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Use of the Stable Isotope Ratio $\delta^{13}C$ to Determine Diet in Humpback Whales (*Megaptera novaeangliae*)

by

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Previous work has shown that stable isotope indicators taken from the muscle tissue of dead, stranded cetaceans can be used to assess diet. Recent advances in biopsy technique have provided a means to collect skin and blubber tissues from live animals. In this study we examine the potential of biopsy samples for isotopic assessments of diet by 1) determining if isotopic differences exist between the two tissues types obtained in a biopsy (skin and blubber) and the traditional source for isotopic analysis, muscle tissue, 2) examining the effects of two different lipid extraction techniques on the removal of the preservative dimethyl sulfphoxide (DMSO) from tissues, and 3) assessing procedural reproducibility for automated isotopic analysis of skin derived from biopsy samples.

Results demonstrate that carbon isotopic values ($\delta^{13}C$) of muscle are not significantly different from those of skin (Scheffé, $p = 0.4985$; $\delta^{13}C = -19.1‰ \pm 0.7$ and $-19.5‰ \pm 0.5$ respectively; mean ± SD). The values for blubber ($\delta^{13}C = -23.7‰ \pm 0.2$) were significantly lower than those of muscle or skin (Scheffé, $p = 0.0001$). This result is consistent with previous studies indicating that the $\delta^{13}C$ of lipids is typically lighter than those tissues with which it is associated.

Analysis also indicate that samples preserved in DMSO have significantly lower $\delta^{13}C$ than the same samples unpreserved (paired t-test, $p = 0.010$). Two methods of lipid extraction - sonication and soxhlet extraction - were successful in removing DMSO from samples. The procedural reproducibility for $\delta^{13}C$ was less than 0.1‰.

In summary, skin tissue yielded from biopsy samples may be used in isotopic assessments of diet. The use of biopsies as a source allows the technique to be used in longitudinal, non-lethal sampling.

Introduction

Studies of cetacean diet and foraging behaviour have traditionally relied upon either analysis of gut content or opportunistic observations of feeding bouts. However, analysis of stomach contents requires subject mortality, and sometimes relies on the identification of partially digested food items and thus can be unreliable. Also, either method only yields information concerning the most recent diet composition.

Isotopic analysis provides an alternative method for identification of diet by examination of storage tissues for isotopic signatures unique to particular food items (Peterson and Fry, 1987). Assuming the tissues can be accessed without subject mortality, the technique is non-lethal and allows for longitudinal assessment via repeated sampling.

The isotope technique is based on the observation that the isotopic composition of a consumer is similar to, or deviates by a consistent amount, from its food source (DeNiro and Epstein, 1981; Peterson and Fry, 1987; Harrigan *et al.*, 1989). Whereas organisms differ by 1‰ in $\delta^{13}C$ from their diet, the $\delta^{15}N$ composition is typically 3‰ greater than its diet (DeNiro and Epstein, 1978; 1981; Harrigan *et al.*, 1989; Wada *et al.*, 1987; Ostrom and Fry, 1993). Thus nitrogen isotope values are excellent indicators of trophic position (Harrigan *et al.*, 1989; Wada *et al.*, 1987), and in the near absence of other reliable dietary information, $\delta^{13}C$ and $\delta^{15}N$ values provide direct evidence of past feeding behaviour[1].

Recent studies on a wide variety of animals have demonstrated that isotope analysis on a number of different tissues (commonly muscle or bone) can be used to successfully delineate diet and trophic relationships. Within marine systems analysis of macrofauna has included seabirds, some fish species, and the polar bear (e.g., McConnaughey and McRoy, 1979; Fry and Sherr, 1984; Ramsay, 1991; Hobson and Welch, 1992; Rau *et al.*, 1992). Only two studies have examined trophic relationships within cetaceans, using either muscle tissue (Ostrom *et al.*, 1992) or baleen (Schell *et al.*, 1989a; 1989b) taken from stranded or harvested animals. In these studies the tissues were sampled *post mortem*.

