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NOthing goes to WAste (NOWA): A protocol to optimise sampling of ancient teeth

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A R T I C L E I N F O

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A B S T R A C T

Advancements in archaeological sciences through innovative scientific techniques applied to ancient human remains have increasingly been transforming the study of the past. Destructive analyses of bioarchaeological or palaeontological specimens such as dental histology, isotopic or elemental analysis of dental mineralised tissues, ^{14}C dating, proteomic analysis or ancient DNA are increasingly being applied to obtain ever more refined past life histories. In tandem with spatially-resolved analyses, mineralised dental tissues, especially enamel, have proven to be ideal archives. The main concern with any of these techniques is the need for some level of destructive sampling and thus damage to specimens. In compliance with the ALARA (As Low [damage] As Reasonably Achievable) principle, we present a standardised protocol (the NOWA protocol) for the optimisation, in terms of minimising the destructive approach, of tooth sampling for histology, biomolecular and biogeochemical analyses. The NOWA protocol enables the collection of multiple datasets through optimised sampling of a single dental specimen, ensuring the preservation of most of the tooth and, to a substantial degree, its morphology. Overall, this approach balances the significant scientific insights from such investigations against the inevitable conservation requirements of valuable archaeological and palaeontological specimens, thus maximising the feasibility of future analyses of individual specimens that need to be evaluated on a case-to-case basis.

1. Introduction

The growing application of cutting-edge techniques in archaeological sciences has increasingly transformed the study of past human populations, offering unrivalled interpretative potential. Advanced analyses of human archaeological and palaeontological specimens are today ever-present in archaeological studies, as evidenced in a plethora of scientific publications and projects, providing detailed reconstructions of individual life histories (e.g., Dupras et al., 2001; Müller et al., 2003; Porr and Alt, 2006; Dupras and Tocheri, 2007; Abbazzi et al., 2008; Melton et al., 2013; Frei et al., 2015; Lugli et al., 2017; Emery et al., 2018; Lugli et al., 2019a; Lugli et al., 2019b; Craig-Atkins et al., 2020; Francisci et al., 2020; Lugli et al., 2020; Nava et al., 2020; Gigante et al., 2021; Richards et al., 2021; Lugli et al., 2022; Esposito et al., 2023; Müller et al., 2024; Nava et al., 2024).

Mineralised dental tissues, and especially enamel, are optimal for opening a window on individual growth trajectories, as well as dietary, mobility, ancestry, and exposome histories with remarkable temporal

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accuracy (e.g., Montgomery, 2010; Dean, 2012; Beaumont et al., 2014; Humphrey, 2014; Beaumont and Montgomery, 2015; Smith et al., 2018; Nava et al., 2020; Smith et al., 2021; Lugli et al., 2022). Furthermore, tooth enamel is considered largely resistant to diagenetic alterations (i.e., post-burial elemental uptake Kohn et al., 1999; Chiaradia et al., 2003; Hoppe et al., 2003; Bentley, 2006; Montgomery, 2010) over both archaeological (e.g., Budd et al., 2000; Nielsen-Marsh and Hedges, 2000; Price et al., 2002; Trickett et al., 2003; Montgomery, 2010; Montgomery et al., 2010; Madgwick et al., 2012) and geological timescales (e.g., Bocherens et al., 1994; Horn et al., 1994; Michel et al., 1996), although complete resistance to diagenesis cannot be unconditionally assured (Nava et al., 2020; Kubat et al., 2023; Kowalik et al., 2023; Higgins et al., 2024).

Despite the undeniable achievements of these techniques, there is a concern about the unavoidable destructiveness of sampling. Techniques such as dental histology, stable and radiogenic isotope analyses — both high-resolution and bulk approaches — serial sampling of dentine, trace element (TE) analysis, radiocarbon dating (^{14}C), high-resolution oxygen isotopes mapping using Secondary Ion Mass Spectrometry (SIMS), proteomics, and ancient DNA (aDNA) analyses are all based on the destructive sampling of specimens, albeit in increasingly smaller quantities with ever less invasive approaches (e.g., Czermak et al., 2020; Harney et al., 2021).

The involvement of valuable ancient specimens, including hominins other than *Homo sapiens*, often leads to reluctance from museums to grant permissions for destructive sampling due to concerns over specimen integrity (Hublin et al., 2008) with notable exceptions (Müller et al., 2003; Austin et al., 2013; Joannes-Boyau et al., 2019; Nava et al., 2020; Kubat et al., 2023). Moreover, awareness regarding the ethical importance of specimen preservation when performing destructive analyses (e.g., Makarewicz et al., 2017; Prendergast and Sawchuk, 2018; Pálsdóttir et al., 2019; Sirak and Sedig, 2019; Squires et al., 2019; Gibbon, 2020; Wagner et al., 2020; Alpaslan-Roodenberg et al., 2021; Roberts, 2022; Squires et al., 2022; Stantis et al., 2024) is constantly increasing given the limited nature of archaeological material (Pálsdóttir et al., 2019; Stantis et al., 2024) and the growing necessity of preserving samples for future analyses with progressively sophisticated techniques.

The entire research process – including research design, data management, storage and publication – raises specific ethical considerations that must be carefully addressed, as highlighted in Stantis et al. (2024). Planning a research project involves careful selection and continual refinement of methodologies, which may vary on a case-by-case basis. It is essential to use the smallest sample necessary to answer specific research questions (Stantis et al., 2024), balancing sample availability within a specific context with the need to address precise research questions. However, without the destructive analyses developed and applied in the past decades, our understanding of the past would not be as refined. As a non-destructive alternative, especially for dental histology, virtual histology using synchrotron light microtomography is available (Tafforeau and Smith, 2008; Smith et al., 2010; Tafforeau et al., 2012; Le Cabec et al., 2015; Nava et al., 2017b, 2022; Hodgkins et al., 2021; Mahoney et al., 2021; Cerrito et al., 2022) but see Immel et al. (2016) for an evaluation of damage to organic material caused by high energy synchrotron-based X-ray beams.

We propose here a standardised protocol (NOthing goes to WAste protocol: NOWA) for the optimisation, in terms of minimising the destructive approach, of tooth sampling, which adopts a conservative approach. NOWA aims to preserve tooth morphology while allowing sampling for histological, biomolecular and biogeochemical analyses and opening the possibility to other analytical pathways. Recognising the diverse requirements and methodologies inherent in this research field, where specific samples are selectively procured to address research objectives in a conservative manner, the NOWA protocol provides a strategy to optimise data acquisition while minimising specimen destruction. It outlines a detailed methodology and sequence of sampling, acknowledging the current absence of a universally recommended protocol. This approach does not prescribe a uniform method of sampling; instead, it allows for the adaptation of the methodology to suit specific research objectives. Moreover, the NOWA protocol enables researchers to extract multifaceted information from a single dental specimen, encouraging a multidisciplinary approach which is increasingly favoured in bioarchaeological studies.

