

Stable isotope profiles in sperm whale teeth: variations between areas and sexes

Sónia Mendes*[§], Jason Newton[†], Robert J. Reid[‡], Alexandros Frantzis[§] and Graham J. Pierce*

*School of Biological Sciences (Zoology), University of Aberdeen, Tillydrone Avenue, Aberdeen, AB24 2TZ, UK. [†]NERC Life Sciences Mass Spectrometry Facility, SUERC, East Kilbride, G75 0QF, UK. [‡]Wildlife Unit, SAC Veterinary Services, Drummondhill, Stratherrick Road, Inverness, IV2 4JZ, UK. [§]Pelagos Cetacean Research Institute, Terpsichoris 21, 16671 Vouliagmeni, Greece. [§]Corresponding author, e-mail: s.mendes@abdn.ac.uk

Sperm whale (*Physeter macrocephalus*) teeth were used to investigate whether variation in the chronological profiles of carbon and nitrogen stable isotope ratios along dentine growth layers could reflect differences in ontogenetic movements and/or dietary shifts in animals from different regions and sexes, as well as to show the differences in the isotopic environments experienced by these animals. Absolute isotopic ratios ranged from -14.1 to -11.0 ‰ for carbon and 10.8 to 18.1 ‰ for nitrogen, with the whale from the Indian Ocean, the two from the Mediterranean Sea and the female from the Azores presenting the most different median isotopic ratios. The Icelandic and the Indian Ocean males showed the expected decrease in $\delta^{13}\text{C}$ around the age of ten, denoting male segregation from natal groups. For the latter, this was larger by almost twofold compared to other teeth, probably due to the much stronger latitudinal gradient in planktonic $\delta^{13}\text{C}$ in the southern hemisphere. The Mediterranean Sea whales exhibited the lowest median $\delta^{15}\text{N}$ values, probably reflecting the oligotrophy of this sea, while the male showed a marked change in isotopes around the age of 20 that could indicate a move to the eastern basin or a temporal change in basal isotopic signatures. The Atlantic females did not show a marked change in $\delta^{13}\text{C}$ as expected since they stay in low latitudes throughout their lives. Stable isotope profiles in whale teeth can be used to investigate differences in the timings of ontogenetic movements and dietary history between individuals and sexes, and the biogeochemistry of different regions inhabited, and have the potential to allow inferences to be made about population substructure.

INTRODUCTION

Variations in the natural abundance of stable isotopes (SIs) of carbon and nitrogen have provided marine mammal ecologists with a tool to study individual and population differences in diet, movements, habitat use, and physiology. Several tissues have been analysed but incremental structures such as baleen and teeth, which are metabolically inert once formed, are particularly useful as they provide an archive of tissue that preserves the isotopic signatures of the foodwebs exploited and the timing of deposition. Thus, dietary, habitat and movement history can be studied by determining isotope ratios for different portions of the structure formed at different ages (Hobson & Sease, 1998; Lee et al., 2005).

The profiles of SI ratios of carbon ($\delta^{13}\text{C}$) and of nitrogen ($\delta^{15}\text{N}$) along teeth of male sperm whales (*Physeter macrocephalus*, Linnaeus 1758) stranded in Scotland showed trends and timings of change which seem to correspond closely with previously suggested ontogenetic changes in dietary habits, schooling behaviour and geographical occurrence (Mendes et al., in press). There was a marked decrease in $\delta^{13}\text{C}$ around the age of ten probably reflecting movement to the ^{13}C -depleted foodwebs of higher latitudes (Mendes et al., in press) as this timing coincides with male dispersal from their natal groups at the onset of puberty. This results in a striking geographical segregation of the sexes, with mixed groups

of female and young animals inhabiting low latitudes and juvenile and adult males ranging up to the edge of the pack ice (Rice, 1989). Adult males return periodically to lower latitudes to breed from around 25–27 years of age (Best, 1979). However, breeding migrations were not reflected in the carbon isotopic profiles, possibly due to short stays in lower latitudes compared to the time spent feeding in high latitudes (Mendes et al., in press).

The $\delta^{15}\text{N}$ profiles on the other hand showed an increase with age, mirroring the C isotopic changes. Although regional and seasonal differences in the basal $\delta^{15}\text{N}$ of marine foodwebs (Wada et al., 1987; Lesage et al., 2001; Post, 2002) may confound the interpretation of this increase, $\delta^{15}\text{N}$ is mostly used as an indicator of trophic position. Enrichments in ^{15}N of approximately 3.4‰ per trophic level result from the isotopic fractionation between diet and consumer, in particular the preferential excretion of the lighter isotope (Minagawa & Wada, 1984; Post, 2002). Hence, it was assumed that males feed at progressively higher trophic levels as they age and grow in size resulting probably from feeding on larger prey (Mendes et al., in press).

