

Assessing Stable Isotope Signature Variation in Cetaceans: An evaluation of skin sampling techniques and correlations with diet for bottlenose dolphins and killer whales

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Summary

We evaluated methods for assessing carbon and nitrogen isotope compositions in the epidermis of two species of odontocetes, the bottlenose dolphin (*Tursiops truncatus*, n = 2) and killer whale (*Orcinus orca*, n = 9). Diet profiles including composition and food intake rates of captive animals were monitored for 5 - 7 months and matched to stable isotope values determined from skin samples obtained immediately after the diet monitoring period. An investigation of four different epidermal sampling sites for dolphins demonstrated that anatomical site (including peripheral fins and body flank) had no effect on isotopic values (mean for all sites = -18.39 ± 0.29 SD for $\delta^{13}\text{C}$ and 18.82 ± 0.25 SD for $\delta^{15}\text{N}$). Values for killer whales differed from the dolphins and averaged -17.69 ± 0.34 for $\delta^{13}\text{C}$ and 14.51 ± 0.15 for $\delta^{15}\text{N}$. These ranges reflected differences in diet between the species, which consisted of an approximate 50/50 mix of Atlantic herring and Icelandic capelin for the dolphins and a mixed fish diet including these species as well as mackerel, salmon, sardine and smelt for the killer whales. The magnitude of the observed effect depended on the difference in isotopic profiles of the fish species comprising routine and new diets, and may be especially evident for some species of salmon.

Introduction

Survival by carnivorous animals depends on the balance between energetic costs and energy input in the form of prey intake (Stephens and Krebs, 1986). Of these two factors, assessing energy input and dietary preferences of marine living mammals is especially difficult due to cryptic foraging behaviors. With the exception of sea otters that consume their prey on the water surface or studies involving novel technology such as animal borne cameras (Davis et al., 1999), foraging events are rarely recorded for marine mammals. This is in contrast to studies on large terrestrial mammals in which the details of foraging may be obtained through a combination of visual observations, radio-collar tracking, and scat analysis (see for example Schaller, 1972). Thus, a missing element in energy budget analyses for marine mammals is information about foraging behavior and prey intake of the animal. As a result, two of the biggest questions for many species of marine mammal remain unanswered: 1) when and where do they forage and 2) what do they eat?

To address these questions investigators have relied on a variety of indirect methods. Through miniaturized video cameras, satellite tags, three dimensional time-depth recorders, and physiological monitors (i.e. heart rate recorders, stomach temperature sensors) scientists have been able to evaluate the range of movements of marine animals, the use of critical habitats, and the potential impact of these top predators on ecosystems. Because these are relatively short duration events less is known about the long-term diets of marine mammals. One relatively new technique to solve this problem is through the evaluation of stable isotopes in animal tissues. Typically, the ratio of stable forms of nitrogen and carbon in tissues is used as a reflection of dietary preferences and has been investigated in a wide variety of predatory consumers including whales (Abend and Smith, 1997).

Although the value of these isotopic methods depends on calibration against known diets, few studies have been able to address this, particularly for large cetaceans. This is especially problematic for animals with diverse and widely changing diets such as killer whales (Jefferson et al., 1991). In view of this and the current interest in killer whale prey preferences we examined the use of stable isotope profiles to predict diet in odontocetes. There were two primary goals of this study. First, we developed a relatively non-invasive method of obtaining tissue samples from the epidermis of cetaceans. Second, we compared the variation in stable isotope signatures in the epidermis to known diets in both bottlenose dolphins and killer whales as a test of the sensitivity of the method.

Methods

Animals. This work was conducted on two species of adult odontocetes: two male Atlantic bottlenose dolphins (*Tursiops truncatus*; mean body mass = 182.2 kg) housed at Long Marine Laboratory in Santa Cruz, California and nine killer whales (*Orcinus orca*) housed at Sea World (San Diego, CA and San Antonio, TX). The killer whales included two adult females (mean body mass = 3158.9 kg), two adult males (mean body mass = 3195.1 kg), two juvenile females (mean body mass = 774.6 kg) and three juvenile males (mean body mass = 1439.2 kg) (Table 1). Skin sample collection was dependent on facility and was limited to November 2003 and 2004 for the bottlenose dolphins, and either December 2005 or March 2006 for the killer whales. A single skin sample was obtained from each animal in the study.

Skin Samples. To determine potential site specific differences in stable isotope values across the body of cetaceans, we collected samples from four different anatomical sites on the bottlenose dolphins (Figure 1A). The body sites included the dorsal fin, a mid-thoracic site parallel with the dorsal fin, the leading edge of the fluke, and the dorsal surface of the fluke. Because we found no differences between stable isotope values for any of the body sites ($p = 0.64$, $F = 0.47$ for the $\delta^{13}\text{C}$ values; $p = 0.75$, $F = 0.29$ for $\delta^{15}\text{N}$ values), we selected the leading edge of the fluke for the subsequent collection of samples from the killer whales. We chose this site because the animals had previously been trained to present their fluke for husbandry purposes and we found that bleeding was minimized in this area relative to the other sites tested.

To collect the skin samples, the area was first wiped with an alcohol swab. Using a sterile scalpel blade, a shallow incision was made approximately 1- 3 mm into the epidermis (Figure 1B). Sterile forceps were then used to peel away an approximately a 10 -15 mg wet weight sample of skin (Figure 1B). The sample was transferred to a clean glass vial and frozen at -20°C until analysis. The healing time associated with this method of skin collection was minimal. We found that within seven days, there was no visible indication of the collection in either species of odontocete.

Isotope Methods. Skin tissue samples [~ 10 milligram (mg)] were stored and prepared in small glass vials. Lipids were extracted with five rinses of a 2:1 chloroform and methanol solution, with sonication of each rinse for 0.5 hours. For stable isotope analyses, skin subsamples (1.0 mg) were weighed into tin capsules. Stable isotope ratios were measured using an elemental analyzer coupled with a mass spectrometer (Finnigan DeltaPlus) at the University of California Santa Cruz Stable Isotope Laboratory. Stable isotope compositions are reported using the standard δ notation, and are referenced to Vienna PeeDee Belemnite and air for carbon and nitrogen, respectively. The standard deviation for replicates of a gelatin standard was $<0.2\%$ for carbon and nitrogen.

Diet Composition and Variation.

Food intake including total amount fed, diet composition and percentage of diet comprised of different fish species was recorded daily for all individual animals for a period of five to seven months prior to skin sampling for stable isotope profiles (Appendix 1). Daily records were averaged for each month and a grand average for diet composition for the study period computed. Average values represent five contiguous months for the bottlenose dolphins and seven months for the killer whales. Variations in diet that occurred over the study period were also noted but not considered a factor in the present study. Isotopic values as reported in the literature for each fish species in the diet were summarized and used for later comparison with the values measured in epidermal samples from the animals.

Statistics

ANOVA was used to test for significant differences between group means (JMP, SAS Institute Inc., Cary, NC, USA). If a significant result was detected a Tukey-Kramer HSD test was used to determine which groups were significantly different from one another.

Results

Diet Composition and Variation

Dolphins: The dolphins were maintained on a diet consisting primarily of adult Atlantic herring and Icelandic capelin consumed in similar amounts (by weight) (Table 2, Figure 2). There was minimal variation in the relative contribution of each species to the diet during the five months prior to when epidermal samples were taken both in 2003 and 2004. However, in 2004 smelt replaced capelin for approximately one month and overall contributed approximately 11% to the summed diet for both animals (Figure 2).

