

Ecological niche segregation among five toothed whale species off the NW Iberian Peninsula using ecological tracers as multi-approach

Paula Méndez-Fernandez · Graham J. Pierce · Paco Bustamante · Tiphaine Chauvelon · Marisa Ferreira · Angel F. González · Alfredo López · Fiona L. Read · M. Begoña Santos · Jérôme Spitz · José V. Vingada · Florence Caurant

Received: 26 January 2013 / Accepted: 30 May 2013 / Published online: 11 June 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract This study aims to assess niche segregation among the five main toothed whales that frequent the NW Iberian Peninsula waters: the common dolphin, the harbour porpoise, the bottlenose dolphin, the striped dolphin and the long-finned pilot whale. We used cadmium (Cd) and stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) as ecological tracers to assess degree of segregation in diet/trophic level and in foraging habitat, over various time-scales. $\delta^{13}\text{C}$ values highlighted different habitats, while Cd concentrations highlighted feeding differences between oceanic and neritic species. Moreover, $\delta^{15}\text{N}$ values suggest different trophic levels of prey targeted within oceanic and neritic species. Hence, results revealed long-term ecological segregation among five toothed whales that coexist in the NWIP and

demonstrated the ability of ecological tracers to discriminate ecological niches among closely related species.

Introduction

Hutchinson (1957) defined the central concept of ecological niche as a set of biotic and abiotic conditions in which a species is able to persist and maintain stable population sizes. These conditions can be structured along three main dimensions: trophic (e.g. diet composition, trophic level and prey quality), spatial (horizontally and vertically) and temporal (from diel activity patterns to yearly migratory cycles); temporal and spatial dimensions of the niche include various environmental influences on distribution. Description of these different dimensions allows estimation of inter-specific overlap *versus* segregation within a community or a guild (Pianka 1974). The first mechanism that

Communicated by C. Harrod.

P. Méndez-Fernandez (✉) · P. Bustamante · T. Chauvelon · J. Spitz · F. Caurant
Littoral Environnement et Sociétés (LIENSs), UMR 7266
CNRS-ULR, 2 rue Olympe de Gouges,
17042 La Rochelle Cedex 01, France
e-mail: paula.mendez_fernandez@univ-lr.fr

P. Méndez-Fernandez · A. López
Coordinadora para o Estudo dos Mamíferos Mariños (CEMMA),
Apdo. 15, 36380 Pontevedra, Spain

P. Méndez-Fernandez · M. Ferreira · J. V. Vingada
Centro de Biologia Molecular e Ambiental (CBMA)/Sociedade
Portuguesa de Vida Selvagem (SPVS), Departamento de
Biologia, Universidade do Minho, Campus de Gualtar,
4710-057 Braga, Portugal

G. J. Pierce
Oceanlab, University of Aberdeen, Main Street, Newburgh,
Aberdeenshire AB41 6AA, UK

A. F. González · F. L. Read
Instituto de Investigaciones Marinas (C.S.I.C),
Eduardo Cabello 6, 36208 Vigo, Spain

M. B. Santos
Instituto Español de Oceanografía, Centro Oceanográfico de
Vigo, Apdo. 1552, 36280 Vigo, Spain

J. Spitz
Marine Mammal Research Unit, Fisheries Centre, University of
British Columbia, 2202 Main Mall, Vancouver, BC V6T 1Z4,
Canada

J. V. Vingada
Centre for Environmental and Marine Studies (CESAM),
Universidade de Aveiro, Campus Universitário de Santiago,
3810-193 Aveiro, Portugal

allows for niche differentiation is resource partitioning (trophic dimension). In this case, different predators living in the same habitat exploit the resources differently, minimizing the overlap in terms of prey composition (Ballance 2002). The second mechanism involves the spatial and/or temporal dimensions of the availability of different resources (Huisman and Weissing 2001).

The study of the ecological niche as well as of the segregation processes can help to advance our understanding of ecosystem functioning (Leibold 1995) although this objective presents added difficulties since the ecological niche in marine ecosystems is hard to measure (Newsome et al. 2007). Renewed interest in this concept has arisen due to technological advances such as the use of chemical or ecological tracers in marine organisms, e.g., stable isotopes, in particular of carbon (C) and nitrogen (N), analysis of which has become a powerful tool in the study of ecological niches (Jardine et al. 2006; Newsome et al. 2007), notably because $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ provide information on trophic level and foraging area (DeNiro and Epstein 1978; Cabana and Rasmussen 1996; Hobson 1999; Fry 2006). Isotopic results are especially relevant in combination with others approaches such as stomach content analysis, habitat modelling or other potential ecological tracers (e.g. trace elements, persistent organic pollutants or fatty acids) to investigate the ecological niches of marine top predators (e.g. Zhao et al. 2004; Herman et al. 2005; Krahn et al. 2007; Cherel et al. 2008; Kiszka et al. 2011).

Together with stable isotopes, ecological and chemical tracers' elements offer an integrated measure of prey assimilation over different periods of time. This contrasts with conventional dietary analyses (e.g. faeces or stomach contents) which provide information on food ingested over a very short time-scale (several days, see e.g. Santos et al. 2001). Depending on the combination of elements and tissues used, the period of integration represented by the specific signature or concentration will differ, relating biological half-life of tracers and tissue turnover rates. Thus, ecological tracers can provide information from a medium time-scale (from several weeks to months, i.e. fatty acids in blubber or stable isotopes in liver and muscle) to long-term time-scales (from several years to the life span of the animals) when ecological tracers are analysed in hard tissues with no turnover such as teeth (Hobson and Clark 1992; Hobson and Sease 1998; Nordstrom et al. 2008; Caurant et al. 2009).

Moreover, ecological tracers can be viewed as proxies of habitat, feeding habits and trophic position. Thus, the relative abundance of heavy to light carbon isotopes ($\delta^{13}\text{C}$) 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 and Epstein 1978; Fry 2006). The relative abundance of heavy to light nitrogen isotopes ($\delta^{15}\text{N}$) is commonly taken as an indicator of trophic level (Cabana and Rasmussen 1996) because $\delta^{15}\text{N}$ increases from prey to predator due to the preferential excretion of the light isotope (Hobson 1999). However, $\delta^{15}\text{N}$ also vary strongly by habitat: inshore and offshore systems, latitudes and between oceanic basins. Thus, Ruiz-Cooley et al. (2012) recently showed that the $\delta^{15}\text{N}$ values from zooplankton and/or prey such as squid from similar sizes exhibited large differences in $\delta^{15}\text{N}$ (>4 ‰) between inshore and offshore systems due to biochemical properties of the habitat. In the same way, Chouvelon et al. (2012) showed marked decreasing $\delta^{15}\text{N}$ values from inshore to offshore into different marine species from the Bay of Biscay, highlighting the role of stable isotopes of N as an indicator of feeding area.

Considering trace elements, elevated concentrations of cadmium (Cd) in marine mammals are usually associated with a predominance of cephalopods or crustaceans in the diet (Honda et al. 1983; Das et al. 2000) due to the bioaccumulation of this element in their digestive gland where most of the detoxification processes take place (Bustamante et al. 1998b) and the highest concentrations of Cd are found in oceanic species (Bustamante et al. 1998a; Lahaye et al. 2005). The high bioavailability of Cd induces a high potential for its trophic transfer to upper level predators such as marine mammals (Bustamante et al. 2002a, b). In addition, cephalopods show large differences in Cd concentrations depending upon their origin (specifically latitude, with highest Cd enrichment found in sub-polar food webs (Bryan 1976; Miramand and Bentley 1992; Bustamante et al. 1998a). Consequently, marine top predators from polar and sub-polar areas feeding on cephalopods should exhibit high Cd concentrations in their tissues.

The Northwest of the Iberian Peninsula (NWIP) is at the northern limit of the East Central Atlantic upwelling system, characterized by high biodiversity and productive fisheries. NWIP represents an important habitat for cetaceans, at least 20 species of marine mammals (16 cetaceans and 4 pinnipeds) have been recorded and the rate of stranding is one of the highest in Europe (López et al. 2002). The most frequent species in the area are toothed whales, specifically common dolphin (*Delphinus delphis*), bottlenose dolphin (*Tursiops truncatus*), harbour porpoise (*Phocoena phocoena*), striped dolphin (*Stenella coeruleoalba*) and long-finned pilot whale (*Globicephala melas*) (Cendrero 1993; López et al. 2002, 2004). The ongoing occurrence of cetaceans in the area has permitted, for many years, systematic boat- and land-based surveys, as well as several studies on diet, distribution, habitat preferences and interactions with human activities including fisheries

among others (López 2003, López et al. 2004; Santos et al. 2007a, b, in press; Pierce et al. 2010; Spyarakos et al. 2011). Several studies have 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, and results suggest the existence of mechanisms allowing a fine-scale partitioning of habitats and/or resources. For instance, stable isotopes of C and N were analysed in muscle tissues (i.e. medium time-scale information) of these five species and in their prey in order to better understand their position in the NWIP food web (Méndez-Fernandez et al. 2012). Toothed whales occupy high trophic positions (TPs from 4.3 to 5.3), and no significant between-species differences have been detected. However, an overlap in TP does not necessarily imply an overlap in diet, since animals may feed on different prey species with similar TPs.

