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Short communication

Differences in stable isotope ratios of carbon and nitrogen between long-finned pilot whales (*Globicephala melas*) and their primary prey in the western north Atlantic

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Carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) stable isotope ratios were measured in skin and muscle samples from free-ranging long-finned pilot whales stranded or caught in fishing gear in two locations in the western north Atlantic. Samples of the principal pilot whale prey species, long-finned squid and a secondarily important species, Atlantic mackerel, were collected for stable isotope analysis from three areas in the western north Atlantic. The stable carbon and nitrogen ratios from the mackerel and squid samples did not differ between areas. However, carbon ratios differed between the two prey species, while the nitrogen ratios did not. The difference between the stable nitrogen isotope ratios for prey and predator suggests trophic enrichment of 1.1 to 1.7‰, values substantially lower than previously assumed for cetaceans. The differences between carbon ratios among prey species and whale tissues suggest that mackerel comprise a significant proportion of the diet of pilot whales.

Key words: pilot whale, stable isotope, diet, mackerel, squid, trophic level.

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Introduction

Stable isotope tracer studies have shown that the isotopic composition of a predator is similar to that of its prey (DeNiro and Epstein, 1978, 1981; Tieszen *et al.*, 1983; Peterson and Fry, 1987). Stable isotope ratios of carbon usually reflect dietary composition, while stable nitrogen isotope ratios usually indicate the trophic level of the prey item (DeNiro and Epstein, 1978, 1981; Minagawa and Wada, 1984). These ratios often provide a more representative, although less specific, indication of diet history than stomach contents since they reflect an integration of all prey items actually assimilated into a predator's tissues over time.

Carbon and nitrogen isotope ratios tend to become more enriched in the process of being incorporated into predator tissue, a process referred to as fractionation or trophic enrichment. Carbon isotope enrichment has been estimated to be roughly +1‰ for desert rodents (DeNiro and Epstein, 1978, 1981; Tieszen *et al.*, 1983). Nitrogen isotope enrichment have been estimated to range from +2–4‰ (DeNiro and Epstein, 1981) for rodents and +3–4‰ for marine fish and crustaceans (Minagawa and Wada, 1984; Dickson, 1986; Fry, 1988). In the absence of estimates for cetaceans, Ostrom *et al.* (1993) assumed a 3‰ nitrogen enrichment in a study of cetacean trophic levels.

An important aspect of diet analysis using tissues such as skin and muscle is that each tissue can be expected to have an isotopic ''memory''. Such ''memory'' is a function of the prey's isotope ratio at the time of consumption, and the biological turnover rate of the specific predator tissue (Tieszen, 1978).

In the present study, we compared stable carbon and nitrogen isotope ratios from long-finned pilot whales (*Globicephala melas*) with those from samples of their

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principal prey species (Overholtz and Waring, 1991), long-finned squid (*Loligo pealei*) and Atlantic mackerel (*Scomber scombrus*). We used differences in the nitrogen isotopic ratios to estimate trophic enrichment. In addition, assuming reported carbon enrichment rates, we estimated the proportion of each prey species in the diet of the whales.

Methods

Carbon and nitrogen stable isotope ratios are expressed in δ notation as parts per thousand (‰) as determined from:

$$\delta X = [(R_{sample}/R_{standard}) - 1]1000 \quad (1)$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The carbon stable isotope ratios are expressed relative to the international PeeDee Belminite (PDB). The nitrogen stable isotope ratios are relative to atmospheric nitrogen (AIR). The stable isotope ratio for an animal's tissue (δ_{tissue}) is directly related to that of its diet (δ_{diet}) as follows:

$$\delta_{tissue} = \delta_{diet} + \Delta_{dt} \quad (2)$$

where Δ_{dt} represents the isotopic fractionation or trophic enrichment factor between dietary and consumer tissue (DeNiro and Epstein, 1978, 1981).

