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Quantifying variability in stable carbon and nitrogen isotope ratios within the skeletons of marine mammals of the suborder Caniformia

Casey T. Clark[a,b,*], Lara Horstmann[a], Nicole Misarti[b]

[a] College of Fisheries and Ocean Sciences, University of Alaska Fairbanks, P.O. Box 757220, Fairbanks, AK 99775-7220, USA
[b] Water and Environmental Research Center, University of Alaska Fairbanks, P.O. Box 755860, Fairbanks, AK 99775-5860, USA

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ABSTRACT

Stable isotope ratios of bone collagen are commonly used to investigate foraging and movement of human and animal populations. This technique is especially valuable for archaeological and paleoecological applications, as bones are among the few tissues that are commonly preserved in archaeological and assemblages. Selection of skeletal elements for stable isotope analysis is typically driven by sample sizes and convenience, with the assumption that each bone is equally likely to be representative of the entire skeleton. This study investigated the degree of variability in stable carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) within the skeletons of individual marine mammals to determine whether any systematic differences in $\delta^{13}C$ and $\delta^{15}N$ exist among skeletal elements. We measured $\delta^{13}C$ and $\delta^{15}N$ in paired crania and mandibles from 11 Pacific walruses (*Odobenus rosmarus divergens*), as well as representative elements from the skeletons of three marine mammals: an adult ringed seal (*Pusa hispida*, n = 10), a juvenile seal of the genus *Phoca* (*Phoca* sp., n = 9), and an adult sea otter (*Enhydra lutris*, n = 8). Differences among the walrus cranium/mandible pairs were not significant, mostly falling within analytical error. Variability across the skeletons of the seals and sea otter was greater, exceeding 1.0‰ in some cases. Hierarchical cluster analysis indicated systematic differences within all three skeletons, with the distal appendicular bones (metatarsal, phalanx, calcaneus) separating from the rest of the skeleton in the two seals, and the scapula and vertebra distinct from all other bones in the sea otter. Removing these bones from analysis greatly reduced overall variability in all three animals. Further study is required to determine whether the patterns observed in this study are consistent across individuals and taxa as sample sizes increase.

1. Introduction

Stable isotope analysis is a powerful tool for investigating animal diet, movement, and physiology (Hobson, 1999; Kelly, 2000). This technique is particularly useful for paleoecological and archaeological studies, where feeding habits and movements of animal and human populations cannot be directly observed and must instead be reconstructed from preserved or fossil remains (Schoeninger and Moore, 1992). Stable carbon and nitrogen isotope ratios of bone collagen are widely used for such reconstructions, as bones are among the few animal parts commonly recovered from archaeological and paleontological sites. Applications of stable isotope analysis of zooarchaeological assemblages include reconstructions of human and animal diet (e.g., Hilderbrand et al., 1996; Katzenberg and Weber, 1999; Richards and Hedges, 1999; Szpak et al., 2012), food web structure (e.g., Misarti et al., 2009; Bocherens et al., 2015), and environmental change (e.g., Ambrose and DeNiro, 1989; Zangrando et al., 2014; Commendador and Finney, 2016), as well as investigations of human and animal distribution, movement, and dispersal patterns (e.g., Sealy et al., 1995; Barberena et al., 2009; Lamb et al., 2014). Analysis of bone collagen can thus provide information about vertebrate diet and movements across thousands of years.

Though bone collagen is commonly used in archaeology and paleoecology, relatively little work has been done to investigate the degree to which stable isotope ratios vary within the skeletons of individual animals. Bone turnover rates vary with age and type of bone (cortical vs. trabecular), thus stable isotope ratios might be expected to vary accordingly among skeletal elements (Snyder et al., 1975; Klepinger, 1984; Libby et al., 1995; Sealy et al., 1995; Lamb et al., 2014). For the sake of convenience, however, it is commonly assumed that the stable isotope ratios of collagen extracted from any single bone will be representative of the entire skeleton, an assumption that is loosely supported by the work of DeNiro and Schoeninger (1983). Decisions about which skeletal elements to use for stable isotope analysis are typically driven by the availability and preservation of elements within an assemblage (Jørkov et al., 2009); however, if systematic

* Corresponding author at: College of Fisheries and Ocean Sciences, University of Alaska Fairbanks, P.O. Box 757220, Fairbanks, AK 99775-7220, USA.
E-mail addresses: ctclark@alaska.edu (C.T. Clark), lara.horstmann@alaska.edu (L. Horstmann), nmisarti@alaska.edu (N. Misarti).

