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Multi-approach analysis to assess diet of harbour porpoises *Phocoena phocoena* in the southern North Sea

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ABSTRACT: Over the past decade, the distribution of harbour porpoises *Phocoena phocoena* has undergone a southward shift in the North Sea, which has led to an increase in the number of stranded porpoises in its southern part. Since the changes in distribution and relative abundance of porpoises may be linked to the changes in prey availability, the aim of the present work was to investigate whether any changes in the feeding habits of harbour porpoises along the North Sea occurred in the past decade. The diet of harbour porpoises stranded along the southern North Sea (northern France and Belgian coast) was assessed through 3 complementary methods: stomach content analysis, stable isotopes (carbon and nitrogen) analysis determined from muscle samples, and fatty acids analysis determined from blubber samples. Fatty acid patterns and stable isotope values from 52 porpoises were compared to 14 potential prey species collected from the southern North Sea. Our results showed that the diet of porpoises along the southern North Sea comprises fish species that are among the most abundant and widely distributed in the area, except for the sardine *Sardina pilchardus* that appeared to be a new potential prey. Moreover, our results suggested that the decline in sandeel (Ammodytidae) in the northern parts of the North Sea along with the re-invasion of the southern North Sea by sardine species might affect the distribution of harbour porpoises.

KEY WORDS: Harbour porpoises · North Sea · Distribution · Foraging ecology · Stomach contents · Stable isotopes · Fatty acids

INTRODUCTION

Harbour porpoises *Phocoena phocoena* are the most common cetacean species in the North Sea (Hammond et al. 2013). A comparison between the results of the 2 major abundance and distribution surveys in European waters revealed a major shift in the distribution of harbour porpoises from the northern

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parts of the North Sea to its southeastern parts rather than a population increase in the English Channel (Hammond et al. 2002, 2013). Also, a clear increase in the number of stranded animals has been observed along the southern North Sea, including the Netherlands, Belgium and northern France, since the beginning of the 21st century (Jauniaux et al. 2008, Haelters & Camphuysen 2009).

In order to meet specific energy requirements, harbour porpoise foraging strategies and diet are shaped by the quality of their prey (Spitz et al. 2012). The distribution of this predator is expected to follow the distribution of its main prey species (Santos et al. 2004). Therefore, changes in prey abundance due to temporal variation, fishing or other anthropogenic impacts may negatively affect porpoise survival by reducing the availability of prey or by inducing its dispersal (Lassalle et al. 2012). In fact, changes in the distribution and relative abundance of porpoises in the southern North Sea over the last few decades have been linked to changes in prey availability (Camphuysen 2004, MacLeod et al. 2007, Hammond et al. 2013). Accordingly, studying the feeding ecology of harbour porpoises serves to investigate their feeding strategy, predator-prey relationships, and responses to changes in food web dynamics, climate change or fishery interactions (Haelters & Camphuysen 2009, Herr et al. 2009). In turn, this information can be used to devise or improve appropriate conservation management for harbour porpoises.

Several techniques are widely used to study the feeding ecology of marine mammals, including stomach content analysis (e.g. Santos & Pierce 2003), bulk stable isotopes analysis (e.g. Hobson et al. 1994) and fatty acid (FA) analysis (e.g. Iverson et al. 2004, Budge et al. 2006). These diet estimation methods have their assumptions and limitations. They differ in the level of information they give on diet (i.e. quantitative versus qualitative and species versus general trophic level) and they also differ in time scale (i.e. more recent diet versus longer-term diet) (reviewed in Bowen & Iverson 2013). To our knowledge, only a few studies have combined the 3 methods to assess the foraging ecology of marine mammals (Hooker et al. 2001, Jansen 2013). Through using all of the above methods, we can gain a more comprehensive insight on predator diet and foraging strategies. The objectives of this paper are (1) to determine the feeding ecology of harbour porpoises along the southern North Sea by using stomach content, stable isotopes and FA analyses, and (2) to investigate changes in the feeding ecology of harbour porpoises by comparing the present results to previous diet studies.

MATERIALS AND METHODS

Sampling and data collection

Muscle and blubber samples were collected from 52 stranded harbour porpoises in the southern North Sea along French and Belgian coasts between 2010 and 2013, with 14, 9, 15 and 14 individuals collected in 2010, 2011, 2012 and 2013, respectively. There were 15 juvenile females, 27 juvenile males, 7 adult females and 3 adult males. Fourteen porpoises were sampled for stomach content analysis: 4 stomachs from 2012 and 10 from 2013. This sampling included 10 juvenile males, 2 adult females, 1 adult male and 1 undetermined porpoise. Muscles, blubbers and stomachs were stored at −20°C prior to analysis. All animals were freshly dead and sampled up to 48 h after death (Jauniaux et al. 2002). Post-mortem investigations were performed according to the protocol from Kuiken & Hartmann (1993) and Jauniaux et al. (2002). Morphometric data such as sex and length were collected, and, according to the length of the animal, age groups were determined (see Table 1). Porpoises with lengths 91–135 cm were considered juveniles and >135 cm were considered adults (Jauniaux et al. 2002).

