Use of Biopsy Samples of Humpback Whale (Megaptera novaeangliae) Skin for Stable Isotope ($\delta^{13}C$) Determination

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Abstract

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 remote biopsy techniques have provided a means to collect skin and blubber tissues from live animals. This study examines the potential of biopsy samples taken from humpback whales (Megaptera novaeangliae) for isotopic assessments of diet by 1) determining if isotopic differences exist between the two tissue 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 were not significantly different from those of skin (Scheffé, $p = 0.4985$; $\delta^{13}C = -19.1\% \pm 0.7$ and $-19.5\% \pm 0.5$ for muscle and skin respectively; mean $\pm$ 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 was consistent with previous studies indicating that the $\delta^{13}C$ of lipids is typically lighter than those tissues with which it is associated. The analysis also indicates that samples preserved in DMSO have significantly lower $\delta^{13}C$ than unpreserved samples (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 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.

Key words: diet, Humpback whale, sampling methods, stable isotopes

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 gut contents usually requires subject mortality, and sometimes relies on the identification of partially digested food items and thus can be unreliable. Also, both methods only yield information concerning the most recent diet composition.

Isotopic analysis provides an alternative method for identification of diet by examination of tissues for isotopic signatures that reflect food eaten (Peterson and Fry, 1987). The technique is
non-lethal, assuming the tissues can be accessed without subject mortality, and thus 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.

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 and Hobson, 1991; Hobson and Welch, 1992; Rau et al., 1992). However, few studies have investigated trophic relationships within cetaceans; such examinations typically used either muscle tissue (Ostrom et al., 1993; Abend and Smith, 1995) or baleen (Schell et al., 1989a; 1989b) taken from stranded or harvested animals. In these studies the tissues were sampled post mortem. In addition, depending on cause of death, stranded animals may represent nutritionally deficient individuals.

The recent development of biopsy techniques for live sampling of large cetaceans has introduced the possibility of isotopic 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 Northwest Atlantic population of humpback whales (Megaptera novaeangliae) – termed YoNAH – provided a number of biopsies associated with identified individuals around the coasts of Newfoundland and Labrador (Mattila et al., MS 1991). The biopsy dart 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).

Borobia et al. (1995) demonstrated that the blubber fraction of biopsy samples taken from humpback and finback (Balaenoptera physalus) whales may be used in stable isotope analysis. In order to assess the potential of YoNAH samples (in particular the skin fraction) for use in a similar isotope analysis program, we sampled stranded humpback whales from Newfoundland to determine, specifically with respect to $\delta^{13}C$:

1. if any isotopic differences exist between the two tissue types obtained in a biopsy (blubber and skin) and the tissue most frequently used for isotopic analysis of diet, (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 of the same size as those obtained by biopsy.

This paper reports the findings of the above three experiments. It is shown that skin tissue taken by biopsy may be an appropriate source for isotope analysis, that 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).

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1 Stable carbon and nitrogen isotope ratios are defined as $\delta X = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000$, 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$_2$. 

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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 as described above without additional treatment to remove lipids. The remaining half were thawed (skin only), dried and ground, and then subjected to lipid extraction. Lipids were removed via sonication using an azeotropic mixture of 87% dichloro-methane 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 by the modified Dumas method (Macko et al., 1987) described 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 subjected 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, Crescent 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 δ13C and δ15N analyses were performed using a Carlo Erba Elemental Analyzer interfaced to a PRISM (VG Istotech) stable isotope ratio mass spectrometer.

Results

Comparison of δ13C Among Tissues

Paired t-tests indicated no difference between males and females for different tissue types (skin, p = 0.406; muscle, p = 0.312; blubber, p = 0.949) (Fig. 1). However, independent of sex, significant differences exist between tissue types (F test, p <0.0001). These differences were primarily between blubber (mean ± SD = -23.7‰ ± 0.2) and muscle/skin considered together (Scheffé, p = 0.0001), but not between muscle (avg. = -19.1‰ ± 0.7) and skin (avg. = -19.5‰ ± 0.5) (Scheffé, p = 0.4985) (Fig. 1).

