

# Diet of the social groups of long-finned pilot whales (*Globicephala melas*) in the Strait of Gibraltar

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**Abstract** The Strait of Gibraltar is inhabited throughout the year by a group of pilot whales (*Globicephala melas*), but their spatial distribution varies between Summer and Autumn. In this paper, we have used carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) stable isotope signatures to investigate the differences in diet amongst seasons, sex and stable social units. Skin samples were collected from 56 individually photo-identified pilot whales during Autumn 2005 and Summer 2006. These individuals were genetically sexed and their isotopic signature determined. The level of inter-individual association both within and between stable social units were compared to Euclidean distances between individual isotopes signatures. No differences in either  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  were found according to the sex of individuals, but significant seasonal differences were found in  $\delta^{15}\text{N}$ , although not in the  $\delta^{13}\text{C}$  values. This suggests that pilot whales are resident year round in the Strait, a finding

supported by independent photo-identification. The variation in  $\delta^{15}\text{N}$  could reflect a shift in pilot whale diet through the year, with pilot whales feeding at a higher trophic level in Autumn compared to Summer. This could also represent a change in the diet of pilot whale prey species. The  $\delta^{13}\text{C}$  values were significantly different amongst the four stable social units sampled and individual  $\delta^{13}\text{C}$  values were significantly related to the level of inter-individual association, while no relationship was found for  $\delta^{15}\text{N}$ . These results suggest that within the same general area (i.e. the Strait of Gibraltar), there is some level of specialisation in habitat or prey choice between pilot whales social units.

## Introduction

The Strait of Gibraltar is the narrow and shallow connection between the Mediterranean Sea and the Atlantic Ocean (Fig. 1). The water circulation in the Strait is characterised by (a) a surface inflow of Atlantic waters which is driven by the excess of evaporation over precipitation in this basin and (b) a deep outflow of dense Mediterranean water (Lacombe and Richez 1982). The Strait is also characterised by mixing processes through a pulsed upwelling induced by the tides and constrained by the bathymetry (Echevarría et al. 2002).

The interface between the Atlantic surface waters and the deep Mediterranean waters generally takes place at a depth between 50 and 200 m, depending on the geographical location and intensity of the tidal flows. The boundary between Atlantic waters and Mediterranean waters is deeper from the Spanish coast to the Moroccan coast (north to south) (Reul et al. 2002) and from the Atlantic to the Mediterranean (east to west), from

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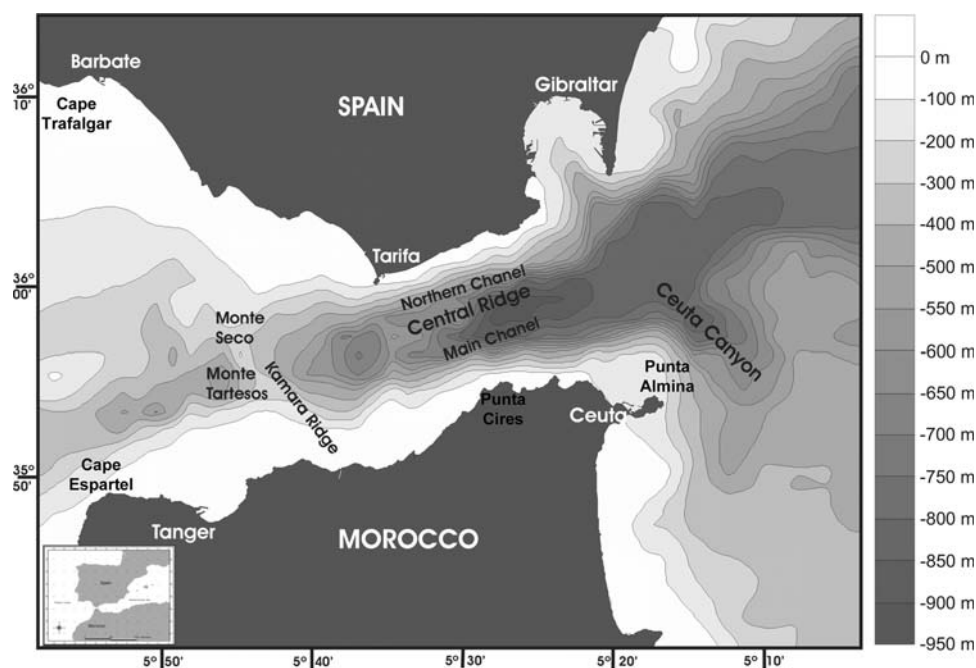
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**Fig. 1** Research area and bathymetry of the Strait of Gibraltar (from Sanz et al. 1988)



approximately 100 m at 5°20W to 300 m at 6°W in the central part of the Strait (Kinder et al. 1988). This Atlantic–Mediterranean interface is considered to be a biogeographical boundary (Sanjuán et al. 1994). Nevertheless, there is substantial transport of organisms across this ecotone. Most of the plankton biomass is transported into the Mediterranean Sea by the Atlantic waters. Reul et al. (2002) estimated that 5,570 tonnes C per day, dominated by autotrophic nanoplankton (42%) and heterotrophic bacteria (37%), were transported towards the Mediterranean Sea, whereas 1,140 tonnes C of heterotrophic organisms (89%) were exported daily towards the Atlantic by the deep Mediterranean outflow.

