Toothed whales in the northwestern Mediterranean: Insight into their feeding ecology using chemical tracers

Emilie Praca [a,b,*], Sophie Laran [b,c], Gilles Lepoint [a], Jean-Pierre Thomé [d], Antoni Quetglas [e], Paola Belcari [f,g], Paolo Sartor [h], Frank Dhermain [i,c], Denis Ody [j,c], Nathalie Tapie [k], Hélène Budzinski [k], Krishna Das [a]

[a] MARE Centre, Laboratory for Oceanology, University of Liège, allée de la Chimie 17, 4000 Liège, Belgium
[b] Centre de Recherche sur les Cétacés – Marineland, 306 avenue Mozart, 06600 Antibes, France
[c] Groupement d'Intérêt Scientifique pour les Mammifères Marins de Méditerranée, Parc national de Port-Cros, Castel Sainte Claire, 83418 Hyères, France
[d] Laboratoire d'Ecologie Animale et Ecotoxicologie, Université de Liège, allée du 6 Août 15, 4000 Liège, Belgium
[e] Instituto Español de Oceanografía, Centre Oceanogràfic de Balears, Moll de Ponent s/n, 07080 Palma de Mallorca, Spain
[f] Dipartimento Scienze Uomo e Ambiente, University of Pisa, via Derna 1, 56100 Pisa, Italy
[g] Department of Physics and Astronomy and London Centre for Nanotechnology, University College London, Gower Street, London WC1E 6BT, UK
[h] Consorzio per il Centro Interuniversitario di Biologia Marina ed Ecologia Applicata "G. Bacci", Viale Nazario Sauro n. 4, 57128 Livorno, Italy
[i] Groupe d'Etude des Cétacés de Méditerranée, 13 boulevard du Redon, 13009 Marseille, France
[j] WWF – France, 6 rue Fabres, 13001 Marseille, France
[k] University Bordeaux 1/CNRS, Laboratory of Physico- and Toxico-Chemistry of the environnement (LPTC), Institute of Molecular Sciences (ISM), UMR 5255, 351 cours de la Libération, 33405 Talence, France

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ABSTRACT

Risso's dolphins, pilot whales and sperm whales rarely strand in the northwestern Mediterranean. Thus, their feeding ecology, through the analysis of stomach contents, is poorly known. The aim of this study was to gain further insight into the segregation/superposition of the diet and habitat of Risso's dolphins, pilot whales and sperm whales using chemical tracers, namely, stable isotopes (δ^{13}C, δ^{15}N) and organochlorines. Significantly different δ^{15}N values were obtained in Risso's dolphins (11.7 ± 0.7‰), sperm whales (10.8 ± 0.3‰) and pilot whales (9.8 ± 0.3‰), revealing different trophic levels. These differences are presumably due to various proportions of Histioteuthidae cephalopods in each toothed whale's diet. Similar δ^{13}C contents between species indicated long-term habitat superposition or corroborated important seasonal migrations. Lower congener 180 concentrations (8.20 vs. 21.73 μg.g^{-1} lw) and higher tDDT/tPCB ratios (0.93 vs. 0.42) were observed in sperm whales compared with Risso's dolphins and may indicate wider migrations for the former. Therefore, competition between these species seems to depend on different trophic levels and migration patterns.

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1. Introduction

Diet and trophic ecology of terrestrial predators can be studied directly by behavioral observation or fecal analysis. For cetaceans, and particularly those preying in deep waters, diet is generally studied by stomach content analysis with hunted, incidentally caught or stranded individuals. The latter case is the most frequent but includes a certain bias that creates limitations of the methodology. The stomach of a stranded individual contains the last food ingested before stranding. However, the stranded individual might be sick or injured and can spend a period of time in waters that are not its usual habitat. Furthermore, the digestion time of different prey varies and can result in an overestimation of some food sources (Santos et al., 2001; Tollit et al., 1997).

Chemical tracers reflect the ecosystem conditions under which organisms live and feed and offer a complementary approach to the examination of stomach contents. The concentration of these persistent natural or man-made molecules represents the dietary inputs over the turnover time of the analyzed tissue (Hobson and Clark, 1992; Tieszen et al., 1983), but not the last meal before stranding. As the chemical analyses require only a small amount of sample, they can be performed using biopsies, which are not lethal to the animal and allow for the study of living healthy animals.