The recent development of biopsy techniques for live sampling of large cetaceans has introduced the possibility of isotope analysis on free-ranging whale species with minimal disturbance (Palsbøll *et al.*, 1991; Weinrich *et al.*, 1991; Lambertson *et al.*, 1994). From 1992 to 1994, an extensive program of sampling of the north-west Atlantic population of humpback whales (*Megaptera novaeangliae*) - termed YoNAH - provided a number of biopsies associated with

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[1]Stable carbon and nitrogen isotope ratios are defined as $\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$, where 'R' is $^{13}C/^{12}C$ or $^{15}N/^{14}N$ for $\delta^{13}C$ and $\delta^{15}N$ respectively. The standard for carbon is the Chicago Peedee Belemnite (PDB) and for nitrogen the standard is atmospheric N_2.

identified individuals around the coasts of Newfoundland and Labrador (Mattila *et al.*, 1991). The biopsy dart used typically sampled a 0.5 cm diameter core of skin and blubber 2-3 cm in depth (the depth depended on angle of dart entry). To preserve the material for genetic analyses, the biopsy material was usually archived in dimethyl sulfphoxide (DMSO).

In order to assess the potential of YoNAH samples (in particular the skin fraction) for use in an isotope analysis program, we sampled stranded humpback whales from Newfoundland to determine 1) if any isotopic differences exist between the two tissue types obtained in a biopsy (blubber and skin) and the common source for isotopic analysis - muscle, 2) the effects of two different lipid extraction techniques on the removal of DMSO from tissue samples, and 3) procedural reproducibility for automated isotopic analysis of skin samples the same size as those yielded by biopsy.

In this paper we report the findings of the above three experiments and conclude that skin tissue taken via biopsy may be an appropriate source for isotope analysis, and may be used investigate trophic relationships and diet in humpback whales.

Methods

Tissue comparison of $\delta^{13}C$

Muscle, skin and blubber tissue from four stranded humpback whales (2 males, 2 females) were taken *post mortem* and frozen for subsequent analysis. Samples were thawed and dried at 40°C to a constant weight. Dried samples were ground to a fine powder. Isotope analyses were performed by a modified Dumas method (Macko *et al.*, 1987). Approximately 5 mg of tissue were combusted in a sealed quartz tube in the presence of copper and copper oxide. Gases of suitable purity were obtained by cryogenic gas separation. Isotopic determinations were performed using an OPTIMA stable isotope ratio mass spectrometer (VG Isotech).

DMSO removal

A second set of samples from one male and one female individual of the above group were soaked in a solution of DMSO and brine prior to freezing. Half of these samples were analyzed directly as described above. The remaining half were thawed, dried and ground, and then subjected to lipid extraction. Lipids were removed via sonication using a azeotropic mixture of 87% dicholoromethane and 13% methanol for a period of three hours under agitation from a sonic probe. The resulting solution was centrifuged, the supernatant decanted and the tissue washed in further solvent, and finally dried at room temperature. After additional homogenization the sample was combusted as above. Isotopic analyses were performed using an OPTIMA stable isotope ratio mass spectrometer (VG Isotech). Lipid removal via soxhlet extraction was performed using the same azeotropic mixture for seven hours. In this procedure, approximately 100 mg of dried tissue were placed in a cellulose filter and subject to repeated washings of clean condensate of the azeotropic solvent mixture.

Procedural Reproducibility

A section of skin collected from a stranded humpback (unknown gender) was dried and divided into 4 aliquots of approximately 50 mg, 100 mg, 250 mg, and 500 mg dry weight. Each aliquot was homogenized using a ball and capsule amalgamator (Wig-L-Bug, Cresent Industries). Lipids were removed from the homogenates in an azeotropic mixture of 87% chloroform and 13% methanol (this mixture is of a similar polarity to that used for the extractions described above) for seven hours. Samples were dried in an evaporatory oven and $\delta^{13}C$ and $\delta^{15}N$ analysis were performed using a Carlo Erba Elemental Analyzer interfaced to a PRISM (VG Istotech) stable isotope ratio mass spectrometer.