2. Methods: protocol presentation

The NOWA protocol consists of nine steps (a-i) which are described below and graphically represented in Fig. 1.

2.1. Selection and documentation of the specimen (Fig. 1a)

Samples should be selected taking into consideration the state of preservation and the stage of development of the teeth according to specific research questions. Additionally, potential curatorial requests and restrictions must be factored into the selection and sampling planning processes.

Prior to destructive sampling, detailed documentation of the specimen is recommended following standard procedures (see Buikstra and Ubelaker, 1994; Hillson, 2024 for comprehensive reviews). Recorded data should include: 1) presence and identification of oral pathologies, enamel developmental defects, masticatory tooth wear, para- and extra-masticatory modifications determined through macro- and microscopic inspection; 2) estimated age-at-death of the individual; 3) dental metric and non-metric traits. Moreover, the specimen is documented (micro)photographically (White et al., 2012; Parsley et al., 2018; Ceccarelli, 2023), recording the labial/buccal, lingual/palatal, occlusal, distal, and mesial views (Fig. 2A). Complementary to the basic photographic records, additional documentation techniques such as Structure-from-Motion photogrammetry (SfM) (Luhmann et al., 2006; Micheletti et al., 2015; Silvester and Hillson, 2020; Scaggion et al., 2022; Lauria et al., 2022), micro-CT acquisition (Marchewka et al., 2014; Higgins et al., 2020; Scaggion et al., 2022), or 3D surface scanning (Fiorenza et al., 2009; Kuzminsky and Gardiner, 2012) are highly recommended. However, the decision to employ these methods should consider a balance between cost and benefit.

2.2. Pre-embedding sampling

Calculus sampling (Fig. 1b). If present, dental calculus can be sampled at this stage following the procedure described by Sabin and Fellows Yates (2020) and Nava et al. (2021). Dental calculus derives from the mineralization of the dental plaque, trapping biomolecules from the oral biome, traces of foodstuff, and inclusions originating from the interaction with the environment (Hardy et al., 2009; Cristiani et al., 2016; Radini et al., 2017; Nava et al., 2021; Ottoni et al., 2021; Quagliariello et al., 2022; Radini and Nikita, 2023). Besides information on dietary practices, information on daily life activities or environmental conditions can be retrieved from the analysis of the mineralised plaque.

Further pre-embedding sampling: Should aDNA and/or ^{14}C analyses be required, the tooth can be sampled prior to specimen embedding (Fig. 1d). The lower third of a root (preferably from a multi-rooted tooth) should be cut using a flexible diamond-edged rotary-mounted blade. As, to our knowledge, aDNA and ^{14}C analyses on embedded teeth have not been previously attempted, it is cautiously suggested to perform these samplings at this stage. For radiocarbon dating, a sample size of around 40–200 mg is typically employed (Gurfinkel, 1987; Beaumont et al., 2010; Santos et al., 2007; Fewlass et al., 2019) with studies using increasingly smaller samples (3–60 mg) (Cersoy et al., 2017), depending on collagen availability. For aDNA analysis ~100–300 mg is generally recommended (Gamba et al., 2014; Ziesemer et al., 2019; Vazzana et al., 2022). It is worth noting that bulk sampling of the tooth root may reduce the effectiveness of specimen

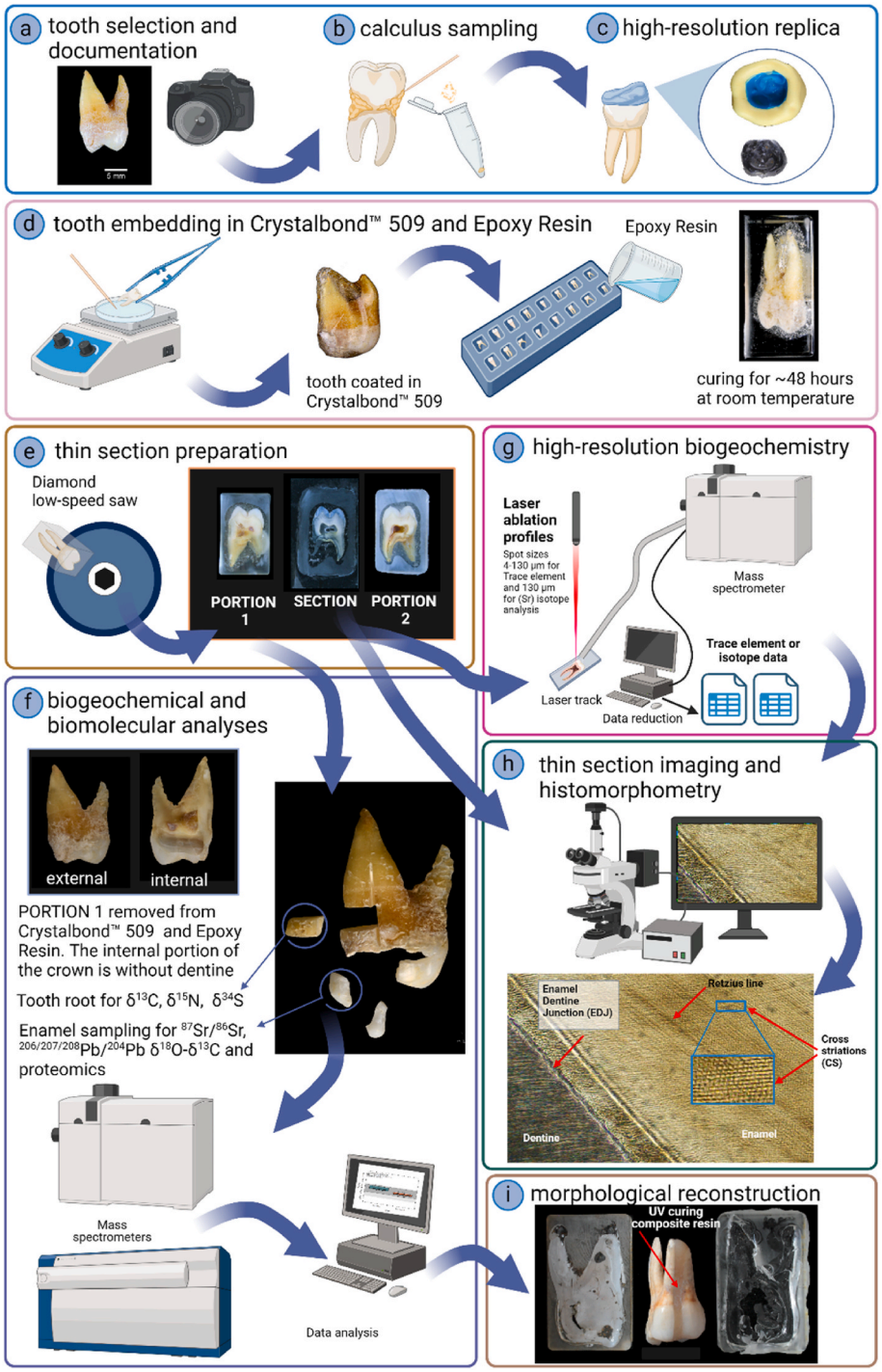

Fig. 1. Summary of the proposed NOWA protocol. Within step f, as an alternative to sampling portions of a tooth root, serial sampling of dentine can be performed to detect dietary shifts occurring during root formation (Beaumont et al., 2013; Eerkens et al., 2019; Czermak et al., 2020; Cheung et al., 2022; Curtis et al., 2022). Should aDNA and/or ^{14}C analyses be required, the tooth root can be sampled prior to specimen embedding (d) and after documentation. Figure created with BioRender.com.

reconstruction (see Fig. 1i). Alternatively, aDNA can be also extracted from the cementum of tooth roots through a minimally destructive extraction method following Harney et al. (2021).