Bathymetry plays an important direct or indirect role on prey distribution (Gil de Sola 1993). This role can be direct on demersal prey species whose distribution can often be related to the topographical features such as depth and slope. For pelagic cetacean prey species such as fishes or cephalopods, bathymetric features act indirectly through topographically induced vertical (upwelling) and horizontal (currents) circulation which can either stimulate primary and secondary production, and act directly on the spatial distribution of prey species through transport and/or aggregating effects (Davis et al. 1998; Cañadas et al. 2005).

Six species of cetaceans occur regularly within the Strait (Cañadas et al. 2005; Guinet et al. 2007; de Stephanis et al. 2008a). Amongst them a community of approximately 216 long-finned pilot whales (*Globicephala melas*) is observed year-round in the strait of Gibraltar (de Stephanis 2008a; Verborgh et al. 2008). Pilot whales have a hierarchical social system and individual lives in stable social groups of

related individuals (Ottensmeyer and Whitehead 2003; Jankowski 2005; de Stephanis et al. 2008b). Pilot whale populations consist of several pods, typically of 14 individuals, which form temporarily associated and interbreeding clans or grinds (de Stephanis et al. 2008b). Pods are stable social subunits which are likely to be matrilineal in structure (Amos et al. 1991a, b, 1993a, b; Fullard 2000; Fullard et al. 2000; de Stephanis et al. 2008b).

Pilot whales are sexually dimorphic with mature males longer (mean 552 ± 1.7 cm) and heavier (mean 1694 ± 39 kg) than mature females (432 ± 0.8 cm; 893 ± 10 kg) (Bloch et al. 1993). Such large sexual dimorphism implies that mature males and females have different absolute metabolic requirements (Flinn et al. 2002). In other highly sexually dimorphic marine mammals, such as sperm whales (*Physeter macrocephalus*) and Weddell Seals (*Leptonychotes weddellii*) (Flinn et al. 2002; Evans and Hindell 2004; Zhao et al. 2004), there is evidence of sex-specific foraging strategies. However, this has not been documented for pilot whales.

In Summer, the spatial distribution of the pilot whale community within the Strait of Gibraltar extends over deep waters with a steep bathymetric gradient in the narrowest part of the strait (de Stephanis 2008). During Autumn and Winter, this community of pilot whales disperses more widely throughout the entire Strait of Gibraltar (de Stephanis 2008; de Stephanis et al. 2008a). Whether this shift is mediated by the whales prey switching or a shift in the distribution of their prey species is unknown. The diet of pilot whales in this community is known only from stomach content of three whales that were found stranded along the Northern coast of the Strait during Autumn 2006. The

stomachs of these whales contained squid (cephalopod) beaks as well as fish otoliths and bones but the species were not identified due to a lack of taxonomic expertise at the moment of the necropsy (De La Fuente, pers. commun.). In other locations, stomach contents from stranded animals or from whaling activities in the Feroe Islands, consisted predominantly of squid and fish species (Desportes and Mouritsen 1993; Clarke and Goodall 1994; Abend and Smith 1995, 1997; Gannon et al. 1997; Bjørke and Gjørseter 1998; Clarke et al. 1998; Aguiar dos Santos and Haimvici 2001; Santos et al. 2001; Beatson et al. 2007a, b). However, these data are subject to several limitations and biases. Animals that strand often have an empty stomach and can be sick, resulting in an inability to prey efficiently on their usual prey. Stomach content of stranded whales may therefore not be representative of the diet of healthy individuals. In addition, stomach content analyses often present a snap shot of the prey items consumed days or hours preceding the death of the animal with no information on seasonal or interannual shifts in diet.

