Title

High-resolution serial sampling for nitrogen stable isotope analysis of archaeological mammal teeth

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Abstract

We present the results of an archaeological application of a rapid method for high-resolution stable nitrogen isotope ($\delta^{15}N$) measurements of time-series samples of tooth dentine. Over 250 analyses of samples of untreated dentine powder taken at continuous millimeter intervals along the growth axis of archaeological pig tusks were compared to results from a subset of tandem $\delta^{15}N$ measurements of extracted and purified tooth collagen from the same teeth. Samples were also taken at 0.25 mm depth intervals to test the effect of depth with respect to temporal resolution of diet. Results show that $\delta^{15}N$ measurements of untreated dentine powder from well-preserved archaeological teeth provide: 1) broadly comparable $\delta^{15}N$ values to extracted and purified collagen, and 2) a rapid method of assessing dietary change over much shorter time intervals than is possible using extracted collagen. Analyses also show that large changes in $\delta^{15}N$ values can occur across the thickness of a tooth due to the inclusion of multiple growth layers and/or secondary dentine, which results in a significant time−averaging lag in dietary representation, as demonstrated by samples that analyze collagen from the full width of the tooth wall. This method will also be useful for initial prescreening of samples to select for specimens of interest before undertaking further, more rigorous, sample pre−treatment and measurement.

Highlights

A fast method for high-resolution sub-millimeter serial dentine sampling for $\delta^{15}N$ analyses was tested

$\delta^{15}N$ values from bulk powder and collagen from the same dentine are comparable

Sampling intervals lower than 1mm provide a new level of temporal resolution for dietary analysis

Shallow sampling depth is critical for maintaining highest temporal resolution

Method is also an inexpensive way of prescreening bone and tooth samples for dietary analyses

Key Words

Dentine, serial samples, weaning, diet transitions, high resolution, stable nitrogen isotopes, pigs

1. Introduction

In recent years there has been a significant increase in the use and development of stable carbon and nitrogen isotope analyses of tooth dentine as a means of investigating archaeological human diet at the level of the individual (e.g., Henderson et al. 2014; Makarewicz 2014; Sandberg et al. 2014). This approach can provide diachronic insights into diet and mobility that address questions about changing resources regimes and weaning age patterns (e.g., Fuller et al. 2003; Fahy et al. 2014).

This paper presents the first archaeological application of a rapid and inexpensive method that is used in other fields (e.g. marine ecology, Ambrose et al. 2013; Borrell et al. 2013; Knox et al. 2014; and primatology, Fahy et al. 2014) for high-resolution stable nitrogen isotope (δ^{15}N) measurements of time-series samples of modern mammal tooth dentine. We measured over 250 individual samples of untreated (i.e., not demineralized) dentine powder taken at continuous millimeter increments along the growth axes of archaeological pig tusks and compared these data to results from tandem δ^{15}N measurements of extracted and purified tooth collagen from the same teeth. We also compared a subset of samples taken at 0.25 mm depth intervals at the same locations on one tooth to test the effect of depth with respect to temporal resolution of diet in tooth dentine δ^{15}N values. This study demonstrates the archaeological potential of this high-resolution standalone method for showing seasonal dietary differences, and also as a method to initially screen samples for further more-rigorous sample pretreatment and measurement. While we test this method on pig teeth, as simplified model, the technique should also be useful for time-series analyses of teeth from humans and other animals.

2. Stable isotope theory and tooth formation processes

Several reviews of stable carbon and nitrogen isotope ecology for archaeological analyses have been published (e.g., Lee Thorp 2008). This section briefly reviews factors of stable isotope theory and dentine formation processes that are relevant to this study. Stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope values are expressed in ‰ relative to VPDB and AIR standards, respectively. The δ^{13}C values of plants broadly differ based on: 1) whether a C_3 or C_4 photosynthetic pathway is used to fix carbon (O'Leary 1981, 1988), and 2) the marine versus terrestrial atmospheric origin of carbon that is used (Chisholm et al. 1982). Because δ^{13}C values do not change significantly as they are passed up the food chain they are useful for distinguishing between diets based on different kinds of plants and plant communities (DeNiro and Epstein 1978) as well as between diets focusing on marine as opposed to terrestrial foods (Chisholm et al. 1982). Unlike δ^{13}C, δ^{15}N values undergo a large change between different trophic levels in a food chain, becoming enriched in ^{15}N by roughly 3 to 5 ‰ at each ascending interval (DeNiro and Epstein 1981). As marine ecosystems tend to have longer foods chains than terrestrial environments, this relationship makes δ^{15}N values a useful indicator of high trophic level marine dietary intake (Schoeninger et al. 1983). It is also important to note that there is a variety environmental and anthropogenic variables which can influence δ^{15}N isotope soil biogeochemistry in pant-soil systems at the base of a given food web (see Szpak 2014 for review). Collagen forms the main organic component in bone and tooth dentine and has isotopic signatures that primarily reflect dietary protein intake (Ambrose and Norr 1993; Tieszen and Fagre 1993). In contrast to bone, which remodels over a lifetime, primary dentine is not thought to undergo turnover after it is laid down (Gage et al. 1989) and therefore preserves a record of isotopic dietary values over the course of its formation. Mammal dentine begins formation at the crown and growth generally proceeds in obliquely angled increments at a gradual pace down to the root where tooth formation is completed (for review see Hillson 2005)

