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NOTE

Sperm whale feeding variation by location, year, social group and clan: evidence from stable isotopes

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ABSTRACT: We studied the diet of sperm whales *Physeter macrocephalus* by measuring carbon and nitrogen isotope ratios in 106 sloughed skin samples and 10 squid beaks (*Histioteuthidae* spp.), the latter collected from sperm whale defecations. Samples were collected during 8 studies conducted between 1989 and 2000 in the South Pacific Ocean. We examined diet variation across region, year, social group and vocal clan. The isotopic signatures of groups and acoustic clans of sperm whales were compared using a nested analysis of variance (ANOVA) and the absolute distances between each pair of samples were calculated. The $\delta^{13}C$ and $\delta^{15}N$ values ranged from –17.8 to –14.5‰ and from 8.5 to 22.3‰, respectively. The $\delta^{15}N$ values of defecated squids were about 3‰ lower than values of the sperm whale, corresponding to a trophic difference of one level. There was a significant difference in both the $\delta^{15}N$ and $\delta^{13}C$ values between groups (nested within clans and the studies) and clans (nested within studies). Most of the variation was between studies. The latitude at which the samples were collected was inversely related to the $\delta^{15}N$ values. We suggest that the differences in diet between the groups from different clans are mainly caused by characteristic behaviour of the clans and differential use of micro-habitats; i.e. groups from a clan with a generally more benthic or inshore distribution had higher $\delta^{13}C$ than groups with a more offshore or pelagic influence, a general characteristic of this isotope in marine habitats.

KEY WORDS: Culture · *Physeter macrocephalus* · Carbon · Nitrogen · *Histioteuthidae* spp. squid

INTRODUCTION

The sperm whale *Physeter macrocephalus* forages in the deep waters of the world's oceans, primarily on meso- and bathy-pelagic cephalopods (Kawakami 1980, Smith & Whitehead 2000, Whitehead 2003). Recently, the analysis of naturally occurring carbon and nitrogen isotope ratios has become an important tool for studying diet (Kelly 2000), and has been successfully applied to several cetacean species (Abend & Smith 1997, Hooker et al. 2001, Lesage et al. 2001, Lee et al. 2005) including sperm whales (Ruiz-Cooley et al. 2004). This technique integrates diet over a period of roughly 2 mo (Ruiz-Cooley et al. 2004).

Sperm whale populations are strongly structured both socially, with females living in long-term social units (Whitehead & Weilgart 2000) and, possibly, culturally, with social units assignable to vocal clans based on their production of short patterns of clicks (termed codas; Rendell & Whitehead 2003). Groups

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from different clans seem to differ in their movement patterns and in the way they use their habitat. Moreover, differences in feeding success between clans have been observed (Whitehead & Rendell 2004), but it is unclear whether these dissimilarities reflect different diets or different ways of hunting prey. We wanted to test whether the dietary variation of South Pacific sperm whales maps onto social-cultural as well as geographic divisions. We used the stable isotopes ratios in sloughed skin samples to examine how the diet of sperm whales varies with region, year, clan, and social group. We also measured the same ratios in a number of squid beaks from fecal samples to understand how sperm whale isotope ratios relate to those of their presumptive, predominant prey in this region of the Pacific Ocean.

MATERIALS AND METHODS

Collection of samples. While in the proximity of sperm whale groups, naturally-sloughed skin samples were collected from 10 to 13 m auxiliary sailing vessels using a dip net, or by snorkellers (Whitehead et al. 1990), off northern Chile in 1993 and 2000, off the Galápagos Islands in 1989, 1991 and 1995, off Peru in 1993, and in the Southwest Pacific in 1992 (Fig. 1). If samples were not from photographically-identified individuals within a group, we used samples of the group from different days or if not possible, from different encounters during the same day (to minimize the chance of analyzing the skin of the same individual). Squid beaks (*Histioteuthis* spp.) were gathered from sperm whale feces off the Galápagos in 1989 and 1991 and Chile 1993 using the same collection techniques as for skin.