2.3. High-resolution replica (Fig. 1c)

High-resolution replicas of the crown aspects are made to allow future microwear analysis following Fiorenza et al. (2009), Ungar (2017), Hodgkins et al. (2021).

Dental crown moulds are made with a light body polyvinylsiloxane silicone (in our case, Provil® Novo Light C.D.2, Heraeus Kulzer GmbH), and supported with a casing made of a silicone impression material (e.g., Zhermack, elite HD+). Transparent repliques are produced using bicomponent epoxy resin (e.g., EpoxiCure 2, Buehler Ltd) (Fig. 3).

2.4. Specimen embedding (Fig. 1d)

The NOWA protocol employs a two-step embedding process

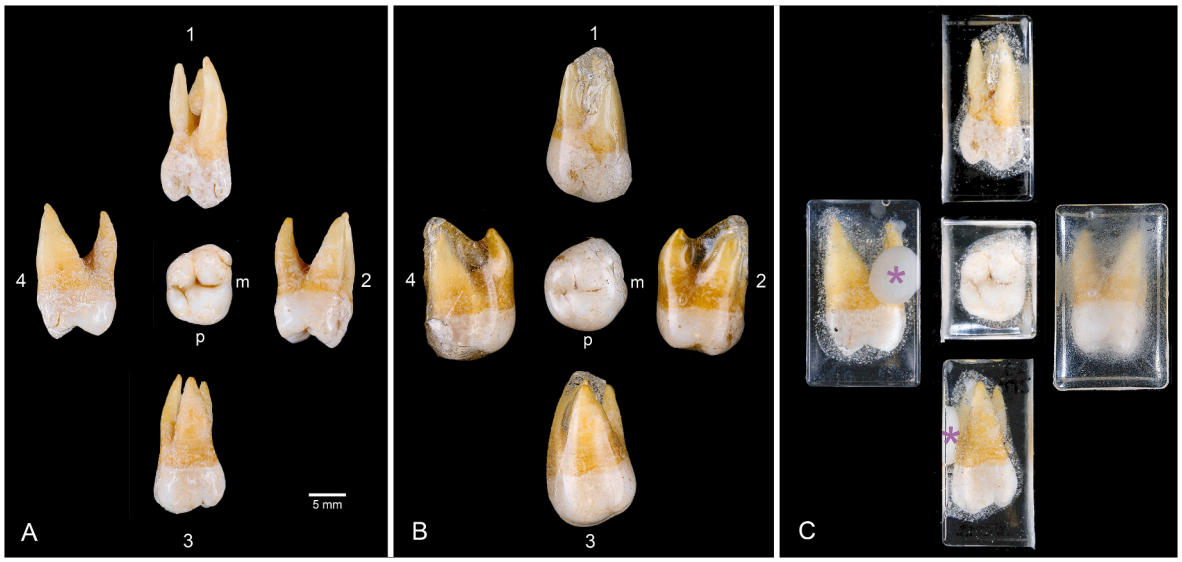

Fig. 2. (A) Upper right second permanent molar (URM2) from an archaeological individual (Tomb 77, Sala Consilina, Iron Age southern Italy). (B) Specimen URM2 from Tomb 77 embedded in Crystalbond™ 509. (C) Specimen URM2 from Tomb 77 embedded in bicomponent epoxy resin. The tooth is shown in buccal (1), distal (2), palatal (3), mesial (4) and occlusal views; m = mesial aspect, p = palatal aspect. In (C), the purple star indicates the UV-light-curing composite support shaped to align the tooth in the mould. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

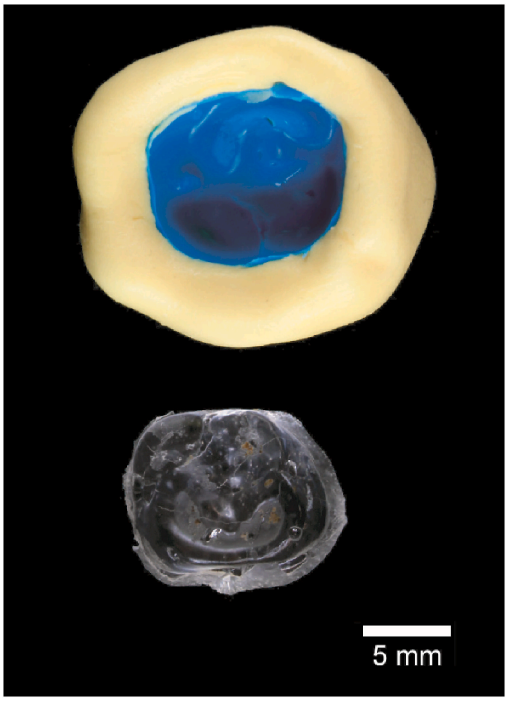

Fig. 3. Example of high-resolution replica of the crown of a molar tooth. Above, polyvinylsiloxane silicone mould (in blue) supported with a casing made of a silicone impression material (in yellow). Below, the occlusal view of the resulting transparent replique made using bicomponent epoxy resin (EpoxiCure 2, Buehler Ltd). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

following Nava et al. (2020) and Esposito et al. (2023). The initial phase involves embedding the tooth in a reversible adhesive (in our case, Crystalbond™ 509, Aremco Products Inc) which is soluble in Crystalbond™ cleaning agent (Crystalbond™ 509-S Stripper, Aremco Products Inc) or acetone (CH_3-CO-CH_3). This first step allows the future extraction of the specimen from the resin block after sectioning. The application of Crystalbond™ 509 entails melting it in a Petri dish on a laboratory hot plate at a temperature of ~80 °C. Once the adhesive reaches its mid-range melting point (74 °C), the tooth is coated with Crystalbond™ 509 using a wooden stick and laboratory tweezers, ensuring the adhesive fully covers the entire surface of the tooth as a thin film, while making contact at the lowest possible temperature (Fig. 2B).

The second step involves embedding the Crystalbond™509-coated tooth in a bicomponent epoxy resin (in our case, EpoxiCure 2, Resin and Hardener Buehler Ltd) to ensure sample stability during the sectioning procedure (Nava et al., 2017a, 2019). The resin block is left to cure for ~48 h at room temperature (Fig. 2C).

