Journal of Archaeological Science 66 (2016) 112–119

Contents lists available at ScienceDirect

Journal of Archaeological Science

journal homepage: http://www.elsevier.com/locate/jas

Alternative method for subsampling annual dentin layers in small mammalian teeth for stable isotope analysis

Florence Chan Evacitas [a], Graham A.J. Worthy [b], Lien-Siang Chou [a, *]

[a] Institute of Ecology and Evolutionary Biology, College of Life Science, National Taiwan University, Taipei City 10617, Taiwan
[b] Physiological Ecology and Bioenergetics Lab, Department of Biology, University of Central Florida, Orlando, FL 32816-2368, USA

ARTICLE INFO

Article history:
Received 27 March 2015
Received in revised form
21 September 2015
Accepted 3 January 2016
Available online xxx

Keywords:
Dentin subsampling
Collagen
$\delta^{15}N$
$\delta^{13}C$
C/N ratio
Risso's dolphin

ABSTRACT

Several techniques have been used to sample annual dentin layers in mammalian teeth for stable isotope analysis. However tooth size in smaller animals and the conical arrangement of the dentin layers have constrained precision of subsampling and collection of adequate sample from each annual growth layer. We tested an alternative subsampling technique using teeth from Risso's dolphins ($n = 15$) that involved cutting out the annual dentin growth layer groups (GLGs) from 300 to 500 μm longitudinal sections from one half of a demineralized tooth and comparing the results to those obtained using a standard micromilling process on the other half of the same tooth. Subsamples were analyzed for elemental C and N content and for stable C and N isotopes. Subsamples obtained from cutting out the GLGs showed more consistent wt%N, wt%C, and atomic C/N ratios that were significantly different ($P<0.0001$) from those obtained by micromilling. Consequently, the $\delta^{15}N$ and $\delta^{13}C$ values differed significantly between methods with values from the former method being more concordant with the expected variations in the early years of Risso's dolphins. Deviations in $\delta^{13}C$ values in the micromilled subsamples were large enough to create possible errors in interpretation of dietary sources. Cutting out the dentin layers reduced sample processing time by 90% and yielded ~10% more collagen than micromilling. These results suggest that cutting out the annual dentin layers can produce greater yield of samples of better collagen quality with a much shorter processing time than the micromilling process and is, therefore, an effective method to subsample small mammalian teeth.

1. Introduction

A growing number of studies, such as in the fields of ecology, archaeology, and zooarchaeology, employ stable carbon and nitrogen isotope analyses (SIA) using dentin to reconstruct feeding patterns in prehistoric and modern mammals from both terrestrial and aquatic environments (Owen et al., 2011; Sandberg et al., 2014; Zazzo et al., 2006; Walker and Macko, 1999). Since dentin is deposited incrementally as growth layers and is neither resorbed nor remodeled once formed, it can reflect the isotopic ratios at the time of its synthesis. Variations in isotopic ratios in sequential dentin layers allow for the reconstruction of ontogenetic dietary changes at the individual level from which population level data can be drawn for comparison of diets across different time periods and geographic areas. Because of the potential of dentin layers to reveal dietary history of individuals, various methods have been employed to obtain sufficient quantities of dentin subsamples at finer time scale. These subsampling methods include the automated process of micromilling (e.g., Newsome et al., 2009; Zazzo et al., 2006) and some manual techniques that use drills (e.g., Fahy et al., 2014), punch (e.g., Burt and Garvie-Lok, 2013; Kirsanow et al., 2008), or scalpels (e.g., Balasse et al., 2001; Eerkens et al., 2011).

In terrestrial mammals with relatively large teeth (e.g., bovines and chimpanzees), subsampling of dentin layers is done with the use of a micromill (Zazzo et al., 2006) or a hand-held electric drill (Fahy et al., 2014). Similar techniques have also been used to subsample acid-etched half tooth sections of bigger marine mammal teeth such as those of elephant seals (Martin et al., 2011; Riofrío-Lazo et al., 2012), fur seals (Hanson et al., 2009), sea lions (Hobson and Sease, 1998; Newsome et al., 2006), and killer whales (Newsome et al., 2009). Mendes et al. (2007) sampled the dentin layers in demineralized half-sections of sperm whale teeth by manually cutting out each layer from apex to pulp cavity by using a

* Corresponding author.
E-mail address: chouls@ntu.edu.tw (L.-S. Chou).

http://dx.doi.org/10.1016/j.jas.2016.01.001
0305-4403/

scalpel.

Despite the availability of high-precision microsampling tools such as the micromill, tooth size in smaller mammals and the conical arrangement of the dentin layers have constrained precision of subsampling and collection of adequate sample from each annual growth layer. Eerkens et al. (2011) modified the method of Fuller et al. (2003) to microsample dentin of human teeth by making horizontal sections from the root to crown. Although their method improved resolution of isotope profiles at the individual scale, it was not able to resolve cross-contamination among adjacent layers. Burt and Garvie-Lok (2013) demonstrated a different method of microsampling human teeth by using a punch on longitudinal tooth sections. However, collection of adequate amount of dentin collagen from small teeth remains a challenge. As such, age-specific isotopic profiles at the individual level have not been reported for most terrestrial mammals and for all smaller delphinid species. Previous studies on smaller delphinid species used the entire tooth of individuals from different age groups (e.g., Niño-Torres et al., 2006) or subsamples of the outer and inner dentin to represent early and latter life stages, respectively (e.g., Knoff et al., 2008).