Storage and preparation of the samples. Most of the skin samples were preserved in dimethyl sulfoxide (DMSO) solution. Squid beaks were preserved in ethanol solution. All samples were prepared following a technique commonly used for marine mammals (e.g. Das et al. 2000, 2003, Van de Vijver et al. 2003). The samples were first rinsed with distilled water, then the pieces of skin were lipid extracted by several rinses with a 2:1 mixture of chloroform and methanol for 24 h. Samples were then dried at 50°C for 48 h and ground into a homogenous mixture. Isotopic analysis was completed by the Sinlab at University of New Brunswick and the G. G. Hatch Isotope Laboratory at University of Ottawa. At both laboratories, the isotopic composition was determined by combustion in a Carlo Erba NC2500 Elemental Analyzer followed by a gas chromatograph separation and an analysis by continuous-flow with a Delta XP or a Thermo-Finnigan Delta Plus isotope ratio mass spectrometer. The standard used for the carbon measurements were Pee-Dee Belemnite carbonate (Craig 1957), and for the nitrogen, atmospheric nitrogen (Mariotti 1983) was used. The typical precision obtained by repeated analyses of primary standards at both laboratories was approximately ±0.2‰, for both $\delta^{13}C$ and $\delta^{15}N$, which represent about 28 and 5.8%, respectively, of the standard deviation in these measures between our samples of different sperm whales. Among the total samples (n = 106), a subset of 15 were analyzed in duplicate. The average differences were 0.2 and 0.17‰ for $\delta^{13}C$ and $\delta^{15}N$,

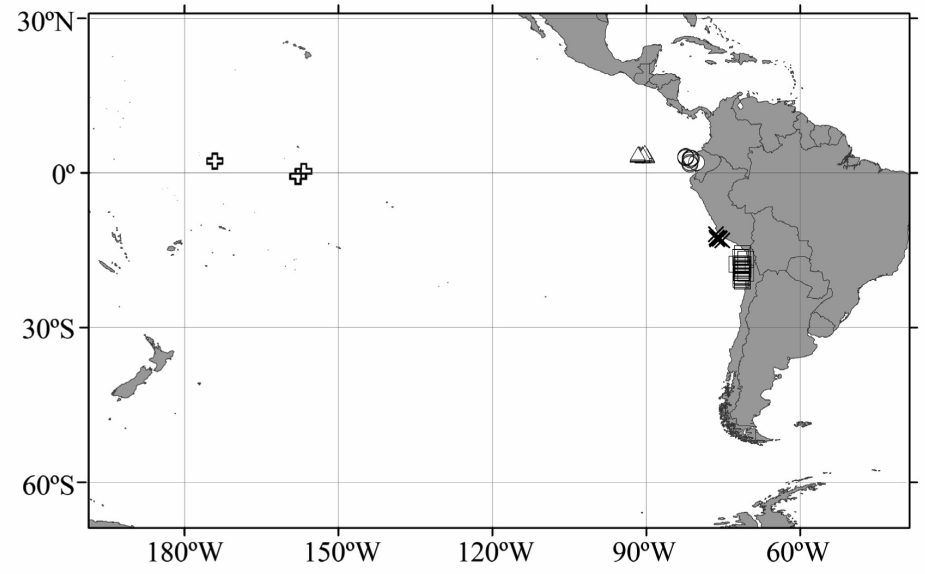

Fig. 1. *Physeter macrocephalus*. Sperm whale skin sample locations in the Galápagos 1989, 1991 and 1995 studies (triangle), the southwest Pacific 1992 study (cross), the Chile 1993 and 2000 studies (square) and the Peru 1993 study ('X')

respectively. In addition, to test for possible biases between the 2 laboratories, 6 samples were analyzed in both.

Statistical analysis. The $\delta^{13}C$ and $\delta^{15}N$ ratios of the samples from females and immature males were compared using a nested analysis of variance (ANOVA) within the general linear model (GLM) procedure in SAS V8. We included the independent categorical variables group, clan and study (a combination of the year and the location); the groups were nested within the clans and the study, and the clans within the study. The replicates were skin samples from different animals. Group and clan memberships were determined by Rendell & Whitehead (2003).

RESULTS

The analysis comprised 106 skin samples representing 31 groups from among 4 clans. We discarded 13 samples as their results were outliers (from a stem and leaf plot, their values were more than 1.5 times the distance between the lower and the upper quartiles; McClave & Sincich 2000) and were probably the result of deterioration due to bad storage. In order to test the effect of storage in DMSO, we divided one single sample of skin (collected in 2004), originally stored in ethanol, into 12 pieces. We soaked half of the pieces in DMSO for 2 wk and compared the measured isotopic ratios of these samples to those stored only in ethanol. There was no significant difference in either the carbon or nitrogen signatures between the DMSO and in those left in ethanol (*t*-test, $t_{2,10} = 2.23$, p = 0.29 and $t_{2,10} = 2.23$, p = 0.93, respectively), a result that concurs with previous studies that found that neither DMSO or ethanol storage significantly affect isotope signatures after lipids are extracted (Hobson et al. 1997, Todd et al. 1997). There was no significant difference between laboratories in the reported isotope ratios of those samples analyzed in both (paired *t*-test, p > 0.1).