Fourteen potential prey species were collected along the southern North Sea in June, October and November 2012. Prey species were previously identified as potential prey for harbour porpoises in the study area (Santos et al. 2004, Haelters et al. 2012). Prey samples were stored at −20°C prior to analysis and selected in order to cover the size-classes found in the stomach contents of the harbour porpoises.

Stomach content analyses

Porpoise stomachs were weighed then rinsed through running water and contents were emptied in a sieve with a mesh size of 0.2 mm. Otoliths and fish vertebrae were stored dry, whereas whole or partly digested prey items and cephalopod beaks were stored in 70% ethanol. All remains were counted, measured and identified to the lowest taxonomic level using our reference collection of specimens and published data from Leopold et al. (2001). Otoliths were identified, sorted into left and right, and measured (right otolith width and length) using a video system fitted to a compound microscope and the image analysis system TNPC 5.0. Fish weight and length estimates were calculated from the measured length and width of otoliths using regressions from

Leopold et al. (2001). No corrections were made for loss or reduction of size of prey remains due to digestion. Extremely eroded or broken otoliths were removed prior to counting and measuring.

Three indices were used to estimate the prey importance in the diet of harbour porpoises:

Percentage frequency of occurrence (%O):

$$\%O_i = \left(n_i / n\right) \times 100 \quad (1)$$

where n_i is the number of stomachs where the particular prey species was found and n is the total number of non-empty stomachs;

Percentage by number (%N):

$$\%N_i = \left(N_i / N\right) \times 100 \quad (2)$$

where N_i is the number of the particular prey species found and N is the total number of prey found;

Percentage by mass (%W):

$$\%W_i = \left(w_i / w\right) \times 100 \quad (3)$$

where w_i is the total weight of the particular prey species and w is the total weight of all prey species.

The %N and the %W were calculated for each prey type in each individual stomach and then averaged across all stomachs in order to avoid the influence of outliers.

Stable isotopes analysis

Stable isotope ratios ($^{15}N/^{14}N$ and $^{13}C/^{12}C$) were determined in the dorsal muscle of harbour porpoises and fish, except for gobies (Gobiidae) where the whole body was ground. Samples were freeze-dried for 48 h then ground into fine powder. An aliquot of approximately 100 mg from each sample was mixed with 4 ml of cyclohexane for lipid extraction, since lipids are highly depleted in ^{13}C compared to other tissue components (Tieszen et al. 1983). Samples were agitated for 1 h at 800 rpm then centrifuged for 5 min at 4000 × g. The upper solution containing the lipids was removed and the samples were dried in an oven at 50°C for 48 h. Subsamples of dried and lipid-free muscle powder (0.35 ± 0.05 mg) were weighed into tin cups. Stable isotope measurements were performed with an elemental analyser coupled to an isotope ratio mass spectrometer (DELTA V ADVANTAGE Isotope Ratio MS, Thermo Scientific). The stable carbon isotope composition of an aliquot of the freeze-dried and powdered sample is referred to as the 'bulk' stable carbon isotope composition in this article.

Stable isotope abundances are expressed in delta notation in parts per thousand (‰) following the equation:

$$\delta X = \left(\frac{R_{Sample}}{R_{Standard}} - 1\right) \times 1000 \quad (4)$$

where X represents ^{13}C or ^{15}N and R_{sample} is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ isotopic ratio of the sample. Ratios are expressed relative to the international standards ($R_{standard}$) VPDB and atmospheric N_2 for ^{13}C and ^{15}N measurements, respectively. Replicate measurements of internal laboratory standards (acetanilide) during each series of measurement indicated errors less than 0.15‰ and 0.20‰ for carbon and nitrogen, respectively.

FA composition

FA compositions were determined from the inner blubber layer of harbour porpoises and the muscle of prey species. Relatively high levels of dietary FA are usually found in the inner layer of harbour porpoise blubber, suggesting that it is more metabolically active than the outer layer (Koopman et al. 1996). Before FAs extraction, an internal standard (23:0) was added to approximately 50 mg of fresh blubber from harbour porpoise or freeze-dried muscle from prey (Bligh & Dyer 1959, modified as in Meziane et al. 2007). Samples were subject to ultrasonication for 20 min with distilled water:$CHCl_3$:MeOH (1:1:2, v:v:v). Afterwards, the addition of a distilled water:$CHCl_3$ mixture (1:1, v:v) and centrifugation (5 min, 1100 × g) of the mixture formed a 2-layer system. The lower $CHCl_3$ layer containing the lipids was retained. This step was repeated one more time with $CHCl_3$ (2 ml). The residue obtained from the consecutive extractions was concentrated under an N_2 flow, then saponified in a mixture of 2 mol NaOH:MeOH (1:2, v:v) and heated for 90 min at 100°C. In order to obtain the total lipids as methyl esters, saponification and methylation were conducted according to Meziane & Tsuchiya (2002). For identification, FA methyl esters (FAMEs) were separated and quantified by gas chromatography equipped with a flame ionization detector (GC; Varian CP-3800). The GC was fitted with a Supelco OMEGAWAX 320 column (30 m, 0.32 mm ID, 0.25 µm film thickness). Helium was the carrier gas. The sample (1 µl) was injected at 60°C and the temperature was raised to 150°C at 40°C min^{-1}, then ramped up to 240°C at 3°C min^{-1} and kept there for