The availability of archived tooth specimens of Risso's dolphins (*Grampus griseus)* in Taiwan provides an opportunity to develop a subsampling technique appropriate for small teeth with conical layering of dentin. Like all other toothed whales, the Risso's dolphins grow only one set of homodont teeth (i.e., all teeth are morphologically the same) throughout life. However, it has only 4–6 pairs of teeth on the lower jaw with the anterior and posterior pairs being small and possibly undeveloped. These make the Risso's dolphin teeth a good model for extracting dentin from limited number of morphologically similar teeth, as would be the case in most mammals with heterodont dentition.

In the present study, initial subsampling using a micromill (ESI New Wave™ Research) on half-tooth sections from Risso's dolphins produced inadequate quantities of dentin powder from each individual GLG. Repeated drilling of the same GLG to produce adequate quantity of dentin powder for SIA would take 8–10 h, which would not be very efficient when processing large quantity of samples. Because of the low sample yield, relatively long processing time required for each GLG, and difficulty in resolving GLGs in older animals when using the micromill, a different subsampling method that involved cutting out the GLGs from demineralized tooth sections was devised. We modified the method used by Mendes et al. (2007) on sperm whale teeth to make it more suitable for smaller teeth, such as those of the Risso's dolphin, and to make it more sensitive to the geometry of dentin layers. We then evaluated the effectivity and efficiency of this method by comparing them with those of the micromilling process. The two methods were compared in terms of yield, length of subsampling time, quality of collagen produced based on C/N ratios, wt%N, and wt%C, as well as the $\delta^{15}N$ and $\delta^{13}C$ values of the samples. We expected the cutting process to reduce subsampling time and sample losses, thus increase collagen yield at much faster subsampling rate. It was also expected that measures on the quality of collagen as well as the isotope ratios will be comparable in both methods.

2. Materials and methods

2.1. Preparation of tooth samples

Teeth were collected from archived specimens of Risso's dolphins (n = 15) held at the Cetacean Laboratory at the National Taiwan University. These animals either stranded or were collected as fisheries by-catch in Taiwan between 1994 and 2008. Adhered soft tissues were cleaned from teeth using a scalpel or a soft bristle brush. Each tooth was mounted in commercial epoxy resin (EpoFix, Struers) and cut into longitudinal halves along the bucco–lingual plane with a low-speed diamond edged saw (Buehler Isomet®). The cut surface of each half was ground with 2000 and 2400 grit silicon carbide and polished with Alumina (aluminum oxide) using Buehler lapidary grinder-polisher. One of the polished halves was mounted on glass slide for micromill subsampling of dentin. The other half tooth was used to make a ~150-μm longitudinal section that was permanently mounted on a glass slide with epoxy resin for subsequent age determination by GLG counts. Sectioning, grinding, and polishing of all tooth samples were performed at the Institute of Oceanography, National Taiwan University.

After the thin section was made, the remaining portion of the half tooth was removed from the resin mount and demineralized in 0.25 N HCl (~15 mL acid solution:400 mg sample) at ambient temperature (~25–29 °C). The acid solution was replaced daily until the half tooth appeared translucent. This process took 5–7 days for teeth of older animals and 2–3 days for younger animals (1–2 yr old).

To avoid possible intra-individual variations in dentin isotope ratios that could influence the comparison of measurements, the two halves of the same tooth were used for the two methods. While teeth of older individuals were also available, only teeth from younger individuals (1–4 yr old) where dentin layers were relatively wider were used to ensure that enough subsample could be produced from each method.

2.2. Subsampling of dentin by micromilling

After counting GLGs to determine age of the individual, dentin from each GLG was extracted using two methods, i.e, by micromilling and by manually cutting out the layers. Subsampling with the use of the Micromill (ESI New Wave™ Research) was performed on the polished half of each tooth at the Department of Geosciences, NTU. Lines were drawn following boundaries of each GLG and the micromill paths were interpolated from such lines at distance of 150–200 μm. Because of the conical arrangement of the dentin layers, each line within the same GLG was drilled at varying depths, starting at 50 μm near the boundary of the preceding layer and increasing incrementally towards the boundary of the succeeding layer until a depth that was half the width of the GLG. Dentin powder obtained from micromilling was demineralized in 0.25 N HCl for 3–4 h at ambient temperature or until dentin powder appeared translucent under the microscope. The acid solution was pipetted out and the sample was rinsed twice in distilled water before drying at 60 °C in a laboratory oven for 15–18 h. Filtration was not performed as it can lower the collagen yield by up to 86% (Jørkov et al., 2007).

2.3. Subsampling of dentin by cutting out the GLGs

Two to four longitudinal sections of 300–500 μm thick were made from the demineralized half tooth with the use of scalpel. Thinner sections were more fragile, which made cutting out the layers right along the boundaries of the GLGs more difficult. Cutting the sections more thickly made the GLGs more difficult to resolve under the stereomicroscope. Each thin section was cut into buccal and lingual halves that were not perfectly symmetrical but with all dentin layers present in both. The longitudinal sections were then examined under the stereomicroscope for presence of portions that were not demineralized (Fig. 1a). In such cases, the sections were further soaked in 0.25 N HCl until fully demineralized (Fig. 1b).

Once completely demineralized, remaining portions of cementum were trimmed from the outer surface while those of the enamel were left in place. The presence of the enamel was