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Quantitative reconstruction of seasonality from stable isotopes in teeth.

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Abstract

Seasonal variation in rainfall and temperature are major determinants of ecosystem structure and productivity and influence physical processes such as erosion rates and glaciation. By extension, seasonality can drive evolutionary change, and is hypothesized to have influenced early human subsistence and technological development. Rainfall patterns are reflected by oxygen isotope ratios ($\delta^{18}\text{O}$ values) in mammalian tooth enamel, which records environmental chemistry as reflected by blood chemistry during mineralization. Fossilized herbivore molars are commonly used to study paleoseasonality, since temporal variation in precipitation $\delta^{18}\text{O}$ results in spatial variation in tooth mineral $\delta^{18}\text{O}$. However, this approach has been hampered by incomplete knowledge of isotope incorporation during tooth mineralization. Here we test a new synchrotron-based mineralization model and demonstrate its potential to quantitatively reconstruct original seasonal input histories under a Bayesian computational framework. To accomplish this, we first integrate the mineralization model with blood water oxygen isotope turnover to produce high-resolution spatial $\delta^{18}\text{O}$ predictions. We test these predictions using fine-scaled tooth enamel phosphate $\delta^{18}\text{O}$ measurements ($n=109$ locations sampled in a 2D grid format) in a sheep (*Ovis aries*) subjected to a controlled water switch (switch $\delta^{18}\text{O}$ magnitude of 12‰). Tooth mineralization, blood oxygen turnover and isotopic measurements demonstrate that enamel secretion and maturation waves advance at nonlinear rates with distinct geometries. Final enamel isotopic composition may be influenced by isotopic shifts during enamel maturation from amorphous precursors. We combine these observations to produce a Bayesian inverse model for reconstructing water $\delta^{18}\text{O}$ inputs from tooth isotopic measurements. The system accurately reproduces the controlled switch of the experimental animal and reveals transient documented meteorological events that were unplanned during the experiment. A simulation approach shows that given appropriate priors and search methodologies, this system also reconstructs precipitation histories at different latitudes with striking fidelity. We show that in some contexts even simple sampling approaches (i.e. 1D transects) can yield accurate reconstructions of seasonality. Finally, we refine our mineralization model further by exploring the possible extent of isotopic resetting during mineral phase transitions. This work demonstrates that tooth isotopic measurements can be employed to reconstruct past seasonal isotopic variation in

rainfall quantitatively, allowing exploration of the relationships among climate, earth history and evolution.

1. Introduction

On a broad scale, seasonal climate patterns influence earth-scale phenomena including glaciation onset and extent (Haug *et al.*, 2005), erosion rates (Wulf *et al.*, 2010), and are an important variable in past environmental reconstructions (Tindall and Haywood 2015). Seasonal precipitation regimes are major determinants of ecosystem structure, regulating grassland, savannah and forest plant communities (Vincens *et al.*, 2007; Good and Caylor 2011; Mayer and Khalyani 2011; Hoetzel *et al.*, 2013). Water and resource availability are thought to influence human and primate behavior, adaptation and evolution (Marshall and Wrangham 2007; Potts 2013; Isler and Van Schaik 2014; Melin *et al.*, 2014). Human subsistence, health and conflict are all sensitive to ecosystem stability, and changes in the seasonality of precipitation are recognized as an important consequence of contemporary climate change (Sellen 2000; Morin and Comrie 2010; Hsiang *et al.*, 2013; Kumar 2013; Watson *et al.*, 2013; Garcia *et al.*, 2014). Rainfall cycles can be inferred from spatial variation in the mineral composition of teeth because the stable oxygen isotope composition ($\delta^{18}\text{O}$ value) of precipitation exhibits characteristic seasonal fluctuations that are recorded in teeth as they grow over time (Bryant *et al.*, 1996; Gat 1996; Bowen and Revenaugh 2003; Balasse *et al.*, 2012). The $\delta^{18}\text{O}$ of body water and blood of animals drinking surface waters reflects the $\delta^{18}\text{O}$ of precipitation, alongside food, air and physiological processes (Longinelli and Padelino 1980; Kohn *et al.*, 1998; Podlesak *et al.*, 2008; Cerling and Sharp 1996). Oxygen in blood plasma phosphate, carbonate, and hydroxyl ions equilibrates isotopically with blood water, and these ions retain an equilibrium isotopic composition as they are incorporated into tooth enamel hydroxyapatite (HAp) (Kohn and Cerling 2002). Thus, tooth $\delta^{18}\text{O}$ values provide an invaluable record of recent and ancient seasonal climatic patterns, bearing in mind that animal behavior, physiology, and local hydrology can also influence the relationship

between environmental and animal isotopic compositions (Kohn *et al.*, 1998; Podlesak *et al.*, 2008).

1.2 Documenting seasonality in teeth. Tooth $\delta^{18}\text{O}$ can be measured for inference about past seasonal water sources using several techniques. While enamel and dentin are both viable tissues for isotopic analysis, enamel is favored for fossil specimens because it is more resistant to diagenetic modification over geological timescales (Kohn and Cerling 2002; Kirsanow *et al.*, 2008). Enamel carbonate $\delta^{18}\text{O}$ is sampled in bulk or through microdrilling, also providing measurements of carbon isotopes ($\delta^{13}\text{C}$) that allow an estimate of feeding upon arid-adapted C4 plants and diet more broadly (Pellegrini *et al.*, 2011; Balasse *et al.*, 2006; Balasse *et al.*, 2012). Laser or ion-based ablative techniques can capture enamel $\delta^{18}\text{O}$ or $\delta^{13}\text{C}$ in much smaller samples, but cannot distinguish between oxygen in phosphate, carbonate and hydroxyl ions. This may be problematic because these ions have different susceptibilities to diagenetic alteration (Passey and Cerling 2006; Sponheimer *et al.*, 2006; Aubert *et al.*, 2012; Blumenthal *et al.*, 2014), and the processes determining their incorporation into mineral vary (Lacruz *et al.*, 2017; Trayler and Kohn 2017).

Enamel phosphate is particularly useful for the study of hydrology as bulk phosphate oxygen fractionation from water into teeth is well understood, and substitution after death and burial is limited (Fricke *et al.*, 1998; Kohn and Cerling 2002; Pellegrini *et al.*, 2011; Longinelli 1984; Daux *et al.*, 2008; Gehler *et al.*, 2011; Kirsanow and Tuross 2011). Accurate $\delta^{18}\text{O}$ measurements of phosphate and carbonate components of enamel can be made from very small enamel samples, which facilitates high-resolution intra-tooth sequential sampling, or repeated isotope measurements across a single tooth (Fricke and O'Neil 1996; Sponheimer *et al.*, 2006; Wiedemann-Bidlack *et al.*, 2008). Sequential sampling is ideal for estimating past seasonality, because the approach of conventional bulk sampling is limited by the problem of time averaging: a single low-resolution sample may include enamel secreted and mineralized over many months or even years.

Because of their large size and abundance in the fossil record, ungulate teeth are regularly sampled for $\delta^{18}\text{O}$ to reconstruct seasonal pastoralist practices, hydrology, feeding and migratory behaviors (Longinelli and Padalino 1980; Zazzo *et al.*, 2010; Stevens *et al.* 2011; Brookman and Ambrose 2012). Despite these efforts, the significance of intra-tooth $\delta^{18}\text{O}$ fluctuations is difficult to evaluate because ungulate tooth mineralization remains poorly understood. In particular, it is unknown how mineralization integrates and dampens seasonal $\delta^{18}\text{O}$ shifts in drinking water during incorporation into blood and ultimately enamel (Passey and Cerling 2002; Passey *et al.*, 2005). Efforts to infer aspects of past climate or diet from sequential isotope sampling rely on models of mineralization developed for ever-growing canines and incisors. It is unclear if these models are appropriate for ungulates, which form their large molars at variable rates over a fixed period (Passey and Cerling 2002; Kohn 2004; Passey *et al.*, 2005; Zazzo *et al.*, 2010; Zazzo *et al.*, 2012), or how developments in the understanding of enamel mineralization affect interpretations of isotopic patterns (Beniash *et al.*, 2009; De Yoreo *et al.* 2015). An accurate understanding of the spatial and temporal pattern of tooth crown mineralization in ungulates is critical to efforts linking seasonal patterns to $\delta^{18}\text{O}$ values in teeth.

1.3 Tooth formation, temporal and spatial pattern of mineralization. Tooth enamel mineralization is conceptualized in two stages: secretion and maturation (Simmer *et al.*, 2012). During the secretion stage, enamel-forming cells (ameloblasts) secrete matrix proteins that are required for proper enamel formation. These control crystal size and shape, as well as the mineral phase, specifically the transformation of amorphous calcium phosphate (ACP) into hydroxyapatite (HAp) (Diekwisch 1998; Smith 1998; Beniash *et al.*, 2009; Simmer *et al.*, 2012). This process begins at the interface between ameloblasts and dentin-forming cells at the location of the future cusp tip. A mineralization front advances towards the future enamel cervix and root (Fig. 1A) by successively activating neighboring ameloblasts to secrete enamel matrix, a process known as tooth crown extension. Simultaneously, through appositional growth organic matrix and mineral are added from the enamel-dentin junction (EDJ) towards the enamel surface in regular increments marking formation time (Boyde 1989; Smith 2006; Smith and Tafforeau 2008). The mineral content of secreted enamel is 20–30 % by weight, that is only about 10% by volume

(Passey and Cerling 2002; Smith *et al.*, 2011; Simmer *et al.*, 2012). The majority of mineral is added during maturation, a more complex and diffuse process that begins once ameloblasts complete secretion and full enamel thickness is reached (Driessens and Verbeeck 1990; Smith 1998; Green *et al.*, 2017).

A number of models have been proposed to explain maturation phase geometry and timing during mineralization. One describes a series of waves of increasing mineralization moving back and forth between the EDJ and the surface of the tooth crown. This model is based on radiographs of primates and ungulates (Fig. 1B) (Suga 1982; Hoppe *et al.*, 2004; Tafforeau *et al.*, 2007). Another model designed for ever-growing teeth, and based upon phosphate concentration measurements, proposes that an initial secretory front is followed immediately by a single, steady maturation wave in the same geometric orientation (Fig. 1C) (Passey and Cerling 2002). This model has been employed using an inverse method to solve for original body fluid isotope composition in an experimentally manipulated rabbit and other domestic animals (Passey *et al.*, 2005; Zazzo *et al.*, 2010). However, a recent mineralization model based on synchrotron X-ray density mapping and Markov Chain Monte Carlo (MCMC) sampling demonstrates that secretion and maturation are temporally and spatially distinct, advancing at differing nonlinear rates (Fig. 1D) (Green *et al.*, 2017). This model is consistent with our current understanding of ameloblast biology and supports observations of mineral density from phosphorus concentration measurements, scanning electron microscopy, and X-ray imaging of mammalian molars (Suga 1982; Passey and Cerling 2002; Hoppe *et al.*, 2004; Tafforeau *et al.*, 2007; Blumenthal *et al.*, 2014; Lacruz *et al.*, 2017; Trayler and Kohn 2017).

Here we address longstanding uncertainty in the temporal and spatial nature of mineralization (Fig. 1B–D) and improve methods for reconstructing seasonal patterns from tooth $\delta^{18}\text{O}$ measurements. To test available models of mineralization, we first conduct an experiment using a sheep subject to a controlled water switch. Measurements of enamel phosphate $\delta^{18}\text{O}$ from this animal demonstrate that a synchrotron-based model from 45 additional sheep (Green *et al.*, 2017) can accurately predict tooth $\delta^{18}\text{O}$ values from known $\delta^{18}\text{O}$ inputs. Next, we show that these results suggest that the timing and geometry of tooth

$\delta^{18}\text{O}$ values may be determined, in part, by phosphate oxygen exchange during mineralization. Lastly, we develop an inverse method that uses a Bayesian optimization framework to estimate original drinking water inputs (Fig. 2). This reconstruction method is able to correctly identify even transient past meteorological events and will ultimately improve the accuracy of environmental reconstruction at key sites where fossil herbivore teeth are abundant.