2.5. *Thin section preparation (Fig. 1e)*

The specimen embedded in the resin block is sectioned following the standard method in dental histology (e.g., Dean, 2012; Mahoney, 2012; Mahoney, 2015; Nava et al., 2020; Peripoli et al., 2023) using a low-speed saw (in our case, IsoMet low-speed diamond blade precision cutter, Buehler Ltd) equipped with a 300 μm thick diamond blade, or a microtome. The cuts are made longitudinally along the buccolingual plane by passing through the tip of the dentine horn. The embedded specimen undergoes two cuts during this step, resulting in three distinct parts: PORTION 1, the SECTION, and PORTION 2 (Fig. 4).

PORTION 1 (block resulting from the first cut)

The first cut is performed adjacent to the cutting plane, sectioning the tooth, and yielding two blocks. This first cut typically serves as a preparatory step for producing thin sections from the embedded tooth intended for histological and biogeochemical analyses. Here, PORTION 1 is used for multi-isotope and proteomics bulk analyses, thereby optimising the use of all specimen parts, and minimising wastage.

The advantages of multi-isotope sampling after embedding are twofold: first, it is possible to assess the quality of the two portions after sectioning, facilitating the selection of the more appropriate one for analysis; secondly, after sectioning the thickness of enamel is clearly visible, thereby reducing the risk of mixing enamel and dentine during subsequent sampling.

SECTION

The exposed inner surface of the tooth embedded in the other epoxy block is gently grinded with abrasive paper (in our case, MicroCut, P2500, Buehler Ltd) and subsequently polished with dye-free polycrystalline diamond suspension (in our case, MetaDi Supreme DF, 1 μm,

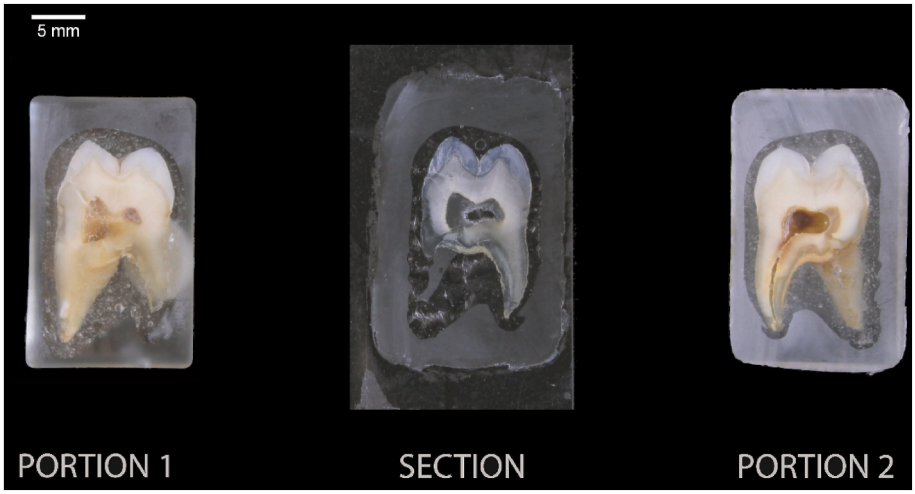

Fig. 4. The resulting three parts of the specimen URM2 from Tomb 77 (see Fig. 2) after cutting: PORTION 1, the SECTION, and PORTION 2.

Buehler Ltd). It is then affixed with a thin layer of bicomponent epoxy resin (in our case, EpoxiCure 2, Buehler Ltd) to a microscope slide pre-treated with silane (in our case, Relyx Ceramic primer, 3M Ltd). Following this, the specimen is sectioned parallel to the microscope slide, obtaining a section thickness of around 120–150 μm. This thickness is necessary to perform laser-based high-resolution biogeochemical analyses (Fig. 1g) following which an image of the laser tracks is taken and the section can be further ground to a thickness of around 100 μm, which is optimal for the visibility of dental microstructures (Fig. 1h) (Caropreso et al., 2000; Smith et al., 2006; Nava et al., 2020). Therefore, the use of cover glasses with permanent mounting is avoided.

PORTION 2 (block resulting from the second cut)

The block resulting from the second cut can be used for further or future analyses.

2.6. PORTION 1: bulk sampling of tooth enamel and dentine for multi-isotope analyses and sex determination through proteomics (Fig. 1f)

PORTION 1 of the tooth is employed for proteomics of dental enamel and multi-isotope analyses, encompassing strontium ($^{87}Sr/^{86}Sr$), oxygen ($\delta^{18}O$), carbon ($\delta^{13}C$) and lead ($^{206/207/208}Pb/^{204}Pb$) of dental enamel, carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulfur ($\delta^{34}S$) of root dentinal collagen.

Proteomic analysis of tooth enamel is a cost-effective, minimally destructive tool enabling unambiguous sex characterisation from amelogenin of poorly preserved skeletal remains of both adult and subadult individuals (Stewart et al., 2017; Lugli et al., 2019b).

Strontium ($^{87}Sr/^{86}Sr$), oxygen ($\delta^{18}O$) and, to a lesser extent, lead ($^{206/207/208}Pb/^{204}Pb$) are established techniques for investigating movement and provide geological and climatic signals for origins (Bentley, 2006; Montgomery, 2010; Lightfoot and O'Connell, 2016; Evans et al., 2018; Pederzani and Britton, 2019; Evans et al., 2022). Sulfur ($\delta^{34}S$) analysis shows whether individuals were raised in coastal, wetland or inland areas and has a geological component useful for exploring origins (Nehlich et al., 2011; Nehlich, 2015; Guiry et al., 2022; Lamb et al., 2023; Madgwick et al., 2023). $\delta^{13}C$ and $\delta^{15}N$ of bone and dentine collagen provide information on past diets (Lee-Thorp, 2008; Britton, 2017). $\delta^{13}C$ is used to understand if a population diet was based on C4 (e.g., millet and sorghum) or C3 (e.g., wheat, root crops) plants. Environmental factors (Hamilton and Thomas, 2012), as well as the consumption of fungi (Hamilton et al., 2009; O'Regan et al., 2016) and marine resources (e.g., seaweed, molluscs, fish, and marine mammals) can also impact $\delta^{13}C$ values (Little and Schoeninger, 1995; Balasse et al., 2006). $\delta^{15}N$ is used to differentiate between herbivorous, omnivorous, and carnivorous diets, reflecting different trophic levels (O'Connell et al., 2012). $\delta^{15}N$ values can increase due to manuring, which raises the $\delta^{15}N$ values in crops, fauna and humans (Hamilton and Thomas, 2012; Bogaard and Outram, 2013). Additionally, marine diets (Schoeninger and DeNiro, 1984), nursing (Fogel, 1989; Reynard and Tuross, 2015) and nutritional stress (Fuller et al., 2005) can result in elevated $\delta^{15}N$ values. The interception of the possible consumption of marine food in an individual's diet enables a better interpretation of $^{87}Sr/^{86}Sr$ isotope ratio analysis. A significant intake of seafood can indeed largely influence $^{87}Sr/^{86}Sr$ values in human individuals, which will be closer to the seawater $^{87}Sr/^{86}Sr$ value, 0.7092 (McArthur et al., 2001). Human teeth form in precise periods of an individual's life (AlQahtani et al., 2010; Hillson, 2024). Bioapatite in tooth enamel starts forming in utero and early childhood and remains stable without undergoing remodeling, while collagen within dentine undergoes minimal remodeling (AlQahtani et al., 2010; Montgomery, 2010; Scorrer et al., 2021). Hence, the isotope compositions in teeth are used as a record of childhood diet and mobility, therefore the choice of specific teeth is strictly related to the research questions. However, we must consider that bulk sampling hinders the precise estimation of temporal ranges in an individual's life as it involves large portions of tissue which cover a wide timeframe.