The isotopic composition of a consumer is a well-established indicator, which is based on the weighted averaging of the isotopic composition of different prey, which is potentially modified by isotopic fractionation. This fractionation is caused by the discrimination of heavy and light isotopes during metabolic reactions (Kelly,

2000; Newsome et al., 2010), and the fractionation results in a slight change of the isotopic ratios between a consumer and its diet. In ecology, the most commonly used isotopic ratios are those of carbon ($^{13}C/^{12}C$, notated $\delta^{13}C$ relative to the Vienna Peedee Belemnite standard) and nitrogen ($^{15}N/^{14}N$, notated $\delta^{15}N$ relative to the atmospheric nitrogen standard). $\delta^{15}N$ values generally increase through the food chain, and the $\delta^{15}N$ value of a consumer's tissue can be used to infer the relative or absolute trophic position, depending on the quality of supporting information (Kelly, 2000; Newsome et al., 2010). The increase of $\delta^{13}C$ values in the food web is usually smaller and may reflect the origin of the primary production. The phytoplankton isotopic composition is indeed influenced by factors such as community composition, temperature, the origin of the carbon used in photosynthesis or photosynthetic rates (Kelly, 2000; Laws et al., 1995; Newsome et al., 2010; Rau et al., 1982). As terrestrial and marine carbon sources differ in their $\delta^{13}C$ values (Kelly, 2000), the $\delta^{13}C$ can indicate offshore/nearshore or benthic/pelagic contribution to food intake (Cherel and Hobson, 2007; Fry and Sherr, 1984). Both $\delta^{15}N$ and $\delta^{13}C$ values also vary among seasons in relation to changes in primary production or seasonal diet shifts.

However, organochlorine (OC) contaminants are less frequently used for dietary reconstruction, but they can allow for segregation of cetacean stocks (Borrell et al., 2006; Herman et al., 2005; Krahn et al., 2007; Pierce et al., 2008). OC accumulates through the food web and increases with the species trophic level (Hoekstra et al., 2003; Marsili and Focardi, 1996b; Ross et al., 2000; Weijs et al., 2009). Species inhabiting different geographical areas accumulate pollutant loads qualitatively and quantitatively and are characteristic of these areas (Aguilar, 1987; Borrell et al., 2006). Each element or congener has its own trophic source, and a whole OC profile may be used as a kind of fingerprint to infer the dietary behavior and habitat (Borrell et al., 2006; Pierce et al., 2008).

In the northwestern Mediterranean Sea (NWMS), toothed whales, including Cuvier's beaked whales (*Ziphius cavirostris*), long-finned pilot whales (*Globicephala melas*), sperm whales (*Physeter macrocephalus*) and Risso's dolphins (*Grampus griseus*), seem to present a certain spatial segregation in the summer. Pilot whales seem to prefer the pelagic area, Risso's dolphins prefer the upper part of the slope, and sperm whales seem to have a habitat on the whole slope and surrounding pelagic waters (Praca et al., 2009; Praca and Gannier, 2008). However, Cuvier's beaked whales seem to be restricted to the northeast Ligurian Sea (Azzelino et al., 2008; Podesta et al., 2006) and will not be included here. On the other hand, the diet of pilot whales, sperm whales and Risso's dolphins has been studied in very few stomach content analyses, as these species rarely strand. In particular, only one stomach content analysis (Roberts, 2003) has been published for the sperm whale, and no studies have been published for the pilot whale. Roberts (2003) showed the stomach content of a single sperm whale was composed of 90% *Histioteuthis bonnellii*. Unpublished data for the pilot whale seems to show a diet with around 50% *Todarodes sagittatus*, 10–20% Histioteuthidae and around 30% other squid and octopus species (Astruc, 2005). Stomach content analysis in Risso's dolphin was performed in 14 specimens stranded on Spanish coasts (Blanco et al., 2006) and in one specimen in Italy (Würtz et al., 1992). In Spain, the stomach contents were mainly composed of *Argonauta argo* (40%), while in Italy, the stomach contents of Risso's dolphins were mainly composed of *Histioteuthis reversa* (80%). The remaining stomach contents were composed of several other squids and octopods (Blanco et al., 2006; Würtz et al., 1992). Therefore, these toothed whale species are strictly cephalopod feeders and may compete for a certain part of their diet.

Regarding the weak knowledge on the toothed whale feeding ecology in the NWMS, chemical tracers offer a good alternative to explore further aspects about feeding ecology. Therefore, this study assessed the long-term segregation or superposition in diet and habitat of Risso's dolphins, pilot whales and sperm whales. This study combined stable isotope (SI) and OC analyses performed in these species, and SI analysis was done in several putative cephalopod prey species.

2. Material and methods

2.1. Sampling

Cetaceans stranded on the Mediterranean French coasts (Fig. 1) between 2003 and 2009 were sampled following the French National Stranding Network (Réseau National Echouage) protocol (see Kuiken and Garcia Hartmann, 1991). Skin, blubber and muscle samples were taken as soon as possible, usually on the day of the stranding, and were frozen immediately after collection.

Biopsies (Fig. 1) were performed using a 150 lb crossbow (Panzer, US) and arrows fitted with stainless steel sampling heads of 0.5×4 cm for sperm whales and of 0.5×2.5 cm for pilot whales (Ceta-Dart, Denmark). To avoid infection of cetaceans and contamination of the samples, the sampling heads were sterilized in boiling water and sprayed with 90% alcohol prior to use. Skin and blubber were separated and cut in two in the height. Sub-samples of skin and blubber, intended for SI and OC analyses, respectively, were directly frozen onboard.