14 min. Peaks were identified by comparing their retention times with those of authentic standards (Supelco™ 37, PUFA Mix – No 1 Marine Source and Bacterial mix). For some samples, peaks of FAs were confirmed with GC-mass spectrometry (GC-MS; ThermoFinnigan TRACE DSQ). Standard nomenclature is used for the identified FAs ($x{:}y\omega z$), where x is the number of carbon atoms, y is the number of double bonds and z is the position of the ultimate double bond from the terminal methyl.

According to Schomburg (1987), the concentration of each FA (C_{FA}, mg) was calculated as:

$$C_{FA} = \frac{A_S}{A_{IS}} \times \frac{C_{IS}}{W_S} \quad (5)$$

where A_S is the peak area of the FA, A_{IS} is the peak area of the internal standard, C_{IS} is the concentration of the internal standard (mg), and W_S is the dry weight (g) of the sample for prey species and fresh weight for the blubber of porpoises.

Data analysis

Data analysis of stable isotopes was performed using XLSTAT – Pro 2013 (Addinsoft). The level of significance was set at $\alpha = 0.05$. We used ANOVA followed by post hoc multiple comparison tests to compare $\delta^{15}N$ and $\delta^{13}C$ values in muscle between age classes (juveniles, adult females and adult males) and among years of stranding (2010–2013). Age class and sex comparisons within each year could not be made due to small sample sizes. Thus, individuals were pooled within each year to investigate interannual variation. We used a Kruskal-Wallis test to compare $\delta^{15}N$ and $\delta^{13}C$ values in the muscle of prey species chosen for SIAR (see next paragraph) followed by a post hoc test for multiple comparisons to check for pairwise differences.

The stable isotope mixing model SIAR (Stable Isotope Analysis in R; R Development Core Team 2010) was used to estimate the relative contribution of each prey species in the diet of harbour porpoises stranded in the southern North Sea. The main prey species contributing to the total diet of porpoises as determined by weight from stomach content analyses and as identified in previous studies were considered as potential sources: gobies (Gobiidae), sandeels (Ammodytidae), different species of Gadidae (whiting *Merlangius merlangus* and pouting *Trisopterus* spp.) and some clupeids (herring *Clupea harengus*, sprat *Sprattus sprattus* and sardine *Sardina pilchardus*) (Santos et al. 2004, Haelters et al. 2012). We ran 2 models: Model 1 based on the trophic enrichment factors (TEFs) for captive harp seals as given by Hobson et al. (1996) (1.3 ± 0.1 [SD]‰ and 2.4 ± 0.3‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively), and Model 2 based on the TEFs for captive bottlenose dolphins and killer whales as given by Caut et al. (2011) (1.26 ± 0.2‰ and 1.23 ± 0.15‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively). Individual isotope ratios of porpoises (n = 52) were entered in the model, whereas for prey species (n = 49), means and standard deviations were used. The mixing model was run using default parameters (iterations: 500 000; burn in: 50 000 and thinning by: 15) without using concentration dependencies.

We used ANOSIM to examine variations in FA composition among groups. In addition, we used SIMPER to determine which FAs contributed to the differences between 2 sets of data. Factors used for the analysis were gender and age class, cause of death and blubber thickness. We used non-metric multi-dimensional scaling (MDS) to display groups according to some specific FAs. FAs are presented as percentage values. Analysis was performed on Bray-Curtis similarity matrices calculated from untransformed FA data in PRIMER 5.

RESULTS

Stomach contents

More than 8000 individual prey remains (mainly otoliths) representing 13 species from 9 families were identified in the 14 stomachs analysed of harbour porpoises. In terms of occurrence (%*O*), Gobiidae, sprat, Ammoditydae, sand smelt *Atherina presbyter* and undetermined Gadidae dominated as prey, at 71, 50, 36, 29 and 29%, respectively (Fig. 1a). In regards to percentage by number (%*N*), Gobiidae were the most important prey with 53%, followed by Ammoditydae, sprat, herring and whiting, with 9, 8, 7 and 6%, respectively (Fig. 1b). Similarly, Gobiidae was the main prey species with a contribution of 37% of the composition by weight (%*W*), followed by whiting (22%) and sprat (14%) (Fig. 1c).

Stable isotopes and SIAR

Stable isotope values measured in the muscle of harbour porpoises ranged from −18.5 to −16.3‰ for $\delta^{13}C$, and from 13.5 to 18.4‰ for $\delta^{15}N$ (Table 1). For both males and females, $\delta^{13}C$ values did not differ between juveniles and adults (ANOVA, $p > 0.05$).

