Chemical Geology 404 (2015) 126–135

Contents lists available at ScienceDirect

Chemical Geology

journal homepage: www.elsevier.com/locate/chemgeo

Tooth enamel sampling strategies for stable isotope analysis: Potential problems in cross-method data comparisons

Hazel Reade [a,c,*], Rhiannon E. Stevens [a,c], Graeme Barker [a,b], Tamsin C. O'Connell [a,b]

[a] McDonald Institute for Archaeological Research, University of Cambridge, Downing Street, Cambridge CB2 3ER, UK
[b] Department of Archaeology and Anthropology, University of Cambridge, Downing Street, CB2 3DZ, UK
[c] UCL Institute of Archaeology, 31-34 Gordon Square, London WC1H 0PY, UK

ARTICLE INFO

Article history:
Received 27 August 2014
Received in revised form 25 February 2015
Accepted 16 March 2015
Available online 9 April 2015

Editor: Michael E. Böttcher

Keywords:
Enamel
Isotope
Seasonal
Bulk
Serial

ABSTRACT

The stable isotope composition of a given sample of tooth enamel is a function of the mineralisation process, the isotopic composition of the input signal, and sampling geometry. As the dominant axis of tooth growth is orientated from the occlusal surface to the enamel–root junction, the isotopic composition of an enamel sample that spans the full length of this axis is often taken to represent a whole-tooth or 'bulk' average, while serial (or 'intra-tooth') samples taken along the same axis are considered to track isotopic variation across the period of tooth formation. A 'bulk' sample of enamel should therefore approximate the mean of the 'serial' samples, and in teeth that form over a complete annual cycle 'bulk' and 'serial' sampling methods are often assumed to possess an annual–seasonal relationship. However, this does not take into account the effect of the mineralisation process and sampling geometry on the derived isotope signals. Here we investigate the reproducibility of both the 'bulk' and 'serial' sampling methods as independent, stand-alone techniques and then explore the assumed relationship between the two. Each method shows good reproducibility in the oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) isotope results across paired left and right molars. However, cross method comparisons reveal a systematic offset between bulk and serial results within a single population, with 19 out of 23 $\delta^{18}O$ bulk-tooth values being lower, and 22 out of 23 $\delta^{13}C$ bulk-tooth values being higher, than the means of their respective intra-tooth sequences. In 4 out of 19 cases for $\delta^{18}O$ and 6 out of 22 cases for $\delta^{13}C$ the bulk-tooth results fall completely out of the range observed in the corresponding intra-tooth sequence. This finding has implications for cross-method data comparisons and questions the assumed temporal relationship between 'serial' and 'bulk' isotope data.

1. Introduction

Variations in the stable carbon and oxygen isotope composition of mammalian tooth enamel have been attributed to variations in climate, diet and animal migration patterns, providing a useful tool to address a diverse range archaeological and palaeoenvironmental questions (e.g. Hoppe and Koch, 2007; Tütken et al., 2007; Wang et al., 2008; Feranec et al., 2009; Towers et al., 2011). This is possible as mammalian tooth enamel forms in isotopic equilibrium with the animal's body fluid, which obtains its isotopic signal from the animal's food and water, which are in turn derived from the environmental conditions under which they form (Longinelli, 1984; Luz and Kolodny, 1985; D'Angela and Longinelli, 1990).

In hypsodont (high-crowned) herbivore teeth, tooth formation progresses over a finite period of time from the occlusal surface to the enamel–root junction (ERJ), such that serial sampling the enamel along this growth axis provides a record of isotopic variation over time, while a 'bulk' sample that spans the length of the growth axis represents an average of the isotopic input across the period of formation (Fricke and O'Neil, 1996; Sharp and Cerling, 1998). Therefore, in teeth that form over one or more complete annual cycles, isotopic data derived from 'bulk' and 'serial' samples should approximate annually averaged and seasonally resolved signals, respectively.

