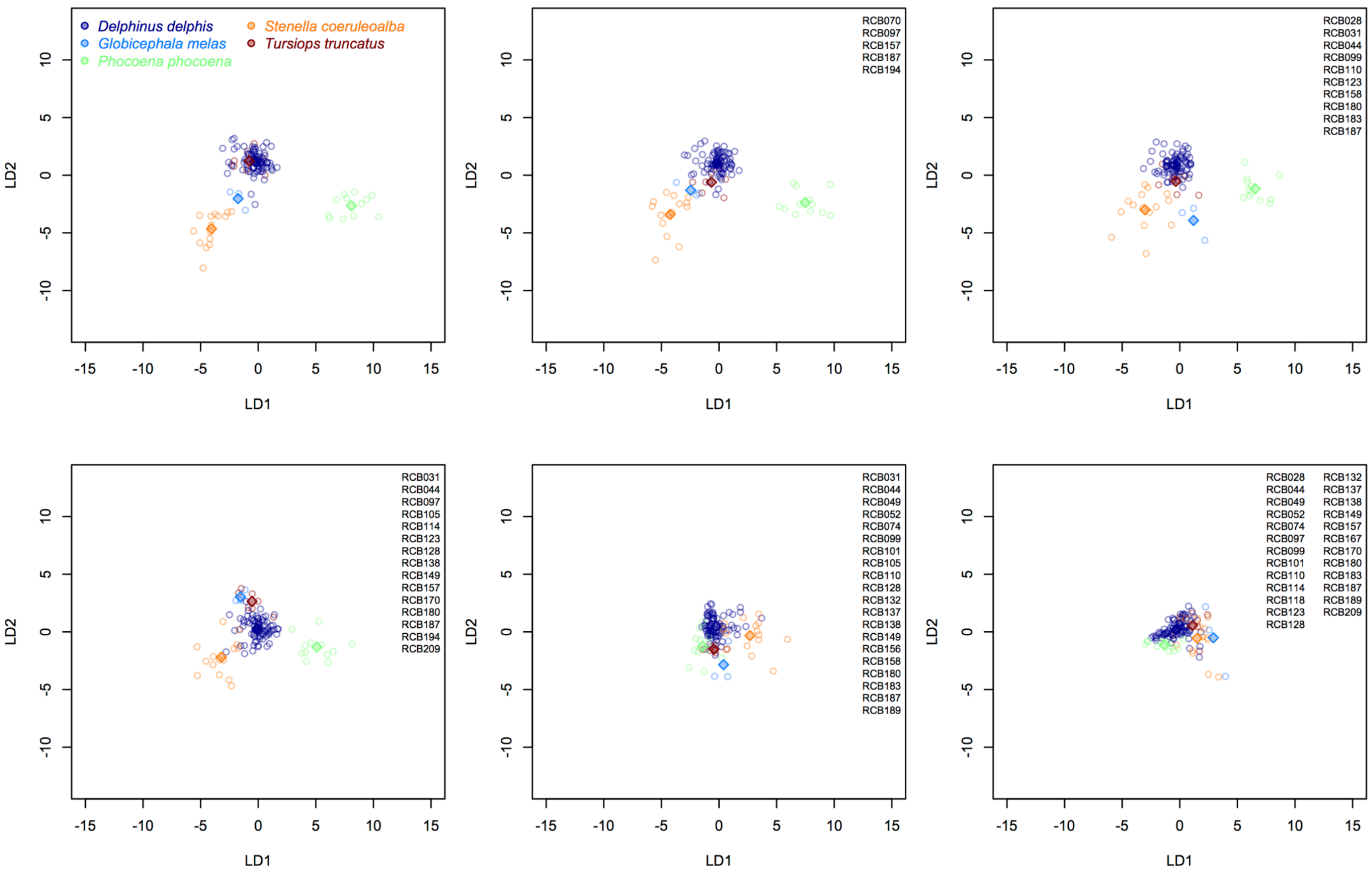

Figure 4.4 Example of 6 discriminant analyses (DA) realized with 0, 5, 10, 15, 20 and 25 congeners randomly removed. The names of removed congeners are given in each panel. Common dolphin *Delphinus delphis*, harbour porpoise *Phocoena phocoena*, bottlenose dolphin *Tursiops Truncatus*, striped dolphin *Stenella coeruleoalba* and long-finned pilot whale *Globicephala melas*.

Finally, the RCBs which apparently are responsible of the well-separation among species in the first DA were used to compute a last DA (i.e. CB157, 137, 114, 149, 118, 158, 189, and 183). This analysis showed once more a well-separation among species and especially for harbour porpoise and striped dolphin (Figure 4.5).

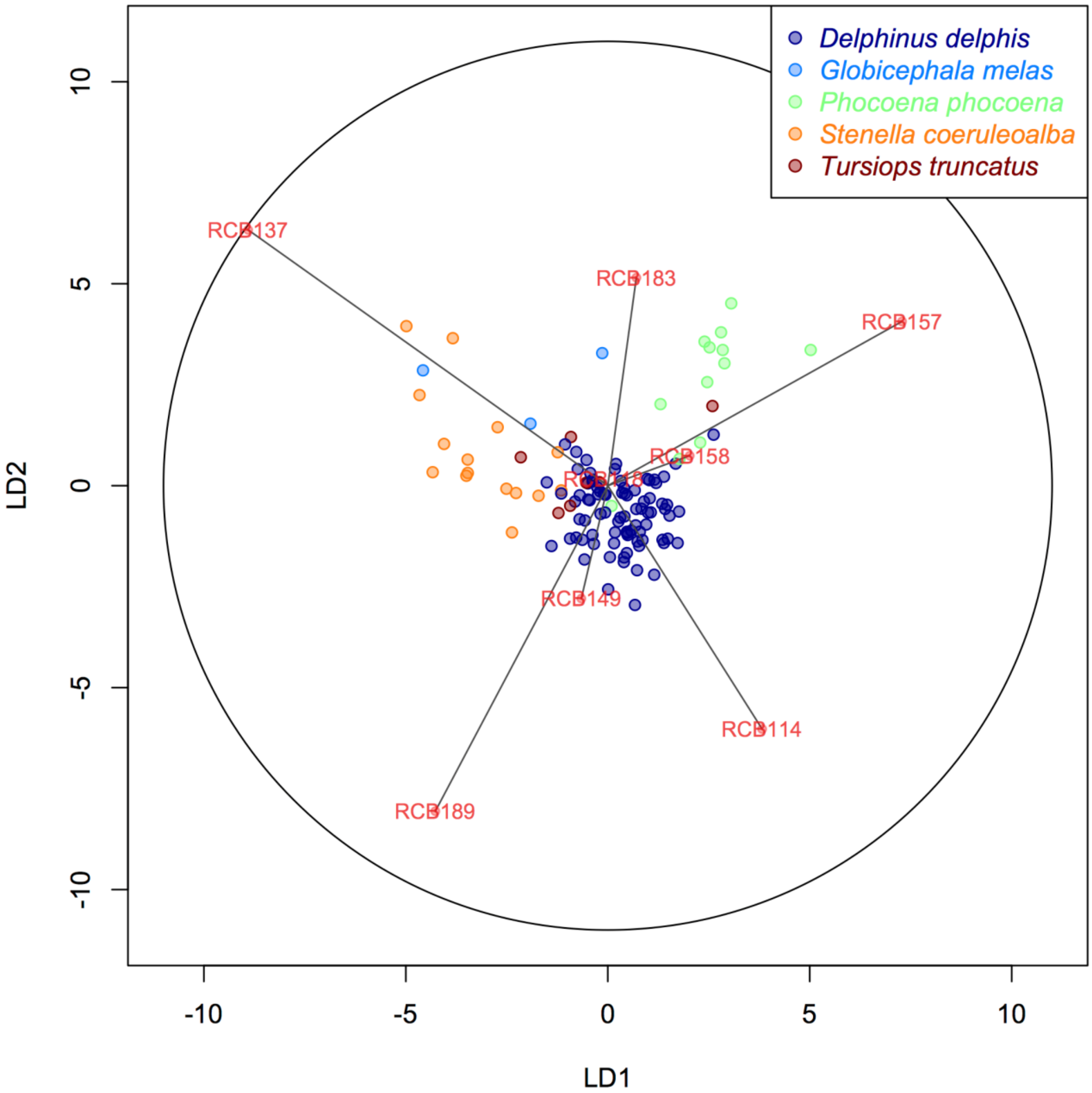

Figure 4.4 Results of discriminant analysis (DA) on selected PCBs (i.e. CB157, 137, 114, 149, 118, 158, 183 and 189) of immature individuals from common dolphin *Delphinus delphis* (Dde), harbour porpoise *Phocoena phocoena* (Pph), bottlenose dolphin *Tursiops Truncatus* (Ttr), striped dolphin *Stenella coeruleoalba* (Sco) and long-finned pilot whale *Globicephala melas* (Gme) from the NWIP: bi-plot for axes 1-2 (i.e. LD1 and LD2). RCB = ratio of each PCB concentration divided by the concentration of CB153 (see Materials and methods).

DISCUSSION

The aim of the present study was to validate the use of PCB patterns to differentiate five sympatric toothed whale species using multivariate analyses. To minimise the influence of biological factors and to avoid biases due to the mature females' transfer of some PCB congeners to their progeny (Tanabe et al. 1982, Addison & Brodie 1987, Aguilar & Borrell 1994), the study was only conducted on immature individuals. The influence of some other biological and ecological parameters on PCB patterns has also been evaluated through redundancy analysis, and the results obtained give us an idea of which parameters have the highest influence on the PCB patterns of the NWIP toothed whales studied here. First of all, the RDA results showed that lipid content and habitat are the variables having the highest influence. Food also significantly influenced the PCB patterns of the five species (Table 4.1) whereas it was not the case for the gender. The absence of influence of the gender is not a surprise considering that only immature individuals were included in this study. The effect of sex on PCB patterns is essentially related to the transfer of congeners to their offspring in mature females, leading to a selective excretion, whereas they continuously accumulate in males (Tanabe et al. 1982, Aguilar & Borrell 1994).

Several PCB congeners had a consistent behaviour in the RDA and appeared separated in two clusters in function of their structural and metabolic groups. Hence, the first cluster included only two PCB congeners (i.e. CB170 and 180) belonging to the structural groups I and II which were positively related with the lipid content of the individuals (Figure 4.1). Boon et al. (1997) overviewed 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 part of those exhibiting a high octanol-water partition coefficient (log Kow ≈ 7) indicating their high liposolubility (Jäntschi & Bolboacà 2006, Walters et al. 2011). The highest percentage of lipid content exhibited by harbour porpoise (75.9% for males and 87.0% for females; Table 4.2) compared to the other four species may thus partly explain the high ratio of these both congeners in all harbour porpoise individuals (Figure 4.1). The geographic distribution of a species influences their blubber lipid content. The harbour porpoise mainly distributes in cold waters, exhibits mean blubber lipid contents of at least 60%, with percentage as high as 80% (Koopman 2007). Yet water temperature is not the only factor associated with lipid content, since all phocoenids possess high quantities of lipids in their blubber, regardless of the thermal regime. However, porpoises are relatively small (< 2.2 m), it is thus likely that the blubber lipid content is constrained in all members of this family to counter their small body size (Koopman 2007). Nonetheless beyond the lipid amounts contend in the blubber tissue, lipid composition plays an important role in the distribution of lipophilic chemicals and is a determinant factor in their bioaccumulation (Weijs et al. 2011). However, more physiological background information on harbour porpoises (e.g. lipid composition of the tissues, enzyme-mediated metabolic transformation) may explain the association found between this species and the persistent congeners CB170 and 180.

The second PCB cluster obtained in the RDA showed that congeners CB101, 110, 52, 187 and 149 are highly correlated between them (Figure 4.1). These congeners belong to structural groups IV and V, i.e. less chlorinated and consequently metabolizable congeners, with the exception of the CB187 which belong to group I, i.e. high chlorinated and consequently less metabolizable. These congeners seem to be highly related to the consumption of cephalopod (teuthophagous feeding) and to the oceanic habitat. These are particularly present in striped dolphin individuals and one pilot whale (Figure 4.1). As we can see in Table 4.3, both toothed whales are mainly teuthophagous and oceanic species in the NWIP, which explains the results obtained by the RDA.

Once the effect of the biological-ecological parameters on PCB patterns was investigated, discriminant analysis was applied in order to visualise the overall differences between species on the basis of their relative congener patterns (i.e. ratios). Figure 4.2 shows the bi-plot for all the species in the data set for the first two discriminant axes. Two groups of animals are clearly separated in this DA plane, the harbour porpoise and the striped dolphin. The predominant PCB congeners found to be the cause of this separation are difficult to interpret since data in the literature on this subject is scarce. In fact, few studies have analyzed an amount of 32 congeners making difficult to compare and interpret certain of them (i.e. congeners). Wells et al. (1996) previously proved the importance of a large number of PCB congeners to describe the patterns of marine mammals. Consequently, in this study we have considered all the 32 congeners in the analysis in order to maximise the discrimination as much as possible. However, the MED calculated between species and in comparison to common dolphin and harbour porpoise (see Figure 4.3) together with the example of DA realized with increasing congeners removed (Figure 4.4), revealed that the separation between species in function of its PCB patterns is still good until 10-12 congeners have been removed. On the contrary, when 20 congeners were removed this separation became poor for most of the species. The congeners indentified to be responsible of the good separation of harbour porpoise and striped dolphin in the first DA (Figure 4.2) where used to make a second DA (i.e. only with these congeners; Figure 4.5) showing the same good separation of harbour porpoise and striped dolphin and confirming their importance in it. The majority of these congeners belong to the most persistent metabolic groups I and II (CB157, 137, 183, 158 and 189) with the exception of CB114, 149 and 118 which belong to the metabolic groups III, IV and V characterized to be more metabolizable by the organisms. Interestingly, Boon et al. (1997) showed that CB114 and 149 behaves like a persistent compound in cetaceans, and can be the reason because these congeners are the main responsible of the inter-species separation together with PCBs belonging to groups I and II in this study.

As we previously explained in the introduction section, the separation of marine mammals as a function of their PCB patterns depends on: 1) their metabolic capacities and different ability to metabolize and/or eliminate certain congeners and 2) the differences in pollutant intakes as a direct consequence of the specific diet composition and feeding habitats (Tanabe et al. 1988, Wells et al. 1996, Boon et al. 1994, 1997, Borrell & Aguilar 2005). Since only immature individuals were analysed in the present study, the differences found in PCB patterns can only be a consequence of a different PCB metabolism among species and/or pollutants exposure *via* the food. Differences in metabolic capacities were specially demonstrated between pinnipeds and cetaceans (Tanabe et al. 1988, Goksøyr et al. 1992, Wells et al. 1996) for 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. Biochemical and immunochemical analyses have revealed the presence of CYP1A in both pinnipeds and cetaceans (Watanabe et al. 1989, White et al. 1994). However, pinnipeds showed an inducible CYP2B (i.e. CYP2B1 and CYP2B2) activity (Goksøyr et al. 1992), whereas CYP2B expression is usually much weaker in cetaceans (Boon et al. 1992, White et al. 1994). In addition, the catalytic capacity of these iso-enzymes is also different allowing the metabolization of different congeners as function of their degree and position of chlorine substitution (i.e. of its metabolic group classification). Indeed, substantial inter-specific variation in the capacity to degrade organochlorine compounds has already been observed between striped and common dolphins (Marsili & Focardi 1996) but also between other taxonomically close vertebrates (Fossi et al. 1995). These previous results indicated that the different metabolic capacities are not related with the taxonomic proximity of the species. In the present study, the recalcitrant congeners CB170 and 180 seem to be more related (showing increased levels) in harbour porpoises (Figure 4.1), whereas the easily metabolizable forms, which were found forming a second cluster in the RDA bi-plot (i.e. CB101, 110, 52 and 149), had the highest values in striped dolphins (Figure 4.1). Given the fact that, in both species the metabolism of the different congeners within different metabolic groups was not consistent and could be the reason of their good separation.

Harbour porpoises from the North Sea (Duinker et al. 1989, Wells & Echarri 1992, Boon et al. 1992, 1994) and the Baltic Sea (Bruhn et al. 1995) showed a metabolism of some group III and group IV type congeners. In the same way a comparison of the PCB patterns in three harbour porpoises from the Baltic Sea with herring as food source, indicated the biotransformation of congeners belonging to group V (Bruhn et al. 1995). In another study (Weijs et al. 2009), harbour porpoises exhibited more difficulties to metabolize lower halogenated and less persistent PCB congeners than harbour seals, probably as consequence of less efficient cytochrome P450 enzymes as previously discussed above. Therefore, a number of studies seem to indicate that harbour porpoises may have a greater ability for metabolizing low chlorinated congeners than striped dolphins, which is consistent with our results. However, further studies in the metabolism of several PCB congeners in toothed whales but also considering those of their prey become necessary and would elucidate our results. However and as explained before, the PCB pattern of a given species is not only the result of its ability to handle, metabolize, or excrete certain congeners, but also of its diet composition and feeding habitat. Diet and habitat are well documented for these five toothed whales in the NWIP and the information is summarised in Table 4.3. In temperate area higher pollutant burden are generally found in species inhabiting coastal regions due to the close proximity of the animals to possible sources of input (Storr-Hansen & Spliid 1993). Moreover, the pattern of the PCBs may differ according to the distance from the source, the more volatile congeners being transported on longer distances (Aguilar & Borrell 2005). In the NWIP, the bottlenose dolphin and harbour porpoise are somewhat more coastal than the common dolphin, striped dolphin and pilot whale (López et al. 2004, Pierce et al. 2010, Spyrakos et al. 2011). However, bottlenose dolphin is also seen in offshore waters (López et al. 2004) and recent studies revealed the existence of both inshore and offshore forms

(Fernandez et al. 2011a,b). Common dolphin and pilot whale are mainly found in waters deeper than 200 m depth (Spyrakos et al. 2011), although they can apparently forage successfully in both oceanic and neritic habitats as was demonstrated by stomach content and stable isotope analyses (Santos et al. 2004a,c, Pierce et al. 2007, Spitz et al. 2011, Méndez-Fernandez et al. submitted). These species have the same trophic level in the NWIP food web (Méndez-Fernandez et al. 2012) but same trophic level does not necessarily mean that these species exploit the same food resources. Thereby, bottlenose dolphin and harbour porpoise are mainly piscivorous, striped dolphin and pilot whale mainly teuthophagous and common dolphin can be considered as mixed feeder due to the presence of important amounts of fish and cephalopods in its diet (see Table 4.3). Any differences of relative biphenyl congener patterns as a result of different sources, i.e. different commercial mixtures, will be effectively integrated through the food web and through the habitat and mobility of species. Additionally, the PCB pattern in fish is dependent on location, seasonal changes in lipid mobilisation and the degree of its migrations (Wells et al. 1996). Storr-Hansen et al. (1995), for example, found PCB pattern differences between plaice from the Wadden Sea and mackerel from the Atlantic Ocean. Therefore, and in agreement with the RDA results obtained in this study which revealed that habitat and food variables have an effect on the PCB patterns of the five toothed whales, animals appear grouped in our DAs bi-plot as a probably result of food source and feeding habitat (Figure 4.2 & 4.5). Consequently, the striped dolphin which is mainly teuthophagous and the more oceanic species in the study is well separated for the rest of the species as well as harbour porpoise which is piscivorous and the more coastal species. The other three species, known to frequent both oceanic and coastal habitats and/or to have some dietary plasticity are grouped in the left side of the DA, being less differentiated. The slight difference found for pilot whale, in spite of its mainly cephalopod-based diet in the area as we could expected, is probably due to the low number of individuals available for this study.

