Journal of Experimental Marine Biology and Ecology 465 (2015) 56–63

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Application of stable isotopes to assess the feeding ecology of long-finned pilot whale (*Globicephala melas*) in the Northeast Atlantic Ocean

Sílvia Monteiro [a,b,*], Marisa Ferreira [a], José V. Vingada [a,c], Alfredo López [d], Andrew Brownlow [e], Paula Méndez-Fernandez [f,1]

[a] CBMA & Departamento de Biologia, Sociedade Portuguesa de Vida Selvagem, Universidade de Minho, Campus de Gualtar, Braga 4710-047, Portugal
[b] School of Biological Sciences (Zoology), University of Aberdeen, Tillydrone Avenue, Aberdeen AB242TZ, UK
[c] CESAM & Departamento de Biologia, Universidade de Aveiro, Campus Universitário de Santiago, Aveiro 3810-193, Portugal
[d] Coordinadora para o Estudio dos Mamíferos Mariños, PO Box 15, Gondomar, Pontevedra 36380, Spain
[e] Wildlife Unit, SAC Veterinary Science Division, Drummond Hill, Stratherrick Road, Inverness IV2 4JZ, UK
[f] Littoral Environnement et Sociétés (LIENSs), UMR 7266 CNRS-ULR, 2 Rue Olympe de Gouges, 17042 La Rochelle Cedex 01, France

ARTICLE INFO

Article history:
Received 28 August 2014
Received in revised form 12 January 2015
Accepted 13 January 2015
Available online xxxx

Keywords:
Isotopic mixing models
Trophic relationships
Feeding habitat
Marine mammals
Northeast Atlantic

ABSTRACT

In order to improve our knowledge on the feeding ecology of long-finned pilot whales (*Globicephala melas*) in Northeast Atlantic waters, skin samples of 68 long-finned pilot whales stranded in Northwest Iberia ($n = 22$) and Scotland ($n = 46$) were analysed using stable isotopes of $\delta^{13}C$ and $\delta^{15}N$. Isotopic mixing models were applied to obtain a quantitative estimate of the proportion of the main prey species in the diet of pilot whales. Stable isotope analysis revealed that 57.8–73.8% of the diet in Northwest Iberia consisted in curled octopus (*Eledone cirrhosa*), followed by European flying squid (*Todarodes sagittatus*), while in Scotland the predominant prey species was either *Histioteuthis* sp. or *T. sagittatus*, depending of the trophic enrichment factor applied. These results are generally in accordance with previous stomach content studies; however, the isotopic analysis may provide new information regarding key prey species and habitat use that could be missed or underestimated if only stomach contents analysis were used. Additionally, considering that the Atlantic Coast of Iberia was responsible for 95% of the landings of the main prey consumed by pilot whales in this area, between 2000 and 2010, these data provide trophic baseline information to be taken into account in fishery impact assessment studies and management decisions.

1. Introduction

As apex predators, it is crucial to improve the knowledge regarding the foraging ecology of cetaceans in order to understand their role in marine food webs. The difficulty associated with the direct observation of the feeding behaviour of cetaceans in their natural habitat has underpinned the development of numerous techniques to acquire information about the diet of these species. The analysis of prey hard parts from stomach contents and/or scats and, more recently, the analysis of prey remains DNA collected from the digestive track or faeces, along with stable isotopes and fatty acids analyses of predator tissues are some examples of diet inference methods (Bowen and Iverson, 2013; Tollit et al., 2010).

Traditional dietary methods, such as stomach and scat analyses, are often the only direct way to obtain baseline knowledge on marine mammals diet, which may further be necessary, in recent dietary methods, to construct prey "signature" libraries for diet quantification (Newsome et al., 2010). However, these traditional techniques provide only a snapshot of the diet of an individual, with reduced integration time (i.e., few days, Pierce and Boyle, 1991), which together with the fact that stomach contents are often collected from stranded animals, potentially biased due to health status of the animal, may not be representative of the population diet (Tollit et al., 2010). Additionally, biases may also occur towards different digestion, retention and recovery rates of hard-bodied prey items (Santos et al., 2001).

The analysis of biogeochemical markers, such as stable isotopes, as methods of dietary intake has shown to be a useful tool to complement and validate the traditional dietary techniques for investigating trophic ecology (e.g., Jansen et al., 2013; Meissner et al., 2012; Méndez-Fernandez et al., 2012). One of the advantages of stable isotope analysis relatively to traditional methods is the potential to provide a temporally integrated picture of assimilated diet in a time scale dependent of the half-life of stable isotopes and the turnover rate of the tissue, which can range from weeks to several years (Abend and Smith, 1995). As an example, stable isotopes analysed in the skin of cetaceans provide longer-term dietary information (1–3 months, Abend

* Corresponding author at: CESAM & Departamento de Biologia, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal.
E-mail address: silvia.sm.monteiro@gmail.com (S. Monteiro).
[1] Current address: Oceanographic Institute of the University of São Paulo, Praça do Oceanográfico, 191, Cidade Universitária, São Paulo 05508-120, SP, Brazil.

http://dx.doi.org/10.1016/j.jembe.2015.01.007
0022-0981/

and Smith, 1995; Browning et al., 2014; Hicks et al., 1985) comparatively to stomach content analysis (few days, Pierce and Boyle, 1991). Another advantage is that this longer-term dietary information provided by stable isotopes may prevent the influence of some of the bias present in traditional dietary methods. Particularly, it may increase the chance that diet estimations based on stable isotopes are representative of the feeding habits of healthy animals, even if analysed in stranded animals. Moreover, since stable isotopes reflect the assimilated diet, there is no influence of potentially different recovery, retention and digestion rates of prey hard-parts in these elements.

Stable isotope analysis has become a powerful tool in the study of trophic and habitat preferences of wild species, where the relative abundance of carbon isotope ($\delta^{13}C$) can be applied to discriminate between benthic/inshore relative to pelagic/offshore food webs (e.g., Fry, 2006; Hobson and Welch, 1992; Newsome et al., 2010), while the relative abundance of nitrogen isotope ($\delta^{15}N$) can be used to evaluate trophic relationships, by analysing the differences among top predators and primary producers (Borrel et al., 2012; Browning et al., 2014; Caut et al., 2011; Vanderklift and Ponsard, 2003). Recent studies suggest that $\delta^{15}N$ values can also be used to indicate feeding habitats since strong variation in this measure has been registered in primary producers and different marine predators, between inshore and offshore systems, due to biochemical properties of the habitat (Chouvelon et al., 2012; Ruiz-Cooley et al., 2012). However, stable isotopes are also able to provide quantitative information about the predator dietary intake. Consumers usually retain the heavier isotope (i.e., ^{13}C or ^{15}N) to synthesise tissues (Owens, 1987), resulting in higher predator isotopic values in comparison to the respective prey. Therefore, stable isotope analysis relies on the fact that stable isotope composition of a consumer is a weighted mixing of the stable isotope composition of its food sources, modified by isotopic fractionation (Newsome et al., 2010). Recently, several isotopic mixing models have been developed to estimate the proportional contribution of each source (prey species) to the isotopic signature (accumulated diet) of the predator, taking into account isotopic fractionation between them (Parnell et al., 2010; Phillips and Gregg, 2003). The difference between the isotopic signal of a consumer relatively to its prey is referred to as diet-tissue discrimination (or fractionation) or trophic enrichment factor (TEF) (Newsome et al., 2010) and reflects the different reactions of the elements to the biochemical processes in the predator, due to their atomic weights (Browning et al., 2014; DeNiro and Epstein, 1978; Hobson and Welch, 1992). The three main causes of TEFs variation are related with the following: (i) the species analysed (e.g., Hobson et al., 1996); (ii) the tissue sampled (e.g., Hobson et al., 1996) and (iii) the diet composition (e.g., Browning et al., 2014; McCutchan et al., 2003), although the nutritional status, the growth rate (especially for ^{15}N enrichment; Vanderklift and Ponsard, 2003) or maternal strategies (i.e., mother to offspring transfer of nutrients; Mendes et al., 2007; Newsome et al., 2009) have also been described. Therefore, in order to obtain accurate diet estimations with isotopic mixing models, the use of accurate, species- and tissue-specific TEFs is one of the most important basic requirements.