Thus, the present study aims to provide further insight into habitat and resource partitioning among these toothed whale species, extending previous stable isotopes of C and N results in muscle (from Méndez-Fernandez et al. 2012) with new analyses on C and N isotopes in teeth as well as Cd in liver and kidney tissues, to cover a wide range of temporal scales. We hypothesized that each toothed whale species occupies its own ecological niche in the area, defined by differences in at least one of the ecological tracers used. Cd and $\delta^{15}\text{N}$ values will determine the trophic dimension of the ecological niche over different time-scales (Bustamante et al. 1998a; Hobson 1999), whereas information on the spatial dimension (offshore vs inshore, pelagic vs benthic) will be provided by $\delta^{13}\text{C}$ and also $\delta^{15}\text{N}$ isotope values (Hansson et al. 1997; Hobson 1999; Sherwood and Rose 2005; Ménard et al. 2007; Chouvelon et al. 2012). An important aspect of the temporal dimension is the possibility that habitat use and trophic position may change over time. Consequently, effects of seasons and years were studied on common dolphin, the species for which the biggest sample size was available.

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; Fig. 1). Stranded and by-caught cetaceans were attended by experienced personnel of the Galician (Coordinadora para o Estudo dos Mamíferos Mariños, CEMMA) or Portuguese (Sociedade Portuguesa de Vida Salvagem, SPVS) stranding networks. Animals

were identified to species, measured (total length and several other standard measurements are routinely taken), 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 and Garcia Hartmann 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 1). 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 $-20\text{ }^{\circ}\text{C}$ until being processed in the laboratory. Since decomposition state can affect results from stable isotope analysis of muscle tissue, individuals selected for analysis were those with condition code 1–3, i.e. originally stranded alive, freshly dead or moderately decomposed (after Kuiken and Garcia Hartmann 1991).

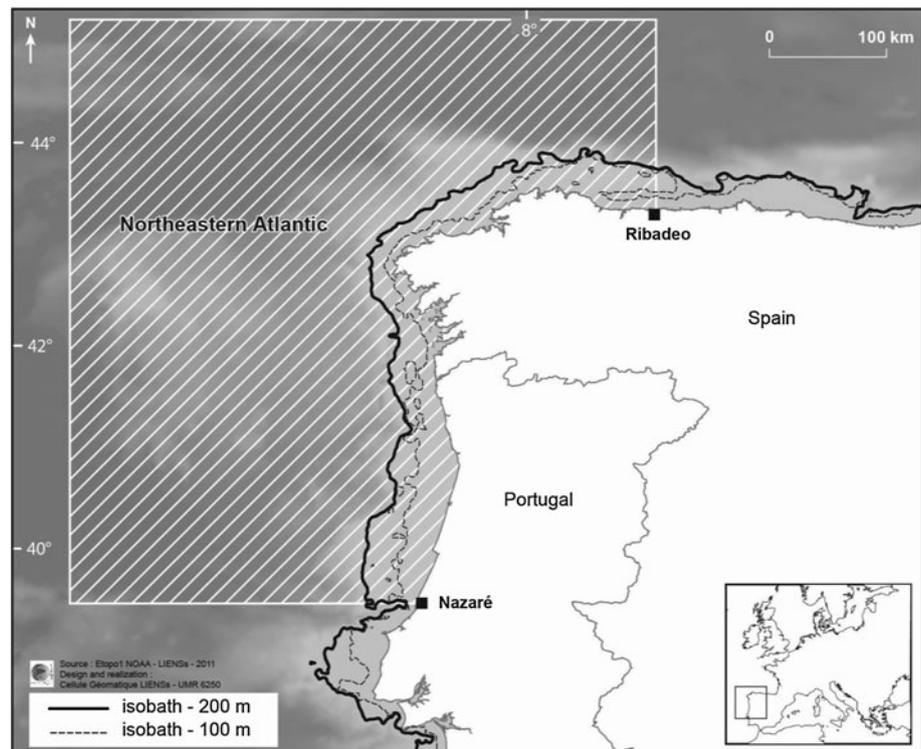
Age determination

At least 5 teeth were collected from the mid-section of the left mandible of each sampled individual, selecting the least worn/damaged and least curved teeth, to ensure sufficient material for replicate preparations. Teeth were preserved frozen, and age was determined by analysing growth layer groups (GLGs) in the dentine of teeth, following adapted methods based on Lockyer (1993), Hohn and Lockyer (1995) 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. If the age estimates obtained by the two readers differed by more than 1 year, readings were repeated. If the increments were difficult to count, both readers discussed the interpretation and either reached an agreed age or judged the tooth to be unreadable.

Stable isotope analyses

Muscle samples were previously analysed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (see Méndez-Fernandez et al. 2012), and, as for whole tooth samples, they were freeze-dried and then

Fig. 1 Map of the sampling area with 100- and 200-m isobaths. The 200-m isobaths is taken as the limit for the shelf-break. The sampling area is framed in *white*, representing the north and south limits for the strandings and the western latitude of the by-catches



ground into a fine powder before isotopic analyses. Since lipids are highly depleted in ^{13}C relative to other tissue components (DeNiro and 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 1 h. Next, the sample was centrifuged for 5 min at 4,000 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 $\delta^{13}\text{C}$ analysis that may contain biogenic carbonates (i.e. inorganic carbon) must be acidified, and carbonates can be removed in the dry powder leaving only the organic portion of the sample, composed of collagen. Thus, carbonates were removed from powdered whole tooth samples by digesting 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 analyser 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 $\delta^{13}\text{C}$ and atmospheric

N_2 for $\delta^{15}\text{N}$, in parts per thousand (‰). Replicate measurements of internal laboratory standards (acetanilide) during each run indicate measurement errors <0.2 ‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

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 and ground to powder, and then, each sample was treated in duplicate.

For Cd analysis, aliquots of the liver and kidney samples (i.e. the main storage tissues; 0.1–0.3 g) were digested with 6 mL 67–70 % HNO_3 and 2 mL 34–37 % HCl . Acid digestion was performed overnight under ambient temperature before being heated in a microwave (ThermoFisher Scientific Milestone Start D) during 30 min with increasing temperature up to 105 °C, and 15 min at 105 °C (1200 W). After the mineralization process, each sample was diluted to 30 or 50 mL with milli-Q quality water, according to the volume of acid added to the mineralization (3.0 mL or 4.5 mL). Thus, 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 complement was 25 mL. Elements were analysed using a Varian Vista-Pro ICP-OES and a Thermo Fisher Scientific XSeries 2 ICP-MS. Three control

Table 1 Summary of numbers of samples collected per species, year and tissue from cetaceans stranded and by-caught in the North West of the Iberian Peninsula

Species	Number of samples			
	Muscle	Teeth	Kidney	Liver
Common dolphin <i>Delphinus delphis</i>	103	98	101	101
2004	4	3	4	4
2005	16	17	16	16
2006	12	12	11	12
2007	41	39	41	40
2008	30	27	29	29
Harbour porpoise <i>Phocoena phocoena</i>	17	15	15	15
2004	5	5	5	4
2005	1	1	1	1
2006	3	3	3	3
2007	5	3	3	4
2008	3	3	3	3
Bottlenose dolphin <i>Tursiops truncatus</i>	8	8	8	8
2004	1	1	1	1
2005	2	2	2	2
2006	3	3	3	3
2007	2	2	2	2
Striped dolphin <i>Stenella coeruleoalba</i>	18	20	18	18
2004	2	1	2	2
2005	4	4	4	4
2006	3	3	3	3
2007	6	9	6	6
2008	3	3	3	3
Long-finned pilot whale <i>Globicephala melas</i>	9	3	9	8
2004	4	0	4	3
2006	1	1	1	1
2007	2	2	2	2
2008	2	0	2	2

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 $0.15 \mu\text{g g}^{-1}$ dry weight (d.wt). Finally, this protocol was validated by an international intercalibration method (Christopher et al. 2007).

Data treatment

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

To investigate the possible role of confounding factors, notably age and season but also interannual differences, on niche segregation, generalized additive models (GAMs; Hastie and Tibshirani 1990) were used. GAM is a semi-parametric extension of generalized linear modelling (McCullagh and Nelder 1989). GAMs are more flexible; they permit both linear and complex nonlinear relationships between species and environmental predictors, as well as combinations of the two within the same model (Wood and Augustin 2002), without the problematic step of postulating response curve shape or parametric response function. GAMs estimate response curves with a nonparametric smoothing function ('smoothers') instead of parametric terms, and as such are said to be data-driven methods. Differences between the sexes were also tested but since there was no effect for any species and tracers, we removed this parameter from the final GAMs.