Killer Whales: The diet composition of captive killer whales consisted of a wide variety of fish species (Table 3, Figure 3, Appendix 1). The primary prey items included adult Atlantic herring and Icelandic capelin. When salmon was present in the diet it consisted of adult salmon (*Oncorhynchus ssp.*) caught in the Gulf of Alaska, specifically near Petersburg, Alaska. There was no apparent trend in dietary variation between male and female killer whales. In contrast, juvenile whales tended to show a 2 - 10% increase in the proportion of the diet consisting of herring compared to adult animals (Figure 3). Each facility utilized slightly different diets. Killer whales housed in the San Diego facility consumed a relatively consistent mixed fish diet of capelin, herring, mackerel, and salmon for the seven months prior to skin sampling. Killer whales in the Texas facility also used this mixed fish diet with the addition of sardines and smelt, although at < 5% levels. The diets of the animals in Texas were also slightly more varied in terms of relative contribution of the different fish species to the overall composition of the diet. In addition, two animals (ID number 7 and 8), did not receive salmon in their diet at any time during the seven months prior to when the samples were collected.

Isotope Values of Prey Items

In view of the number of factors that can affect isotopic signatures (see Discussion and Appendix 2), an estimation of isotope values for the various dietary items using previously published values could include a margin of error. To account for this we used values from a study that examined isotopic profiles for fish examined for captive northern fur seals (Kurle, 2002). These isotopic values for Atlantic herring and Icelandic capelin provide reasonable estimates for the animals in our study as they represent the same species residing in the same ocean basins as the fish fed to the captive odontocetes. Note that the dolphins were fed smelt instead of capelin for approximately one month during 2004 and this occurred in the first of the five months analyzed prior to skin sample collection. Given the high epidermal turnover in these animals (discussed below), and the relatively low contribution the smelt made to the overall diet, the addition of the smelt was assumed to have a minimal impact on the isotopic signature. Stable nitrogen isotope values reported previously for smelt range from approximately 13.5 to 16.5 (estimated from Hansson et al., 1997). Hobson et al. (1994) also reported both carbon and nitrogen isotope values for smelt, and these values were used to estimate smelt isotope values for the present study. Lastly, isotope signatures for various species of salmon (from Herman et al., 2005) and mackerel (from Abend and Smith, 1997) are summarized along with the other fish species in Table 4.

Epidermal Isotope Values

Dolphins: Epidermal isotope values for the two Atlantic bottlenose dolphins were similar between animals in both 2003 and 2004, and were thus pooled for the remainder of the comparisons. There were no significant differences between animals for either stable isotope $\delta^{13}\text{C}$ ($p = 0.65$, $F = 0.22$) or $\delta^{15}\text{N}$ ($p = 0.11$, $F = 2.96$). We found that isotope values averaged -18.39 ± 0.29 SD for $\delta^{13}\text{C}$ and 13.82 ± 0.25 SD for $\delta^{15}\text{N}$ (Figure 4).

Killer Whales: Despite slight differences in diet composition including species of fish, we found no significant differences in stable isotopes $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between whales housed at SeaWorld, San Diego and San Antonio ($\delta^{13}\text{C}$: $p = 0.21$, $F = 1.93$; $\delta^{15}\text{N}$: $p = 0.86$, $F = 0.04$). Thus, the values for all whales were pooled for the remainder of the comparisons. The mean \pm SD for stable isotopes was -17.69 ± 0.32 for $\delta^{13}\text{C}$ and 14.50 ± 0.15 for $\delta^{15}\text{N}$ for killer whales fed a mixed fish diet (Figure 4).

Because two killer whales were not fed salmon for the entire study period, we were able to opportunistically compare isotope values between adult whales that were fed salmon ($n = 2$) and those that were not ($n = 2$). Although the sample sizes were too small to quantitatively test these data, qualitatively there appears to be no difference in stable isotope $\delta^{13}\text{C}$ values between salmon eaters (-17.61 ± 0.390 SD) and non-salmon consumers (-17.45 ± 0.35 SD). However, there was a slightly greater difference in stable isotope $\delta^{15}\text{N}$ values between the salmon consumers (14.37 ± 0.09 SD) and non-consumers (14.63 ± 0.09 SD). Similarly, we found that stable isotope $\delta^{15}\text{N}$ values for five juvenile whales with the highest proportion of herring in the diet averaged 14.502 ± 0.14 SD while those with low herring diets ranged from 14.61 to 14.65 (Table 5). No difference in stable isotope $\delta^{13}\text{C}$ values was found for these two groups. However, both age and the presence of smelt in the diet of the low herring diet whales should be considered when evaluating the cause of the observed differences.

Comparisons Between Species: There were significant differences in both stable isotope $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between Atlantic bottlenose dolphins and killer whales. In general, both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were greater in killer whales relative to bottlenose dolphins and these differences were highly significant ($\delta^{13}\text{C}$: $p < 0.0001$, $F = 24.79$; $\delta^{15}\text{N}$: $P < 0.0001$, $F = 53.47$).

Discussion

The first goal of the present study was to refine methods for obtaining skin samples for isotope evaluation in odontocetes. Most importantly, we found that there was no statistical difference in isotope composition for skin samples taken from either peripheral or central sampling sites in bottlenose dolphins (Figures 1 and 4). Assuming similar skin characteristics for the mammals comprising this group, the implication is that typical biopsy sample sites for odontocetes (e.g., dorsal fin, flank and back) obtained in less controlled environments such as at sea will likely yield similar results for carbon and nitrogen isotopic values.

The similarity between anatomical sites may be due in part to the relatively fast turnover rates of the upper epidermis of cetaceans (Hicks et al., 1985). However, a question arises regarding the effect of this skin turnover rate on the incorporation of dietary components into the epidermal layers. Previous studies have noted that the rate at which the diet is integrated into tissues varies

between tissue type and species. Generally, incorporation occurs over 2-3 half-lives of the isotope of interest (Hobson, 1993). For example, for skin tissue, a carbon isotope half-life of approximately 30 days has been reported for rats (*Rattus norvegicus*) and lambs (Family Bovidae) (Attaix et al., 1988; Cherel et al., 1991; Mays et al., 1991; summarized in Welle, 1999). Consequently, if the animals have been maintained on a consistent diet, skin samples from these species will likely be representative of the animals' diets from approximately 60 - 90 days prior to sampling. With a half-life of approximately 13 days for dogs (*Canis familiaris*) (Welle, 1999), skin samples would reflect the diet from 26-39 days prior to sampling for stable isotopes.

Similar determinations for marine mammals are difficult due to variation in epidermal growth among groups. Kurle and Worthy used tissue rates from gerbils (Tieszen et al., 1983) to estimate a carbon half-life of 6.4 - 27.6 days for Northern fur seals (*Callorhinus ursinus*), with the result that dietary components were assumed to be effectively incorporated into the skin within 13 - 83 days. By evaluating the mean rate of epidermal cell migration from the basal lamina/germinal layer to the stratum externum ($0.19 \text{ mm}\cdot\text{day}^{-1}$) in bottlenose dolphins, Hicks et al. (1985) found that most cells had reached the skin surface by 73 days. If the departure time from the germinal layer is considered, figure 5 from Hicks et al. (1985) also indicates that half of the epidermal cells are mobilized within approximately 5.5 days in the bottlenose dolphin. Hence, the diet of bottlenose dolphins would be incorporated into the topmost skin layers within 11-16 days. Similarly, Toperoff et al. (in prep.) estimated a rate of several days to weeks for the incorporation of dietary components into the skin tissue of harbor porpoises (*Phocoena phocoena*), a value identical to that provided by Abend and Smith (1997) for long-finned pilot whales (*Globicephala melas*). Assuming turnover rates in killer whales are similar to those of other odontocetes, it is likely that the five to seven month collection period for dietary data for the animals in the current study represents an adequate time period to ensure incorporation of the dietary isotopes into the skin samples.