3. Overview of tooth and bone time-series analyses in archaeology and ecology

Recent publication trends show that isotope time-series analyses involving tooth dentine are becoming an important contributor to archaeological research (Figure 1). There has been long-standing archaeological interest in obtaining dietary information for different periods of individual lives (e.g., Sealy et al. 1993). Such diachronic, intra-individual data provide an important means of addressing more-detailed questions about human and animal biology such as weaning age (e.g., Burt and Garvie-Lok 2013; Fuller et al. 2003) as well as the interplay between individual life-histories and wider social and economic processes (e.g., Cox and Sealy 1997; Szpak et al. 2014). Analyses of incrementally forming tissues such as hair and nails, when available, provide ideal sequential isotopic archives for human and animal diets (e.g., Fuller et al. 2006; Szpak et al. 2014). The most commonly preserved tissues in archaeological contexts are skeletons and, for this reason, most archaeological analyses have focused on bones and teeth. Figure 2 provides an overview of methods for dentine time-series analyses in archaeology, ecology, and paleontology. The simplest approach for obtaining diachronic data at the intra-individual level has been analyses of bone materials with differing turnover rates (e.g., Cox and Sealy 1997; Sealy 1993; Sealy et al. 1995; White 1993). These studies compare dietary values from collagen extracted from dense long bones (e.g., femora) and less robust bones (e.g., ribs) as representatives of relatively long- and short-term diets. Early archaeological and paleontological studies (Cox and Sealy 1997; Drucker et al. 2001; Sealy 1993; Sealy et al. 1995) moved this work a step further by comparing collagen stable isotope values from bones with those of whole teeth and reasoning that because dentine does not remodel after it is laid down, it will capture a record of an earlier time in the individual's life. A logical next step was to analyze collagen from a sequence of teeth formed at differing times in the life of an individual (e.g., Bocherens et al. 1994, 1995, 1997; Richards et al. 2002; Wright and Schwarcz 1999). In subsequent work, researchers began to take multiple samples in series along the growth axis of an individual tooth to track diet change at a finer scale (e.g., Balasse and Tresset 2002; Balasse et al. 2001; Koch et al. 1995; Fuller et al. 2003; Walker and Macko 1999). These analyses usually proceed by partitioning a tooth at regular intervals either before or after it has been demineralized and have been widely used in archaeology, (e.g., Beaumont et al. 2013; Mongomery et al. 2013), paleontology (Fisher et al. 2014; Metcalfe et al. 2010; Rountrey et al 2007), and ecology (Mendes et al 2007ab; Knoff et al. 2008; Newsome et al. 2009). More recent analyses in archaeology and especially ecology have further developed this serial-sectioning technique by taking into account histological observations during sampling (e.g., Ambrose et al. 2013; Burt and Garvie Lok 2013; Elorriaga-Verplancken et al. 2013; Hanson et al. 2009; Newsome et al. 2006, 2007).

Despite these advances, archaeological studies (Figure 1, 1994 to present, *n*=55) have invariably focused on materials with relatively course temporal resolution and, for this reason, there remains significant potential for improvements in temporal control of dietary data. In particular, commonly used methods analyze collagen that has been extracted from dentine that spans multiple growth layer increments (e.g., Eerkens et al. 2011; Montgomery et al. 2013). This is due, in part, to the irregular contour followed by growth layer increments during the formation processes of most teeth. While it would be ideal to collect material only from individual growth layer increments, for most archaeologically relevant species (including humans), practical concerns with respect to tooth histology (i.e., a lack of visible annuli) and, more importantly, the need to collect sufficient sample material (usually at least 50-100 mg) have prevented previous archaeological work for tackling this issue.

High-resolution analyses have, however, been established in the field of ecology for almost two decades. Hobson and Sease (1998) conducted the first in-depth $\delta^{15}N$ measurements on untreated dentine samples taken from between annulated growth features in seal teeth. They were able to overcome the issue of sample size by omitting collagen extraction procedures and, instead, directly

analyzing untreated dentine powder samples. They reasoned that, because the primary nitrogen-bearing material in tooth dentine would be collagen, analyses that concentrate on $\delta^{15}N$ only (i.e., those that do not require concurrent $\delta^{13}C$ analyses) should not need to undergo pretreatment. This method has found traction in subsequent ecological work and can allow for nitrogen isotopic analysis of as little as 1 mg of untreated (or minimally treated) dentine powder (e.g., Ambrose et al. 2013; Authier et al. 2012; Borrell et al. 2013; Ciotti et al. 2014; Fahy et al. 2014; Hanson et al. 2009; Hobson et al. 2004; Knox 2014; Martin et al. 2011; Newsome et al. 2006, 2007; York et al. 2008). Recent work has also established the comparability of $\delta^{15}N$ from bulk dentine powder and extracted collagen in modern specimens (Borrell et al. 2013; Brault et al. 2014). Because this method usually does not separate mineral and protein phases of dentine, it cannot be used to measure $\delta^{13}C$ values that are comparable to those of extracted collagen. Nonetheless, a capacity to analyse minute masses of dentine powder could allow for significant methodological advances in stable nitrogen isotope analyses in archaeological research. Despite this potential, and a recent surge in ancient and modern tooth dentine analyses (Figure 1), the archaeological applicability of this method has not yet been assessed.