The inter-species separation obtained in this study through the PCB patterns, is then highly in concordance with the previous study carried out in the NWIP (i.e. spatial segregation, habitat preferences) by using cadmium, carbon and nitrogen isotopes as ecological tracers (Méndez-Fernandez et al. submitted). Thus, we could confirm from these results, or at least for harbour porpoise and striped dolphin, that PCB patterns can be consider as a good tracer of feeding habits to study a possible ecological segregation among sympatric toothed whale species.

Conclusions

Understanding the bioaccumulation of pollutants and associated risks in marine mammals is a challenging problem. The results presented in this study demonstrate that the PCB patterns can be used as tracers to discriminate among sympatric species of toothed whales. Among them, harbour porpoise and striped dolphin seem to be the species that can be better separated as a consequence of their different feeding habits, and consequently of their different pollutant intakes, but also of their metabolic features. Additionally, around 22 PCB congeners seem to be enough to discriminate the species and for this study we identified persistent and recalcitrant congeners belonging to metabolic groups I and II (i.e. CB157, 137, 114, 149, 118, 158, 183 and 189) as the main responsible of the fine separation among the toothed whales studied. Moreover, a large sampling could improve and facilitate the comprehensiveness of these results, since for some of the species very few animals were available. Additionally in order to better understand their specific capacity to metabolize PCBs, it will be important to know the isomer and congeners compositions in their foods. Therefore, the understanding of these differences can lead to a better knowledge of the relative sensibility of these species towards the more toxic PCB congeners and/or metabolites or other kind of pollutants (e.g. Polybrominated diphenylethers PBDEs, dichlorodiphenyltrichloroethane DDT, hexabromocyclododecane HBCD).

Table 4.2 Arithmetic means ± SD of biological data (age and sexual maturity in years), percentage of lipid content (%) and the sum of the 32 congener concentrations (ΣPCB_{32} in µg/g lw and range in parenthesis) measured in blubber of the toothed whale species from the NWIP. n = sample sizes for each species and sex.

	n	Age	Sexual maturity[a]	Lipid	ΣPCB_{32}
Male					
Common dolphin	27	2.5 ± 1.4	-	65.4 ± 16.3	17.9 ± 13.6
Delphinus delphis					(3.9 - 71.8)
Harbour porpoise	2	3.0 ± 0.0	3 - 4	75.9 ± 4.8	7.8 ± 1.6
Phocoena phocoena					(6.7 - 9.0)
Bottlenose dolphin	4	4.9 ± 1.7	10 - 15	66.3 ± 12.4	62.1 ± 41.8
Tursiops truncatus					(36.3 - 124.4)
Striped dolphin	7	1.6 ± 2.0	14	58.2 ± 27.6	20.9 ± 24.4
Stenella coeruleoalba					(3.4 - 68.7)
Long-finned pilot whale	1	2.0	~14	56.0	38.7
Globicephala melas					
Female					
Common dolphin	18	2.5 ± 1.6	6 - 7	68.3 ± 12.7	13.8 ± 6.3
Delphinus delphis					(4.5 - 27.6)
Harbour porpoise	4	2.2 ± 1.2	3 - 4	87.0 ± 15.9	11.2 ± 3.0
Phocoena phocoena					(6.9 - 13.5)
Bottlenose dolphin	3	2.3 ± 1.6	~10	71.8 ± 0.8	49.0 ± 30.9
Tursiops truncatus					(13.9 - 72.2)
Striped dolphin	5	3.8 ± 3.5	14	65.7 ± 5.5	9.9 ± 5.4
Stenella coeruleoalba					(4.1 - 15.4)
Long-finned pilot whale	1	2.0	~8	62.3	7.9
Globicephala melas					

[a] Adapted from Reproduction in Marine Mammals (Boyd et al. 1999)

Table 4.3 Background of the foraging ecology data 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 NWIP, studied through: stomach contents analysis, land-based and sea surveys and ecological tracer analysis (stable isotopes and trace elements).

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

Adapted from Santos et al. 2004a,b,c, 2007a,b, Spitz et al. 2006, 2011, Pierce et al. 2007, 2010, Fernández et al. 2011a,b, Spyrakos et al. 2011, Méndez-Fernandez et al. Submitted

CHAPTER V

An assessment of trace element and persistent organic pollutant (PCBs and PBDEs) concentrations in toothed whale species from the North West Iberian Peninsula

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O Caldeirón (Globicephala melas)

In preparation for Science of the Total Environment

An assessment of trace element and persistent organic pollutant (PCBs and PBDEs) concentrations in toothed whale species from the North West Iberian Peninsula

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ABSTRACT

In order to complete the baseline data of contaminants for the five most common toothed whales from the Northwest Iberian Peninsula (NWIP), including common dolphin (*Delphinus delphis*), long-finned pilot whale (*Globicephala melas*), harbour porpoise (*Phocoena phocoena*), striped dolphin (*Stenella coeruleoalba*) and bottlenose dolphin (*Tursiops truncatus*), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) were determined in their blubber, while trace elements (Ag, As, Cd, Co, Cu, Cr, Fe, Hg, Mn, Ni, Pb, Se, V, Zn) were determined in liver and kidney. Samples were obtained from animals stranded or by-caught between 2004 and 2008. The study confirmed that contaminant concentrations are highly dependent on two biological factors (age and sex) but also on ecological factors i.e. the trophic level at which the species feed, the type of prey eaten and the habitat used. Among the five toothed whales, bottlenose dolphin and harbour porpoise showed the greatest concentrations of PCBs while the pilot whale and striped dolphin exhibited the highest Cd concentrations, and harbour porpoise the lowest Hg concentrations. Arsenic was only detected in pilot whales and bottlenose dolphin. A slightly and significantly decreasing temporal trend was found for PCBs in blubber during the time period studied. Finally, in order to evaluate the contaminant status of the five species over the Northeast Atlantic waters, a geographic comparison was carried out and it showed that Iberian toothed whales are less contaminated.

KEY WORDS: Toothed whales · Persistent Organic Pollutants · Trace elements · Bioaccumulation · Temporal trend

INTRODUCTION

Several studies have investigated marine mammal ecology in the Northwest Iberian Peninsula (NWIP) since the nineteen nineties (e.g. López et al. 2002, 2004, Pierce et al. 2007, 2008, 2010, Santos et al. 2004a,b,c, 2007a,b, Fernández et al. 2011a,b, Spyrakos et al. 2011). The NWIP is situated at the northern limit of the NW African upwelling system (Figueiras et al. 2002). During the summer, the prevalent northerly winds favour the upwelling of nutrient-rich Eastern Atlantic Central Water (ENACW), these nutrient-rich waters sustaining high productivity which results in a considerable biodiversity. This includes nearly 300 species of fish (Solórzano et al. 1988), 78 species of cephalopods (Guerra 1992) and at least 16 species of marine mammals (Fernández de la Cigoña 1990, Penas-Patiño & Piñeiro-Seage 1989). During the last decades, industrial development and an increase in human activities have disturbed the natural environment of the NWIP. Several studies were thus carried out with the primary objective of assessing pollution through analysing sediments and the water column as well as monitoring the contamination of commercial species such as shellfish (see Carro et al. 2002, Prego & Cobelo-García 2003). Potentially toxic substances were routinely investigated in marine mammals since the 1970s, however only a previous study (Bioaccumulation of persistent organic pollutants in small cetaceans in European waters: transport pathways and impact on reproduction, BIOCET) was provided data on contaminants of two species of cetaceans in the NWIP: the common dolphin *Delphinus delphis* and harbour porpoise *Phocoena phocoena*.

The persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs) and pesticides (e.g. dichlorodiphenyltrichloroethane, DDT) are among the main pollutants of concern in marine ecosystems being cited on the OSPAR list of Chemicals for Priority Action (OSPAR 2010). They are a group of lipophilic synthetic organic compounds that have been produced for industrial and agricultural purposes, or are by-products of other industrial processes since the 1940s. Even though the use of some harmful organic compounds has decreased, or ceased, since the early 1970s, concentrations of POPs remain high enough to cause a threat to aquatic ecosystems (Jonsson et al. 1996). In addition, new classes of organic chemicals are of concern nowadays, notably the brominated diphenyl ether formulations (PBDEs) (DeBoer et al. 1998). The other contaminants (potential pollutants) are trace elements, either the non essential ones (e.g. Cd, Hg, Pb) or the essential ones (e.g. Cu, Se, Zn). As long-lived and apex predators, marine mammals may bioaccumulate high concentrations of contaminants and are at particular risk from the effects of POPs and methyl-Hg which both biomagnify in the food webs. Moreover, compared to most terrestrial mammals, marine mammals appear to have a lower capacity to metabolize and excrete lipophilic organochlorine compounds (Tanabe et al. 1988, Duinker et al. 1989, Boon et al. 1992). Metabolic capacity might be lower in toothed whales (odontocetes) than in pinnipeds (seals and sea lions) (Tanabe et al. 1988). Marine mammals are, therefore, especially vulnerable to POPs which enter their body almost exclusively *via* the diet and tend to accumulate efficiently in the lipid-rich blubber because of the lipophilic characteristics of POPs (Tanabe et al. 1988, Duinker et al. 1989). However, the amount of POPs in marine mammal tissues varies in relation to intake (reflecting concentrations of environmental pollutants, trophic position and the type of prey eaten), elimination in faeces and transformation to non-toxic forms (Aguilar et al. 1999). Several biological factors have been also found to be responsible for variation in POP concentrations in marine mammals, such as body size, body composition, nutritive condition, age, sex, disease state, duration of lactation and transfer from mother to offspring during pregnancy and lactation (Aguilar et al. 1999). These factors have to be taken into account when interpreting concentrations and the potential related effects. Some of the harmful consequences of POP in marine mammals include depression of the immune system (Ross 1995), increased risk of infection (Hall et al. 2006) and reproductive failure (Reijnders 1986). The toxic effects of PBDEs are less well known, but sub-lethal effects reported include neurodevelopmental toxicity and altered thyroid hormone homeostasis (Darnerud 2003).

However, there is no evidence that pollutants cause direct mortality in any marine mammal population (Reijinders 1986, DeSwart 1995, Ross 1995).

Similarly to POPs, investigations on trace elements and their distribution in the sea has increased over the past few decades. This is as a result of rising concern about environmental pollution (Das et al. 2003b). However, contrary to organic chemicals, trace elements occurring in the marine environment are derived from both natural and anthropogenic (mining and industrial discharges) sources. They are divided into essential (when they participate in biological function) and non-essential elements, the latter being potentially toxic even at low concentrations (Chappuis 1991). Nevertheless, major studies focus on non-essential elements because of their threat and toxicity to mammals (O'Shea 1999), and consequently, they are more frequently monitored in marine mammal science. Trace element concentrations also vary greatly among marine mammals. This is a consequence of the various diets as well as a result of the numerous ecological and physiological factors that might affect their concentrations: e.g. geographical location, diet, age, sex and toxicodynamic and toxicocinetic processes. The actual toxic effects of trace elements on marine mammals remain unclear since marine mammals have been exposed to these natural compounds for a very long time in evolutionary terms and have developed mechanisms either to control or to mitigate their toxic effects (Law et al. 1996). As an example, the detoxification process of methylmercury by selenium through the formation of tiemanite in the liver (Koeman et al. 1973, Martoja & Berry 1980) leads to high concentrations of Hg, especially in toothed whales, without any obvious toxic effects in the individuals or populations.

In this context, the aim of the present work was to complete the baseline data initiated with BIOCET project setting for more trace elements as well as PCBs and PBDEs, and in a total of five toothed whale species being the most common inhabiting the waters of the NWIP: the common dolphin (*Delphinus delphis*), the long-finned pilot whale (*Globicephala melas*), the harbour porpoise (*Phocoena phocoena*), the striped dolphin (*Stenella coeruleoalba*) and the bottlenose dolphin (*Tursiops truncatus*). The blubber of a total of 172 individuals was analyzed for 32 PCB congeners and 9 PBDE congeners while 14 trace elements were investigated in liver and kidney. The selection of trace elements was based on both their biological function and/or essentiality As, Co, Cr, Cu, Fe, Mn, Ni, Se, V and Zn and their toxicity Ag, Cd, Hg and Pb. Furthermore, a geographical comparison was made between the five toothed whales species from the NWIP and those from the adjacent waters of the Northeast Atlantic. Any temporal trend of these contaminants was also investigated.

MATERIALS AND METHODS

Sampling and study area

Sampling was carried out in the North West 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) (Figure 1.4). 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 attending stranded and by-caught cetaceans for over ten years. Animals were identified to species, measured, sexed and, if the state of preservation of the carcass allowed, full necropsies were performed and samples collected whenever possible. All procedures follow the standard protocol defined by the European Cetacean Society (after Kuiken 1991).

A total of 172 stranded and by-caught individuals were selected for this study covering five toothed whale species (common dolphin, long-finned pilot whale, harbour porpoise, striped dolphin and bottlenose dolphin) and the period 2004 to 2008. The animals selected were in a good state of preservation. The common dolphin is the most represented species as a result of the great number of individuals by-caught in the NWIP fisheries. Teeth were collected for age determination, liver and kidneys for trace elements analyses and blubber samples for POPs determination. Blubber samples were taken from the left side in front of the dorsal fin. Samples were complete vertical cross-sections so as to prevent any possible effects of stratification of the blubber. The samples were wrapped separately in aluminium foil. Samples of liver and kidneys for trace element analyses were removed and stored in polyethylene bags. After necropsies, all samples for contaminant analyses were frozen at –20°C until required for analysis.

Analysis of POPs is costly and was budget-limited. As such, effort was focused on the best sample sets (i.e. individuals for which most data on other variables were available). Thus a total of 120 blubber samples were analyzed for PCBs and 20 for PBDEs.

Determination of age

At least 5 teeth were collected from each sampled individual, selecting the least worn/damaged and least curved teeth, to ensure sufficient material for replicate preparations. Teeth were preserved frozen or in 70% alcohol. Age was determined by analyzing growth layer groups (GLGs) in the dentine of the teeth, following adapted methods based on Hohn & Lockyer (1995), Lockyer (1993) and Rogan et al. (2004). In summary, teeth were decalcified and then sectioned using a freezing microtome. The most central and complete sections (including the whole pulp cavity) were selected from each tooth, stained with Mayer's haematoxylin (modified by Grue) and 'blued' in a weak ammonia solution, mounted on glass slides, and allowed to dry. GLGs were counted under a binocular microscope. All readings were initially made blind (without access to individual biological data) and replicate counts were made by at least two readers.

Determination of trace elements

All the equipment used in the sample processing was cleaned, and subsequently decontaminated for 24 h in a solution composed of 35 mL HNO_3 (65% of concentration) and 50 mL HCl (36% of conentration) mixed into 1 L of Milli-Ro quality water. Liver and kidney frozen samples were freeze-dried, ground to powder using a Planetary Ball Mills Retsch PM 200 and 2 replicates for each sample were prepared for trace element analyses.

Trace element analyses were performed with a Varian Vista-Pro ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrophotometry) and a Thermofisher Scientific XSeries 2 ICP-MS (Inductively Coupled Plasma Mass Spectrometry) for Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, V and Zn. Total Hg was determined using an Advanced Mercury Analyser (Altec AMA 254).

For ICP-AES and ICP-MS measurements, aliquots of samples (0.1 - 0.3 g) were digested with 6 ml 67-70% HNO_3 and 2 mL 34-37% HCl. Acid digestion of the samples was carried out overnight at room temperature and then in a Milestone microwave oven (30 min with constantly increasing temperature up to 120°C, then 15 min at this maximal temperature). Each sample was completed to 50 ml with milli-Q water. For samples with a weight < 0.1 g, the mixture was 3 ml 67-70% HNO_3/2 mL 34-37% HCl and the milli-Q water added was 25 mL. Two certified reference materials (CRMs) and one blank treated and analyzed identically as the samples, were included in each analytical batch. CRMs were DOLT-4 (dogfish liver; National Research Council, Canada [NRCC]) and TORT-2 (lobster hepatopancreas; NRCC). The limits of detection (LoDs) (µg/g wet weight, w.wt) were 0.07 for Ag, Cd, Co, Cr, Pb; 0.67 for As; 0.2 for Ni; 0.35 for Se; 1.0 for V; 2.5 for Cu and Mn; 13 for Fe and Zn.

For determination of total mercury, aliquots of 5 ± 0.5 mg dried-material were analyzed directly in an Advanced Mercury Analyser spectrophotometer (Altec AMA 254) as described in Bustamante et al. (2006). Mercury analyses were run according to a thorough quality control program including the analysis of the CRM TORT-2, CRM aliquots being treated and analyzed under the same conditions as the samples. The LoDs was 0.002 µg/g w.wt. Trace element concentrations in tissues are reported in µg/g w.wt.