This leads to the second goal of this study, an examination of the effect of diet on stable isotope values for skin samples obtained from odontocetes. As suggested for wild odontocetes (Abend and Smith, 1997; Herman et al., 2005), our data demonstrate that the carbon and nitrogen isotopic signatures of captive bottlenose dolphins and killer whales reflect diet composition within limits (Figure 4). In general, the mixed fish diets of killer whales, including those containing salmon, showed higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values than the herring-capelin diet of dolphins, thus reflecting the isotopic enrichment of the individual fish (Table 4; Herman et al., 2005). This was particularly evident for nitrogen (Figure 4).

Admittedly, the level of stable isotopes can be confounded by numerous factors including age and reproductive state of the animal, diet quality, body size as well as the age of prey fish and many others. Indeed, we find that the isotopic profiles observed in the present study for captive killer whales fed a relatively mixed diet, showed less variation than observed for a wide sampling of wild killer whale subpopulations in Alaska (Figure 5). For example, when compared to values in Herman et al. (2005) we find that the range of carbon and nitrogen isotope values for dolphins and killer whales fed a mixed fish diet in captivity was remarkably narrow. This difference remains even when comparing captive animals to fish eating resident ecotypes of wild killer whales.

Overall, within the limits of the present study, it appears that relatively small changes in the diet composition may affect stable isotope profiles in the epidermis of odontocetes. The magnitude of the effect will depend upon the difference in profiles of the fish species comprising the routine and new diet. In the case of Chinook salmon, elevated $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values relative to many other common prey items (Table 4) may make dietary changes involving this specific prey species especially evident though stable isotope methodologies.

References

- Abend, A.G. and Smith, T.D. (1997) Differences in stable isotope ratios of carbon and nitrogen between long-finned pilot whales (*Globicephalus melas*) and their primary prey in the western north Atlantic. *ICES J Mar Sci* 54: 500-503.
- Attaix, D, E Aourousseau, A Manghbaty and M Arnal. (1988) Contribution of liver, skin and skeletal muscle to whole-body protein synthesis in the young lamb. *Brit J Nutrit* 60: 77-84.
- Cherel, Y, D Attaix, D Rosolowska-Huszcz, R Belkhou, JP Robin, M Arnal et al. (1991) Whole-body and tissue protein synthesis during brief and prolonged fasting in the rat. *Clin. Sci.* 81: 611-619.
- Davis, R.W., Fuiman, L.A., Williams, T.M., Collier, S.O., Hagey, W.P., Kanatous, S.B., Kohin, S., and Horning, M. (1999) Hunting behavior of a marine mammal beneath the Antarctic fast ice. *Science* 283: 993-996.
- Dickson, M.L. (1986) A comparative study of the pelagic food webs in two Newfoundland fjords using stable carbon and nitrogen isotope tracers. MSc Thesis, Memorial Univ. Newfoundland.
- Fry, B. (1988) Food web structure on Georges Bank from stable C, N, and S isotopic compositions. *Limnol. Oceanogr.* 33: 1182-1190.
- Hansson, S., Hobbie, J.E., Elmgren, R., Larsson, U., Fry, B., and Johansson, S. (1997) The stable nitrogen isotope ratio as a marker of food-web interactions and fish migration. *Ecology* 78: 2249-2257.
- Harvey, C.J., Hanson, P.C., Essington, T.E., Brown, P.B., and Kitchell, J.F. (2002) Using bioenergetics models to predict stable isotope ratios in fishes. *Can. J. Fish. Aquat. Sci.* 59: 115-124.
- Harvey, C.J. and Kitchell, J.F. (2000) A stable isotope evaluation of the structure and spatial heterogeneity of a Lake Superior food web. *Can. J. Fish. Aquat. Sci.* 57: 1395-1403.

- Herman, DP, DG Burrows, PR Wade, JW Durban et al. (2005) Feeding ecology of eastern North Pacific killer whales *Orcinus orca* from fatty acid, stable isotope, and organochlorine analyses of blubber biopsies. *Marine Ecology Progress Series* 302:275-291.
- Hicks, BD, DJ St Aubin, JR Geraci and WR Brown. (1985) Epidermal growth in the bottlenose dolphin, *Tursiops truncatus*. *J Invest Dermat* 85: 60-63.
- Hirons, A.C., Schell, D.M., and Finney, B.P. (2001) Temporal records of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in North Pacific pinnipeds: inferences regarding environmental change and diet. *Oecologia* 129: 591-601.
- Hobson, K.A. (1993) Trophic relationships among high Arctic seabirds: insights from tissue-dependent stable isotope models. *MEPS* 95: 7-18.
- Hobson, K.A. and Montevecchi, W.A. (1991) Stable isotopic determinations of trophic relationships of great auks. *Oecologia* 87: 528-531.
- Hobson, K.A., Piatt, J.F., and Pitocchelli, J. (1994) Using stable isotopes to determine seabird trophic relationships. *The Journal of Animal Ecology* 63: 786-798.
- Hobson, K.A., Schell, D.M., Renouf, D., and Noseworthy, E. (1996) Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. *Can. J. Fish. Aquat. Sci.* 53: 528-533.
- Hobson, K.A., Sease, J.L., Merrick, R.L., and Piatt, J.F. (1997) Investigating trophic relationships of pinnipeds in Alaska and Washington using stable isotope ratios of nitrogen and carbon. *Mar. Mamm. Sci.* 13: 114-132.
- Hooker, S.K., Iverson, S.J., Ostrom, P., and Smith, S.C. (2001) Diet of northern bottlenose whales inferred from fatty-acid and stable-isotope analyses of biopsy samples. *Can. J. Zool.* 79: 1442-1454.
- Jefferson, T.A., Stacey, P.J., and Baird, R.W. (1991) A review of killer whale interactions with other marine mammals: predation to co-existence. *Mammal Review* 21:151-180.
- Jennings, S., Greenstreet, S.P.R., Hill, L., Piet, G.J., Pinnegar, J.K., and Warr, K.J. (2002) Long-term trends in the trophic structure of the North Sea fish community: evidence from stable-isotope analysis, size-spectra and community metrics. *Marine Biology* 141: 1085-1097.
- Kurle, C.M. (2002) Stable-isotope ratios of blood components from captive northern fur seals (*Callorhinus ursinus*) and their diet: applications for studying the foraging ecology of wild otariids. *Can. J. Zool.* 80: 02-909.