Cephalopod samples were collected in hauls carried out between 104 m and 762 m in depth during bottom trawl surveys off the Balearic Islands in June of 2006 and in the north Tyrrhenian Sea in January of 2007 (Fig. 1). Cephalopod species were selected because of their occurrence in the stomach contents of toothed whale species studied here or are known to spend a part of their life in deep waters (Mangold and Boletzky, 1987). In addition, two freshly dead specimens of *H. reversa* and one species of *H. bonnellii* were found at the surface during cetacean surveys. Cephalopod mantle samples (around 4 cm^2) were taken and frozen onboard.

All cetacean and cephalopod samples were stored at $-20\ ^\circ C$ until further analyses.

2.2. Stable isotope analyses

SI analyses were performed with the whole skin sub-sample of the biopsies. For stranding samples, a sub-sample of skin was used by taking a whole height of the skin.

Fig. 1. Map of the study area and sampling locations. Species are denoted by the following symbols: sperm whales (triangles), pilot whales (dots), Risso's dolphins (squares) and cephalopods (crosses).

Samples were dried at 50 °C for 48 h and were grounded into a fine homogenous powder. An isotope ratio mass spectrometer (Isoprime, UK) coupled to an N–C–S elemental analyzer (Thermo Scientific, USA) was used to perform automated measurements of isotope ratios. The isotope ratios were expressed using the classical δ notation:

$$\delta X = \left(\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}}\right) \times 1000 \qquad (1)$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of samples or of standards. The latter are the Vienna Peedee Belemnite (v-PDB) for carbon and atmospheric nitrogen. The reference materials were IAEA CH-6 (sucrose, $\delta^{13}C = -10.4 \pm 0.2‰$) and IAEA-N1 ($\delta^{15}N = 0.4 \pm 0.2‰$). The precision of measurements, evaluated by the standard deviation of the replicates, were 0.4‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$.

Lipids have been shown to be depleted in ^{13}C in comparison to the isotopic composition of the food source (Tieszen et al., 1983). However, chemical lipid extraction affects $\delta^{15}N$ and requires separation of $\delta^{13}C$ and $\delta^{15}N$ analyses (Sweeting et al., 2006). As our subsamples that were intended for stable isotope analysis were very small (around 0.5 cm³), the separation of $\delta^{13}C$ and $\delta^{15}N$ was not feasible. Therefore, we applied a lipid normalization equation (McConnaughey and McRoy, 1979), adapted by Post et al. (2007) for aquatic animals:

$$\delta^{13}C_{\text{normalised}} = \delta^{13}C_{\text{untreated}} - 3.32 + 0.99 \times (C:N) \qquad (2)$$

Post et al. (2007) recommend the use of a lipid normalization equation when C:N > 3.5, which corresponds to a lipid content >5% in samples. However, other authors also warn that such an equation could lead to bias if the lipid content is too high, for example, C:N > 6.5 in cormorants (Doucette et al., 2010), C:N > 7 in zooplankton (Smyntek et al., 2007) or C:N > 8 in deep-sea fish (Hoffman and Sutton, 2010). The majority of our samples contain C:N between 3.4 and 4.4. Only two of our samples have a C:N > 6. Therefore, we considered the conditions to be satisfactory to apply lipid a normalization equation.

2.3. Organochlorine contaminant analyses

For OC analyses, whole blubber sub-samples from biopsies were used. In stranding samples, blubber sub-samples were done in the outermost layer to correspond to the layer sampled by biopsies (around 2 cm height for sperm whales and 1 cm for pilot whales and Risso's dolphins).

2.3.1. 2005 to 2007 samples

The samples obtained from 2005 to 2007 were analyzed using the following procedures: (1) fat extraction from blubber was microwaved at 650 W for 3 × 20 s, (2) the surrogate standard (PCB congener IUPAC n°112) was added, (3) acid purification using 2 ml of a sulfuric acid mixture (fuming sulfuric acid at 30% and concentrated sulfuric acid at 98%, 1:3, v:v), followed by an extraction with 2 ml of n-hexane, (4) reconcentration of the sample to 1 ml under nitrogen flow, (5) clean-up was performed with Florisil© solid phase cartridges (Supelco, USA), (6) evaporation of eluates to just dryness under a gentle nitrogen flow and reconstitution of residues with 125 μl of hexane and 125 μl of Mirex (50 pg.μl⁻¹), (7) dilution of the samples from 5 to 30 times in an hexanic solution of Mirex (50 pg.μl⁻¹) after the first test analyses were carried out to perform analysis in the linear response of the ECD detector, and (8) analyses of purified extracts by high resolution gas chromatography using a Thermo Quest Trace 2000 gas chromatograph equipped with a ^{63}Ni ECD detector and a Thermo Quest AS 2000 autosampler for liquids (Thermo Quest, Italy). The details of the clean-up, extraction and analysis procedures can be found in Schnitzler et al. (2008).