Results

Comparison of $\delta^{13}C$ among tissues

Figure 1 gives the $\delta^{13}C$ values for 2 males and 2 females for the three types of tissue, prior to treatment with DMSO. Paired t-tests indicate no difference between males and females for different tissue types (skin, $p = 0.406$; muscle, $p = 0.312$; blubber, $p = 0.949$). However, significant differences do exist between tissue types (F test, $p < 0.0001$), specifically between blubber (mean ± SD = -23.7‰ ± 0.2) and muscle/skin combined (Scheffé, $p = 0.0001$), but not between muscle (avg. = -19.1‰ ± 0.7) and skin (avg. = -19.5‰ ± 0.5) (Scheffé, $p = 0.4985$).

The addition of DMSO significantly decreased values of $\delta^{13}C$ (paired t-test, $p = 0.010$) for muscle and skin pooled, but not when values for blubber were included ($p = 0.055$) (Figure 2).

DMSO removal

Figure 3 demonstrates the effect of lipid extraction on DMSO and non-DMSO treated samples. Lipid extraction significantly increased $\delta^{13}C$ values of DMSO treated samples (Scheffé, $p = 0.0001$) values when compared to non-lipid extracted DMSO samples. There was no significant difference between a lipid extracted DMSO treated sample and a lipid extracted non-DMSO treated sample (Scheffé, $p = 0.8282$).

The soxhlet and sonication techniques resulted in slight, but significant, differences in terms of final $\delta^{13}C$ (Scheffé, $p = 0.0282$), although these differences approach the level of procedural reproducibility (see below).

Reliability Test

Figure 4 demonstrates that sample size does not significantly effect values for either $\delta^{13}C$ ($r = 0.375$, $p = 0.407$) or $\delta^{15}N$ ($r = 0.568$, $p = 0.183$). In fact, the technique produces highly consistent results for both $\delta^{13}C$ (mean ± SD: -18.8‰ ± 0.05) and $\delta^{15}N$ (mean ± SD: 14.2‰ ± 0.07).

Discussion

Within the literature, there are few studies that report isotopic data for large cetaceans. Ostrom *et al.* (1992) presented data for muscle tissues collected post mortem for a number of cetaceans using non-lipid extracted tissues. The soxhlet-lipid extracted $\delta^{13}C$ value for skin of -18.6‰ ± 0.02

(mean ± SD) found in the present study closely agrees with their quoted $\delta^{13}C$ for humpback whale muscle of 18.7‰, and falls within a range typical of many of the baleen whales sampled in that *same report*. It is also similar to previous estimates of lipid-extracted muscle ($\delta^{13}C$ = -17.8‰ ± 0.4, n = 11) from humpback whales taken around Newfoundland (Ostrom, unpub. data). Variability among whales and studies is likely a function of geographic area, time of year, and diet, as demonstrated by Schell *et al.*, (1989a) for bowhead whales.

The lipid fraction of a sample tends to be isotopically light (DeNiro and Epstein, 1977). It is therefore expected that a non-lipid extracted sample would yield a lower $\delta^{13}C$ than a lipid extracted sample. Results from the present study indicating that an aliquot of lipid-extracted muscle or skin tissue has a higher $\delta^{13}C$ (approx. 1‰) than the non-extracted aliquot demonstrate this. *Weights of the lipids* extracted from the skin samples indicate that the lipid fraction accounts for only 10 - 15 % of the sample by mass (Todd, unpub. data).