4. Research Design and Materials

We conducted an experiment to investigate the archaeological utility of $\delta^{15}N$ isotope analyses of untreated dentine powder taken at short intervals. The technique was applied in two dimensions relative to the growth axis (i.e., distance from root and depth from enamel-dental junction) on a model substrate – continuously-growing pig tusks (lower canine teeth; Frémondeau et al. 2012). This sampling strategy was designed to address three methodological questions. First, do $\delta^{15}N$ analyses of untreated archaeological dentine power provide comparable results to established collagen extraction protocols? Second, what sampling intervals are optimal for resolving rapid dietary changes? And third, what effect does sampling depth have with respect to temporal resolution of diet?

Pig tusks were expected to provide an ideal experimental model for a number of reasons. Pig tusks grow continuously (Frémondeau et al. 2012; Tonge and McCance 1973), meaning that the interpretation of isotopic change versus linear sample distance will be more straightforward. Their relatively fast growth (roughly 6 cm per year; Frémondeau et al. 2012) also makes it more likely that dietary changes will be identifiable. Pig tusks begin formation between 1 and 3 months of age (McCance et al. 1961) and therefore we do not expect to observe a weaning signal in tusk $\delta^{15}N$ values. Finally, pig tusks have a simplified morphology, lacking the irregular growth layer contours that are present in other teeth such as molars.

The tusks used in this study come from seventeenth-century deposits at the English fishing settlement of Ferryland in Newfoundland, Canada (Figure 3). The settlement functioned as a permanent base for domestic and agricultural activities, including large scale pig husbandry, and as a seasonal station for processing cod for export to European markets (Gaulton 2013; Guiry and Gaulton 2015; Hodgetts 2006; Pope 2004). Between the spring and fall months fish offal was abundant at Ferryland and was used as a feed source for adjacent piggery enterprises (Guiry et al. 2012a,b). Marine-derived offals would not have been available during the winter and, for this reason, we anticipated that pig tusk specimens from Ferryland would exhibit the degree of isotopic contrast (over a short time interval) needed to examine the effects of sampling strategy on dietary temporal resolution.

5. Methods

We prescreened 11 tusks (Table 1) by conducting δ^{15}N measurements on dentine powder in order to identify individuals for in-depth analyses (i.e., those which had likely been raised at the settlement on oscillating marine and terrestrial diets). Tusks were selected based on minimum number of individual counts per archeologically distinct context. Prescreening consisted of serially sampling rasters (1 cm long, 1.00-1.25 mm wide, 0.25-0.50 mm depth) at approximate 1.5 cm intervals perpendicular to the growth axis of each tooth. Powder samples were collected using a 1 mm wide diamond coated spheroidal dental bur head.

Unlike bone collagen, quality criteria have not been defined for archaeological δ^{15}N measurements on undemineralized dentine powder. We recommend the analysis of a subset of paired samples from dentine powder and extracted collagen in order to test the comparability of results from dentine powder with methods common in archaeological investigations. We cut 150 mg samples (n=17) from the same areas from a subset of teeth. Collagen extraction followed well-established techniques (Brown et al. 1988; Richards and Hedges 1999). Briefly, tooth dentine pieces were demineralized in 0.5 M hydrochloric acid at 4 °C and then gelatinized in 10^{-3} M HCl on a heating block (75 °C) for 48 hours. Gelatins were subsequently 45-90 µm mesh Ezee filtered (Elkay Laboratory Products, Hampshire, UK), 30 kD ultrafiltered (Pall Corporation, Port Washington, NY, USA), and lyophilized. Collagen integrity was assessed by %C and %N (> 18 and 6 %, respectively), carbon to nitrogen ratio (between 2.9 and 3.6), and % collagen yield (>2 %) (DeNiro 1985; Van Klinken 1999).

From the prescreening group, three tusks (no. 12, 20, and 58) were selected to undergo comprehensive experimental analyses comparing fine-grained adjoining samples along and perpendicular to tusk growth axes (at 1.25 mm intervals) and across tusk thickness (approximately 0.25 mm depth intervals from external to internal surfaces). All samples were taken in the same shape and relative orientation (i.e., rasters were about 1.00-1.25 mm wide x 1 cm long at a depth of approximately 0.25 mm) from the surface of the enamel-dentine juncture.