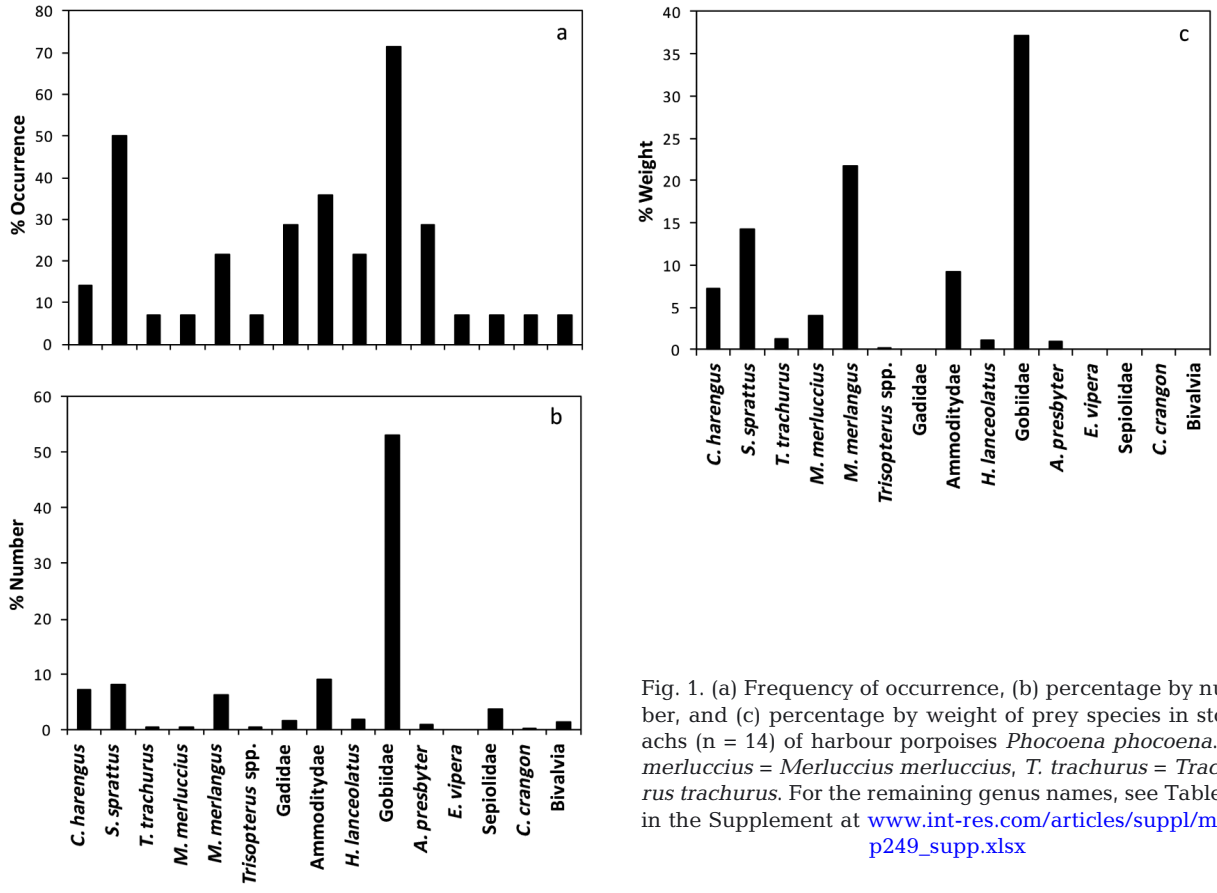

Fig. 1. (a) Frequency of occurrence, (b) percentage by number, and (c) percentage by weight of prey species in stomachs (n = 14) of harbour porpoises *Phocoena phocoena*. *M. merluccius* = *Merluccius merluccius*, *T. trachurus* = *Trachurus trachurus*. For the remaining genus names, see Table S1 in the Supplement at www.int-res.com/articles/suppl/m563p249_supp.xlsx

Table 1. Size, and $\delta^{13}C$ and $\delta^{15}N$ values in muscle of harbour porpoise *Phocoena phocoena* and selected fish species from the southern North Sea. n: number of individuals