The long-finned pilot whale (*Globicephala melas*), hereafter referred to as pilot whale, is one of the largest odontocetes. It is commonly known as a deep-diving oceanic species (e.g., Bloch et al., 2003; Macleod et al., 2007), with maximum diving depths reaching approximately 700 m (Baird et al., 2002). However, pilot whales can also occur over the continental shelf since this species has occasionally been observed during land- and boat-based sightings surveys in the Northwest Spain (Galicia) (Fernández et al., 2013; Pierce et al., 2010). A recent study that analysed stomach contents of stranded pilot whales from Portugal, Northwest Spain and Scotland found evidence of geographical, seasonal and ontogenetic differences in the diet of this species (Santos et al., 2014). Specifically, this study revealed that oceanic cephalopods from the Ommastrephidae family represented the main prey species at higher latitudes (Scotland), contrasting with the predominance of coastal species, such as the curled octopus (*Eledone cirrhosa)* in lower latitudes (Portugal and Galicia). A stable isotope analysis on several cetacean species and respective prey from Northwest Iberia also suggests that, in this region, pilot whales may be feeding on coastal and/or benthic species or occupying coastal habitats (Méndez-Fernandez et al., 2012).

In this context, the present study aims to assess the feeding ecology of pilot whales stranded in the Northwest Iberia and Scotland, at a medium time scale (1–3 months, Abend and Smith, 1995; Browning et al., 2014; Hicks et al., 1985), through the analysis of skin $\delta^{13}C$ and $\delta^{15}N$ stable isotopes. Specifically, it is intended to obtain quantitative information about pilot whale foraging habits in both areas, by using isotopic mixing models to estimate the proportional contribution of each prey source on this predator species diet.

2. Materials and Methods

2.1. Sample collection

A total of 68 pilot whale skin samples were collected from animals stranded in Northwest Iberia ($n = 22$) and Scotland ($n = 46$) (Fig. 1), from 1992 to 2012. Strandings were attended in all cases by experienced personnel from three stranding networks operating in the study area: Sociedade Portuguesa de Vida Selvagem (SPVS) in Portugal,

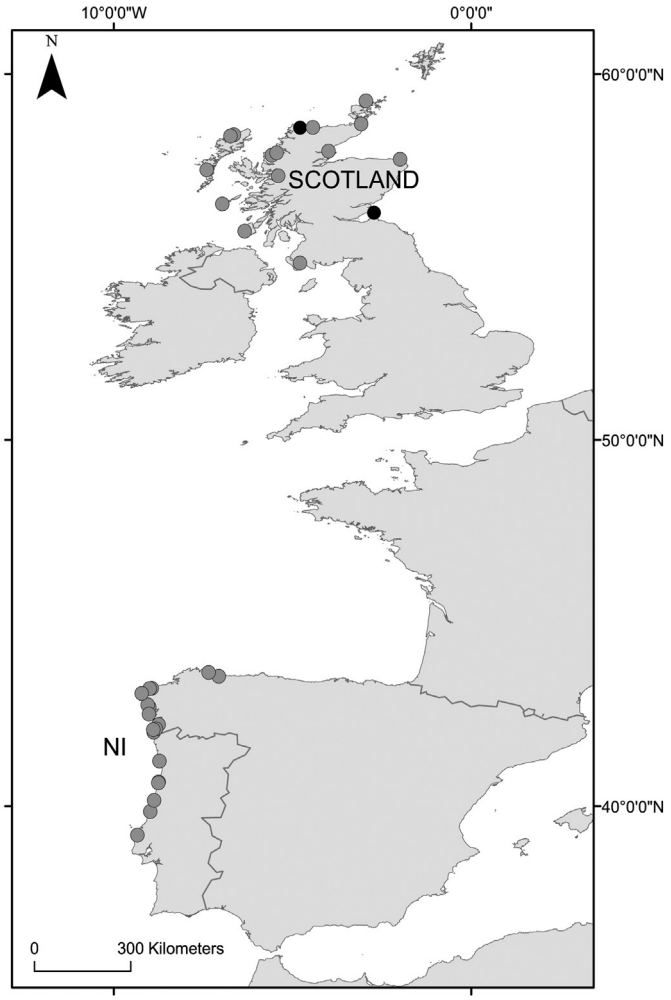


Fig. 1. Map of the study area showing the stranding locations of the pilot whales (*Globicephala melas*) in Northwest Iberia (NI) and Scotland, on which stable isotope analyses were performed. Grey circles represent single strandings, while black circles represent mass stranding events.

Coordinadora para o Estudio dos Mamíferos Mariños (CEMMA) in Galicia (northwest Spain) and Scottish Agriculture College Veterinary Science Division (SAC) in Scotland. Samples collected from two mass stranding events that occurred in Scotland were also included in the present study. In total, 70 animals were stranded in both events, of which 29 animals (out of the 37 animals necropsied) were analysed. When the condition of the animal permitted, detailed necropsies were performed. Otherwise, basic measurements/information (i.e., length, sex, decomposition state) and samples were collected. After collection, only skin samples preserved frozen (−20 °C) were analysed in the present study since recent studies suggest that other preservation methods (DMSO, ethanol) may influence stable isotope values (Kiszka et al., 2014a; Lesage et al., 2010). To prevent biases associated to the decomposition state of the animals, only animals recently dead (decomposition state ≤3, moderate decomposition; Kuiken and Hartmann, 1991) were used in this analysis.

Mantle tissue of three species of cephalopods previously identified in the literature as prey of pilot whales from Scotland (Santos et al., 2014) was also analysed, while the remaining data regarding prey isotopic signatures was obtained from previous studies in the areas (Table 1.; Chouvelon et al., 2012; Fernández et al., 2011; Mendes, 2008; Méndez-Fernández et al., 2012). Individuals of the three prey species analysed in the present study (Table 1) were measured and dissected to obtain portions of lateral mantle, which were also stored at −20 °C prior to analysis.