Untreated dentine powder samples (4 mg) and collagen samples (0.5 mg) were weighed into tin capsules and analyzed on an Elementar vario MICRO cube elemental analyzer coupled to an Isoprime isotope ratio mass spectrometer in continuous flow mode. The δ^{13}C and δ^{15}N values were calibrated relative to VPDB and AIR, respectively, using USGS40 and USGS41. Accuracy was assessed using internal methionine (n=44) and modern seal collagen (n=31) standards. The average observed δ^{13}C value for methionine and modern seal collagen over six runs were −28.59±0.14 ‰ and −13.74±0.16 ‰, which compares well to their long−term average values of −28.57 ‰ and −13.73 ‰, respectively. Average observed δ^{15}N values of methionine and modern seal collagen were −5.0±0.2 ‰ and +17.4±0.2 ‰, which also compare well to their expected values of −5.0 ‰ and +17.4 ‰, respectively. All collagen and a subset of dentine samples were analyzed in duplicate. The average difference between duplicates (n=24) of both materials was less than 0.05 ‰ and 0.16 ‰ for δ^{13}C values and δ^{15}N values, respectively.

6. Results and Discussion

6.1 Treated and untreated dentine samples

Data from all analyses are presented in Supplementary Table 1. To visualize the data Figure 4 plots all analyses for the most intensively analyzed tooth, tusk no. 12. Supplementary Figures 1 and 2 plot analyses from tusks no. 58 and 20, respectively. All samples produced collagen of acceptable quality. Analyses of untreated dentine samples (n=268) produced an average %N value of 3.5 % (ranging between 0.9 % and 5.0 %). Treated and untreated dentine samples (n=18) taken at the same locations produced comparable δ^{15}N values for all samples with a mean difference of 0.6±0.5 ‰ (range of <0.1 to 1.5 ‰). The largest differences systematically occurred at sample locations where tandem sequences of

δ^{15}N analyses of untreated dentine identified a pronounced change in diet. It is therefore likely that these larger offsets reflect inclusion of a larger number of growth layer intervals during sampling for collagen extractions (see below Section 6.4.2).

6.2 Prescreening and diet

Prescreening activities showed that a majority of tooth samples derived from animals that had maintained terrestrial diets consistent with known historical pig husbandry practices (Guiry et al. 2014, 2015). In the context of Ferryland, where terrestrial foods would have been in limited supply, these individuals probably represent animals raised in Europe and then imported to the site in the form of salt pork (Guiry et al. 2012a). These tusks are not discussed further. Three individuals (tusks 12, 20, and 58) produced highly elevated δ^{15}N values suggesting overall diets focused on marine foods and are consistent with local pig husbandry practices. For these samples, δ^{13}C value elevations co−vary with δ^{15}N values, confirming this interpretation.

6.3 Seasonality of husbandry at Ferryland

Large sinusoidal variation is apparent with δ^{15}N values, ranging up to 6‰ within a single tooth. The pattern in all teeth is similar. At the gross scale, values are consistently high towards the root (later life), preceded by a relatively steep decline in the middle, and finally a gradual climb in values moving towards the cusp. This sequence is in good correspondence with the anticipated pattern of pigs being born in the spring, growing over the summer when fish offal is in good supply, living through one winter on limited terrestrial feeds, and then fattening on fish offal again during their second spring in preparation for slaughter. The consistency in this pattern among all three teeth also provides indirect evidence for a single season, rather than two (Hodgetts 2006; Tourigny 2009), of pig rearing at Ferryland. The archaeological provenience of these individuals (from deposits spanning the seventeenth century) suggests that this practice may have persisted for a relatively long period of time. Analyses of a larger sample of locally raised pigs would be needed to further investigate this pattern.

6.4 Exploring methodological utility

6.4.1 Do 1 mm increments fully resolve dentinal isotopic records?

One millimeter sampling intervals produced a very high-resolution time series of pig diets. Particular sections of these series, especially those where large scale dietary shifts occur, can provide insight on: 1) the capacity for tooth dentine growth layers to record isotopic information about diet, as well as 2) the technique's capacity to resolve them. Taking the most complete tusk (no. 12) as an example, during the main dietary shift, smooth gradual change is observed (Region 2, Figure 4; average change in δ^{15}N is 0.14 ‰ between samples increments 63 through 85) suggesting that 1.00−1.25 mm sampling intervals are adequately capturing dietary change. This observation could be explained in two ways. First, the smooth δ^{15}N transition could reflect a gradual shift in diet over a relatively short period of time. Second, it could be registering a more rapid shift from marine to terrestrial foods that has been recorded as an attenuated δ^{15}N decline due to lagging processes in nitrogen mixing pools. It is worth pointing out that given the small amount of time represented by the largest shift in δ^{15}N values, occurring over a distance of 7 mm (increment 49 through 55), which may represent only a short period of growth (Frémondeau et al. 2012), the difference between these explanations may have less significance for archaeological interpretation. In either case, given that the average shift in δ^{15}N values between increments (Regions 2 in Figure 4) is less than instrumental error (±0.2 ‰, see Section 5) 1.25 mm sampling intervals appear to provide ample detail for analyses of general dietary trends.