For common dolphin, Gaussian GAMs with an identity link function were fitted to stable isotope values and Cd concentrations (log-transformed) (response variables) with age, season and year as potential explanatory variables. 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. Season was defined as a categorical variable with 4 values: spring (March, April and May), summer (June, July and August), autumn (September, October and November) and winter (December, January and February). The age smoother was constrained to take a relatively simple form, avoiding overfitting, by setting to the number of knots to $k = 4$. Overfitting occurs when the model learns the training data extremely well but is not able to generalize well. To test for significant interactions between age-season and age-year effects, models were fitted both with single age smoothers and with separate age smoothers for each season or year and, in both cases, the two versions were compared using a 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:

$$\text{Tracer value} \sim s(\text{age}, k = 4) + s(\text{month}, k = 4) \\ + \text{year} [+ \text{interaction terms}]$$

In principle, this approach could be extended to species comparisons, but sample sizes for all species except common dolphin were too small to allow fitting a common model (with relevant interaction terms), 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):

$$\text{Tracer value} \sim \text{species} \\ + s(\text{age}, k = 4, \text{by} = \text{as.factor}(\text{species})) \\ + s(\text{month}, k = 4, \text{by} = \text{as.factor}(\text{species})) \\ + \text{year} [+ \text{other interaction terms}]$$

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, a stepwise procedure was used to select the most parsimonious model using the Akaike Information Criterion (AIC; Akaike 1981). The best model was found using backwards selection (successive eliminations), removing the least important nonsignificant

terms sequentially until all remaining effects were significant. Stepwise procedures have been criticized by several authors (e.g. Whittingham et al. 2006), for example, because they effectively update the hypothesis tested at each step. However, resulting models can be viewed more as a refinement of the original hypothesis than a hypothesis test per se. Thus, all such models require tests with independent data sets and we therefore argue that stepwise procedures remain a useful tool to guide future work. The percentage of deviance explained by the final model was calculated as follows:

$$\text{Explained deviance} = ((\text{Null model deviance} - \text{final model residual deviance}) / \text{Null model deviance}) * 100,$$

with the null model that only contains the intercept terms.

Final models were checked for normality and trends in residuals and for outliers and other influential data points (Zuur et al. 2007). The models performed for common dolphin, including respective AIC values, are summarized in Table 2.

To provide a quick and easily understandable summary of between-species differences in accumulation/assimilation of trace elements and stable isotopes, ANOVA (analysis of variance) with Tukey's tests was also performed, specifically to test for differences in Cd concentrations ($\mu\text{g g}^{-1}$ wet weight, w.wt) and stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) (log-transformed) between species.

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

Results

The number of samples available by tissue, species and year are summarized in Table 1.

Cadmium concentration in kidney

Concentrations of Cd were higher in the kidney than in the liver for all species; ranges and values (mean \pm SD) are summarized in Table 3.

ANOVA confirmed that Cd concentrations ($\mu\text{g g}^{-1}$ w.wt, log-transformed) in kidney varied significantly between species (ANOVA, $F_{(4)} = 5.31$, $p < 0.001$), being significantly higher in striped dolphin than in common dolphin and harbour porpoise and significantly higher in pilot whale than in harbour porpoise (Tukey's tests). However, no significant differences were found between pilot whale and striped dolphin ($p > 0.05$, Tukey's test). The final GAM for Cd concentrations ($\mu\text{g g}^{-1}$ w.wt, log-transformed) in the kidney of common dolphins explained 37.9 % of deviance ($N = 96$, AIC = 133.6; Table 2) and included only a significant effect of age ($p < 0.0001$,

Table 2 Results of the GAM models for explaining common dolphin (*Delphinus delphis*) tracers of the North West of the Iberian Peninsula

Tracer	Variables	Significance	AIC	Deviance explained (%)
Renal Cd	CdK ~ s(Age, k = 4) + s(Month, k = 4) + Year	ns	135.2	39.1
	CdK ~ s(Age, k = 4) + s(Month, k = 4)	ns	133.9	38.7
	CdK ~ s(Age, k = 4)	***	133.6	37.9
Hepatic Cd (CdL)	CdL ~ s(Age, k = 4) + s(Month, k = 4) + Year	ns	114.2	47.5
	CdL ~ s(Age, k = 4) + s(Month, k = 4)	ns	112.2	47.5
	CdL ~ s(Age, k = 4)	***	110.3	47.4
$\delta^{13}\text{C}$ Muscle ($\delta^{13}\text{CM}$)	$\delta^{13}\text{CM} \sim \text{s(Age, } k = 4) + \text{s(Month, } k = 4) + \text{Year}$	ns	119.8	34.9
	$\delta^{13}\text{CM} \sim \text{s(Age, } k = 4) + \text{s(Month, } k = 4)$	ns	121.1	32.4
	$\delta^{13}\text{CM} \sim \text{s(Month, } k = 4)$	***	122.8	29.8
$\delta^{13}\text{C}$ Teeth ($\delta^{13}\text{CT}$)	$\delta^{13}\text{CT} \sim \text{s(Age, } k = 4) + \text{s(Month, } k = 4) + \text{Year}$	ns	111	26.9
	$\delta^{13}\text{CT} \sim \text{s(Age, } k = 4) + \text{s(Month, } k = 4)$	ns	109.5	26.5
	$\delta^{13}\text{CT} \sim \text{s(Month, } k = 4)$	***	115.8	20
$\delta^{15}\text{N}$ Muscle ($\delta^{15}\text{NM}$)	$\delta^{15}\text{NM} \sim \text{s(Age, } k = 4) + \text{s(Month, } k = 4) + \text{Year}$	ns	151.5	26
	$\delta^{15}\text{NM} \sim \text{s(Age, } k = 4) + \text{s(Month, } k = 4)$	ns	149.8	25.9
	$\delta^{15}\text{NM} \sim \text{s(Age, } k = 4)$	***	151.9	19.7
$\delta^{15}\text{N}$ Teeth ($\delta^{15}\text{NT}$)	$\delta^{15}\text{NT} \sim \text{s(Age, } k = 4) + \text{s(Month, } k = 4) + \text{Year}$	ns	162	52.4
	$\delta^{15}\text{NT} \sim \text{s(Age, } k = 4) + \text{s(Month, } k = 4)$	ns	163.1	50.8
	$\delta^{15}\text{NT} \sim \text{s(Age, } k = 4)$	***	161.3	50.7

Explanatory variables, deviance explained and Akaike Information Criterion (AIC) value for each model are given. Significance level of variables is indicated by asterixes: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns not significance. Final models chosen are highlighted in bold

Table 3 Age (mean \pm SD, in years), carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope values (mean \pm SD, ‰) and cadmium (Cd) concentrations (mean \pm SD, $\mu\text{g g}^{-1}$ wet weight, w.wt) in different tissues of the toothed whale species collected along the North West of the Iberian Peninsula

Species	Age	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		Cd	
		Muscle	Teeth	Muscle	Teeth	Liver	Kidney
Common dolphin	6.1 \pm 5.3	-17.0 \pm 0.5	-14.3 \pm 0.5	11.7 \pm 0.6	13.0 \pm 0.8	0.4 \pm 0.5	2.3 \pm 2.7
<i>Delphinus delphis</i>	(0, 20)	(-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	5.4 \pm 6.1	-16.5 \pm 0.7	-13.7 \pm 0.7	13.0 \pm 1.2	15.5 \pm 0.6	0.1 \pm 0.1	2.2 \pm 5.3
<i>Phocoena phocoena</i>	(0, 18)	(-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	4.3 \pm 2.0	-16.5 \pm 0.8	-13.8 \pm 0.8	12.6 \pm 0.9	13.7 \pm 1.0	1.2 \pm 2.8	5.7 \pm 13.8
<i>Tursiops truncatus</i>	(0.5, 7)	(-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	4.1 \pm 5.0	-17.6 \pm 0.6	-14.6 \pm 0.4	10.8 \pm 0.7	12.4 \pm 0.8	3.9 \pm 3.8	10.3 \pm 11.0
<i>Stenella coeruleoalba</i>	(0, 15)	(-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	7.4 \pm 5.0	-16.5 \pm 0.5	-13.5 \pm 0.8	12.2 \pm 0.6	13.6 \pm 0.5	8.3 \pm 8.4	30.0 \pm 26.9
<i>Globicephala melas</i>	(0, 14)	(-17.3, -15.7)	(-14.4, -13.0)	(11.0, 13.3)	(13.3, 14.3)	(0.01, 22.2)	(<0.01, 79.3)

Ranges (minimum, maximum) are presented in parentheses. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values in muscle tissue are from Mèndez-Fernandez et al. (2012)

$df = 2.81$). Cd concentrations increased with increasing age, although levelling off after around 10 years (Fig. 2a).

A GAM model for all species which included separate age smoothers for each 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 and showed different trends

among species (Fig. 3), except for bottlenose dolphin ($p = 0.060$). This was probably a result of the low number of samples and high proportion of young (immature) animals of this species. Significant between-species variation is confirmed, with pilot whale, striped dolphin and common dolphins all showing higher age-corrected accumulation of Cd in the kidney than harbour porpoise and striped