Sperm whales are the most sexually dimorphic in size of all cetaceans with males attaining 18 m in body length while female maximum size reaches 12 m (Rice, 1989). This sexual dimorphism is probably responsible for the geographical segregation of the sexes either due to different nutritional

Table 1. *Estimated age and number of samples of dentinal collagen taken for each sperm whale tooth.*

Sperm whale/ tooth ID	Year of capture/ stranding	Location	Sample source	Sex	Length		No. samples
					(m)	Final age estimate (years)	
MIce73	1973	Iceland, Atlantic Ocean 64°N 22°W	Captured	M	–	40	35a
MIce72	1972	Iceland, Atlantic Ocean 64°N 22°W	Captured	M	16.8	40	33a
MSt1905	1905	Shetland, Atlantic Ocean 61°N 01°W	Captured	M	–	33+2 (section not central)	33
FGa03	2003	Galicia, Atlantic Ocean 42°N 09°W	Stranded	F	11.0	40	40
FAz81	1981	Azores, Atlantic Ocean 39°N 29°W	Captured	F	–	22+2 (section not central)	22
MMed01	2001	Crete, Mediterranean 35°N 24°E	Stranded	M	12.9	42	42
FMed00	2000	Zakynthos, Mediterranean 37°N 20°E	Stranded	F	10.0	24	24
MSh15	1915	Seychelles, Indian Ocean 04°S 55°E	?	M	–	33+2 (section not central)	31
Scottish (Mendes et al., in press)	1993– 1998	Scotland (several locations)	Stranded	M	12.30– 15.65	19–48	–

a, lower resolution profiles as samples obtained after first age estimate, which was considerably lower than subsequent estimates.

and energetic demands between males and females, with males searching richer feeding grounds at higher latitudes (Best, 1979), or due to a disadvantage for the males, being excluded from superior quality habitats by the better adapted females (Whitehead, 2003). Sperm whales feed mainly on mesopelagic cephalopods and stomach content analyses have indeed indicated that diet composition may vary between sperm whale sexes, ages or body lengths and also between regions, seasons and years (e.g. Clarke et al., 1993). Profiles of SI ratio variation along teeth can potentially be used to discriminate between individuals or groups of whales with different dietary and habitat histories, in addition to the snap-shot information on recently ingested food that stomach contents provide. Variation in the isotopic signatures will be caused by two main factors: (a) animals foraging in habitats with different isotopic signatures reflecting biogeochemical processes occurring at the base of the food web; (b) animals foraging in isotopically similar habitats but where prey availability and/or dietary preferences may be different.

Sperm whales are distributed in all oceans of the world. Attempts were made, during whaling times, to sort sperm whale populations into distinct stocks, for managing exploitation (Dufault et al., 1999). This was made difficult by the complex social organization and behaviour of the species and by the extensive movements, particularly by adult males, which have resulted in a remarkable global genetic uniformity (Lyrholm et al., 1999). Although sperm whaling has mostly ended, it remains important, from an evolutionary, ecological and conservation perspective, to discern any existing geographical structure (Whitehead, 2003). Biogeographical variation in predation, environmental or anthropogenic conditions and food availability could cause differences in group sizes and distribution, growth rates and ontogenetic benchmarks, which could be reflected in the stable isotopic profiles along teeth. For example, differential growth in young males from different regions could cause different timings of segregation, since their departure from natal groups seems to be linked to a burst in growth rate and the attainment of a certain body size (Best, 1979).

Here we analysed the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ along teeth of male and female sperm whales from different geographical

regions. We investigated whether: (1) isotopic ratios and their trends along the teeth varied between regions and individuals; (2) ontogeny-related movements and trophic ecology shifts, as were found in males stranded in Scotland (Mendes et al., in press), could be detected in whales from other regions/sexes; (3) the biogeochemical environment inhabited throughout an individual's life would be reflected in the isotopic composition of this top-predator's teeth.

MATERIALS AND METHODS

Sperm whale teeth ($N=8$) were obtained from museums and institute collections. Teeth were of three adult males from the high latitudes of the North Atlantic, one from the Mediterranean and one from the Indian Ocean and of two females from the North Atlantic and one from the Mediterranean, caught or stranded between 1905 and 2003 (Table 1). Teeth were sampled along the growth layers and analysed for C and N stable isotope ratios in order to obtain chronological profiles with age.

Tooth preparation

Each tooth was mounted onto a wood base using epoxy adhesive and cut in half along the longitudinal axis using a slow rotating diamond saw. A thin section (1–3 mm) was sliced off one of the halves and further bisected with a bandsaw to yield two symmetrical portions. One portion was used for age-estimation and was accordingly etched with formic acid (Evans & Robertson, 2001). The other portion was used for SI measurements, and was decalcified to remove inorganic C using HCl (0.5 N) for at least a week, until it became flexible. At that point only the organic portion of the tooth remained, composed of collagen.