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differences in stable isotope ratios do exist within the skeletons of individual animals, these differences are important to consider when selecting elements for analysis and reconstructions.

The purpose of this study was to quantify variability of stable isotope ratios among the bones of individual, free-ranging marine Caniformia. To accomplish this, we analyzed the stable carbon and nitrogen isotope ratios of bone collagen extracted from the crania and mandibles of Pacific walruses (*Odobenus rosmarus divergens*), as well as from a variety of bones selected to represent the skeletons of two phocid seals and a sea otter (*Enhydra lutris*). The resulting estimates of variability within the skeletons of individual animals, as well as the identification of systematic differences in stable carbon and nitrogen isotope ratios among skeletal elements, will improve future studies by providing researchers with a better understanding of intra-skeletal stable isotope variability. Furthermore, these data will allow for the exclusion of skeletal elements that are not representative of the entire skeleton.

2. Materials and methods

Eleven Pacific walrus cranium/mandible pairs and representative elements from three marine mammal skeletons (n = 8–10) were on loan from the University of Alaska Museum, Fairbanks, AK. Skeletons used for this study were from an adult ringed seal (*Pusa hispida*), a juvenile seal of the genus *Phoca*, and an adult sea otter. Representative bones were selected from all parts of the skeleton including the skull (cranium/mandible), axial skeleton (vertebra, rib), and appendicular skeleton (scapula, innominate, humerus, femur, metatarsal, calcaneus and/or phalanx). All samples were from animals that died after 1930, thus were historical or modern and not of archaeological origin.

Bones were sampled using handheld cutting tools, and ~0.4 g of bone was used for collagen extractions, which were carried out according to the methods described by Misarti et al. (2009), as modified from Matheus (1995). Briefly, bones were cleaned in a sonic bath, then lipids were extracted by soaking bone in 2:1 choloroform:methanol for eight hours. Hydrochloric acid was used to remove the mineral component of the bone. The organic component was then gelatinized in a mildly acidic solution at 65 °C, filtered through a 0.45 μm filter to remove any insoluble particles and non-collagen organic compounds, and freeze dried to produce purified collagen. A subsample of 0.2–0.4 mg of collagen was then submitted for stable isotope analysis. Collagen was extracted from walrus crania and mandibles once. Three replicate subsamples were cut from the bones of the seals and sea otter to incorporate some of the variability in stable isotope ratios of collagen within each bone. Replicate subsamples were taken from locations directly adjacent to one another and collagen was extracted from each subsample separately. The phalanx of the ringed seal and the metatarsal and rib of the *Phoca* sp. could only be subsampled and collagen extracted twice due to limitations in the amount of available material.

Stable carbon and nitrogen isotope ratios of collagen samples were analyzed by the Alaska Stable Isotope Facility at the Water and Environmental Research Center, University of Alaska Fairbanks, using a Costech ECS 4010 elemental analyzer and ThermoScientific Conflo IV, interfaced with a ThermoScientific Delta V mass spectrometer. Stable isotopic compositions were calibrated relative to Vienna Pee Dee Belemnite and atmospheric nitrogen gas (air) scales using USGS40 and USGS41. Results were reported in parts per thousand (‰) using δ notation. A commercially available peptone standard (No. P-7750 Bovine based protein. Sigma Chemical Company, lot #76f-0300; $\delta^{13}C$: − 15.8‰, $\delta^{15}N$: 7.0‰) was analyzed as a check standard after every 10 samples to measure uncertainty. Precision of these analyses was determined to be ± 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$, based on repeated measurements of this check standard across all analytical runs (n = 48). Measurements were accurate to within less than ± 0.01‰ for both $\delta^{13}C$ and $\delta^{15}N$, based on differences between observed and known values of the check standard. Collagen yield (percent of dry bone weight) and sample composition (weight percent carbon, weight percent nitrogen, and C/N ratio) were assessed to evaluate the quality of the collagen samples.