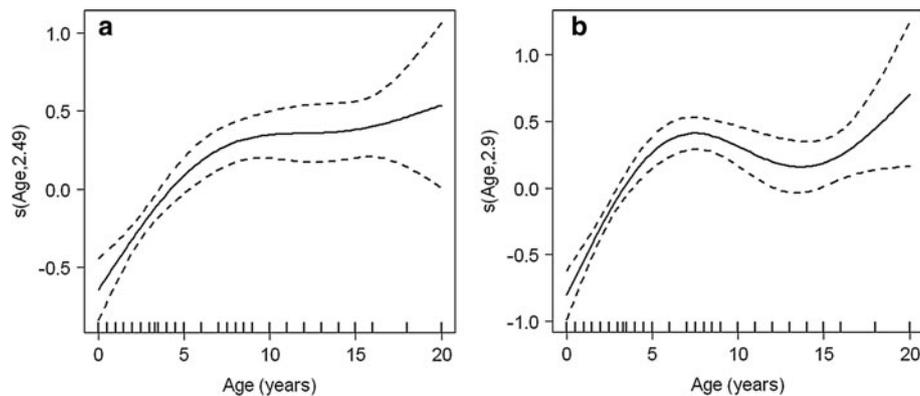
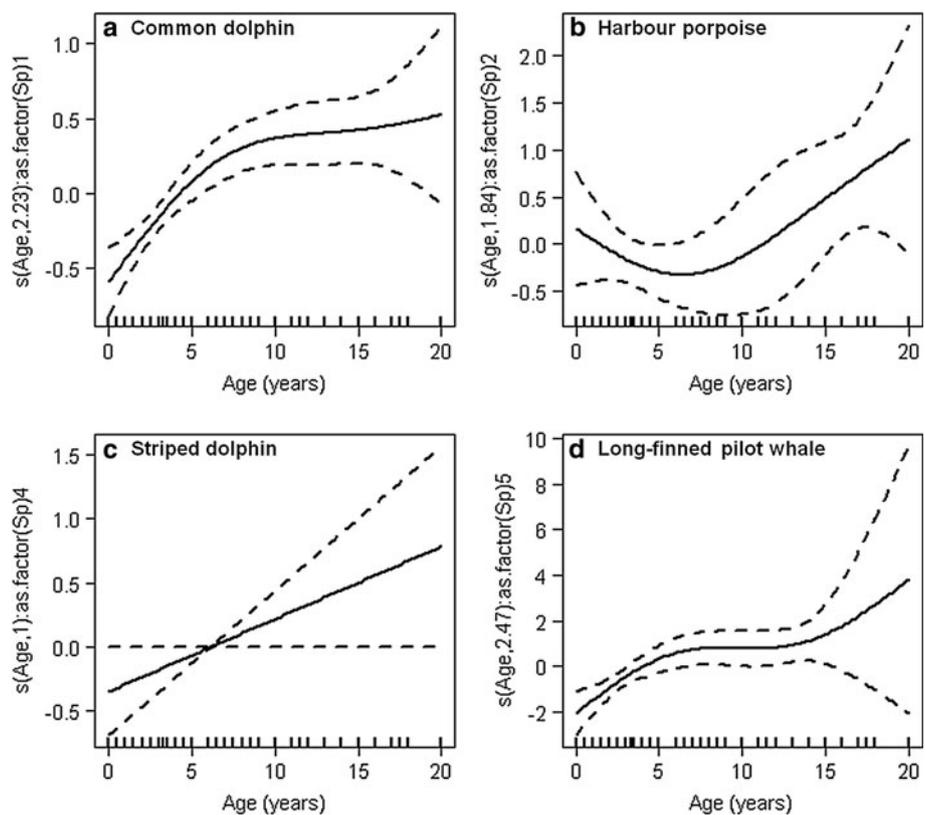


Fig. 2 Smoothers for effects of age on Cd concentrations ($\mu\text{g g}^{-1}$ w.wt) in kidney (a) and in liver (b) of common dolphin *Delphinus delphis*. The y-axis shows the contribution of the smoother to the predictor function (in arbitrary units). Smoothers illustrate the partial

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 individuals. Dashed lines represent 95 % confidence bands for the smoothers

Fig. 3 Smoothers for between-species differences of age-corrected accumulation of Cd concentrations in kidney ($\mu\text{g g}^{-1}$ w.wt) of common dolphin *Delphinus delphis* (a) harbour porpoise *Phocoena phocoena* (b) striped dolphin *Stenella coeruleoalba* (c) and 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 individuals. Dashed lines represent 95 % confidence bands for the smoothers



dolphin having a higher concentration than common dolphin ($p < 0.0001$ in all cases).

Cadmium concentration in liver

In liver, Cd concentrations also varied significantly between species (ANOVA, $F_{(4)} = 13.85$, $p < 0.0001$), 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's tests). The final model for Cd in liver of common dolphin explained 47.4 % of deviance ($N = 95$, $AIC = 110.3$; Table 2). Cd concentrations in liver increased with increasing age ($df = 2.99$, $p < 0.0001$) up to around age 7 (Fig. 2b). As for Cd in kidney, seasonal and between-year differences were nonsignificant.

The final GAM for Cd concentrations in liver for all species included separate age smoothers for each species

and explained 52.4 % of deviance ($N = 143$, $AIC = 253.9$). The age effects were significant for all the species except for striped dolphin and pilot whale. Again, differences between species were highly significant. 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 ($p < 0.0001$ in all cases).

$\delta^{13}\text{C}$ values in muscle and teeth

A summary of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the different tissues (muscle and tooth) of the five toothed whales from the NWIP is presented in Table 3. Results for muscle are based on data from Mèndez-Fernandez et al. (2012).

$\delta^{13}\text{C}$ values (‰) in muscle and teeth varied significantly between species, as shown by ANOVA (ANOVA, $F_{(4)} = 11.1$ and 10.91 , respectively, $p < 0.0001$). Striped dolphin showed significantly lower $\delta^{13}\text{C}$ values in both muscle and teeth (Tukey's tests) compared to all other species, except for common dolphin in teeth. Common dolphin also showed significantly lower $\delta^{13}\text{C}$ values than harbour porpoise in both muscle and teeth, and lower $\delta^{13}\text{C}$ values than pilot whale and bottlenose dolphin in teeth (Tukey's tests) (Fig. 4).

The final GAM for $\delta^{13}\text{C}$ values (‰) in the muscle of common dolphin explained 34.9 % of deviance ($N = 104$, $AIC = 119.8$; Table 2). There was no effect of age but the effect of season was highly significant ($p < 0.0001$, $df = 2.3$), with the highest values in April and the lowest in September (Fig. 5a).

GAM results for $\delta^{13}\text{C}$ in muscle of all species confirmed clear differences between species and the effect of season also differed between species, with the seasonal trends for

common dolphin, harbour porpoise and bottlenose dolphin all being statistically significant (Fig. 5a–c). The model explained 45.7 % of deviance. Seasonally adjusted $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ in teeth gave similar results, although deviance explained was only 26.5 % (Table 2).

$\delta^{15}\text{N}$ values in muscle and teeth

ANOVA confirmed that $\delta^{15}\text{N}$ values (‰) in muscle and teeth also varied significantly between species (ANOVA, $F_{(4)} = 25.92$ 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 teeth of all the other species (Tukey's tests). Finally, $\delta^{15}\text{N}$ values in muscle of common dolphin were significantly lower than in muscle of bottlenose dolphin (Tukey's tests) (Fig. 4).

The final GAM for $\delta^{15}\text{N}$ values in muscle of common dolphin explained 19.7 % of deviance ($N = 97$, $AIC = 151.9$; Table 2). There was no effect of season or year but a strong negative and linear effect of age ($p < 0.001$, $df = 1.0$) where $\delta^{15}\text{N}$ values decreased with increasing age (Fig. 6).

GAM results for the all-species analysis of $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ data produced no marked improvement in fit. The final model therefore contained a significant age effect

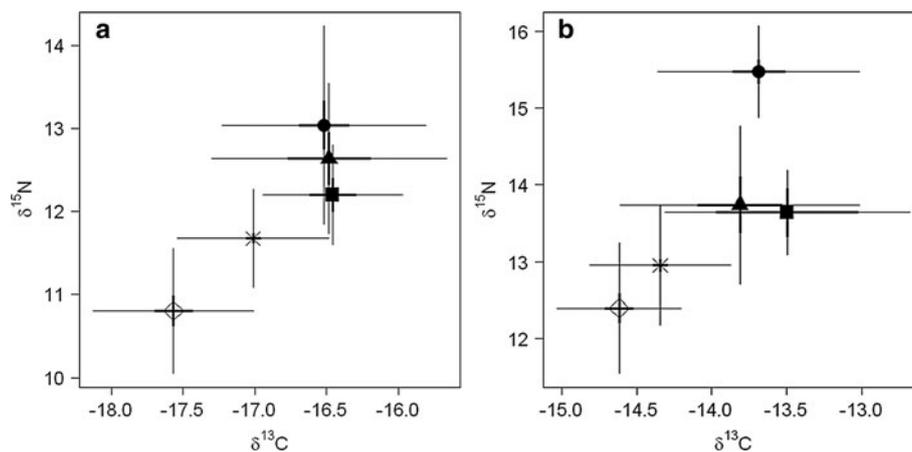


Fig. 4 Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope values in muscle tissue (a) and in teeth (b) of common dolphin *Delphinus delphis* (asterisk), harbour porpoise *Phocoena phocoena* (filled circle), bottlenose dolphin *Tursiops truncatus* (filled triangle), Striped

dolphin *Stenella coeruleoalba* (open diamond) and long-finned pilot whale *Globicephala melas* (filled square). Data are mean (‰) \pm SD and SEM (bold). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values in muscle from Mèndez-Fernandez et al. (2012)

Fig. 5 Smoothers for between-species differences of season-adjusted effects on $\delta^{13}\text{C}$ values in muscle of common dolphin *Delphinus delphis* (a) harbour porpoise *Phocoena phocoena* (b) and bottlenose dolphin *Tursiops truncatus* (c). The y-axis shows the contribution of the smoother to the predictor function (in arbitrary units). Smoothers illustrate the partial effect of season once the effects of all other explanatory variables in the model have been taken into account. Tick marks in x-axis represent sampled individuals. Dashed lines represent 95 % confidence bands for the smoothers