Measurements of naturally occurring stable isotopes of carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) can provide information to trace sources of primary productivity and relative trophic level of organisms in marine ecosystems (Michener and Schell 1994). These methods are based on the principle that the composition of stable isotopes in an animal's diet will be reflected in its tissues (Klem 1935; Ackman and Eaton 1966; Rouvinen and Kiiskinen 1989; Hobson 1990; Wada et al. 1991; Iverson et al. 1995; Kirsch et al. 1998, 2000). Thus, compared with the short-term record of diet provided by stomach contents, diet information from stable isotope analyses reflects the average composition of food resources that have been assimilated over periods of days to months, depending on tissue turnover (Tieszen 1978; Kirsch et al. 1998, 2000). Carbon isotope values are found to vary between benthic or inshore food webs relative to pelagic food webs (Hobson and Welch 1992; Hobson et al. 1994). In pelagic habitat carbon isotopes vary according to depleted high latitude (i.e. temperature) waters relative to carbon enriched low latitude waters. There is little (i.e. approx. 1‰) or no change in the relative abundance of  $^{13}\text{C}$  between trophic levels following the primary producer to primary consumer link (Hobson and Welch 1992). For nitrogen, significant enrichment in  $^{15}\text{N}$  occurs with trophic level, an effect that typically ranges from 1.7 and 4‰ with a mean value close to 3‰ (Michener and Schell 1994; Abend and Smith 1997). The combined use of carbon and nitrogen isotope measurements of marine organisms, together with more conventional dietary approaches, can provide important new information on trophic relationships and feeding ecology, including spatial use of habitat (Hobson and Welch 1992; Hobson et al. 1994; Michener and Schell 1994; Kelly 2000; Lawson and Hobson 2000).

The aims of this study were to analyse possible seasonal variation in diet of the long-finned pilot whales occurring in the Strait of Gibraltar. We also wanted to assess possible inter-sexual and inter-group differences in diet. To our knowledge such comparisons have never been conducted for pilot whales, and the investigation of possible diet differences between stable social units belonging to the same community have not been investigated in any cetaceans.

## Materials and methods

### Study area and surveys

The study area is the area involving of the Strait of Gibraltar and contiguous waters, comprised between 5° and 6° of longitude west, and including Spanish and Moroccan waters. The Strait of Gibraltar (Fig. 1) is nearly 60 km long. Its western border is located between Cape Trafalgar (Europe) and Cape Espartel (Africa), 44 km apart. The Strait then narrows to the East to reach a minimum width of 14 km between Tarifa (Europe) and Punta Cires (Europe). Its eastern border is located between Gibraltar and Punta Almina (Africa) 23 km apart (Parrilla et al. 1988). The bathymetry of the Strait is characterised by a west to east canyon, with shallower waters (200–300 m) found on the Atlantic side and deeper waters (800–1,000 m) on the Mediterranean side (Fig. 1).

Survey transects were conducted between 1999 and 2006 from the CIRCE (Conservation, Information and Research on Cetaceans) research motorboat “Elsa” (11 m). The observation post of this vessel was a platform elevated 4 m above sea level. Two trained observers occupied the lookout post in 1 h shifts (during daylight) and covered an arc of 180° ahead of the vessel with a minimum of 3 nm (5.6 km) visibility (assisted by 8 × 50 binoculars). When a group of pilot whales were seen, the location of the ship, as well as the distance and bearing of the group were recorded, to enable the whales to be found on approach. The location of the animals was also recorded when they were approached by the vessel to photo-identify individuals and perform skin biopsies of known individuals (i.e. those individuals indexed in the strait of Gibraltar pilot whales photo-identification catalogue (de Stephanis 2008; Verborgh et al. 2008).

### Pilot whales sampling strategy

The skin biopsies were performed using a 67 kg draw crossbow (Barnett Wildcat XL) at distances ranging from 5 to 15 m from the animal, and the dart was fired at the mid-lateral region, near the dorsal fin of the whale. A stop collar attached to the tip of the bolt prevented penetration deeper

than the biopsy tip and caused the bolt to rebound upon impact with the whale. The darts were designed to float and were collected using a dip net. The skin biopsies collected included epidermis and dermis layers with biopsy arrows with 1.5 cm long and 0.6 cm in internal diameter. Both the tips and the arrows were designed and fabricated by Finn Larsen of the Danish Institute for Fisheries Research, Charlottenlund, Denmark. Samples were obtained under a permit issued by the Spanish Ministry of Environment.

Prior to sampling, animals were identified using the natural marking of their dorsal fin (Auger-Méthé and Whitehead 2007; Verborgh et al. 2008) to avoid double sampling and allow samples to be analysed according to individual identity and the social units to which they belong. In each biopsy session, the crew of the *Elsa* made sure that there was no calf present in the area before firing the crossbow, therefore, no calves were biopsied. The individuals were sampled independently of their size (excluding calves).