Differences among crania and mandibles were examined using linear regression analyses. The structure of the data did not allow for parametric comparisons of variability within the skeletons of the seals and sea otter, thus this information was summarized using primarily descriptive statistics. Pooled standard deviations were calculated for measures that averaged variability across all three skeletons to account for differences in the number of skeletal elements analyzed for each individual. Systematic differences in stable isotope ratios within the skeleton were investigated using Ward's method of hierarchical clustering, an agglomerative method that generates clusters based on smallest squared Euclidean distances between cluster centers. All statistical analyses were conducted using R version 3.2.3 (R Core Team, 2014) with RStudio version 1.0.136 (RStudio Team, 2015).

3. Results

Stable carbon and nitrogen isotope ratios of walrus crania exhibited linear correlations with those of mandibles from the same individuals ($\delta^{15}N$: $F_{1,9} = 153.8$, $P \leq 0.001$; $\delta^{13}C$: $F_{1,9} = 86.6$, $P < 0.001$). These correlations had slopes close to one, y-intercepts close to zero, and explained the majority of the variability in the data ($\delta^{13}C$: $R^2 = 0.90$; $\delta^{15}N$: $R^2 = 0.94$), indicating that $\delta^{13}C$ and $\delta^{15}N$ values of the one skeletal element (cranium/mandible) from an individual walrus can be used to accurately predict values of the other element and that these values are essentially identical (Fig. 1). The mean differences (± 1 SD) between the two bones were 0.0 ± 0.2‰ for $\delta^{13}C$ and 0.0 ± 0.3‰ for $\delta^{15}N$. The maximum differences between the cranium and mandible of an individual walrus were 0.2‰ for $\delta^{13}C$ and 0.4‰ for $\delta^{15}N$ (Table 1).

Variability in $\delta^{13}C$ and $\delta^{15}N$ was low across multiple skeletal elements from each individual marine mammal. The mean range (± 1 pooled SD) of stable carbon and nitrogen isotope ratios for the three animals was 0.9 ± 0.6‰ for $\delta^{13}C$ and 0.9 ± 0.4‰ for $\delta^{15}N$. Stable carbon and nitrogen isotope ratios from all ringed seal elements exhibited a maximum range of 1.2‰ for $\delta^{13}C$ and 1.3‰ for $\delta^{15}N$ within a single replicate analysis. The range of all values across all three replicate analyses was 1.6‰ for $\delta^{13}C$ and 1.5‰ for $\delta^{15}N$, and the range of the mean values of the three replicate analyses was 0.6‰ for $\delta^{13}C$ and 1.1‰ for $\delta^{15}N$ (Table 2). Hierarchical cluster analysis indicated the presence of two groups within the ringed seal bones, with the metatarsal, phalanx, and calcaneus grouping separately from the rest of the bones in the skeleton (Fig. 2). With these bones removed from analysis, stable carbon and nitrogen isotope ratios from the ringed seal exhibited a maximum range of 0.9‰ for $\delta^{13}C$ and 0.8‰ for $\delta^{15}N$ within a single replicate analysis. The range of all values across all three replicate analyses with these bones removed was 1.2‰ for both $\delta^{13}C$ and $\delta^{15}N$, and the range of mean values across all three replicate analyses was 0.2‰ for $\delta^{13}C$ and 0.5‰ for $\delta^{15}N$ (Table 2).

The stable carbon and nitrogen isotope ratios of the juvenile seal of the genus *Phoca* exhibited a maximum range of 0.7‰ for $\delta^{13}C$ and 1.3‰ for $\delta^{15}N$ within a single analytical replicate analysis. The range of all values across all three replicate analyses was 0.9‰ for $\delta^{13}C$ and 1.4‰ for $\delta^{15}N$, and the range of the mean values across the three replicate analyses was 0.6‰ for $\delta^{13}C$ and 1.2‰ for $\delta^{15}N$ (Table 3). Hierarchical cluster analysis also indicated the presence of two groups in the bones of the juvenile seal, with the metatarsal and phalanx grouping separately from the rest of the skeleton (Fig. 2). With these bones removed from analysis, stable carbon and nitrogen isotope ratios from this animal exhibited a maximum range of 0.6‰ for $\delta^{13}C$ and 0.8‰ for $\delta^{15}N$ within a single replicate analysis. The range of all values across all three replicate analyses with these bones removed was 0.8‰ for $\delta^{13}C$ and 0.9‰ for $\delta^{15}N$, and the range of mean values across all three replicate analyses was 0.4‰ for $\delta^{13}C$ and 0.7‰ for $\delta^{15}N$ (Table 3).