2. Methods

2.1 Experimental water switch. We raised a male high-percentage Dorset sheep at the Concord Field Station in Bedford, Massachusetts, USA, following weaning at two months of age. It was fed from a single batch of dry feed (Central Connecticut Co-op Farmers Association) and provided Bedford drinking water (ranging from -7.4 to -5.9 ‰ throughout the experiment; all $\delta^{18}\text{O}$ values are reported on V-SMOW scale) originating primarily (85% or more) from the 1.5km^3 Quabbin Reservoir, and to a lesser extent from Bedford ground water (Green *et al.*, 2018). Between 201 and 263 days of age we provided the sheep with glacial melt water (-19.38 ‰, 1 s.d. = 0.05 over the duration of the switch) from Lake Fork Creek in the Beartooth Mountains near Red Lodge, Montana, USA (Fig. 3). At 201, 221, 263 and 283 days of age we gave the animal 8mg/kg subcutaneous calcein injections. Drinking water, feed, and jugular IV blood samples were collected at regular intervals prior to, during and after the water switch. At 1.4 years of age we sacrificed the sheep by 180mg/kg IV sodium pentobarbital injection. Animal care and data collection protocols were approved by the Harvard University Faculty of Arts and Sciences Institutional Animal Care and Use Committee.

2.2 Water, blood, and feed $\delta^{18}\text{O}$ measurements. Sheep blood (300 μl) and drinking water samples (100 μl) were aliquoted into He-flushed exetainer tubes. The exetainers receiving water standards and samples contained 0.3% CO_2 in He for standard $\text{CO}_2\text{-H}_2\text{O}$ equilibrations; blood samples were allowed to outgas and equilibrate endogenous CO_2 . Equilibration incubations were conducted for 24 hours at 26°C on a Thermo GasBench II,

followed by headspace sampling and analysis using the GasBench II and Delta V Plus isotope ratio mass spectrometer (IRMS) at the stable isotope facility in the Department of the Geophysical Sciences, University of Chicago. Samples were referenced to lab standards (HH and HL), which are stored in flame-sealed glass ampoules and processed and analyzed in parallel with samples. The HH and HL standards have $\delta^{18}\text{O}$ values of +1.59 and -23.8 ‰ as determined through repeat analysis against VSMOW, GISP, and SLAP2 standards. The precision of replicate analyses was better than 0.03 ‰ (1 s.d.), and accuracy for water samples is better than 0.1 ‰ (1 s.d.) based on repeat analyses on different days and participation in international laboratory intercalibration exercises .

Aliquots of animal feed were dried at 60 °C and analyzed for oxygen isotope analysis on the TCEA-Conflo IV-Delta V Plus IRMS system at the University of Chicago. Feed $\delta^{18}\text{O}$ was measured to be +25.3 (+/- 0.8 1 s.d.) ‰. Results were assessed relative to standards IAEA-601 benzoic acid (+23.14 ‰) and IAEA-CH-6 sucrose (+36.20 ‰; also referred to as “ANU sucrose”) in addition to the HH and HL encapsulated waters (Qi *et al.*, 2010). The larger scatter for organic $\delta^{18}\text{O}$ may represent feed heterogeneity and variable contributions of a small amount of residual moisture.

2.3 Enamel sample dicing. The lower left M2 was extracted, embedded in methacrylate, and the mesial loph sectioned with a Buehler Isomet saw to produce two 1 mm-thick sections at the maximum buccal-lingual loph breadth. One section, fixed to a silica wafer, was diced with a Disco DAD3240 dicing saw and zinc alloy blade (DZAD1150 Z09-SD2000-Y1-60, Disco). The tooth was diced at 1.500 mm x 0.150 mm increments, with long axis cuts (1.500 mm) proceeding from the cusp tip to root, and short axis cuts proceeding from the EDJ to the enamel surface (Fig. 4A). Cut depth was 0.600 mm, and blade advance and water jet speeds were reduced to minimize sample loss. This method yields 80 – 130 individual block samples in columns and rows of blocks throughout the thickness and length of enamel, weighing c. 500 µg each. In this case we produced 106 block samples from the enamel of the buccal loph. Blade cut precision is < 1µm and sample loss is limited to the 30 µm blade width, thus spatial resolution using this method is

primarily constrained by the mass required for chemical processing and analysis. The second section was polished to a thickness of c. 100 μm and mounted using UV curing resin (Logitech, resin 358) and DPX mounting media onto a glass slide for calcein fluorescence imaging (Fig. 4B).

2.4 Silver phosphate $\delta^{18}\text{O}$ measurements. After dicing, blocks were removed under a microscope using fine forceps and placed into separate 2ml microcentrifuge tubes. Following established protocols for silver phosphate microprecipitation (Colman 2002; Wiedemann-Bidlack *et al.*, 2008), samples were pre-treated overnight with a 2.5% NaOCl solution to remove organic matter, centrifuged, rinsed in DI water five times, and dissolved overnight in 100 μl 2M HNO_3 . The Ca^{2+} in solution was removed through precipitation as CaF_2 by adding 100 μl 2M HF and 150 μl 2M NaOH to each sample for 1–4 hours. The resultant suspension was centrifuged to pellet CaF_2 , and the supernatant transferred to the cut off bulb of a disposable transfer pipette. The solution was reacted uncovered at 50°C in a heat block placed in a chemical hood with 750 μl silver-amine solution (0.067M AgNO_3 , 0.12M NH_4NO_3 , 0.43M NH_4OH), 450 μl 1.25M NH_4NO_3 buffer and 90 μl 1:1 NH_4OH until solution volume was brought below 250 μl (ca. 12 hours). All reagents were from Fisher Chemical (Fairlawn, NJ, USA) or Sigma Aldrich (St. Louis, MO, USA) and were ACS grade or higher. Laboratory deionized (DI) water was 18.2 M Ω , produced by a Thermo Barnstead Nanopure system.

Ag_3PO_4 crystals were then rinsed six times with 1000 μl DI to remove silver nitrate solution, dried, and weighed into silver foil capsules. A single apatite sample yielded sufficient Ag_3PO_4 for one to three 250–300 μg aliquots of Ag_3PO_4 for analysis. The oxygen isotope analysis was completed using a ThermoFisher (Bremen, Germany) TCEA system operated at 1450°C to thermally decompose the phosphate and produce CO from reaction between the released O and the graphite and glassy carbon reactor. The CO was entrained in a He carrier gas and conveyed to a Delta V Plus IRMS via a Conflo IV open split. Replicate analyses of Ag_3PO_4 samples derived from a single diced block gave an analytical precision of 0.15 ‰. Samples were analyzed relative to the following internal standards of

crystalline Ag_3PO_4 from Strem Chemicals (Newburyport, MA, USA), Aldrich (Sigma-Aldrich, St. Louis, MO, USA), and Elemental Microanalysis (Okehampton, UK) with $\delta^{18}\text{O}$ values of 8.2, 10.8, and 21.87 ‰ (VSMOW), respectively through repeat evaluation against YR-1, YR-2, and YR3-1 Ag_3PO_4 standards (Vennemann *et al.*, 2002; Wiedemann-Bidlack *et al.*, 2008) and checked against VSMOW, SLAP2, UC03, and UC04 silver-tube encapsulated waters (USGS, Reston, VA, USA).

For columns 2–6 (15 blocks), some Ag_3PO_4 samples were lost after weighing and prior to measurement; the remaining Ag_3PO_4 within each of these columns was combined for columns 2–4, for three out of four blocks in columns 5–6 and 12, and for two blocks in column 12 (SI).

2.5 Code structure and optimization. Blood physiological, isotopic, and mineralization modeling was conducted using Python2.7. Optimization of all physiological and mineralization parameters, and reconstructions of drinking water history, were performed with the aid of optimization algorithms hosted by the python non-linear optimization module (NLOpt, Johnson, 2017). For each optimization, local parameter searches were conducted using constrained optimization by linear approximation (COBYLA) (Powell 1998). Global searches were conducted using the quasi-Monte Carlo multi-level single linkage algorithm (MLSL) (Rinnooy Kan and Timmer 1987; Kucherenko and Sytsko 2005). Parameter goodness of fit was evaluated using a likelihood framework.

3. Results and Modeling

3.1 Blood and tooth $\delta^{18}\text{O}$ measurements. The blood water $\delta^{18}\text{O}$ value was -5.71 ‰ when the animal arrived in Bedford in mid-March 2013 at 65 days of age. Blood values in Bedford from April through August prior to the start of the experimental water switch were on average -4.30 ‰ (± 0.44 1 s.d.) (Fig. 3). During the water switch, blood water $\delta^{18}\text{O}$ value dropped from -4.96 ‰ to -13.16 ‰ (a change of 8.2 ‰) in 20 days. Average blood water $\delta^{18}\text{O}$ while the animal was at steady state with switch water was -13.24 ‰ (± 0.22 1 s.d.). After returning to Bedford water, average blood water $\delta^{18}\text{O}$ was -4.83 ‰

(± 0.45 1 s.d.) until the completion of the experiment in June, with the exception of two excursions related to snow consumption in early January and mid-March 2014 (-9.47 ‰ and -6.55 ‰, respectively).

The M2 phosphate $\delta^{18}\text{O}$ value is $+14.30$ ‰ at the cuspal tip, and values steadily decline toward the first third of the enamel crown until they reach a minimum of $+9.47$ ‰ (a change of 4.8 ‰) (Fig. 4C). In the region of the tooth with lower $\delta^{18}\text{O}$ values, values increase from near the EDJ to the enamel surface. Toward the middle of the tooth, $\delta^{18}\text{O}$ values increase, more rapidly near the surface and more slowly near the EDJ. In the last third of the tooth $\delta^{18}\text{O}$ values return to higher values, with $+14.9$ ‰ measured at the cervical margin of the enamel.

To evaluate available physiological and mineralization models using these data, we compare results to expectations given model parameters and known experimental $\delta^{18}\text{O}$ inputs in the sections that follow.

3.2 Blood-water $\delta^{18}\text{O}$ modeling. Existing blood $\delta^{18}\text{O}$ and turnover models were adapted to predict sheep blood $\delta^{18}\text{O}$ changes given known water and feed ingestion histories. Following frameworks used for humans and rodents under controlled conditions (Gretebeck *et al.*, 1997; Podlesak *et al.*, 2008), we model the steady-state $^{18}\text{O}/^{16}\text{O}$ ratio in body water, or R_{bw} , as contingent upon the ratios of inputs and outputs:

$$R_{bw} = \frac{(R_{dw} * F_{dw}) + (R_{O_2} * F_{O_2} * \alpha_{O_2}) + (R_{fd} * F_{fd})}{F_{H_2O-un} + (F_{H_2O-fr} * \alpha_{H_2O-fr}) + (F_{CO_2} * \alpha_{CO_2})} \quad (1)$$

where in the numerator, R_{dw} and F_{dw} are the isotope ratio and fractional contribution of drinking water to body water oxygen, R_{O_2} and F_{O_2} are those for inspired oxygen and α_{O_2} is the fractionation factor between gaseous and dissolved O_2 at the gas-liquid boundary in the lungs, and R_{fd} and F_{fd} are the ratio and fractional contribution of feed. In the denominator, F_{H_2O-un} is the fraction of water oxygen leaving the body in water without fractionation, F_{H_2O-}

f_r is that fraction of water oxygen leaving the body in water that fractionates according to the factor $\alpha_{H_2O-f_r}$, and F_{CO_2} is the fraction of body water oxygen leaving as CO_2 , fractionated by the factor α_{CO_2} (Gretebeck *et al.*, 1997; Podlesak *et al.*, 2008). We fit all of these parameters simultaneously using observed blood, drinking water and feed $\delta^{18}O$. Changing blood $\delta^{18}O$ values over time are defined by:

$$\frac{d\delta_b}{dt} = -\lambda_b [\delta_b(t) - \delta_{b,ss}(t)], \quad (2)$$

where λ_b is the decay constant, $\delta_b(t)$ is the blood $\delta^{18}O$ value at time t , and $\delta_{b,ss}(t)$ is the theoretical blood $\delta^{18}O$ steady state at that same time, determined by physiological parameters and feed and air $\delta^{18}O$ values. Importantly, from equations (1) and (2) we can predict both the $\delta^{18}O$ value of body water, and its rate of change, from environmental sources and physiological conditions (Green *et al.*, 2018).