Isotope sequences towards the root of each tooth show a different pattern, with relatively large changes in δ^{15}N values of up to 2.5 ‰ over a single sample interval. Again, taking the most complete tusk (no. 12)

as an example, a series of three small oscillations occurs between sample increments 1 and 50 (Region 1 in Figure 4) which appears to capture dietary changes over very short intervals. Two hypotheses can explain this. First, they could reflect sporadic high trophic level additions to pig diet (e.g., occasional consumption of seal carcasses in a diet otherwise dominated by cod refuse). Second, they might also be the outcome of normal periodic dietary variability which has become resolvable due to a period of increased tooth growth and/or the presence of fewer growth intervals bands towards the leading edge of tooth development (see below Section 6.4.2). In this latter scenario, tooth length growth is slower during early life and increases as the animal ages. Because this rapid fluctuation between high and low values is observed at the same point, towards the latest forming part in multiple teeth (which come from different time periods), we believe the latter hypotheses to be most likely. In either case, these data indicate that sub−millimeter incremental analyses could provide a new degree of temporal resolution in cases where tooth growth is fastest.

6.4.2 How important is sampling depth?

Analyses of untreated dentine powder from different depths were undertaken at two locations, depth sampling group (DSG) A and B, on one tusk (no. 12). It is important to note that DSG A and B are located at points of rapid and gradual dietary change, respectively, and are thus well situated to record the sensitivity of sampling depth with respect to temporal resolution of dietary shifts. Differences in δ^{15}N values between samples taken at the 0.00−0.25 and 0.25−0.50 mm intervals were within analytical error for both DSGs. This suggests that sampling depths of up to 0.5 mm have not included and averaged significant differences between different growth layer intervals. Large δ^{15}N differences of up to 4 ‰ were found between samples from above and below 1 mm in depth. Deeper samples (0.50−1.50mm) tended towards the tooth average. This could reflect inclusions of materials from multiple growth layer increments or secondary dentine growth and helps explain what appears to be a temporal lag between untreated dentine powder (taken at 0.25 mm depth) and collagen (i.e., including material from the full thickness of the tooth) samples.

Histological observation (Figure 5) of periodic growth features in an archaeological pig canine shows that the relationship between increasing sampling depth and temporally-skewed stable isotope values can be explained by the averaging of many successive growth intervals in primary dentine apposition. Figure 5 depicts sampling depth relative to long-period growth features known as Andresen's lines (Dean et al. 1993) which are known to occur in the process of normal mammalian dentine apposition. These long-period lines represent the successive positions of the dentine-forming front and are composed of a regular series of short-period lines known as Von Ebner's lines which develop with circadian rhythm in the teeth of many species, including humans and pigs (particularly pig canines; Yilmaz 1976; Dean 1998). The number of days represented by long-period lines varies between species and is correlated with body mass (Bromage 2009). For instance, whereas a repeat interval of up to 14 days has been observed between long-period lines in larger taxa such as Proboscideans, most other species that have been analyzed tend to have values between 1 and 9 days (for review see Smith 2008; Bormage 2009). Because the width and quantity of Andresen's lines varies between different areas on the same tooth (e.g., for developing teeth there will be progressively fewer lines towards the roots relative to the cusp) sampling location, in addition to depth, could also have a large impact on temporal-skewing of stable isotope values. For example, based on the number of Andresen's lines that are crossed by samples taken at different depths in Figure 5, stable isotope values from raster depth interval 1 (crossing 4 Andresen's lines) might produce an average of about 20 days whereas samples that take materials from the full width of the tooth (28 Andresen's lines) would average about 140 days (assuming a repeat interval of 5 days/ Von Ebner's lines).

These results indicate that optimal sampling for serial analyses of tooth dentine will be obtained when isotopic measurements are performed on material from as shallow a raster as possible. Though these analyses have been performed on pig tusks, as a simplified model substrate, findings should also hold for more complex tooth morphologies, such as in humans, where growth layers form at oblique angles to the enamel–dentine junction.

7. Summary and Conclusion

We have investigated the utility of a method for serial sampling archaeological mammal tooth dentine for stable nitrogen isotope analyses. These analyses represent the first systematic application of this method to an archaeological context and have provided new evidence for the highly seasonal nature of aquatic resource use and historic pig husbandry practices, both in terms of feeding and reproduction, at the archaeological site of Ferryland.

Where preservation is adequate, δ^{15}N isotope analyses of untreated dentine powder is readily adaptable to archeological teeth and provides broadly comparable δ^{15}N values to traditional archaeological methods. For this reason, when δ^{15}N analyses are of primary concern (i.e., in studies of trophic shifts such as weaning age analyses and marine versus terrestrial seasonal dietary oscillations), this method (e.g., Fahy et al. 2014; Hobson and Sease 1998) offers advantages over common techniques (e.g., Beaumont et al. 2013; Eerkens et al. 2011; Montgomery et al. 2013) for serial dentine analyses. In particular, we have shown that, by sampling only a thin (approximately 0.25 mm deep) raster at the dentine–enamel junction, analyses of untreated dentine powder provides dietary data from significantly shorter time intervals than concurrent analyses that rely on collagen which has been extracted and purified from whole dentine samples (which span the thickness of a tooth). Analyses of untreated dentine powder from different depths also provide further positive evidence for this by demonstrating that significant changes in δ^{15}N can occur across the thickness of a tooth due to the inclusion of multiple growth layers and/or secondary dentine and result in a time–averaging lag in dietary representation for samples that analyse collagen from the full width of the tooth wall.