Determination of persistent organic pollutants

Lipid determination

The total lipid content was determined using a modified Folch et al. (1957) method. The samples were weighed (100-150 mg), homogenized then extracted 3 times with a mixture of chloroform:methanol (1:2, 2:1 and 4:1, v/v). A volume of 6.5 mL of sodium chloride 1% was added and the mixture separated into 2 phases. The lower layer containing the lipid and lipophilic compounds was collected and traces of water removed by addition of dry sodium sulphate. These extracts were shaken and stored at 4°C for 1h. Centrifugation for 10 minutes at 3000 g was used to separate the organic extract from the particulate material and the solvent was removed under a stream of nitrogen in a water bath at 40°C. When all solvent had evaporated, the weight of residue was determined and the lipid content calculated by gravimetry.

Extraction and clean-up for PCB and PBDE analyses

Samples were extracted by Pressurised Liquid Extraction (PLE) (Walsham et al. 2006). For each extraction an approximately 200 mg of blubber was cut (in vertical section), homogenised and mixed with sodium sulphate (~ 20 g). This was spiked with appropriate internal standards (PCBs by GC-MS: ^{13}C-CB28, ^{13}C-CB52, ^{13}C-CB101, ^{13}C-CB153, ^{13}C-CB138, ^{13}C-CB156, ^{13}C-CB180, ^{13}C-CB189, ^{13}C-CB194 and ^{13}C-CB209; PBDEs: FBDE160[1]). Samples were then refrigerated overnight before being ground to a fine powder using a mortar and pestle. Solvent washed PLE cells (100 mL) were packed as follows: solvent washed filter paper, pre-washed sodium sulphate (10 g), 5% deactivated alumina (30 g), solvent washed filter paper and the samples/sodium sulphate mixture prepared as above.

Samples were extracted by PLE using an ASE 300 (Dionex Ltd., Camberley, Surrey, UK) under elevated temperature (100°C) and pressure (1500 psi). Five minutes of heating was followed by 2 x 5 min static cycles. The cell flush was 50% total cell volume (i.e. 25% of the cell volume for each flush = 25 mL per flush) with a 120 s purge (using nitrogen) at the end of each sample extraction. The extraction solvent was *iso*-hexane.

Special precautions were required when analyzing PBDEs due to their sensitivity to UV light. Specifically, incoming light was minimized in the laboratory by placing UV filters over the windows.

Following PLE, the extract for PCB analyses was concentrated by Syncore (fitted with flushback module) to ~ 0.5 mL and passed through silica columns, before transferring with washing to amber glass GC vials. For 20 samples the extract was split in two, before being concentrated by syncore, one half for PBDE analysis and the other one for PCB analysis. The concentrated extracts were analyzed for PCBs by GC-EIMS (Gas Chromatography Electron Impact Mass Spectrometry) and for PBDEs by GC-ECNIMS (Gas Chromatography Electron Capture Negative Ionization Mass Spectrometry).

Determination of PCBs by GC-EIMS

The concentration and composition of 32 PCB congeners (IUPAC PCB numbers 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, 209) were determined by GC– EIMS using an HP6890 series gas chromatograph interfaced with an HP5975 MSD, fitted with a cool on-column injector and a 50 m x 0.22 mm x 25 µm SGE HT-8 column (SGE, Milton Keynes, UK). The initial oven temperature was 80°C, which was held for 1 minute. The temperature was raised at 20°C min^{-1} up to 170°C and held at this temperature for 7.5 minutes. This was followed by a ramp of 3°C min^{-1} up to a final temperature of 290°C at which it was held for 10 minutes. The MS was set for selective ion monitoring (SIM) with a dwell time of 50 ms. Calibration standards containing all 32 PCB congeners and covering the concentration range 0.6-500 ng/mL were analyzed in triplicate, and the average response used to compute the calibration curve. Correlation coefficients of at least 0.99 were achieved for all PCBs.

Determination of PBDE by GC-ECNIMS

The concentration and composition of the PBDEs, specifically BDE 28, 47, 66, 85, 99, 100, 153, 154 and 183, were determined by GC–ECNIMS using an HP6890 Series gas chromatograph interfaced with an HP5973N MSD, fitted with a cool on-column injector.

A Thames Restek STX-500 column (STX-500, 30 m × 0.25 mm i.d., 0.15 µm film thickness, Thames Restek, Buckinghamshire, UK) was utilised, fitted with a Thames Restek Siltek (0.53 mm i.d.) 5 m guard column. The injector temperature was initially at 120°C and after 2 min the temperature was elevated at 100°C min^{-1} to 300°C where it was maintained until the end of the run. The carrier gas was helium, set at a constant pressure of 15 psi. Methane was used as the reagent gas at a pressure of 1.6 bar. The transfer line was held at 280°C and the ion source at 150°C. Injections were made at 120°C and the oven temperature held constant for 2 minutes. Thereafter, the temperature was raised at 15°C min^{-1} up to 205°C. This was followed by a ramp of 6°C min^{-1} up to a final temperature of 330°C. The MS was set for selective ion monitoring with a dwell time of 50 ms. Ions monitored were m/z 78.9 and 80.9 (ions equating to bromine) for all PBDEs.

Quality control

All methods were validated by the replicate analysis of standards and samples, and through spiking experiments or analysis of certified reference materials (CRMs). Limits of detection (LoDs) were determined through the repeated analysis of a low spiked sample and the LoDs calculated from 4.65 x SD (standard deviation) of the mean concentration. LoDs were dependent on the sample size. The replicate analysis of standards on separate days gave coefficient of variation (CV%) of ~ 3% for PCBs analyzed by GC–EIMS. Recoveries greater than 75% were achieved for PCB and PBDE spiked samples and CRMs.

Internal quality control procedures incorporated the use of a laboratory reference material (LRM) for all determinants, and also a CRM for PCBs, in each batch of samples. Procedural blanks were performed with each batch of samples, and the final concentration adjusted accordingly. The data obtained from the LRM were transferred onto NWA Quality Analyst and Shewhart charts were produced with warning and action limits being drawn at ± 2x and ± 3x the standard deviation of the mean, respectively. CRM data were accepted if recoveries were between 75% and 120% of the certified concentration. Quality assurance was further demonstrated through successful participation in the QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) Laboratory Performance Studies.

Data treatment

Prior to the statistical analyses, concentration data for trace elements were logarithmic transformed to reduce skewness, and to fit parametric requirements. Concentration data for PCBs showed a marked departure from normality which was substantially improved by log-transformation. Although concentration data for PBDEs were not exactly normally distributed, transformation did not improve the distribution and untransformed data were therefore used in the statistical analysis.

Differences in trace element concentrations (µg/g w.wt), in both tissues (liver and kidneys) were tested between species on log-transformed data using ANOVA with Tukey tests, once the differences in biological parameters (i.e. age and sex) between species had been checked (ANOVA, $P > 0.05$). In order to identify any trends in the relationship of trace element concentration with sex and age, Gaussian GAMs were fitted for common dolphin, the species exhibiting the most numerous individuals with the larger range of ages. In these GAMs, age was considered as a continuous explanatory variable, while sex was treated as a categorical explanatory variable. The general form of the model performed for each trace element and tissue was expressed as follow:

$$\text{Trace element} \sim s(\text{age}) + \text{factor}\ (\text{sex})$$

For each GAM performed, the best model was established using backwards selection, removing the least important non-significant terms sequentially until all remaining effects were significant. Final selection of the "best model" was based upon the Akaike Information Criterion (AIC). Final models were checked for normality and trends in residuals and for outliers and other influential data points (Zuur et al. 2007).

In addition, Pearson's correlation analyses were calculated in order to determine any interaction between trace elements. There was a focus on trace elements known to participate in Cd and Hg detoxification process (i.e. Cu, Se and Zn).

With respect to the treatment of the concentration data for POPs (µg/g lipid weight, lw), an age-gender classification of the individuals based upon their age at sexual maturity was carried out for each species (Boyd et al. 1999); the individuals were divided into four groups: adult male (AM), adult female (AF), juvenile male (JM) and juvenile female (JF). All concentrations were normalized to the lipid content (%) of the blubber. Hence, differences in the sum of the 32 PCB congeners (ΣPCB_s) were tested between species and age-gender groups using ANOVA with Tukey tests on log-transformed data. A Kruskal-Wallis test followed by pairwise comparison tests was performed for testing differences in the sum of the 9 PBDE congeners (ΣBDEs) between age-gender groups in common dolphin.

Temporal trends analysis of ΣPCBs was first evaluated with scatterplots using LOWESS smoothing. Then, Log-linear regression analysis was applied to describe the linear component. Only males were selected in order to avoid biases caused by the effect of reproduction in sexually mature females; there may be a transfer of POPs to the foetus or pup. In addition, bottlenose dolphin males were excluded for the investigation of temporal trends because of their significant different concentrations of ΣPCB_s compared to the other species.

All statistical analyses were performed using the free software R, version 2.11.1 (R Development Core Team 2010).

RESULTS

Trace elements

Concentration of trace elements and tissue distribution

Mean trace element concentrations in liver and kidney from the five toothed whales are presented in Table 5.1 together with mean age and the number of tissue samples available for each species. Co, Pb and V were below the detection limit in all species for both kidney and liver while the concentrations of Ag and Mn were below the limit of detection in all species for kidney only. For the remaining elements, they could be categorized into two distinct groups where the pattern was generally similar across all species. Four of the elements (Cu, Fe, Hg and Se) exhibited higher concentrations in liver than in kidney (see Hg shown as an example in Figure 5.1a). In contrast, Cd, Cr and Ni all showed lower concentrations in the liver compared to kidney (see example of Cd, Figure 5.1b).

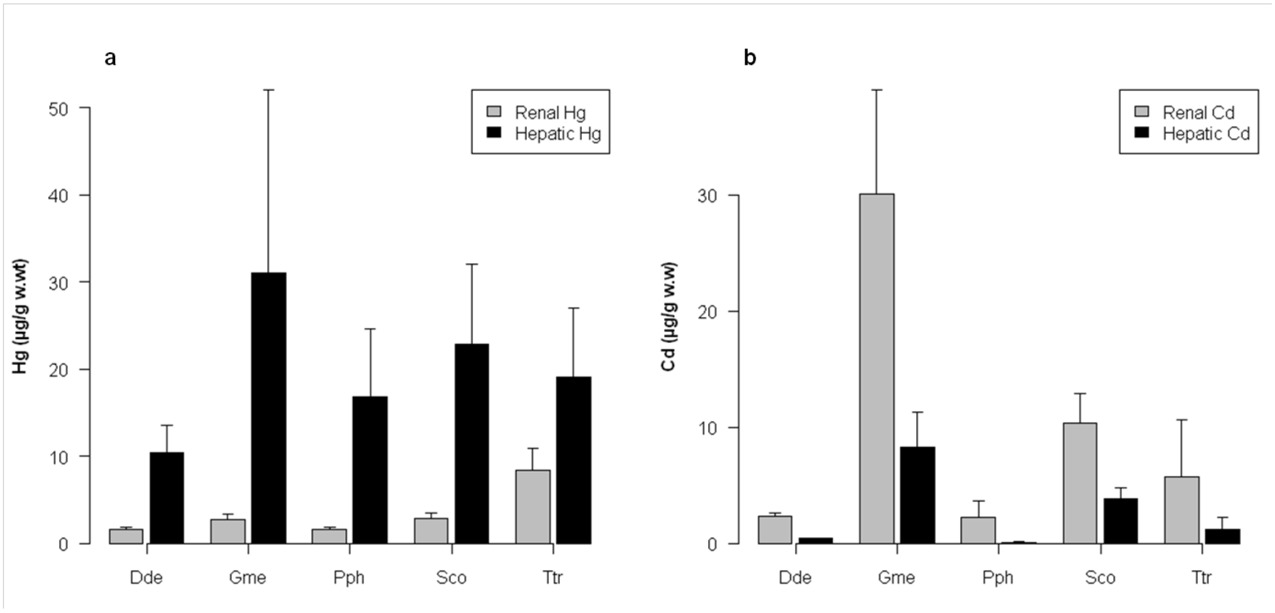

Figure 5.1 Concentrations (mean ± standard error, µg/g w.wt) of Hg (a) and Cd (b) in the liver and kidneys of common dolphin *Delphinus delphis* (Dde), harbour porpoise *Phocoena phocoena* (Pph), bottlenose dolphin *Tursiops Truncatus* (Ttr), striped dolphin *Stenella coeruleoalba* (Sco) and long-finned pilot whale *Globicephala melas* (Gme) from the NWIP. Hg and Cd were chosen since each of them represented one of the two patterns of tissue distribution of the 14 trace elements analysed.

Across all species, the ranking of highest to lowest mean trace element concentration, on the basis of absolute values presented on a wet weight basis, was Fe > Zn > Hg > Se > Cu > Cd and Mn in liver and Fe > Zn > Cd > Hg = Se > Cu in kidneys. Once biological parameters i.e. age and sex were found to have the same effect for all species (ANOVA, $P > 0.05$), significant differences in trace element concentrations were found among species both in liver and kidney (ANOVA, $P < 0.05$), with the exception of Mn and Se in liver, and Cu, Fe and Hg in kidney (ANOVA, $P > 0.05$). The significant differences found among species for trace elements using the Tukey test are summarized in Table 5.2, with the exception of Co, Pb and V whose concentrations were below the detection limit for both tissues in all species. This table illustrates the difficulty in highlighting a general trend regarding differences in trace elements concentrations among species. However the following remarks can be made. As was only detected in pilot whale and bottlenose dolphin. Harbour porpoise and common dolphin seem to be the two species most often involved in significant differences in trace element concentrations. Harbour porpoise exhibited significantly higher Ag and Cu concentrations in liver when compared to common dolphin and pilot whale. However, harbour porpoise exhibited significantly lower Cd and Hg concentrations in liver and also lower Cd concentrations in kidney compared to all other species (Tables 5.1 & 5.2). The concentrations of Cr and Ni in common dolphin kidneys were significantly higher when compared to all species except bottlenose dolphin. The same species exhibited lower Fe concentrations in liver compared to harbour porpoise and striped dolphin. Finally, striped dolphin exhibited significant higher hepatic concentrations of Zn compared to common dolphin and harbour porpoise as well as significantly higher renal concentrations of Zn compared to common dolphin. In considering the non essential elements, pilot whales and striped dolphins showed higher Cd concentrations in both tissues (Tables 5.1 & 5.2) and, together with bottlenose dolphin, also the higher Hg concentrations in liver compared to harbour porpoise and common dolphin.

Age trends of trace element concentrations and sex differences

Gaussian GAMs were fitted in order to identify any trends in trace element concentrations with age and sex in common dolphins. The trace elements showing an age effect ($P < 0.05$) were: hepatic Ag and Se, renal Fe, and Cd and Ni in both tissues. Only hepatic Ni in both tissues had a sex effect ($P < 0.05$), with greater bioaccumulation for males than for females.

Three different patterns were identified for bioaccumulation of trace elements with age (Figure 5.2). Hepatic Se and Ni showed one trend, renal Fe and Ni showed a second trend while Cd (in both tissues) and hepatic Ag, displayed a third trend. The final GAMs of hepatic Se, renal Fe and renal Cd concentrations (µg/g w.wt, log-transformed) were selected for graphic representation as the models which explained the higher variability of the data (Figure 5.2). The final GAM for hepatic Se concentrations explained 37.9% of deviance ($N = 80$, AIC = 7.52). Se concentrations increased linearly with age, this being a highly significant effect ($P < 0.001$, F = 47.7) (Figure 5.2a). The final GAM for renal Cd concentrations explained 34.2% of deviance ($N = 81$, AIC = 122.6); Cd concentrations show an initial increase with age ($P < 0.001$, F = 11.54). However, there is limited increase in concentration after about 7 years of age (Figure 5.2b). The final model for renal Fe explained 13.5% of deviance ($N = 78$, AIC = -19.5). The effect of age was slightly significant on renal Fe concentrations ($P < 0.05$, F = 3.72). Concentrations increased with age up to around 10 years after which a significant decrease in concentration was observed (Figure 5.2c).

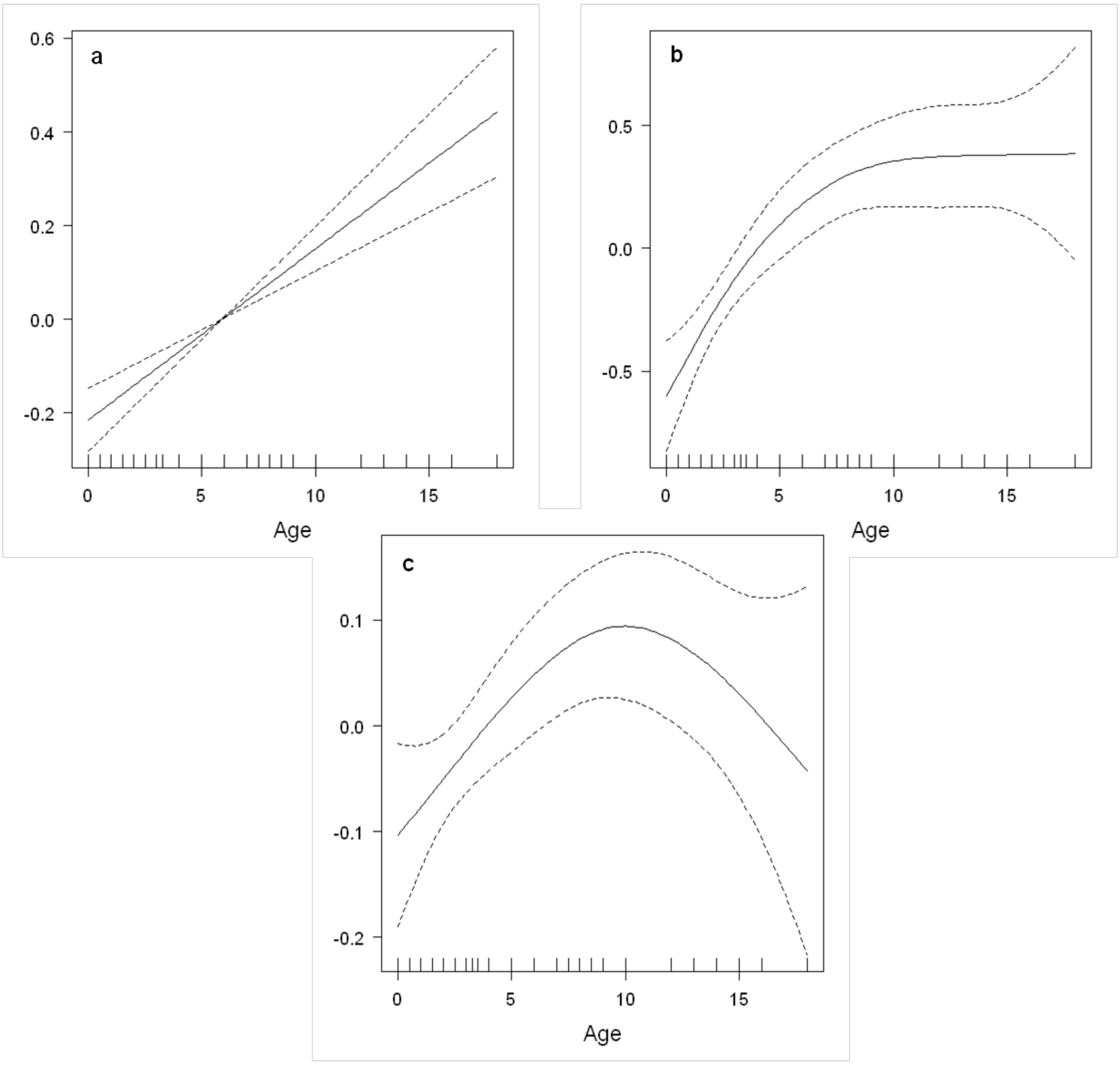

Figure 5.2 Smoother for the effect of age (years) on hepatic Se (a), renal Cd (b) and renal Fe concentrations (µg/g w.wt) (c) for common dolphin *Delphinus delphis* from the NWIP. The Y-axis represents the trend (positive or negative) of trace elements in relation to age. Tick marks on the X-axis represent sampled common dolphins. Dotted lines are the approximate 95% confidence limits.