The $\delta^{13}C$ ratios of all the sperm whale skin samples (n = 106) ranged from −17.6 to −14.2‰ (mean = −16.0‰, SD = 0.7‰) and the $\delta^{15}N$ ratios, from 8.5 to 22.3‰ (mean = 16.8‰, SD = 3.5‰) (Fig. 2). These results show substantial variation compared with the results of Ruiz-Castro (2002), but the present study covered much larger space and time scales.

In the ANOVA analysis, residuals in the $\delta^{13}C$ model for the sperm whale isotope results were normally distributed but those for the $\delta^{15}N$ model were not. Consequently, p-values for tests of statistical significance for the $\delta^{15}N$ values should be treated cautiously. Both the $\delta^{13}C$ and the $\delta^{15}N$ ratios differed significantly among groups (nested within clan and study; Table 1). Often, samples from the same group were closely clustered for both isotopes,

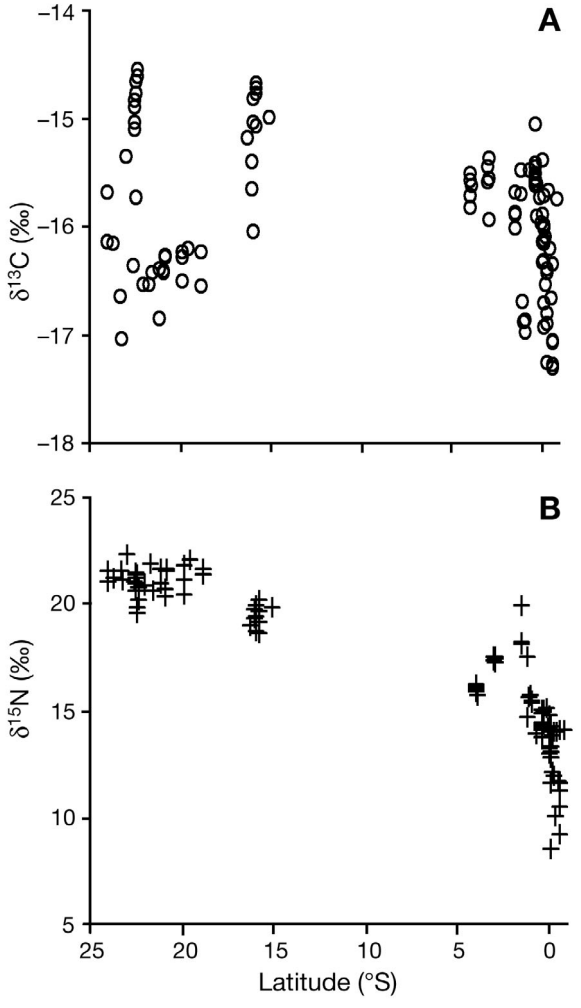

Fig. 2. *Physeter macrocephalus*. (A) $\delta^{13}C$ and (B) $\delta^{15}N$ values (‰) of sperm whale skin samples, vs. latitude (°) of sampling location

Table 1. *Physeter macrocephalus*. ANOVA table for the $\delta^{13}C$ and ^{15}N values measured in South Pacific sperm whale skin samples

Source (nesting)	df SS	Types IV	*F*	p	% var
$\delta^{13}C$					
Group (within clan & study)	16	6.6	3.8	<0.001	20.1
Clan (within study)	7	3.45	4.48	<0.001	10.5
Study	6	22.82	34.62	<0.001	69.4
$\delta^{15}N$					
Group (within clan & study)	16	90	8.88	<0.001	8.8
Clan (within study)	7	22.82	5.14	<0.001	2.2
Study	6	909.1	239.0	<0.001	89