Initially, the crown dentine from PORTION 1 is sampled, and any dentine adhering to the enamel is thoroughly removed using a dental burr and stored in a microcentrifuge tube for future analysis. This step is critical to eliminate any traces of dentine since it is more susceptible to diagenesis which would influence $^{87}Sr/^{86}Sr$ isotope values in dental samples (Chiaradia et al., 2003; Bentley, 2006).

Subsequently, PORTION 1 is immersed in the Crystalbond™ cleaning agent – or alternatively, acetone (CH_3-CO-CH_3), as it has been shown not to affect the biogeochemical properties of the samples (Reynaga et al., 2023) – to dissolve the Crystalbond™ 509 coating and release the tooth from the resin block. The specimen is subsequently thoroughly rinsed in a clean dissolving agent and then with deionised water and dried at room temperature.

Enamel sampling

Tooth enamel is sampled (Fig. 5) for $^{87}Sr/^{86}Sr$ (approximately 5–20 mg, as low as 0.1 mg; Müller et al., 2003), $\delta^{18}O$-$\delta^{13}C$ (approximately 2–3 mg) and amelogenin for sex determination (approximately 10 mg; Lugli et al., 2019b, 2020). If $^{206/207/208}Pb/^{204}Pb$ analysis is performed, approximately 20–100 mg of tooth enamel is necessary for both $^{87}Sr/^{86}Sr$ and $^{206/207/208}Pb/^{204}Pb$ analyses – depending crucially on Pb concentrations, which can be < 0.1 μg/g in pre-metallurgy specimens. A flexible diamond-edged rotary-mounted blade is used to sample the crown as in Esposito et al. (2023), selecting specific portions of the tooth to answer specific research questions. All equipment is thoroughly cleaned between each sampling to prevent cross-contamination.

Fig. 5. The specimen URM2 from Tomb 77 (see Fig. 2) sampled for multi-isotope analysis and proteomics.

Alternatively, a microsampling device can be used to increase the precision of the chronological interval covered by the sampling. For $^{87}Sr/^{86}Sr$ analysis, the sample undergoes ultrasonic cleaning in deionised water and is processed in a clean working area following the protocol described in Scorrer et al. (2021). The same solution and same resin (SrSpec) can be used for sequential elution of strontium and for lead for subsequent isotope analysis (Müller et al., 2003). $^{87}Sr/^{86}Sr$ (and $^{206/207/208}Pb/^{204}Pb$ if required) ratios are measured using a multi-collector inductively coupled plasma mass spectrometer (MC-ICPMS). For $\delta^{18}O$-$\delta^{13}C$ analyses, a cut parallel to the section previously sampled for $^{87}Sr/^{86}Sr$ to provide the same chronological timeframe is performed. It is suggested to avoid early developing enamel (e.g., first permanent molars) so that no nursing signal would impact the analysis (Wright and Schwarcz, 1998). Enamel samples are powdered using an agate pestle and mortar. The isotope composition of the carbonate moiety ($\delta^{13}C$ and $\delta^{18}O$) of enamel bioapatite is measured (without further pre-treatment) following the protocol described in Scorrer et al. (2021) using a dual inlet mass spectrometer coupled to a carbonate preparation device. In the case of hypsodont (high-crowned) teeth, as in species such as cattle and sheep, sequential sampling through rotary abrasion of enamel for $\delta^{13}C$ and $\delta^{18}O$ analyses can be undertaken to provide seasonal resolution on diet and management (e.g., Trentacoste et al., 2020; Kowalik et al., 2023; Messana et al., 2023). Oxygen isotopes have been measured by high-resolution ion microprobe (e.g., Krzemińska et al., 2017; Smith et al., 2018; Smith et al., 2024). It is worth noting that a thin section containing Crystalbond™ 509 cannot be analyzed by a high-resolution ion microprobe, as it requires cleaning with detergents that could dissolve Crystalbond™ 509 and may release volatiles in the high vacuum. The remaining tooth enamel is sampled for proteomics (Lugli et al., 2019b, 2020) and analysed by liquid-chromatography mass spectrometry (LC-MS/MS) after protein extraction using 5% HCl. Alternatively, dental proteins can be extracted by immerging the resin-embedded tooth with the cut surface exposed in 1.2 M HCl as reported by Peripoli et al. (2023).

Tooth root sampling

Root dentine (approximately 20–100 mg) is sampled for bulk $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ collagen analyses (Fig. 5). Targeting specific sections of the root informs on different periods of an individual's life, depending on the research questions being addressed. Alternatively, for a more time-specific approach, serial sampling of dentine can be performed to detect dietary shifts that occurred throughout the formation of the root (Beaumont et al., 2013; Eerkens et al., 2019; Czermak et al., 2020; Cheung et al., 2022; Curtis et al., 2022). Collagen is extracted following a modified Longin method (Brown et al., 1988; Madgwick et al., 2023) and analysed with a continuous-flow isotope ratio mass spectrometer (CF-IRMS) coupled to an elemental analyser.

2.7. SECTION: high-spatial resolution biogeochemical analysis (Fig. 1g)

High-spatial resolution Sr isotope and trace element (TE) analyses are performed on the thin section. Time-resolved Sr isotopic compositions are measured using a laser ablation system coupled to a multi-collector ICPMS (see Müller and Anczkiewicz, 2016; Nava et al., 2020; Lugli et al., 2022). TE data is acquired through LA coupled to a SF-ICPMS or a (T)Q-ICPMS. After a pre-ablation cleaning step, acquisitions are performed in slow continuous profiling mode in the enamel, closest to (and <100 μm from) the enamel-dentine junction (EDJ), in the direction of tooth growth, with a spot size ranging from 4 to 25 μm for TE and from 100 to 130 μm for Sr isotopes, and a scan speed of 5 μm/s (Müller et al., 2019; Nava et al., 2020; Kubat et al., 2023). TE data can be used to understand (early-life) dietary changes and exposure history (specifically through Sr/Ca, Ba/Ca and Pb/Ca ratios), and decipher the overall chemical-preservation status (e.g., through Mn, REE and U concentrations) of dental specimens (Müller et al., 2019; Nava et al., 2020; Kubat et al., 2023). In addition, information on enamel mineralization can be obtained through specific elemental signatures, as Zn (Müller et al., 2019). 2D mapping can also be performed from which compositional profiles can be extracted (Müller et al., 2024). The NOWA protocol was successfully applied by Nava et al. (2020), Lugli et al. (2022) and Higgins et al. (2024) to detect individual mobility at high resolution.