Correlations between trace elements

Correlations between Cd, Cu, Hg, Se and Zn were tested within species separately (Table 5.3). Renal Hg and Se were positively correlated in harbour porpoise and bottlenose dolphin (Pearson correlation, $r = 0.54$ and $r = 0.75$, $P < 0.05$, respectively; Table 5.3). Cd and Zn were positively correlated in liver and negatively correlated in the kidney of striped dolphin (Pearson correlation, $r = -0.50$ and $r = 0.60$, $P = 0.05$, respectively). For common dolphin, only hepatic Cd and Cu showed a positive correlation (Pearson correlation, $r = 0.23$, $P = 0.05$). Finally, no significant correlations between these trace elements were shown for pilot whales (Table 5.3).

Persistent organic pollutants (POPs)

POP concentrations

PCBs were detected in all five species of toothed whale across age-gender groups. Significant differences were found for ΣPCB mean concentrations (µg/g lw, log-transformed) among the five species (ANOVA, $P < 0.05$), with bottlenose dolphin showing the highest ΣPCB_s concentrations followed by harbour porpoise (Table 5.4). However the adult male (AM) groups (none represented in the bottlenose dolphins or pilot whales) showed no significant differences among species (ANOVA, $P = 0.517$) whereas the adult female (AF) groups (none represented in the bottlenose dolphins) exhibited significant differences between harbour porpoise and striped dolphin (Tukey post-hoc, $P < 0.05$). Significant differences were found between bottlenose dolphin and the other species (Tukey post-hoc, $P < 0.05$), except with pilot whale (Tukey post-hoc, $P = 0.993$ and $P = 0.102$ for JM and JF, respectively) for both juvenile males (JM) and juvenile females (JF). CB153 was the congener, which showed the highest concentration in all species and in the same proportion as the ΣPCB concentrations (Table 4). Significant differences within species were only observed for common dolphin (ANOVA, $P < 0.05$), specifically between AM-AF, JF-AF and JM-AF groups (Tukey post-hoc, $P < 0.05$).

PBDE concentrations for common dolphin and harbour porpoise (one juvenile male) were lower than their blubber PCB concentrations (Table 5.5). The sum of the 9 PBDE congener (ΣBDE_s) concentrations was higher but not significantly in the one harbour porpoise analysed compared to the common dolphin individuals. No differences between age-gender groups were observed for common dolphin, even though adult females showed lower ΣBDE_s (Table 5.5) compared with other groups. The BDE47 showed the highest concentrations for both species and in all age-gender groups, following the same proportion as the ΣBDE_s concentrations.

PCBs composition of age-gender specific groups

The PCB congeners were classified into several groups according to the number and position of chlorine atoms as introduced by Boon et al. (1997) and recently developed by Weijs et al. (2009) and Yordy et al. (2010) (see Table 5.6). There is no AM group for pilot whale and there are no adult groups for bottlenose dolphin due to the lack of individuals of these species in the sampling. PCB congener metabolic groups I and II reached the highest proportions in all age-gender groups across the five species with combined percentage composition of between 63 and 86%. One exception is juvenile male pilot whales for which the proportion is only 52% (Figure 5.3). Common dolphin, harbour porpoise and bottlenose dolphin showed almost the same pattern for the juveniles' age-gender groups. Juveniles of striped dolphin and pilot whale showed clear differences with adults for both sexes, juveniles having a higher proportion of the metabolic groups III, IV and V than the adults (Figure 5.3).

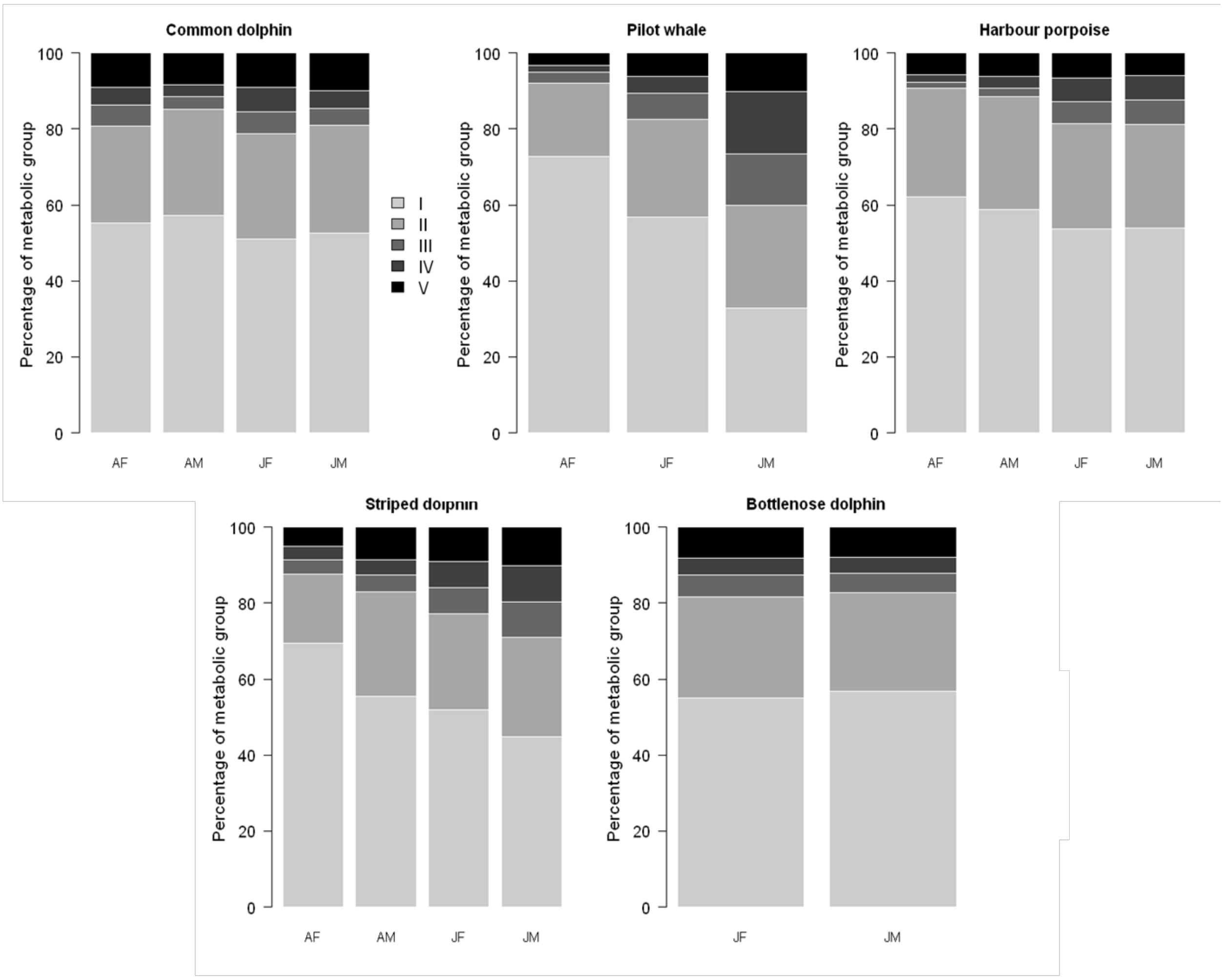

Figure 5.3 Percentages of the metabolic groups (see Table 5.6) in the four age-gender groups of common dolphin *Delphinus delphis*, harbour porpoise *Phocoena phocoena*, *bottlenose dolphin Tursiops truncatus,* striped dolphin *Stenella coeruleoalba* and long-finned pilot whale *Globicephala melas* from the NWIP.

Geographic comparison and temporal trends

Table 5.7 reports the concentrations of PCBs, PBDEs, Hg in liver and Cd in kidneys present in the five toothed whales analyzed in this study and from previous studies for the NWIP and adjacent areas of the Northeast Atlantic. These contaminants were selected as they are the most commonly reported to occur at high levels in marine mammal tissues.

The ΣPCB$_s$ concentrations in blubber showed a slight increase between 2004 and 2006. However, between 2006 and 2008 there was a significant decrease ($P < 0.01$; Figure 5.4).

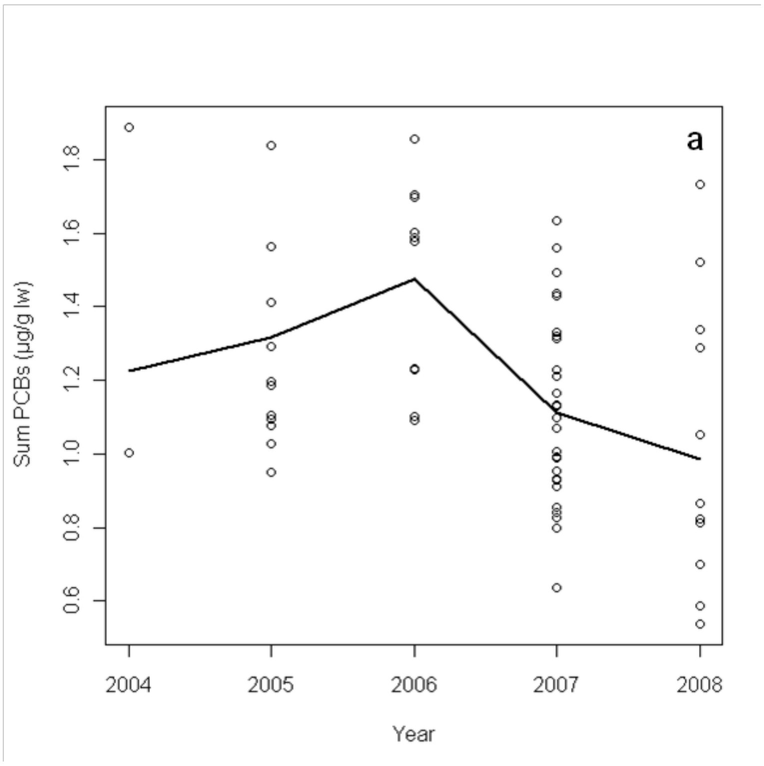

Figure 5.4 Temporal trends of the sum of PCBs in blubber (µg/g lw) of common dolphin *Delphinus delphis*, harbour porpoise *Phocoena phocoena*, striped dolphin *Stenella coeruleoalba* and long-finned pilot whale *Globicephala melas* from the NWIP collected between 2004 and 2008. The smoothing lines (robust, locally weighted scatterplot smoothing system based on the LOWESS algorithm) represent the fitted non-linear trends of the values. The y-axis (ΣPCB concentrations) is a Logarithmic scale.

DISCUSSION

Trace elements

Marine mammals may be potentially valuable indicators of the contamination of the marine environment by trace elements. This is because of both their long life span, and also the fact that they are apex predators in food webs. Consequently, trace element concentrations in the tissues of marine mammals are well documented on a global basis, with a focus on Hg and Cd, which are non essential, and the essential trace elements including Cu and Zn which are recognised for their role in the detoxification of Cd through metallothioneins and Se, for its role in the detoxification of Hg. However, very few studies have investigated the wide range of trace elements in the liver and kidney of marine mammals as studied here (Bustamante et al. 2003, Bryan et al. 2007, Griesel et al. 2008, Stavros et al. 2008). The reason is probably that the trace elements analyzed are either not bioaccumulated in large amounts in mammalian tissues (Thompson 1990); or liver and kidney are not the organs of storage for some of them. In this study, the five species of toothed whales investigated did contain Ag in the liver, Mn in kidney and Co and Pb concentrations in both tissues below their limit of detection (see Material and Methods).

The trace elements analysed have different biological characteristics. Ag, Cd, Hg and Pb are non essential elements for which no biological function has so far been demonstrated. In contrast, Co, Cr, Cu, Fe, Ni, Se and Zn are essential elements whose deficiency induces pathology in humans and more generally in mammals (Chappuis 1991, Underwood 1977, Muyssen et al. 2004). Finally, Mn and V are present in low concentrations in humans, but no pathological consequence resulting from their deficiency has been demonstrated. The status of As depends on the physico-chemical form to which the mammal is exposed. In humans, the toxicological risk of As is related to the inorganic form to which individuals are exposed, specifically in occupational situations. In the marine environment, tissues from marine invertebrates and fish have been shown to contain high concentrations of arsenic, usually in the range of 1 to 100 μg/g dry weight. Most of the As is in the form of organoarsenic compounds, particularly arsenobetaine (Neff 1997). Therefore, marine As generally represents a low risk to predators. In our study, only pilot whale and bottlenose dolphin exhibited detectable As concentrations, probably as a consequence of a higher exposure through their diet compared to the other species. Pb has a strong affinity for calcified tissues while concentrations are often very low (less than 1 μg/g w.wt) in the soft tissues of marine mammals (Honda et al. 1983, Wagemann & Muir 1984, Cardellicchio 1995, Storelli et al. 1999, Cardellicchio et al. 2002). Pb concentrations were particularly low in the five species studied from the NWIP. Considering the other non essential elements, pilot whale and striped dolphin exhibited the highest Cd concentrations in both liver and kidneys. This result is not surprising since both species primarily feed in offshore waters and have a high proportion of oceanic squids in their diet (Pierce et al. 2007, 2010, Spitz et al. 2006); squid are known to accumulate large amounts of Cd (Bustamante et al. 1998, Lahaye et al. 2005). None of the species contained high Hg concentrations, which is probably related to the young age of the individuals in this study.

Considering essential trace elements, their distribution can be explained by toxicodynamic processes and liver was largely revealed as the main storage tissues in mammals (Underwood 1977, Chappuis 1991). This is the case in this study for most of them. The exceptions were Cr and Ni, which exhibited higher concentrations in kidneys than in liver for all species except pilot whales. The other characteristic of essential elements is that they are closely regulated through homeostasis. Several different routes of entry of trace elements have been suggested in marine mammals: uptake from the atmosphere, absorption through the skin, across the placenta and *via* milk, by ingestion of seawater and ingestion of food (Caurant et al. 1994, Wagemann et al. 1995, Law 1996, Lahaye et al. 2007). However, the major route seems to be *via* food (André et al. 1990, Law 1996). The processes leading to homeostatic control include regulation of intestinal absorption and/or biliary and urinary excretion. As a consequence, essential trace elements are generally not normally bioaccumulated with age. This is the case for almost all the trace elements analyzed for common dolphin in this study; the majority of them revealed no significant effect of age ($P > 0.05$) and for those having an effect of age (Ni, Se and Fe) their GAM models only explained a small proportion of the data variability (< 20%). In this study, hepatic Ni showed the same behaviour as hepatic Se; their concentration increased linearly with age (Figure 5.1a). Se exhibited the same increasing concentrations with age as anticipated for Hg. This is explained on the basis of the detoxification process which leads to the fossilization of Hg through the formation of tiemannite in liver of marine mammals (Koeman 1973, Martoja & Berry 1980). Across the individual common dolphins from this study, Hg did not accumulate with age. However, the Hg data included 3 outliers. If they were removed from the dataset then there was an effect of age, there being a linear increase in Hg concentration in liver across the full age range of the individuals. Renal Cr, Fe and Ni exhibited an increase followed be a decrease after 10-years (Figure 5.1c). These three elements are all considered as essential in humans. They are involved in the regulation of very different metabolic processes as cofactors of enzymes, either activated by a metal ion or as specific components of them. However the physiological needs probably differ over the life of an individual, possibly explaining the acute absorption during the first years. Moreover, the significant differences among species probably reflect different physiological and/or metabolic needs rather than an influence of the diet.

Conversely, non essential trace elements have been reported to increase with age in several species and across various geographical areas, especially the most studied trace elements, Hg and Cd (Honda et al. 1983, Caurant et al. 1994, Bustamante et al. 2004, Lahaye et al. 2006, 2007). Both elements accumulate probably because uptake exceeds the ability of the animal to excrete Hg and Cd. As a consequence of this, concentrations in their respective storage tissues, liver for Hg and kidney for Cd, will increase with age throughout life. However, the kinetic seems to differ between Hg and Cd, but remaining similar across species: Hg increases throughout the life of individuals while Cd reaches a plateau after several years, more or less rapidly according to the species (Caurant et al. 1994, Bustamante et al. 2004, Lahaye et al. 2006, 2007). In this study, the same patterns were observed: for Hg in liver, when the outliers were removed as discussed above, and for Cd (in liver and kidney) as illustrated in Figure 5.1.

Sex is another biological factor that can have an influence on trace element burdens in marine mammals. Specifically, reproductive activities such as pregnancy, parturition and lactation can modify these levels (Honda et al. 1987). In addition, differences in prey preferences and in feeding areas between females and males may also affect trace element burdens (Gochfeld 1997). Several studies have demonstrated these sex differences (Honda et al. 1987), while numerous others concluded that gender did not affect the levels of trace elements in marine mammals (Wagemann et al. 1995). Consequently, the sex influence on trace element concentrations is not that predictable in marine mammals as it seems to vary with the element, the tissue and the species analyzed (Aguilar et al. 1999). However, as has been observed previously, females show greater concentrations than males, due to commonly noted sexual dimorphism. This phenomenon could indeed lead to: 1) a dilution process in males as a result of their large size (Caurant et al. 1994, Bustamante et al. 2004), 2) high input rate for females due to the energetic cost of the reproduction (gestation and lactation) and 3) as a result of a lower transfer of trace elements from females to their progeny as it is known to occur for other pollutants (e.g. POPs, see discussion below). The sex-related differences found for common dolphin in this study revealed that there was no clear pattern with few elements being affected by sex, as described in the literature. This is probably explained by the fact that common dolphin do not show an important sexual dimorphism. Moreover, the sampled individuals for this species were very young, many of them being sexually immature, thus excluding the potential effect of the increased needs of females during their reproduction period.