Blood $\delta^{18}O$ data from our experimental animal indicate an overall single-pool blood water oxygen half-life of 2.6 days, calculated by fitting λ_b with data. Fitting of physiological parameters with blood and input $\delta^{18}O$ data suggest that the ratio of drinking water oxygen to metabolic oxygen (feed and inspired O_2) intake is approximately 2:1, a value that closely corresponds with measured water and feed intake from August-October during the experimental switch. Modeling results suggest evaporation accounts for approximately 19% of the water (as opposed to total oxygen) lost from the body (Table 1).

Table 1: Modeled sources of oxygen inputs and outputs, and fractionation factors for $\delta^{18}O$.

	<u>Fraction</u>	<u>α</u>
Drinking water influx	.69	
O_2 influx	.18	.990
Feed influx	.13	
O efflux (unfractionated)	.69	
O efflux (fractionated)	.13	.990

CO ₂ O efflux	.18	1.040
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3.3 Blood $\delta^{18}\text{O}$ integration with mineralization for 2D forward modeling of $\delta^{18}\text{O}$.

Based on knowledge of enamel formation and body water oxygen fluxes, tooth $\delta^{18}\text{O}$ values can be predicted from any given drinking water history. Tooth $\delta^{18}\text{O}$ maps generated from combining either measured or modeled blood or drinking water histories with mineralization modeling are described as “forward models”, because they predict spatial patterns in tooth $\delta^{18}\text{O}$ (Passey and Cerling 2002). Here, we produce forward models of tooth spatial $\delta^{18}\text{O}$ distributions by using given body water histories (modeled, or measured, with missing data points filled by interpolation) with a synchrotron-based mineralization model (Green *et al.*, 2017). The model discretizes tooth mineralization into daily increments. On any day d , newly added mineral at pixel location $px_{x,y}$ would be in equilibrium with blood $\delta^{18}\text{O}$ on that day. The accumulated (i.e., time integrated) mineral phosphate at a specific location in the tooth would then have an oxygen isotope composition:

$$\delta^{18}\text{O}_{px,d} = \Delta_{PO4-H2O} + \sum_{i=init}^d \delta^{18}\text{O}_i * \rho_i^{\Delta} / \rho_d^t$$

(3)

where *init* denotes the first day of mineral density increase for that pixel, $\delta^{18}\text{O}_i$ is the blood $\delta^{18}\text{O}$ value for day i since mineralization began, ρ_i^{Δ} is the density increase for each day, and ρ_d^t is the total cumulative mineral density through day d over that same period. $\Delta_{PO4-H2O}$ represents the equilibrium offset between phosphate and water $\delta^{18}\text{O}$. This offset is a function of temperature and was originally established by Longinelli and Nuti (1973), although we evaluate more recent assessments below.

Because we test a synchrotron mineralization model built using sheep lower M1s with a sheep lower M2, we convert between spatial landmarks and tooth formation timing using transfer functions (Green *et al.*, 2017). This transformation is analogous to the application

of a mineralization model designed for ever-growing teeth to a variety of taxa, and to molars which are not ever-growing (Passey and Cerling 2002; Passey *et al.*, 2005; Zazzo *et al.*, 2010). Briefly, enamel formed at time t in the M2 is deposited at tooth length l as a function of the M2 extension curve:

$$l = a * \operatorname{erf}(s * (t - o)) + (l_{\max} - a) \quad (4)$$

where a is the wave amplitude (mm), erf is the Gaussian error function, s is the wave slope (mm/day), o is the offset (days) from birth, and $l_{\max}$ (mm) is the mature length of the M2. In the M1, equivalent spatial positions are distributed over a smaller area, due to the smaller size of the tooth. To predict the M2 $\delta^{18}\text{O}$ spatial patterns resulting from a given drinking history using the M1 model, a new drinking water history characterized by events at times t_{m1} is generated:

$$t_{m1} = \frac{\operatorname{erf}^{-1}((a_{m1} + l_{m1} - l_{\max m1})/a_{m1}) + (o_{m1} * s_{m1})}{s_{m1}} \quad (5)$$

in this case with parameters describing M1 extension and maximum length (Green *et al.*, 2017). This procedure is able to predict tooth isotopic values in an M2 from an M1 mineralization model given knowledge of extension in both molars. Uncertainties in both M1 and M2 extension rates are incorporated into model estimates as a source of error during drinking water reconstructions (described in greater detail by eq. 12).

Uncertainty in our parameterization of $\Delta_{\text{pO4-H2O}}$ arises in part from uncertainty in sheep oral temperature. Based on temperature measurements in our animal and differences between rectal, tympanic, and oral temperatures in sheep, we adopt a value of 38.0°C (+/- 0.5°C) as being broadly representative of mean sheep oral temperature over a diurnal cycle (Refinetti and Menaker 1992; daSilva and Minomo 1995; Goodwin 1998). This temperature uncertainty converts to an uncertainty in $\Delta_{\text{pO4-H2O}}$ and therefore $\delta^{18}\text{O}_{\text{px,d}}$ of approximately

+/- 0.13‰. A greater source of uncertainty in $\Delta_{\text{PO4-H2O}}$ stems from different thermometric equations, possibly resulting from differing source materials, analytical methods, and isotopic standards used (Longinelli and Nuti 1973; Longinelli 1984; Pucat *et al.*, 2010; Lcuyer *et al.*, 2013; Chang and Blake 2015). The calculated $\Delta_{\text{PO4-H2O}}$ at a temperature of 38.0C with different available equations is 17.1 (Longinelli and Nuti 1973), 17.6 (Lcuyer *et al.*, 2013), 18.0-18.9 (Pucat *et al.*, 2010, depending on the accepted value of NBS120c) and 19.8 ‰ (Chang and Blake 2015).

Evolving water flux magnitude and turnover time throughout the course of the experiment further complicate the modelling of blood water $\delta^{18}\text{O}$ values (O’Grady *et al.*, 2010). We test values ranging from 17.5 – 19.5 ‰ by using these offsets in our forward model and comparing resulting model $\delta^{18}\text{O}$ spatial patterns with our data using a likelihood score. Best fit offsets are sensitive to spatial uncertainty at the model perimeter and show a range of possible values from 18.6 – 19.3 ‰. We find a most likely value for $\Delta_{\text{PO4-H2O}}$ to be 19.1 ‰, close to the value found by Pucat *et al.*, (2010), and use this offset throughout this study. In summary, equations (3-5) permit the prediction of tooth $\delta^{18}\text{O}$ values from any given mineralization and blood $\delta^{18}\text{O}$ history, including for teeth with differing patterns of extension over time.

3.4 Modeled and observed secretion and maturation. It is expected that the geometry and timing of tooth mineralization should be reflected by $\delta^{18}\text{O}$ values in the tooth, if mineralization was ongoing during a large, transient shift in blood isotopic values. This is because a short-term large excursion in blood $\delta^{18}\text{O}$ value can serve as a label for mineral density increases at a specific narrow interval of time, and therefore provide a snapshot of mineralization. The phosphate that mineralizes in enamel HAp during this pulse of isotopically distinct drinking water will also be isotopically distinct, tracking the magnitude of the excursion in blood water $\delta^{18}\text{O}$ (Fig. 1B-D).

Integrating the drinking water history of our experimental animal (Fig. 3) with mineralization modeling (Green *et al.*, 2017), we predict that depleted $\delta^{18}\text{O}$ values from

Montana switch water should appear in two primary waves corresponding to observations of secretion and maturation (Fig. 4E). In the predicted tooth $\delta^{18}\text{O}$ spatial pattern resulting from equations (3-5), the secretory wave appears at a steep angle to the EDJ and is separated from the maturation wave by a gap that is visible near the EDJ but not the enamel surface (Fig. 4C). Isotopically, the maturation wave occupies more space in the tooth compared to the secretory wave and has an orientation more perpendicular to the EDJ. Predicted switch $\delta^{18}\text{O}$ values are far lower in the maturation wave than the secretory wave. This is due to the greater proportion of HAp mineral deposited during maturation phase (Passey and Cerling 2002; Passey *et al.*, 2005; Smith *et al.*, 2011; Simmer *et al.*, 2012; Green *et al.*, 2017).

Phosphate $\delta^{18}\text{O}$ values measured from the experimental sheep broadly correspond to synchrotron model predictions (Fig. 4E). In the measured tooth $\delta^{18}\text{O}$ values, the experimental switch is also expressed in two separate waves – secretion and maturation – that are even more spatially distinct than in predictions. Predicted and observed $\delta^{18}\text{O}$ values corresponding to secretion and maturation (Fig. 4C, E) occupy a broader space within the tooth than transient, instantaneous mineral addition (Fig. 4D) because the switch occurred over a 40-day period (Fig. 3). While these predicted and measured $\delta^{18}\text{O}$ spatial patterns show a similar range of $\delta^{18}\text{O}$ variation, the experimental animal shows lower $\delta^{18}\text{O}$ values in the cuspal region, which return to higher $\delta^{18}\text{O}$ values more gradually than the model prediction. This small discrepancy between modeled and measured $\delta^{18}\text{O}_\text{p}$ maps is diminished if we assume that exchange occurs between a portion of the mineralized phosphate with phosphate in the extracellular enamel matrix fluid. The latter is replenished through the duration of enamel crown formation and is in isotopic equilibrium with oxygen in blood. We therefore present a model that incorporates this hypothetical delayed partial resetting of enamel phosphate oxygen isotopes, as supported by evidence that phosphate may exchange during the secretory transformation of amorphous precursors to HAp, and during dissolution-reprecipitation processes during maturation (Diekwisch 1998; Beniash *et al.*, 2009; Josephsen *et al.*, 2010; Simmer *et al.*, 2010; Simmer *et al.*, 2012; Damkier *et al.*, 2014; De Yoreo *et al.*, 2015; Green *et al.*, 2017).

3.5 Phosphate $\delta^{18}\text{O}$ resetting modeling. We have constructed a refined model that uses three variables to describe delayed partial isotopic resetting of mineralized phosphate within the context of the overall progressive mineralization model. These variables are: 1) the delay between onset of initial mineral deposition and the onset of oxygen or phosphate ion exchange (during phase transformation and crystallite growth), 2) the proportion of phosphate that exchanges, and 3) the rate of exchange. In this model, we take into account the delay between initial mineral deposition and later changes in mineral phase and crystal size through mineral addition. Both are regulated by enzymatically driven changes in protein matrix composition and protein-mineral interactions. The dynamic sequence of events from initial ACP deposition in secretory stage, phase transformation to HAp and crystal expansion, is reflected in the re-equilibration of oxygen isotopic compositions in a fraction of already precipitated mineral with blood water. Parameter results will be described below.