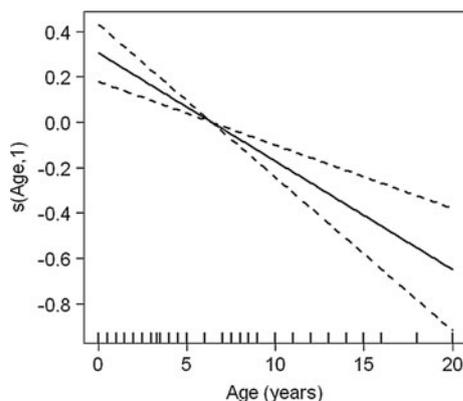
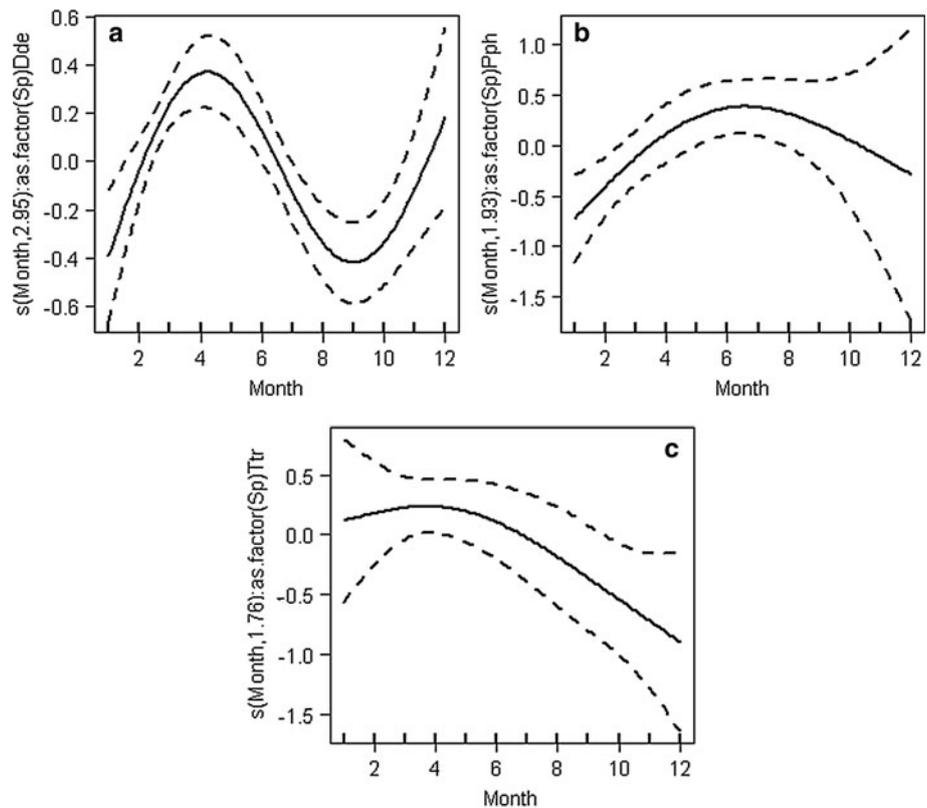


Fig. 6 Smoothers for effects of age on $\delta^{15}\text{N}$ values in muscle of common dolphin *Delphinus delphis*. The y-axis shows the contribution of the smoother to the predictor function (in arbitrary units). Smoothers illustrate the partial effect of age once the effects of all other explanatory variables in the model have been taken into account. Tick marks in x-axis represent sampled individuals. Dashed lines represent 95 % confidence bands for the smoothers

($p < 0.0001$, $df = 2.8$), depicting a decline in $\delta^{15}\text{N}$, 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 (age-corrected) $\delta^{15}\text{N}$ values than common dolphin and striped dolphin, while values for common dolphin were also higher than for striped dolphin.

Finally, for $\delta^{15}\text{N}$ in teeth of all species, the best model included separate age effects for each 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. $\delta^{15}\text{N}$ 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

We have shown how the application of a multi-tracer approach has allowed the evaluation of the foraging niche of the five most common toothed whales in the NWIP. This approach has permitted the investigation of the foraging niche in two dimensions (i.e. trophic and spatial) covering a range of different time spans. Moreover, this approach also provided complementary information to the studies using conventional methods previously carried out in the area (e.g. based on stomach contents analysis or direct observations). Overall, the main finding in this work is that none of the individual ecological tracers examined alone reveals complete ecological segregation amongst the five

species studied, whereas the integration of results from all these tracers indicates segregation of all five species in the long term (conceptually represented in Fig. 7). The NWIP represents only a part of the range of these species within the NE Atlantic, and each individual may not spend a substantial proportion of its time within this area. This is why a possible segregation found by tracers may attest to a different distribution of the species on a broader geographic scale out of the NWIP. A further and important consideration is that all the ecological tracers are mainly transmitted via food with only differences in tracer signatures are really informative (similar signatures could arise from different combinations of different prey).

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 it is very unlikely that sufficient individuals covering all seasons over several consecutive years are available. However, and despite the potential biases of the sampling regime, the stranded and by-caught animals used in this study reflect the ecology of the different species in the study area and provide evidence of their distinct ecological niches.

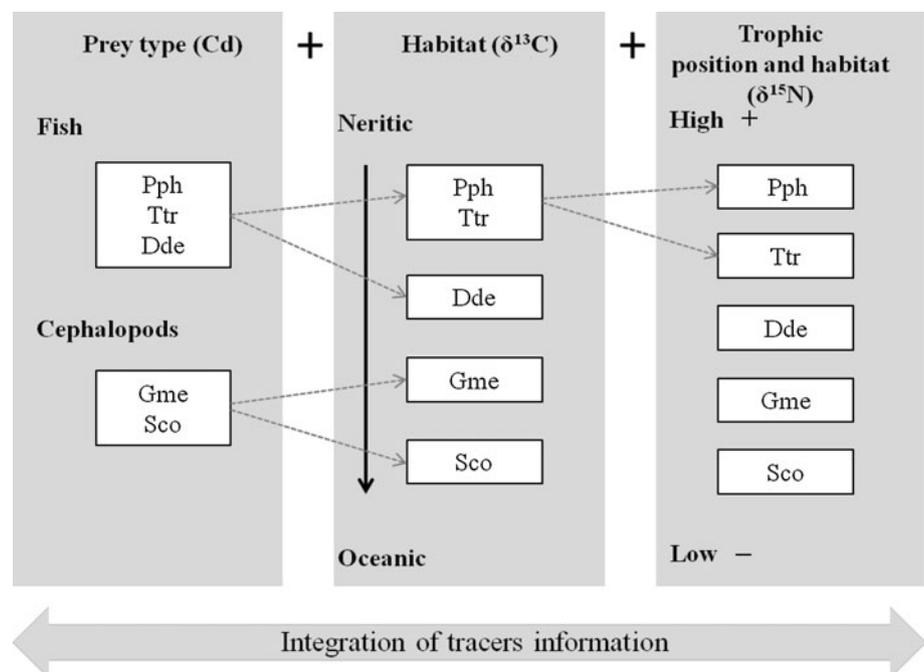
Resource partitioning inferred from Cd concentrations

It is well known that diet, size, weight, longevity and metabolic rates (i.e. ingestions and assimilation) have an influence on Cd bioaccumulation and variations of concentrations among species (Caurant et al. 1994; Aguilar et al. 1999; Das et al. 2003a). Thus, as shown (Figs. 2 and

3), Cd is a metal which accumulates in cetacean tissues, but with different accumulation rates according to the species. In common dolphin, the species for which we had the largest sample size, concentrations increased with age up to around 10 years old, after which concentrations reached a plateau (Fig. 2a). Similar results are well documented in the literature for several marine mammal species (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. These results emphasize the need to take age into account when carrying out comparison between species.

Differences in Cd concentrations between 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 the use of Cd as a tracer confirmed the existence of dietary segregation between oceanic and neritic common dolphins over a long time-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 (Table 3). High Cd concentrations in tissues of both species are consistent with their teuthophagous feeding habits. In the NWIP, pilot whales mainly feed on neritic and/or benthic cephalopods such as common and curled octopuses *Octopus vulgaris* and *Eledone cirrhosa* (González et al. 1994; Pierce et al. 2007; Santos et al. in press). However, striped dolphin feeds on both cephalopods (mainly *Gonatus* sp. and ommastrephids) and fish (including blue whiting

Fig. 7 Conceptual scheme of foraging niche segregations obtained by the sum of the information given by ecological tracers' in the long term (i.e. several years) and in the five toothed whale species. Dde: common dolphin *Delphinus delphis*, Pph: harbour porpoise *Phocoena phocoena*, Ttr: bottlenose dolphin *Tursiops truncatus*, Sco: striped dolphin *Stenella coeruleoalba* and Gme: pilot whale *Globicephala melas*. Ecological tracer values of species lumped together were not significantly different (Tukey's test, $p > 0.05$)



Micromesistius poutassou, scads *Trachurus trachurus*, sand smelt *Atherina presbyter* and silvery lightfish *Maurolicus muelleri*, Santos et al. 2007b) and crustaceans (specifically pelagic shrimp species, Jose Cedeira pers comm). Therefore, their fish consumption could explain their lower Cd concentrations (Table 3) in comparison with pilot whales.