		Size (cm)		$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)	
Family/species	n	Mean ± SD	(min, max)	Mean ± SD	(min, max)	Mean ± SD	(min, max)
Harbour porpoise	52	120 ± 18	(92, 161)	−17 ± 0.5	(−18.5, −16.3)	15.8 ± 1	(13.5, 18.4)
Juvenile females	15	115 ± 10	(98, 133)	−17.4 ± 0.5	(−18.2, −16.8)	16.1 ± 1.0	(14.1, 18.4)
Juvenile males	27	110 ± 7	(92, 120)	−17.3 ± 0.5	(−18.3, −16.3)	15.9 ± 1.1	(13.8, 18.0)
Adult females	7	156 ± 6	(145, 161)	−17.5 ± 0.5	(−18.5, −17.0)	14.7 ± 1.1	(13.5, 16.3)
Adult males	3	146 ± 4	(143, 150)	−17.3 ± 0.5	(−17.6, −16.9)	15.4 ± 0.6	(14.7, 15.9)
Fish							
Gadidae							
Whiting *Merlangius merlangus*	10	23 ± 0.7	(22, 24.2)	−16.9 ± 0.3	(−17.3, −16.3)	16.3 ± 0.4	(15.6, 17.0)
Pouting *Trisopterus luscus*	9	12 ± 6.7	(4.7, 19.3)	−17.8 ± 0.6	(−18.7, −16.7)	14.5 ± 1.1	(13.7, 17.0)
Clupeidae							
Herring *Clupea harengus*	5	29 ± 1	(27.8, 30)	−18.5 ± 0.5	(−19.0, −17.6)	10.8 ± 0.7	(9.9, 11.4)
Sprat *Sprattus sprattus*	10	7.5 ± 1.3	(6.2, 9.5)	−17.7 ± 0.7	(−18.3, −16.2)	13.5 ± 0.7	(12.4, 14.5)
Sardine *Sardina pilchardus*	5	22.8 ± 0.9	(22, 24)	−18.5 ± 0.4	(−19.0, −18.0)	12.4 ± 0.7	(11.4, 13.2)
Ammodytidae							
Sandeel *Hyperoplus lanceolatus*	5	24.3 ± 5.5	(20.3, 32.6)	−17.7 ± 0.6	(−18.4, −16.9)	13.5 ± 0.7	(13.1, 14.6)
Gobiidae							
Gobies	5	6.2 ± 0.6	(5.6, 7.3)	−18.2 ± 1.4	(−20.3, −16.9)	15.5 ± 1.6	(14.1, 17.9)

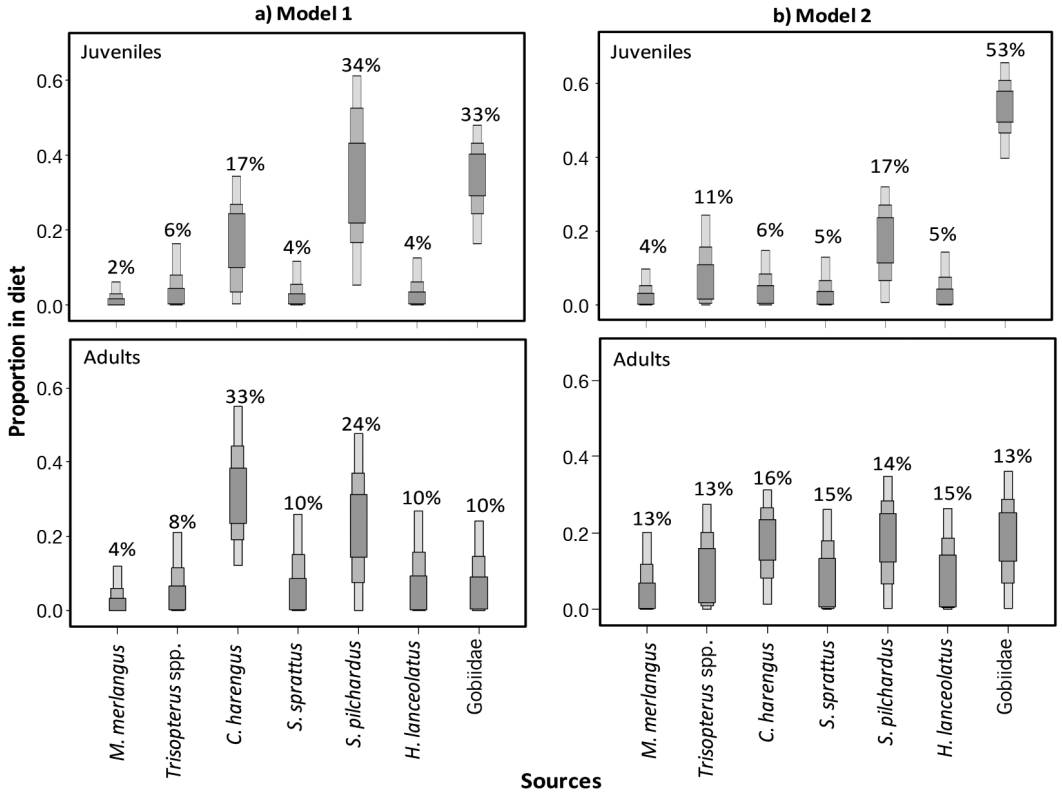

Fig. 2. Stable Isotope Analysis in R (SIAR) modeling: boxplots of estimated prey contributions in diet of harbour porpoises *Phocoena phocoena* (upper panels: juveniles, lower panels: adults). (a) Model 1 and (b) Model 2 (see 'Materials and methods' for model details). Credibility intervals (CI): CI_{95}: light grey; CI_{75}: medium grey and CI_{50}: dark grey. See Table 1 for genus names

However, $\delta^{15}N$ values were higher in juvenile females than in adult females (ANOVA, post hoc Tukey's test, $p = 0.03$), whereas comparing the other age classes, $\delta^{15}N$ values showed no significant differences. No significant differences were found in $\delta^{13}C$ and $\delta^{15}N$ values in the muscle of porpoises stranded among years (ANOVA, $p > 0.05$). For the prey species, selected for SIAR model, $\delta^{13}C$ values ranged between −20.3 and −16.2‰ for Gobiidae and *Sprattus sprattus*, respectively. For $\delta^{15}N$, values ranged between 9.9 and 17.9‰ for *Clupea harengus* and Gobiidae, respectively (Table 1). The $\delta^{13}C$ and $\delta^{15}N$ values were significantly different in the muscle of prey species (Kruskal-Wallis, $p < 0.001$).