We define M_A as the mass of mineral formed at time t_A , and we track the isotopic composition of M_A within the 2D grid of our combined stable isotope-mineralization model. The initial δ_{PO_4} of M_A is represented by δ_A , which represents equilibration with blood water $\delta^{18}\text{O}$ at t_A . Phosphate exchange in a labile fraction of M_A begins after a pause of duration Δt_{ex} following initial deposition. We have little direct information on the extent to which M_A resets isotopically. In this simple model, we treat the fraction that resets as a constant, f , regardless of whether M_A was formed during secretion or maturation. We define the amount of labile or exchangeable M_A as $M_{A,\text{ex}}$. For the interval $t_A < t < (t_A + \Delta t_{\text{ex}})$, $M_{A,\text{ex}} = f \times M_A$, and we let $M_{A,\text{ex},0}$ represent this initial amount of exchangeable M_A . After the exchange process begins, $M_{A,\text{ex}}$ diminishes according to:

$$\frac{dM_{A,\text{ex}}}{dt} = -\lambda_{\text{PO}_4} M_{A,\text{ex}} \quad (6)$$

in which the rate of isotopic resetting is governed by the first order decay constant λ_{PO_4} . Once this portion of mineral exchanges phosphate or phosphate oxygen, it is considered reset to a new isotopic composition, denoted by $\delta_{A,reset}$, that reflects equilibration with blood water at the time of the resetting. Solving for $M_{A,ex}$, we have,

$$M_{A,ex} = M_{A,ex,0} e^{-\lambda_{PO_4} (t - (t_A + \Delta t_{ex}))} \quad (7)$$

where the term $(t - (t_A + \Delta t_{ex}))$ measures the elapsed time since the resetting process began. The $\delta^{18}O_p$ of this fraction of M_A that undergoes resetting, δ'_A , is calculated as a two-component mixture of unreset and reset phosphate:

$$\delta'_A = (1 - x)\delta_A + x \delta_{A,reset} \quad (8)$$

where $1 - x$ = the fraction of $M_{A,ex,0}$ that has not yet reset = $e^{-\lambda_{PO_4} (t - (t_A + \Delta t_{ex}))}$ for the time interval corresponding to resetting. The $\delta^{18}O$ of the cumulative exchanged, or reset, enamel phosphate, $\delta_{A,reset}$, is then:

$$\delta_{A,reset} = \frac{\int_{(t_A + \Delta t_{ex})}^t \lambda_{PO_4} M_{A,ex} \delta_{t,eq} dt}{x M_{A,ex,0}} \quad (9)$$

which integrates the $\delta^{18}O$ of the phosphate in the incremental additions of exchanged mineral, averaging over the total amount of exchanged mineral. The model with this delayed partial resetting then treats the above equations numerically to allow for complex histories of $\delta_{t,eq}$, which represents the $\delta^{18}O$ of mineral phosphate equilibrated with blood water at t . The overall $\delta^{18}O_p$ for each location within the tooth is then calculated through combining equations 3, 8, and 9 to monitor the progression of original mineralization and delayed partial resetting.

We estimate f to be 30%, Δt_{ex} to be 35 days (the average delay between secretion and maturation onset throughout the crown) (Green *et al.*, 2017), and λ_{PO4} to be 3 days based upon the rate of phase transition observed in mouse molars (Beniash *et al.*, 2009).

Accounting for possible phosphate oxygen exchange, the new predicted $\delta^{18}\text{O}$ values match experimental observations more closely. Modeling delayed partial phosphate exchange has the effect of elongating the predicted spatial distribution of the switch in the tooth, as is observed in our experimental animal (Fig. 4F). This model also follows the pattern observed in the measurements where the water switch is evident more cusally in the tooth due to the partial resetting, during the switch, of enamel originally mineralized earlier.

3.6 Seasonal drinking water reconstruction: general approach. The principle goal of sequential isotope sampling in teeth is to reconstruct the original seasonal physiological, behavioral or environmental process that produced observed tooth values. Reconstruction of drinking water $\delta^{18}\text{O}$ histories from the spatial array of $\delta^{18}\text{O}$ values of a “target” tooth can be achieved by iteratively proposing drinking water $\delta^{18}\text{O}$ histories and generating associated forward tooth $\delta^{18}\text{O}$ maps until these closely match the target (Fig. 2). This iterative approach to identify a drinking water or body chemistry history that explains measured $\delta^{18}\text{O}$ values in a tooth is described as an inverse method (as opposed to a forward model) (Passey *et al.*, 2005).

We use a Bayesian framework to reconstruct drinking water histories from tooth $\delta^{18}\text{O}$ values that takes into account model-target tooth $\delta^{18}\text{O}$ differences, and expectations for likely water $\delta^{18}\text{O}$ values in nature. In general, we wish to calculate the probability p of a drinking water history, δ^w , given tooth $\delta^{18}\text{O}$ observations δ^d :

$$p(\delta^w|\delta^d)$$

(10)

Per Bayes' theorem, this is equal to

$$\frac{p(\delta^d|\delta^w)p(\delta^w)}{p(\delta^d)} \quad (11)$$

where the prior $p(\delta^w)$ is the probability of the drinking water history given prior knowledge of what a reasonable drinking water history should look like. Here, we evaluate $p(\delta^w)$ based upon an assumed maximum likely rate of change in drinking water δ^{18O} (expressed in ‰ per day), denoted by d_{rate} . The probability of measured data over all of theoretical parameter space $p(\delta^d)$ is for practical purposes difficult to calculate, but is constant and can therefore be ignored when evaluating the relative posterior probability p of drinking water histories δ^w :

$$p(\delta^w|\delta^d, d_{rate}) \propto p(\delta^d|\delta^w)p(\delta^w) \quad (12)$$

This probability can be calculated from the data δ^d and model δ^m tooth isotopic values resulting from δ^w for all i of n tooth locations (px), and from the expected (d_{rate}) and observed ($\delta^{w_i} - \delta^{w_{i+1}}$) rates of drinking water change for all i of n days during tooth formation:

$$p(\delta^w|\delta^d, d_{rate}) \propto \prod_i^{n_{px}} \frac{1}{\sigma_i} e^{-\frac{(\delta_i^m - \delta_i^d)^2}{2\sigma_i^2}} * \prod_i^{n_{days}-1} e^{-\frac{(\delta_i^w - \delta_{i+1}^w)^2}{2d_{rate}^2}} \quad (13)$$

For computational purposes, we employ a modified version of this equation that reduces search times, and is adequate for parameter optimization:

$$\ln p(\delta^w | \delta^d, d_{rate}) \propto sw \sum_i^{n_{px}} \left[\frac{(\delta_i^m - \delta_i^d)^2}{2\sigma_i^2} + \ln \sigma_i \right] + pw \sum_i^{n_{days}-1} \frac{(\delta_i^w - \delta_{i+1}^w)^2}{2d_{rate}^2} \quad (14)$$

where sw is the score weight equal to $n_{days} / (n_{px} + n_{days})$, and pw the prior weight equal to $1 - sw$. When using this equation, tooth $\delta^{18}O$ likelihood scores are weighted proportionally to the number of evaluated locations (i.e., to the sampling resolution). Total error per tooth location σ is estimated as the root of the sum of squared error sources:

$$\sigma_{px} = \sqrt{(\sigma_m)^2 + (\sigma_\delta)^2 + (\sigma_z)^2} \quad (15)$$

where σ_m is model error, σ_δ is the overall PO_4 $\delta^{18}O$ IRMS measurement error, and σ_z is a minimum error set to 0.05. Model error σ_m is estimated for each location in the tooth, and is derived from 30 possible $\delta^{18}O$ values at that location: ten possible values drawn from the synchrotron-based model to account for population-level variability in the mineralization process, for each of three possible overall growth (extension) rates that might account for taxonomic differences. Here, rapid and slow extension curve amplitudes, slopes and offsets are set to 62.00, .0037, 0.00, and 74.00, .0031, -48.00, respectively, based upon mean extension rates for sheep lower M2s (Green *et al.*, 2017).

Proposed drinking water histories are composed of linearly interpolated $\delta^{18}O$ value parameters spaced at 14 days, spanning the duration of tooth formation. For each proposed drinking water history, resulting blood, PO_4 , and tooth $\delta^{18}O$ values are also calculated to produce tooth spatial isotopic compositions δ^m (see above, **3.3 Blood $\delta^{18}O$ integration with mineralization**). Minimization of the likelihood estimator L is implemented using the MLSL and COBYLA algorithms. Reconstructions are performed using a mineralization model that is spatially downsampled to the scale of sampling for isotopic measurements.

To shorten search times during reconstructions and capture input signal detail, for all reconstructions, optimizations are run in series of three with d_{rate} progressively relaxed for each optimization, using values of $d_{\text{rate}} = 1/5, 1/2$ and $1 \text{ } \text{‰}/\text{day}$. Within a reconstruction trial, the first optimization is started with a $\delta^{18}\text{O}$ drinking water history estimate, based upon target tooth $\delta^{18}\text{O}$ averages moving from the cusp to the root. The results of each optimization are used as first guesses for the next. These techniques that modify priors and initial parameter start positions dramatically decrease the search times required for accurate reconstructions.

3.7 Drinking water reconstruction: model results. Before testing our method of drinking water reconstruction experimentally, we test its ability to reconstruct drinking water histories from modeled forward tooth $\delta^{18}\text{O}$ spatial distributions. This should represent a best-case scenario, because the inverse model uses approximately the same representation of animal physiology and mineralization as the forward model that was used to generate the tooth isomap. By using this approach, we are able to determine features of drinking water histories that are robustly recovered and those that are difficult to reconstruct. We can also determine how the spatial resolution of measurements in a tooth impacts the quality of drinking water $\delta^{18}\text{O}$ reconstructions. This extends to consideration of the high resolution 2D sampling that we conducted (many samples collected in a grid pattern throughout the enamel) vs. traditional 1D serial sampling with high or low spatial resolution (many or few samples collected in a line from enamel).

We first attempt to reconstruct drinking water history from modeled tooth $\delta^{18}\text{O}_p$ values produced from water with sinusoidally varying oxygen isotope composition. We tested periods of 45, 90, 180 and 360 days to determine the sensitivity of reconstructions to different time scales of variation. When nonlinear extension and growth processes are accounted for, reconstruction of high frequency (45 and 90 day) inputs is possible using either sampling regimes with high or low resolution (Fig. 5). This result is particularly important, since many studies employ only a limited number of samples extending from the cusp down the tooth crown to the cervix (1D sampling along the tooth). It does appear

that sampling with a 2D sampling regime (at varying tooth crown heights and depths within the enamel) produces slightly more detailed reconstructions, recovering more of the 45-day frequency compared to 1D sampling (Fig. 5).

Reconstructions are most accurate early in tooth growth, when temporal signals are spread out over larger spatial scales due to rapid extension rates. The fidelity of drinking water isotopic reconstructions degrades later in tooth formation, and this degradation occurs earlier and is more pronounced with lower sampling resolution (when fewer samples are taken from the tooth to inform the reconstruction). We find that a direct interpretation of sequential sample analyses as reflecting time variation in drinking water inputs, without a model reconstruction based on patterns and timing of tooth mineralization, does not accurately reflect either the magnitude or the timing of original inputs in any of the tested circumstance. This stems from both dampening of the drinking water input signal due to animal physiology as well as time averaging during the mineralization process. Coarser spatial resolution leads to enamel samples that mineralized over larger spans of time. The coarser the sampling, the more the drinking water input variation is dampened in the tooth $\delta^{18}\text{O}_p$ measurements.

We also find that the assumption of a linear growth rate, as might be expected for an ever-growing tooth, results in reconstructions that distort the timing of the input signal (Fig. 5). However, when nonlinear growth and the complexity of the mineralization process are accounted for in the inverse model, both high and low resolution sampling regimes are able to effectively reconstruct the original water input $\delta^{18}\text{O}$ values over the majority of tooth formation time. Nevertheless, when input signals are especially complex, higher resolution sampling results in more faithful reconstructions (Fig. 6).

We then test this reconstruction system using forward maps generated from published and open-access precipitation $\delta^{18}\text{O}$ records at high and low latitude sites (Harvey and Welker 2000; Moerman *et al.*, 2013; IAEA 2017), setting the drinking water composition to match that of the precipitation record. Reconstructions with high-resolution and low-resolution

sampling are fairly accurate, and their accuracy decreases only marginally with lowered sample resolution (Fig. 7). Importantly, if a linear growth model is used along with conventional 1D sampling of the tooth, the inferred timing of seasonal variation in drinking water isotopic composition can be off by many months. The reconstruction of the time variation in original input signal timing, and in many cases the accurate reconstruction of the amplitude of the variation, requires an inverse method that incorporates nonlinear growth, a result consistent with previous findings (Passey and Cerling 2002; Kohn 2004; Passey *et al.*, 2005; Zazzo *et al.*, 2010).