However, the observed isotopic signal is complicated by the cumulative effect of the tooth formation process, variations in the isotopic composition of the input signal, and sampling geometry. With regard to tooth formation, while the primary axis of formation progresses from the occlusal surface to the ERJ, mineralisation is a discontinuous process that possesses both horizontal and vertical components (Suga, 1982; Robinson et al., 1983; Aoba and Moreno, 1990). This produces an isotopic signal with a temporal axis advancing both from the occlusal surface to the ERJ and from the enamel–dentine junction (EDJ) to the outer enamel surface (Zazzo et al., 2005) (Fig. 1a). As the orientation of mineralisation is different to the orientation of the commonly followed 'bulk' and 'serial' sampling axis, the temporal resolution of each sampling method, and relationship between them, may be different to that that is typically assumed.

* Corresponding author at: UCL Institute of Archaeology, 31-34 Gordon Square, London WC1H 0PY, UK.
E-mail address: h.reade@ucl.ac.uk (H. Reade).

http://dx.doi.org/10.1016/j.chemgeo.2015.03.026
0009-2541/

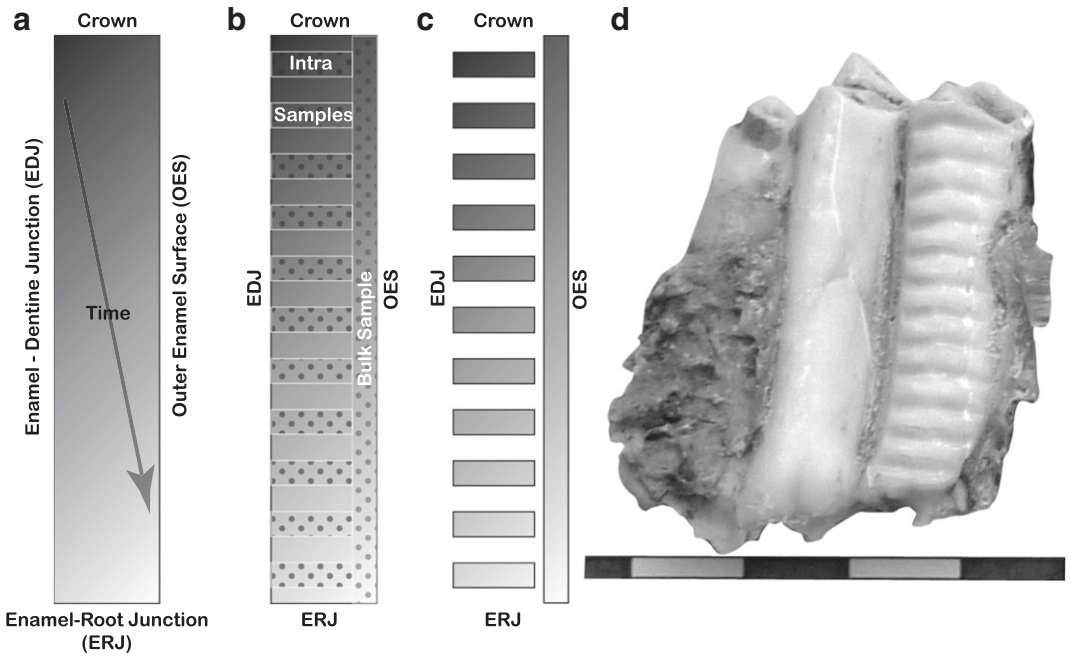

Fig. 1. Schematic cross section of tooth enamel from inner enamel–dentine junction (EDJ) to outer enamel surface (OES). a) Diagram showing the progression of enamel mineralisation from the crown to the enamel–root junction (ERJ) and from the EDJ to the OES, after Zazzo et al. (2005). b and c) Position of bulk and serial samples in relation to the enamel mineralisation process showing that the earliest mineralising enamel to be most heavily representing in the uppermost serial samples and the later mineralising enamel to be most heavily represented in the bulk sample and lowermost serial samples. d) Photograph of a sampled *Ammotragus lervia* lower right M3.