This study shows the potential of this high-resolution but relatively rapid and simple method for investigating short-term seasonal dietary, weaning, or other isotopic differences for well-preserved archaeological samples. It can also be used as a rapid prescreening method for less well-preserved samples, to help researchers select specimens that have isotope variation that is of interest, that can then be further prepared using traditional, but lower resolution, collagen extraction methods.

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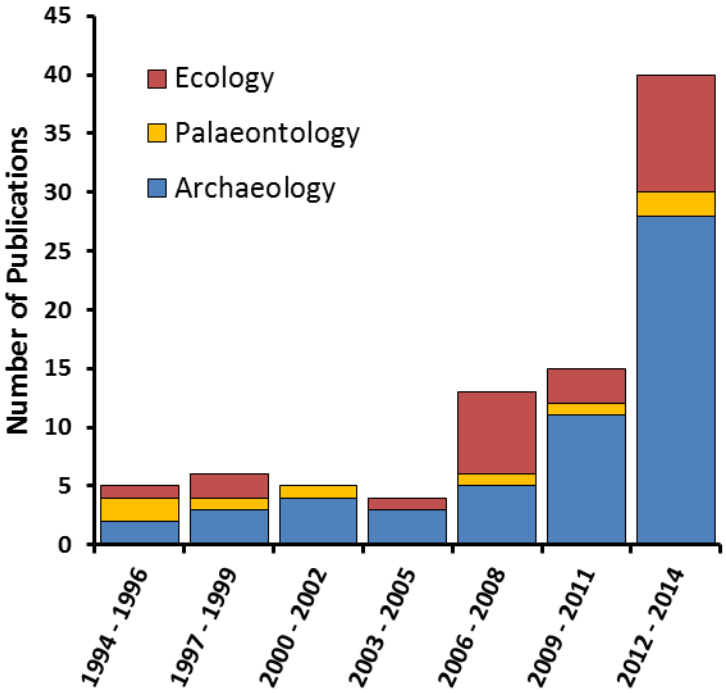
526

Figure Captions

Figure 1. Publication date versus number of papers (n=90) that use intra-individual time-series information from dentine. These data were gathered through a systematic key word search ("dentine", "isotope", and "nitrogen") of over 150 archaeology, biology, and paleontology journals.



532 **Figure2.** Procedural flowchart of methods for dentine time-series analyses in archaeology, ecology, and Paleontology.