Trace element concentrations vary greatly among marine mammals. This is a result of the differences in dietary uptake of the trace elements and is also a function of the habitat used. This study revealed significant differences ($P < 0.05$) between species for all trace elements at least in one of the two tissues analyzed, except for Mn. These species differences are more intriguing for essential trace elements than the non essential ones, since it indicates that the physiological concentrations due to homeostatic processes would be different depending on the species. However, these different essential trace element concentrations may also be the consequence of the different exposure of these species to the non essential elements, and the complex interactions that exist either for absorption or because of the competition for binding to proteins in the different tissues. As essential elements for the organisms, it could be postulated that the concentration of these elements was dependent on the physiology and the metabolism of the specimens. In addition, it has been shown that Cu and Zn are homeostatically controlled in liver of marine mammals (Law et al. 1991) and their concentrations are probably homogeneous in these tissues due to their metabolic importance. In this study Zn was significantly correlated with Cd in both tissues of striped dolphins (Table 5.3), one of the species exhibiting the highest Cd concentrations which could be interpreted as a disturbance in this species.

Persistent organic pollutants

Numerous studies have investigated the concentrations of POPs in a wide range of marine mammals mainly because they occupy the top of aquatic food webs and thus, they accumulate considerable amounts of these contaminants in their tissues through biomagnification. Nevertheless, as we discussed above, few studies were carried out in the NWIP (Pierce et al. 2008) and none in five different toothed whale species. The significant human activity and industrial development in this area make this study very important, not only to provide the contaminant status of the POPs for these species but also since marine mammals are considered as "pollution indicators for organochlorines" (Mössner & Ballschmiter 1997).

POPs enter marine mammals almost exclusively *via* the diet and their amounts in tissues vary greatly with intake factors (Aguilar 1989), i.e. trophic level, type of prey eaten, and with the environmental pollution, all these factors having thus to be taken into consideration when interpreting POP concentrations in marine mammals. The five toothed whale species studied showed relatively close and not significantly different trophic levels in the NWIP, ranging from 4.3 to 5.3 (Méndez-Fernandez et al. 2012). However, it was demonstrated by both stomach content analyses and stable isotopic signatures (Santos et al. 2004a,b,c, 2007a,b, Pierce et al. 2007, Méndez-Fernandez et al. 2012, submitted), that these species feed on different prey types and have different feeding habitats. In addition, marine mammal organochlorine loads tend to increase with age during the juvenile stage of both genders, because the uptake of pollutant usually exceeds metabolism and excretion. In adult males, this pattern continues throughout their life span with their pollutant levels increasing with age, while in adult females, the transfer of pollutants to offspring during gestation and lactation reduce progressively pollutant concentrations to decline progressively with age (Borrell et al. 1996). Therefore, these biological factors have to be considered together with the proper ecological factors of the species to interpret inter- and intra-specific differences.

For common dolphin, harbour porpoise and striped dolphin, the AM group exhibited the highest ΣPCB concentrations probably due to bioaccumulation of these pollutants with age (Table 5.4). However, there is no significant difference in concentrations among species ($P > 0.05$). This may be because of the low number of individuals available for each species and the absence of individuals of bottlenose dolphin and pilot whale in this group. In contrast, the AF group displayed significant differences in ΣPCB concentrations between harbour porpoise and striped dolphin, with higher concentrations for harbour porpoise (Table 5.4). JM and JF bottlenose dolphins showed significantly greater ΣPCB concentrations compared to the other species, except for pilot whale. These results are not surprising since harbour porpoise and bottlenose dolphin from the NWIP are characterized by their coastal habitat and a fish-feeding habit (López et al. 2004, Pierce et al. 2010, Méndez-Fernandez et al. 2012). These species are usually observed from the coast, which is an indicator of their proximity to areas with greater anthropogenic impact (López et al. 2004, Pierce et al. 2010). In addition, both species are more frequently seen in the southern part of the study area, which is more populated and industrialized than the northern part (López et al. 2004, Pierce et al. 2010). Furthermore, fish are known to accumulate greater amounts of POPs than cephalopods (O'Shea & Tanabe 2003). Thus these ecological factors explain the high ΣPCB concentrations found for AF, JF and JM of these species in the NWIP. In contrast, pilot whale is mainly associated with oceanic habitats, remote from land-based sources of contamination (López et al. 2004, Pierce et al. 2010, Méndez-Fernandez et al. 2012) and mainly feeds on cephalopods. However, pilot whale species in the

NWIP also frequents neritic habitats, especially for feeding (Pierce et al. 2007, Méndez-Fernandez et al. submitted), and only two JM and two JF individuals were available in this study. The small sample size could well be the reason for there being no significant difference between pilot whale and bottlenose dolphin ΣPCB concentrations for the JM and JF groups. Differences within species were only found for common dolphin, especially between AM-AF, JF-AF and JM-AF groups, which suggests that has adult females have a different accumulation pattern from the other groupings in common dolphin, probably due to the well described transfer during gestation and lactation (Covaci et al. 2002, Wolkers et al. 2004).

PBDE concentrations (i.e. ΣBDE_s) were determined for common dolphin and harbour porpoise; the concentrations were almost 10 times lower than PCBs in blubber for both species and age-gender groups (Tables 5.4 & 5.5). This agrees with several previous studies (Nyman et al. 2002, Pierce et al 2008, Yogui et al. 2011). In common dolphin, juveniles tended to have higher PBDE concentrations than adults suggesting that the capacity for metabolic breakdown increases with age or with body burden. These findings were, however, not statistically significant but were in agreement with results from previous studies (Law et al. 2006, Weijs et al. 2009). Similar to PCBs, PBDE concentrations in the AF group were lower than for the other age-gender groups supporting the hypothesis that AF animals reduce their pollutants load through gestation and lactation (Covaci et al. 2002).

The proportion of higher chlorinated PCBs (≥ 6 chlorines) increases up the food chain as these compounds are less volatile, more lipophilic and more resistant to metabolic and microbial degradation (Storelli & Marcotrigiano 2003). As a consequence, top predators, such as marine mammals, potentially will have a greater proportion of the higher chlorinated PCBs (hexa- to deca- CBs) in their tissues than of the less chlorinated (tri- to penta- CBs). This study supports the hypothesis; the superhydrophobic metabolic groups I and II (Table 5.6) appear to reach the highest proportions, with percentages of between 63-99% for all species except for pilot whale

(52%) (Figure 5.3). The higher proportion of highly chlorinated PCBs is in agreement with other published studies on marine mammals (Boon et al. 1997, Weijs et al. 2009) and on other biological models such as fish and bivalve molluscs (Carro et al. 2006, Webster et al. 2011), which confirms the increase of highly chlorinated PCBs through the food chain, and the lower capacities that these organisms (especially the marine mammals; Tanabe et al. 1988) have to metabolize this group of PCBs, leading therefore to bioaccumulation. Common dolphin and harbour porpoise did not show important differences between age-gender patterns. These differences appear clearer for striped dolphin and pilot whale species, which showed a higher proportion of the metabolic groups III, IV and V in juvenile than in adult groups and in males than in females (Figure 5.3). This is probably the consequence of the PCB transfer from females to their offspring during the gestation and lactation, exposing neonates to some of the highest concentrations of these compounds they will encounter during their lifetime (Addison & Brodie 1987). Moreover, since the calculated transfer rates from total blubber burden demonstrate the more ready transfer of lower chlorinated congeners to the fetus compared to higher chlorinated congeners (Tanabe et al. 1997, Desforges et al. 2012), this can explain the higher proportions of the lower chlorinated metabolic groups in juvenile groups (Figure 5.3).

CB153 was the dominated PCB congener in all individuals across the five species and BDE47 the most concentrated PBDE congener for common dolphin and harbour porpoise. As revealed in this study, these highly chlorinated and low volatile congeners usually follow the same pattern as the ΣPCBs and ΣPBDEs in organisms (Tables 5.4 & 5.5), and are the main constituents of industrial mixtures. Thus, their presence in nearly all organisms and areas of aquatic ecosystems, allow its use to compare levels among different organisms and to study concentration trends.

Geographic comparison and temporal trends

In order to evaluate the contaminant status of the NWIP toothed whales within the Northeast Atlantic waters a geographic comparison was made (see Table 5.7) by selecting previous studies carried out in the NWIP (Galicia and in Portugal) and those in the Northeast Atlantic adjacent areas. For this purpose, the main contaminants studied in marine mammals were selected and include: PCBs and PBDEs in blubber, Hg in liver and Cd in kidneys. The differences in concentrations among marine mammals from various locations might be due to variations in biological parameters i.e. age, sex, metabolic capacity and nutritive and reproductive status, but also in feeding and habitat preferences of the species in the areas or in the environmental chemistry of the water mass. Therefore all these factors were considered when comparing results among areas.

Bottlenose dolphin and harbour porpoise, which tend to be the more coastal species in the NE Atlantic waters (López et al. 2004, Pierce et al. 2010), and consequently the more exposed to industrial and human activities, showed the highest PCB concentrations in this study and slightly higher than individuals from others areas, with the exception of one bottlenose dolphin from England (a young individual of 10 months), and Baltic porpoises. Concentrations of renal Cd, one of the non essential elements, seem higher for Iberian bottlenose dolphins and slightly higher for Iberian porpoises in comparison with those from French waters. This could be a consequence of high inputs of Cd result of the upwelling waters of NWIP. In terms of feeding ecology, a high consumption of cephalopod species and/or a more oceanic habitat for the Iberian specimens could explain these high Cd concentrations (Bustamante et al. 1998, Lahaye et al. 2005), since the existence of oceanic forms in the northern part of the NWIP was recently demonstrated for bottlenose dolphin (Fernández et al. 2011a,b). The only important variation for hepatic Hg was found between Iberian and French bottlenose dolphins (Holsbeek et al. 1998; Table 5.7), the latter showing high Hg concentrations, likely suggesting a high proportion of aging individuals.

For common dolphin, although the POPs comparison was difficult due to the scarcity of studies on NE Atlantic waters, it revealed a few differences between Iberian common dolphins and the individuals from other areas. Renal Cd concentrations are higher in oceanic common dolphins from France and Ireland, explained by their oceanic habitat (Lahaye et al. 2005). Concentrations of Hepatic Hg were also higher in French and Irish common dolphins than in Iberian ones, and again probably due to a major proportion of aging individuals.

Few data were found for pilot whale, which showed in this study lower PCB, hepatic Hg and renal Cd concentrations than those reported in individuals from the Faroe Islands, one of the most studied areas for this species. Although the Faroe Islands are relatively far from major sources of anthropogenic pollution, marine top predators from this area, such as the long-finned pilot whale, exhibit much higher Cd and Hg concentrations in their tissues than those reported for individuals from temperate areas (Julshamn et al. 1987, Caurant et al. 1994).

Finally, for striped dolphin, most of the PCBs studies have been carried out on animals from the Mediterranean Sea, which showed generally greater concentrations than individuals from this study (Borrell & Aguilar 2005). Our PCB results are similar to what was found for the specimen from England but much lower than was measured in the French one; however caution is needed since only one young individual was analysed in each study. Regarding trace elements, renal Cd concentrations are generally low, which was slightly unexpected given that this species is known to be oceanic and because of the important inputs of Cd in the NWIP as mentioned above. For hepatic Hg, concentrations in this study are lower than those from French studies but higher than those from the Celtic Sea. This shows once more the high hepatic Hg concentrations in French dolphins in comparison with Iberian ones.

A decrease of PCBs in the marine environment has been observed in Spain and more generally in Europe over the last decades due to restrictions and bans on their industrial use and emission (Gómara et al. 2005, González-Quijano et al. 2006, O'Connor & Lauenstein, 2006). Specifically, a study carried out by Carro et al. (2010) revealed decreasing PCB concentrations in mussel samples (*Mytilus galloprovincialis*) during the past 10 years in most parts of Galicia (northern part of the NWIP). A significant decreasing trend of the PCB concentrations between 2004 and 2008 was also observed in our study ($P < 0.05$; Figure 5.4), showing that this decrease is now evident in apex predators as well. This suggests that the decrease might have been effective for more than four years in the marine food webs.

Five years is a short-term time series but accepted for temporal trend conclusions (AMAP, 2001). Monitoring temporal trends of contaminants is essential to any programme concerned with assessing the possible effects of contamination on wildlife. It is recommended that a temporal monitoring programme of these compounds in marine mammals from the NWIP is established.

Toxicological aspect

Reliable toxicity data for predating marine mammals are scarce and threshold levels are often extrapolated from terrestrial species. Indeed, potential effects of toxic compounds cannot be tested in free-living animals because experimental manipulations are undesirable (Das et al. 2003b) and epidemiological studies nearly impossible to carry out. This is why, the contamination status of the five toothed whales from the NWIP can only be evaluated through a comparison of the non-essential elements and POP concentrations with the thresholds found in the literature, or with the same species from other geographical areas. The levels of Cd in liver and kidney of all species are below the suggested threshold levels of toxic effects in mammals (Law 1996, Ma 1996), except for pilot whale, for which 12% of the individuals were above the Cd levels of 20-200 µg/g w.wt in liver and 50-400 µg/g w.wt in kidney. For total hepatic Hg concentrations, several individuals from this study exceeded the threshold level of 60 µg/g w.wt for liver damage in mammals (Law 1996, AMAP 1998). This was again pilot whale and bottlenose dolphin with 12.5% of the individuals exceeding the threshold value in each species.

There are several other examples of very high concentrations of total Hg in the liver of wild marine mammals, but no signs of Hg intoxication have been reported, due to their detoxification mechanism, as discussed above. However, it has been proposed that chronic Hg accumulation may result in increased active liver disease (Law 1996). In this study, Se in liver is the element which most exceeded the threshold level established for hepatic lesions (> 7 µg/g w.wt; WHO 1997) and for all species, being higher in pilot whale and striped dolphin with 37 and 33% of the individuals, respectively. However, this threshold was obtained for terrestrial mammals and very few samples were available for pilot whale; therefore we must be cautious when interpreting these results.

A total PCB concentration of 17 µg/g lw has been reported as a threshold level for health effects, including immunosuppression and reproductive disease (Kannan et al. 2000). Since this value was based on comparison with the main peaks in the commercial PCB mixture Aroclor 1254, this level cannot be directly compared with our ΣPCB_s levels. Following Jepson et al. (2005), we derived the summed concentration of the ICES (International Council for the Exploration of the Sea) 7 PCBs in each sampled animal. The ICES7 PCBs were recommended by the European Union Community Bureau of Reference; these PCBs (CB28, 52, 101, 118, 138, 153 and 180) were selected as indicators due to their relatively high concentrations in technical mixtures and their wide chlorination range. In addition, their concentrations usually followed the same pattern as ΣPCB_s concentrations. Multiplying the sum of the ICES7 PCB (i.e. ΣICES7 PCB) by three gives a figure that is equivalent to the Aroclor 1254 value reported by Kannan et al. (2000). On this basis, the threshold value was frequently exceeded for all species in the present study, especially by bottlenose dolphin (100% of individuals) and harbour porpoise (75% of individuals). PBDE concentrations in common dolphin and harbour porpoise are at least 10 times lower than PCBs, being slightly higher for harbour porpoise. Although there is no information on the toxic threshold for PBDEs in marine mammals, experimental exposure studies revealed that

PBDEs induce a wide variety of disorders including cancer, reproductive and developmental toxicity, endocrine disruption and central nervous system effects (ATSDR 2004).

However, some caution is needed when applying these thresholds to cetaceans, since the published experimental data all derive from mammals of the order Carnivora (mink, otters and seals). Another issue is the extent to which the sampled animals were representative of the population. Thus, there probably were a higher proportion of animals that had died due to disease or parasitic infection and together with the small number of some of the toothed whale species available for analyses, it is difficult to know whether this does reflect the condition of animals in the extant populations of the NWIP.

Conclusions

This study has provided baseline data on trace elements and POP compounds in five of the most frequent toothed whales inhabiting the NWIP. Despite the interpretation of concentrations in the light of biological or ecological factors, the bioaccumulation of trace elements in marine mammals has been shown to be less predictable than POPs. The considerable influence of the biological factors, especially age, makes the consideration of these factors essential to correctly interpret the contaminant concentrations. However, the assessment of ecological factors, including feeding ecology and habitat used, is also indispensable as well as the influence of the sex of the animals. Consequently, biological and ecological factors are difficult to separate when interpreting and comparing trace element and POP concentrations, and these have to be taken together when making assessments.

Bottlenose dolphin and harbour porpoise seem to be the more contaminated species in terms of PCBs as shown by their higher concentrations when compared to the rest of the NWIP toothed whales and with those of adjacent NE Atlantic waters. At the same time, a greater number of individuals of both species exceeded the PCB toxic thresholds. The bottlenose dolphin and harbour porpoise are recorded in the EU Habitats Directive as a Species of Special Interest

(Directive 92/43/CEE), the protection of which requires the designation of Special Areas of Conservation (SACs) by the EU Member States. Knowledge of contamination status and specific monitoring programmes are therefore required to be able to design and apply effective conservation measures for these species in the NWIP.

With regard to future research priorities in the NWIP, a bigger sample sizes, the identification of additional contaminants, especially organochlorine compounds (e.g. DDT), as well as a probabilistic risk-assessment approach, are also advisable to better resolve the contamination status of the cetacean species in the NWIP and their situation with respect to adjacent areas of the NE Atlantic waters. Finally, to guarantee the establishment of adequate management measures, efforts should be directed towards the development of a continuous monitoring program of contaminants in cetaceans from the NWIP (i.e. as it is carried out on others biological models in the area) to provide regular basis data, and to improve temporal trends assessment, allowing the detection of ongoing changes and to evaluate the impact of future protected areas for these species in the NWIP

Table 5.1 Trace element concentrations (mean ± SD, µg/g w.wt) in kidney and liver 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 NWIP. 'n' is the total number of samples analyzed per species, with the values on the left side being the number of kidney samples analyzed and those on the right side the number of liver samples analyzed. Age values are expressed as years (mean ± SD).