- Kurle, CM and GAJ Worthy (2001) Stable isotope assessment of temporal and geographic differences in feeding ecology of northern fur seals (*Callorhinus ursinus*) and their prey. *Oecologia* 126: 254-265.
- Mays, PK, RJ McAnulty and GJ Laurent (1991) Age-related changes in rates of protein synthesis and degradation in rat tissues. *Mech Aging Dev* 59: 229-241.
- Schaller, G. (1972) *The Serengeti Lion*. Univ. of Chicago Press, Chicago, IL.
- Schmutz, J.A. and Hobson, K.A. (1998) Geographic, temporal, and age-specific variation in diets of glaucous gulls in western Alaska. *The Condor* 100: 119-130.
- Smith, R.J., Hobson, K.A., Koopman, H.N., and Lavigne, D.M. (1996) Distinguishing between populations of fresh- and salt-water harbour seals (*Phoca vitulina*) using stable-isotope ratios and fatty-acid profiles. *Can. J. Fish. Aquat. Sci.* 53: 272-279.
- Stephens, D.W., and Krebs, J.R. (1986) *Foraging Theory*. Princeton University Press, Princeton, NJ.
- Sydeman, W.J., Hobson, K.A., Pyle, P., and McLaren, E.B. (1997) Trophic relationships among seabirds in Central California: combined stable isotope and conventional dietary approach. *The Condor* 99: 327-336.
- Thompson, D.R., Lilliendahl, K., Solmundsson, J., Furness, R.W., Waldron, S., and Phillips, R.A. (1999) Trophic relationships among six species of Icelandic seabirds as determined through stable isotope analysis. *The Condor* 101: 898-903.
- Tieszen, LL, TW Boutton, KG Tesdahl and NA Slade (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: implications for delta ¹³C analysis of diet. *Oecologia* 57: 32-37.
- Toperoff, AK, RK Burton, JT Harvey and PL Koch (2007) Dietary fractionation of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ within three tissues of harbor porpoise (*Phocoena phocoena*) from Monterey Bay, California. Draft.
- Welle, S. (1999) *Human protein metabolism*. Springer-Verlag: New York. Pp. 288.

Table 1: Life history data for the dolphins and whales sampled in this study. Details concerning housing and diet are provided in the Methods.

Species	Animal ID	Mass (kg)	Sex	Age Class
<i>O.orca</i>	1	3688.0	F	Adult
<i>O.orca</i>	2	1270.1	M	Juvenile
<i>O.orca</i>	3	2629.9	F	Adult
<i>O.orca</i>	4	1835.7	M	Juvenile
<i>O.orca</i>	5	4219.3	M	Adult
<i>O.orca</i>	6	2170.9	M	Adult
<i>O.orca</i>	7	985.5	F	Juvenile
<i>O.orca</i>	8	563.6	F	Juvenile
<i>O.orca</i>	9	1211.8	M	Juvenile
<i>T.truncatus</i>	A	168.7	M	Adult
<i>T.truncatus</i>	B	195.6	M	Adult

Table 2: Time-averaged diet composition for bottlenose dolphins during the five months prior to skin sampling for stable isotope analyses. Two adult dolphins (A and B) were used in the study and sampled on two occasions.

Fish Species	Dolphin A 11/13/03	Dolphin B 11/13/03	Dolphin A 11/23/04	Dolphin B 11/23/04
Atlantic herring	56.3%	47.4%	47.5%	45.8%
Icelandic capelin	43.7%	52.6%	41.3%	43.2%
Smelt	0.0%	0.0%	11.3%	11.0%

Table 3: Time-average diet composition for killer whales during the seven months prior to skin sampling for stable isotope analyses. Nine whales including adults and juveniles (listed in Table 1) from two different facilities were used in the study. Each was sampled on one occasion.

Animal ID	capelin %	herring %	mackerel %	salmon %	sardines %	smelt %	Food Total (kg/day)
1	31.0	49.0	15.0	5.0	0	0	86.4
2	32.0	52.0	8.0	8.0	0	0	41.7
3	29.0	52.0	14.0	5.0	0	0	50.1
4	32.0	52.0	11.0	5.0	0	0	48.0
5	30.0	52.0	12.0	6.0	0	0	80.5
6	45.3	29.4	9.2	9.7	0.0	6.3	63.0
7	49.3	44.0	1.6	0.0	5.1	0.0	44.1
8	48.2	48.5	0.0	0.0	3.3	0.0	27.9
9	55.5	26.6	5.8	7.6	0.0	4.5	40.0

Table 4. Mean ratios of carbon and nitrogen stable isotopes for common dietary items of captive dolphins and killer whales. Values are from Appendix 2, Kurle (2002), Herman et al. (2005), Hansson et al, (1997) and Abend and Smith (1997) as described for each prey type in the text. Geographic area of the items was matched to those fed to the animals when known.

Fish species	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
Atlantic herring (<i>Clupea harengus</i>)	11.8 ± 0.2	-18.5 ± 0.3
Icelandic capelin (<i>Mallotus villosus</i>)	11.1 ± 0.2	-19.8 ± 0.1
Smelt (<i>Thaleichthys pacificus</i>)	14.4 ± 0.2	-16.7 ± 0.1
Chinook salmon (<i>Oncorhynchus tshawytscha</i>)	15.2	-17.2
Sockeye salmon (<i>O. nerka</i>)	11.1	-20.8
Coho salmon (<i>O. kisutch</i>)	13.8	-19.1
Mackerel (<i>Scomber australicus</i>)	12.21 ± 0.2	-21.28 ± 0.4

Table 5: Stable isotope values for carbon and nitrogen for the individual killer whales used in this study. Life history characteristics of the individuals are listed in Table 1. Table 3 and Appendix 1 provide the corresponding diet composition for each animal.

Whale ID	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
1	-17.58	14.68
2	-17.94	14.55
3	-18.12	14.34
4	-18.00	14.39
5	-17.40	14.55
6	-17.89	14.65
7	-17.70	14.25
8	-17.21	14.49
9	-17.33	14.61

Figure Legends

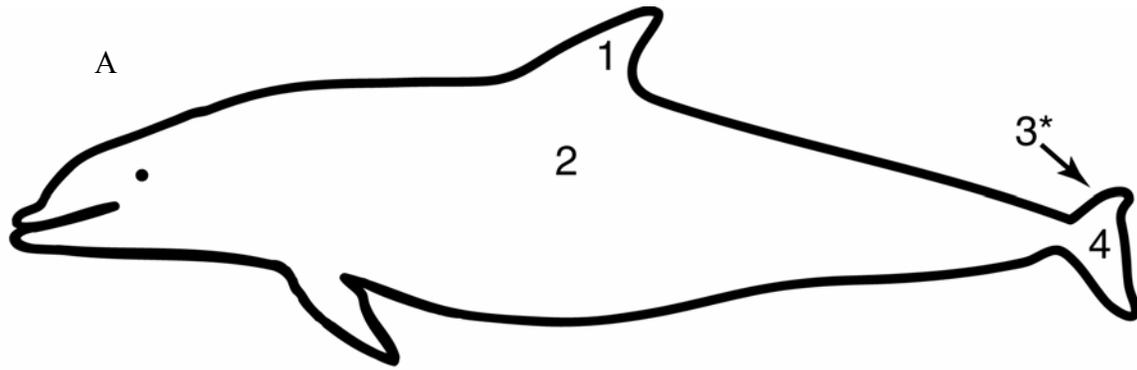
Figure 1. Procedures for obtaining skin samples for stable isotope evaluation in the epidermis of cetaceans. (A) Location of body sites that were sampled to test for site specific differences in epidermal stable isotopic signatures. Two adult Atlantic bottlenose dolphins were used in this evaluation with the following sites: dorsal fin (1), a mid-thoracic site parallel with the dorsal fin (2), the leading edge of the fluke (3), and the dorsal surface of the fluke (4). (B). Photographs illustrating a simple procedure for epidermal sampling in cetaceans. Steps included (i) a shallow incision into the epidermis, (ii) sterile forceps being used to pull back the sample, and (iii) cutting the sample free from the epidermis with minimal bleeding.