2.3.2. 2008–2009 samples

For the samples of 2008 and 2009, the applied procedures involved the following methods: (1) extraction-purification performed by accelerated solvent extraction (ASE) by the ASE 200 Dionex system, (2) addition of internal standards (PCB congeners IUPAC n° 30, 103, 155 and 198), (3) addition of 1 ml of isooctane in the extract, (4) reconcentration of the extract using a Rapid-Vap vacuum evaporation system (Labconco, USA), (5) a second purification on an acidic silica gel column, elution with 3 × 5 ml of a pentane-dichloromethane mixture (90:10 v/v), (6) reconcentration and transfer of the samples in 90 μl of isooctane, (7) addition of a syringe of a standard (Octachloronaphthalene), and (8) analyses of the extracts on an HP 5890 series II gas chromatograph (Hewlett–Packard, USA) coupled to a ^{63}Ni electron-capture detector (ECD). These procedures are detailed in Tapie et al. (2008).

2.3.3. Contaminants analyzed

We analyzed polychlorinated biphenyls (PCB) congeners IUPAC n° 28, 44, 52, 101, 118, 128, 138, 153, 170, 180, 187, 195, 206, and 209, dichlorodiphenyl- trichloroethane (pp'-DDT), pp'-DDT metabolites, dichloro-diphenyl-dichloroethylene (pp'-DDE) and dichloro-diphenyl-dichloroethane (pp'-DDD), dieldrine and lindane. All results are presented in μg.g⁻¹ lipid weight (lw).

In this paper, tPCB is the total sum of the 14 PCB congeners analyzed, and ΣICES7 is the sum of the seven PCB congeners (i.e., congeners 28, 52, 101, 118, 138, 153, and 180) listed by the International Council for the Exploration of the Sea for International Comparisons, and tDDT is the total sum of pp'-DDT, pp'-DDE, pp'-DDD and dieldrine. We also calculated the following ratios: ΣICES7 in tPCB (ICES/PCB), pp'-DDE + dieldrine in tDDT (DDE/tDDT) and tDDT compared to tPCB (tDDT/tPCB).

2.4. Statistical analyses

All data were log-transformed. Negative $\delta^{13}C$ values were converted to absolute values before being log transformed. The normality of the data was checked by a Shapiro–Wilk normality test, while the homogeneity of variances between species was assessed using a Bartlett test.

Interspecific comparisons were performed using a one-way ANOVA for all chemical tracers separately and for tPCB, ΣICES7 and tDDT. If a significant difference was obtained, that is, for p-value < 0.05, a post hoc Tukey multiple comparison of means was done to perform individual comparisons of species.

All statistical analyses were performed with R 2.10.1 statistical software, using a *stats* package (R Development Core Team, 2009).

3. Results

The whale and cephalopod samples that were analyzed, are summarized in Tables 1 and 2, respectively. Mean ± SD of $\delta^{13}C$, $\delta^{15}N$, OC concentrations and ratios and the results of the different ANOVA tests are presented in Table 3. The log-transformed data followed a normal distribution, and homogeneity of variance was observed between species. For sperm and pilot whales, 12 and 4 biopsies were performed, respectively. Four samples for Risso's dolphin, 2 samples for the sperm whale and one sample for the pilot whale were taken on stranded individuals.

Table 1
Summary of analysed toothed whale samples - Species, sample type (B: biopsy, S: stranding), sampling date, measured or estimated size of the animal in meter (U: undetermined), sex (M: male, F: female, U: undetermined) and analyses performed (SI: stable isotopes in skin, OC: organochlorine contaminants in blubber).

Species	Sampletype	Date	Size(m)	Sex	SI	OC
Pilot whale	B	12/06/2007	6,0	M	√	-
	B	22/09/2007	4,0	U	√	√
	S	12/10/2007	2,5	F	√	√
	B	22/09/2008	4,0	U	√	√
	B	22/09/2008	4,0	F	√	√
Sperm whale	S	21/03/2006	12,5	M	√	√
	B	07/09/2007	9,0	U	√	√
	B	09/09/2007	11,5	U	√	√
	B	14/09/2007	11,0	U	√	√
	B	16/09/2007	10,0	U	√	√
	B	20/09/2007	10,0	U	√	√
	B	20/09/2007	10,0	U	√	-
	B	20/08/2008	U	U	√	√
	B	10/09/2008	12,0	U	√	√
	B	16/09/2008	10,0	U	√	-
	B	10/06/2009	12,0	U	√	√
	B	22/06/2009	6,0	U	√	√
	S	02/07/2009	U	U	√	√
	B	07/08/2009	U	U	√	√
Risso's dolphin	S	28/07/2005	3,1	M	√	√
	S	01/04/2007	2,2	M	√	√
	S	01/05/2007	2,4	M	√	√
	S	03/09/2009	1.6	M	√	√

Table 2
Summary of analysed cephalopod samples - Species, area of sampling (BI: Balearic Islands, TS: Tyrrhenian Sea, LS: Ligurian Sea) and number of individuals (n).