The estimates from SIAR Model 1 showed that *Sardina pilchardus* and Gobiidae contributed the most to the diet of juvenile porpoises, at 34 and 33%, respectively, whereas *C. harengus* and *S. pilchardus* contributed the most to the adults' diet, at 33 and 24%, respectively (Fig. 2a). SIAR Model 2 estimated that Gobiidae contributed 53% to the diet of juvenile porpoises, while in the adult diet, the proportional contribution of each prey item was similar (range: 13–16%) (Fig. 2b).

FA composition

A common spectrum of marine FAs was obtained for the harbour porpoises, with 40 compounds present in relative amounts >0.1%. FA profiles of the analysed porpoises were not significantly different between age classes and years of stranding (ANOSIM, $p > 0.05$). Within prey species, sandeel showed the highest amounts of polyunsaturated FAs (PUFAs) at 55.32 ± 5.07%, while herring exhibited the lowest amounts at 24.18 ± 12.55% (Table S1 in the Supplement at www.int-res.com/articles/suppl/m563p249_supp.xlsx). In contrast, herring had the highest amounts of monounsaturated FAs (MUFAs) (51.16 ± 12.80%), whereas sandeel had the lowest amounts (11.78 ± 2.88%) (Table S1).

Non-metric MDS of FA proportions from total FAs (Fig. 3) in the blubber of all harbour porpoises showed that according to some specific FAs, porpoises may be divided into 3 groups. Porpoises in which the proportion of the FA 16:1ω7 is the highest (>19%) are marked as Group a in Fig. 3; porpoises where the FAs 22:1ω11/9 and 20:1ω9 were relatively dominant (>3%) are marked Group b; and those who