Age estimation

The age of each individual was estimated by counting dentinal growth layer groups (GLGs, tissue laid down in one year) in the etched half of the section. Three or more (if the first readings were inconsistent) independent age estimates were carried out by the same reader, spaced at intervals of over a week between readings. The final age-estimate for

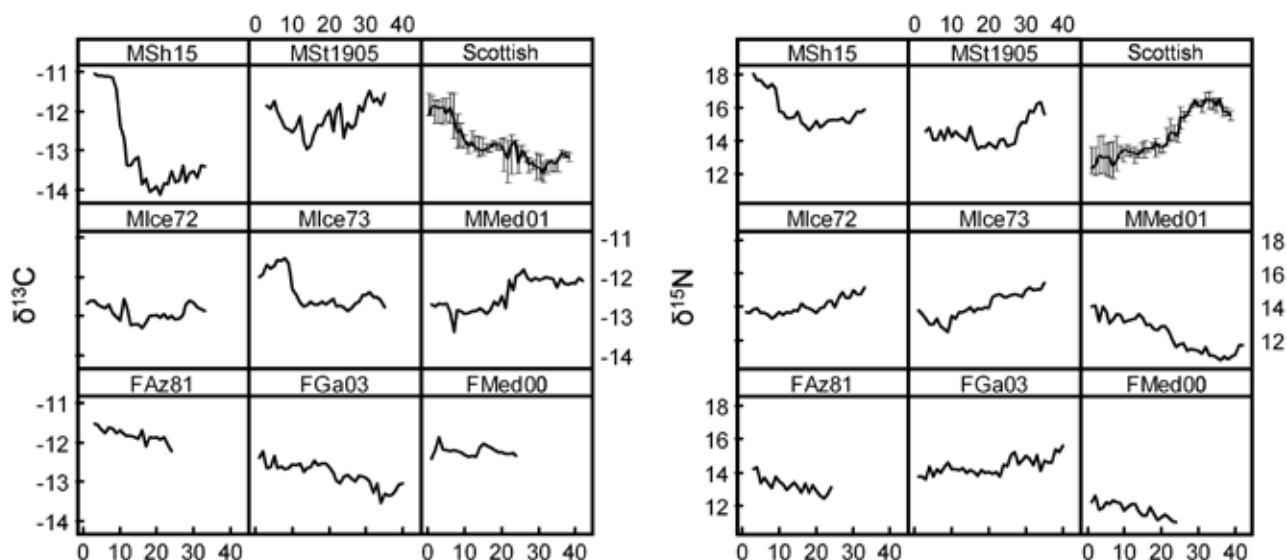


Figure 1. Profiles of stable isotope ratios in teeth with age for each individual whale. The profiles of Mice72 and Mice73 were stretched in the older layers to account for the discrepancy between the number of samples and the age estimated. The profiles labelled 'Scottish' show the median isotope ratios of all Scottish teeth analysed (Mendes et al., in press); the error bars represent the IQR.

each tooth (Table 1) was either the most repeated GLG count (3 teeth) or the mean of the three most similar counts (5 teeth) (following Evans et al., 2002). There was typically a difference of 1–3 y between successive individual tooth readings. Some tooth sections (3 teeth) did not present the whole axis because the tooth was cut slightly off-centre, hence losing some of the apex layers representing the earliest ages. By visually inspecting the apex of both the thin section and the other tooth half it was estimated that at least two layers had been missed out. Thus the age estimates of these sections consisted of the number of GLGs counted plus two. The estimated ages for sperm whales investigated here ranged between 34 and 42 y for males and were 24 for two of the females and 40 for the other (Table 1).

There is a degree of subjectivity in the interpretation of growth layers in teeth (Evans et al., 2002) due to poor definition, accessory layers, mineralization interferences and anomalies (Lockyer, 1995), which will inevitably result in variation of estimates between readers and between readings by the same reader. For the purpose of this study, any biases would have been consistent as teeth were read by the same person and so we assume that the profiles will be directly comparable.

Teeth sampling and lipid extraction

The dentinal collagen in the decalcified half-section of each tooth was cut from the apex to the pulp cavity with a scalpel, to obtain one sample per GLG. For the decalcified half-sections where we could not distinguish the GLGs clearly, we used the etched half-section as a guide to where to cut the samples. All teeth were sampled based on a single age reading, i.e. the number of GLGs counted at the time of sampling and prior to all subsequent readings and final age estimate, which resulted, for three teeth, in a discrepancy between the number of samples and the age estimated. If the final estimate were assumed to be closer to the true age of the animal, then some GLGs along the tooth profile would

have been either sampled together or sampled twice. This translated into five and seven samples short for teeth Mice73 and Mice72, respectively and two samples short for MSh15. For the former two teeth, the last few isotopic values were assigned to two ages each in order to complete the gaps up to the estimated age for a more realistic profile. These two teeth were difficult to read due to the amount of accessory lines, particularly for older ages, hence the large discrepancy. In addition, the first sample taken from the three sections that did not present the whole tooth axis was considered to have been deposited at three years of age.

Samples were delipidified by placing them in glass vials and washing in successive rinses in a 1.0:2.0:0.8 ratio solution of chloroform: methanol: water, following the method of Bligh & Dyer (1959), until the chloroform phase was clear. The samples were then rinsed and sonicated several times with deionized water and freeze-dried.

Stable isotope analysis

Approximately 0.8 mg of each sample was placed into a tin capsule and the C and N isotope analyses performed simultaneously using continuous-flow isotope ratio mass spectrometry (CF-IRMS). All stable isotope ratios are expressed in permil (‰) deviations from primary international standards, using the delta (δ) notation. Replicate measurements of internal laboratory standards (gelatine) indicate a precision of 0.1 and 0.2‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. The C:N ratio for all samples varied between 3.03 and 3.43—well within the acceptable values that guarantee the purity of collagen and allow for comparison between samples (De Niro, 1985).