Species	Sampling area	n
Octopoda		
Bathypolypus sponsalis	BI	2
Eledone cirrhosa	BI	2
Octopus salutii	BI	2
Pteroctopus tetracirrhus	BI	2
Scaeurgus unicirrhus	BI	2
Sepioidea		
Heteroteuthis dispar	BI	2
Rossia macrosoma	BI	1
Teuthoidea		
Ancistroteuthis lichtensteinii	BI	2
Alloteuthis media	BI	3
Abralia veranyi	BI	3
Histioteuthis bonnellii	BI	1
	LS	1
Histioteuthis reversa	BI	2
	LS	2
Illex coindetii	BI	2
	TS	13
Todaropsis eblanae	TS	15
Todarodes sagittatus	BI	2
	TS	2

3.1. Stable isotopes

The highest $\delta^{15}N$ values were measured in Risso's dolphins (11.7 ± 0.7‰), followed by sperm whales (10.6 ± 0.5‰) and by pilot whales (9.9 ± 0.3‰). The differences between the three species were significant. $\delta^{13}C$ values were −16.8 ± 0.8‰ for Risso's dolphins, −17.4 ± 0.7‰ for sperm whales and −17.5 ± 0.7‰ for pilot whales. The interspecific difference for $\delta^{13}C$ was not statistically significant.

The $\delta^{15}N$ and $\delta^{13}C$ values for cephalopods ranged from 6.7 to 10.4‰ and from −22.8 to −16.8‰, respectively. The mean differences between cephalopods and toothed whales were 2.4‰ for

$\delta^{15}N$ and 1.2‰ for $\delta^{13}C$ (Fig. 2), which is within the enrichment range usually observed between marine mammals and their prey (Newsome et al., 2010).

3.2. Organochlorine contaminants

For Risso's dolphins, tPCB concentrations were 114.24 ± 68.62 $\mu g.g^{-1}$ lw, while tPCB concentrations were 107.81 ± 108.72 $\mu g.g^{-1}$ lw for sperm whales and 66.02 ± 57.91 $\mu g.g^{-1}$ lw for pilot whales. The concentrations of tDDT were 87.59 ± 52.95 $\mu g.g^{-1}$ lw for Risso's dolphins, 107.81 ± 108.72 $\mu g.g^{-1}$ lw for sperm whales and 66.02 ± 57.91 $\mu g.g^{-1}$ lw for pilot whales. ICES7/tPCB and DDE/DDT ratios ranged from 0.55 to 0.80 and from 0.64 to 1, respectively.

Congener 180 concentrations were significantly higher in Risso's dolphins (21.73 ± 10.66 $\mu g.g^{-1}$ lw) than in sperm whales (8.20 ± 7.62 $\mu g.g^{-1}$ lw), while in pilot whales (10.78 ± 8.46 $\mu g.g^{-1}$ lw) no significant difference was detected compared with the two previous species. For the tDDT/tPCB ratio, significantly higher values were obtained for sperm whales (0.93 ± 0.33) compared with Risso's dolphins (0.42 ± 0.09), and they were not significantly different than pilot whales (0.55 ± 0.23) or the two former. For all other OC concentrations and ratios, nonsignificant interspecific differences were found.

4. Discussion

The SI and OC compositions reflect the dietary input over the turnover time of the type of tissue analyzed (Hobson and Clark, 1992; Tieszen et al., 1983). In tissues such as skin, hair or feathers, isotopes reflect the diet incorporated during the growth of the tissue (Hobson and Clark, 1992). The turnover rate for skin has been evaluated for the beluga *Delphinapterus leucas* (St Aubin et al., 1990) and the bottlenose dolphin *Tursiops truncatus* (Hicks et al., 1985). These authors estimated that cell migration from the basal lamina to the outermost surface ranged from 70 days to 75 days for both species. The skin of the species studied here are supposed to be at least as thick as the skin of bottlenose dolphins and belugas, and isotopic ratios in this tissue were presumed to reflect the diet over two months at least.

Cetacean blubber is the major location of body fat storage and is structured in several layers, having different composition and mobilization degrees (Koopman, 2001). The outermost layer, which is sampled during biopsy, is generally not remobilized during pregnancy and lactation in females (Koopman, 2001) or during starvation periods (Koopman et al., 2002). Therefore, the composition of this layer could reflect the OC accumulated through the entire life of the animal.

Temporal variations in $\delta^{13}C$ and $\delta^{15}N$ baselines can occur in relationship to seasonal change in primary production, such as phytoplankton blooms. However, such variations seem to be attenuated by the increase of trophic levels, in particular in long-turnover tissues. The species studied in this study are top predators, and their skin presents a turnover of at least two months. Consequently, we considered that such seasonal variations were not significant here.