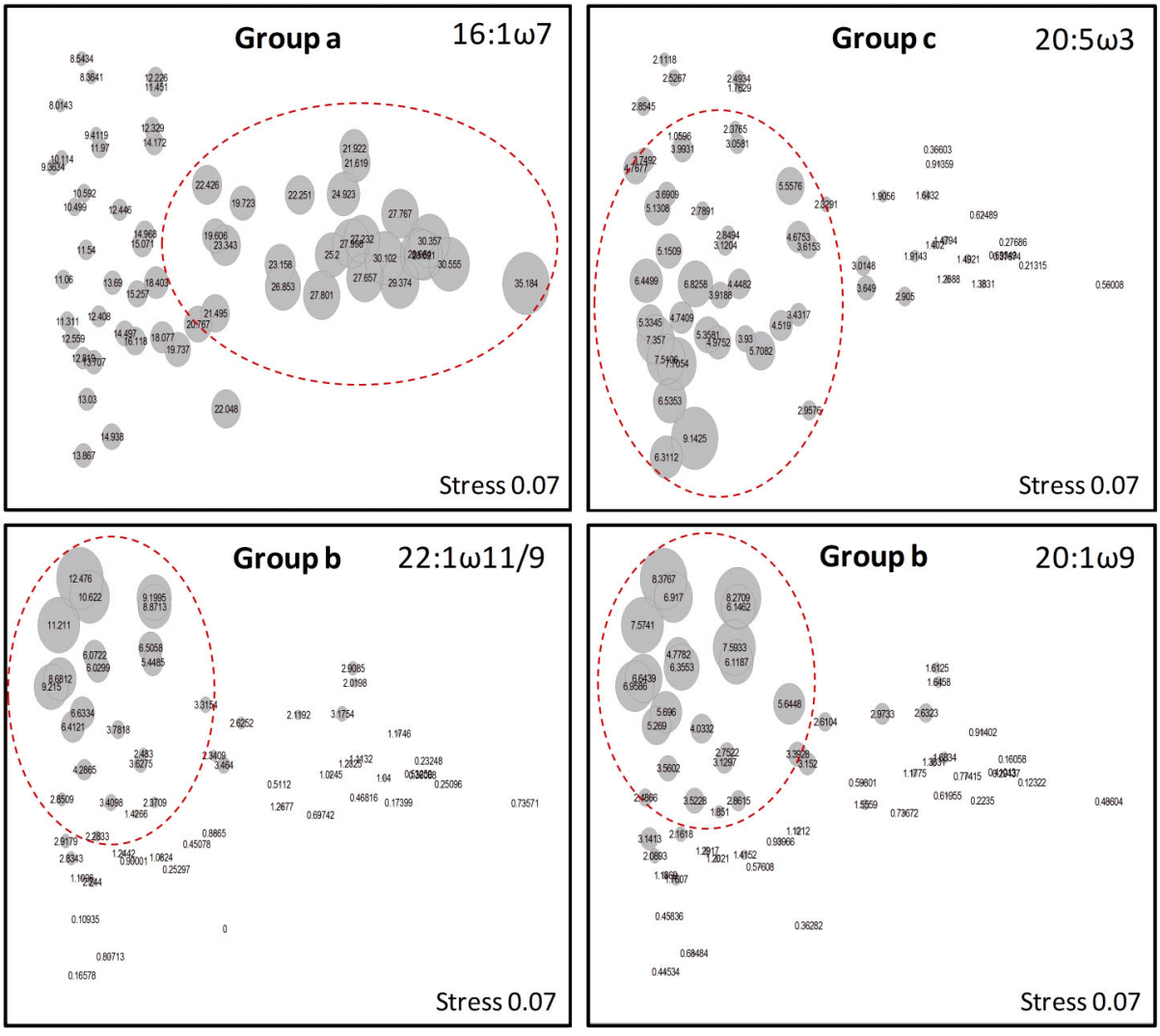

Fig. 3. Non-metric multi-dimensional scaling of fatty acid (FA) proportions (numerical data shown) from total FAs (%) in harbour porpoise *Phocoena phocoena* (each grey ellipse represents an individual porpoise) blubber. Group a: the proportion of the FA 16:1ω7 was the highest (>19%), Group b: the FAs 22:1ω11/9 and 20:1ω9 were relatively dominant (>3%), and Group c: the proportion of the FA 20:5ω3 was the highest (>5%)

exhibited the largest proportion of 20:5ω3 (>5%) are called Group c in Fig. 3.

DISCUSSION

Comparison of dietary assessment methods

Gobies, whiting and sprat primarily dominated the ingested biomass according to the stomach content analysis. Previous diet studies on harbour porpoises' stomachs in the North Sea found that these prey also contributed to the diet of this species (Leopold & Camphuysen 2006, Haelters et al. 2012). Whiting and gobies are demersal and coastal species, which can explain their presence in the stomach content analysis and lead to the limitation of the method, since stranded individuals have stomach contents biased towards inshore prey species (Pierce & Boyle 1991). The strength of stomach content analysis lies in the determination of prey size and number of individuals in the diet. It allows the identification of prey to species level, whereas stable isotope analysis often has less taxonomic resolution due to a lack of adequate separation between prey sources (an overlap in isotope signatures of similar prey species). However, even hard parts such as otoliths may be degraded by stomach gastric acids, leading to false negatives or to an over-representation with large and robust hard parts (Pierce & Boyle 1991).

The estimates from SIAR Model 1 showed that gobies, herring and sardine contributed the most to the diet of harbour porpoises, whereas Model 2 showed that only gobies contributed to the diet. The stable isotope results from the 2 models using different TEFs were quite different, suggesting some limitations to this approach in estimating proportions in the porpoise diet. These limitations include selecting the most appropriate TEFs and the absence of potential prey from the model. In fact, the TEFs used in the models are those of captive harp seals (Hobson et al. 1996) and captive bottlenose dolphins and killer whales (Caut et al. 2011); unfortunately, there were no available TEFs for harbour porpoises in the literature. Moreover, one should take into consideration the lack of sources; preferential and abundant prey could be missing and not included in our sampling data or we might have included a prey that harbour porpoises did not eat. In addition, if source signatures overlap considerably, the model can also have trouble distinguishing their contributions.

Variations in FA signatures may likely be due to differences in the diet of animals regardless of gender and age class. Elevated levels of 20:1ω9 and 22:1ω11/9 in the blubber of harbour porpoises from the southern North Sea reflected that some individuals may be feeding on zooplanktonivorous fish such as herring (Tocher 2003). Other FAs such as 16:1ω7 markers of diatoms and present in high levels in the blubber of porpoises may be explained by indirect ingestion via herbivorous species with a diatom-based diet (Dalsgaard et al. 2003). The FA 20:4ω6 marker of benthic littoral algae (Dalsgaard et al. 2003) is present in elevated amounts in the muscle of prey benthic species such as gobies, whiting and pouting (Table S1 in the Supplement). However, these amounts were not found in porpoise blubber, even though according to the stomach content analysis, these 3 species contributed to the diet of porpoises. Gobies, whiting and pouting are benthic species, and their presence in the stomachs of porpoises may be explained by the last meal consumed before stranding (inshore), whereas the FA signatures reflect the diet of porpoises over a period of up to several months (Budge et al. 2006). It should be noted that, in our study, we only used FA profiles of the muscle of prey species, whereas porpoises consume the entire prey. In order to determine which specific species were present in the diet of harbour porpoises, a quantitative FA signature analysis (QFASA) needs to be applied (Iverson et al. 2004).

Combining techniques that integrate diet over days and weeks allowed the gaining of a more complete understanding of harbour porpoises' diet relative to stomach content. However, the interpretation of the results is limited by a lack of parameters which are necessary for each technique.