Samples were collected during two sampling periods. Five individuals from three distinctive social units (de Stephanis et al. 2008b) were sampled during Winter 2005 (25–27 October 2005). Fifty-one individuals belonging to different social units were sampled during mid-Summer 2006 (25 July–1 August).

Immediately after collection, the skin samples were preserved in two different ways. One part was immediately put in a 2 ml tube containing a solution of 20% dimethylsulphoxide (DMSO) in saturated salt (NaCl) (Amos and Hoelzel 1991) and frozen at  $-20^{\circ}\text{C}$ . This part of the sample was used to perform genetic sexing of individuals. The second part was frozen to  $-20^{\circ}\text{C}$  without any treatment, and was used to assay  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotope values.

#### Sex determination

The sex of 56 individuals was assessed genetically by polymerase chain reaction (PCR) of introns within the *Zfx* and *Zfy* genes (Shaw et al. 2003). To double check the results obtained with the *Zfx/Zfy* specific primers and avoid false positives, we reamplified the same samples with primers specific for males only, targeting the *SRY* gene (Rosel 2003). DNA was extracted and purified from a 20-mg aliquot of skin with the Qiagen “DNeasy Blood and Tissue Kit”. PCR was performed on 30 ng of purified DNA in a 20- $\mu\text{l}$  reaction volume otherwise containing 2  $\mu\text{l}$  of  $10\times$  *Taq* polymerase reaction buffer (supplied by the manufacturer), 1.5 mM  $\text{MgCl}_2$ , 0.2 mM dNTPs, 0.1 mg/ml BSA, 1 U of *Taq* polymerase (Invitrogen), and either 0.2  $\mu\text{M}$  of primers LGL331-forward and LGL335-reverse (for *Zfx/Zfy* amplification), or 0.2  $\mu\text{M}$  of primer *SRY*-forward and 0.04  $\mu\text{M}$  of primer *SRY*-reverse (for *SRY*

amplification). The PCR reactions were performed as follows: 1 cycle at  $94^{\circ}\text{C}$  for 3 min, then 35 cycles of denaturation for 30 s at  $94^{\circ}\text{C}$ , annealing for 30 s at  $52^{\circ}\text{C}$  (for *Zfx/Zfy* primers) or  $56^{\circ}\text{C}$  (for *SRY* primers), and amplification at  $72^{\circ}\text{C}$  for 45 s. Then each sample was loaded on a 1% agarose gel and the fragments were separated by electrophoresis. *Zfx/Zfy* specific primers allowed for the differentiation between males (2 bands,  $\sim 930$  bp for the X-specific band and  $\sim 1,000$  bp for the Y-specific fragment) and females (1 band). Confirmation of the results was obtained with the primers specific for the *SRY* gene which is positive for males (1 band,  $\sim 350$  bp) and negative for females (no band). The sequences of the primers are presented in Tables 1 and 2.

#### Isotopic relationship determination

Skin samples were dried at  $60^{\circ}\text{C}$  for 48 h and were then pulverised. High lipid concentration can mask a large part of the isotopic signature, preventing comparison (Sotiropoulos et al. 2004; Hobson et al. 1996, 1997), to avoid this problem samples (0.2 g) were rinsed repeatedly with a 2:1 chloroform:methanol solution to remove lipids following the protocol recommended by Morin and Lesage (2003).

In the Laboratory of Isotopic Mass Relationship Spectrometry of the Autonomous University of Madrid, each sample was reduced to a purified gas ( $\text{CO}_2$ ,  $\text{N}_2$ ,  $\text{SO}_2$ ,  $\text{SH}_6$  and  $\text{H}_2$ ) that was analysed by a mass spectrometer. The spectrometer used was a Micromass Cf-Isochrom of magnetic sector. The isotopic relationship of  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$  were determined in  $\text{CO}_2$  and  $\text{N}_2$  using a continuous flow elemental analyser Carlo Erba 1108-Chns. The analytic precision was 0.1 and 0.2‰ for C and N, respectively. We refer to the isotope ratios in terms of delta values ( $\delta$ ) (Coleman and Fry 1991).