Skin and muscle tissue samples were obtained from free-ranging long-finned pilot whales that had stranded or had been incidentally killed in commercial fishing activities. Samples came from: (1) three whales incidentally killed in one school by the foreign mackerel fishery located off the New Jersey coast in the Mid-Atlantic Bight (MAB) during April 1990; (2) three whales from one school stranded on Cape Cod, Massachusetts in December 1991; and, (3) long-finned squid and Atlantic mackerel collected during Northeast Fisheries Science Center fishery resource surveys from Cape Cod and Mid-Atlantic Bight areas between autumn 1991 and spring 1992. All whale and prey samples were frozen after collection.

Each of the whale tissues were subsampled to 3 to 5 g before drying. Prey samples consisted of five similarly-sized specimens collected from the same area. Whale and fish samples were fully dried at 70°C for approximately 24 h, then finely powdered. Lipids were not removed prior to analysis. To reduce variation amongst individual prey, dried specimens of the same species were pooled by mixing the individual specimens collected from the same area. Approximately 2 mg of each powdered tissue were loaded into a quartz combustion tube along with 2.5 g pre-combusted cupric oxide and 5 g copper wire to fully combust the sample by pyrolytic decomposition. Carbon dioxide and nitrogen gas were isolated by cryogenic distillation and analysed using a gas isotope ratio mass spectrometer (Finnigan Delta S) for carbon and nitrogen stable isotope ratios. The replication error for the mass spectrometer is 0.1‰ for carbon and 0.2‰ for nitrogen.

Because lipids are lighter than proteins in ^{13}C by as much as 6‰ (McConnaughey and McRoy, 1979) and mackerel contains more lipid than either pilot whales or squid, our measured values of $\delta^{13}C$ for mackerel are downwardly biased. The magnitude of this bias, following Alexander *et al.* (1996), is roughly 0.3‰ assuming whole mackerel are composed of 5% lipids (Bernard and Ullrey, 1989).

The isotope ratios for the pilot whale tissues were compared among areas and tissues and the ratios for prey species were compared among areas and species, both via two-way analysis of variance (ANOVA). Mean ratios for the whales and prey were compared to estimate enrichment. Standard error of the means were used to compute estimates of variability for enrichment.

In the case when two prey species differ isotopically, it is possible to estimate the proportion of each prey required to obtain the observed predator isotopic ratio. If the isotope ratio for the whale tissue is denoted by δ_w and by δ_m and δ_s for mackerel and squid, respectively, then the proportion of the diet which is mackerel can be estimated by solving the following equation for f (fraction of prey) between 0 and 1:

$$\Delta = \delta_w - (f\delta_m + (1 - f)\,\delta_s). \quad (3)$$

Rearranging the equation, one obtains

$$f = (\Delta - (\delta_w - \delta_s))/(\delta_s - \delta_m). \quad (4)$$

The statistical uncertainty of f can be estimated using bootstrap procedures. A set of values of f were computed from sets of samples of the measured isotope ratios drawn without replacement for the predator and two prey species. That set of resampled values of f was used to estimate limits on confidence intervals using the bias-corrected accelerated method recommended by Efron and Tibshirani (1993). The variance of the estimate of f was estimated as the variance of the set of resampled f values.

Results and discussion

The $\delta^{13}C$ and $\delta^{15}N$ values for the skin and muscle samples from the pilot whales are shown in Table 1. ANOVA comparing $\delta^{13}C$ suggest difference between tissues ($p<0.01$), but no difference between areas ($p=0.80$), with no interaction. ANOVA comparing $\delta^{15}N$ similarly showed differences between tissues ($p<0.01$),

Table 1. Skin and muscle stable isotope ratios of six long-finned pilot whales sampled in the Mid-Atlantic Bight (MAB) in spring 1990 and Cape Cod in winter 1991.

Location	Skin $\delta^{13}C$ (‰)	Muscle $\delta^{13}C$ (‰)	Skin $\delta^{15}N$ (‰)	Muscle $\delta^{15}N$ (‰)
MAB	− 18.7	− 18.1	14.0	13.4
MAB	− 18.5	− 17.6	13.5	13.2
MAB	− 19.4	− 18.0	14.2	13.6
Cape Cod	− 18.4	− 18.4	13.9	13.3
Cape Cod	− 18.8	− 17.9	13.5	13.2
Cape Cod	− 18.7	− 17.8	14.2	13.2

Table 2. Stable isotope ratios of Atlantic mackerel and long-finned squid collected during fishery resource surveys from the Mid-Atlantic Bight (MAB) and Cape Cod between autumn 1991 and spring 1992.