Figure 2. Percentage diet composition for two Atlantic bottlenose dolphins (designated A and B) for the five months prior to when epidermal samples were collected. Two separate years are presented. Height of each bar denotes the total proportion of the diet with the gradation in color indicating the specific proportion of capelin, herring and smelt in the diet.

Figure 3. Percentage diet composition for nine killer whales (designated 1- 9) for the seven months prior to when epidermal samples were collected. Height of each bar denotes the total proportion of the diet with the gradation in color indicating the specific proportion of capelin, herring, mackerel, salmon, sardine and smelt in the diet. Note that whales identified number 7 and 8 received no salmon in their diet. Isotope profiles for these adults were compared to those of adults 6 and 9 to assess the effects of differences in diet composition (described in the text).

Figure 4: Epidermal stable isotope values for odontocetes. Two Atlantic bottlenose dolphins (four anatomical sites, triangles) and nine killer whales (one anatomical site, circles) as described in Table 1 are shown. Each point represents the values for an individual animal. Isotope values for killer whales fed salmon (black circles) are compared to two whales in which salmon was absent in the diet (gray circles). Numbers next to the killer whale points indicate animal ID number given in Table 5.

Figure 5: Comparison of mean stable isotope ratios for captive and wild odontocetes. Circles and lines denote mean values ± 1 SD for each odontocete group. Values for Atlantic bottlenose dolphins and killer whales from Figure 4 in this study are plotted in comparison to the range of values reported by Herman et al. (2005). The previous study includes a variety of killer whale ecotypes from the North Pacific Ocean. Note that the animals in the present study were only fed fish and are most comparable in diet composition to the fish eating resident ecotypes.



B



Figure 1. Procedures for obtaining skin samples for stable isotope evaluation in the epidermis of cetaceans. (A) Location of body sites that were sampled to test for site specific differences in epidermal stable isotopic signatures. Two adult Atlantic bottlenose dolphins were used in this evaluation with the following sites: dorsal fin (1), a mid-thoracic site parallel with the dorsal fin (2), the leading edge of the fluke (3), and the dorsal surface of the fluke (4). (B). Photographs illustrating a simple procedure for epidermal sampling in cetaceans. Steps included (i) a shallow incision into the epidermis, (ii) sterile forceps being used to pull back the sample, and (iii) cutting the sample free from the epidermis with minimal bleeding.

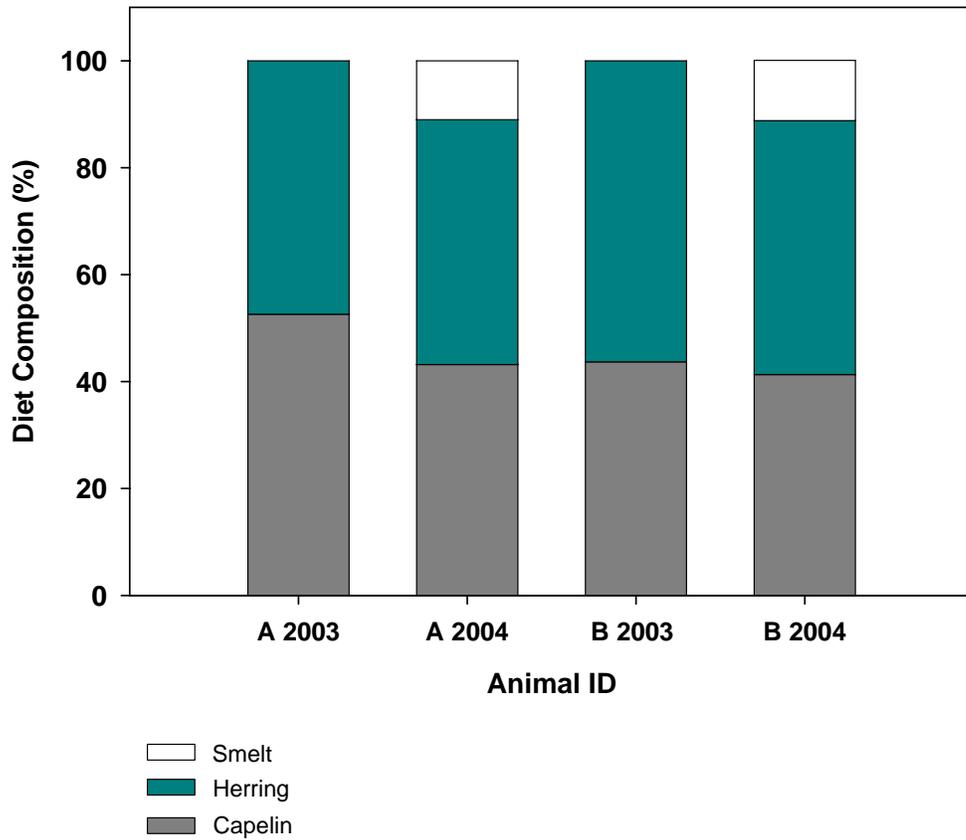


Figure 2. Percentage diet composition for two Atlantic bottlenose dolphins (designated A and B) for the five months prior to when epidermal samples were collected. Two separate years are presented. Height of each bar denotes the total proportion of the diet with the gradation in color indicating the specific proportion of capelin, herring and smelt in the diet.