2.2. Stable isotope analysis

According to Méndez-Fernandez et al. (2012), skin samples of cetaceans were dried in an oven at 50 °C for 48 h, while mantle samples from cephalopods were freeze-dried. In both cases, samples were then ground into a fine powder. Afterward, a lipid extraction was performed on cetacean and prey samples, by agitating approximately 100 mg of powder with 4 ml of cyclohexane, for 1 h, followed by a centrifugation at 4000 g for 5 min and discard of the supernatant. Then samples were dried in an oven at 45 °C for 48 h, and subsamples of lipid-free powder were then weighed in tin cups for stable isotope analyses

The stable isotope analyses were performed on 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 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) indicated that measurement errors were ±0.15 and ±0.2‰ for δ^{13} C and δ^{15} N, respectively.

2.3. Statistical analysis

The mean isotopic composition and the respective standard deviation were calculated for pilot whales and their prey.

To estimate the proportional contribution of sources (prey species) within the isotopic mixture of pilot whales (consumer tissue), isotopic mixing models were applied, as implemented in the R package SIAR (Stable Isotope Analysis in R, Parnell et al., 2010). This model accounts for uncertainty in input parameters, such as isotopic variation of dietary sources and discrimination factors, and estimates probability distributions of source contributions (Parnell et al., 2010).

Separate analyses were run for each geographical location (Northwest Iberia and Scotland). The main prey species to be included in the models for the distinct locations were selected based on their contribution to the total diet by weight of pilot whales, determined from previous stomach contents analysis (Santos et al., 2014). Therefore, the prey species to be considered as sources were: European flying squid (*Todarodes sagitattus),* curled octopus (*E. cirrhosa*), common octopus (*Octopus vulgaris*), lesser flying squid (*Todaropsis eblanae*), for Iberian pilot whales and *T. sagitattus, T. eblanae*, as well as squids belonging to the genus *Gonatus* and *Histioteuthidae* for pilot whales from Scotland. Iberian cephalopod prey samples used in this study were collected from previous studies performed in this region (Table 1; Chouvelon et al., 2012; Fernández et al., 2011; Méndez-Fernandez et al., 2012). Additionally, stable isotopes results of *Gonatus* sp. from Scotland, analysed by Mendes (2008), were also included in the analysis. The only prey species analysed in the present study consisted of oceanic cephalopod species from Scottish waters, such as *T. sagitattus*, *T. eblanae* and *Histiotheuthis* sp., which were collected during the annual survey of monkfish (*Lophius* sp.) and megrim (*Lepidorhombus whiffiagonis*) stocks, on Scottish waters (Rockall bank and Hebridean-Malin slope down to 1000 m), conducted by the *Marine Scotland Science* (Aberdeen), in October of 2013 (Table 1). Although there was an effort to obtain prey samples with similar length ranges to the ones previously reported to be consumed by pilot whales (Santos et al., 2014; Table 1), the difficulty of capturing oceanic cephalopods prevented that achievement for some prey species (e.g., *Gonatus* sp. and *Histioteuthis* sp. in Scotland).

Isotopic mixing models also require that the isotopic values for food sources must be adjusted by appropriate enrichment factors between diet and consumer tissue (Phillips and Gregg, 2003). To date, there are few published estimates of enrichment factors of carbon and nitrogen for marine mammals and no published results for pilot whales. The use of proxy discrimination factors may not be appropriate for species or tissues for which the specific trophic enrichment factors (TEFs) are unknown (Bond and Diamond, 2011); however, obtaining this information is particularly challenging for marine mammals because individuals

Table 1
Number of samples, length (cm) and δ^{15}N and δ^{13}C stable isotopes (‰) of pilot whales (*Globicephala melas*) and prey species, from Northwest Iberia (NI) and Scotland. Sample length represents the total length for cetaceans and dorsal mantle length for cephalopods, included in this study. Reported length represents the prey lengths reported in previous pilot whale stomach content analysis (Santos et al., 2014). [1] Méndez-Fernandez et al., 2012; [2] Chouvelon et al., 2012; [3] Fernández et al., 2011; [4] Mendes, 2008.

	Code	Location	*n*	length	Reported length	δ^{15}N	δ^{13}C	Source
Predator								
Globicephala melas	Gm	NI	22	190–532		12.0 ± 0.7	−17.7 ± 0.7	This study
		Scotland	46	264–576		11.3 ± 0.6	−18.7 ± 0.7	This study
Prey								
Eledone chirrosa	Ec	NI	9	20.8–49.5	6.81–16.3	10.8 ± 0.2	−17.3 ± 0.2	[1]
Octopus vulgaris	Ov	NI	5	67.0–81.0	–	11.2 ± 0.2	−15.9 ± 0.3	[1]
Todarodes sagittattus	Ts	NI	36	19.1–40.5	10.1–55.4	11.9 ± 0.7	−17.9 ± 0.4	[2]
Todaropsis eblanae	Te	NI	7	10–16.6	15.4–19.9	13.9 ± 0.3	−17.1 ± 0.4	[3]
Todarodes sagittattus	Ts	Scotland	11	26–45.5	21.3–54.4	9. 7 ± 1.2	−19.2 ± 0.4	This study
Todaropsis eblanae	Te	Scotland	4	6.5–10.2	–	12.9 ± 0.5	−18.7 ± 0.6	This study
Histioteuthis sp.	Hs	Scotland	2	8–17.5	4.2–12.2	9. 9 ± 1.6	−19.9 ± 0.2	This study
Gonatus sp.	Gs	Scotland	2	12–14.5	11–21.4	13.3 ± 0.4	−17.7 ± 2.0	[4]

must be held on an isotopically fixed diet in controlled conditions that allow for regular sampling, over prolonged periods of time. For this reason, in an attempt to cover a high diversity of enrichment factors analysed in marine mammals, four mixing models for skin tissue using different and specific TEFs from the literature were performed (Table 2). The different species used to estimate the TEFs applied in the mixing models were harp seal (*Pagophilus groenlandicus*; TEF 1, Hobson et al., 1996), killer whale (*Orcinus orca;* TEF 2, Caut et al., 2011), fin whale (*Balaenoptera physalus*, TEF 3, Borrell et al., 2012) and bottlenose dolphin (*Tursiops truncatus*, TEF 4, Browning et al., 2014) (Table 2). Afterward, the mixing models were run using default parameter (iterations = 500,000, burnin = 50,000, thinby = 15; Parnell et al., 2010). All models and statistical tests mentioned above were performed using the free software R v.3.1.1 (R Development Core Team, 2014).

3. Results

Isotopic compositions of pilot whales and cephalopods used in the present study are summarised in Table 1 and Fig. 2. Regarding pilot whales, higher values of $\delta^{13}C$ and $\delta^{15}N$ were observed in Northwest Iberia. Considering the prey, in this region, $\delta^{15}N$ values ranged between 10.8 ± 0.2 for *E. cirrhosa* and 13.9 ± 0.3 for *T. eblanae*, while $\delta^{13}C$ varied between −15.9 ± 0.3 for *O. vulgaris* and −17.9 ± 0.4 for *T. sagitattus* (Table 1; Fig. 2). In Scotland, *T. sagitattus* exhibited the lowest levels of $\delta^{15}N$ (9.7 ± 1.2), contrasting with *Gonatus sp.* (13.3 ± 0.4). In this location, *T. eblanae* showed also the highest levels of $\delta^{13}C$ (−18.7 ± 0.6), contrarily to *Histioteuthis sp.* (−19.9 ± 0.2) (Table 1; Fig. 2).