Given that lipids tend to have low $\delta^{13}C$ values, our observation that blubber has lower $\delta^{13}C$ values (approximately 4.5‰) than either muscle or skin is expected (DeNiro and Epstein, 1977). Muscle and skin $\delta^{13}C$ values appear similar, suggesting that the two tissues reflect similar dietary information. Consequently, our finding suggest that $\delta^{13}C$ of biopsied skin tissue would likely provide similar dietary information to muscle, but blubber would retain lower $\delta^{13}C$ values that reflect their unique metabolism rather than diet information.

It is clear that the addition of DMSO dramatically changes the $\delta^{13}C$ value for muscle and skin, and that its effects should be removed before isotopic analysis. Sonication and soxhlet extraction are effective methods of removing DMSO and provide similar isotopic results. The reduced handling time associated with soxhlet extraction is an appealing attribute of this method. In addition to its ability to remove DMSO, lipid extraction has the advantage of removing isotopically unique component of tissues (lipids) that can obscure isotopic information retained in the tissues (Ostrom *et al.*, 1992).

Results from the reproducibility tests indicate that working with small sample sizes, such as those obtained from dart biopsies, does not effect the reproducibility or accuracy of $\delta^{13}C$ or $\delta^{15}N$. Combined with the observation of no significant difference between isotopic values of skin and muscle, this indicates that the analysis of skin biopsies would be a reliable technique for dietary analysis of cetaceans.

In conclusion, the above results indicate that skin biopsies may be used in isotope analysis. Moreover, it should be possible to analyze biopsies archived in DMSO, using extraction techniques that will also remove noise created by lipid fractions with unique $\delta^{13}C$ values. Stable isotope analysis provides a non-lethal alternative to examination of stomach contents to delineate diet. As a further advantage, dietary evaluations based on analyses of assimilated tissues implies that the data reflects dietary information integrated over a longer period of time (defined as a function of

turnover of the tissue), as opposed to the instantaneous sampling of recently digested food items. With the added possibility of resampling photo-identified individuals within or between seasons, isotope analysis may also be used in longitudinal studies of foraging behaviour.

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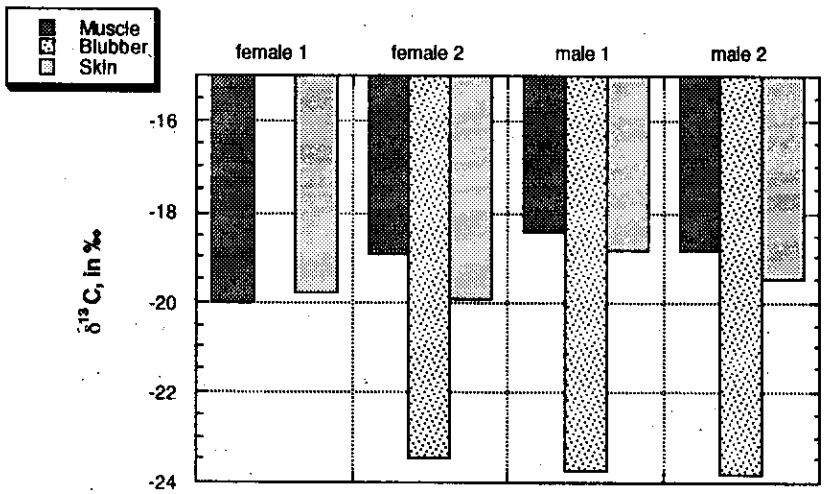

Figure 1. Values for the isotope ratio $\delta^{13}C$ for three tissue types across males (n = 2) and females (n = 2). A $\delta^{13}C$ value for blubber in female 1 was not available.