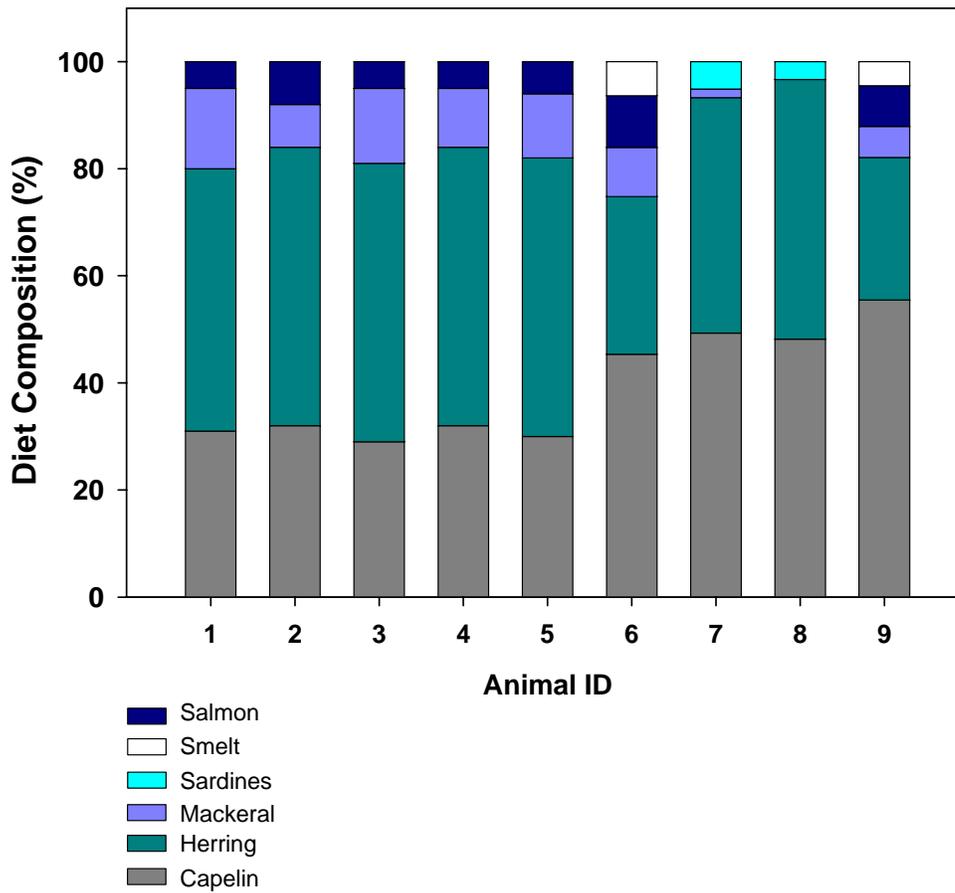


Figure 3. Percentage diet composition for nine killer whales (designated 1- 9) for the seven months prior to when epidermal samples were collected. Height of each bar denotes the total proportion of the diet with the gradation in color indicating the specific proportion of capelin, herring, mackerel, salmon, sardine and smelt in the diet. Note that whales identified number 7 and 8 received no salmon in their diet. Isotope profiles for these adults were compared to those of adults 6 and 9 to assess the effects of differences in diet composition (described in the text).

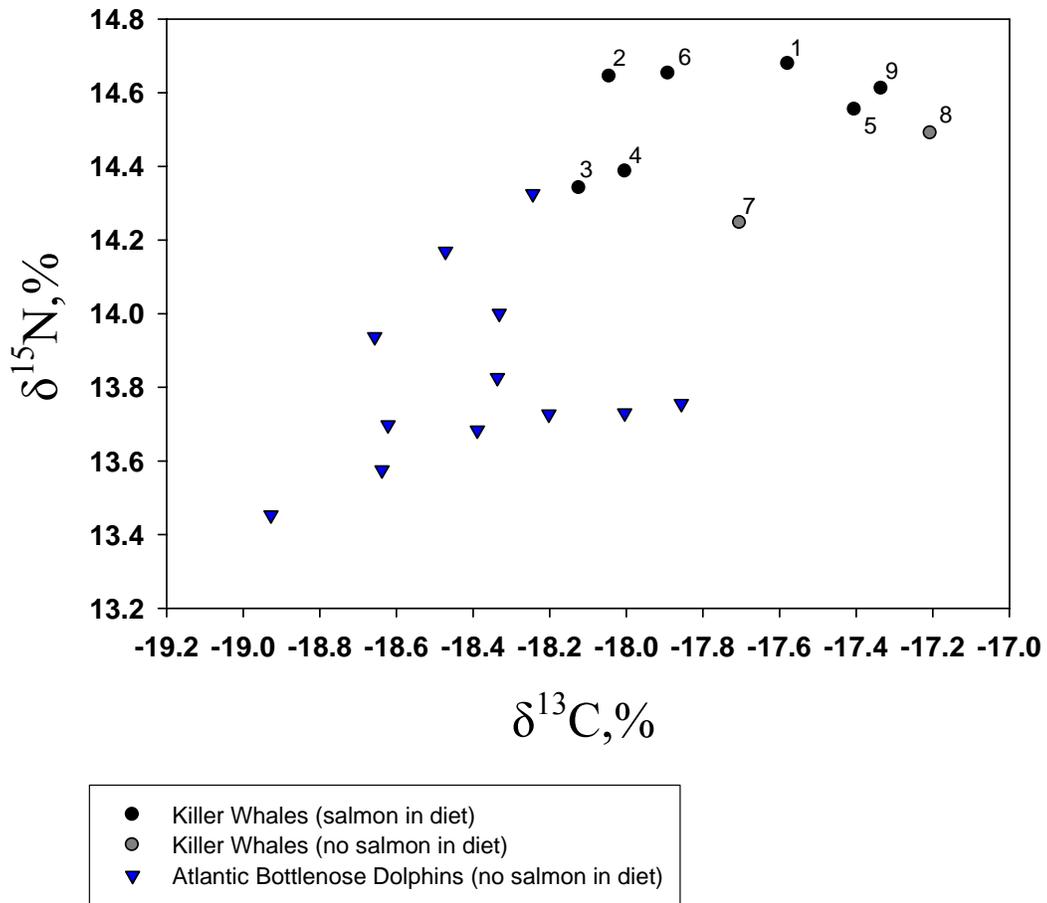


Figure 4: Epidermal stable isotope values for odontocetes. Two Atlantic bottlenose dolphins (four anatomical sites, triangles) and nine killer whales (one anatomical site, circles) as described in Table 1 are shown. Each point represents the values for an individual animal. Isotope values for killer whales fed salmon (black circles) are compared to two whales in which salmon was absent in the diet (gray circles). Numbers next to the killer whale points indicate animal ID number given in Table 5.

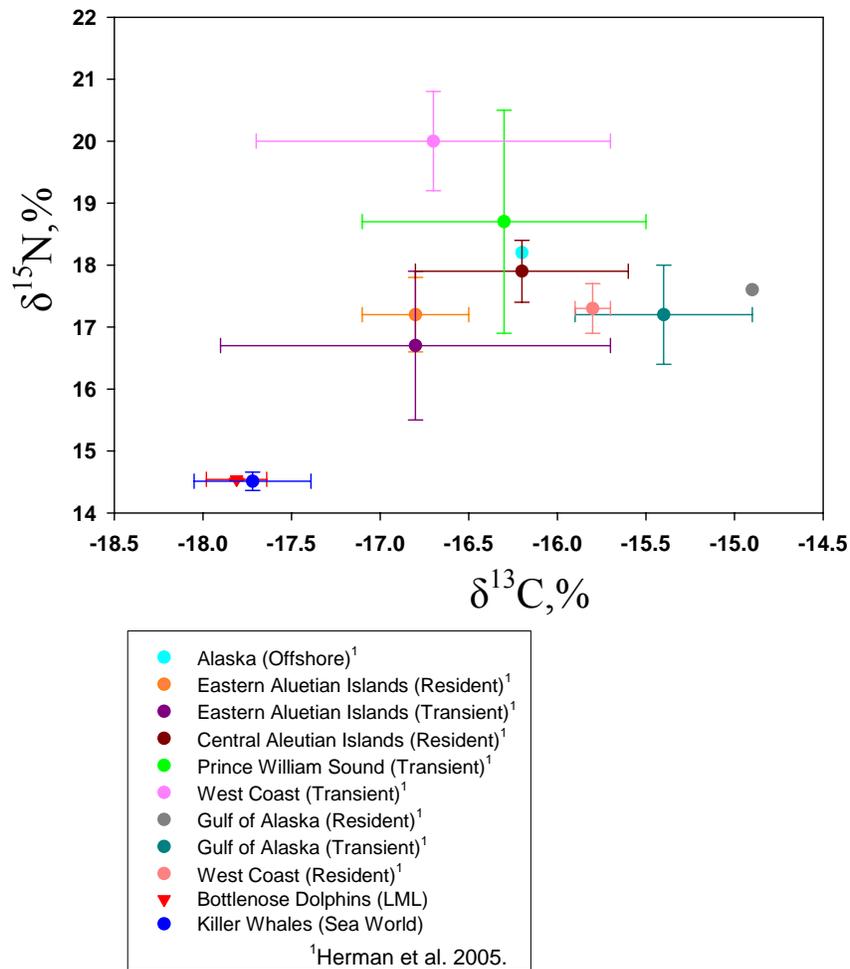


Figure 5: Comparison of mean stable isotope ratios for captive and wild odontocetes. Circles and lines denote mean values ± 1 SD for each odontocete group. Values for Atlantic bottlenose dolphins and killer whales from Figure 4 in this study are plotted in comparison to the range of values reported by Herman et al. (2005). The previous study includes a variety of killer whale ecotypes from the North Pacific Ocean. Note that the animals in the present study were only fed fish and are most comparable in diet composition to the fish eating resident ecotypes.