#### Social structure of the pilot whales of the Strait of Gibraltar

The social structure of the pilot whales in the Strait was obtained using a long term photo-identification catalogue. The social structure was obtained using two different approaches. Firstly, the degree of association between pair of known individuals was analysed using the methodology

**Table 1** Sequence of the primers used in the genetic sexing of the pilot whales of the Strait of Gibraltar during 2005–2006

Primers	Sequence
LGL331-forward	5'-CAAATCATGCAAGGATAGAC-3'
LGL335-reverse	5'-AGACCTGATTCCAGACAGTACCA-3'
<i>SRY</i> -forward	5'-ACCGGCTTTCCATTTCGTGAACG-3'
<i>SRY</i> -reverse	5'-CATTGTGTGGTCTCGTGATC-3'

**Table 2** Summary of the sampling events on *Globicephala melas* in the Strait of Gibraltar, and results of the levels of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in skin

Season	Dates	n	$\delta^{15}\text{N}$		$\delta^{13}\text{C}$	
			Mean (SD) (‰)	Range (‰)	Mean (SD) (‰)	Range (‰)
Autumn	25–27 October 2005	5	12.70 ± 0.32	12–13.85	-16.20 ± 0.22	-16.37 to 15.87
Summer	25 July–1 August 2006	51	11.29 ± 0.38	10.12–12.05	-16.37 ± 0.40	-17.26 to 15.25

used by Ottensmeyer and Whitehead (2003), Jankowski (2005) and de Stephanis (2008). The strength of the behavioural relationships between dyads of individuals was represented using one index of the frequency of co-occurrence, the half-weight association index (Ginsberg and Young 1992; Ottensmeyer and Whitehead 2003) that gave a Matrix of Association between each pair of individuals (MAI). Secondly, social units of pilot whales were described using the methodology of Christal et al. (1998) for sperm whales, and Ottensmeyer and Whitehead (2003) and de Stephanis et al. (2008b) for long-finned pilot whales. The term unit was defined to investigate the stability of groups over time. *Key individuals* were defined as individuals sighted in at least 5 different years, where each sighting was at intervals of  $\geq 180$  days. *Constant companions* were defined as individuals sighted in the same group as the *key individual* during a day for at least 5 days, where each sighting was at intervals of  $\geq 60$  days, and in approximately 2 different years. A *unit* was defined as a *key individual* and all of its *constant companions* (if a unit consisted of more than two individuals, all individuals had to be *constant companions* of at least two of the others). The eight long lasting social units previously identified in de Stephanis et al. (2008b) were used in this study.

#### Comparing the social structure and the diet

To compare the social structure and the diet, two approaches were used. First, three matrices of Euclidean distances were built to assess the distances in the levels of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  between individuals. The first matrix ( $\text{MED}_1$ ) took into consideration both the levels of carbon and nitrogen, and their distance between each pair of individuals A and B according to the formula:

$$\text{Dist}(A,B) = \sqrt{(\delta^{13}\text{C}_A - \delta^{13}\text{C}_B)^2 + (\delta^{15}\text{N}_A - \delta^{15}\text{N}_B)^2}$$

Two other matrices were constructed using the differences in levels of  $\delta^{13}\text{C}$  ( $\text{MED}_2$ ) and  $\delta^{15}\text{N}$  ( $\text{MED}_3$ ), respectively, between each pair of individuals. Only the samples collected during mid-Summer 2006 were used for this analysis. The matrix of association index rates (MAI) was compared with the three matrices of Euclidean distances using a Mantel Test.

For the second approach, the results obtained using the first approach were verified by comparing the  $\delta^{13}\text{C}$  and

$\delta^{15}\text{N}$  signatures of individuals between social units defined in de Stephanis et al. (2008b).

## Results

### Isotopic relationship determination with sex and season

Amongst the 56 pilot whales sexed genetically, 23 were females and 33 were males. No statistical differences in either  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  was found according to the sex of individuals during the Summer ( $\delta^{15}\text{N}$ : females  $11.51 \pm 0.15\text{‰}$ , males  $11.38 \pm 0.09\text{‰}$ , Mann–Whitney  $U$  test = 351,  $Z = -0.356$ ,  $P = 0.72$ ;  $\delta^{13}\text{C}$ : females  $-16.37 \pm 0.09\text{‰}$ , males  $-16.45 \pm 0.09\text{‰}$ ,  $U = 296$ ,  $Z = -1,290$ ,  $P = 0.20$ ). No comparison was possible during the Autumn due to the small sample size. When comparing the results by season, significant differences were found in the levels of  $\delta^{15}\text{N}$  (Summer  $11.27 \pm 0.38\text{‰}$ , Winter  $12.70 \pm 0.72\text{‰}$ , Mann–Whitney  $U$  tests = 1,  $Z = -3.616$ ,  $P < 0.005$ ) but not in  $\delta^{13}\text{C}$  (Summer  $16.37 \pm 0.40\text{‰}$ , Winter  $-16.20 \pm 0.22\text{‰}$ ,  $U = 92.50$ ,  $Z = -0.78$ ,  $P = 0.44$ ; Fig. 2). Additionally the range of  $\delta^{15}\text{N}$  was larger in Winter (10.12–12.05‰) compared to Summer (12–13.85‰). In Summer  $\delta^{13}\text{C}$  ranged from -17.26 to -15.25‰.