3.8 Experimental test of reconstruction method. Finally, we test this inverse method by reconstructing drinking water $\delta^{18}\text{O}$ from the tooth phosphate $\delta^{18}\text{O}$ measurements of our experimental animal. We compare the results with measured drinking water, feed, and air $\delta^{18}\text{O}$ inputs in addition to blood $\delta^{18}\text{O}$ values. In the special case of drinking water reconstruction from the tooth of our experimental animal, water histories are fitted using either naturalistic conditions (model as described earlier) or using an additional three parameters that allow the model to identify the step-function experimental switch in water source. This switch model is structured so that it is challenged to identify: the day of switch onset, the duration of the switch, and the $\delta^{18}\text{O}$ value of the switch water. Rate of change penalization employed is either $d_{\text{rate}} = 1/5, 1/3$ and $3/4$ ‰/day (naturalistic scenario), or $1/5, 1/3$ and $1/2$ ‰/day (experimental scenario). For the experimental scenario, penalization does not include the days of switch onset and conclusion, and drinking water $\delta^{18}\text{O}$ does not change during the switch.

Inversion trials reconstruct the approximate location and magnitude of the experimental switch (Fig. 8), although many solutions propose variable switch morphologies that preserve the integrated weight of the switch history. Interestingly, inverse trials reliably estimate depleted $\delta^{18}\text{O}$ input values following the experimental water switch. These excursions were unplanned in the experimental design. However, local meteorological records from Hanscom Air Force base and news reports indicate that reconstructions track two unanticipated local snowstorms to which the sheep was exposed in an open pen. Video records show another sheep consuming snow during this post-switch interval. Additional

blood and snow $\delta^{18}\text{O}$ measurements from February and March (Fig. 8) in our experimental animal and the snow in its environment confirm that blood water was isotopically light relative to expectations from drinking only municipal water and consistent with the ingestion of snow with its low $\delta^{18}\text{O}$ value. Thus, the reconstructions accurately recovered the timing of meteorological events from tooth $\delta^{18}\text{O}$ measurements.

3.9 Optimization of PO_4 resetting. We make use of available blood and tooth phosphate $\delta^{18}\text{O}$ measurements to better constrain our estimate of possible phosphate resetting parameters by employing the optimization architecture used for inversion. Estimation of parameters f , Δt_{ex} , and λ_{PO_4} that govern PO_4 exchange during mineralization is conducted using the code architecture developed for drinking water reconstruction. In this case, resetting parameters are varied to minimize the likelihood estimator L given known blood values over time, δ^b :

$$L(f, \Delta t_{\text{ex}}, \lambda_{\text{PO}_4} | \delta^d, \delta^b) \propto \prod_i^{n_{\text{px}}} \frac{1}{\sigma_i} e^{\left(-\frac{(\delta_i^m - \delta_i^d)^2}{2\sigma_i^2} \right)} \quad (16)$$

Where error σ is as described above (equation 13), and flat priors are employed for all resetting parameters. Observing the likelihood of parameter space for f , Δt_{ex} , and λ_{PO_4} , we find that higher Δt_{ex} values are associated with lower f and λ_{PO_4} values. Δt_{ex} is optimized at 55 days, though values of 40–65 days are found to be similarly probable. Resetting half-life (derived from λ_{PO_4}) is optimized at 8 days, with probable values constrained to 5–15 days. Interestingly, some likely $t_{1/2}$ values are higher, approaching 40 days, though we consider these unlikely. The fraction f that exchanges is optimized to 0.38, with likely values from 0.3–0.5. Our modeling framework is not ideal for fully quantifying resetting for a number of reasons. One limitation of our approach is that we treat phosphate deposited during secretion and maturation equally, though it is likely that enamel deposited during secretion is more prone to resetting than that deposited during maturation over the full duration of mineralization. Another limitation is the empirical nature of the mineralization model,

which includes uneven sampling across the enamel formation process. Nevertheless, resetting dramatically improves model-data enamel $\delta^{18}\text{O}$ mismatch (Fig. 4). Overall, these optimizations provide some support for the concept of mobility of oxygen in phosphate following deposition.

4. Discussion

Sequential isotopic sampling from herbivore teeth remains a favored and potentially powerful tool for reconstructing seasonality of climate and variation in animal physiology, behavior, diet, and environment. Despite longstanding interest, it has been difficult to interpret the implications of sequential enamel $\delta^{18}\text{O}$ measurements for seasonality. A principle cause of this difficulty has been the recognition that the process of enamel mineralization, and in particular enamel maturation, dampens and distorts original inputs to an uncertain extent (Passey and Cerling 2002; Passey *et al.*, 2005; Zazzo *et al.*, 2010). Another obstacle to interpreting $\delta^{18}\text{O}$ values is accounting for further attenuation of input drinking water signals within the body by physiological processes (Kohn 1996). Until recently, the only mathematical model describing mineralization was built for ever-growing teeth (Passey and Cerling 2002; Passey *et al.*, 2005). Tests in the most controlled of circumstances, for large herbivores, have been conducted using dietary $\delta^{13}\text{C}$ manipulation with forward but not inverse modeling (Zazzo *et al.*, 2010).

The observation of rapid body water turnover in our experimental animal, and the appearance of transient meteorological events as an isotopic imprint in the teeth, suggest that this turnover pool may have a smaller dampening effect in some animal contexts (Podlesak *et al.*, 2008; Zazzo *et al.*, 2010). Modeling of oxygen throughput in our experimental animal, using available blood water isotopic measurements and $\delta^{18}\text{O}$ values for sources of O to blood water, produces results consistent with observations of drinking water and feed intake through the duration of the experimental switch. Our results are also within the range of body water half-lives (2.2 – 9.0 days) previously reported for domesticated sheep using deuterated and tritiated water experiments (MacFarlane *et al.*, 1971; Dawson, 1978; Nicol, 1978; Green *et al.*, 2018). Nevertheless, it is reasonable to

assume that parameters such as the magnitude of drinking water intake, evaporative water loss, and oral temperature will vary between breeds, species, and over seasonal and even diurnal cycles (Kohn *et al.*, 1998; Zazzo *et al.*, 2010; Gretebeck *et al.*, 1997). In natural settings, changes in feed source and composition, water budgeting, and movement over the landscape represent significant complications for interpreting seasonal changes in body $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values (Kohn 1996; Fricke *et al.*, 1998; Kohn *et al.*, 1998; Levin *et al.*, 2009; Quinn *et al.*, 2015). These obstacles highlight the importance of developing inferential models that link seasonal body isotopic compositions to complex foraging behaviors and landscape hydrology (Fricke *et al.*, 1998).

Despite these difficulties, the close match in the present study between measured phosphate $\delta^{18}\text{O}$ values in teeth and predictions from the coupled stable isotope and synchrotron-based mineralization models is striking (Fig. 4). Most importantly, in both modeled and observed $\delta^{18}\text{O}$ values, secretory and maturation waves appear as distinct events with predictable magnitudes and geometries. With modeling, the complex spatial and temporal patterns of signal attenuation can be used to reconstruct seasonal body $\delta^{18}\text{O}$ values. An equally striking phenomenon is the efficacy of the mineralization component of the model, built using M1 teeth, when used in an M2 with somewhat different extension rates. The geometry of secretion and maturation waves recorded during the drinking water switch by the 2D $\delta^{18}\text{O}_\text{p}$ maps is similar to the geometry observed in other herbivores with multiple imaging platforms and analytical techniques (Suga 1982; Passey and Cerling 2002; Hoppe *et al.*, 2004; Tafforeau *et al.*, 2007; Jordana and Köhler 2011; Kierdorf *et al.*, 2013; Blumenthal *et al.*, 2014; Bendrey *et al.*, 2015; Trayler and Kohn 2017). Similar reconstruction methods may be employed with modifications in a variety of taxa, as has been attempted in a few cases using linear estimates of tooth extension (Passey *et al.*, 2005; Zazzo *et al.*, 2010).

A critical result is that sequential $\delta^{18}\text{O}$ values, when viewed without modeling or reconstruction, may have little similarity to original seasonal inputs or body water compositions. This is consistent with prior forward and inverse modeling work (Passey and Cerling 2002; Passey *et al.*, 2005; Zazzo *et al.*, 2010). Our experimental observations

show that conventional sequential tooth $\delta^{18}\text{O}$ values capture less than half of the total magnitude of drinking water variation over the duration of the experiment (Fig. 8). Modeling further suggests that the discrepancy between measured and reconstructed values would be especially pronounced in the tropics, where more rapid seasonal changes will result in apparently invariant conventional sample values (Figs. 6–7). However, with modeling, even a small number of samples can be sufficient for reconstructing both the magnitude and timing of inputs (Figs. 5–7). In theory, faithful reconstruction of a signal requires a minimum of two samples per signal period (Nyquist 1928). The fidelity of low-resolution sampling in some contexts explored here underscores the value of the information gained from detailed study of tooth mineralization. In the case of our experimental animal, reconstruction recovered snowstorms that were not wholly apparent from sequential enamel $\delta^{18}\text{O}$ measurements, informing us of unanticipated but real events that occurred during the experiment (Fig. 8).

There are some cases where increased sample resolution improves the observable fidelity of reconstruction. At some low latitude sites, inverse methods reconstruct input signal amplitudes more fully through the use of higher sample resolution (Figs. 6–7). Increased resolution sampling may therefore be important for reconstructing seasonal patterns from some low-latitude ecosystems. Nevertheless, even reduced sampling resolution can quantitatively reconstruct the timing and magnitude of isotopic inputs from a wide range of locations, provided that nonlinear extension is accounted for.

In sheep and in mammals more broadly, several aspects of mineralization remain unknown. These include the possibility of multiple (or more complex) maturation waves. Our experimental study coupled with detailed modeling work provides evidence that the phosphate oxygen isotopic signal acquired during initial mineral deposition is partially overprinted by a delayed isotope exchange or recrystallization (and phosphate ion exchange) process. This leads to greater time averaging, and it also suggests that the three oxygen-bearing constituents of HAp (phosphate, carbonate, and hydroxyl ions) may all record different time intervals in the early life of the animal (Trayler and Kohn 2017). The inclusion within the isotope model of limited exchange of mineral phosphate oxygen (re-

equilibrating with blood water at a later time), or limited exchange of phosphate ions between the mineral lattice and the dissolved (isotopically equilibrated) phosphate in the mineralizing fluid, is a simple mechanism for improving the spatial and numerical alignment of model with measured $\delta^{18}\text{O}_p$ values (Fig. 4).

It is probable that our modeling of phosphate resetting does not fully reflect exchange processes occurring in both secretory and maturation phases, which we here treat equally. Nevertheless, by modeling the resetting of $\delta^{18}\text{O}$ values we appear to find experimental support for some phosphate oxygen exchange after mineral deposition. There are a number of specific mechanisms, supported by recent advances in the field of biomineralization, by which enamel $\delta^{18}\text{O}_p$ values may be re-equilibrated with later body water. Upon initial secretion of matrix and ACP, the most abundant enamel matrix protein amelogenin is cleaved by the proteolytic enzyme matrix metalloproteinase 20. It has been shown that both amelogenin phosphorylation and cleavage are critical for its ability to stabilize ACP, regulate mineral phase transition, and control crystallite growth and orientation (Caterina *et al.* 2002; Kwak *et al.* 2009, 2014; Wright *et al.* 2009; Pugach *et al.* 2013; Le Norcy *et al.*, 2018). Amorphous mineral phosphate may substitute with matrix fluid phosphate during the transformation of ACP to HAp prior to maturation. This process could be facilitated by matrix protein cleavage by a second proteolytic Kallikrein 4, and calcium and phosphate concentration regulation by ameloblast cells (Beniash *et al.*, 2009; De Yoreo *et al.* 2015; Lacruz *et al.*, 2017). Substantial pH fluctuations during maturation, driven by proton generation during HAp precipitation and variations in solute carrier and anion channel activity, are further avenues for repeated dissolution and reprecipitation of existing enamel mineral (Smith *et al.*, 2005; Simmer *et al.* 2009; Josephsen *et al.*, 2010; Hu *et al.* 2011; Smith *et al.* 2011; Damkier *et al.*, 2014; Kawasaki *et al.* 2014; Lacruz *et al.*, 2017). The diversity of ion channel activity during maturation suggests that the matrix abundance, and therefore the resetting of oxygen-bearing species in HAp mineral (PO_4^{3-} , CO_3^{2-} , OH^-), will differ slightly. Despite these complexities, our modeling and observations overall appear to support the finding that amorphous calcium phosphate phases, protein-mineral interactions, and pH fluctuations are a potentially important part of mammalian tooth mineralization, and may affect isotopic compositions (Beniash *et al.*, 2009; Josephsen *et al.*,

2010; Simmer *et al.*, 2010; Simmer *et al.*, 2012; Omelon *et al.*, 2013; Damkier *et al.*, 2014; Robinson 2014; De Yoreo *et al.* 2015; Lacruz *et al.*, 2017).