RESULTS AND DISCUSSION

Previous studies have used the phenotypic record provided by marine mammal teeth to study habitat, diet and, particularly, life history events, and to infer variation between

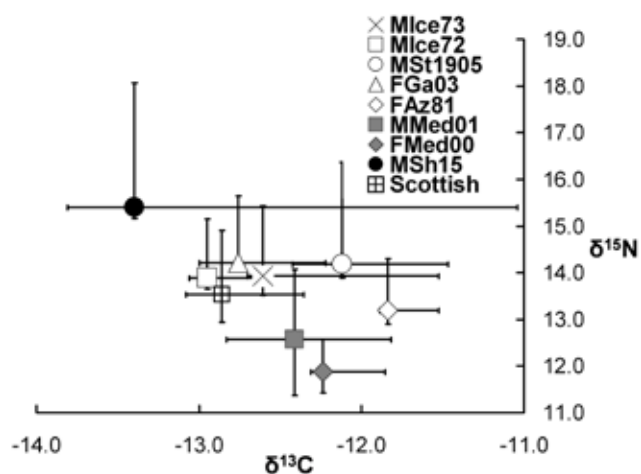


Figure 2. Carbon and nitrogen stable isotope ratio signatures for each sperm whale tooth (‰; Median \pm IQR). For the Scottish teeth a combined median of all teeth is represented.

animals from different geographical regions (Lockyer, 1995, 1999). In this study we have used the geochemical record preserved in sperm whale teeth, and looked for variation in C and N SI ratios that could reflect ontogenetic movements and/or dietary shifts in animals from different regions and sexes as well as indicate differences in the isotopic environment experienced by these animals. The SI ratios showed relevant differences amongst individuals, regions and within different life stages for some individuals; these are discussed below.

All of the teeth showed very different trends in the C and N isotope ratio profiles with age (Figure 1). Absolute isotopic ratios ranged from -14.1 to -11.0 ‰ and 10.8 to 18.1 ‰, for C and N respectively, with a larger range of values for the latter element, both within each tooth and amongst teeth (Figure 2; Table 2). The teeth from the Indian Ocean and from the two Mediterranean whales and the female caught in the Azores presented the most different median signatures of all teeth, at either side of the spectrum, while the other teeth had similar medians to the teeth of the whales stranded in Scotland (Mendes et al., in press; Figure 2).

Indian Ocean male (MSh15)

The Indian Ocean male had the highest values for $\delta^{15}\text{N}$ and the lowest median for $\delta^{13}\text{C}$ of all teeth (Figure 2; Table 2). The large inter-quartile intervals (IQR) in its isotopic values denote also the greatest spread of values. It showed the highest value of all teeth for $\delta^{15}\text{N}$ in the oldest GLG, representing the collagen deposited in the year before death, even though it declined 2.2 ‰ from its early age. It also presented the highest value of $\delta^{13}\text{C}$ for the youngest GLG, but conversely presented the lowest value of $\delta^{13}\text{C}$ for the oldest GLG, with a 2.4 ‰ difference. The sharp depletion in ^{13}C and ^{15}N occurred between the ages of 8 to 13 y, which is consistent with the pattern found for the 'Scottish teeth' (Mendes et al., in press) (Figure 1), and probably reflects the onset of pubertal male dispersal from the natal groups into higher latitudes. This decrease was larger by almost twofold than seen for the other teeth analysed here or in the previous study. Lower-latitude plankton food bases tend to be enriched in both ^{13}C and ^{15}N relative to higher-latitude waters in the Southern

Ocean (Wada et al., 1987), with a much stronger latitudinal gradient in planktonic $\delta^{13}\text{C}$ than in the northern hemisphere (Goericke & Fry, 1994). In the Indian Ocean in particular, both ^{13}C and ^{15}N of particulate organic matter (POM) show major depletion from north to south, between 40°S and 45°S , in the vicinity of the Subtropical Front (Francois et al., 1993). In addition, higher ^{15}N enrichment in nitrate is expected in the Indian Ocean when compared to the Atlantic due to a more prevalent denitrification in the former, which could then be reflected in enriched values for foodwebs, and could explain the higher overall $\delta^{15}\text{N}$ values presented by this whale's tooth.

Mediterranean male (MMed01) and female (FMed00)

The whales from the Mediterranean exhibited the lowest median $\delta^{15}\text{N}$ values of all whales in the present study. The female showed slightly decreasing $\delta^{15}\text{N}$ values (12 to 11‰) throughout life until it stranded at the age of 24 y, and a fairly steady profile for $\delta^{13}\text{C}$ with values ranging mostly between -12.0 and -12.5 ‰. Both isotopic profiles seem to suggest that this whale did not undertake large-scale movements, possibly remaining in the same region and feeding at a similar trophic level throughout life.