### Social structure of long-finned pilot whales sampled in the Strait of Gibraltar

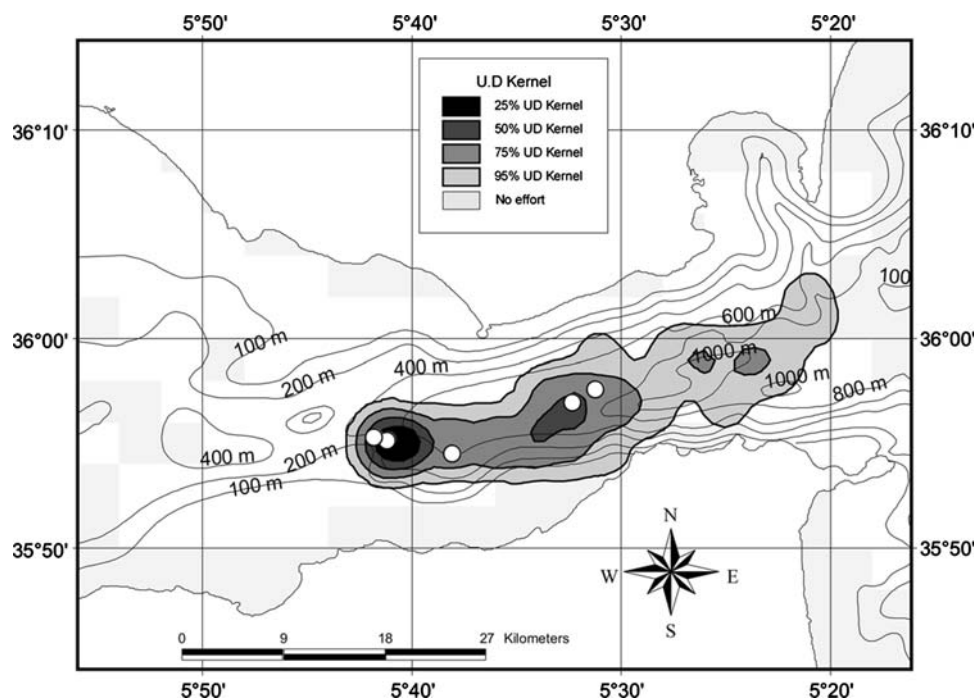
A total of 32 *key individuals* and 8 *units* could be defined in de Stephanis et al. 2008b. Biopsies were obtained from 4 of the stable social units: A (4 individuals out of the 8 individuals photo-identified and belonging to unit A), D (3/3), F (3/9), H (2/7). The units A, D, F and H had a mean association index rate of 0.82 (SD = 0.1), 0.76 (SD = 0.07), 0.39 (SD = 0.18) and 0.60 (SD = 0.23), respectively.

### Comparing the social structure and the diet

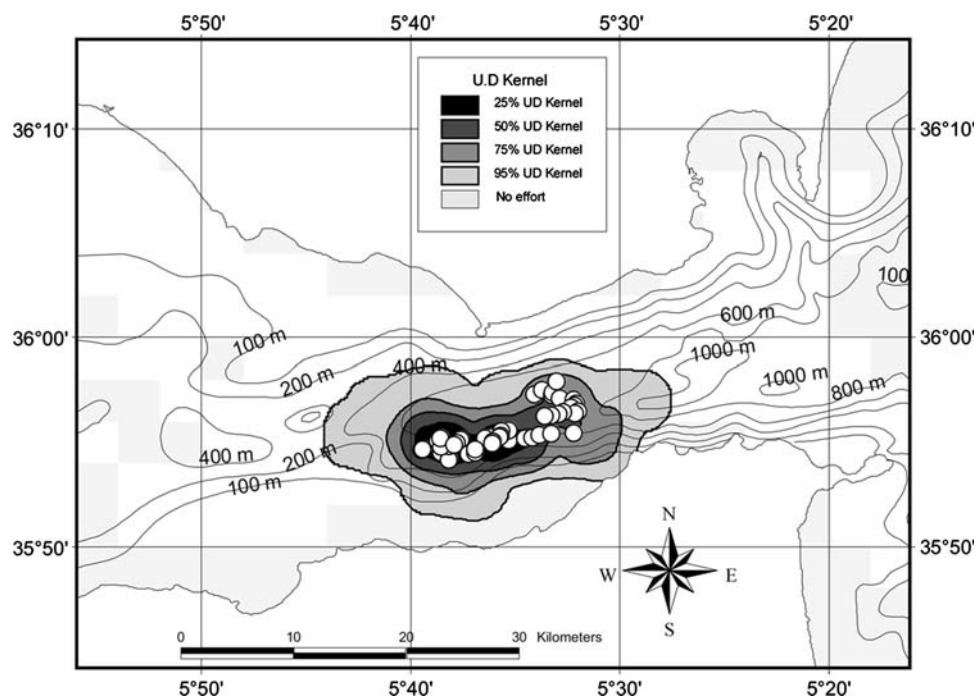
#### Comparison between social units and stable isotopes signature