In the NWMS, $\delta^{15}N$ and $\delta^{13}C$ coastal baselines seem to greatly vary at fine-scale (a few kilometers), mainly in relation to river inputs. However, at a large spatial scale (>500 km), these punctual spatial variations seemed mitigated for $\delta^{15}N$. Similar $\delta^{15}N$ values have been obtained for zooplankton or fish in the Balearic Islands, Corsica or Sicily. These findings are consistent with our results for cephalopods that did not seem to present discrepancies in $\delta^{15}N$ between the different sampling areas. Our findings also agree with the results of Takai et al. (2000), which did not find a significant

Table 3

Mean ± SD of nitrogen (δ^{15}N) and carbon (δ^{13}C) isotopic ratios (‰), organochlorine contaminant concentrations (g.g^{-1} lw) and organochlorine contaminant ratios in the three toothed whale species, and ANOVA results (F, p-value). Mean values flanked by different letters (a, b and ab for differences in 2 species out of 3, and a, b and c for dissimilarities between all 3 species) indicate significant differences in post hoc Tukey tests (p-value < 0.05).

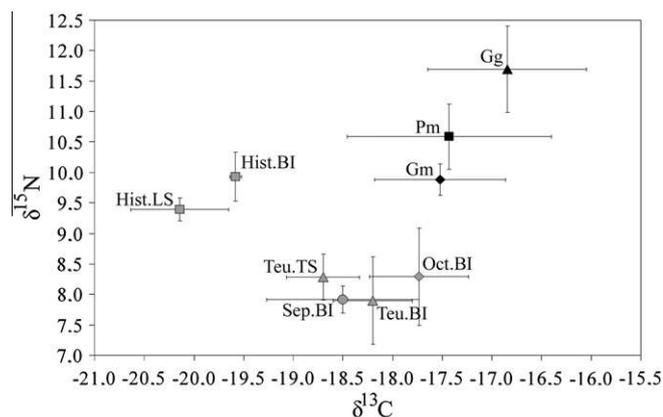| Tracers	Risso's dolphin	Pilot whale	Sperm whale	F	*p*-value
δ^{15}N	a 11.7 ± 0.7	b 9.8 ± 0.3	c 10.8 ± 0.3	13.28	0.0002
δ^{13}C	−16.9 ± 0.8	−17.5 ± 0.7	−17.7 ± 1.3	0.78	0.47
Congener 28	0.25 ± 0.14	0.04 ± 0.06	0.01 ± 0.01	3.21	0.07
Congener 44	0.92 ± 0.72	5.67 ± 10.23	6.14 ± 16.71	1.92	0.18
Congener 52	0.22 ± 0.21	0.17 ± 0.22	0.14 ± 0.16	0.24	0.79
Congener 101	6.39 ± 4.99	2.95 ± 3.61	2.60 ± 4.06	1.60	0.23
Congener 118	6.18 ± 4.03	3.24 ± 3.50	2.63 ± 3.52	0.52	0.61
Congener 128	3.94 ± 2.29	1.70 ± 1.64	0.80 ± 0.72	0.82	0.46
Congener 138	24.11 ± 14.00	13.35 ± 12.38	11.26 ± 14.23	0.52	0.61
Congener 153	28.72 ± 19.50	15.56 ± 17.88	13.22 ± 18.11	1.74	0.21
Congener 170	8.36 ± 4.92	5.36 ± 4.01	2.03 ± 3.01	1.61	0.23
Congener 180	a 21.73 ± 10.66	ab 10.78 ± 8.46	b 8.20 ± 7.62	3.94	0.04
Congener 187	12.51 ± 7.78	6.67 ± 7.53	4.71 ± 6.05	2.64	0.10
Congener 195	0.79 ± 0.28	0.42 ± 0.39	0.13 ± 0.14	1.61	0.23
Congener 206	0.13 ± 0.16	0.07 ± 0.08	0.04 ± 0.05	0.03	0.97
Congener 209	0.00 ± 0.00	0.06 ± 0.08	0.02 ± 0.04	1.29	0.30
tPCB	114.24 ± 68.62	66.02 ± 57.91	107.81 ± 108.72	2.52	0.11
ΣICES7	87.59 ± 52.95	46.07 ± 45.50	76.31 ± 68.08	2.56	0.11
pp'-DDT	2.12 ± 1.40	2.59 ± 2.41	2.98 ± 3.46	0.28	0.76
pp'-DDE + dieldrine	43.09 ± 23.32	31.56 ± 30.61	47.10 ± 72.73	0.29	0.76
pp'-DDD	2.01 ± 1.08	1.23 ± 1.24	0.83 ± 0.89	0.70	0.51
tDDT	47.20 ± 25.21	35.38 ± 33.63	115.98 ± 112.35	0.26	0.78
lindane	0.00 ± 0.00	0.17 ± 0.29	0.19 ± 0.37	1.20	0.33
ICES7/tPCB	0.77 ± 0.00	0.69 ± 0.13	0.78 ± 0.07	2.35	0.13
DDE/DDT	0.90 ± 0.03	0.85 ± 0.12	0.86 ± 0.13	0.32	0.74
tDDT/tPCB	a 0.42 ± 0.09	ab 0.55 ± 0.23	b 0.93 ± 0.33	7.66	0.004