		Delphinus delphis	*Globicephala melas*	*Phocoena phocoena*	*Stenella coeruleoalba*	*Tursiops truncatus*
	n	98/100	6/8	12/14	16/18	6/8
	Age	5.8 ± 5.1	4.5 ± 4.3	7.4 ± 6.5	4.1 ± 5.0	4.8 ± 1.8
	Tissue	mean ± SD	Mean ± SD	mean ± SD	mean ± SD	mean ± SD
Ag	Kidney	< 0.07	< 0.07	< 0.07	< 0.07	< 0.07
	Liver	0.21 ± 0.21	0.13 ± 0.21	1.04 ± 1.06	0.3 ± 0.3	0.20 ± 0.19
As	Kidney	< 0.67	1.0 ± 0.9	< 0.67	< 0.67	0.7 ± 0.4
	Liver	< 0.67	1.2 ± 0.8	< 0.67	< 0.67	0.99 ± 0.94
Cd	Kidney	2.3 ± 2.7	30.0 ± 26.9	2.2 ± 5.3	10.3 ± 11.0	5.7 ± 13.8
	Liver	0.4 ± 0.5	8.3 ± 8.4	0.08 ± 0.1	3.4 ± 3.8	1.2 ± 2.9
Co	Kidney	< 0.07	< 0.07	< 0.07	< 0.07	< 0.07
	Liver	< 0.07	< 0.07	< 0.07	< 0.07	< 0.07
Cr	Kidney	1.8 ± 2.1	0.12 ± 0.25	0.25 ± 0.8	0.4 ± 0.8	1.3 ± 2.7
	Liver	1.2 ± 1.4	0.12 ± 0.15	< 0.07	0.2 ± 0.5	0.27 ± 0.6
Cu	Kidney	2.8 ± 0.7	2.9 ± 0.7	2.97 ± 0.83	3.3 ± 1.50	2.7 ± 0.5
	Liver	5.08 ± 2.1	3.4 ± 1.2	8.7 ± 5.90	7.3 ± 2.16	4.5 ± 2.7
Fe	Kidney	123 ± 111	128 ± 66	132 ± 40	131 ± 50	131 ± 22
	Liver	195 ± 88	323 ± 232	398 ± 250	321 ± 177	258 ± 158
Hg	Kidney	1.6 ± 2.1	2.7 ± 1.9	1.6 ± 0.8	2.8 ± 2.6	8.4 ± 7.2
	Liver	10.4 ± 31.8	31.0 ± 59.5	16.8 ± 30.0	22.9 ± 39.1	19.1 ± 22.4
Mn	Kidney	< 2.5	< 2.5	< 2.5	< 2.5	< 2.5
	Liver	2.6 ± 1.1	< 2.5	3.4 ± 1.6	3.2 ± 1.35	< 2.5
Ni	Kidney	0.8 ± 0.99	< 0.2	0.2 ± 0.5	< 0.2	0.6 ± 1.2
	Liver	0.5 ± 0.6	< 0.2	< 0.2	< 0.2	< 0.2
Pb	Kidney	< 0.07	< 0.07	< 0.07	< 0.07	< 0.07

Table 5.1 (*Continued*)

	Liver	< 0.07	< 0.07	< 0.07	< 0.07	< 0.07
Se	Kidney	2.7 ± 1.1	2.9 ± 1.6	3.0 ± 0.95	3.2 ± 1.4	4.6 ± 3.1
	Liver	5.0 ± 5.8	16.9 ± 30.1	8.0 ± 11.6	12.3 ± 17.2	10.8 ± 13.0
V	Kidney	< 1	< 1	< 1	< 1	< 1
	Liver	< 1	< 1	< 1	< 1	< 1
Zn	Kidney	18.8 ± 8.7	22.6 ± 5.4	19.1 ± 1.7	24.2 ± 7.5	19.6 ± 5.4
	Liver	40.5 ± 19.5	42.3 ± 14.5	32.7 ± 7.8	53.0 ± 21.1	33.8 ± 8.6

The less than values are where the values were less than the limit of detection

Table 5.1 ANOVA and Tukey test results for log-transformed trace element concentrations of liver and kidney of 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) from the NWIP.

	Overall model *P* < value		Tukey test	
Trace element	Liver	Kidney	Liver	Kidney
Ag	0.0002	–	Pph > Dde* Pph > Gme**	
Cd	< 0.0001	< 0.0001	Pph < Gme** Pph < Sco*** Pph < Dde* Dde < Sco*** Dde < Gme* Ttr < Sco*	Pph < Gme* Pph < Sco* Dde < Sco*
Cr	< 0.0001	< 0.0001	Dde > Sco*	Dde > Gme* Dde > Pph *** Dde > Sco**
Cu	< 0.0001	–	Pph > Dde* Pph > Gme* Sco > Dde* Sco > Gme*	
Fe	< 0.0001	–	Dde < Pph * Dde < Sco*	
Hg	< 0.0001	–	Pph > Dde** Pph < Ttr* Pph < Sco* Pph < Gme**	
Mn	–	–		
Ni	–	< 0.0001		Dde > Gme* Dde > Pph* Dde > Sco*
Se	–	0.02		Dde < Ttr*
Zn	0.012	0.009	Sco > Dde* Sco > Pph*	Sco > Dde*

*0.01 < *P* < 0.05
**0.001 < *P* < 0.01
****P* < 0.001

Table 5.2 Correlation matrix of Pearson's correlation coefficient between non-essential and essential elements in the liver (L) and kidney (K) 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 NWIP.

	HgL/SeL	HgK/SeK	CdL/CuL	CdK/CuK	CdL/ZnL	CdK/ZnK
Delphinus delphis	-0.022	-0.086	**-0.235***	-0.01	-0.075	-0.136
Globicephala melas	-0.514	-0.274	-0.255	-0.076	-0.021	0.331
Phocoena phocoena	0.527	**0.544***	0.116	0.295	0.081	0.242
Stenella coeruleoalba	0.242	-0.007	-0.036	0.196	**-0.506***	**0.600***
Tursiops truncatus	0.651	**0.755***	-0.057	0.290	-0.173	0.589

*$0.01 < P < 0.05$

Table 5.4 Age-gender specific PCB concentrations (mean ± SD, μg/g lw), lipid content (%) and age values expressed in years (mean ± SD) 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 NWIP. AF: adult female, AM: adult male, JF: juvenile female and JM: juvenile male.

	n	Age	Lipid	CB153	ΣPCBs[a]	I[b]	II[b]	III[b]	IV[b]	V[b]
Delphinus delphis	81	6.6 ± 5.4	60.6	5.2 ± 5.0	17.2 ± 14.1	9.4 ± 8.6	4.8 ± 3.9	0.7 ± 0.4	0.7 ± 0.7	1.6 ± 1.5
AF	13	13.9 ± 3.2	55.6	2.3 ± 2.4	8.5 ± 7.5	4.7 ± 4.2	2.2 ± 1.9	0.5 ± 0.5	0.4 ± 0.4	0.8 ± 0.7
AM	24	10.1 ± 3.8	53.1	7.3 ± 6.6	22.7 ± 18.3	13.0 ± 11.3	6.3 ± 5.0	0.8 ± 0.4	0.7 ± 0.3	1.9 ± 1.4
JF	18	2.5 ± 1.6	68.3	3.9 ± 1.9	13.8 ± 6.3	7.0 ± 3.3	3.8 ± 1.8	0.8 ± 0.3	0.9 ± 1.3	1.3 ± 0.6
JM	26	2.2 ± 1.4	64.4	5.3 ± 5.0	18.3 ± 13.7	9.6 ± 8.5	5.2 ± 4.0	0.8 ± 0.3	0.7 ± 0.4	2.0 ± 2.1
Globicephala melas	3	5.2 ± 5.5	65.2	3.6 ± 4.1	16.2 ± 19.7	6.2 ± 5.8	4.3 ± 5.4	1.9 ± 2.8	2.2 ± 3.5	1.5 ± 2.1
AF	1	11.5	77.3	0.4	2.0	1.4	0.4	0.1	0.0	0.1
JF	1	2.0	62.3	2.2	7.9	4.5	2.0	0.5	0.3	0.5
JM	1	2.0	56.0	8.3	38.7	12.7	10.5	5.2	6.3	4.0
Phocoena phocoena	12	7.0 ± 6.5	77.9	6.3 ± 6.7	20.5 ± 20.4	12.1 ± 13.0	5.9 ± 6.0	0.6 ± 0.2	0.7 ± 0.3	1.3 ± 1.1
AF	4	11.5 ± 6.7	79.4	9.6 ± 9.3	30.3 ± 28.9	18.8 ± 18.7	8.7 ± 8.4	0.4 ± 0.1	0.6 ± 0.3	1.8 ± 1.5
AM	2	11.5 ± 9.2	58.6	10.2 ± 9.0	31.7 ± 27.0	18.7 ± 16.4	9.4 ± 8.3	0.7 ± 0.1	1.0 ± 0.3	2.0 ± 1.8
JF	4	2.3 ± 1.2	87.0	3.0 ± 0.9	11.2 ± 3.0	6.0 ± 1.7	3.1 ± 0.8	0.6 ± 0.2	0.7 ± 0.2	0.8 ± 0.3
JM	2	3.0 ± 0.0	75.9	2.2 ± 0.5	7.8 ± 1.6	4.2 ± 0.9	2.1 ± 0.5	0.5 ± 0.03	0.5 ± 0.1	0.5 ± 0.1
Stenella coeruleoalba	15	4.9 ± 5.5	61.1	4.2 ± 5.1	15.7 ± 18.6	7.6 ± 8.5	4.1 ± 5.0	1.2 ± 1.7	1.3 ± 1.8	1.5 ± 1.9
AF	2	14.0 ± 0.0	65.6	0.3 ± 0.0	1.8 ± 0.3	1.3 ± 0.2	0.3 ± 0.03	0.1 ± 0.00	0.1 ± 0.01	0.1 ± 0 01
AM	1	15.0	50.1	10.7	36.4	20.2	10.0	1.6	1.4	3.2
JF	5	3.8 ± 3.5	65.7	2.6 ± 1.6	9.9 ± 5.4	5.2 ± 3.1	2.5 ± 1.4	0.7 ± 0.2	0.7 ± 0.3	0.9 ± 0.5
JM	7	1.6 ± 2.0	58.2	5.5 ± 6.6	20.9 ± 24.4	9.3 ± 10.7	5.5 ± 6.4	1.9 ± 2.4	2.0 ± 2.5	2.1 ± 2.6

Table 5.4 (*Continued*)

Tursiops truncatus	7	3.8 ± 2.0	68.6	15.2 ± 10.1	56.4 ± 35.2	31.7 ± 22.5	14.8 ± 8.6	3.0 ± 1.4	2.5 ± 1.0	4.5 ± 2.2
JF	3	2.3 ± 1.6	71.8	13.3 ± 8.8	48.9 ± 30.9	26.9 ± 17.0	13.0 ± 8.4	2.8 ± 1.9	2.2 ± 1.3	4.0 ± 2.5
JM	4	4.9 ± 1.7	66.3	16.7 ± 12.1	62.1 ± 41.8	35.3 ± 27.9	16.0 ± 9.7	3.1 ± 1.2	2.6 ± 0.9	4.9 ± 2.2

[a] ΣPCBs includes 32 congeners. See Material and methods section for full list.

[b] I, II, III, IV and V represent different compound groupings, grouped by degree of halogen substitution, see Table 5 for congener details. n = sample size

Table 5.5 Age-gender specific PBDE concentrations (mean ± SD, µg/g lw), lipid content (%) and age values expressed in years (mean ± SD) in blubber of common dolphin *Delphinus delphis* and harbour porpoise *Phocoena phocoena* from the NWIP. AF: adult female, AM: adult male, JF: juvenile female and JM: juvenile male.

	n	Age	Lipid	BDE47	ΣBDEs[a]
Delphinus delphis	19	7.4 ± 5.1	59.9	0.23 ± 0.19	0.35 ± 0.25
AF	2	12.0 ± 5.7	52.2	0.04 ± 0.00	0.09 ± 0.01
AM	11	9.1 ± 4.5	54.2	0.21 ± 0.13	0.34 ± 0.21
JF	4	3.5 ± 2.5	78.2	0.29 ± 0.30	0.39 ± 0.36
JM	2	1.3 ± 1.1	62.2	0.39 ± 0.24	0.55 ± 0.32
Phocoena phocoena	1	3.0	40.3	0.42	0.57
JM	1	3.0	40.3	0.42	0.57

[a] ΣBDEs includes 9 congeners. See Material and Methods section for full list. n = sample size

and V represent different compound groupings, grouped by degree of halogen substitution, see Table 5 for congener details. n = sample size

Table 5.6 Classification of the PCB congeners analyzed in the present study according to Boon et al. (1997), Weijs et al. (2009) and Yordy et al. (2010).

Metabolic group	Description of group	PCB congeners in this study
I	No vicinal H-atoms	153, 180, 183, 187, 194, 189, 209
II	Vicinal H-atoms exclusively in *ortho*- and *meta*-positions in combination with ≥ 2 *ortho*-Cl substituents	99, 128, 138, 170, 157, 137, 97, 123, 132, 158
III	Vicinal H-atoms in the *ortho*- and *meta*-positions in combination with ≤ 1 *ortho*-Cl	28, 31, 74, 105, 118, 156
IV	Vicinal H-atoms in the *meta*- and *para*-positions in combination with ≤ 2 *ortho*-Cl	52, 101, 110 , 114, 44, 70, 49
V	Vicinal H-atoms in the *meta*- and *para*-positions in combination with ≥ 3 *ortho*-Cl	149, 167

Table 5.7 Concentrations of PCBs, PBDEs, hepatic total Hg and renal Cd in µg/g lw for POPs and in µg/g w.wt for non-essential elements in selected NE Atlantic adjacent waters to the NWIP.

Area		n	ΣPCB	ΣPBDE	Total Hg	Cd	References
Delphinus delphis							
NWIP		83/19/100	17.2 ± 14.1	0.3±0.2	10.4 ± 31.8	2.3 ± 2.7	This study
	Galicia	23/30	11.0	0.4	8.2 ± 12.9	1.6 ± 1.7	Pierce et al. 2008, Lahaye 2006
	Portugal	nd	–	–	5.7 ± 9.0	–	Carvalho et al. 2002
	Portugal	4	–	–	11.0 ± 18.3	0.5 ± 0.3	Zhou et al. 2001
Bay of Biscay	France	72	–	–	21 ± 35	2.7 ± 4.1	Lahaye et al. 2005
	France	20			34 ± 44	3.0 ± 4.4	Holsbeek et al. 1998
	Oceanic	nd/10	–	–	27 ± 30	16.3 ± 14.0	Das et al. 2000, Holsbeek (unpub. data)
Celtic Sea	English Channel	5	–	–	35 ± 44	2.0 ± 1.9	Das et al. 2003a
	English Channel	3	–	–	9.2 ± 9.4	2.6 ± 1.9	Lahaye 2006
	Ireland	28	–	–	27 ± 30	9.4 ± 9.1	Lahaye 2006
	Ireland	12	–	–	12.9 ± 15.4	7.6 ± 5.8	Das et al. 2003a
	Ireland	nd	–	–	15.3 ± 23	nd	Law et al. 1992
	England	nd	–	0.4	–	–	Law et al. 2003
Globicephala melas							
NWIP		3/8/6	16.2 ± 19.7	–	31.0 ± 59.5	23.9 ± 26.9	This study
Faroe Islands		8	–	–	852 ± 776	344.0	Caurant et al. 1996
		211/14	37.5 ± 23.1	–	61.9	72.7	Borrell 1993, Julshamn et al. 1987
Phocoena phocoena							
NWIP		12/14/12	20.5 ± 20.4	0.6	16.8 ± 30.0	2.3 ± 5.3	This study
	Galicia	3	5.3	0.3	1.2 ± 0.3	0.1	Pierce et al. 2008
	Galicia	2	–	–	1.2 ± 0.3	0.09	Lahaye et al. 2007
Bay of Biscay	France	2	–	–	19.8 ± 24	1.5 ± 1.4	Lahaye et al. 2007

Table 5.7 (*Continued*)

Celtic Sea	English Channel	8	–	–	10.7 ± 14.4	0.6 ± 0.7	Lahaye et al. 2007
	English Channel	4	–	–	2.6 ± 3.2	0.3 ± 0.6	Das et al. 2003a
	Ireland	6	8.0	–	–	–	Smyth et al. 2000
	Ireland South	15	–	–	15.1 ± 20	1.0 ± 0.8	Lahaye et al. 2007
	Ireland and Scotland	6/14	6.2	–	28 ± 47	0.7 ± 0.6	Smyth et al. 2000, Lahaye et al. 2007
	Scotland East	21/17	16.0	–	3.4 ± 3.3	1.1 ± 0.8	Wells et al. 1994, Falconer et al. 1983
		15/6	6.6	–	6.0 ± 5.9	2.7 ± 2.8	Wells et al. 1994, Falconer et al. 1983
		19/1	–	–	8.7 ± 10.1	2.3 ± 2.6	Lahaye et al. 2007
	England	60	–	2.3	–	–	Law et al. 2002
	England	2/4	49	–	0.62	–	Morris et al. 1989
			–	–	–	–	
North Sea		33/34	29.6 ± 14.1	1.2±0.9	–	–	Weijs et al. 2009
Baltic Sea	Germany	12/14	–	–	1.3 ± 1.0	0.2 ± 0.3	Das et al. 2004
	Poland	4	11.8	–	nd	1.4 ± 1.1	Kawano et al. 1997, Szefer et al. 1994
		3	33.93	–	–	–	Kannan et al. 1993
Stenella coeruleoalba							
NWIP		15/18/16	15.7 ± 18.6	–	22.9 ± 39.1	9.9 ± 11.0	This study
	Galicia	7	–	–	61 ± 70	15.4 ± 8.5	Lahaye et al. 2006
Bay of Biscay	France	32	–	–	63 ± 89	11.9 ± 9.9	Lahaye et al. 2006
	France	nd	–	–	10.2	31.3	Holsbeek et al. 1998
	France	nd	–	–	52 ± 29	–	André et al. 1991
	France	1	270	–	–	–	Alzieu & Duguy 1979
Celtic Sea	English Channel	3	–	–	10.4 ± 7	16.3 ± 24	Das et al. 2003a
	Oceanic	23	–	–	nd	21 ± 13	Das et al. 2000
	Ireland	4	–	–	11.50	35 ± 8.1	Das et al. 2003a
	British islands	3	–	–	8.9 ± 2.8	nd	Law et al. 1992

Table 5.7 (*Continued*)

	England	1	22	–	–	–	Morris et al. 1989
Tursiops truncatus							
NWIP		7/8/6	56.4 ± 35.2	–	19.1 ± 22.4	5.8 ± 13.8	This study
	Galicia	4	–	–	43 ± 25	3 ± 1.6	Lahaye et al. 2006
	Portugal	nd	–	–	37 ± 34	nd	Carvalho et al. 2002
Bay of Biscay	France	4/5	–	–	118 ± 104	1.12 ± 1.15	Holsbeek et al. 1998
	France	12	–	–	47 ± 67	0.7 ± 0.7	Lahaye et al. 2006
Celtic Sea	English Channel	3	–	–	7.9 ± 3.7	0.1 ± 0.1	Lahaye 2006
	Ireland West	2	–	–	96.0	1.7	Lahaye 2006
	Ireland Est	nd	–	–	21	nd	Law et al. 1992
	Scotland	1	30.7	–	–	–	Wells et al. 1994
	Scotland	1	16.5	–	–	–	Wells & Echarri 1992
	England	1	290	–	–	–	Morris et al. 1989

All values are arithmetic mean ± SD when SD is available. nd: not detailed data. ‘n’ correspond to the number of samples analyzed for POPs i.e. in order PCBs and PBDEs and to the number of samples analyzed for toxic elements i.e. in order Hg and Cd.