2.8. SECTION: thin section imaging and histomorphometry (Fig. 1h)

Several partially overlapping, high-resolution micrographs of the polished section, captured under polarised light at various magnifications (40x, 100x) using a transmitted light microscope are merged to generate a single image of the whole crown. In our case study, an Axio Imager 2 (Carl Zeiss AG) equipped with an Axiocam 807 color camera (Carl Zeiss AG) is used to acquire the micrographs, while the ZEN core version 3.8 software (Carl Zeiss AG) performed the automatic stitching of the images (Fig. 6). Histomorphometric analysis is performed using ImageJ 1.54f (National Institute of Health USA) (Schneider et al., 2012) software to perform the required measurement and to calculate the dental growth parameters. The timing of dental crown formation is derived following the method described by (Guatelli-Steinberg et al., 2012; Birch and Dean, 2014). The chronological reconstruction utilises enamel's circadian and circaseptan rhythms to reconstruct the developmental history and timing of the crown, identifying crown growth parameters such as daily secretion rates (DSRs; the speed of enamel vertical growth along its prisms; Reid et al., 1998), enamel extension rate (EER; the speed of the crown's longitudinal growth along the EDJ; Dean and Smith, 2009), crown initiation (Ci), crown completion (CrC), and crown formation time (CFT), as well as identifying life history markers such as the neonatal line (NNL; which marks the moment of birth; Sabel et al., 2008; Dean et al., 2019) and accentuated lines (ALs; which mark systemic stress events occurred within the timeframe of crown formation; Witzel et al., 2008; Nava et al., 2019).

2.9. Morphological reconstruction (Fig. 1i)

The NOWA protocol aims to optimise the amount of material

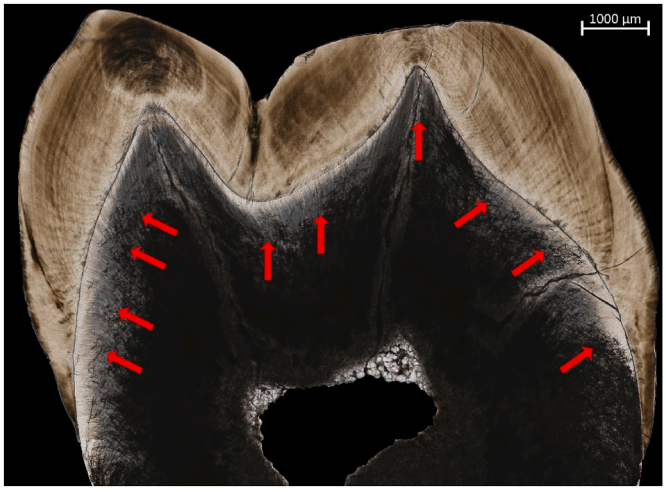

Fig. 6. Photomosaic of a permanent molar tooth crown from an archaeological horizon showing extensive diagenetic alteration of dentine indicated by the strong presence of fungus hypha branching (red arrows). The micrographs captured under polarised light using a transmitted light microscope are merged to generate a single image of the whole crown. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

sampled to minimise 'wastage' and allow reconstruction of the dental specimen after sampling. However, more analyses necessitate more extensive sampling, which can complicate the specimen's reconstruction.

There are two primary approaches to the reconstruction of a specimen. The first is the method described by Vazzana et al. (2022), consisting of the use of microCT scanning, reverse engineering techniques and rapid prototyping to replicate the sampled portion to be integrated. The second approach relies on modern dental reconstruction techniques commonly applied by professionals in dental care environments (Fidalgo-Pereira et al., 2022). Restorative materials used in dental practice are chosen because they have the same quality and appearance as natural teeth. Dentistry polymer-based restorative materials allow a simple reconstruction while permitting the recognition between restored and unrestored portions. An example of a restored Neanderthal tooth following life history analysis (Nava et al., 2020) is given in Müller et al. (2024).

The reconstruction of the entire tooth is carried out using the adhesive techniques typical of conservative dentistry with a light-curing, radiopaque composite. This technique involves neither the use of orthophosphoric acid as a conditioning agent nor the use of an adhesive in a subsequent step. Instead, it entails the pre-treatment of the two exposed surfaces of the specimen with 99% ethanol, drying and bonding through the application of a small amount of pre-heated (using light manual pressure) composite resin which is then consolidated using light curing with a dental light curing lamp. Before the final curing of the composite, the alignment of the tooth portions is carefully checked under a stereomicroscope and the dimensions of the reconstructed tooth are checked against the original.

3. Discussion and conclusions

The application of advanced scientific methodologies to ancient dental specimens, both human and fauna, has undeniably reshaped our understanding of the past, revealing intricate details about individual life histories and societal dynamics. Indeed, teeth are biological records of an individual's life, yielding a wealth of information and providing insights into wide-ranging aspects of the past (Fleming, 2009).

Non-destructive methods, such as macro-morphological analysis (Buikstra and Ubelaker, 1994; Ungar, 2017; Hillson, 2024) and virtual anthropological techniques (Tafforeau and Smith, 2008; Smith et al., 2010; Tafforeau et al., 2012; Le Cabec et al., 2015; Nava et al., 2017b, 2022; Hodgkins et al., 2021; Mahoney et al., 2021; Cerrito et al., 2022), can reveal a wide range of information. Though employing destructive advanced analytical techniques, researchers can further reconstruct the lives of past individuals and communities, addressing with great accuracy questions such as weaning practices, dietary changes, exposure to pollutants, mobility patterns, refining chronological information and ancestry (Müller et al., 2024; Nava et al., 2024).

The rationale behind the proposal of the NOWA protocol is to maximise the information retrievable from a single specimen through a multi-analytical approach while minimising as much as possible damages of fossil and archaeological dental remains. Following the ALARA principle (As Low [damage] As Reasonably Achievable), commonly adopted in radiographic and dental medical practices (Yeung, 2019) we suggest that destructive analyses should adhere to stringent guidelines: 1) analyses should only be conducted if they yield a well-documented net benefit; 2) all destructive sampling must be minimised as reasonably achievable, considering economic and social factors; 3) the extent of destructive sampling should not exceed the limits recommended for the specific circumstances, following relevant literature and curatorial standards. Moreover, in specific contexts, it is prudent to further mitigate assumed risks, even below recommended thresholds, and the definition of acceptable risk may vary substantially between cases (Nava, 2024). The NOWA protocol proposed here takes into consideration the preservation of fossils and archaeological dental remains, while advocating for a holistic and multidisciplinary approach to reconstructing the lives of past communities. This approach not only safeguards the integrity of the specimens but also fosters a collaborative environment, where diverse expertise converges to enrich our understanding of the complexities inherent to these remnants of human history.