Comparing isotopic values of pilot whales from distinct regions and the respective prey reveal that both in Northwest Iberia and Scotland, pilot whales showed the highest values, being only overcome by *T. eblanae* (in Northwest Iberia and Scotland) and *Gonatus sp.* (in Scotland), for $\delta^{15}N$ values (Fig. 2).

In both locations, pairs of different TEFs showed similar relative contributions of prey species (TEF2 / TEF3 vs. TEF1 / TEF4) (Table 2). Although slight variations occurred when using the different TEFs, all the estimates of dietary contributions of isotopic mixing models (SIAR) identified both *E. cirrhosa* and *T. sagitattus* as the major dietary contributors to the diet of pilot whales in Northwest Iberia. In this region, there is a prevalence of octopods in the diet of pilot whales, with *E. cirrhosa* being the most common prey with a mean estimated contribution of 57.8 ± 20.0 to 73.8 ± 18.3%, followed by the *T. sagitattus* (14.5 ± 13.6 to 31.7 ± 20.3%) (Fig. 3 a, c, e, g and Table 2). The contrast between the pairs of TEFs used in the models was more evident in Scotland. In this region, most models (b, d, f) showed a higher contribution of *Histioteuthis sp*. in pilot whales diet (ranging from 56.6 ± 15.0 to 86.9 ± 11.7%), followed by the *T. sagitattus* (11.1 ± 11.6 to 41.4 ± 15.2%). However, when using the TEF of Browning et al. (2014) (model h), the *T. sagitattus* showed a higher dietary contribution (61.7 ± 8.5%) than *Histioteuthis sp.* (34.8 ± 8.4%) (Fig. 3h and Table 2).

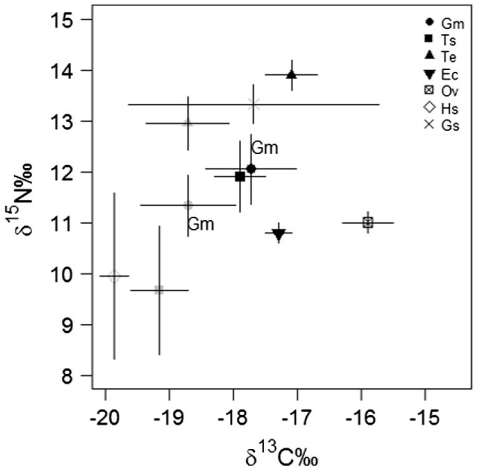


Fig. 2. Mean ± SD of $\delta^{15}N$ and $\delta^{13}C$ values of prey species and pilot whales (*Globicephala melas*) from Northwest Iberia (NI) and Scotland. Black symbols: NI; grey symbols: Scotland. Species codes correspond to those in Table 1.

All mixing models suggested that other prey species were less important in the diet of pilot whales, since the remaining prey species all accounted for less than 10% of the estimated diet, except for *O. vulgaris*, which showed contributions of 10.1 ± 9.7% and 10.3 ± 9.8% in the diet of Iberian pilot whales, in models a and b, respectively (Fig. 3 and Table 2).

4. Discussion

The combination of diet inference approaches is important to comprehend the feeding ecology of marine mammals in order to recognise their role in the ecosystem and support conservation or management strategies, especially in geographical locations where the impact of anthropogenic activities, such as fishery bycatch mortality (Leeney et al., 2008; López et al., 2002, 2003) or prey depletion due to overfishing, may represent the main threats to cetacean populations. Recently, in order to obtain a more robust knowledge about the feeding ecology of marine mammals, at different timescales, some studies combined the information collected from stomach contents and stable isotopes analyses (Jansen et al., 2013; Meissner et al., 2012; Méndez-Fernandez et al., 2012). As examples, Jansen et al. (2013) used this combination of

Table 2
Summary of estimated contributions (mean values) of potential prey species in the diet of pilot whales (*Globicephala melas*) from Northwest Iberia (NI) and Scotland, from stable isotope mixing models using different trophic enrichment factors (TEFs: $\Delta^{13}C$ and $\Delta^{15}N$) taken from the literature.

	Stable isotopes							
TEFs	1.Hobson et al. (1996)		2.Caut et al. (2011)		3.Borrel et al. (2012)		4.Browning et al. (2014)	
$\Delta^{13}C$ (‰)	2.8 ± 0.1		2.4 ± 0.2		1.3 ± 0.4		0.7 ± 0.1	
$\Delta^{15}N$ (‰)	2.3 ± 0.3		3.2 ± 0.1		2.8 ± 0.3		1.9 ± 0.1	
	NI	**Scotland**	**NI**	**Scotland**	**NI**	**Scotland**	**NI**	**Scotland**
Models	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**
Proportional contribution (%)								
Eledone chirrosa	72.5 ± 17.4		57.8 ± 20.0		58.2 ± 21.1		73.8 ± 18.3	
Octopus vulgaris	10.1 ± 9.7		10.3 ± 9.8		6.4 ± 6.4		4.5 ± 4.5	
Todarodes sagittattus	14.5 ± 13.6	41.4 ± 15.2	27.4 ± 18.0	11.1 ± 11.6	31.7 ± 20.3	14.3 ± 10.9	19.3 ± 17.6	61.7 ± 8.5
Todaropsis eblanae	2.8 ± 2.8	1.1 ± 1.0	4.4 ± 4.5	1.1 ± 1.1	3.6 ± 3.6	1.0 ± 0.97	2.3 ± 2.3	1.9 ± 1.6
Histioteuthis sp.		56.6 ± 15.0		86.9 ± 11.7		83.8 ± 10.8		34.8 ± 8.4
Gonatus sp.		0.08 ± 0.07		0.08 ± 0.08		0.08 ± 0.08		1.6 ± 1.3

approaches to analyse the diet of harbour porpoise (*Phocoena phocoena*) off the Netherlands, revealing a higher importance of pelagic, schooling prey species in porpoises' diet based on stable isotopes relatively to stomach contents analysis. Likewise, Méndez-Fernandez et al. (2012) applied it to assess the feeding ecology of common dolphins (*Delphinus delphis*) in the Northwest of the Iberian Peninsula, where the results of isotopic mixing models or signatures supported previous results of stomach contents analysis.