CHAPTER VI

GENERAL DISCUSSION

O Arroaz (Tursiops truncatus)

BACKGROUND TO THE OBJECTIVES

All forms of life modify their environment (White 1967) and marine mammals are not an exception to this. We have much to learn about their ecological roles, but we have evidences that the abundance and distribution of marine mammals can have important effects on the structure and functioning of ecosystems (Bowen 1997, Estes et al. 1998, DeMaster et al. 2006). At a time when several worldwide marine legislations have been developed in order to protect and restore ecological quality or integrity, within estuarine, coastal and offshore systems (e.g. Oceans Act in USA, Australia or Canada; Water Framework Directive [WFD, 2000/60/ EC] and Marine Strategy Framework Directive [MSFD, 2008/56/ EC] in Europe, and National Water Act in South Africa; Borja et al. 2011), scientists must be able to provide the necessary knowledge to manage and maintain the integrity of marine ecosystems and to provide diagnosis tools to assess changes in the structure and functioning of these marine ecosystems, including changes that may be related to anthropogenic activities. Developing a better understanding of the ecology of marine mammals as apex predators of the marine ecosystems can greatly benefits this. As we previously introduced, the marine mammals' trophic ecology can be studied through an "alternative" and relatively new approach, that is the chemical parameters, which allow two main perspectives: 1) gathering information at different levels, from the individual level, through the social structure dynamics, to the population level by identifying segregations over a long-time scale 2) evaluating the contamination status of the populations and the identification of the most vulnerable species or populations, through its second perspective.

The study area of this PhD thesis, the NWIP, as we previously described, has special oceanographic features that support a high biodiversity and is an important habitat for marine mammals. This richness entails a high impact of the anthropogenic activities such as fisheries, traffic boats and industries among others. Consequently, local government is presently formulating conservation plans for some cetacean species and especially for toothed whales due to their important presence in the area and their high interaction with human activities such as fisheries and boat traffic as a result of their more coastal habitat in comparison with baleen whales for example. Data is needed to better assess which areas are consistently used by cetaceans, particularly feeding areas, and/or the seasonal uses of coastal waters, feeding preferences (i.e. target prey species). In this respect, it is also important to identify which species show a high contamination in the area and therefore can be more vulnerable with respect to the contamination of the marine environment. This PhD study raised these questions and tried to reply by using “chemical parameters” as presented in the introduction. The main results of this study, which will be more discussed and detailed below, can contribute to the planning and implementation of management plans or mitigation measures for these five species of toothed whales in the NWIP, through a better understanding and knowledge of their feeding habits, degree of ecological segregation, and status of contamination in the area.

CHEMICAL PARAMETERS AS ECOLOGICAL TRACERS IN THE STUDY OF MARINE MAMMALS ECOLOGY

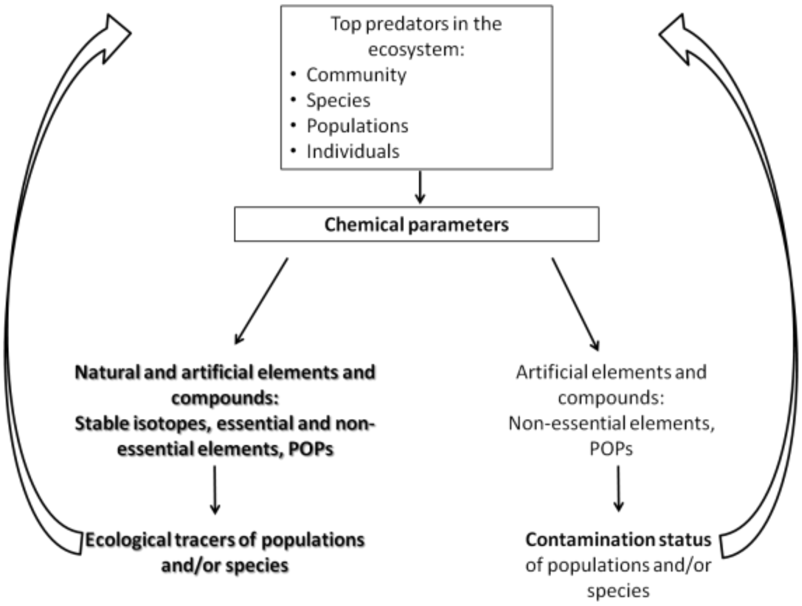

Figure 6.1 Scheme of the two perspectives applied in the marine mammals' ecology through chemical parameter analyses.

The use of chemical parameters, such as stable isotopes, trace elements and persistent organic pollutants, as ecological tracers has undergo an important increase in the last years inside the scientific community, and especially in the study of the marine mammal's ecology (Aguilar 1987, Jeffrey 2000, Herman et al. 2005, Krahn et al. 2007, Newsome et al. 2010). Probably the relative easiness in the analyses and their consistent decreasing costs thanks to increasing technologic performances has enhanced this growing interest. These approaches constitute however indirect methods as reflected by the term "tracer" and may constitute a signature of either the trophic position and dietary preferences or the habitat and geographical area of marine mammals. Therefore, the combination of different tracers at the same time may provide complementary results than direct methods such as stomach content and faeces analyses and/or direct observations.

Besides this, according to the tissue and tracer analyzed, the specific signature and/or concentration can cover a short or long time-scale period of integration, unlike to what happens with the so called “direct methods”. In this part of the PhD, we have used these particularities and advantages of the chemical parameters as ecological tracers to come back with one of our main objectives. **Is there inter-specific ecological niche segregation or a large overlapping among the sympatric toothed whale species inhabiting the NWIP waters (objective 1)?** To this end, we firstly studied the potential prey of the five toothed whales from the NWIP food web as well as their trophic relationships and the position they occupied (i.e. trophic position, TP), through the analysis of the stable isotopes (i.e. $\delta^{13}C$ and $\delta^{15}N$) in muscle tissues (a medium term integrator) (Chapter II). This intended to get an overall image of this food web considering spatial variations (i.e. neritic *vs* oceanic) and the position of these top predators in it. This work allowed us to determine that the NWIP food web exhibited five trophic levels, with the toothed whales occupying the highest levels but within a narrow range (4.3 to 5.3), not significantly different between them. However, an overlap in TP does not necessarily imply an overlap in diet since marine mammals may feed on different prey species with also similar TPs (see Chapter II for details), and this fact was subsequently demonstrated by stables isotopes of carbon and nitrogen and Cd concentrations (Chapter III).

The main prey of the toothed whales were identified in the literature by previous studies based on stomach contents analyses (Santos et al. 2004a,b,c 2007a,b). Since common dolphin is the most abundant cetacean species in the area and also the best represented in the samples available in this PhD, isotopic mixing models (i.e. Stable Isotope Analysis in R, SIAR) were applied to confirm that in the NWIP this species mostly feed on important economical species such as blue whiting, sardine and mackerel, which is completely consistent with the information previously given by stomach content analyses. By exploiting the same sources as the fisheries, toothed whales could be at risk in the NWIP from both prey depletion and incidental captures in fishing gear (by-catch) and, indeed there is abundant evidence that by-catch of cetaceans is a common occurrence in the area (see Table 1.2, Chapter I for details). The recent Bayesian model (SIAR) is now widely used in ecological studies and more specifically to study the diets and trophic relationships in food webs, as we did for common dolphin. One advantage of this new statistical tool is the possibility to incorporate variability in discriminating factors (also called trophic enrichment factors, TEF), which are the differences in isotopic composition between an animal and its diet (Caut et al. 2009). This fact is of a major importance since it was largely reported in the literature that these parameters depend on several sources of variation (e.g. taxon, environment, tissue) (Caut et al. 2009). In addition, only few studies were able to determine TEF experimentally due to the difficulty to carry out such experiments with animals in captivity, and especially for marine mammals (see Hobson et al. 1996, Caut et al. 2011). Caut et al. (2009) recently conducted an extensive review of the literature concerning estimates of animal-diet TEF for both $\delta^{13}C$ and $\delta^{15}N$. They determined an overall mean of 2.7‰ for $\delta^{15}N$ and 0.7 for $\delta^{13}C$, which is greatly lower than the values previously found in animals, especially in marine mammals. However, our results were even lower (for $\delta^{15}N$) than these values being 1.4‰ and 0.8‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively. However, our TEF values should be interpreted with caution, as they were estimated on the basis on rather few samples and only the main prey were selected for the TEF calculations. These differences found in the literature confirm, as discussed above, the importance for researchers either to have an accurate estimate of TEF or to evaluate the influence of the most likely values on the results, when wishing to use mixing models (Bond & Diamond 2011) in the study of ecological trophic relationships.

In the aim of a better understanding of the possible segregation or overlap of the ecological niche of these sympatric species in this restricted area (i.e. the NWIP), which was not possible to carried out in the first work (Chapter II), we combined in a second work (Chapter III) the stable isotope analysis with other ecological tracers (specifically cadmium) analyzed in other tissues (kidneys and teeth). This multi-tracer approach gave us the possibility to cover a range of different time spans and allowed us to investigate the foraging niche (i.e. subset of the ecological niche) in their two dimensions (i.e. trophic and spatial dimension). Combining the results of two or more of these techniques increase the information gained and the multi-approach has been largely used by scientists in the study of the trophic niche in marine mammals. For example, Hooker et al. (2001) showed that fatty acid profiles and stable isotope compositions in biopsy samples, when used in combination, provided useful information about the diet of northern bottlenose whales *Hyperoodon ampullatus*. In addition, Fisk et al. (2002) demonstrated the utility of combining organochlorine contaminant results with stable isotope data in assessing the feeding ecology of Greenland sharks *Somniousus microcephalus*. Another well-known example is the case of the North Pacific killer whales *Orcinus orca* presented in the introduction part, where ecological tracers (specifically stable isotope ratios of carbon and nitrogen, persistent organic pollutants and fatty acids in biopsy samples) were used to complement genetic, acoustic and morphologic studies to better understand the different types of killer whales inhabiting these waters. Because potential ambiguities exist in interpreting the results from each of these individual "alternative" methods of feeding ecology estimation, employing multiple independent chemical methods may provide more accurate results than those obtained from measurements using a single biometric. A further consideration is that all the ecological tracers are mainly transmitted *via* food and only differences in tracer signatures are really informative, since similar signatures may in fact sometimes result from different combinations of different prey. Another advantage of this multi-tracer approach is that the chemical parameters can be analyzed in several tissues with different turnover rates giving us information on different periods of integration. This is all the more important since the foraging niche segregation probably depends on the availability of resources in the area, which may varies with time (e.g. season, year).

Additionally, our main finding in this work (Chapter III) is that none of the ecological tracers examined alone revealed complete ecological segregation amongst the five species studied whereas the integration of results from all these tracers did indicate segregation of all five species in the long-term, and was hypothesized in a conceptual scheme (Figure 6.2).

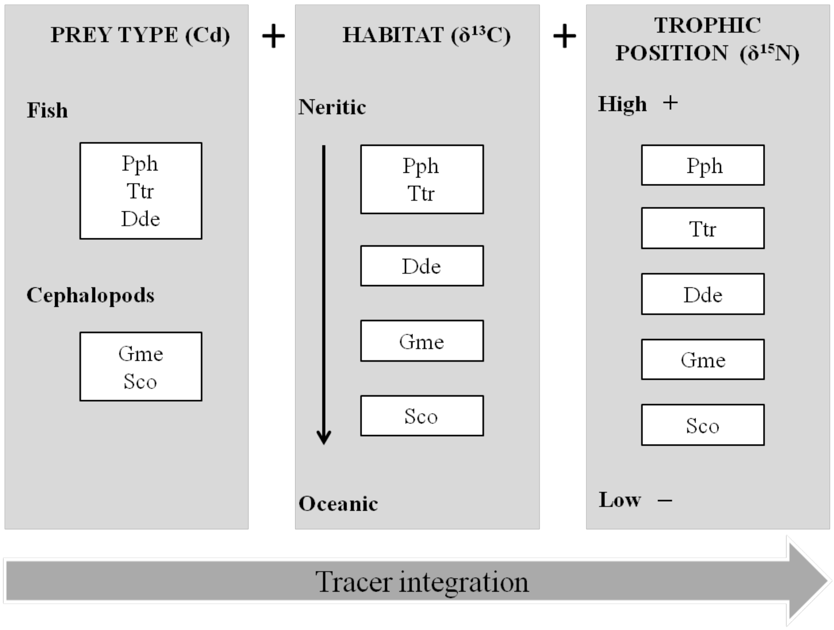

Figure 6.2 Conceptual scheme of long-term foraging niche segregations obtained by the information given by all ecological tracers analyzed in the five toothed whale species. Common dolphin *Delphinus delphis* (Dde), harbour porpoise *Phocoena phocoena* (Pph), bottlenose dolphin *Tursiops truncatus* (Ttr), striped dolphin *Stenella coeruleoalba* (Sco) and long-finned pilot whale *Globicephala melas* (Gme).

However an overlap cannot be excluded sometimes, i.e. during the day or some years and/or season, as it was the case for common and striped dolphin for which their different prey type preferences (given by renal Cd concentrations) could avoid the potential competition at some periods of the year when common dolphin mostly exploited oceanic waters as striped dolphin (see Figure 3.4, Chapter III for details).

Stable isotopes of carbon and nitrogen, fatty acids and trace elements (especially Cd and Hg) are the ecological tracers that are the most commonly used by scientists in the study of the trophic ecology. The persistent organic pollutants (POPs), which include several different types (e.g. Polychlorinated biphenyls, PCBs, dichlorodiphenyltrichloroethane, DDT, polybrominated diphenyl ethers, PBDEs, hexachlorobutadiene, HCBD) are less frequently used for dietary or feeding ecology reconstruction, but they can allow determining segregation of cetacean stocks (as in Borrell et al. 2006, Herman et al. 2005, Krahn et al. 2007, Pierce et al. 2008). In addition, POPs are bioamplificated in the food webs since they bioaccumulation in all organisms and their concentration increases with the species trophic level (Tanabe et al. 1988, Wagemann & Muir 1984, Weijs et al. 2009). Species inhabiting different geographical areas and having different feeding habits accumulate different pollutant loads, both qualitatively and quantitatively, which are characteristic of these areas and the prey eaten (Aguilar 1987, Borrell et al. 2006). Therefore, each element or congener has its own trophic source, and a whole POPs profile may be used as a kind of fingerprint to infer the dietary behaviour and habitat (Wells et al. 1996, Borrell et al. 2006, Pierce et al. 2008). But additionally, the POPs patterns observed in marine mammals differ both from the patterns seen in the technical formulations originally released to the environment and from those in their prey (Muir et al. 1988) as a result of the enzymatic metabolism during their passage through the food chain and especially after uptake by marine mammals (Boon et al. 1987, 1992). This enhances the difficulty of using POPs profiles as a signature or fingerprint of a diet or a geographical area.

For these reasons, most of the studies on POPs as ecological tracers used only one type of compounds to discriminate between species which are taxonomically close as the five toothed whales studied here are. Therefore it was necessary to consider differences in toothed whales' diet, body condition, age, sex and possibly location as well as their ability to metabolize the congeners. In a third work (Chapter IV), we thus analyzed 32 PCB congeners in the blubber of these same five species of toothed whales. In order to reduce the within-species variability and to allow a more critical evaluation of the differences between the species, all congeners (i.e. concentrations in μg/g lw) were normalizing to a single congener, the CB153 (2,2′, 4,4′, 5,5′-hexachlorobyphenyl) since it is one of the most recalcitrant chlorobiphenyl congener with respect to biotransformation and since it reaches the highest concentrations among PCB congeners in the great majority of samples of aquatic mammals (Boon et al. 1987, 1992, 1994, Duinker et al. 1989, Wells & Echarri 1992). Many factors may affect the chemical burden of individual animals or of entire population components. These factors include, among others, biological parameters such as nutritional state, sex, age, excretion, metabolism, tissue-composition, and factors related with their ecology such as trophic level, distance of habitat from mainland, and then pollution source. In our work only immature animals were used to minimize the influence of age and sex and especially the influence of the gestation and lactation on POPs burden in mature females. We tested the effects of some other factors (i.e. lipid content, habitat, food and sex) on PCB patterns applying multivariate analyses (specifically redundancy analysis, RDA). Another multivariate method, the discriminant analysis (DA) was finally used to assess whether or not all the analyzed individuals could be categorized into (their) species on the basis of their PCB patterns. Data reduction is essential to identify patterns, to be more readily and to provide a descriptive overview, but differences in these patterns are commonly imperceptible when graphing the data directly from the chemical analysis and the descriptive interpretation is usually convoluted. For this reason, several studies already used multivariate analysis as a tool to describe possible POPs pattern differences between species. As an example, principal component analyses (PCA) was used to identify differences between three harbour seal (*Phoca vitulina*) populations around the coast of Denmark (Storr-Hansen & Spliid 1993). PCA was also used by Borrell and Aguilar (2005) to discriminate between common and striped dolphins from the Mediterranean Sea. DA carried out with only three congeners (CB105, 151 and 99) analyzed in blubber allowed Herman et al. (2005) to show separation of killer whales ecotypes based on their PCB patterns. Wells et al. (1996) showed differences between 12 species of marine mammals from the Scottish coast using both methods, DA to differentiate between species patterns and PCA to investigate within species pattern variability. Our results showed a good-separation of overall species on the basis of their PCB patterns (i.e. the 32 PCB congeners normalizing to CB153). Harbour porpoise and striped dolphin seem to be the species more clearly separated from the others. This would be the consequence of their ecological characteristic, harbour porpoise being a piscivorous and coastal species, striped dolphin being mainly a teuthophagous and oceanic species (see Chapter IV for details), but also of their special and distinct capacity to metabolize some congeners. Indeed, harbour porpoises from the NWIP seem to better metabolize low chlorinated congeners than NWIP striped dolphins do.