The male had a marked shift in isotopic ratios around the age of 20 y with values becoming close to those recorded in the female thereafter and until death at 42. After starting with the lowest $\delta^{13}\text{C}$ value of all teeth in the youngest GLG, the values ranged mostly between -13.00 and -12.5 ‰ until the age of 20 when a steep increase of around 1 ‰ occurred. This was concurrent with depletion in ^{15}N , which resulted in the strikingly low value of $\delta^{15}\text{N}$ in the last GLG, having decreased 2.3 ‰ from the youngest GLG to the oldest (Figure 2; Table 2). This timing and trend differs from the profiles of all other males in the present and previous study (Mendes et al., in press). The marked isotopic change in the male around 20 years of age could indicate a movement from the western to the eastern basin of the Mediterranean Sea. There is a high regional heterogeneity of water temperature, salinity and primary production in this Sea, which is likely to result in geographical variability in the basal isotopic signatures of foodwebs. The $\delta^{15}\text{N}$ of phytoplankton, for example, is higher in the western compared to the eastern basin (Pantoja et al., 2002), which could account for the decrease in $\delta^{15}\text{N}$ if the whale had moved between basins. The depletion of ^{15}N in organic matter of oligotrophic waters is one of the strongest patterns of nitrogen isotopic variation in marine systems (Wada & Hattori, 1976). The Mediterranean Sea, and particularly the eastern basin, is considered to be one of the most oligotrophic regions in the world (Psarra et al., 2000), which could also explain both animals' depleted signature in comparison to all others analysed here and in the previous study. The concomitant ^{13}C enrichment in the male profile could also reflect east/west differences in the isotopic composition of the C source (Pierre, 1999).

Although sperm whales in the Mediterranean Sea show some genetic differentiation from sperm whales of the Atlantic, which is consistent with the notion of a resident sperm whale population in the Mediterranean Sea (Drouot, 2003), information on feeding ecology and migratory habits within this Sea is very scarce. In particular, the ontogenetic dispersal of males, generally observed in different oceans,

Table 2. Results of stable isotope ratio analyses of carbon and nitrogen on dentinal collagen of sperm whale teeth.

Sperm whale/ tooth ID	$\delta^{13}\text{C}$ (‰)				$\delta^{15}\text{N}$ (‰)			
	First layer	Last layer	Last layer–first layer	Median (IQR)	First layer	Last layer	Last layer–first layer	Median (IQR)
MIce73	–12.0	–12.8	–0.8	–12.6 (–12.7 to –11.5)	13.8	15.4	1.6	13.9 (13.5 to 15.4)
MIce72	–12.7	–12.9	–0.2	–12.9 (–13.1 to –12.6)	13.7	15.1	1.4	13.9 (13.7 to 15.1)
MSt1905	–11.9	–11.6	0.3	–12.1 (–12.4 to –11.5)	14.6	15.6	1.0	14.2 (13.9 to 16.4)
FGa03	–12.4	–13.0	–0.6	–12.8 (–13 to –12.2)	13.8	15.7	1.9	14.2 (14.0 to 15.6)
FAz81	–11.5	–12.2	–0.7	–11.8 (–11.9 to –11.5)	14.2	13.1	–1.1	13.2 (12.9 to 14.3)
MMed01	–12.7	–12.1	0.6	–12.4 (–12.8 to –11.8)	14.0	11.7	–2.3	12.6 (11.4 to 14.1)
FMed00	–12.4	–12.3	0.1	–12.2 (–12.3 to –11.9)	12.2	11.0	–1.2	11.9 (11.4 to 12.6)
MSh15	–11.0	–13.4	–2.4	–13.4 (–13.8 to –11.0)	18.1	15.9	–2.2	15.4 (15.2 to 18.1)
Scottish (all teeth)	–	–	–	–12.86 (–13.17 to –12.36)	–	–	–	13.53 (12.94 to 14.91)

is not obvious in this population and it might even vary a lot based on different sub-regions of this Sea and so it is not possible to infer timing and geographical direction of movements based on only one male and one female. Another plausible explanation for the trends observed could be that the animal remained in the same area throughout life but the basal planktonic isotopic signatures in the area varied through time, with a particularly strong shift around 1980 (when the male was 20–21). When we compare the isotopic values in the dentine samples of both whales since the birth year of the female (1977) until 2000–2001 when the whales stranded, which for the male it will correspond to 18–41 y of age, we see some similarity in general trends and absolute values although not such marked changes occur for the female around 1980 (Figure 1). Studies on salmon scales and whale baleen across several years have suggested that temporal changes in N and C isotopic compositions at the base of foodwebs caused by changes in primary productivity or nutrient cycling might be responsible for the trends observed in the isotopic signatures of predators (Schell, 2000; Satterfield & Finney, 2002). In order to be able to discriminate between the two potential causes for the marked isotopic change, more teeth from males and females that stranded in the Mediterranean at ages older than 20 y, having lived through different decades, should be analysed.

Icelandic males (MIce73, MIce72)

The two males caught in Iceland in the 1970s had similar isotopic profiles, with identical median isotope ratios, particularly for N, presenting a gradual enrichment in ^{15}N with age, larger than 1‰ (Figure 1), which is consistent with what was found in the previous study (Mendes et al., in press) and the expected increase in trophic level as the animal grows in size. Sperm whales caught off Iceland

were found to have mostly fish in their stomachs (Martin & Clarke, 1986). However, this apparent difference in diet does not seem to be reflected in differences in the N isotopic signature compared to other teeth, which might indicate that these animals were feeding at the same trophic level as the animals occurring off Scotland. Because the timings of change in $\delta^{15}\text{N}$ did not coincide with those in $\delta^{13}\text{C}$ it is unlikely that changes in $\delta^{15}\text{N}$ are related to movements between different basal signature foodwebs.