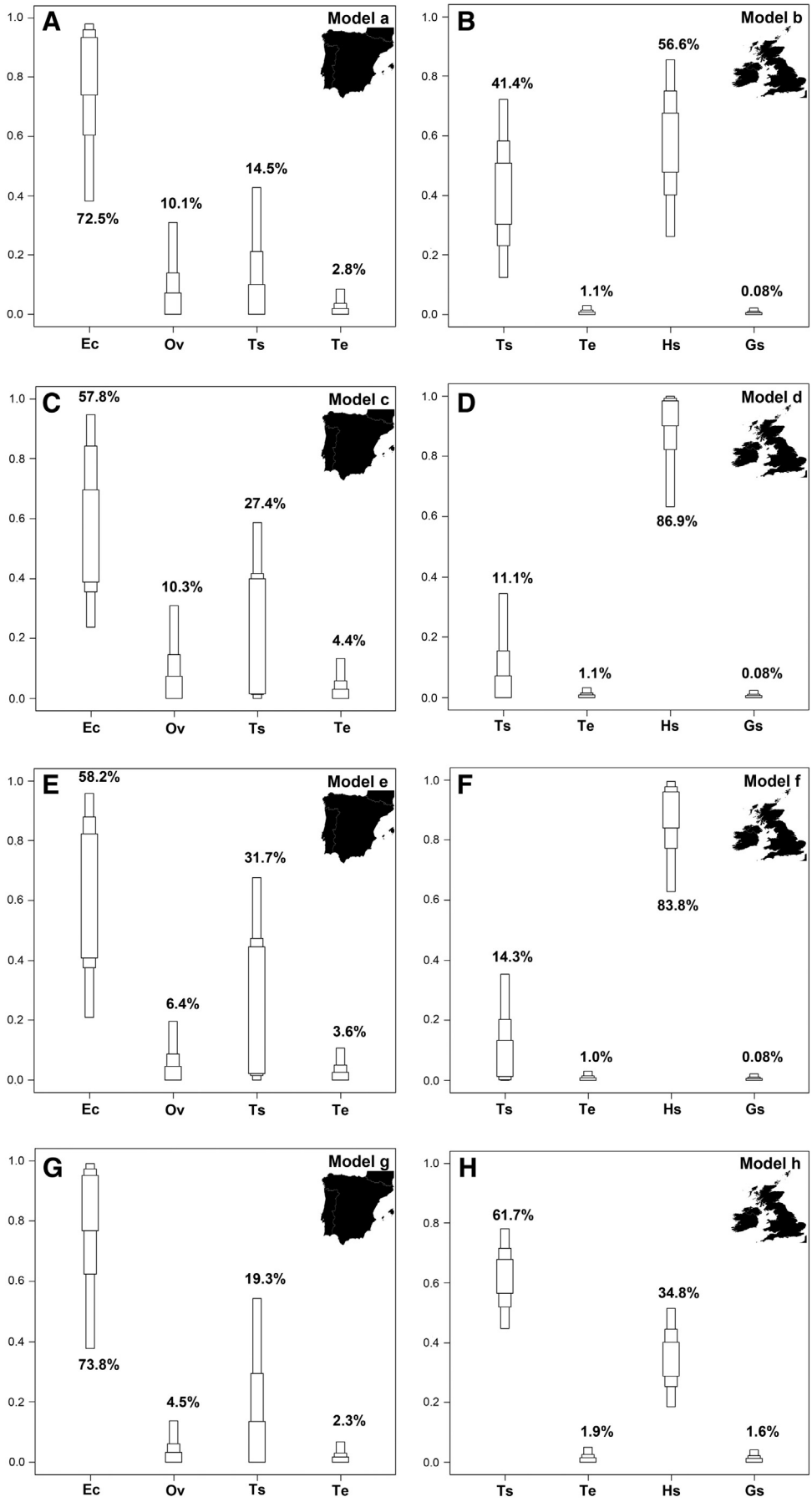
The diet estimation based on stable isotopes obtained in the present study, which is generally in agreement with previous stomach contents analysis (Santos et al., 2014), suggested that a higher proportion of benthic and neritic octopus species (*E. cirrhosa*) were ingested by pilot whales in Northwest Iberia, along with some *T. sagitattus*. In contrast, in Scotland, the predominant prey species varied between the oceanic pelagic squids *Histioteuthis* sp. and *T. sagitattus*, depending of the model used. The high levels of consistency in the results of both methodologies suggest that pilot whales exhibit a preference for particular prey species in a longer-term scale than the one described in stomach contents analysis. The ingestion of oceanic species seem to be in accordance with the commonly known oceanic distribution of pilot whales (e.g., Macleod et al., 2007), while the consumption of neritic species in Northwest Iberia may be associated with the occurrence of pilot whales close to the coastline, as already reported in land and boat surveys in this region (Fernández et al., 2013; Pierce et al., 2010). The different feeding habits and habitats of pilot whales in Northwest Iberia and Scotland may influence the behaviour of this species. Deeper dives performed by pilot whales seem to function mainly for foraging, which reflects an association between the diving behaviour of pilot whales and the prey ingested (Baird et al., 2002; Desportes and Mouritsen, 1993; Gannon et al., 1997). The preference for a more coastal habitat and prey in Northwest Iberia results in a need to perform shallower dives to capture prey, comparatively to the animals stranded in Scotland, which may be reflected in the energy expenditure for foraging.

The results related with the feeding habits of pilot whales obtained in the present study highlight the potential conservation issue to be considered in future management decisions, concerning the overlap between fisheries and pilot whale resource exploitation. Both geographical areas analysed (Northwest Iberia and Scotland) represent the major fishing forces of both the Atlantic coast of Iberia (e.g., López et al., 2002) and the United Kingdom (Elliot et al., 2012). This may increase the potential interaction between fisheries and pilot whales in these areas, but particularly in Northwest Iberia, since between 2000 and 2010, Iberia was responsible for 95% of the octopus landings (*E. cirrhosa* and *Octopus vulgaris*) in ICES area (ICES, 2012), which represent the preferred prey of pilot whales in that region. Additionally, this study emphasise the ability of stable carbon and nitrogen isotope ratios, together with mixing models, to complement stomach content analysis results and obtain information regarding trophic ecology of wild species when this traditional method cannot be used due to its invasive approach or to inaccessibility to stranded animals (Kiszka et al., 2014b).

The slight variations between stable isotope diet estimations and previously published stomach contents analysis may be due to several methodological and ecological reasons. First, only 14 pilot whales ($n = 8$ and $n = 6$ from Northwest Iberia and Scotland, respectively) were common to stable isotope and stomach contents studies which could, in part, explain the slight differences between dietary methodologies in Scotland. Additionally, although it cannot be discarded that the similarity between both methodologies may reflect similar bias associated to the use of stranded animals, the longer integration of stable isotopes increase the chance of obtaining a diet representative of healthy animals, which may also be responsible for the differences between methodologies. Second, stable isotope mixing models can either be influenced by the number of prey sources included in the models, the isotopic distinctiveness of the sources (Parnell et al., 2013) and/or the TEFs used (Bond and Diamond, 2011; Gannes et al., 1997). To achieve some balance between the number of prey described in stomach content analysis and the ratio between sources and number of isotopes used in SIAR, only the major importance prey identified in stomachs were included in the models. Although this decision could influence the quantitative diet estimation, since one of the assumptions of mixing models is that all the food sources are included in the analysis (Parnell et al., 2013; Phillips and Gregg, 2003), having in mind the prey diversity found previously in pilot whale stomachs (Santos et al., 2014), it would not be statistically feasible to include them all in the models. Therefore, a reduced set of prey sources was used. Nevertheless, there was still some overlap in $\delta^{13}C$ and $\delta^{15}N$ values between prey species. A requisite for the reconstruction of diet, based on stable isotopes, is the use of isotopically distinct sources (Gannes et al., 1997; Parnell et al., 2013). In the present study, although some of the SIAR results may be confounded by relatively high levels of correlation between some prey species (*E. cirrhosa* vs. *Todarodes sagittatus* in Iberia and *T. sagittatus* vs. *Histioteuthis* sp. in Scotland), the ability of the SIAR models to incorporate standard deviations of source values reduces the impact of some of this overlap.