Our model performs strikingly well both on the experimental study of a drinking water shift in sheep and in theoretical reconstructions of real-world precipitation records. The experimental work confirms the broad patterns of mineralization observed using synchrotron density mapping. This is evidenced by the inverse model's ability to recover not only the experimental switch but also transient meteorological events using the high spatial resolution measurement of $\delta^{18}\text{O}$ values in teeth. This work will be extended to a wider range of contexts by adapting the mineralization and isotopic modeling to a range of taxa, including wild herbivores commonly found in archeological and fossil assemblages. The quantitative reconstruction of paleoseasonality is poised to become a more common feature of climatic and environmental studies.

5. Conclusions

Paleoseasonality reconstructions from teeth can play a critical role in resolving debates about the onset of tropical monsoon patterns, shifting ecological pressures, or the magnitude of temperature changes at high latitudes (Haug *et al.*, 2005; Nelson, 2005; Retallack, 2005). In human evolutionary history, climatic variability has been proposed as a driver of behavioral innovation (Potts 2013; Antón *et al.*, 2014), and seasonality may have influenced hominin foraging patterns, fallback food choice and extractive stone tool use (Isler and Van Schaik 2014; Melin *et al.*, 2014). Yet given the substantial geographic heterogeneity and ecological diversity in Africa, legitimate questions remain about the importance of seasonality in driving evolutionary change. Here, we have approached seasonality reconstruction using new sampling strategies, experimentation, a more complex mineralization model, and a Bayesian inferential approach. Employing high-resolution dicing ($n=106$ locations with single, duplicate or triplicate analyses within the buccal enamel crown), we show that enamel phosphate $\delta^{18}\text{O}$ values reflect both secretory and maturation wave geometries, and nonlinear enamel extension rates. We have demonstrated how large amplitude environmental drinking water changes (12 ‰ range)

over time are dampened in an experimental animal's blood water (8 ‰ range) and tooth enamel measurements (5 ‰ range).

By observing isotopically light values associated with our experimental switch in a wider region of enamel than predicted by synchrotron-based density measurements, we find evidence for possible resetting of a portion of phosphate oxygen after initial mineral deposition. We develop an inverse method for reconstructing $\delta^{18}\text{O}$ from abundant bovid molars, and through a simplified modeling context, show that this method can distinguish between seasonal $\delta^{18}\text{O}$ rainfall patterns globally, including at different sites in the tropics. The method demonstrates that in the context of less variable seasonality regimes, low-resolution, 1D serial sampling strategies can recover most of the original seasonal $\delta^{18}\text{O}$ signal. The method also highlights the importance of accounting for nonlinear mineralization patterns and the dampening of input signals during biomineralization when characterizing seasonality from spatial variation in tooth enamel oxygen isotope composition.

These methods hold real promise for evaluating whether increased seasonal variability is observed at sites of early hominin stone tool manufacturing (Potts 2013). These results will also aid with the extension of general circulation models to paleoclimate applications, as seasonal variation in oxygen isotopes in precipitation is a sensitive indicator of moisture sources and air mass trajectories (Sturm et al., 2010; Jones et al., 2016). Finally, we demonstrate that our inverse method can quantitatively reconstruct the drinking water history of our experimental animal, and reveals transient meteorological events that were unplanned during the experiment. This work, alongside further study of mineralization among diverse taxa, continued improvements in seasonal reconstruction methods, and ongoing efforts to describe isotope hydrology will help to resolve the complex relationship between climate, behavior and evolution.

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References

- Antón SC, Potts R, Aiello LC (2014) Evolution of early Homo: An integrated biological perspective. *Science* 345:1236828.
- Aubert M, Williams IS, Boljkovac K, Moffat I, Moncel MH, et al. (2012) In situ oxygen isotope micro-analysis of faunal material and human teeth using a SHRIMP II: a new tool for palaeo-ecology and archaeology. *J Arch Sci* 39:3184-3194.
- Balasse M, Tresset A, Ambrose SH (2006) Stable isotope evidence ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$) for winter feeding on seaweed by Neolithic sheep of Scotland. *Journal of Zoology*. 270(1):170-6.
- Balasse M, Obein G, Ughetto-Monfrin J, Mainland I (2012) Investigating seasonality, birth in herds: a reference set of sheep enamel stable oxygen isotope ratios. *Archaeom* 54:349.
- Bendrey R, Vella D, Zazzo A, Balasse M, Lepetz S (2015) Exponentially decreasing tooth growth rate in horse teeth: implications for isotopic analyses. *Archaeometry*.
- Beniash E, Metzler RA, Lam RSK, Gilbert P (2009) Transient amorphous calcium phosphate in forming enamel. *J Struc Bio* 166:133-143.
- Blumenthal S, Cerling T, Chritz K, Bromage T, Kozdon R, Valley J (2014) Stable isotope time-series in mammalian teeth: In situ $\delta^{18}\text{O}$ from the innermost enamel layer. *Geochim Cosmochim Acta* 124:223236.

- Bowen GJ, Revenaugh J (2003) Interpolating the isotopic composition of modern meteoric precipitation. *Water Resources Res* 39.
- Boyde A (1989) Enamel. In *Teeth* 309-473. Springer Berlin Heidelberg.
- Brookman TH, Ambrose SH (2012) Seasonal variation in kangaroo tooth enamel oxygen and carbon isotopes in southern Australia. *Quat Res* 78:256-265.
- Bryant, JD, Froelich PN, Showers WJ, Genna BJ (1996) A tale of two quarries: biologic and taphonomic signatures in the oxygen isotope composition of tooth enamel phosphate from modern and Miocene equids. *Palaios* 397-408.
- Caterina JJ, Skobe Z, Shi J, Ding Y, Simmer JP, Birkedal-Hansen H, Bartlett JD (2002) Enamelysin (matrix metalloproteinase 20)-deficient mice display an amelogenesis imperfecta phenotype. *J Biol Chem*. 277(51):49598-604.
- Cerling TE, Sharp ZD (1996) Stable carbon and oxygen isotope analysis of fossil tooth enamel using laser ablation." *Palaeogeo, Palaeoclim, Palaeoecology* 126:173-186.
- Chang SJ, Blake RE (2015) Precise calibration of equilibrium oxygen isotope fractionations between dissolved phosphate and water from 3 to 37° C. *Geochimica et Cosmochimica Acta*. 150:314-29.
- Colman, AS (2002) The oxygen isotope composition of dissolved inorganic phosphate and the marine phosphorus cycle. PhD Dissertation, Yale University.
- Damkier HH, Josephsen K, Takano Y, Zahn D, Fejerskov O, Frische S (2014) Fluctuations in surface pH of maturing rat incisor enamel are a result of cycles of H(+)-secretion by ameloblasts and variations in enamel buffer characteristics. *Bone* 60:227-34.

- Daux V, Lécuyer C, Hérán MA, Amiot R, Simon L, et al. (2008) Oxygen isotope fractionation between human phosphate and water revisited. *J Hum Ev* 55:1138-1147.
- Dawson (1978) Osmotic and volume regulation during dehydration in desert kangaroos. *Proceedings of the Alfred Benzon Symposium XI held at the premises of the Royal Danish Academy of Sciences and Letters, Copenhagen 5-9 June 1977.*
- De Yoreo JJ, Gilbert PUPA, Sommerdijk NAJM, Lee Penn R, et al. (2015) Crystallization by particle attachment in synthetic, biogenic, geologic environments. *Science* 349.
- Diekwisch TG (1998) Subunit compartments of secretory stage enamel matrix. *Connect Tissue Res* 38:101-11.
- Driessens F, Verbeeck RK (1990) *Biomaterials*. CRC Press.
- Fricke H, O'Neil J (1996) Inter-and intra-tooth variation in the oxygen isotope composition of mammalian tooth enamel phosphate: implications for palaeoclimatological and palaeobiological research. *Palaeogeogr, Palaeoclim, Palaeoecology* 126:91-100.
- Fricke HC, Clyde WC, O'Neil JR (1998) Intra-tooth variations in $\delta^{18}\text{O}$ (PO₄) of mammalian tooth enamel as a record of seasonal variations in continental climate variables. *Geochimica et Cosmochimica Acta*. 62(11):1839-50.
- Garcia RA, Cabeza M, Rahbek C, Araújo MB (2014) Multiple dimensions of climate change and their implications for biodiversity. *Science* 344:1247579.
- Gat J (1996) Oxygen and hydrogen isotopes in the hydrologic cycle. *Ann. Rev. Earth Plan. Sci.* 24:225262.
- Gehler A, Tütken T, Pack A (2011) Triple oxygen isotope analysis of bioapatite as tracer for diagenetic alteration of bones and teeth. *Palaeogeogr, Palaeoclim, Palaeoecology* 310:84-91.

- Gomes daSilva R, Minomo FR (1995) Circadian and seasonal variation of the body temperature of sheep in a tropical environment. *International journal of biometeorology*. 39(2):69-73.
- Good SP, Caylor KK (2011) Climatological determinants of woody cover in Africa. *Proc Natl Acad Sci USA* 108:4902-4907.
- Goodwin SD (1998) Comparison of body temperatures of goats, horses, and sheep measured with a tympanic infrared thermometer, an implantable microchip transponder, and a rectal thermometer. *Journal of the American Association for Laboratory Animal Science* 37:51-55.
- Green DR, Green GM, Colman AS, Bidlack FB, Tafforeau P, Smith TM (2017) Synchrotron imaging and Markov Chain Monte Carlo reveal tooth mineralization patterns. *PLOS ONE* <https://doi.org/10.1371/journal.pone.0186391>.
- Green DR, Olack G, Colman AS (2018) Determinants of blood water $\delta^{18}\text{O}$ variation in a population of experimental sheep: Implications for paleoclimate reconstruction. *Chemical Geology*. 485:32-43.
- Gretebeck RJ, Schoeller DE, Socki RA, Davis-Street J, Gibson EK, Schulz LO, Lane HW (1997) Adaptation of the doubly labeled water method for subjects consuming isotopically enriched water. *Journal of Applied Physiology* 82(2), 563-570.
- Harvey FE, Welker JM (2000) Stable isotopic composition of precipitation in the semi-arid north-central portion of the US Great Plains. *Journal of Hydrology* 238:90-109.
- Haug GH, Ganopolski A, Sigman DM, Rosell-Mele A (2005) North Pacific seasonality and the glaciation of North America 2.7 million years ago. *Nature*. 433(7028):821.