Conversely, bottlenose dolphins and harbour porpoises are mainly fish feeders (Santos et al. 2007a, b), and in this area, harbour porpoise is mainly a coastal species (López et al. 2002; Pierce et al. 2010). This can explain the significantly lower Cd concentrations found in harbour porpoise compared to the main teuthophagous species over a medium and long time-scale, and the absence of differences between porpoises and bottlenose dolphin at any time-scale. This last result suggests that trophic competition can occur between these two species as reported in adjacent areas of the Northeast Atlantic by Spitz et al. (2006). However, consumption of different prey species, revealed by stomach contents analyses carried out on stranded Iberian Peninsula porpoises and bottlenose dolphins (Santos et al. 2001, 2007a, b), would mitigate the competition in this area (discussed below).

The low hepatic and renal Cd concentrations recorded for common dolphin suggest a lower proportion of cephalopods in its diet than expected (Evans 1994; Santos et al. in press; Table 3). Significant differences were found between common dolphin and pilot whale, over a medium time-scale (hepatic concentrations), and with striped dolphin, over both medium (hepatic) and long (renal) time-scales, indicating that segregation occurs among these species even though common dolphin also frequents oceanic habitats.

Spatial and trophic segregation inferred from stable isotopes

Results on $\delta^{13}\text{C}$ values obtained in the present study are generally in agreement with the habitat preferences inferred from direct observations and/or from habitat modelling carried out in the study area for some of the study species (López et al. 2004; Pierce et al. 2010; Spyarakos et al. 2011).

The $\delta^{13}\text{C}$ values in the tissue analysed (i.e. muscle and teeth) allowed a good differentiation of the most oceanic from the most coastal species. Thus, the significantly lower $\delta^{13}\text{C}$ values exhibited in striped and common dolphins compared to the other species confirm, over medium and long time-scales, the mostly oceanic distribution observed for both 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; Spyarakos et al. 2011). Common dolphin is the most abundant

cetacean in the NWIP (Aguilar 1997; López et al. 2004) and mainly found in waters deeper than 200 m depth (Spyrakos et al. 2011). Despite this, common dolphin is the second most frequently sighted species from the coast, albeit seen most often in areas where the shelf is narrower (Pierce et al. 2010). This characteristic would explain their low and intermediate $\delta^{13}\text{C}$ values, between those of striped dolphin and the other toothed whales studied (Fig. 4). This information could suggest that common dolphins move following pelagic schooling fish in the area (such as sardine and blue whiting; Moura et al. 2012; Santos et al. in press) and, as a consequence, seasonal overlap and/or competition or habitat with striped dolphins may occur.

Based on the $\delta^{13}\text{C}$ values, bottlenose dolphin, harbour porpoise and pilot whale did not show significant differences from each other over medium and long time-scales but showed significantly high $\delta^{13}\text{C}$ values than common and striped dolphins (Fig. 4). 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 groups of bottlenose dolphin (Fernández et al. 2011a, b). Thus, the high variability obtained in our isotopic $\delta^{13}\text{C}$ values for bottlenose dolphin may be due to the presence of some offshore individuals in our sampling (Fig. 4). Again based on coastal sightings, harbour porpoise tend to be seen where the shelf is narrower, while boat-based sightings studies suggest association with water depths of 100–200 m (Spyrakos et al. 2011).

Although the pilot whale is essentially known as oceanic and deep water feeder, it shows some degree of dietary plasticity and can apparently forage successfully in both oceanic and neritic habitats (Spitz et al. 2011). This fact, together with the age/maturity of sampled animals, might explain the high muscular and dental $\delta^{13}\text{C}$ values obtained in this study. Nitrogen isotopic signatures are the main source of information on feeding habitat and on resource utilization and trophic positions (TP) of the marine mammals in the food web. Here, we used the TP obtained in Mèndez-Fernandez et al. (2012) complementing $\delta^{15}\text{N}$ values obtained from hard tissues, i.e. long-term information, to compare species showing overlap in $\delta^{13}\text{C}$ values and/or Cd concentrations, i.e. living in the same habitat and feeding in the same type of prey.

The age effect on muscular $\delta^{15}\text{N}$ values was previously tested for common dolphin (Fig. 6) and showed a negative relationship. Several studies found the same negative effect of age (or body length) on $\delta^{15}\text{N}$ values. In younger animals,

this is consistent with individuals switching from primarily feeding on milk to individuals primarily feeding on living prey (Hobson et al. 1997; Das et al. 2003b; Knoff et al. 2008; Fernández et al. 2011a; Meissner et al. 2011). Additionally, it was demonstrated that the consumption of blue whiting, one of the main prey of common dolphin in the Iberian Peninsula, increased with (post-weaning) dolphin body length (Fernández et al. 2011a, Santos et al. in press). Since blue whiting have low $\delta^{15}\text{N}$ values (Méndez-Fernandez et al. 2012), the decrease in $\delta^{15}\text{N}$ values with common dolphin age makes sense (Fig. 6).

The comparison between species showed that striped dolphins exhibited the lowest $\delta^{15}\text{N}$ values in muscle and teeth, revealing the low TP of this species and its oceanic habitat (Table 3). 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 (Fig. 4). The higher $\delta^{15}\text{N}$ values in common dolphin compared to striped dolphin, over medium and long time-scales, are consistent with its more strongly piscivorous feeding habits as its higher presence in coastal waters. However, these values were still low in comparison with the other toothed whale species (Table 3), probably as the consequence of feeding on fish with a low TP such as blue whiting and sardine *Sardina pilchardus* (Méndez-Fernandez et al. 2012), the most important prey of common dolphin in Galician waters (Santos et al. in press). According to its $\delta^{15}\text{N}$ values, common dolphin is not significantly different from pilot whale and one might conclude that there is a large overlap between their diets, if Cd concentrations (this study) did not contradict this, by confirming the higher occurrence of cephalopods in pilot whale diet (Pierce et al. 2007; Spitz et al. 2011; Santos et al. in press). Furthermore and as discussed above, pilot whales exhibit some degree of dietary plasticity allowing them to forage successfully in both oceanic and neritic habitats (Spitz et al. 2011). This dietary plasticity can also explain their relatively high $\delta^{15}\text{N}$ values and the variability obtained, 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 2003–2007 were during the months September to November, which would be consistent with seasonal use of coastal waters (Pierce et al. 2010; unpubl data).

Bottlenose dolphin and harbour porpoise exhibited elevated $\delta^{15}\text{N}$ values, with harbour porpoise showing significantly higher values in teeth compared to the other species (Table 3), indicating that they feed on prey with a high TP

and located at more coastal areas on a long-term basis. Thus, scad, pouting *Trisopterus luscus* and garfish *Belone belone* were identified as the most important prey in their diet (Santos et al. 2004, 2007b; Pierce et al. 2010), and these prey also exhibit high $\delta^{15}\text{N}$ values (Méndez-Fernandez et al. 2012). Bottlenose dolphin is slightly more generalist and its main prey, in terms of both biomass and number, are blue whiting and hake *Merluccius merluccius* of less than 30 cm length (Mahe et al. 2007; Santos et al. 2007a), which exhibit lower $\delta^{15}\text{N}$ values than harbour porpoise prey (Méndez-Fernandez et al. 2012).

Time integration of ecological tracers

Ecological tracers were analysed 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, except for the common dolphin, and the variations which may occur between seasons. Foraging niche segregation probably depends on the availability of resources in the area. The NWIP 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, which 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 possible seasonal movements demonstrated for common dolphin (Fig. 5a), this species is well separated from striped dolphin by its $\delta^{13}\text{C}$ isotopic composition over a medium time-scale, $\delta^{15}\text{N}$ isotopic composition and Cd concentrations. Thus, over a long time-scale, both species mostly share the same habitat but feed on different resources. The other species for which results were highly influenced by the time period of integration is the harbour porpoise. Only dental $\delta^{15}\text{N}$ isotopic composition discriminates 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

In this work, we studied the three main 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 which

frequent the NWIP waters. Over a medium time-scale period of integration, ecological tracers did not reveal a complete segregation among the five species. Thus, the harbour porpoise and bottlenose dolphin could face competition for habitat in waters off the NW Iberian Peninsula. However, integrating information given by long-term ecological tracers showed complete ecological niche segregation among all species (see Fig. 7). We hypothesize the following conceptual scheme to sum up our results:

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 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ 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 that frequent the NWIP and demonstrates that ecological tracers provide a relatively simple way to quantify ecological niche differences over different time-scales, especially when looking at closely related species within a common area. However, there is a need for a larger sample sizes and a stronger representation of older animals to strengthen these conclusions.

Acknowledgments The authors gratefully acknowledge the assistance of volunteers from the Galician (CEMMA) and Portuguese (SPVS) stranding networks. They also thank P. Richard and G. Guillou (UMR LIENSs) for running stable isotope measurements, C. Churlaud and M. Kalombo from the Centre Commun d'Analyses (Fédération de Recherche en Environnement pour le Développement Durable FR 3097 CNRS-IFREMER-Université de La Rochelle) for running part of the metal analyses, and P. Brunello from the Cellule Géomatique (UMR LIENSs) for providing the map of the study area. We also wish to thank B. Simon-Bouhet and Géraldine Lassale (UMR LIENSs) for useful comments during statistical analysis and T. Berthuin for her assistance during laboratory work. This work was supported through the PhD grant to P. Méndez-Fernandez from the Portuguese Foundation for Science and Technology of the Government of Portugal (SFRH/BD/36766/2007). G.J. Pierce acknowledges support from the EU under the ANIMATE project (MEXC-CT-2006-042337). M.B. Santos acknowledges support from the LOTOFPEL project (Plan Nacional de I + D + I, CTM 2010-16053) and from the Spanish Ministry of Education, Programa Nacional de Movilidad de Recursos Humanos de Investigación (PR-2010-0518). Two anonymous reviewers and the associate editor C. Harrod are thanked for helpful suggestions and comments on an earlier form of this manuscript.