Finally, the isotopic values for food sources must be adjusted by appropriate enrichment factors between diet and consumer tissue (Phillips and Gregg, 2003). Stable isotope mixing models are sensitive to variation in trophic enrichment factors (TEFs), potentially leading to misinterpretation of the SIAR results when general TEFs are applied in the models due to unknown information regarding species- and tissue-specific TEFs (Bond and Diamond, 2011). Given that specific TEFs are not available for pilot whales, several discrimination factors previously described for marine mammals were included in this study in order to cover a wide range of possible TEFs (Borrell et al., 2012; Browning et al., 2014; Caut et al., 2011; Hobson et al., 1996). The discrimination factors estimated from odontocetes (bottlenose dolphin and killer whale) were expected to perform better in the isotopic mixing models of pilot whales, due to the closest phylogenetic relationship between the species that could possibly result in a stronger similarity in the physiological integration of stable isotopes. Therefore, it was not surprising that the models using the TEF of adult bottlenose dolphins with a mixed diet (Browning et al., 2014) showed the highest similarity with the results from stomach contents, both in Northwest Iberia and Scotland. Although the dissimilarity of results between stomach contents and stable isotope analyses, when the TEF of another odontocete was used (killer whale; Caut et al., 2011) would not be expected, it is noteworthy that this discrimination factor was based on a single killer whale that died during the diet experiment due to a bacterial infection, which could have physiological alterations in the integration of stable isotopes. A surprising result was that the model with the TEF estimated from a species belonging to a different phylogenetic order (harp seal; Hobson et al., 1996) provided a diet more similar with stomach content analysis, than the model that applied a TEF of a cetacean (fin whale; Borrel et al., 2012). This result may be due to similarity between the isotopic signatures of the diet of harp seals used in the experiments and the diet of pilot whales in this study, which surely contrasts with the isotopic signature of the monodiet based on krill, ingested by fin whale (Borrel et al., 2012). In recent years, a strong effort has been oriented towards the elucidation of the adequate application of TEFs in marine mammal studies, particularly for odontocetes. However, the variation in diet estimation models found in the present study, due to the enrichment factors used, highlights the need for more investigation in order to develop diet-, tissue- and species-specific isotope discrimination studies, which would likely improve the accuracy of SIAR outputs.

It was not possible to investigate the extent of the temporal differences in isotopic signatures of pilot whales, which could also be responsible for the slight differences between mixing models and stomach contents results. Considerable seasonal or annual variability seems to exist in the isotopic composition of primary producers and their


A
Model a
72.5%
10.1%
14.5%
2.8%
Ec
Ov
Ts
Te
B
Model b
41.4%
1.1%
56.6%
0.08%
Ts
Te
Hs
Gs
C
Model c
57.8%
10.3%
27.4%
4.4%
Ec
Ov
Ts
Te
D
Model d
11.1%
1.1%
86.9%
0.08%
Ts
Te
Hs
Gs
E
Model e
58.2%
6.4%
31.7%
3.6%
Ec
Ov
Ts
Te
F
Model f
14.3%
1.0%
83.8%
0.08%
Ts
Te
Hs
Gs
G
Model g
73.8%
4.5%
19.3%
2.3%
Ec
Ov
Ts
Te
H
Model h
61.7%
1.9%
34.8%
1.6%
Ts
Te
Hs
Gs

consumers (Bode et al., 2007; Trueman et al., 2012), which may be particularly evident in areas with strong oceanographic phenomena, such as the upwelling in the Atlantic shelf of the Iberian Peninsula (Bode et al., 2007) or the Subpolar Gyre in the North Atlantic (Trueman et al., 2012), where the isotopic baseline seem to be strongly influenced by these seasonal events. Samples of stranded pilot whales were collected over a period of 21 years, in different seasons, but sample size prevented the investigation of potential seasonal differences. Additionally, some level of spatial variation may influence the SIAR results since the isotopic data of *T. sagittatus* is from animals of the Bay of Biscay. Currently, there is no information regarding the isotopic signature of this prey species in the Atlantic coast of Iberia, which in addition with the difficulty of collecting samples from this species prevented us from analysing local animals for the present study.

Despite the potential methodological and ecological sources of variation that may have influenced the results of SIAR, the present study provides useful information regarding the trophic ecology of pilot whales. Since stomach content analysis provides only a snapshot of the diet (i.e., few days, Pierce and Boyle, 1991), it could be argued that those dietary results were likely biased towards neritic prey species that were ingested shortly before the stranding, as could be the case for the predominance of *E. cirrhosa* in Iberian whales stomachs (Santos et al., 2014). However, skin stable isotopes supply information on longer-term diet comparatively to stomach contents (1–3 months, Abend and Smith, 1995; Browning et al., 2014; Hicks et al., 1985), which increases the chance to include prey ingested during offshore foraging events. This could help explain the higher proportion of oceanic species in Northwest Iberia (*T. sagittatus*) and Scotland (*Histioteuthis* sp.), when compared to stomach content analysis, highlighting the ability of isotopic analysis providing new information regarding key prey species and habitat use that could be missed or underestimated if only stomach contents analysis were used. Nevertheless, stable isotope analysis supports the possibility that pilot whales are feeding mainly in coastal waters in Northwest Iberia due to the levels of $\delta^{13}C$ found and the predominance of *E. cirrhosa* and, at some level, *T. sagittatus* (which are known to perform inshore-offshore migrations; Hastie et al., 2009). In contrast, the high proportion of *Histioteuthis* sp. in Scotland suggests that, in this region, pilot whales can be feeding mainly offshore, although some caution is needed due to the high correlation between *Histioteuthis* sp. and *T. sagittatus*.

5. Conclusions

In the present study, stable isotope analysis suggests that pilot whales seem to occur in coastal habitats and/or ingest coastal or benthic preys in Northwest Iberia, while animals frequenting Scottish waters reveal more oceanic preferences. These results were supported by the isotopic mixing models which revealed that the main prey of pilot whales in Northwest Iberia were *E. cirrhosa*, followed by *T. sagittatus*, while in Scotland the predominant prey species was either *Histioteuthis* sp. or *T. sagittatus*. The dietary information is generally in agreement with previous stomach contents analysis, although skin stable isotopes reflect a longer-term understanding about the assimilated diet. Therefore, the results of this study support that the best approach for the analysis of the feeding ecology of wild animals consists in the combination of the snapshots of detailed diet estimation obtained by stomach contents with the longer-term and relatively coarse trophic information obtained with stable isotopes or other biogeochemical markers, allowing for the acquirement of knowledge on dietary ecology over a range of time spans, depending of the type of tissue used.