Fig. 2. Mean ± SD of stable isotope ratios in toothed whales and their putative prey. Risso's dolphins (Gg), sperm whales (Pm), pilot whales (Gm), Histioteuthidae (Hist), other Teuthoidea (Teu), Sepioidea (Sep) and Octopoda (Oct) in the Tyrrhenian Sea (TS), Ligurian Sea (LS) and off the Balearic Islands (BI).

correlation between δ^{15}N and latitude in cephalopods. Our analysis allows us to perform a simple comparison of δ^{15}N between cephalopods and toothed whales. Pantoja et al. found an eastward decrease of around 3‰ in the δ^{15}N values of surface particulate organic nitrogen from the Alboran Sea (western basin) to the Ionian Sea (eastern basin). Such difference could then lead to different δ^{15}N values for species at higher trophic levels in the eastern and western basins. However, δ^{13}C is negatively correlated with latitude (Takai et al., 2000). Therefore, cephalopods from the Tyrrhenian Sea and Balearic waters could present higher δ^{13}C values than those preyed on by toothed whales in the Ligurian Sea and Provençal Basin. Subsequently, δ^{13}C in cephalopods and toothed whales will not be compared.

We found a significant difference in δ^{15}N between the three toothed whale species, with higher values for Risso's dolphin compared to the sperm whale and to the pilot whale. Eastern basin shows lower δ^{15}N baseline values than western basin. Therefore, our δ^{15}N results cannot be explained by different potential migrations of the species from the western basin to the eastern basin. Indeed, species presenting the highest δ^{15}N values, namely, the sperm whale and Risso's dolphin, are both frequently observed in both basins. The pilot whale, which presents the lowest δ^{15}N, is quite rare in the Ionian Sea and off western Greece, and a priori not present in the eastern Mediterranean.

The δ^{15}N toothed whale values are more reliable with regard to differences in their diets and, in particular, to different diet proportions of cephalopods from the Histioteuthidae family. Indeed, Histioteuthidae, from both the Ligurian Sea and Balearic Islands, seem to present higher δ^{15}N values (9.2–10.4‰) than other squids (6.7–9.2‰), octopods (6.7–9.3‰) or cuttle-fishes (7.7–8.2‰). Few gastric analyses previously performed in the study area seem to corroborate such a hypothesis. The stomach contents of pilot whales were composed of 10–20% Histioteuthidae (Astruc, 2005). The stomach contents of Risso's dolphins were very variable, containing between 15% and 80% Histioteuthidae (Astruc, 2005; Blanco et al., 2006; Clarke, 1996; Würtz et al., 1992). However, individuals stranded in France and Italy presented a higher percentage of Histioteuthidae, from 40% to 80% (Astruc, 2005; Würtz et al., 1992). The stomach content of the single sperm whale analyzed was made up of 90% Histioteuthidae (Roberts, 2003) and could be unrepresentative of the species diet.

Concerning δ^{13}C values, nonsignificant mean differences were found between the three toothed whale species. This result could highlight a possible superposition in their habitat over a long-term period. However, habitat models in the NWMS showed spatial segregation between the three species in summer (Praca et al., 2009; Praca and Gannier, 2008). While Risso's dolphin seems to prefer the upper part of the slope (mean depth around 650 m), the pilot whale presents the most oceanic habitat at 2500 m mean depth (Praca and Gannier, 2008). The sperm whale has a wider habitat

on the whole slope and surrounding oceanic water (mean depth around 1800 m) (Praca et al., 2009; Praca and Gannier, 2008). These findings may indicate that $\delta^{13}C$ variability is not adapted for a study of this type of habitat segregation because $\delta^{13}C$ baselines do not differ enough between different offshore habitats. However, SI analyses in skin reflect a period of at least two months. Thus, our samples covered a period between January and October. The species studied are suspected to perform important seasonal movements within the study area (Drouot-Dulau and Gannier, 2007; Laran, 2005; Laran and Drouot-Dulau, 2007; Moulins and Würtz, 2005) and towards other regions of the Mediterranean Sea. In particular, SI analyses in sperm whale dentine layers, illustrating SI variations through animal life, could indicate a movement between western and eastern basins of the Mediterranean (Mendes et al., 2007). Our results could corroborate these suspected important seasonal migrations and, therefore, the integration of isotopic signal from different areas. Such a hypothesis could be supported by the larger variation of $\delta^{13}C$ values in sperm whales (1.3‰) compared with Risso's dolphin and pilot whales (0.8% and 0.7‰, respectively).