533

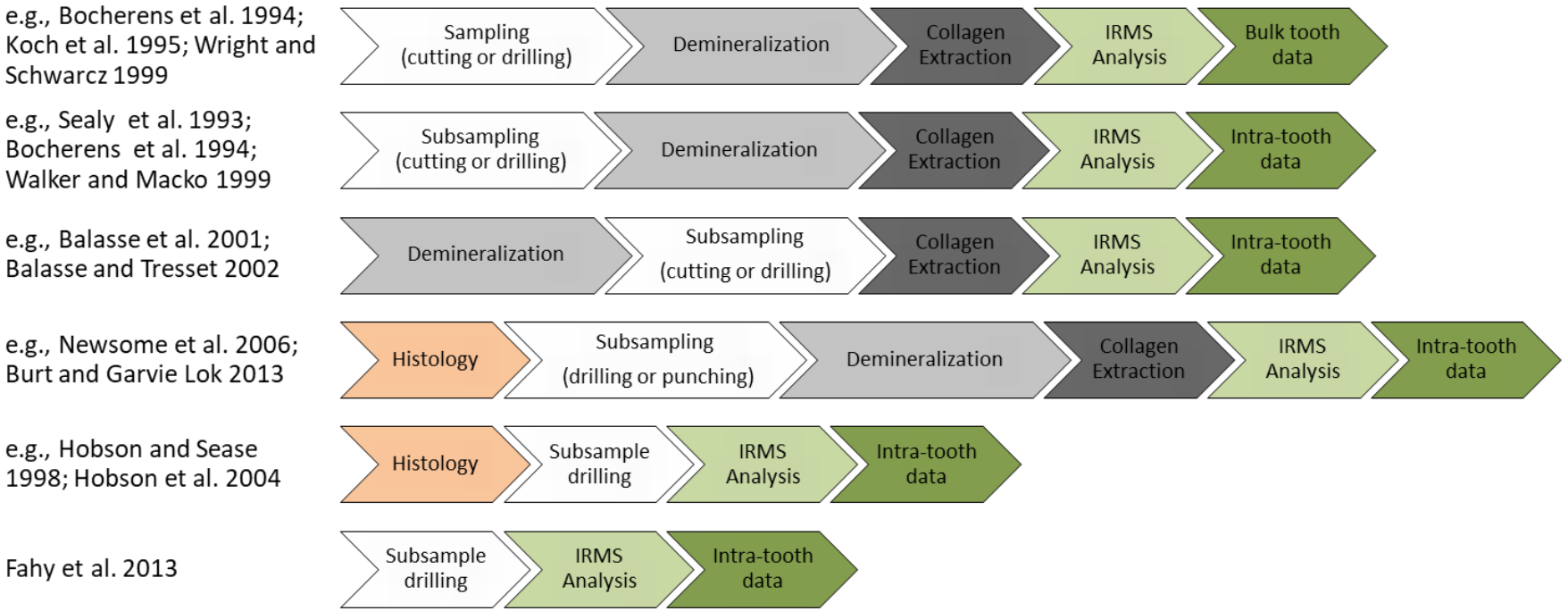


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Figure 3. Map showing location of Ferryland.

Figure 4. Top. Pig tusk no. 12 showing sampling increments (actual size). Bottom. Stable nitrogen isotope values from untreated dentine powder and collagen versus sample increment for tusk no. 12.

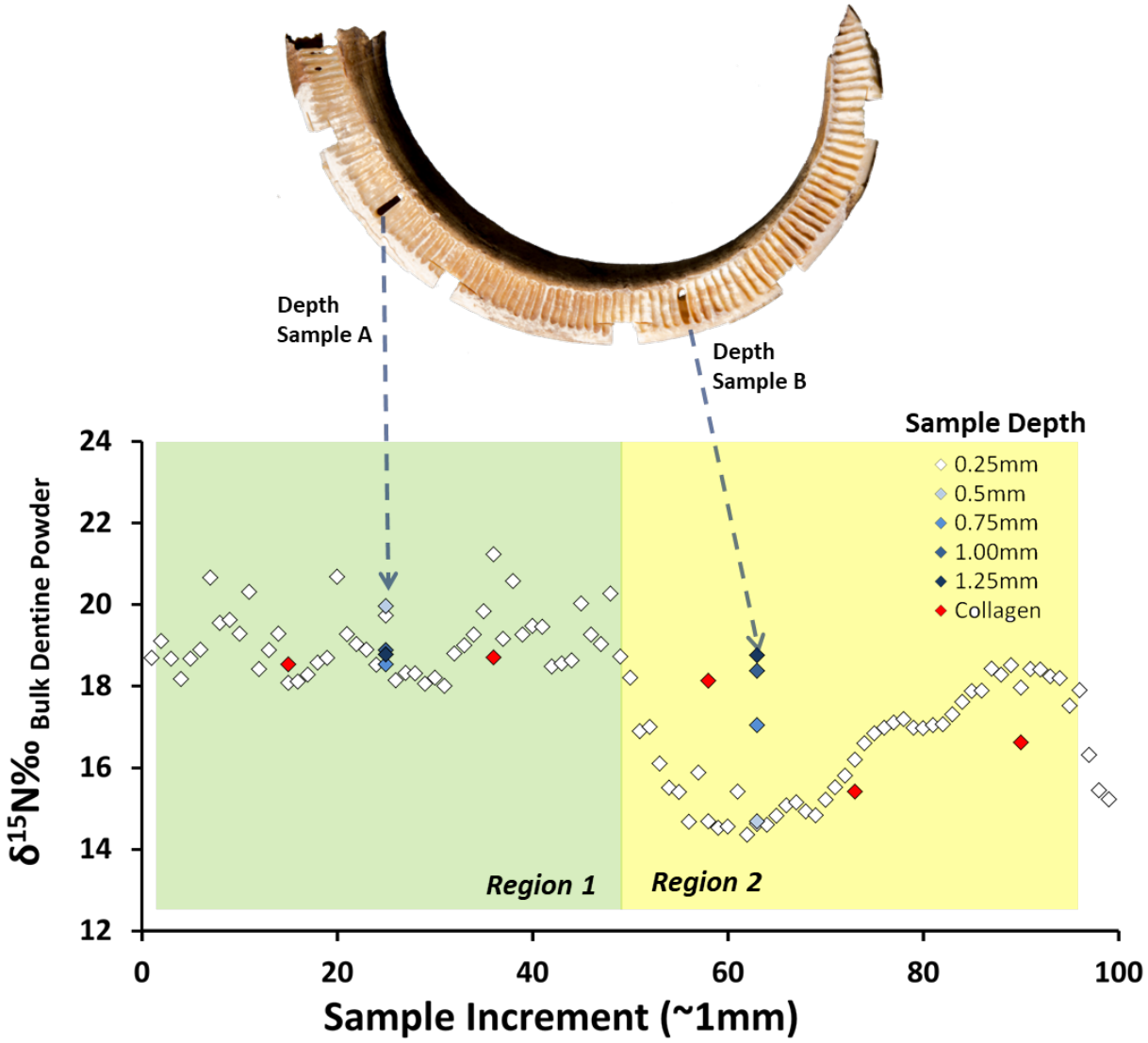
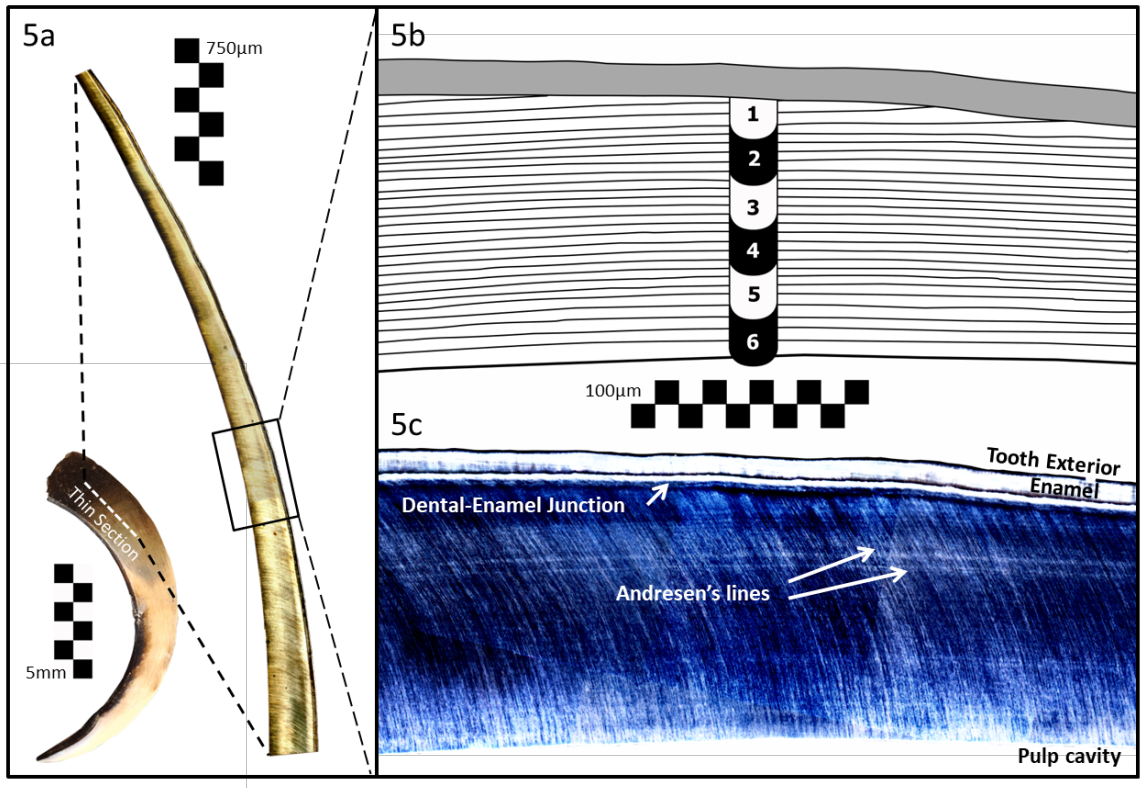