The  $\delta^{13}\text{C}$  values were significantly different between social units (Kruskal–Wallis chi-squared = 8.51,  $df = 3$ ,  $P < 0.05$ ), while no differences were found in  $\delta^{15}\text{N}$  values

**Fig. 2** Biopsies made in Autumn (*white dots*), and spatial distribution in Autumn/Winter (between 2001 and 2006) of long-finned pilot whales in the Strait of Gibraltar (from de Stephanis 2008)



**Fig. 3** Biopsies made in Summer (*white dots*), and spatial distribution (between 2001 and 2006) in Summer of long-finned pilot whales in the Strait of Gibraltar (from de Stephanis 2008)



(Kruskal–Wallis chi-squared = 3.95,  $df = 3$ ,  $P = 0.27$ ) (Fig. 5).

#### Comparison between social structure and isotopes signature

When comparing the matrix  $MED_1$  and MAI, the social structure and the stable isotope signature were marginally

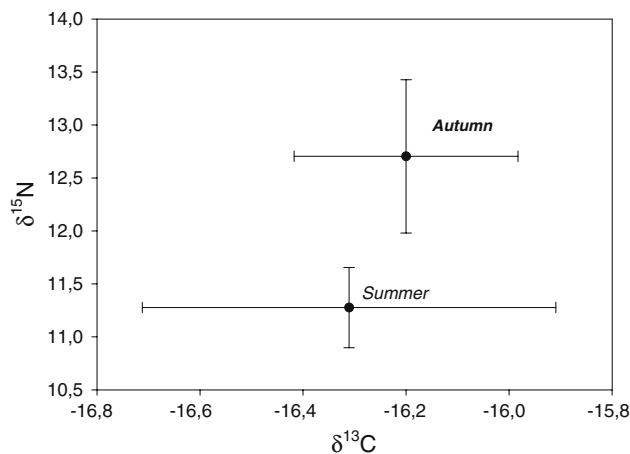
correlated (Mantel  $r$  statistic = 0.13,  $P = 0.07$ ; 10,000 permutations). When comparing the levels of  $\delta^{13}C$  and  $\delta^{15}N$  independently with the association index rates matrix (MAI), the matrices were significantly related for  $\delta^{13}C$  values ( $MED_2$ ) (Mantel  $r$  statistic = 0.17,  $P < 0.05$ ; 10,000 permutations), but not for  $\delta^{15}N$  values ( $MED_3$ ) (Mantel  $r$  statistic =  $-0.07$ ,  $P = 0.79$ ; 10,000 permutations).

## Discussion

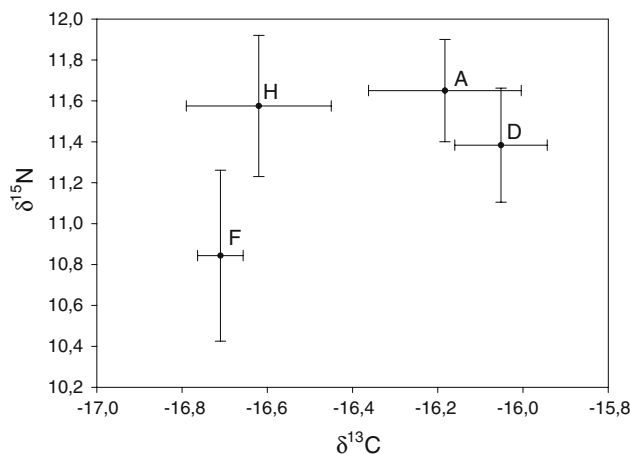
The absence of any change in the  $\delta^{13}\text{C}$  values between Summer and Autumn supports the hypothesis that long-finned pilot whales remain in the Strait of Gibraltar throughout the Summer and Autumn. This result is consistent with previous photo-identification work establishing that this community of pilot whales is resident year-round in the Strait of Gibraltar (de Stephanis 2008).