- Hoetzel S, Dupont L, Schefuß E, Rommerskirchen F, Wefer G (2013) The role of fire in Miocene to Pliocene C4 grassland and ecosystem evolution. *Nat Geosci* 6:1027-1030.
- Hoppe KA, Stover SM, Pascoe JR, Amundson R (2004) Tooth biomineralization in horses: implications for isotopic microsampling. *Palaeogeogr, Palaeoclim, Palaeoecology* 206:355–365.
- Hsiang SM, Burke M, Miguel E (2013) Quantifying the influence of climate on human conflict. *Science* 341:1235367.
- Hu Y, Hu JC, Smith CE, Bartlett JD, Simmer JP (2011) Kallikrein-related peptidase 4, matrix metalloproteinase 20, and the maturation of murine and porcine enamel. *Eur J Oral Sci.* 119 Suppl 1:217-25. PMID: PMC3281808.
- IAEA/WMO (2017). Global Network of Isotopes in Precipitation. The GNIP Database. Accessible at: <http://www.iaea.org/water>.
- Isler K, Van Schaik CP (2014) How Humans Evolved Large Brains: Comparative Evidence. *Ev Anthr* 23:65-75.
- Jones GB, Potter BJ, Reid CS (1970) The effect of saline water ingestion on water turnover rates and tritiated water space in sheep. *Australian Journal of Agricultural Research*, 21(6):927-32.
- Jones MD, Dee S, Anderson L, Baker A, Bowen G, Noone DC (2016) Water isotope systematics: Improving our palaeoclimate interpretations. *Quaternary Science Reviews*, 131(B), pp.243-249
- Jordana X, Köhler M (2011) Enamel microstructure in the fossil bovid *Myotragus balearicus* (Majorca, Spain): implications for life-history evolution of dwarf mammals in insular ecosystems. *Palaeogeogr, Palaeoclim, Palaeoecology*, 300:59-66.

- Josephsen K, Takano Y, Frische S, Praetorius J, Nielsen S, et al. (2010) Ion transporters in secretory and cyclically modulating ameloblasts: a new hypothesis for cellular control of preeruptive enamel maturation. *Am J Physiol Cell Physiol.* 299:C1299-307.
- Kawasaki K, Hu JC, Simmer JP (2014) Evolution of *Klk4* and enamel maturation in eutherians. *Biol Chem.* 395(9):1003-13. PMID: PMC4252046
- Kierdorf H, Kierdorf U, Frölich K, Witzel C (2013) Lines of Evidence-Incremental Markings in Molar Enamel of Soay Sheep Revealed by Fluorochrome Labeling, Backscattered Electron Imaging Study. *PLoS ONE* 8.9:e74597.
- Kirsanow K, Makarewicz C, Tuross N (2008) Stable oxygen ($\delta^{18}\text{O}$) and hydrogen (δD) isotopes in ovicaprid dentinal collagen record seasonal variation. *J Arch Sci* 35:3159-3167.
- Kirsanow K, Tuross N (2011) Oxygen and hydrogen isotopes in rodent tissues: Impact of diet, water and ontogeny. *Palaeogeogr, Palaeoclim, Palaeoecology* 310:9-16.
- Kohn M (2004) Comment: Tooth Enamel Mineralization in Ungulates: Implications for Recovering a Primary Isotopic Time-Series, by B. H. Passey and T. E. Cerling (2002). *Geochim Cosmochim Acta* 68:403405.
- Kohn M, Cerling T (2002) Stable Isotope Compositions of Biological Apatite. *Rev Min Geochem* 48:455-488.
- Kohn MJ (1996) Predicting animal $\delta^{18}\text{O}$: accounting for diet and physiological adaptation. *Geochimica et Cosmochimica Acta.* 60(23):4811-29.

- Kohn MJ, Schoeninger MJ, Valley JW (1998) Variability in oxygen isotope compositions of herbivore teeth: reflections of seasonality or developmental physiology? *Chem Geo* 152:97.
- Kucherenko S, Sytsko Y (2005) Application of deterministic low-discrepancy sequences in global optimization. *Computational Optimization and Applications*. 30(3):297-318.
- Kumar P (2013) Hydrology: Seasonal rain changes. *Nature Climate Change*, 3(9):783-784.
- Kwak SY, Wiedemann-Bidlack FB, Beniash E, Yamakoshi Y, Simmer JP, Litman A, Margolis HC (2009) Role of 20-kDa amelogenin (P148) phosphorylation in calcium phosphate formation in vitro. *J Biol Chem*. 284(28):18972-9. PMID: PMC2707234.
- Kwak SY, Kim S, Yamakoshi Y, Simmer JP, Beniash E, Margolis HC (2014) Regulation of calcium phosphate formation by native amelogenins in vitro. *Connect Tissue Res*. 55 Suppl 1:21-4. PMID: PMCPMC4145609.
- Lacruz RS, Habelitz S, Wright JT, Paine ML (2017) Dental Enamel Formation and Implications for Oral Health and Disease. *Physiological Reviews*. 2017 Jul 1;97(3):939-93.
- Lécuyer C, Amiot R, Touzeau A, Trotter J (2013) Calibration of the phosphate $\delta^{18}\text{O}$ thermometer with carbonate–water oxygen isotope fractionation equations. *Chemical Geology*. 347:217-26.
- Levin NE, Zipser EJ, Cerling TE (2009) Isotopic composition of waters from Ethiopia and Kenya: insights into moisture sources for eastern Africa. *Journal of Geophysical Research: Atmospheres*. 114(D23).
- Longinelli A 1984. Oxygen isotopes in mammal bone phosphate: a new tool for paleohydrological and paleoclimatological research? *Geochim Cosmochim Acta* 48:385-390.

- Longinelli A, Nuti S (1973) Revised phosphate-water isotopic temperature scale. *Earth and Planetary Science Letters*. 19(3):373-6.
- Longinelli A, Padalino AP (1980) Oxygen isotopic composition of water from mammal blood: first results. *Eur J Mass Spectrom Biochem Med Env Res* 1:135-139.
- Macfarlane WV, Howard B, Haines H, Kennedy PJ, Sharpe CM (1971) Hierarchy of water and energy turnover of desert mammals. *Nature*. 234(5330):483.
- Marshall AJ, Wrangham RW (2007) Evolutionary consequences of fallback foods. *Int J Prim* 28:1219-1235.
- Mayer AL, Khalyani AH (2011) Grass trumps trees with fire. *Science*, 334:188-189.
- Melin AD, Young HC, Mosdossy KN, Fedigan LM (2014) Seasonality, extractive foraging and the evolution of primate sensorimotor intelligence. *J Hum Ev* 71:77-86.
- Moerman JW, Cobb KM, Adkins JF, Sodemann H, Clark B, Tuen AA. (2013) Diurnal to interannual rainfall $\delta^{18}\text{O}$ variations in northern Borneo driven by regional hydrology. *Earth and Planetary Science Letters* 369:108-119.
- Morin CW, Comrie AC (2010) Modeled response of West Nile virus vector *C. quinquefasciatus* to climate using dynamic mosquito simulation. *Int J Biometeor*. 54:517-529.
- Nelson SV (2005) Paleoseasonality inferred from equid teeth and intra-tooth isotopic variability. *Palaeogeography, Palaeoclimatology, Palaeoecology*. 222(1):122-44.

- Nicol SC (1978) Rates of Water Turnover in Marsupials and Eutherians: a Comparative Review With New Data on the Tasmanian Devil. *Australian Journal of Zoology*, 26(3):465-73.
- Nyquist H (1928) Certain topics in telegraph transmission theory. *Transactions of the American Institute of Electrical Engineers*. 47(2):617-44.
- O'Grady SP, Wende AR, Remien CH, Valenzuela LO, Enright LE, Chesson LA, Abel ED, Cerling TE, Ehleringer JR (2010) Aberrant water homeostasis detected by stable isotope analysis. *5(7):e11699*.
- Omelson S, Ariganello M, Bonucci E, Grynpas M, Nanci A (2013) A review of phosphate mineral nucleation in biology and geobiology. *Calcified tissue international*. 93(4):382-96.
- Passey B, Cerling T, Schuster G, Robinson T, Roeder B, Krueger S (2005) Inverse methods for estimating primary input signals from time-averaged isotope profiles. *Geochim Cosmochim Acta* 69:4101-4116.
- Passey BH, Cerling TE (2002) Tooth enamel mineralization in ungulates: implications for recovering a primary isotopic time-series. *Geochim Cosmochim Acta* 66:3225-3234.
- Passey BH, Cerling TE (2006) In situ stable isotope analysis ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$) of very small teeth using laser ablation GC/IRMS. *Chem Geo*, 235:238-249.
- Pellegrini M, Lee-Thorp JA, Donahue, RE (2011) Exploring the variation of the $\delta^{18}\text{O}_\text{p}$ and $\delta^{18}\text{O}_\text{c}$ relationship in enamel increments. *Palaeogeogr, Palaeoclim, Palaeoecology* 310:71-83.
- Podlesak DW, Torregrossa AM, Ehleringer JR, Dearing MD, Passey BH, Cerling TE (2008) Turnover of oxygen and hydrogen isotopes in the body water, CO_2 , hair, and enamel of a small mammal. *Geochim Cosmochim Acta*, 72:19-35.

Potts R (2013) Hominin evolution in settings of strong environmental variability. *Quat Science Rev* 73:1-13.

Powell, M. J. D. (1998) "Direct search algorithms for optimization calculations." *Acta numerica* 287-336.

Pucat E, Joachimski MM, Bouilloux A, Monna F, Bonin A, Motreuil S, Morinire P, Hnard S, Mourin J, Dera G, Quesne D (2010) Revised phosphate–water fractionation equation reassessing paleotemperatures derived from biogenic apatite. *Earth and Planetary Science Letters*. 298(1):135-42.

Pugach MK, Suggs C, Li Y, Wright JT, Kulkarni AB, Bartlett JD, Gibson CW (2013) M180 amelogenin processed by MMP20 is sufficient for decussating murine enamel. *J Dent Res*. 92(12):1118-22. PMID: PMC3834654.

Qi HP, Grning M, Coplen TB, Buck B, Mroczkowski SJ, Brand WA, Geilmann H, Gehre M (2010) Novel silver-tubing method for quantitative introduction of water into high-temperature conversion systems for stable hydrogen and oxygen isotopic measurements, *Rapid Communications in Mass Spectrometry*, v. 24, no. 13, p. 1821–1827.

Quinn RL (2015) Influence of Plio-Pleistocene basin hydrology on the Turkana hominin enamel carbonate $\delta^{18}\text{O}$ values. *Journal of human evolution*. 86:13-31.

Refinetti R, Menaker M (1992) The circadian rhythm of body temperature. *Physiology & behavior* 51:613-637.

Retallack GJ (2005) Pedogenic carbonate proxies for amount and seasonality of precipitation in paleosols. *Geology*. 33(4):333-6.

- Rinnooy Kan AH, Timmer GT (1987) Stochastic global optimization methods Part II: multi level methods. *Mathematical Programming*. 39(1):57-78.
- Robinson C (2014) Enamel maturation: background, implications for enamel dysplasias. *Front Physiol* 5.
- Sellen DW (2000) Age, sex and anthropometric status of children in an African pastoral community. *Ann Hum Bio* 27:345-365.
- Simmer J, Papagerakis P, Smith C, Fisher D, Rountrey A, et al. (2010) Regulation of dental enamel shape and hardness. *J Dent Res* 89:1024–38.
- Simmer JP, Hu Y, Lertlam R, Yamakoshi Y, Hu JC (2009) Hypomaturation enamel defects in *Klk4* knockout/*LacZ* knockin mice. *J Biol Chem*. 284(28):19110-21. PMCID: PMC2707199.
- Simmer J, Richardson A, Hu Y-Y, Smith C, Hu J (2012) A post-classical theory of enamel biomineralization... and why we need one. *Int J Oral Sci* 4:129–134.
- Smith CE (1998) Cellular and Chemical Events During Enamel Maturation. *Crit Rev Oral Bio Med* 9:128-161.
- Smith CE, Chong DL, Bartlett JD, Margolis HC (2005) Mineral acquisition rates in developing enamel on maxillary and mandibular incisors of rats and mice: implications to extracellular acid loading as apatite crystals mature. *J Bone Miner Res*. 20(2):240-9.
- Smith CE, Hu Y, Richardson AS, Bartlett JD, Hu JC, Simmer JP (2011) Relationships between protein and mineral during enamel development in normal and genetically altered mice. *European journal of oral sciences*. 119(s1):125-35.
- Smith T, Tafforeau P (2008) New visions of dental tissue research: Tooth development, chemistry, and structure. *Ev Anthro* 17:213–226.