References

- Aguilar A (1997) Inventario de los cetáceos de las aguas atlánticas peninsulares: aplicación de la directiva 92/43/CEE. Memoria Final del Departamento de Biología Animal, University of Barcelona, Barcelona
- Aguilar A, Borrell A, Pastor T (1999) Biological factors affecting variability of persistent pollutant levels in cetaceans [special issue]. *J Cetac Res Manage* 1:83–116
- Akaike H (1981) Likelihood of a model and information criteria. *J Econom* 16:3–14
- Ballance LT (2002) Cetacean ecology. In: Perrin WF, Wiirsig B, Thewissen JGM (eds) *Encyclopedia of marine mammals*. Academic Press, San Diego, pp 196–201
- Bryan GW (1976) Heavy metal contamination in the sea. In: Johnston R (ed) *Marine pollution*, ch 3. Academic Press, London, pp 185–302
- Bustamante P, Caurant F, Fowler SW, Miramand P (1998a) Cephalopods as a vector for the transfer of cadmium to top marine predators in the north-east Atlantic Ocean. *Sci Total Environ* 220:71–80
- Bustamante P, Cherel Y, Caurant F, Miramand P (1998b) Cadmium, copper and zinc in octopuses from Kerguelen Islands, Southern Indian Ocean. *Pol Biol* 19:264–271
- Bustamante P, Teyssié JL, Fowler SW, Cotret O, Danis B, Miramand P, Warnau M (2002a) Biokinetics of zinc and cadmium accumulation and depuration at different stages in the life cycle of the cuttlefish *Sepia officinalis*. *Mar Ecol Progr Ser* 231: 167–177
- Bustamante P, Cosson RP, Gallien I, Caurant F, Miramand P (2002b) Cadmium detoxification processes in the digestive gland of cephalopods in relation to accumulated cadmium concentrations. *Mar Environ Res* 53:227–241
- Bustamante P, Morales CF, Mikkelsen B, Dam M, Caurant F (2004) Trace element bioaccumulation in grey seals *Halichoerus grypus* from the Faroe Islands. *Mar Ecol Progr Ser* 267:291–301
- Cabana G, Rasmussen JB (1996) Comparison of aquatic food chains using nitrogen isotopes. *Proc Nat Acad Sci USA* 93:10844–10847
- Caurant F, Amiard JC, Amiard-Triquet C, Sauriau PG (1994) Ecological and biological factors controlling the concentrations of trace elements (As, Cd, Cu, Hg, Se, Zn) in delphinids *Globicephala melas* from the North Atlantic Ocean. *Mar Ecol Progr Ser* 103:207–219
- Caurant F, Chouvelon T, Lahaye V, Méndez-Fernandez P, Rogan E, Spitz J, Ridoux V (2009) The use of ecological tracers for discriminating populations: the case of the short-beaked common dolphin *Delphinus delphis* in the European Atlantic waters. *RIWC Madeira*
- Cendrero O (1993) Nota sobre los hallazgos de cetáceos en el norte de España. *Bol Inst Esp Oceanogr* 9:251–255
- Cherel Y, Le Corre M, Jaquemet S, Ménard F, Richard P, Weimerskirch H (2008) Resource partitioning within a tropical seabird community: new information from stable isotopes. *Mar Ecol Progr Ser* 366:281–291
- Chouvelon T, Spitz J, Caurant F, Méndez-Fernandez P, Chappuis A, Laugier F, Le Goff E, Bustamante P (2012) Revisiting the use of $\delta^{15}\text{N}$ in meso-scale studies of marine food webs by considering spatio-temporal variations in stable isotopic signatures—the case of an open ecosystem: the Bay of Biscay (North-East Atlantic). *Progr Oceanogr* 101:92–105
- Christopher SJ, Pugh RS, Ellis MB, Mackey EA, Spatz RO, Porter BJ, Bealer KJ, Kucklick JR, Rowles TK, Becker PR (2007) Description and results of the NIST/NOAA 2005 interlaboratory

- comparison exercise for trace elements in marine mammals. *Accred Qual Assur* 12:175–187
- Das K, Lepoint G, Loizeau V, Debacker V, Dauby P, Bouquegneau J-M (2000) Tuna and dolphin associations in the North-east Atlantic: evidence of different ecological niches from stable isotope and heavy metal measurements. *Mar Poll Bull* 40:102–109
- Das K, Debacker V, Pillet S, Bouquegneau J-M (2003a) Heavy metals in marine mammals. In: Vos JG, Bossart GD, Fournier M, O'shea TJ (eds) *Toxicology of marine mammals, volume 3 systems*. Taylor & Francis Inc, New York, pp 135–167
- Das K, Lepoint G, Leroy Y, Bouquegneau J-M (2003b) Marine mammals from the southern North Sea: feeding ecology data from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements. *Mar Ecol Progr Ser* 263:287–298
- DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochim Cosmochim Acta* 42:495–506
- Evans WE (1994) Common dolphin, white-bellied porpoise *Delphinus delphis* Linnaeus, 1758. In: Ridgway S, Harrison R (eds) *Handbook of marine mammals, vol 5*. Academic Press, London, pp 191–224
- Fernández R, García-Tiscar S, Santos MB, López A, Martínez-Cedeira JA, Newton J, Pierce GJ (2011a) Stable isotope analysis in two sympatric populations of bottlenose dolphins *Tursiops truncatus*: evidence of resource partitioning? *Mar Biol* 158:1043–1055
- Fernández R, Santos MB, Pierce GJ, Llavona A, López A, Silva MA, Ferreira M, Carrillo M, Cermeño P, Lens S, Piertney SB (2011b) Fine-scale genetic structure of bottlenose dolphins, *Tursiops truncatus*, in Atlantic coastal waters of the Iberian Peninsula. *Hydrobiologia* 670:111–125
- Fraga F (1981) Upwelling off the Galician Coast, Northwest Spain. In: Richards FA (ed) *Coastal upwelling*. American Geophysical Union, Washington, pp 176–182
- Fry B (2006) *Stable isotope ecology*. Springer, New York
- González N, González JJ, García C, Cabanas JM (1979) Dinámica de nutrientes en las Rías de Arosa y Muros. *Bol Inst Esp Oceanogr* 1:51–81
- González AF, López A, Guerra A, Barreiro A (1994) Diets of marine mammals stranded on the northwestern Spanish Atlantic coast with special reference to Cephalopoda. *Fish Res* 21:179–191
- Hansson S, Hobbie JE, Elmgren R, Larsson U, Fry B, Johansson S (1997) The stable nitrogen isotope ratio as a marker of food-web interactions and fish migration. *Ecology* 78:2249–2257
- Hastie TJ, Tibshirani RJ (1990) *Generalized additive models*. Chapman & Hall, New-York
- Herman DP, Burrows DG, Wade PR, Durban JW, Matkin CO, LeDuc RG, Barrett-Lennard LG, Krahn MM (2005) Feeding ecology of eastern North Pacific killer whales *Orcinus orca* from fatty acid, stable isotope, and organochlorine analyses of blubber biopsies. *Mar Ecol Progr Ser* 302:275–291
- Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* 120:314–326
- Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes: I. Turnover of carbon-13. *Condor* 94:181–188
- Hobson KA, Sease JL (1998) Stable isotope analyses of tooth annuli reveal temporal dietary records: an example using stellar sea lions. *Mar Mam Sci* 14:116–129
- Hobson KA, Sease JL, Merrick RL, Piatt JF (1997) Investigating trophic relationships of pinnipeds in Alaska and Washington using stable isotope ratios of nitrogen and carbon. *Mar Mam Sci* 13:114–132
- Hohn AA, Lockyer C (1995) Protocol for obtaining age estimates from harbour porpoise teeth. Appendix 3, Report of the harbour porpoise age determination workshop. In: Bjørge A, Donovan GP (eds) *Biology of phocoenids*. RIWC Cambridge
- Honda K, Tatsukawa R, Itano K (1983) Heavy metal concentrations in muscle, liver and kidney tissue of striped dolphin, *Stenella coeruleoalba*, and their variations with body length, weight, age and sex. *Agric Biol Chem* 47:1219–1228
- Huisman J, Weissing FJ (2001) Biological conditions for oscillations generated by multispecies oscillations. *Ecology* 82:2682–2695
- Hutchinson GE (1957) *A treatise on limnology*. Wiley, New York
- Jardine TD, Kidd KA, Fisk AT (2006) Applications, considerations, and sources of uncertainty when using stable isotope analysis in ecotoxicology. *Env Sci Technol* 40:7501–7511
- Kiszka J, Simon-Bouhet B, Martínez L, Pusineri C, Richard P, Ridoux V (2011) Ecological niche segregation within a community of sympatric dolphins around a tropical island. *Mar Ecol Progr Ser* 433:273–288
- Knoff A, Hohn A, Macko S (2008) Ontogenetic diet changes in bottlenose dolphins (*Tursiops truncatus*) reflected through stable isotopes. *Mar Mam Sci* 24:128–137
- Krahn MM, Hanson MB, Baird RW, Boyer RH, Burrows DG, Emmons CK, Ford JKB, Jones LL, Noren DP, Ross PS, Schorr GS, Collier TK (2007) Persistent organic pollutants and stable isotopes in biopsy samples (2004/2006) from Southern Resident killer whales. *Mar Pollut Bull* 54:1903–1911
- Kuiken T, Garcia Hartmann M (1991) Proceedings of the first European Cetacean Society workshop on 'Cetacean pathology: dissection techniques and tissue sampling'. *ECS Newsletter* 17:1–39
- Lahaye V, Bustamante P, Spitz J, Dabin W, Das K, Pierce GJ, Caurant F (2005) Long-term dietary segregation of common dolphins *Delphinus delphis* in the Bay of Biscay, determined using cadmium as an ecological tracer. *Mar Ecol Progr Ser* 305:275–285
- Lahaye V, Bustamante P, Dabin W, Van Canneyt O, Dhermain F, Cesarini C, Pierce GJ, Caurant F (2006) New insights from age determination on toxic element accumulation in striped and bottlenose dolphins from Atlantic and Mediterranean waters. *Mar Pollut Bull* 52:1219–1230
- Leibold MA (1995) The niche concept revisited: mechanistic models and community context. *Ecology* 76:1371–1382
- Lockyer C (1993) A report on patterns of deposition of dentine and cement in teeth of pilot whales, genus *Globicephala*. *RIWC Special Issue* 14:138–161
- López A (2003) *Estatus dos Pequenos cetaceos da Plataforma de Galicia*. Dissertation, University of Santiago de compostela, Santiago de compostela
- López A, Santos MB, Pierce GJ, González AF, Valeiras X, Guerra A (2002) Trends in strandings of cetaceans on the Galician coast, NW Spain, during the 1990s. *J Mar Biol Assoc UK* 82:513–521
- López A, Pierce GJ, Valeiras X, Santos MB, Guerra A (2004) Distribution patterns of small cetaceans in Galician waters. *J Mar Biol Assoc UK* 84:283–294
- Mahe K, Amara R, Bryckaert T, Kacher M, Brylinski JM (2007) Ontogenetic and spatial variation in the diet of hake (*Merluccius merluccius*) in the Bay of Biscay and the Celtic Sea. *ICES J Mar Sci* 64:1210–1219
- McCullagh P, Nelder JA (1989) *Generalized linear models*. Chapman & Hall, London
- Meissner AM, MacLeod CD, Richard P, Ridoux V, Pierce G (2011) Feeding ecology of striped dolphins, *Stenella coeruleoalba*, in the north-western Mediterranean Sea based on stable isotope analyses. *J Mar Biol Assoc UK* 92(8):1677–1687
- Ménard F, Lorrain A, Potier M, Marsac F (2007) Isotopic evidence of distinct feeding ecologies and movement patterns in two migratory predators (yellowfin tuna and swordfish) of the western Indian Ocean. *Mar Biol* 153:141–152
- Méndez-Fernandez P, Bustamante P, Bode A, Chouvelon T, Ferreira M, López A, Pierce GJ, Santos MB, Spitz J, Vingada JV,