Pilot whales seem to exploit the same resources as fisheries, especially in Iberia which was responsible for 95% of the octopus landings between 2000 and 2010 in ICES area (ICES, 2012), suggesting that this cetacean species may be at risk both from prey depletion and incidental capture in fishing gear (bycatch). Therefore, the data gathered in this study through the combination of traditional and recent dietary methods is particularly relevant for fishery and ecological impact assessment studies and can help sustain wildlife management decisions.

Acknowledgments

Cetacean samples were collected under the auspices of strandings monitoring programs run by Sociedade Portuguesa de Vida Selvagem (SPVS) (Portugal), Coordinadora para o Estudio dos Mamíferos Mariños (CEMMA) (Galicia) and the Scottish Agriculture College (SAC) Veterinary Science Division (Scotland). The authors thank all the members of SPVS, CEMMA and SAC for their assistance with data and sample collection. The authors would also like to thank Jim Drewery from *Marine Scotland Science* (Aberdeen) for the collection of cephalopod samples in Scotland and Sónia Mendes for kindly providing the *Gonatus sp*. data used in this study. SSM and MF were supported by PhD grants from Fundação para a Ciência e Tecnologia (POPH/FSE ref SFRH/BD/38735/2007 and SFRH/BD/30240/2006, respectively). AL was supported by a postdoctoral grant from Fundação para a Ciência e Tecnologia (ref. no. SFRH/BPD/82407/2011). The work related with strandings and tissue collection in Portugal was partially supported by the SafeSea Project EEAGrants PT 0039 (supported by Iceland, Liechtenstein and Norway through the EEA Financial Mechanism), by the Project MarPro-Life09 NAT/PT/000038 (funded by the European Union—Program Life +) and by the Project CetSenti FCT RECI/AAG-GLO/0470/2012; FCOMP-01-0124-FEDER-027472 (funded by the Program COMPETE and Fundação para a Ciência e Tecnologia). The Galician network is supported by the regional government Xunta de Galicia. **[SS]**

References

Abend, A.G., Smith, T.D., 1995. Differences in ratios of stable isotopes of nitrogen in long-finned pilot whales (*Globicephala melas*) in the western and eastern North Atlantic. ICES J. Mar. Sci. 52, 837–841.

Baird, R.W., Borsani, J.F., Hanson, M.B., Tyack, P.L., 2002. Diving and night-time behavior of long-finned pilot whales in the Ligurian Sea. Mar. Ecol. Prog. Ser. 237, 301–305.

Bloch, D., Heide-Jorgensen, M.P., Stefansson, E., Mikkelsen, B., Ofstad, L.H., Dietz, R., Andersen, L.W., 2003. Short-term movements of long-finned pilot whales Globicephala melas around the Faroe Islands. Wildl. Biol. 9 (1), 47–58.

Bode, A., Alvarez-Ossorio, M.T., Cunha, M.E., Garrido, S., Peleteiro, J.B., Porteiro, C., Valdés, L., Varela, M., 2007. Stable nitrogen isotope studies of the pelagic food web on the Atlantic shelf of the Iberian Peninsula. Prog. Oceanogr. 74, 115–131.

Bond, A.L., Diamond, A.W., 2011. Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. Ecol. Appl. 21 (4), 1017–1023.

Borrell, A., Abad-Oliva, N., Gómez-Campos, E., Giménez, J., Aguilar, A., 2012. Discrimination of stable isotopes in fin whale tissues and application to diet assessment in cetaceans. Rapid Commun. Mass Spectrom. 26, 1596–1602.

Bowen, W.D., Iverson, S.J., 2013. Methods of estimating marine mammal diets: a review of validation experiments and sources of bias and uncertainty. Mar. Mamm. Sci. 29 (4), 719–754.

Browning, N.E., Dold, C., I-Fan, J., Worthy, G.A.J., 2014. Isotope turnover rates and diet–tissue discrimination in skin of ex situ bottlenose dolphins (*Tursiops truncatus*). J. Exp. Biol. 217, 214–221.

Caut, S., Laran, S., Garcia-Hartmann, E., Das, K., 2011. Stable isotopes of captive cetaceans (killer whales and bottlenose dolphins). J. Exp. Biol. 214, 538–545.

Chouvelon, T., Spitz, J., Caurant, F., Mèndez-Fernandez, P., Chappuis, A., Laugier, F., Goff, E.L., Bustamante, P., 2012. Revisiting the use of $\delta^{15}N$ 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.

DeNiro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta 42, 495–506.

Fig. 3. Results of SIAR mixing model (50%, 75% and 95% confidence intervals) showing the probable prey species proportion (%) in the diet of pilot whales (*Globicephala melas*) from Northwest Iberia (NI) and Scotland, using skin stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$). The species codes and trophic enrichment factors (TEFs) used to run models are summarized in Tables 1 and 2.