The change in the isotopic values of  $\delta^{15}\text{N}$  of pilot whales between the Summer and the Autumn could be due to pilot whales shifting prey species between Summer and Autumn. It is also possible that their prey species could experience a shift to a higher trophic level between Summer and Autumn due to a change in their prey's diet. The isotopic signature of the pilot whales could therefore reflect the change of diet of their consumed prey, and would explain the significant differences in  $\delta^{15}\text{N}$  amongst seasons. For nitrogen, significant enrichment in  $^{15}\text{N}$  occurs with trophic level, an effect that appears to be typically ranging between 1.7 and 4‰ with a mean value (generally skin, muscle or bones) close to 3‰ for cetaceans (Michener and Schell 1994; Abend and Smith 1997). The fact that the difference in the  $\delta^{15}\text{N}$  is only 1.41‰ suggests that if the change reflects a change in the prey species the whales are consuming, the shift is not a complete trophic level. However, the higher range observed in the  $\delta^{15}\text{N}$  isotopic signatures in Autumn compared to Summer, despite the smaller sample size in Autumn, could suggest that pilot whales feed on a broader range of prey species in Autumn compared to Summer. This possible change in diet is concomitant to a change in the spatial distribution of pilot whales through the Strait, with pilot whales foraging over a broader area in Autumn compared to Summer (de Stephanis 2008; Figs. 2, 3). Nevertheless, the possibility of a change in diet of the prey species of the pilot whales between seasons cannot be ruled out (Fig. 4).

Differences in isotope signature between social units of the same community and the fact that no differences between sexes were found suggest segregation by diet specialisation, or some differences in the water masses exploited, independently of their sexes. We can rule out the possible effect of the timing between biopsies on the stable isotope values obtained as samples were collected within 7 days and the stable isotope turnover rate of the skin is unlikely to vary much over such a short period of time. Closely associated animals were more likely to share a similar  $\delta^{13}\text{C}$  signature compared to individuals belonging to the same clan (de Stephanis et al. 2008b), but which associate less often. No information on  $\delta^{13}\text{C}$  is available regarding the possible prey species of the pilot whales in the Strait. Abend and Smith (1997) analysed the  $\delta^{13}\text{C}$  of possible preys of pilot whales in the Atlantic Ocean, and



**Fig. 4**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values with SD for long-finned pilot whales (both sexes) in the Strait of Gibraltar during the Summer 2006 and the Autumn 2005



**Fig. 5**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values with SD for the long-finned pilot units (for both sexes) A (4 samples; 50% of the unit sampled), D (3 samples; 100% of the unit sampled), F (3 samples; 33% of the unit sampled) and H (2 samples; 29% of the unit sampled) in the Strait of Gibraltar. The units are defined in de Stephanis et al. 2008b. The samples were taken between 25 July and 1 August 2006

found  $\delta^{13}\text{C}$  ranges  $-19.8$  to  $-22.5$ ‰ for Atlantic mackerel (*Scomber scombrus*) and  $-17.8$  to  $-19.7$ ‰ for long-finned squid (*Loligo pealei*) in the South mid-Atlantic. Those ranges are higher than the differences between groups observed in Fig. 5. Carbon isotope values are found to vary between benthic or inshore food webs relative to pelagic food webs (Hobson and Welch 1992; Hobson et al. 1994). The pilot whales are expected to have a matrilineal structure (Amos et al. 1991a, b, 1993a, b; Fullard 2000; Fullard et al. 2000); therefore, the age structure is expected to be similar for all groups. As the individuals were sampled independently to their size we do not expect any inference in the results due to the age of the individuals. The fact that no calf was sampled will eliminate possible bias due to a

maternal effect on our results. Therefore, these results tend to indicate that individuals of the same social units tend to exploit prey species in the same water masses, whereas the absence of differences in the  $\delta^{15}\text{N}$  values suggest that during Summer the type of preys did not vary between long lasting social units. One possible interpretation of these results would be that social groups tend to target different geographical locations or depths along a bathymetric gradient. As the Strait of Gibraltar is a highly stratified environment (Lacombe and Richez 1982; Echevarría et al. 2002) this may reveal that some pilot whale social units target prey species living in the different water masses (either Mediterranean, either Atlantic) within the Strait of Gibraltar.

The possible horizontal vs. vertical variation in the spatial distribution of pilot whales according to their social units should be investigated in future studies. The horizontal variation according to social units would be the easiest to investigate by looking at the horizontal distribution of individuals belonging to these different social units. If no differences are found, this would suggest that either there is some kind of a vertical segregation between social units, or that different social units tend to select different prey types (more likely to be different species, but may be targeting different age classes of the same prey species). The results of this study suggest that there is some level of social specialisation in habitat or prey choice between social units of pilot whales. Future studies should investigate if those differences between social units persist through the year and in particular in periods like Winter when diet appear to be much more diverse.

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