- Caurant F (2012) Foraging ecology of five toothed whale species in the Northwest Iberian Peninsula, inferred using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures. *J Exp Mar Biol Ecol* 413:150–158
- Miramand P, Bentley D (1992) Concentration and distribution of heavy metals in tissues of two cephalopods, *Eledone cirrhosa* and *Sepia officinalis*, from the French coast of the English Channel. *Mar Biol* 114:407–414
- Moura AE, Sillero N, Rodrigues A (2012) Common dolphin (*Delphinus delphis*) habitat preferences using data from two platforms of opportunity. *Acta Oecol* 38:24–32
- Newsome SD, Martínez del Rio C, Bearhop S, Phillips DL (2007) A niche for isotopic ecology. *Front Ecol Environ* 5:429–436
- Nordstrom CA, Wilson LJ, Sara IJ, Tollit DJ (2008) Evaluating quantitative fatty acid signature analysis (QFASA) using harbour seals *Phoca vitulina richardsi* in captive feeding studies. *Mar Ecol Prog Ser* 360:245–263
- Pianka ER (1974) Niche overlap and diffuse competition. *Proc Nat Acad Sci USA* 71:2141–2145
- Pierce GJ, Santos MB, Ross HM, Reid RJ, Patterson AIP, López A, Cedeira J (2007) Diets of teuthophagous small cetaceans from the Scottish and Galician coasts. Poster, European Cetacean Society, San Sebastian
- Pierce GJ, Caldas M, Cedeira J, Santos MB, Llavona Á, Covelo P, Martínez G, Torres J, Sacau M, López A (2010) Trends in cetacean sightings along the Galician coast, north-west Spain, 2003–2007, and inferences about cetacean habitat preferences. *J Mar Biol Assoc UK* 90:1547–1560
- R Development Core Team (2010) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <http://www.R-project.org>
- Rogan E, Murphy S, Learmonth JA, González A, Dabin W (2004) Age determination in small cetaceans from the NE Atlantic. Appendix 4. In: Pierce GJ, Santos MB, Learmonth JA, Smeenk, C and others (eds) Bioaccumulation of persistent organic pollutants in small cetaceans in European waters: transport pathways and impact on reproduction. Final Report to the European Commission's Directorate General for Research on Project EVK3-2000-00027, Aberdeen
- Ruiz-Cooley RI, Engelhaupt DT, Ortega-Ortiz JG (2012) Contrasting C and N isotope ratios from sperm whale skin and squid between the Gulf of Mexico and Gulf of California: effect of habitat. *Mar Biol* 159:151–164
- Santos MB, Clarke MR, Pierce DGJ (2001) Assessing the importance of cephalopods in the diets of marine mammals and other top predators: problems and solutions. *Fish Res* 52:121–139
- Santos MB, Pierce GJ, Learmonth JA, Reid RJ, Ross HM, Patterson IAP, Reid DG, Beare D (2004) Variability in the Diet of Harbor porpoises (*Phocoena phocoena*) in scottish waters 1992–2003. *Mar Mam Sci* 20:1–27
- Santos MB, Fernández R, López A, Martínez JA, Pierce GJ (2007a) Variability in the diet of bottlenose dolphin, *Tursiops truncatus*, in Galician waters, north-western Spain, 1990–2005. *J Mar Biol Assoc UK* 87:231–241
- Santos MB, Pierce GJ, Ross HM, Reid RJ, Patterson AIP, López A, Cedeira J (2007b) Diets of piscivorous small cetaceans from the Scottish and Galician coasts. Poster, European Cetacean Society, San Sebastian
- Santos MB, Monteiro SS, Vingada, JV, Ferreira M, López A, Cedeira JAM, Reid RJ, Brownlow A, Pierce GJ (in press) Patterns and trends in diet of long-finned pilot whales (*Globicephala melas*) based on the analysis of stomachs contents of animals stranded on Northeast Atlantic coasts. *Mar Mam Sci*
- Santos MB, Pierce GJ, López A, Martínez JA, Fernández MT, Ieno E, Mente E, Porteiro C, Carrera P, Meixide M (in press) Variability in the diet of common dolphins (*Delphinus delphis*) in Galician waters 1991–2003 and relationship with prey abundance. *Mar Ecol Progr Ser*
- Sherwood GD, Rose GA (2005) Stable isotope analysis of some representative fish and invertebrates of the Newfoundland and Labrador continental shelf food web. *Estuar Coast Shelf Sci* 63:537–549
- Spitz J, Rousseau Y, Ridoux V (2006) Diet overlap between harbour porpoise and bottlenose dolphin: an argument in favour of interference competition for food? *Estuar Coast Shelf Sci* 70:259–270
- Spitz J, Chérel Y, Bertin S, Kiszka J, Dewez A, Ridoux V (2011) Prey preferences among the community of deep-diving odontocetes from the Bay of Biscay, Northeast Atlantic. *Deep-Sea Res Pt I* 58:273–282
- Spyrakos E, Santos-Diniz TC, Martínez-Iglesias G, Torres-Palenzuela JM, Pierce GJ (2011) Spatiotemporal patterns of marine mammal distribution in coastal waters of Galicia, NW Spain. *Hydrobiologia* 670:87–109
- Tenore KR, Alonso-Noval M, Álvarez-Ossorio M, Atkinson LP, Cabanas JM, Cal RM, Campos HJ, Castillejo F, Chesney EJ, González N, Hanson RB, McClain CR, Miranda A, Roman MR, Sanchez J, Santiago G, Valdes L, Varela M, Yoder J (1995) Fisheries and oceanography off Galicia, NW Spain: mesoscale spatial and temporal changes in physical processes and resultant patterns of biological productivity. *J Geophys Res* 100:10943–10966
- Whittingham MJ, Stephens PA, Bradburry RB, Freckleton RP (2006) Why do we still use stepwise modelling in ecology and behavior? *J Anim Ecol* 75:1182–1189
- Wood S, Augustin N (2002) GAMs with integrated model selection using penalized regression splines and applications to environmental modelling. *Ecol Model* 157:157–177
- Zhao L, Castellini MA, Mau TL, Trumble SJ (2004) Trophic interactions of Antarctic seals as determined by stable isotope signatures. *Polar Biol* 27:368–373
- Zuur AF, Ieno EN, Smith GM (2007) Analysing ecological data. Springer, New York