- Smith TM (2006) Experimental determination of the periodicity of incremental features in enamel. *J Anat* 208: 99-113.
- Sponheimer M, Passey B H, de Ruiter D J, Guatelli-Steinberg D, et al. (2006) Isotopic Evidence for Dietary Variability in the Early Hominin *P. robustus*. *Science* 314:980-982.
- Steven G. Johnson (2017) The NLOpt nonlinear-optimization package, <http://ab-initio.mit.edu/nlopt>
- Stevens R, Balasse M, O'Connell T (2011) Intra-tooth oxygen isotope variation in a known population of red deer: Implications for past climate and seasonality reconstructions. *Palaeogeogr, Palaeoclim, Palaeoecology* 301:64-74.
- Sturm C, Zhang Q, Noone D (2010) An introduction to stable water isotopes in climate models: benefits of forward proxy modelling for paleoclimatology. *Climate of the Past* 6, no. 1: 115-129. <http://www.clim-past.net/6/115/2010/cp-6-115-2010.pdf>
- Suga S 1982. Progressive mineralization pattern of developing enamel during the maturation stage. *J Dent Res* 1532-1542.
- Tafforeau P, Bentaleb I, Jaeger JJ, Martin C (2007) Nature of laminations and mineralization in rhinoceros enamel using histology and X-ray synchrotron microtomography. *Palaeogeogr, Palaeoclim, Palaeoecology* 246:206-227.
- Tindall JC, Haywood AM (2015) Modeling oxygen isotopes in the Pliocene: Large - scale features over the land and ocean. *Paleoceanography*. 30(9):1183-201.

- Trayler RB, Kohn MJ (2017). Tooth enamel maturation reequilibrates oxygen isotope compositions and supports simple sampling methods. *Geochimica et Cosmochimica Acta* 198:32-47.
- Vennemann TW, Fricke HC, Blake RE, O'Neil JR, Colman A (2002) Oxygen isotope analysis of phosphates: a comparison of techniques for analysis of Ag_3PO_4 . *Chem Geol* 185:321-336.
- Vincens A, Garcin Y, Buchet G (2007) Influence of rainfall seasonality on African vegetation in the Late Quaternary: pollen evidence, Lake Masoko, Tanzania. *J Biogeogr* 34:1274-1288.
- Watson JE, Iwamura T, Butt N (2013) Mapping vulnerability and conservation adaptation strategies under climate change. *Nat Clim Change* 3:989-994.
- Wiedemann-Bidlack FB, Colman AS, Fogel ML (2008) Phosphate oxygen isotope analysis on microsamples of bioapatite: removal of organic contamination and minimization of sample size. *Rapid Comm Mass Spectrom* 22:1807-1816.
- Wright JT, Hart TC, Hart PS, Simmons D, Suggs C, Daley B, Simmer J, Hu J, Bartlett JD, Li Y, Yuan ZA, Seow WK, Gibson CW (2009) Human and mouse enamel phenotypes resulting from mutation or altered expression of AMEL, ENAM, MMP20 and KLK4. *Cells Tissues Organs*. 189(1-4):224-9. PMID: PMC2754863.
- Wulf H, Bookhagen B, Scherler D (2010) Seasonal precipitation gradients and their impact on fluvial sediment flux in the Northwest Himalaya. *Geomorphology*. 118(1):13-21.
- Zazzo A, Balasse M, Passey B, Moloney A, Monahan F, Schmidt O (2010) The isotope record of short- and long-term dietary changes in sheep tooth enamel: Implications for quantitative reconstruction of paleodiets. *Geochim Cosmochim Acta* 74:3571-3586.

Zazzo A, Bendrey R, Vella D, Moloney A, Monahan F, Schmidt O (2012) Refined sampling strategy for intra-tooth stable isotope analysis of enamel. *Geochim Cosmochim Acta* 84:113.

Figure Legends

Figure 1 Models of tooth growth. (A) Growing herbivore molar sectioned longitudinally across lingual and buccal (colored) loph. Mineralization initiates at the dentin horns (left) and proceeds towards the cervix crown completion. Above, solid lines represent formed enamel and dentin increments added during secretion, while dotted lines depict future incremental addition. Colors indicate the progress of maturation extent from low (green) to high (red), as measured by synchrotron density mapping. (B-C) Expected intensity of mineral density at a single, near-instantaneous moment of enamel mineralization. No addition is shown in dark blue, and increasing addition shown in warmer colors. (B) Wavelike pattern of maturation after secretion inferred from radiographs (Suga 1982). (C) Steady, continuous pattern of maturation immediately following secretion, proposed for ever-growing teeth (Passey *et al.*, 2002). (D) Diffuse maturation wave following secretion after a pause, proposed by synchrotron imaging of sheep molars (Green *et al.*, 2017). (E) For a single location in enamel (white star in tooth schematics B-D), the increase in mineral density over time predicted by each model is shown.

Figure 2 Schematic of general approach to the reconstruction of drinking water history from tooth $\delta^{18}\text{O}$ values. (A) Hypothetical $\delta^{18}\text{O}$ rainfall pattern over time (black dashed line) reflecting environmental seasonal hydrology (landscape, gray). (B) Samples (white circles) drawn from the tooth of an animal (black schematic) that lived on that landscape. (C) Resultant sequential $\delta^{18}\text{O}$ values (black dots), whose amplitude and spatial pattern correspond poorly to original rainfall. (D) During reconstructions, hypothetical rainfall histories are proposed (light blue lines) and integrated with mineralization to produce modeled tooth $\delta^{18}\text{O}$ values. The probability p of each hypothetical water history δ^w is calculated given real tooth $\delta^{18}\text{O}$ values δ^d and expectations e . (E) Ultimately, a best fit

rainfall history (dark blue line) is estimated and can be used to make inferences about past seasonal landscape hydrology (landscape, color).

Figure 3 Experiment architecture. (A) Experimental set-up and blood $\delta^{18}\text{O}$ data for the sheep water switch experiment, which holds feed (green line) and inspired air (light blue line) $\delta^{18}\text{O}$ constant, while switching measured drinking water (blue stars) and therefore measured blood (red stars) to an isotopically lighter source from 201-263 days. (B) Estimated and measured first (M1) and second (M2) molar mineralization initiation and completion times are shown below, with dashed lines representing uncertainty in the timing. The experimental water switch (blue, “switch”) was planned to occur during the middle of M2 formation.

Figure 4 Predicted and observed tooth $\delta^{18}\text{O}$ values. Validation of mineralization model using (A) the buccal enamel section diced into 1.50 x 1.00 x 0.15 mm blocks. Both the diced samples and (B) the adjacent histological section are compressed along the cuspal-cervical axis for ease of visualization in this graph. Calcein labels (bright green lines in the histological section) were given to the sheep at 201, 221, 263 and 283 days of age from left to right. (C) Experimental tooth data shows phosphate $\delta^{18}\text{O}$ values measured from the diced tooth blocks. White dotted lines indicate the positions of calcein labels and the extent of extension at the start (201 days) and end (263 days) of the experimental water switch. Black squares show where multiple blocks were combined for a single measurement due to sample loss. Data $\delta^{18}\text{O}$ scale bar is provided at the bottom of (F), and raw values are available in the SI. (D) The mineralization model used to predict tooth spatial $\delta^{18}\text{O}$ values predicts high mineral addition (orange) corresponding to secretion (thin band, right) and maturation (diffuse wave, left) at any moment in time. This prediction, based on synchrotron density estimates from an ontogenetic series of sheep teeth, influences the expected tooth $\delta^{18}\text{O}$ observations resulting from the drinking water switch in our experimental animal. (E) Modeled data represents the predicted tooth $\delta^{18}\text{O}$ values from the measured blood $\delta^{18}\text{O}$ compositions integrated into the mineralization model. (F) A second model produces expected tooth $\delta^{18}\text{O}$ values resulting from the experimental switch in the event of phosphate oxygen exchange during mineralization.

Figure 5 Reconstruction fidelity and signal complexity. Inverse method reconstructions of sine wave rainfall functions at different frequencies and amplitudes, with variable sampling rates. Optimum (blue) and a sample of best fit (light blue) inverse reconstructions of sinusoidal input drinking water $\delta^{18}\text{O}$ histories (black dashed line) with 180 and 45, and 360 and 90 day periods. Reconstructed inputs are compared to conventional serial samples measured from enamel at regular increments from the cusp tip (black circles), converted to water input values using known feed and air inputs; column values are averaged in the case of 2D sampling. Best fits are achieved using nonlinear extension for all panels except the bottom right 180 and 45-day-period plot.

Figure 6 Reconstruction fidelity and sample resolution. Reconstructing a Bornean $\delta^{18}\text{O}$ record at high and low sampling resolution. (A) Forward tooth $\delta^{18}\text{O}$ values resulting from monthly rainfall records from Mulu, Borneo (Moerman *et al.*, 2013) shown at 2D, 1D high resolution, medium and low sample resolutions. These values correspond to sampling strategies choosing to take more or fewer samples from a tooth of interest. (B) The monthly rainfall records from Mulu (dashed black line) used generate forward tooth $\delta^{18}\text{O}$ values in (A). Reconstructions of rainfall from tooth values can produce a seasonal rainfall $\delta^{18}\text{O}$ best fit estimate (blue line) and a sample of most likely trials (light blue lines). In this case, the seasonal rainfall $\delta^{18}\text{O}$ reconstructed using 2D tooth sampling better recovers the original input than sampling at lower resolution.

Figure 7 Modeling seasonality globally. Inverse method reconstructions of drinking water $\delta^{18}\text{O}$ history (black, dashed) using 2D high-resolution (left) or 1D low-resolution (right) forward model $\delta^{18}\text{O}$ values derived from precipitation $\delta^{18}\text{O}$ records in North Platte (United States) (Harvey and Welker 2000), Entebbe (Central Uganda) (IAEA 2017) and Mulu (Borneo) (Moerman *et al.*, 2013). The reconstructed optimum drinking water inputs (blue) and a sample of the most likely 30% of discovered solutions (light blue) are estimated at 14-day intervals. The 2D and 1D tooth $\delta^{18}\text{O}$ data used for reconstructions are shown at the bottom of each panel. Drinking water estimation from conventional serial

sampling using finely spaced (1.5mm, left) or coarsely spaced (5mm, right) enamel samples yields the precipitation history represented by black dots.

Figure 8 Experimental validation of reconstruction. Inverse method reconstructions of drinking water $\delta^{18}\text{O}$ for sheep 962 subject to a water switch, using experimental switch (top) and naturalistic (bottom) models. Optimum drinking water predictions (blue), most likely 30% of associated solutions (light blue), and optimum blood (red) predictions are shown alongside measurements of drinking water, snowfall and blood (blue, light blue and red stars). Tooth $\delta^{18}\text{O}$ measurements as they would have been sampled conventionally are shown as black dots. The dots represent column averages, offset by -19.1 permil to shift the measured phosphate values to equivalent equilibrium blood water values (spatial sampling scale above in mm). The experimental solution employs dampening priors $d_{rate} = 1/5, 1/3$ and $1/2$, while the naturalistic solution employs priors $d_{rate} = 1/5, 1/3$ and $3/4$.

A. Mineralization overview

B. Mineral addition: waves

C. Mineral addition: ever-growing

D. Mineral addition: synchrotron-based

E. Mineralization history, one location

B. Sampled tooth

C. Sequential samples

D. Reconstruction via probability

$$p(\delta^w | \delta^d, e) =$$

E. Best fit

A. Experimental water switch

B. Sheep molar initiation and completion times

A. Diced tooth

B. Histological section

C. Experimental tooth $\delta^{18}\text{O}$ data

D. Synchrotron-based instantaneous mineral addition

E. Modeled $\delta^{18}\text{O}$

F. Modeled $\delta^{18}\text{O}$ - PO_4 resetting

A. Tooth $\delta^{18}\text{O}$ values at differing resolutions

B. Input water $\delta^{18}\text{O}$ reconstructions at differing resolutions