Figure 5. 5a shows an overview and the location of thin a section of the labial side of an archaeological male pig canine. 5b is a schematic drawing that traces the Andresen's lines that were observed under transmitted light and cross polarized light in both true and inverted color. The relative locations of 6 samples taken in sequential 0.25mm deep rasters are also depicted to illustrate the method used for sampling pig teeth in this study. 5c shows the same section of tooth used to draw 5b under cross polarized light with inverted color. Pronounced examples of Andresen's lines are identified.



548 **Table Captions**

549 **Table 1.** List of details for pig tusks that underwent prescreening.

Tusk no.	Event	Time Period	Sex	Side	Length (cm)	Condition	Catalogue no./Provenience
12	520	1620 - 1650	M	R	13.5	Complete	479089
20	545	1620 - 1696	M	R	6.5	75%; Ends missing	418077
58	287	1640 - 1696	M	R	4.0	50%; Ends Missing	265898
33	339	1620 - 1650	M	R	12.0	Complete	355971
25	467	1650 - 1696	M	R	9.5	80%; Mising ends	S7E72-73
31	736	1650 - 1696	M	R	7.5	80%; Mising ends	S6E87
14	656	1700 - 1750	M	R	7.5	90%; Cusp missing	E55S3
17	649	1700 - 1800	M	R	10.5	Complete	E62N01
30	694	1750 - 1800	M	R	8.0	90%; Parital root missing	E39S3
77	846	1700 - 1800	M	L	13.5	Complete	S18E55
56	630	1620 - 1696	M	L	9.0	90%; Parital root missing	E59S15

550

Supplementary Figure Captions

Supplementary Figure 1. Stable nitrogen isotope values from untreated dentine powder and collagen versus sample increment for tusk no. 58. Data from collagen extractions are show as red diamonds.

Supplementary Figure 2. Stable nitrogen isotope values from untreated dentine powder and collagen versus sample increment for tusk no. 20. Data from collagen extractions are show as red diamonds. Grey diamonds mark samples from rasters that were drilled too deeply.

Supplementary Table Captions

Supplementary Table 1. Stable carbon and nitrogen isotope and other data for all analyses of pig tusks in this study.