The male caught in 1973 (MIce73) showed a marked decrease in $\delta^{13}\text{C}$ between 8 and 12 years of age, a similar pattern to the ‘Scottish teeth’ (Mendes et al., in press) (Figure 1), and again probably reflecting the onset of dispersal from the natal groups into higher latitudes. The other male (MIce72) showed a highly variable $\delta^{13}\text{C}$ profile, with a slight decrease in $\delta^{13}\text{C}$ from 7 to 10 years of age but tended towards an increase as the animal aged. This smaller decrease perhaps indicates a shorter latitudinal movement and suggests that this animal might have been born at higher latitudes than the other animal caught in Iceland, as it also presents a more depleted ^{13}C value in the youngest GLG (Table 2). These animals had similar C signatures in the last GLG, representative of the oldest age, reflecting the fact that they were caught in the same area.

Both whales showed a slight increase in $\delta^{13}\text{C}$ around the age of 25. This could be related to the animals attaining full sexual maturity and starting to migrate back to breeding latitudes, residing and foraging for sufficient time at lower latitudes as to enrich their isotopic signature. This is in contrast with the previous study, where $\delta^{13}\text{C}$ signatures remained low, possibly due to breeding migrations being very short in duration and animals not feeding regularly (Mendes et al., in press). Because these animals were of similar age when caught, other factors such as a change in the basal

$\delta^{13}\text{C}$ could be responsible for the trend observed. Long-term trends in basal $\delta^{13}\text{C}$ can be related to variations in phytoplankton growth rate or species composition and in the isotopic composition of dissolved inorganic C (Schell, 2000).

Shetland male (MS1905)

The male caught in Shetland in 1905 showed very different trends in profiles although the absolute values were comparable to other animals. It had a highly variable $\delta^{13}\text{C}$ profile (Figure 1). It started with a $\delta^{13}\text{C}$ value close to those of the other whales, and began gradually decreasing after the age of six, but the pattern thereafter does not correspond to any other teeth's patterns, with its highly variable profile and an increase back to values seen at early ages. It presented a sharp enrichment in ^{15}N after the age of 28 years. This whale had higher values of both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the youngest GLG than the other four Atlantic whales, apart from the female caught in the Azores (Table 2), which had similar values. It is possible that these two whales were born at the same latitude and fed at a similar trophic level in the younger ages.

The tooth of this animal had a striking morphology; large and numerous pulp stones were present in the dentine all along the tooth's axis. These mineralization anomalies are discrete nodules originating in the pulp cavity and becoming embedded in the dentine (Perrin & Myrick, 1980), and their existence might be related to environmental factors, physiological or nutritional anomalous events (Lockyer, 1995). Investigations linking both phenotypic and geochemical records could provide further insights into the hypothesis that the anomalous isotopic trends found here could be the reflection of those events.

Atlantic females (FAz81, FGa03)

Both females showed quite constant $\delta^{13}\text{C}$ profiles, with a slight depletion in ^{13}C occurring for the female stranded in Galicia (FGa03) after 20 years of age. The fairly constant $\delta^{13}\text{C}$ profiles of the two females analysed here confirm that indeed the ^{13}C depletion observed with age in males corresponds to the known ontogenetic latitudinal move, since females are not generally expected to disperse from the natal groups and, if they do, it is only temporary and they remain in the same area (Best, 1979). Female choice of habitat might be influenced by reproductive state, predation and prey availability, and although extensive movements of females within oceans have been documented, they seem to be much less predominant than in males and even some between-year fidelity to local areas has been shown (Best, 1979). However, long-term fidelity by groups to very specific geographical areas is not the pattern for sperm whales in general (Gordon, 1987) and the small variation seen in the $\delta^{13}\text{C}$ profiles might indicate small-scale movements. The female caught in the Azores (FAz81) had the highest median $\delta^{13}\text{C}$ of all whales, reflecting the fact that this animal was caught in lower latitudes than the others, and probably lived all its life in subtropical and tropical areas which are comparatively enriched in ^{13}C .

These females presented very different $\delta^{15}\text{N}$ profiles in the first 24 years of age, with that of the Galician female remaining fairly constant and the one from the Azorean female showing a depleting trend (Figure 1). The

Galician female showed ^{15}N enrichment after the age of 23 accompanied by an increased variability, and presented the highest median value for $\delta^{15}\text{N}$ of all Atlantic whales (Table 2). The Azorean female presented the third lowest median $\delta^{15}\text{N}$, after the Mediterranean whales. It is found, especially in species that are sexually dimorphic in body size with males growing larger, that males are more ^{15}N -enriched than females, with the difference between the sexes increasing with age and reflecting a difference in trophic level and possibly of size of prey consumed (Lesage et al., 2001). Although the female caught in the Azores did indeed present the lowest $\delta^{15}\text{N}$ values of all the Atlantic whales, the other female had the highest median $\delta^{15}\text{N}$. This was mainly due to higher values at younger ages, when compared to the male whales. Nevertheless, this whale, after a marked enrichment in ^{15}N occurring after the age of 23, attained comparable values to males of similar ages. This enrichment occurred after a slight depletion in ^{13}C was observed; possibly indicating a small-scale movement towards a ^{15}N enriched foodweb. Another plausible explanation could be that the prey consumed in this area was enriched in ^{15}N due to it belonging to a higher trophic level.