According to the research questions and the intrinsic value of the specimen, the NOWA protocol offers an array of possible analytical pathways using an optimised sampling approach, focused on histology and biogeochemical analyses and the restoration of the specimen, to the "NOthing goes to WAste" approach, which enables the extraction of multiple high-resolution pieces of information from a single dental specimen. This protocol aims to become a guideline for optimal destructive sampling, advocating for a responsible and ethical approach to bioarchaeological research.

Data availability

The information on the protocol is provided in the manuscript.

CRediT authorship contribution statement

Carmen Esposito: Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Owen Alexander Higgins:** Writing – review & editing, Writing – original draft. **Alessia Galbusera:** Writing – review & editing, Writing – original draft, Visualization. **Melania Gigante:** Writing – review & editing, Writing – original draft, Visualization, Methodology. **Federico Lugli:** Writing – review & editing, Writing – original draft, Validation, Methodology. **Wolfgang Müller:** Writing – review & editing, Validation, Methodology, Conceptualization. **Richard Madgwick:** Writing – review & editing, Resources, Methodology. **Alfredo Coppa:** Writing – review & editing, Resources, Methodology. **Stefano Benazzi:** Writing – review & editing, Resources, Methodology. **Luca Bondioli:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Data curation, Conceptualization. **Alessia Nava:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abbazzi, L., Delfino, M., Gallai, G., Trebini, L., Rook, L., 2008. New data on the vertebrate assemblage of fiume santo (North-west sardinia, Italy), and overview on the late miocene tusco-Sardinian paleobioprovince. Palaeontology 51, 425–451.

Alpaslan-Roodenberg, S., Anthony, D., Babiker, H., Banffy, E., Booth, T., Capone, P., Deshpande-Mukherjee, A., Eisenmann, S., Fehren-Schmitz, L., Frachetti, M., Fujita, R., Frieman, C.J., Fu, Q., Gibbon, V., Haak, W., Hajdinjak, M., Hofmann, K.P., Holguin, B., Inomata, T., Kanzawa-Kiriyama, H., Keegan, W., Kelso, J., Krause, J., Kumaresan, G., Kusimba, C., Kusimba, S., Lalueza-Fox, C., Llamas, B., Maceachern, S., Mallick, S., Matsumura, H., Morales-Arce, A.Y., Matuzeviciute, G. M., Mushrif-Tripathy, V., Nakatsuka, N., Nores, R., Ogola, C., Okumura, M., Patterson, N., Pinhasi, R., Prasad, S.P.R., Prendergast, M.E., Punzo, J.L., Reich, D., Sawafuji, R., Sawchuk, E., Schiffels, S., Sedig, J., Shnaider, S., Sirak, K., Skoglund, P., Slon, V., Snow, M., Soressi, M., Spriggs, M., Stockhammer, P.W., Szecsenyi-Nagy, A., Thangaraj, K., Tiesler, V., Tobler, R., Wang, C.C., Warinner, C., Yasawardene, S., Zahir, M., 2021. Ethics of DNA research on human remains: five globally applicable guidelines. Nature 599, 41–46.

AlQahtani, S.J., Hector, M., Liversidge, H., 2010. Brief communication: the London atlas of human tooth development and eruption. Am. J. Phys. Anthropol. 142, 481–490.

Austin, C., Smith, T.M., Bradman, A., Hinde, K., Joannes-Boyau, R., Bishop, D., Hare, D. J., Doble, P., Eskenazi, B., Arora, M., 2013. Barium distributions in teeth reveal early-life dietary transitions in primates. Nature 498, 216–219.

Balasse, M., Tresset, A., Ambrose, S., 2006. Stable isotope evidence ($\delta^{13}C$, $\delta^{18}O$) for winter feeding on seaweed by Neolithic sheep of Scotland. J. Zool. 270, 170–176.

Beaumont, J., Gledhill, A., Lee-Thorp, J., Montgomery, J., 2013. Childhood diet: a closer examination of the evidence from dental tissues using stable isotope analysis of incremental human dentine. Archaeometry 55, 277–295.

Beaumont, J., Gledhill, A., Montgomery, J., 2014. Isotope analysis of incremental human dentine: towards higher temporal resolution. Bull. Int. Assoc. Paleodontol. 8, 212–223.

Beaumont, J., Montgomery, J., 2015. Oral histories: a simple method of assigning chronological age to isotopic values from human dentine collagen. Ann. Hum. Biol. 42, 407–414.

Beaumont, W., Beverly, R., Southon, J., Taylor, R., 2010. Bone preparation at the KCCAMS laboratory. Nucl. Instrum. Methods Phys. Res. Sect. B Beam Interact. Mater. Atoms 268, 906–909.

Bentley, R.A., 2006. Strontium isotopes from the earth to the archaeological skeleton: a review. J. Archaeol. Method Theor 13, 135–187.

Birch, W., Dean, M.C., 2014. A method of calculating human deciduous crown formation times and of estimating the chronological ages of stressful events occurring during deciduous enamel formation. J. Forensic Leg. Med. 22, 127–144.

Bocherens, H., Brinkman, D.B., Dauphin, Y., Mariotti, A., 1994. Microstructural and geochemical investigations on Late Cretaceous archosaur teeth from Alberta, Canada. Can. J. Earth Sci. 31, 783–792.

Bogaard, A., Outram, A.K., 2013. Palaeodiet and beyond: stable isotopes in bioarchaeology. World Archaeol. 45, 333–337.

Britton, K., 2017. A stable relationship: isotopes and bioarchaeology are in it for the long haul. Antiquity 91, 853–864.

Brown, T.A., Nelson, D.E., Vogel, J.S., Southon, J.R., 1988. Improved collagen extraction by modified Longin method. Radiocarbon 30, 171–177.

Budd, P., Montgomery, J., Barreiro, B., Thomas, R.G., 2000. Differential diagenesis of strontium in archaeological human dental tissues. Appl. Geochem. 15, 687–694.

Buikstra, J.E., Ubelaker, D.H., 1994. Standards for data collection from human skeletal remains. In: Arkansas Archeological Survey Research Series. Arkansas Archeological Survey, Fayetteville, AR.

Caropreso, S., Bondioli, L., Capannolo, D., Cerroni, L., Macchiarelli, R., Condo, S., 2000. Thin sections for hard tissue histology: a new procedure. J. Microsc. 199, 244–247.

Ceccarelli, D., 2023. Handling and photographing fossils: bringing out diagnostic details in paleontological photography, 1900–1960. Nuncius 38, 583–607.

Cerrito, P., Nava, A., Radovčić, D., Borić, D., Cerrito, L., Basdeo, T., Ruggiero, G., Frayer, D.W., Kao, A.P., Bondioli, L., Mancini, L., Bromage, T.G., 2022. Dental cementum virtual histology of Neanderthal teeth from Krapina (Croatia, 130–120 kyr): an informed estimate of age, sex and adult stressors. J. R. Soc. Interface 19, 20210820.