In the NWMS, only one other study analyzed SI in toothed whale species stranded in Italy (Capelli et al., 2008). The results from three Risso's dolphins were consistent with ours ($\delta^{13}C$ from −17.2‰ to −16.9‰, $\delta^{15}N$ from 10.5‰ to 11.9‰), and the results from one sperm whale calf presented a higher $\delta^{15}N$ (13.1‰), due to a mother-calf enrichment during pregnancy (Nelson et al., 1998). In comparison with other species analyzed by Capelli et al. (2008), toothed whales seem to present trophic levels superior to the two fin whales Balaenoptera physalus ($\delta^{15}N$ = 8.3‰ and 9.3‰) and the three striped dolphins Stenella coeruleoalba ($\delta^{15}N$ from 8.9‰ to 9.5‰), similar to one Cuvier's beaked whale ($\delta^{15}N$ = 10.6‰) and inferior to two bottlenose dolphins Tursiops truncatus ($\delta^{15}N$ = 12.6 and 13.5‰). Such results are in accordance with previous stomach content analyses (Blanco and Raga, 2000; Orsi Relini and Giordano, 1992; Würtz and Marrale, 1993). In the Strait of Gibraltar (connecting the Mediterranean Sea and the Atlantic Ocean), pilot whales showed higher $\delta^{15}N$ and $\delta^{13}C$ values of 11.3 ± 0.4‰ and −16.4 ± 0.4‰, respectively (De Stephanis et al., 2008). These differences could mean that populations from the NWMS and the Strait of Gibraltar have different food sources and could be, at least partially, segregated. Similarly, sperm whales from the eastern North Atlantic presented $\delta^{13}C$ and $\delta^{15}N$ values of −19.0 ± 0.9‰ and 14.6 ± 0.6‰, respectively (Das et al., 2003), which was very different from our results. These findings agree with the isolation of Mediterranean sperm whales from the Atlantic population, as shown by mitochondrial DNA studies (Drouot et al., 2004).

Risso's dolphins presented concentrations of congener 180 that were significantly higher and tDDT/tPCB ratios that were significantly lower than sperm whales. Congener 180 belongs to the PCB congener class I, that is, no vicinal hydrogen (Boon et al., 1994), which is very recalcitrant and was one of the most commonly used PCBs in industrial mixtures (UNEP, 2002). However, DDT and dieldrine are pesticides. They have been used in agriculture since the 1980s in Europe, and they are still being use against the vector of malaria in some African countries (UNEP, 2002). Risso's dolphin could then inhabit areas more contaminated with industrial rejects (NWMS), while the sperm whale also moves to areas that have been contaminated with more important agricultural rejects, that is, south and east Mediterranean (Gómez-Gutiérrez et al., 2007). This explanation could corroborate sperm whale movements between the eastern and western Mediterranean, indicated by SI changes in dentine layers (Mendes et al., 2007). Congener 180 concentrations and tDDT/tPCB ratios in pilot whales were similar to those in sperm whales and Risso's dolphins. Therefore, this species could have an intermediate migration

pattern compared to sperm whales and Risso's dolphins. All other OC concentrations and ratios presented nonsignificant differences between the three species. These findings are consistent with previous studies that show the difficulty of pollutant concentrations in segregating populations because of high individual variability (Aguilar, 1987; Borrell et al., 2006). The concentration variability in individuals is caused by numerous factors including nutritional state, sex, age, excretion, metabolism or tissue composition (Aguilar, 1987). On the contrary, ratios, such as the tDDT/tPCB ratio, reflect population structures more precisely due to their lesser variability (Aguilar, 1987).

The ecological niche of a species can be defined by a multidimensional hyperspace principally composed of habitat and diet components and their use according to time (Hutchinson, 1957). Species that share close niches are in competition, and species that exploit resources less efficiently are partially or totally excluded from the area. Specialization can occur and results in different seasonality and/or diet. Toothed whales in the NWMS seem to fit into such a feature. Indeed, carbon stable isotopes seem to show that toothed whale habitats could remain superposed. Nitrogen stable isotopes pointed out specialization in diet, while congener 180 and tDDT/tPCB ratios could show a wider migration for sperm whales.

Besides the ecological interest in the use of chemical tracers, the results presented here revealed a very high OC contamination, between 5 and 10 times higher than in the Atlantic Ocean, for sperm and pilot whales (Dam and Bloch, 2000; Law et al., 1996). In the Mediterranean, a similar important contamination was previously reported in a few Risso's dolphins (Corsolini et al., 1995; Marsili and Focardi, 1996a) and in striped and bottlenose dolphins (Aguilar and Borrell, 2005; Borrell et al., 2006; Borrell and Aguilar, 2007). Such findings can be explained by the high trophic level of the species and the overall contamination of the Mediterranean Sea (Gómez-Gutiérrez et al., 2007; UNEP, 2002). These concentrations are higher than a threshold ($3 \times \Sigma ICES7 > 17$ µg.g^{-1} lw), up to which OC could have a toxicological effect on immune and reproductive systems (Jepson et al., 2005; Kannan et al., 2000). Furthermore, an important contamination of the whole body, and not only in the outer most blubber layer, is suspected because high concentrations have been found in muscle samples (Praca, 2008). To assess the toxicological impact of OC contamination in toothed whales, extensive biopsy sampling and analysis are highly recommended. This sampling could also allow the analyses of recent pollutants found in marine mammals, such as brominated flame-retardants (Ross et al., 2009) or polycyclic aromatic hydrocarbons (Marsili et al., 2001).

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