Stomach content analyses of whales caught in the Azores (2 females and 15 males) (Clarke et al., 1993), and off Galicia (Clarke & MacLeod, 1974) (1 male) showed that the large-sized species *Taningia danae* Joubin, 1931 contributed to 83% of the total weight in the latter sample but only comprised 39% overall in the whales off Azores, which mainly consumed smaller sized prey. If we assume that the sample from Galicia is representative of the food eaten by whales in the area, then it is possible that these animals might be feeding at higher trophic levels due to feeding on squid of a species that has large specimens. Additionally, the very low $\delta^{15}\text{N}$ values found for FAz81 are characteristic of the oligotrophic tropical and subtropical waters of the North Atlantic (Montoya et al., 2002), so it is likely that the differences observed between the two females reflect mainly a regional difference in the foodwebs' basal $\delta^{15}\text{N}$.

CONCLUSIONS AND FUTURE WORK

Here we have provided a preliminary investigation into how variation in C and N stable isotope profiles with age in whale teeth collagen might reflect individual and regional diversity in ontogeny, physiology and geochemical environment inhabited, even though some of the variability found remains unexplained and the main source of isotopic variation is often difficult to pinpoint. For animals that might move between isotopically distinct foodwebs throughout their lives, the comparison of trophic levels between animals or life-stages of an individual needs to be made with caution. Stable isotope profiles in whale teeth can be used to investigate differences in the timings of ontogenetic movements and dietary history between individuals and sexes, and to detect spatial and temporal differences in the biogeochemistry of different regions inhabited. It will be interesting to expand this preliminary study to a wider analysis of male and female teeth from different oceans, including animals that lived in different decades. Stable isotopes in teeth might help to infer population substructure in sperm whales, by looking for differences in ontogenetic movements and trophic ecology.

Ultimately, the linkage between region and life-stage of different portions of the sperm whale population might allow for a better assessment of the consequences of human impact and the development of more specific conservation measures.

We thank Colin MacLeod, Patricia Lastra, Ruth Fernandez, Caroline Weir and Lisa Pritt at the University of Aberdeen, for help with sample preparation and useful comments on the manuscript. We also thank two anonymous referees for their comments. We thank Ana Fernandes (IPIMAR, Lisbon), Jerry Herman (National Museums of Scotland), Juan Ignacio (Sociedade Galega de História Natural), Richard Sabin (The Natural History Museum, London), and Gísli Víkingsson (Marine Research Institute, Iceland) for the provision of some of the teeth analysed. S.M. was supported through a PhD studentship by the Portuguese Foundation for Science and Technology (grant SFRH/BD/5466/2001), and the stable isotope analyses were carried out at the NERC Life Sciences Mass Spectrometry Facility (application no. EK74-11/04).