Moreover, the PCB congeners, which contribute to the highest differences between species, were also identified (i.e. CB157, 137, 114, 149, 118, 158, 183 and 189). These congeners belong to the structural groups I and II (i.e. no vicinal H-atoms and vicinal H-atoms exclusively in *ortho*- and *meta*-positions in combination with ≥ 2 *ortho*-Cl substituents, respectively) that Boon et al. (1997) identified as metabolically type I i.e. “resistant to biotransformation by a given species”. Few studies have analyzed an amount of 32 congeners and the heterogeneity in the congeners analysed according to the studies makes difficult the comparisons among them. Wells et al. (1996) previously demonstrated the importance to consider a large number of PCB congeners in order to describe and differentiate the patterns among marine mammals. Our results revealed that 22 congeners out from the 32 analysed may be enough to determine patterns able to trace the feeding habits and also to discriminate the five toothed whales species of the NWIP (see Chapter IV). The results obtained in this study strongly encourage to continue this research whose results would be strongly improved by a greater number of samples for all the species, by a detailed study of the metabolic characteristic of the congeners in each species and finally by the analysis of the PCB patterns in their main prey in order to identify the likely origin of these congeners.

The work carried out in this first perspective of the PhD revealed the usefulness of the chemical parameters as ecological tracers when they are applied to a same biological model (i.e. marine mammals) as a complement of the direct or traditional methods such as stomach content and faeces analyses, and/or direct observations. Besides this, the chemical tracers ingested *via* food, become integrated into tissues and are the reflection of the environment in which the species evolve. Finally, a multi-approach method is all the more relevant given that the lack of information of one tracer is compensated by the information given by another.

CHEMICAL PARAMETERS TO EVALUATE THE CONTAMINATION STATUS OF MARINE MAMMALS

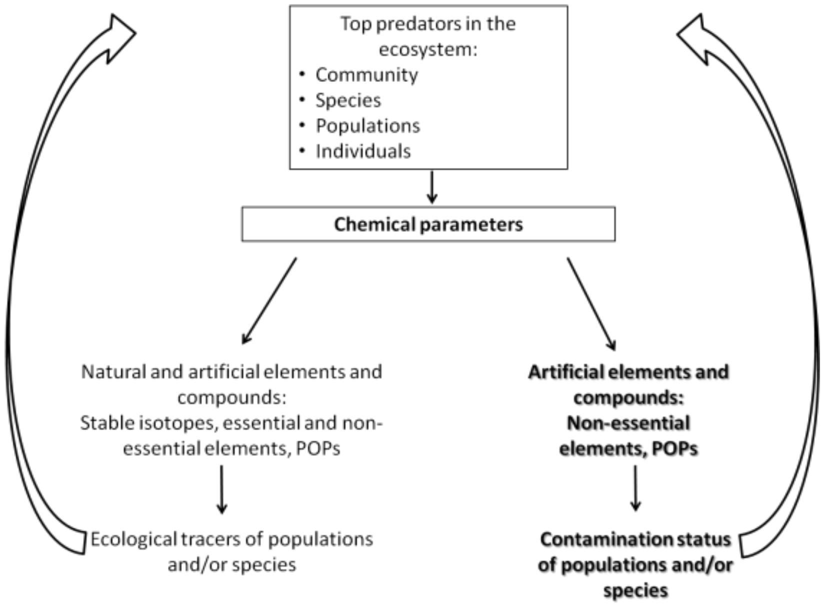

Figure 6.3 Scheme of the two perspectives applied in the marine mammals' ecology through chemical parameter analyses.

Long-lived apex predators are particularly at risk from effects of persistent organic pollutants (POPs), e.g. PCBs, DDTs, PBDEs, due to both the processes of bioaccumulation, i.e. increasing concentration with age in individuals, and of biomagnification, i.e. higher levels higher up the food chain, especially when moving from gill-breathing animals like fish and cephalopods to air-breathing animal like marine mammals. Non essential trace elements and especially Cd and Hg are also known to bioaccumulate in the tissues of marine mammals. Even essential trace elements (e.g. Cu, Mn, Se, Zn) can become toxic when their concentrations exceed a given threshold in biota. Nevertheless it has not yet been confirmed in the marine mammals. The marine environment is highly polluted by these chemical compounds and by trace elements, and even if the non essential trace elements enter the marine environment naturally (for instance, as a result of the weathering of rocks or volcanic activity) they are mainly the consequence of anthropogenic discharges (Law 1996, Nriagu 1996). The increase of human activity and industrial development in the NWIP intensively disturbs the natural marine environment in the area. Its progressive deterioration is mainly caused by contributions of various different industrial discharges that reach the coast without previous treatment or purification, by the intensive boats traffic and in some way by the intensive fishery activity in the area (Carro et al. 2001, López et al. 2002, 2003). As a consequence, it becomes necessary to carry out a baseline analysis of the contamination of marine mammals as apex predators of the food web, as it was already performed in other organisms, representatives of the lower trophic levels in this food web and with a commercial, but also biological, interest in the area (e.g. shellfish, fish and cephalopods) (Prego & Cobelo-García 2003, Carro et al. 2001, 2006, Gómara et al. 2005). This fact is all the more important since despite the importance of these species few studies on contamination were carried out in the area (e.g. program BIOCET EC: EVK3-CT-2000-00027).

In the first part of the PhD, our results and complemented information from the literature, gave us a clear image of the feeding ecology of the most common toothed whales in the NWIP, including their possible competition for resources, main habitats and PCB patterns. Coming back to the first two questions raised in this PhD, we then focused on the study of their **contamination status attempting to determine the potentially more vulnerable species (objective 3).** To this end, several contaminants were analyzed in the internal tissues involved in their storage and in the blubber of animals belonging to the five toothed whales. Specifically, a total of 14 trace elements (i.e. essential and non-essential elements) were analyzed in the liver and kidneys and two types of POPs (i.e. the PCBs and PBDEs) in the blubber.

Many factors unrelated with geographical distribution may affect the chemical burden of contaminants of individual animals or of the whole populations even within a homogeneously distributed population. These factors include, among others, sex, age, trophic level, distance of habitat from mainland and pollution source, excretion, metabolism and tissue composition

(Aguilar et al. 1999). In consequence, biological and ecological factors are difficult to separate when interpreting and comparing trace element and POP concentrations. Thus they were taking into consideration together in this study. For trace elements, sex was not an important parameter for the five species. The influence of gender on trace element concentrations is hardly predictable in marine mammals and seems to vary with the element, the tissue and the species analyzed (Aguilar et al. 1999). However, as a usual difference, females show greater concentrations than males, as a consequence of common sexual dimorphism but also as a result of a lower transfer of trace elements from females to their progeny (Aguilar et al. 1999). In opposition to sex, age is an important factor affecting the bioaccumulation of trace elements, especially for the non-essential ones since they are not regulated in the organisms as it occurs for essential elements. As we expected, our results showed that the effect of age on trace element accumulation is not always related to it essential or non essential character. Thereby some essential elements showed an accumulation with age, as for example it was the case of Fe in kidneys, and Cr and Ni in both liver and kidneys (see Chapter V for details). Among the non-essential elements, Cd and Hg, the elements most often studied in marine mammals, showed a significant bioaccumulation with age as expected. However, this trend is different between both elements as consequence of their different accumulation kinetic and even between different species. Hg increases throughout the life of individuals while Cd reaches a plateau after several years, more or less rapidly according to the species (Caurant et al. 1994, Bustamante et al. 2004). In this study, the bioaccumulation of Cd exhibited this plateau roughly at 7 years old for common dolphins (see Figure 5.2 in Chapter V).

Overall, the concentrations obtained for trace elements were generally low, except for Cd, Fe, Hg, Se, and Zn, with nearly six elements being under the limit of detection of the analytical method used (i.e. Inductively Coupled Plasma Mass Spectrometry, ICP-MS and Inductively Coupled Plasma Atomic Emission Spectrophotometry, ICP-AES). Even when comparing with toothed whales of NE Atlantic waters, Cd and Hg concentrations are also low in the NWIP. The high presence of immature animals in our sampling can be the main reason of the low values obtained for these elements. Despite this, the results revealed significant differences between species for all trace elements at least in one of the tissues analyzed (i.e. liver and kidney). These differences are more intriguing for essential trace elements than non essential ones, since it indicates that the physiological concentrations due to homeostasis processes would be different according to the species. But these different essential trace element concentrations may also be the consequence of the difference exposure to non essential elements to these species, and the complex interactions that exist either for absorption or because of the competition for binding to proteins in the different tissues. As essential elements for the organisms, it could therefore be supposed that the concentrations of these elements were dependent on the physiology and the metabolism of the specimens.

Concerning POPs, both sex and age have a strong influence on their concentrations in internal tissues in a given specimen. As for trace elements, irrespective of sex, pollutants loads tend to increase with age, because uptake usually exceeds the metabolism and/or excretion capacities of the animals. However, they often decrease in sexually mature females, because they transfer appreciable quantities of lipophilic xenobiotics to their offspring during pregnancy and lactation, thus reducing their own pollutant burdens (Reijinders 1980, Gaskin 1982, Tanabe et al. 1982). Consequently, in order to avoid possible bias in the data interpretation, all the individuals from this study were classify in function of their sexual maturity in four different groups (i.e. AM: adult male, AF: adult female, JM: juvenile male and JF: juvenile female).

Despite the interpretation of concentrations in the light of biological and/or ecological factors, the bioaccumulation and differences of POPs in the five species has been shown to be more predictable than trace elements. Generally, the AF of harbour porpoises displayed significantly higher ΣPCB (sum of PCBs) concentrations than striped dolphins, and JM and JF of bottlenose dolphins significantly higher ΣPCB concentrations than the other species, except pilot whale. These results are not surprising since harbour porpoises and bottlenose dolphins from the NWIP are characterized by their preferential coastal habitat and their fish-feeding habits (López et al. 2004, Pierce et al. 2010, Méndez-Fernandez et al. 2012). Thus, these species are usually observed from the coast, which is an index of their proximity to areas with great anthropogenic impact. Besides this, both species are more frequently seen in the southern part of the study area, which is more populated and industrialized than the northern part (López et al. 2004, Pierce et al. 2010). In addition, both species also showed higher PCB concentrations than those of the NE Atlantic adjacent waters (see Table 5.7 in Chapter V for details).

The interpretation of potential toxicity of trace element data in marine mammals is difficult. Unlike POPs, trace elements occur naturally and levels can vary widely in the environment depending upon the regional geology (Becker 2000). Moreover, direct effects of trace elements are not obvious in marine mammals and probably less important than other factors that obviously impact populations such as by-catch in fisheries, degradation of habitat and other ecosystem changes induced by human activities. Furthermore, the detoxification processes, determining direct link between trace element concentrations and effects in marine mammals through dose-response relationship may require searching for endpoints that would be liminal effects and would necessitate many additional studies in epidemiology (Caurant 2012).

Even trace elements should not be a problem but probably constitutes an additional stress to marine mammals exposed nowadays to multiple chemicals in a high variable marine environment (Caurant 2012), we basically do not really know what the natural baseline levels of many trace elements are in marine mammal species (Becker 2000). Conversely, a number of POPs pose a more direct and significant global threat to the health status and viability of marine mammal populations, with PCBs generally posing the greatest concern (Reijinders 1994, Tanabe et al. 1994, Colborn & Smolen 1996, Jepson et al. 2005, Hall et al. 2006, Law et al. 2010).

Recently, experimental data regarding aquatic mammals have been collated to derive dose-response relationships for the adverse health effects of PCB exposure (Kannan et al. 2000, Schwacke et al. 2002). The resulting dose-response relationships, based on experimental studies of PCB-induced immunological and reproductive effects in mink, seals, and otters, have led to a proposed blubber total PCB (based on the Aroclor 1254 formulation) threshold concentration for adverse health effects (including immunosuppression) in all marine mammals of 17 µg/g lw (Kannan et al. 2000). In our study 100 % of the bottlenose dolphin and 75% of the harbour porpoise individuals exceeded this threshold value, which raised the question of toxic effects in these individuals. Despite this, few evidence of toxicological effect for one or another species can be affirmed through this study. However liminal effects cannot be excluded in these populations, which require further studies probably including probabilistic risk assessment.

CONCLUSION

The present study provides some useful data on the feeding habits and habitat areas as well as a baseline of potential contaminants in tissues of five of the most common toothed whales inhabiting the NWIP waters. These results provide a clear indication that these species are not evenly distributed along the NWIP coast and have not the same foraging needs, although it is well known that they are present all year round in the area. Consequently, the main threats at which the species will be exposed are different; therefore the future conservational plans that will be carrying out in the area have to better take this point into account. Bottlenose dolphin and harbour porpoise revealed by their bio-ecological characteristics and their high contaminant concentrations that they may be greatly affected by interactions with human activities, including fishing and boat traffic in general, but also more exposed to contaminants sources or polluted areas. Besides this, common dolphin and pilot whales may be mostly affected by fishing interactions, or in other words by-catch. Striped dolphin due to its preference for deeper waters and low rate of by-catch gathered in the area, make difficult to evaluate which are their major threats in the NWIP; however this species is well known in many other parts of the world to be highly threatened by fisheries, hunting and pollution (Reeves et al. 2003, Aguilar & Borrell 2005). Therefore a survey of this species is still highly relevant in the area.

Consequently, in the NWIP we have to consider two or even three different management units or conservational plans for these five species, which could be enriched by further studies.

PERSPECTIVES AND FUTURE IMPROVEMENTS

Several perspectives and improvements to this study arise after the work performed; they are detailed below in what we consider the order of importance.

More ecological tracers and more time periods covering

In the aim of improving the dynamics of predator-prey relationships initiated in the first work of this PhD (Chapter II) future analyses of fatty acids in these five species but also in their main prey would be relevant. They would add detailed information on the species diet on different time scale according to the tissue analyzed (i.e. liver, muscle or blubber). Therefore, new segregation or on the contrary overlap in the trophic niche of the five species may be identified.

The hard tissues and especially teeth and bones, that do not remobilise accumulated elements, preserve isotopic and elemental records of habitat use and dietary patterns of the individuals covering long periods of time (as showed in chapter III). Thus, these tissues can be used to show the existence of spatial and trophic segregation or overlap, among species, while it is less clear for medium-term integrator tissues. Besides this, these samples are routinely and easily collected, and do not give rise to problems of preservation. Therefore, they also should be a good matrix in order to analyze these chemical parameters but also others and thus allow showing long-term changes (Lee et al. 1999, Outridge et al. 2009); determining stocks (Jay et al. 2008) or reconstructing movements (Stewart et al. 2003, Tabouret 2005). Therefore, they would probably a relevant way to get more information on either population structures or ecological segregation within the NWIP toothed whale species over a long time-scale.

Temporal dimension of the foraging niche

It is evident that these highly mobile populations probably change their feeding habitat in function of the resources availabilities in the area, and therefore in function of the inter-annual and/or seasonal variations of resources. The lack of samples was our main problem to study the temporal trend of the toothed whales foraging niche, with the exception of common dolphin, which is the best represented species. Thus, a bigger sample size for the majority of these species is advisable to improve the dimension of this study and our knowledge on the possible seasonal or even year-to-year overlapping of their niches.

Temporal trends in the context of a climate change

In the context of global change, top predators may have to adapt to a variable environment in the NWIP. The joint analysis of trace elements and stable isotopes (as an example) could allow detecting a change in the feeding behavior, by distinguishing a shift in the use of their habitats or in their feeding ecology as a consequence of modifications in prey availability both in terms of species and/or abundance. In the context of global change, the study of temporal trends of contaminants can be highly informative. Additionally, it could also complement and confirm the short trend detected for PCBs in this PhD, which concentrations have showed a decrease in the toothed whales between 2004 and 2008.

More than a simple baseline of the contaminants

Now that we have roughly completed baseline of contaminant concentrations in five of the most common and frequent species of cetaceans in the NWIP, it would become essential to continue with a monitoring program in order to be able to follow the variations in the contamination status along time. And it would be an important advance, to extend this study to the rest of the species of marine mammals (i.e. whales and seals) and, why not, marine turtles, present in the area and for which a great bank of samples already exists waiting to be valued.

Modelling the drift of cetacean carcasses and the ecological tracers

Very often the lack of information about the origin of carcasses makes difficult our interpretation of the ecological tracers. Recently a study was carried out in the Bay of Biscay (Peltier et al. 2012) using cetaceans found by-caught in fishery observation projects that were subsequently released dead with a numbered tag fitted to the tail fluke. The drift of the carcasses were predicted by using the *Météo-France* drift model MOTHY and the proportion of dead dolphins recovered by volunteers of the French stranding network. The modelling allowed mapping the likely origin of stranded cetaceans (i.e. shelf break-continental shelf, north-south). It would be of a great interest to apply such a model to toothed whales in the NWIP, and so couple the origin of the stranding carcasses with our values of the ecological tracers.

Finally, it is important to underline that these perspectives cannot be consider without the support of monitoring observatories and stranded networks on which depends the acquisition of the majority of the data, as it was the case for this PhD work. This point highlights the necessity of a long-term and continuous monitoring for the observation and collection of data and samples. Thus, the existence of these monitoring observatories appears to be a major necessity for these charismatic's species, and for a comprehensive study of the functioning of the marine ecosystems.

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