APPENDIX 1. Monthly Diet Composition for Captive Killer Whales

Life history data for the individual animals is presented in Table 1.

DATE	ANIMAL I.D.	ANIMAL WEIGHT (kg)	CAPELIN (lbs)	CAPELIN (%)	HERRING (lbs)	HERRING (%)	MACKEREL (lbs)	MACKEREL (%)	SALMON (lbs)	SALMON (%)	TOTAL FOOD AVG (lbs per day)
Jun-05	1	3708	56.4	31.0	89.1	49.0	27.3	15.0	9.1	5.0	181.9
Jul-05	1	3697	56.2	31.0	88.9	49.0	27.2	15.0	9.1	5.0	181.4
Aug-05	1	3708	55.8	31.0	88.2	49.0	27.0	15.0	9.0	5.0	180.0
Sep-05	1	3674	56.0	31.0	88.6	49.0	27.1	15.0	9.0	5.0	180.7
Oct-05	1	3658	55.9	31.0	88.3	49.0	27.0	15.0	9.0	5.0	180.2
Nov-05	1	3658	73.3	31.0	115.9	49.0	35.5	15.0	11.8	5.0	236.4
Dec-05	1	3658	58.9	31.0	93.1	49.0	28.5	15.0	9.5	5.0	190.0
Jun-05	2	1259	25.9	32.0	42.0	52.0	6.5	8.0	6.5	8.0	80.8
Jul-05	2	1259	27.0	32.0	43.9	52.0	6.7	8.0	6.7	8.0	84.4
Aug-05	2	1260	25.9	32.0	42.1	52.0	6.5	8.0	6.5	8.0	81.0
Sep-05	2	1256	25.5	32.0	41.5	52.0	6.4	8.0	6.4	8.0	79.8
Oct-05	2	1279	29.5	32.0	47.9	52.0	7.4	8.0	7.4	8.0	92.1
Nov-05	2	1279	39.7	32.0	64.5	52.0	9.9	8.0	9.9	8.0	124.1
Dec-05	2	1279	32.1	32.0	52.2	52.0	8.0	8.0	8.0	8.0	100.4
Jun-05	3	2687	30.5	29.0	54.6	52.0	14.7	14.0	5.3	5.0	105.0
Jul-05	3	2660	30.5	29.0	54.6	52.0	14.7	14.0	5.3	5.0	105.0
Aug-05	3	2653	30.4	29.0	54.5	52.0	14.7	14.0	5.2	5.0	104.9
Sep-05	3	2612	27.8	29.0	49.8	52.0	13.4	14.0	4.8	5.0	95.7
Oct-05	3	2585	30.1	29.0	54.0	52.0	14.5	14.0	5.2	5.0	103.8
Nov-05	3	2585	40.8	29.0	73.2	52.0	19.7	14.0	7.0	5.0	140.8
Dec-05	3	2585	33.8	29.0	60.6	52.0	16.3	14.0	5.8	5.0	116.6
Jun-05	4	1798	32.1	32.0	52.2	52.0	11.0	11.0	5.0	5.0	100.4
Jul-05	4	1798	31.9	32.0	51.9	52.0	11.0	11.0	5.0	5.0	99.8
Aug-05	4	1825	32.0	32.0	52.0	52.0	11.0	11.0	5.0	5.0	100.0
Sep-05	4	1841	33.0	32.0	53.7	52.0	11.4	11.0	5.2	5.0	103.2
Oct-05	4	1852	33.8	32.0	54.9	52.0	11.6	11.0	5.3	5.0	105.5
Nov-05	4	1852	41.8	32.0	68.0	52.0	14.4	11.0	6.5	5.0	130.7
Dec-05	4	1852	32.0	32.0	52.0	52.0	11.0	11.0	5.0	5.0	100.0
Jun-05	5		49.5	30.0	85.8	52.0	19.8	12.0	9.9	6.0	165.0
Jul-05	5	4255	49.5	30.0	85.8	52.0	19.8	12.0	9.9	6.0	165.0
Aug-05	5	4243	49.5	30.0	85.8	52.0	19.8	12.0	9.9	6.0	165.0
Sep-05	5	4191	49.5	30.0	85.8	52.0	19.8	12.0	9.9	6.0	165.0
Oct-05	5	4191	50.0	30.0	86.7	52.0	20.0	12.0	10.0	6.0	166.8
Nov-05	5	4191	66.5	30.0	115.2	52.0	26.6	12.0	13.3	6.0	221.6
Dec-05	5	4191	57.4	30.0	99.5	52.0	23.0	12.0	11.5	6.0	191.4