When muscle and skin were considered, the addition of DMSO significantly reduced values of δ13C (paired t-test, p = 0.010). However, because values for DMSO treated and non-DMSO treated blubber were similar, inclusion of blubber δ13C values to this dataset reduced the difference between DMSO and non-DMSO treated samples to being non-significant (p = 0.055) (Fig. 2).

DMSO removal

Lipid extraction significantly increased δ13C values of DMSO treated skin samples (δ13C = -18.3‰) values when compared to non-lipid extracted DMSO samples (δ13C = -21.2‰) (Scheffé, p = 0.0001) (Fig. 3). There was no significant difference between a lipid extracted DMSO treated sample and a lipid extracted non-DMSO treated sample (δ13C = -18.5‰) (Scheffé, p = 0.8282).

The Soxhlet and sonication techniques resulted in slight, but significant, differences in terms of final δ13C (Scheffé, p = 0.0282). Although these differences approach the level of procedural reproducibility (see below), additional experimentation is needed to quantify the reasons for this difference.

The Effects of Sample Size on Reproducibility

Sample size did not significantly affect values for either δ13C (r = 0.375, p = 0.407) or δ15N (r = 0.568, p = 0.183), expressed as deviations from the sample mean (Fig. 4). In fact, the technique produced highly consistent results for both δ13C (mean ± SD: -18.8‰ ± 0.1) and δ15N (mean ± SD: 14.2‰ ± 0.1).

Discussion

Within the literature, there are few studies that report isotopic data for large cetaceans. Ostrom et al. (1993) presented data for muscle tissues collected post mortem for a number of cetaceans using non-lipid extracted tissues. The Soxhlet-lipid extracted δ13C value for skin of -18.6‰ ± 0.02 (mean ± SD) found in the present study closely agrees with their quoted δ13C for humpback whale
Fig 1. Stable carbon isotope values ($\delta^{13}C$) for skin, muscle and blubber tissues sampled from 2 male and 2 female humpback whales. The $\delta^{13}C$ value for blubber in female 1 was not available.

Fig 2. Stable carbon isotope values ($\delta^{13}C$) for DMSO ($n = 2$) and non-DMSO ($n = 4$) treated tissues (skin, muscle and blubber), averaged across gender.

Fig 3. Stable carbon isotope values ($\delta^{13}C$) for skin biopsy material treated as follows: a) control, b) lipid extracted using sonication, c) lipid extracted using Soxhlet, d) treated with DMSO (no lipid extraction), and e) DMSO treatment plus lipid extraction using sonication.

Fig. 4. Reproducibility of $\delta^{13}C$ ($\times$) and $\delta^{15}N$ (+) values as effected by sample mass, expressed as deviations from their respective sample means.

muscle of $-18.7^{\circ}\text{C}$, 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 ($\delta^{13}C = -17.8^{\circ}\pm 0.4$, $n = 11$) from humpback whales taken around Newfoundland (Ostrom, Michigan State Univ., Michigan, USA, unpubl. data). Minor variation 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. $1^{\circ}\text{C}$) 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, Memorial University, St. John’s, NF, Canada, unpubl. data).

Given that lipids tend to have low $\delta^{13}C$ values, our observation that blubber has lower $\delta^{13}C$ values (approximately $4.5^{\circ}\text{C}$) than either muscle or skin is expected (DeNiro and Epstein, 1977). Muscle and skin $\delta^{13}C$ values differ by less than $0.4^{\circ}\text{C}$, suggesting that the two tissues reflect similar dietary information. Consequently, our findings suggest that $\delta^{13}C$ of biopsied skin tissue would likely provide similar dietary information to muscle. Blubber would have lower $\delta^{13}C$ values than
that of muscle, reflecting its unique metabolism more so than diet information.

It is clear that the addition of DMSO dramatically changes the δ13C 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., 1993).

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 δ13C or δ15N. 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 and thus provide a non-lethal alternative to examination of stomach contents to delineate diet. 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 δ13C values. 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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