Desportes, G., Mouritsen, R., 1993. Preliminary results on the diet of long-finned pilot whales off the Faroe Islands. Report of International Whaling Commission (special issue 14)pp. 305–324.
Elliott, M., Hargreaves, J., Pilgrim, S., 2012. UK Sea Fisheries Statistics 2011. Marine Management Organisation, Newport, p. 160.
Fernández, R., García-Tiscar, S., Santos, M.B., López, A., Martínez-Cedeira, J.A., Newton, J., Pierce, G.J., 2011. Stable isotope analysis in two sympatric populations of bottlenose dolphins Tursiops truncatus: evidence of resource partitioning? Mar. Biol. 158, 1043–1055.
Fernández, R., MacLeod, C.D., Pierce, G.J., Covelo, P., López, A., Torres-Palenzuela, J., Valavanis, V., Santos, M.B., 2013. Inter-specific and seasonal comparison of the niches occupied by small cetaceans off north-west Iberia. Cont. Shelf Res. 64, 88–98.
Fry, B., 2006. Stable Isotope Ecology. Springer, New York (316 pp.).
Gannes, L.Z., O'Brien, D.M., Rio, C.M.D., 1997. Stable isotopes in animal ecology: assumptions, caveats and a call for more laboratory experiments. Ecology 78 (4), 1271–1276.
Gannon, D.P., Read, A.J., Craddock, J.E., Fristrup, K.M., Nicolas, J.R., 1997. Feeding ecology of long-finned pilot whales Globicephala melas in the western north Atlantic. Mar. Ecol. Prog. Ser. 148 (1–3), 1–10.
Hastie, L.C., Pierce, G.J., Wang, J., Bruno, I., Moren, A., kowski, U.P., Robin, J.P., 2009. Cephalopods in the North-Eastern Atlantic: species, biogeography, ecology, exploitation and conservation. Oceanogr. Mar. Biol. Annu. Rev. 47, 111–190.
Hicks, B.D., Aubin, D.J.S., Geraci, J.R., Brown, W.R., 1985. Epidermal growth in the bottlenose dolphin, Tursiops truncatus. J. Invest. Dermatol. 85 (1), 60–63.
Hobson, K.A., Welch, H.E., 1992. Determination of trophic relationships within a high Arctic marine food web using C^{13} and N^{15} analysis. Mar. Ecol. Prog. Ser. 84, 9–18.
Hobson, K.A., Schell, D.M., Renouf, D., Noseworthy, E., 1996. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. Can. J. Fish. Aquat. Sci. 53, 528–533.
ICES, 2012. Report of the Working Group on Cephalopod Fisheries and Life History (WGCEPH), Cadiz, Spain. p. 241.
Jansen, O.E., Michel, L., Lepoint, G., Das, K., Couperus, A.S., Reijnders, P.J.H., 2013. Diet of harbor porpoises along the Dutch coast: a combined stable isotope and stomach contents approach. Mar. Mamm. Sci. 29 (3), E295–E311.
Kiszka, J., Lesage, V., Ridoux, V., 2014a. Effect of ethanol preservation on stable carbon and nitrogen isotope values in cetacean epidermis: Implication for using archived biopsy samples. Mar. Mamm. Sci. 30, 788–795.
Kiszka, J.J., Méndez-Fernandez, P., Heithaus, M.R., Ridoux, V., 2014b. The foraging ecology of coastal bottlenose dolphins based on stable isotope mixing models and behavioural sampling. Mar. Biol. 161, 953–961.
Kuiken, T., Hartmann, M.G., 1991. Cetacean pathology: dissection techniques and tissue sampling. European Cetacean Society Newsletter No. 17(Special Issue)p. 43.
Leeney, R.H., Amies, R., Broderick, A.C., Witt, M.J., Loveridge, J., Doyle, J., Godley, B.J., 2008. Spatio-temporal analysis of cetacean strandings and bycatch in a UK fisheries hotspot. Biodivers. Conserv. 17 (10), 2323–2338.
Lesage, V., Morin, Y., Rioux, È., Pomerleau, C., Ferguson, S.H., Pelletier, É., 2010. Stable isotopes and trace elements as indicators of diet and habitat use in cetaceans: predicting errors related to preservation, lipid extraction, and lipid normalization. Mar. Ecol. Prog. Ser. 419, 249–265.
López, A., Santos, M.B., Pierce, G.J., González, A.F., Valeiras, X., Guerra, A., 2002. Trends in strandings and by-catch of marine mammals in Northwest Spain during the 1990. J. Mar. Biol. Assoc. U. K. 82 (3916), 1–9.
López, A., Pierce, G.J., Santos, M.B., Gracia, J., Guerra, A., 2003. Fishery by-catches of marine mammals in Galician waters: results from on-board observations and an interview survey of fishermen. Biol. Conserv. 111 (1), 25–40.
MacLeod, C.D., Weir, C.R., Pierpoint, C., Harland, E.J., 2007. The habitat preferences of marine mammals west of Scotland (UK). J. Mar. Biol. Assoc. U. K. 87 (1), 157–164.
McCutchan Jr., J.H., W.M.L, Kendall, C., McGrath, C.C., 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102, 378–390.
Meissner, A.M., Macleod, C.D., Richard, P., Ridoux, V., Pierce, G., 2012. Feeding ecology of striped dolphins, *Stenella coeruleoalba*, in the north-western Mediterranean Sea based on stable isotope analyses. J. Mar. Biol. Assoc. U. K. 92 (Special issue 8), 1677–1687.
Mendes, S., 2008. Foraging ecology and distribution of sperm whales in the North Atlantic, Zoology. University of Aberdeen, Aberdeen.
Mendes, S., Newton, J., Reid, R.J., Frantzis, A., Pierce, G.J., 2007. Stable isotope profiles in sperm whale teeth: variations between areas and sexes. J. Mar. Biol. Assoc. U. K. 87, 621–627.
Méndez-Fernandez, P., Bustamante, P., Bode, A., Chouvelon, T., Ferreira, M., López, A., Pierce, G.J., Santos, M.B., Spitz, J., Vingada, J.V., Caurant, F., 2012. Foraging ecology of five toothed whale species in the Northwest Iberian Peninsula, inferred using carbon and nitrogen isotope ratios. J. Exp. Mar. Biol. Ecol. 413, 150–158.
Newsome, S.D., Etnier, M.A., Monson, D.H., Fogel, M.L., 2009. Retrospective characterization of ontogenetic shifts in killer whale diets via $\delta^{13}C$ and $\delta^{15}N$ analysis of teeth. Mar. Ecol. Prog. Ser. 374, 229–242.
Newsome, S.D., Clementz, M.T., Koch, P.L., 2010. Using stable isotope biogeochemistry to study marine mammal ecology. Mar. Mamm. Sci. 26 (3), 509–572.
Owens, N.J.P., 1987. Natural variations in ^{15}N in the marine environment. Adv. Mar. Biol. 24, 389–451.
Parnell, A.C., Inger, R., Bearhop, S., Jackson, A.L., 2010. Source partitioning using stable isotopes: coping with too much variation. PLoS ONE 5 (3), e9672.
Parnell, A.C., Phillips, D.L., Bearhop, S., Semmens, B.X., Ward, E.J., Moore, J.W., Jackson, A.L., Inger, R., 2013. Bayesian Stable Isotope Mixing Models. Environmetrics 24 (6), 387–399.
Phillips, D.L., Gregg, J.W., 2003. Source partitioning using stable isotopes: coping with too many sources. Oecologia 136, 261–269.
Pierce, G.J., Boyle, P.R., 1991. A review of methods for diet analysis in piscivorous marine mammals. Oceanogr. Mar. Biol. Annu. Rev. 29, 409–486.
Pierce, G.J., Caldas, M., Cedeira, J., Santos, M.B., Llavona, Á., Covelo, P., Martinez, 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. U. K. 90 (8), 1547–1560.
R Development Core Team, 2014. R: a language and environment for statistical computing, www.R-project.org. R Foundation for Statistical Computing, Vienna.
Ruiz-Cooley, R.I., Engelhaupt, D.T., Ortega-Ortiz, J.G., 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, M.B., Clarke, M.R., Pierce, G.J., 2001. Assessing the importance of cephalopods in the diets of marine mammals and other top predators: problems and solutions. Fish. Res. 52 (1–2), 121–139.
Santos, M.B., Monteiro, S.S., Vingada, J.V., Ferreira, M., López, A., martinez-Cedeira,, J., Reid, R.J., Brownlow, A., Pierce, G., 2014. Patterns and trends in the diet of long-finned pilot whales (*Globicephala melas*) in the northeast Atlantic. Mar. Mamm. Sci. 30 (1), 1–19.
Tollit, D.J., Pierce, G., Hobson, K., Bowen, W.D., Iverson, S.J., 2010. Diet. In: Boyd, I.L., Bowen, W.D., Iverson, S.J. (Eds.), Marine Mammal Ecology and Conservation: A Handbook of Techniques. Oxford University Press, Cambridge UK, pp. 191–221.
Trueman, C.N., MacKenzie, K.M., Palmer, M.R., 2012. Stable isotopes reveal linkages between ocean climate, plankton community dynamics, and survival of two populations of Atlantic salmon (*Salmo salar*). ICES J. Mar. Sci. 69 (5), 784–794.
Vanderklift, M.A., Ponsard, S., 2003. Sources of variation in consumer-diet $\delta^{15}N$ enrichment: a meta-analysis. Oecologia 136, 169–182.