Area	$\delta^{13}C$ (‰) Mackerel	$\delta^{13}C$ (‰) Squid	$\delta^{15}N$ (‰) Mackerel	$\delta^{15}N$ (‰) Squid
North MAB	− 21.3	− 18.2	12.3	11.5
	− 19.4	− 18.2	11.5	11.4
	− 21.7	− 18.1	12.5	11.6
South MAB	− 22.1	− 19.7	12.7	11.0
	− 19.8	− 19.7	12.3	10.9
	− 22.5	− 17.8	12.7	13.4
Cape Cod	− 22.2	− 20.2	13.0	11.9
	− 22.3	− 19.5	12.5	14.2
	− 20.2	− 18.1	13.1	11.2

but no differences between areas ($p = 0.30$), and no interactions. Although the power of these tests is not great given the small sample sizes, there is little suggestion of differences among the values, as reflected by the high p values.

The $\delta^{13}C$ and $\delta^{15}N$ values for the prey are shown in Table 2. ANOVA comparing $\delta^{13}C$ suggest differences by species ($p < 0.001$) but not by area ($p = 0.33$), with no interactions. ANOVA comparing $\delta^{15}N$ values suggest no differences by areas or species ($p = 0.54$ and $p = 0.18$, respectively), with no interaction.

The mean $\delta^{13}C$ and $\delta^{15}N$ values are shown in Table 3 for pilot whale and prey tissues. The differences in the mean $\delta^{15}N$ between the two prey species combined, and the pilot whale tissues were 1.7‰ (s.d. ± 0.24) and 1.1‰ (s.d. ± 0.22) for skin and muscle, respectively. These enrichment values are substantially less than the 3–4‰ reported for other marine organism (Minagawa and Wada, 1984; Dickson, 1986; Fry, 1988), and we are unaware of any other estimated values of nitrogen enrichment for cetaceans.

If Ostrom *et al.* (1993) in their evaluation of the trophic levels of cetaceans had used such low values instead of their assumed 3‰, the trophic levels they assigned would have been higher. Despite the reported predominance of squid and mackerel in pilot whale diet

Table 3. Mean ratios of carbon and nitrogen stable isotopes for six samples of skin and muscle tissues from long-finned pilot whales, and nine samples of two species of prey, Atlantic mackerel and long-finned squid, with standard error of the means (S.E.). The nitrogen values for mackerel and squid are pooled as they are not statistically different.

Sample	n	$\delta^{13}C$ (‰) Mean	$\delta^{13}C$ (‰) S.E.	$\delta^{15}N$ (‰) Mean	$\delta^{15}N$ (‰) S.E.
Pilot whale:					
Skin	6	− 18.75	0.143	13.88	0.130
Muscle	6	− 17.97	0.112	13.32	0.065
Prey:					
Mackerel	9	− 21.28	0.393	12.21	0.212
Squid	9	− 18.83	0.306		

(Overholtz and Waring, 1991), pilot whales probably do not feed exclusively on squid and mackerel as we have assumed. However, it is unlikely that they could be feeding on organisms at lower trophic levels sufficiently to account for the differences between our estimate of trophic enrichment and the previously reported values of 3–4‰.

The difference in mean $\delta^{13}C$ between the pilot whale tissues and prey samples suggests that recent and earlier diets were composed of different mixtures of these two species. Using an enrichment for carbon of 1.0‰, the difference in skin using Equation (4) suggests that the more recent diet was most likely to be composed of 35% mackerel (s.d. = 12%) with a 95% confidence interval of 13% to 64%. The difference for muscle, reflecting an earlier diet, suggest a diet of 3.3% mackerel (s.d. = 8%), with a 95% confidence interval of 0 to 27%. Correcting these values for lipid content of mackerel would increase them slightly, from 35 to 40% and from 3.3 to 3.6%, respectively. Our analyses support the conclusion of Overholtz and Waring (1991) that pilot whales consume mackerel in the spring at a significant level.

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