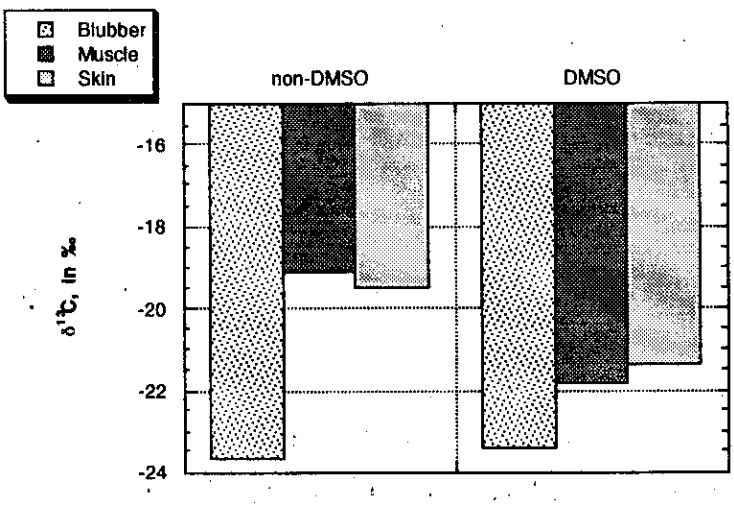

Figure 2. Differences between DMSO (n = 2) and non-DMSO (n = 4) treated tissues, averaged across gender.

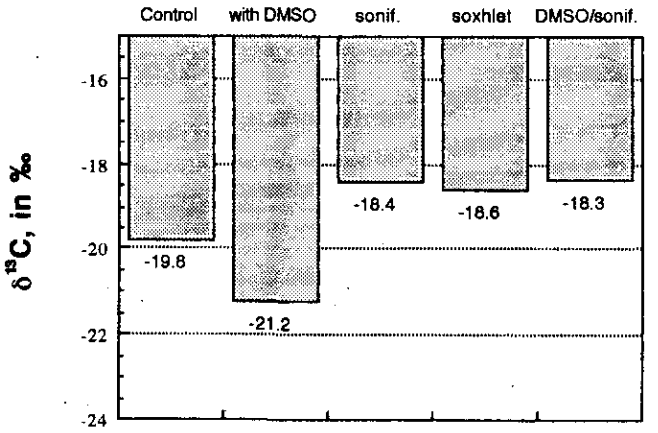

Figure 3. Effects of extraction methods on skin biopsy material

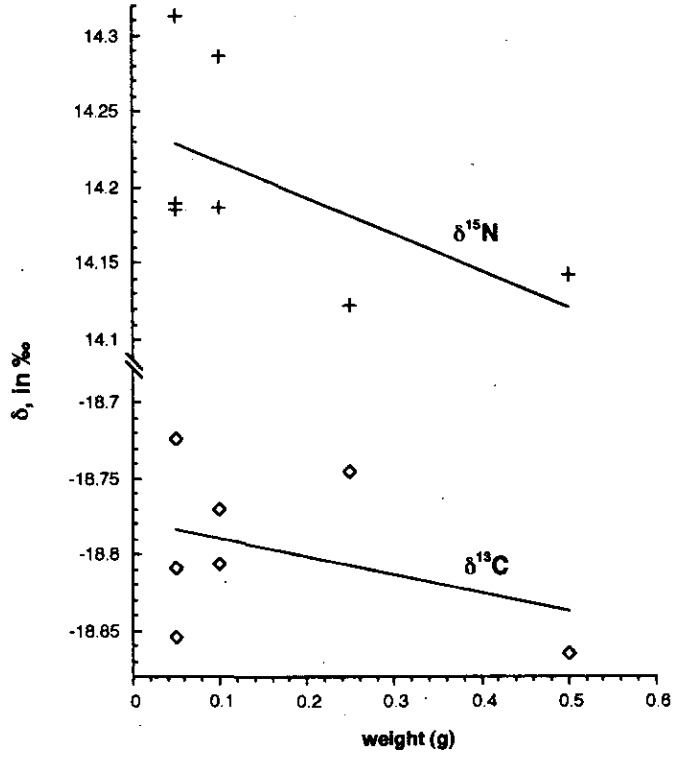

Figure 4. Effects of sample size on repeated measures of $\delta^{13}C$ and $\delta^{15}N$