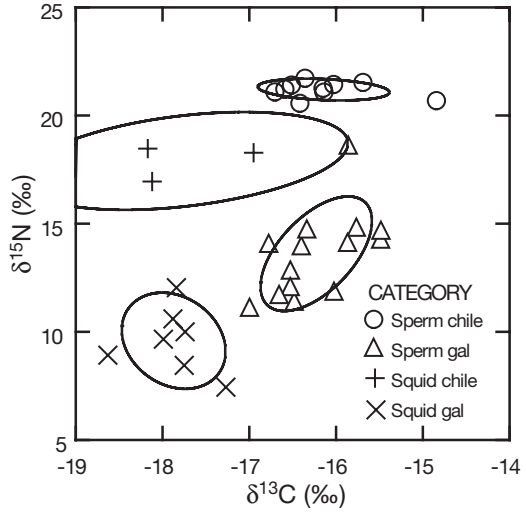

Fig. 3. $\delta^{13}N$ vs. $\delta^{15}C$ values (‰) of *Histioteuthis* spp. beak samples from sperm whale defecations off Chile (+) and Galápagos (x) plotted along with mean $\delta^{13}C$ vs. $\delta^{15}N$ values (‰) per group of female sperm whales off Chile (○) and Galápagos (△), with confidence interval ellipses with probability of 0.50

although there are some instances in which samples from the individuals within the same group were quite different, especially in $\delta^{15}N$. There was significant variation among clans for both the $\delta^{13}C$ and the $\delta^{15}N$ values (nested within the study, Table 1). The sperm whale samples from the 'Regular' clan had lower $\delta^{13}C$ values than those from the 'Short' clan (*t*-test, $t = 6.359$, $p < 0.001$). This difference is consistent across the 2 studies in which the 2 clans are both present (Chile 2000 and Galápagos 1991).

Both the $\delta^{13}C$ and the $\delta^{15}N$ values differed significantly among studies (Table 1) and this single factor explains a large part of the variation observed in the $\delta^{13}C$ and the $\delta^{15}N$ values. Conceivably, some of the inter-study variation observed in the $\delta^{15}N$ and $\delta^{13}C$ values might in part be caused by the preservative effect of storage in DMSO. However, this preservative effect is largely minimized, and therefore does not affect the above-mentioned results, because the variables 'clan' and 'group' are both nested within the variable 'study'.

There was a significant negative relationship between the latitude at which the samples were collected and the $\delta^{15}N$ (coefficient= −0.325, $r = 0.896$, $p < 0.001$, Fig. 2) values, and a significant but very weak relationship with the $\delta^{13}C$ values (coefficient = −0.018, $r = 0.259$, $p = 0.007$). The negative relationship with $\delta^{15}N$ is still present when all the samples from the Galápagos (which have the lowest $\delta^{15}N$ values and are located on the equator) are excluded.

Seven squid beaks from squids taken from near the Galápagos and 3 off Chile were included in the analysis. The squid samples from the Galápagos ranged from −18.6 to −17.3‰ (mean = −17.9‰, SD = 0.4‰) for $\delta^{13}C$ and from 7.4 to 12.0‰ (mean = 9.6‰, SD = 1.5‰) for $\delta^{15}N$, while the samples off Chile ranged from −18.2‰ to −16.95‰ (mean = −17.7‰, SD = 0.7) for $\delta^{13}C$ and from 17.0 to 18.5‰ (mean = 17.9‰, SD = 0.8‰) for $\delta^{15}N$ (Fig. 3). The squid samples off Chile had significantly higher $\delta^{15}N$ ratios ($t_{2,8} = 8.82$, $p < 0.001$) but did not differ in their $\delta^{13}C$ ratios ($t_{2,8} = 0.37$, $p = 0.723$).

DISCUSSION

We have shown here that stable isotope ratios (both nitrogen and carbon) in sperm whales vary among groups, clans and geographic areas. These results suggest that sperm whale feeding can vary even within the same general area, and that the differences in foraging behaviour observed between clans (Whitehead & Rendell 2004) are reflected in the prey consumed. These stable isotope results also make sense given what we already know about the social structure in this species.

Some of our data also differ in interesting ways from the results of previous studies, notably ^{13}C levels in general, and ^{15}N levels in females and immatures sampled around the Galápagos (c.f. Ruiz-Cooley et al. 2004). The $\delta^{13}C$ values in the sperm whale skin samples measured in this study were lower (*t*-test, $t = 8.288$, $p < 0.001$) than those reported by Ruiz-Castro (2002) for sperm whales in the Gulf of California. The Gulf of California is a very productive area with a complex hydrographical system (Santamaría-del-Angel & Alvarez-Borrego 1994) and its organic pool is likely different to the ones in the areas of this study resulting in different $\delta^{13}C$ values for the sperm whale skin samples.