Harbour porpoise distribution and prey species availability

The diet of harbour porpoises stranded along the North Sea since the early 1990s to the present time primarily comprises 7 prey species: gobies, whiting, *Trisopterus* spp., sandeels, sprat, herring and sardine (Table 2). Besides the sardine, these species are among the most abundant fish and widely distributed in the North Sea (Daan et al. 1990, ICES 2013). Before the mid-1960s, clupeids constituted an impor-

Table 2. Harbour porpoise (*Phocoena phocoena*) diet inferred from stomach content analysis in the southern North Sea and adjacent areas. n: number of stomachs analysed

Area (year of stranding)	n	Main prey	Reference
Southern North Sea (2010–2013)	14	Gobies, whiting, sandeel	Present study
Belgian coast (1997–2011)	64	Gobies, sandeels, whiting, *Trisopterus* sp.	Haelters et al. (2012)
Dutch coast (2006)	64	Gobies, sandeels, sprat, herring, whiting, twait shad	Leopold & Camphuysen (2006)
Northeast Atlantic French coast (1988–2003)	29	Sardine, whiting, blue whiting, scad	Spitz et al. (2006)
English Channel (1998–2003)	7	Pouting, gobies	De pierrepont et al. (2005)
Scotland (1992–2003)	188	Whiting, sandeels, gadids, *Trisopterus* sp.	Santos et al. (2004)
UK (1989–1994)	100	Gadids, sandeels, gobies	Martin (1996)
Germany	34	Sandeels, sole	Benke & Siebert (1996)
Denmark, Sweden, Norway	197	Herring, gadids	Aerefjord et al. (1995)
Germany	36	Sole, cod	Lick (1991)
France	8	Blue whiting, scad, hake	Desportes (1985)
Scotland (1959–1971)	93	Herring, sprat, whiting	Rae (1965, 1973)

tant part of the diet of harbour porpoises, but this has apparently changed after the collapse of the herring stock in the northeast Atlantic (Santos & Pierce 2003). In recent years, herring and sardine are becoming more common again (Ifremer 2014), and their return could explain why clupeids, relatively fatty fish presenting an energetic prey in term of energy density (Spitz & Jouma'a 2013), formed an important part of the diet of harbour porpoises in our study.

Gobies and *Trisopterus* spp. are important prey species in porpoises' diet (Table 2), but few data are available on their distribution and abundance. However, data from the International Bottom Trawl Survey (IBTS) in the North Sea showed that the relative abundance of *Trisopterus* spp. decreased since the year 2000, whereas gobies showed no clear temporal variations (Ifremer 2014). These species were accounted for in almost all previous studies (Table 2), and therefore they may be considered as traditional common prey in the diet of harbour porpoises in the North Sea. Herring, sprat, sandeel and whiting are important forage fish species in the North Sea, of economic interest and exploited by target fisheries (ICES 2013). It is well known that forage fish species display fluctuations in their distribution and abundance (Reid et al. 2001, Rijnsdorp et al. 2009). The relative abundance of herring, sprat and whiting in the North Sea from 1983 to 2010 showed no clear temporal variations, but that of the sandeel has undergone a pronounced decrease since the year 2001 (Ifremer 2014). Also, a decrease in the total landings of this species in the north and the centre of the North Sea has been noticeable. In many ecosystems, sandeel is a key prey fish linking trophic levels (Hain et al. 1995, Frederiksen et al. 2007). Since forage fish can exert bottom-up control on top predators, variability in the abundance of sandeels in response to fisheries pressure and climate change is likely to affect porpoise populations in the North Sea by increasing the likelihood of starvation (MacLeod et al. 2007) and changing their abundance and distribution (Reijnders 1992).

In the SIAR results of the present study, the sardine appeared to be a potential prey in the diet of harbour porpoises from the southern North Sea. This species did not figure in previous studies that analysed the stomachs of porpoises stranded in the North Sea (Santos et al. 2004, Haelters et al. 2012). Little information is known about the abundance of sardine in the North Sea; it is considered an occasional occupant and rarely occurs at a biomass large enough to attract fisheries exploitation (Engelhard et al. 2014). This small pelagic fish with more southern distribution is an important commercial species in southern Europe. However, sardine distribution has increased in the North Sea over time. Since the mid-1990s, the re-invasion of the North Sea by sardines has been highlighted and has been associated with climate change (Beare et al. 2004a,b, Montero-Serra et al. 2015). Hence, sardines may be considered a 'new' potential prey species in the diet of porpoises in the North Sea, or it may be a 'backup' prey replacing the sandeel decline.

Results also suggest that porpoises may prey in offshore waters on pelagic shoaling species such as sardine in order to compensate for the decrease in abundance of demersal coastal species. It is necessary to find out whether the decline in one prey species could be counterbalanced by other species in the same area. It has been suggested that the lower survival or emigration of harbour porpoises in the central and southern North Sea in the late 1980s was either the result of food depletion or food lower in caloric content (Reijnders 1992). In fact, the foraging strategies and diet of harbour porpoises are guided by the quality of prey rather than the large quantity of food in order to meet their specific energy requirements (Spitz et al. 2012). Therefore, it is important to identify whether the quality of prey in the potentially new area of distribution of harbour porpoises can cover their energy requirements and consequently lead to the shift of this species towards the new area of distribution.

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