In light of this, we re-examine the temporal relationship between bulk-tooth and intra-tooth data derived from the commonly used 'bulk' and 'serial' sampling methodologies. To add confidence to the comparison, we first investigate the reproducibility of each sampling method as independent, stand-alone techniques by comparing oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) isotope results derived from the respective methods across paired left and right (L–R) molars. Teeth occupying the same position in the jaw (e.g. the mandibular M2) but on opposite sides are assumed to have the same isotopic composition as one another as they form during the same period of time and have the same isotopic source (the animal's body water). Differences observed between L and R paired molars, sampled using the same technique, may therefore indicate inconsistencies in the sampling methodology. We then explore the relationship between the two sampling techniques by comparing the results acquired from 'bulk' and 'serial' sampling the same tooth.

1.1. Enamel mineralisation and sampling strategies

Mature dental enamel is a carbonated hydroxyapatite, which contains both carbonate and phosphate minerals (Aoba, 1996). Enamel mineralisation is a progressive and discontinuous process that begins with the deposition of a protein-rich matrix (the secretion stage) which then gradually mineralises over an extended period of time (the maturation stage) (Robinson et al., 1981, 1983). During the maturation stage mineralisation advances through the developing enamel in different directions, and the degree to which the enamel mineralises varies through the enamel thickness (Suga et al., 1970; Suga, 1982; Robinson et al., 1995). The innermost portion of the enamel mineralises early in the maturation phase, while the highest degree of mineralisation, achieved over a longer period of time, occurs in a narrow layer (~5–20 μm thick in caprids (Balasse, 2003)) just below the outer surface of the enamel (Suga, 1979, 1982). The chemical composition of the enamel also varies by location, with the carbonate content decreasing from ~5% at the EDJ to ~3% at the outer surface (Zazzo et al., 2005).

Traditionally, enamel isotope studies have focused on sampling the part of the tooth where the enamel is thickest, as the analytical procedure commonly required a relatively large sample size (e.g. Bryant et al., 1996; Fricke and O'Neil, 1996; Bocherens et al., 2001). However, such sampling maximises the amount of enamel homogenisation, time-averaging the derived isotopic results. While time averaging is not necessarily a problem when a bulk-tooth average is sought, for serial sampling the larger the degree of sample homogenisation, the greater the degree of attenuation of the measured amplitude of the within-tooth isotopic variation. Sampling only the innermost portion of enamel, which mineralises rapidly soon after the secretion phase, can reduce signal homogenisation (Balasse, 2003; Zazzo et al., 2005; Tafforeau et al., 2007; Blumenthal et al., 2014), but in reality such a sampling strategy is difficult to implement, in part due to the difficulty in isolating this innermost portion, which is only ~20 μm thick in bovids, from the rest of the enamel (Zazzo et al., 2012). Therefore, most isotope studies continue to sample from the enamel surface down through the enamel thickness. The depth to which the enamel is sampled typically depends on the required sample size, and the nature of the sampling permission that has been given by the body responsible for curation of the samples. For fossil specimens, sampling permission is commonly granted on the stipulation that as little enamel as possible is removed from the tooth. Thus for bulk-tooth samples it is common that only the outer portion of enamel is collected, although for intra-tooth studies it is more likely that samples will be taken through the majority of the enamel depth (e.g. Balasse et al., 2006; Merceron et al., 2006; Arppe et al., 2011). Given the varying rates and levels of mineralisation in different portions of the enamel, it is important to understand what part of the mineralisation signal each sampling strategy is capturing.

2. Material and methods

Seven mandibles of *Ammotragus lervia* (an arid-adapted caprid) were collected from a modern, wild population, as part of hunting expeditions unrelated to this study, during 2009/10 from a private range near Hondo New Mexico, USA (approximately 33°23′15″N 105°16′14″ W). All individuals were female, were collected from the same geographical area, and based on tooth wear, were aged at death from