REFERENCES

- Best, P.B., 1979. Social organization in sperm whales, *Physeter macrocephalus*. In *Behaviour of marine animals* (ed. H.E. Winn and B.L. Olla), pp. 227–289. New York: Plenum Press.
- Bligh, E.G. & Dyer, W.J., 1959. A rapid method for total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, **37**, 911–917.
- Clarke, M.R. & MacLeod, N., 1974. Cephalopod remains from sperm whales caught off Vigo, Spain. *Journal of the Marine Biological Association of the United Kingdom*, **54**, 959–968.
- Clarke, M.R., Martins, H.R. & Pascoe, P., 1993. The diet of sperm whales (*Physeter macrocephalus* Linnaeus 1758) off the Azores. *Philosophical Transactions of the Royal Society B*, **339**, 67–82.
- De Niro, M.J., 1985. Post-mortem preservation and alteration of *in vivo* bone collagen isotope ratios in relation to paleodietary reconstruction. *Nature, London*, **317**, 806–809.
- Drouot, V., 2003. *Ecology of sperm whale (Physeter macrocephalus) in the Mediterranean Sea*. PhD thesis, University of Wales, Bangor, UK.
- Dufault, S., Whitehead, H. & Dillon, M., 1999. An examination of the current knowledge on the stock structure of sperm whales (*Physeter macrocephalus*) worldwide. *Journal of Cetacean Research and Management*, **1**, 1–10.
- Evans, K., Hindell, M., Robertson, K., Lockyer, C. & Rice, D., 2002. Factors affecting the precision of age determination of sperm whales (*Physeter macrocephalus*). *Journal of Cetacean Research and Management*, **4**, 193–202.
- Evans, K. & Robertson, K., 2001. A note on the preparation of sperm whale (*Physeter macrocephalus*) teeth for age determination. *Journal of Cetacean Research and Management*, **3**, 101–107.
- Francois, R., Altabet, M.A., Goericke, R., McCorkle, D.C., Brunet, C. & Poisson, A., 1993. Changes in the $\delta^{13}\text{C}$ of surface-water particulate organic matter across the subtropical convergence in the SW Indian Ocean. *Global Biogeochemical Cycles*, **7**, 627–644.
- Goericke, R. & Fry, B., 1994. Variations of marine plankton $\delta^{13}\text{C}$ with latitude, temperature, and dissolved CO_2 in the World Ocean. *Global Biogeochemical Cycles*, **8**, 85–90.
- Gordon, J.C.D., 1987. *Behaviour and ecology of sperm whales off Sri Lanka*. PhD thesis, University of Cambridge, Cambridge, UK.
- Hobson, K.A. & Sease, J.L., 1998. Stable isotope analyses of tooth annuli reveal temporal dietary records: an example using Steller sea lions. *Marine Mammal Science*, **14**, 116–129.
- Lee, S.H., Schell, D.M., McDonald, T.L. & Richardson, W.J., 2005. Regional and seasonal feeding by bowhead whales *Balaena mysticetus* as indicated by stable isotope ratios. *Marine Ecology Progress Series*, **285**, 271–287.
- Lesage, V., Hammill, M.O. & Kovacs, K.M., 2001. Marine mammals and the community structure of the Estuary and Gulf of St Lawrence, Canada: evidence from stable isotope analysis. *Marine Ecology Progress Series*, **210**, 203–221.
- Lockyer, C.H., 1995. A review of factors involved in zonation in odontocete teeth, and an investigation of the likely impact of environmental factors and major life events on harbour porpoise tooth structure. *Report of the International Whaling Commission, Special Issue* **16**, 511–529.
- Lockyer, C.H., 1999. Application of a new method to investigate population structure in the harbour porpoise, *Phocoena phocoena*, with special reference to the North and Baltic Seas. *Journal of Cetacean Research and Management*, **1**, 297–304.
- Lyrholm, T., Leimar, O., Johannesson, B. & Gyllenstein, U., 1999. Sex-biased dispersal in sperm whales: contrasting mitochondrial and nuclear genetic structure of global populations. *Proceedings of the Royal Society B*, **266**, 347–354.
- Martin, A.R. & Clarke, M.R., 1986. The diet of sperm whales (*Physeter macrocephalus*) captured between Iceland and Greenland. *Journal of the Marine Biological Association of the United Kingdom*, **66**, 779–790.
- Mendes, S., Newton, J., Reid, R.J., Zuur, A.F. & Pierce, G.J., in press. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ profiling of sperm whale teeth reveals ontogenetic movements and trophic ecology. *Oecologia*, DOI 10.1007/s00442-006-0612-z
- Minagawa, M. & Wada, E., 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochimica et Cosmochimica Acta*, **48**, 1135–1140.
- Montoya, J.P., Carpenter, E.J. & Capone, D.G., 2002. Nitrogen fixation and nitrogen isotope abundances in zooplankton of the oligotrophic North Atlantic. *Limnology and Oceanography*, **47**, 1617–1628.
- Pantoja, S., Repeta, D.J., Sachs, J.P. & Sigman, D.M., 2002. Stable isotope constraints on the nitrogen cycle of the Mediterranean Sea water column. *Deep-Sea Research I—Oceanographic Research Papers*, **49**, 1609–1621.
- Perrin, W.F. & Myrick, A.C., 1980. *Age determination of toothed whales and sirenians*. Cambridge: International Whaling Commission.
- Pierre, C., 1999. The oxygen and carbon isotope distribution in the Mediterranean water masses. *Marine Geology*, **153**, 41–55.
- Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods and assumptions. *Ecology*, **83**, 703–718.
- Psarra, S., Tselepidis, A. & Ignatiades, L., 2000. Primary productivity in the oligotrophic Cretan Sea (NE Mediterranean): seasonal and interannual variability. *Progress in Oceanography*, **46**, 187–204.
- Rice, D., 1989. The sperm whale *Physeter macrocephalus* Linnaeus 1758. In *Handbook of marine mammals* (ed. S.H. Ridgway and R. Harrison), pp. 177–233. London: Academic Press.
- Satterfield, F.R. & Finney, B.P., 2002. Stable isotope analysis of Pacific salmon: insight into trophic status and oceanographic conditions over the last 30 years. *Progress in Oceanography*, **53**, 231–246.
- Schell, D.M., 2000. Declining carrying capacity in the Bering Sea: isotopic evidence from whale baleen. *Limnology and Oceanography*, **45**, 459–462.
- Wada, E. & Hattori, A., 1976. Natural abundance of ^{15}N in particulate organic matter in the North Pacific Ocean. *Geochimica et Cosmochimica Acta*, **40**, 249–251.
- Wada, E., Terazaki, M., Kabaya, Y. & Nemoto, T., 1987. ^{15}N and ^{13}C abundances in the Antarctic Ocean with emphasis on the biogeochemical structure of the food web. *Deep-Sea Research Part a—Oceanographic Research Papers*, **34**, 829–841.
- Whitehead, H., 2003. *Sperm whales: social evolution in the ocean*. Chicago: University of Chicago Press.

Submitted 6 October 2006. Accepted 3 December 2006.