DATE	NAME	WEIGHT (kg)	CAPELIN (lbs)	CAPELIN (%)	C.R. SMELT (lbs)	C.R. SMELT (%)	HERRING (lbs)	HERRING (%)	MACKEREL (lbs)	MACKEREL (%)	SALMON (lbs)	SALMON (%)	SARDINES (lbs)	SARDINES (%)	TOTAL FOOD AVG (lbs per day)
Aug-05	6	2073	65.3	50.8	17.8	13.8	6.6	5.1	17.4	13.5	21.6	16.8	0.0	0.0	128.7
Sep-05	6	2073	71.1	51.1	29.0	20.8	10.2	7.4	9.4	6.7	19.5	14.0	0.0	0.0	139.2
Oct-05	6	2150	65.5	46.0	30.1	21.2	16.9	11.9	9.9	6.9	20.0	14.0	0.0	0.0	142.4
Nov-05	6	2177	66.3	45.3	2.7	1.8	47.7	32.6	9.8	6.7	19.8	13.6	0.0	0.0	146.3
Dec-05	6	2208	70.0	46.6	0.0	0.0	50.2	33.4	9.9	6.6	19.9	13.3	0.0	0.0	150.1
Jan-06	6	2223	67.7	43.6	1.0	0.6	55.8	35.9	10.6	6.8	20.2	13.0	0.0	0.0	155.3
Feb-06	6		64.9	0.5	0.0	0.0	60.9	0.4	15.0	0.1			0.0	0.0	140.8
Mar-06	6		37.4	0.4	0.0	0.0	40.3	0.4	19.1	0.2			0.0	0.0	96.8
Jul-05	7	907	47.5	55.9	0.0	0.0	37.4	44.1	0.0	0.0	0.0	0.0	0.0	0.0	84.9
Aug-05	7	898	49.7	53.0	0.0	0.0	42.7	45.6	0.0	0.0	0.0	0.0	1.3	1.4	
Sep-05	7	925	52.5	51.3	0.0	0.0	45.0	44.0	0.0	0.0	0.0	0.0	4.8	4.7	102.3
Oct-05	7	997	55.3	52.5	0.0	0.0	45.0	42.7	0.0	0.0	0.0	0.0	5.0	4.8	105.3
Nov-05	7	997	48.9	44.2	0.0	0.0	52.7	47.7	0.0	0.0	0.0	0.0	9.0	8.1	110.6
Dec-05	7	997	51.7	46.8	0.0	0.0	48.8	44.2	0.0	0.0	0.0	0.0	10.0	9.1	110.5
Jan-06	7	997	49.8	46.2	0.0	0.0	48.0	44.5	0.0	0.0	0.0	0.0	10.0	9.3	107.9
Feb-06	7		40.0	0.5	0.0	0.0	35.0	0.5	0.0	0.0			0.0	0.0	75.0
Mar-06	7		34.5	0.5	0.0	0.0	25.9	0.4	7.6	0.1			0.0	0.0	68.0
Jul-05	8	526	16.7	65.1	0.0	0.0	8.9	34.9	0.0	0.0	0.0	0.0	0.0	0.0	25.6
Aug-05	8	526	20.9	56.0	0.0	0.0	15.6	41.7	0.0	0.0	0.0	0.0	0.8	2.3	
Sep-05	8	544	21.8	43.7	0.0	0.0	25.2	50.5	0.0	0.0	0.0	0.0	2.9	5.8	50.0
Oct-05	8	567	25.1	41.8	0.0	0.0	31.9	53.2	0.0	0.0	0.0	0.0	3.0	5.0	60.0
Nov-05	8	567	30.9	44.9	0.0	0.0	35.0	50.8	0.0	0.0	0.0	0.0	3.0	4.4	68.9
Dec-05	8	567	35.7	49.9	0.0	0.0	32.9	46.0	0.0	0.0	0.0	0.0	3.0	4.2	71.7
Jan-06	8	567	38.0	51.4	0.0	0.0	33.0	44.6	0.0	0.0	0.0	0.0	3.0	4.1	74.0
Feb-06	8		32.4	0.5	0.0	0.0	29.3	0.5	0.0	0.0			0.0	0.0	61.7
Mar-06	8		23.2	0.5	0.0	0.0	20.5	0.5	0.0	0.0			0.0	0.0	43.7
Aug-05	9	1134	61.6	68.3	9.6	10.7	0.3	0.3	8.7	9.7	9.9	11.0	0.0	0.0	90.2
Sep-05	9	1134	65.0	0.7	14.4	0.2	0.1	0.0	5.0	0.1	10.0	0.1	0.0	0.0	94.5
Oct-05	9	1216	58.8	64.4	13.1	14.3	4.7	5.2	5.0	5.5	9.7	10.6	0.0	0.0	91.3
Nov-05	9	1211	47.3	52.1	1.0	1.1	27.5	30.3	5.0	5.5	10.0	11.0	0.0	0.0	90.8
Dec-05	9	1229	50.0	52.5	0.0	0.0	30.2	31.7	5.0	5.3	10.0	10.5	0.0	0.0	95.2
Jan-06	9	1256	48.4	49.2	0.6	0.7	33.7	34.3	5.3	5.4	10.3	10.5	0.0	0.0	98.4
Feb-06	9		40.9	0.5	0.0	0.0	34.7	0.5	0.0	0.0			0.0	0.0	75.5
Mar-06	9		33.0	0.5	0.0	0.0	27.2	0.4	9.6	0.1			0.0	0.0	69.9

APPENDIX 2. Isotopic values for common prey items of marine mammal predators.

Appendix 2. Isotopic values for common prey items of marine mammal predators. The list is arranged according to reference source.

Prey species	δ15N	SD	δ13C	SD	Reference
Icelandic capelin (medium)	11.1	0.2	-19.8	0.1	Kurle 2002
Atlantic herring (medium)	11.8	0.2	-18.5	0.3	Kurle 2002
herring, 6-7cm, YOY	11, 14.5, 15.5				Hansson et al 1997 estimates from Figure 3
herring, 11-12cm, age 1	12, 12.5, 13.5				Hansson et al 1997 estimates from Figure 3
herring, 15-17cm	10.5, 13, 13.5				Hansson et al 1997 estimates from Figure 3
herring, >21cm	11, 11.5, 12 13.5, 16, 16.5				Hansson et al 1997 estimates from Figure 3
smelt	7.23	0.12	-25.04	0.11	Harvey & Kitchell 2000
Lake herring	5.45	0.11	-26.90	0.14	Harvey & Kitchell 2000
Rainbow smelt	6.41	0.42	-20.21	0.24	Harvey et al 2002
Rainbow smelt	9.51	0.47	-17.94	0.21	Harvey et al 2002
Atlantic herring (<i>Clupea harengus</i>)	11.4	0.2			Fry (1988), Dickson (1986), cited in Hobson & Montevecchi 1991 Dickson (1986), cited in Hobson & Montevecchi 1991
Capelin (<i>Mallotus villosus</i>)	12.7	0.9			Hobson & Montevecchi 1991
Capelin (<i>Mallotus villosus</i>)	13.0	0.6			Hobson & Montevecchi 1991
Squid spp.	11.0	0.3			Fry (1988), cited in Hobson & Montevecchi 1991
Short-finned squid (<i>Illex illecebrosus</i>)	11.9	0.1			Hobson & Montevecchi 1991 carbon estimated from Figure 2
smelt	14.4	0.2	-16.8	0.1	Hobson et al 1994
squid	13.9				Hobson et al 1994 estimate from Figure 5
herring	13.7				Hobson et al 1994 estimate from Figure 5
capelin	13.3				Hobson et al 1994 estimate from Figure 6
Herring (<i>Clupea</i> sp.)	11.2				Fry (1988), cited in Hooker et al 2001
Herring (<i>Clupea</i> sp.)	14.5	0.1			Hobson et al (1997), cited in Hooker et al 2001
Herring	11.68				Jennings et al 2002 (predicted value)
Pacific herring (small)	15.3	0.2	-19.3	0.5	Kurle & Worthy 2001

Pacific herring (medium)	13.5	0.1	-17.6	0.1	Kurle & Worthy 2001
Squid (<i>Gonatopsis borealis</i>) (small)	11.1	0.2	-18.4	0.5	Kurle & Worthy 2001
Squid (<i>Berryteuthis magister</i>) (small)	11.4	0.2	-18.1	0.2	Kurle & Worthy 2001
Squid (<i>Berryteuthis magister</i>) (medium)	11.4	0.2	-18.3	0.2	Kurle & Worthy 2001
Market squid	12.3	0.4	-17.1	0.2	Sydeman et al 1997
Icelandic capelin	10.8	0.1	-21.3	0.3	Thompson et al 1999
Squid (<i>Gonatus fabricii</i>) - adult female	13.60		-19.60		Hooker et al 2001
Squid (<i>Gonatus fabricii</i>) - adult female	14.23		-19.22		Hooker et al 2001
Squid (<i>Gonatus fabricii</i>) - adult male	13.40		-19.55		Hooker et al 2001
Squid (<i>Gonatus fabricii</i>) - juvenile	9.73		-19.92		Hooker et al 2001
Squid (<i>Gonatus fabricii</i>) - juvenile	9.22		-19.59		Hooker et al 2001