The $\delta^{15}N$ values for the sperm whale skin samples measured in this study generally decrease with increasing latitude. This is consistent with the latitudinal gradient of the $\delta^{15}N$ values in phytoplankton shown by Wada & Hattori (1991) caused by the low concentration of ammonia and nitrite in tropical areas, and in temperate areas during the summer. The $\delta^{15}N$ values from the females and immatures of the 3 study years in the Galápagos appear very low (from 8.5 to 20.0‰, mean of 13.9‰) compared to the other locations in this study (e.g. in Chile, from 19.6 to 22.3‰, mean of 21.1‰) and to mean $\delta^{15}N$ values reported for females and immatures in the Gulf of California (19.6‰; Ruiz-Castro 2002). In this study, the average $\delta^{15}N$ values of the squid prey (*Histioteuthis* spp.) off the Galápagos (9.6‰) were also lower (*t*-test, $t = 11.192$, $p < 0.001$) than the $\delta^{15}N$ values for the same squid genus off Chile (17.9‰; Fig. 3). In both cases, the mean $\delta^{15}N$ values of the sperm whales were about 3.5‰ higher than values

of the squid beaks (Chile: difference in means = 3.28‰, t = 6.60, p = 0.009; Galápagos: difference in means = 4.27‰, t = 6.44, p < 0.001), which corresponds to a trophic enrichment of one level (Hobson & Welch 1992). Thus, our results for the squid are consistent with the values of the sperm whales and suggest that the same general squid species may be part of the diet of the sperm whales in the 2 locations. The Chilean coast is characterized by upwellings and a very productive ecosystem (Hebbeln et al. 2000). This is likely to result in high nitrogen ratios as found by Takai et al. (2000) in squid samples.

Clarke & Paliza (2001) argue that *Histioteuthid* beaks, and beaks of other relatively small squids, found in the stomachs and feces of southeast Pacific sperm whales are 'prey-of-prey', the food of the bigger squid (primarily *Dosidicus gigas*), which are themselves eaten by the sperm whales. Our results, on the surface, suggest only one trophic level between the sperm whale and the *Histioteuthid* squid beaks taken from their feces. The possibility remains that there might be depletion in ^{15}N of the beaks of the *Histioteuthis* spp. compared to its muscle, due to differences in metabolic rates in different tissue types, just as there is in *Psychroteuthis glacialis* (3 to 4‰; Cherel & Hobson 2005). Thus, the *Histioteuthid* squids might be only a part of the sperm whale diet feeding on lower trophic levels. Data on enrichment in the various tissues of *Histioteuthis* spp. is needed to fully evaluate the trophic relationship with sperm whales. However, our results do not support the 'prey-of-prey' hypothesis.

The observed differences in isotopic signature of skin samples are consistent with what is known of the social structure of female sperm whales. The significant differences in both the $\delta^{13}C$ and $\delta^{15}N$ values between the groups and the clans within most study locations indicate that the diets differ in composition at the 2 levels consistently over periods of more than 2 mo (Ruiz-Cooley et al. 2004). In general, members of the same group had similar signatures, which is reasonable since they travel together and so are expected to feed on similar prey and thus have similar isotopic signatures.

Previous work has shown that 'Regular' clan groups have had generally more inshore distribution patterns than the 'Plus-one' clan groups (Whitehead & Rendell 2004). This is consistent with the higher $\delta^{13}C$ values of the 'Regular' clan groups compared to the 'Plus-one' clan groups found in this study (Galápagos 1989, Fig. 3) and the benthic-coastal gradient of carbon stable isotopes found in marine ecosystems (France 1995). Thus, behavioural differences between the groups of the 2 clans are probably at the origin of this difference. There are many examples in the literature of differences in diet within a species; most are explained by environmental, genotypic or class-specific effects, but in the few cases that are not, cultural transmission acts to maintain the difference (Estes et al. 2003). We propose that the differences in diet between the 4 clans of sperm whales found here reflect cultural variation in foraging behaviour between the clans. Traditions affecting habitat use and food choice can affect the fitness of a group. Different habitats have different prey availability and the type of prey eaten influences the fitness of a consumer because each prey has a particular cost and benefit to the consumer (Estes et al. 2003). Major shifts in the food chain are thus likely to affect sperm whale groups and clans differently. Therefore, it is important to include culture in considerations of the management and conservation of this species (Whitehead et al. 2004).

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