Cersoy, S., Zazzo, A., Rofes, J., Tresset, A., Zirah, S., Gauthier, C., Kaltnecker, E., Thil, F., Tisnerat-Laborde, N., 2017. Radiocarbon dating minute amounts of bone (3–60 mg) with ECHoMICADAS. Sci. Rep. 7, 7141.

Cheung, C., Fernández-Crespo, T., Mion, L., DI Giusto, M., Goude, G., Macdonald, R.A., Richards, M.P., Herrscher, E., 2022. Micro-punches versus micro-slices for serial sampling of human dentine: striking a balance between improved temporal resolution and measuring additional isotope systems. Rapid Commun. Mass Spectrom. 36, e9380.

Chiaradia, M., Gallay, A., Todt, W., 2003. Different contamination styles of prehistoric human teeth at a Swiss necropolis (Sion, Valais) inferred from lead and strontium isotopes. Appl. Geochem. 18, 353–370.

Craig-Atkins, E., Jervis, B., Cramp, L., Hammann, S., Nederbragt, A.J., Nicholson, E., Taylor, A.R., Whelton, H., Madgwick, R., 2020. The dietary impact of the Norman Conquest: a multiproxy archaeological investigation of Oxford, UK. PLoS One 15, e0235005.

Cristiani, E., Radini, A., Edinborough, M., Borić, D., 2016. Dental calculus reveals Mesolithic foragers in the Balkans consumed domesticated plant foods. Proc. Natl. Acad. Sci. USA 113, 10298–10303.

Curtis, M.J., Beaumont, J., Elamin, F., Wilson, A.S., Koon, H.E.C., 2022. Method of micro-sampling human dentine collagen for stable isotope analysis. Rapid Commun. Mass Spectrom. 36, e9305.

Czermak, A., Fernández-Crespo, T., Ditchfield, P.W., Lee-Thorp, J.A., 2020. A guide for an anatomically sensitive dentine microsampling and age-alignment approach for human teeth isotopic sequences. Am. J. Phys. Anthropol. 173, 776–783.

Dean, M.C., 2012. A histological method that can be used to estimate the time taken to form the crown of a permanent tooth. Methods Mol. Biol. 915, 89–100.

Dean, M.C., Smith, B.H., 2009. Growth and development of the Nariokotome youth, KNM-WT 15000. In: Grine, F.E., Fleagle, J.G., Leakey, R.E. (Eds.), The First Humans–Origin and Early Evolution of the Genus Homo. Springer, pp. 101–120.

Dean, M.C., Spiers, K.M., Garrevoet, J., LE Cabec, A., 2019. Synchrotron X-ray fluorescence mapping of Ca, Sr and Zn at the neonatal line in human deciduous teeth reflects changing perinatal physiology. Arch. Oral Biol. 104, 90–102.

Dupras, T.L., Schwarcz, H.P., Fairgrieve, S.I., 2001. Infant feeding and weaning practices in Roman Egypt. Am. J. Phys. Anthropol. 115, 204–212.

Dupras, T.L., Tocheri, M.W., 2007. Reconstructing infant weaning histories at Roman period Kellis, Egypt using stable isotope analysis of dentition. Am. J. Phys. Anthropol. 134, 63–74.

Eerkens, J.W., Bartelink, E.J., Bartel, J., Johnson, P.R., 2019. Isotopic insights into dietary life history, social status, and food sharing in American Samoa. Am. Antiq. 84, 336–352.

Emery, M.V., Stark, R.J., Murchie, T.J., Elford, S., Schwarcz, H.P., Prowse, T.L., 2018. Mapping the origins of Imperial Roman workers (1st–4th century CE) at Vagnari, Southern Italy, using $^{87}Sr/^{86}Sr$ and $\delta^{18}O$ variability. Am. J. Phys. Anthropol. 166, 837–850.

Esposito, C., Gigante, M., Lugli, F., Miranda, P., Cavazzuti, C., Sperduti, A., Pacciarelli, M., Stoddart, S., Reimer, P., Malone, C., Bondioli, L., Müller, W., 2023. Intense community dynamics in the pre-Roman frontier site of Fermo (ninth–fifth century BCE, Marche, central Italy) inferred from isotopic data. Sci. Rep. 13, 3632.

Evans, J., Pashley, V., Madgwick, R., Neil, S., Chenery, C., 2018. Tracking natural and anthropogenic Pb exposure to its geological source. Sci. Rep. 8, 1–9.

Evans, J.A., Pashley, V., Mee, K., Wagner, D., Parker Pearson, M., Fremondeau, D., Albarella, U., Madgwick, R., 2022. Applying lead (Pb) isotopes to explore mobility in humans and animals. PLoS One 17, e0274831.

Fewlass, H., Tuna, T., Fagault, Y., Hublin, J.-J., Kromer, B., Bard, E., Talamo, S., 2019. Pretreatment and gaseous radiocarbon dating of 40–100 mg archaeological bone. Sci. Rep. 9, 5342.

Fidalgo-Pereira, R., Carpio, D., Torres, O., Carvalho, O., Silva, F., Henriques, B., Özcan, M., Souza, J.C., 2022. The influence of inorganic fillers on the light transmission through resin-matrix composites during the light-curing procedure: an integrative review. Clin. Oral Invest. 26, 5575–5594.

Fiorenza, L., Benazzi, S., Kullmer, O., 2009. Morphology, wear and 3D digital surface models: materials and techniques to create high-resolution replicas of teeth. J. Anthropol. Sci. 87, 211–218.
Fleming, R., 2009. Writing biography at the edge of history. Am. Hist. Rev. 114, 606–614.
Fogel, M.L., 1989. Nitrogen isotope tracers of human lactation in modern and archaeological populations. In: Annual Report of the Director of the Geophysical Laboratory, vol. 88. Carnegie Institution, Washington, p. 111.
Francisci, G., Micarelli, I., Iacumin, P., Castorina, F., DI Vincenzo, F., DI Matteo, M., Giostra, C., Manzi, G., Tafuri, M.A., 2020. Strontium and oxygen isotopes as indicators of Longobards mobility in Italy: an investigation at Povegliano Veronese. Sci. Rep. 10, 11678.
Frei, K.M., Mannering, U., Kristiansen, K., Allentoft, M.E., Wilson, A.S., Skals, I., Tridico, S., Louise Nosch, M., Willerslev, E., Clarke, L., Frei, R., 2015. Tracing the dynamic life story of a bronze age female. Sci. Rep. 5, 10431.
Fuller, B.T., Fuller, J.L., Sage, N.E., Harris, D.A., O'Connell, T.C., Hedges, R.E., 2005. Nitrogen balance and $\delta^{15}N$: why you're not what you eat during nutritional stress. Rapid Commun. Mass Spectrom. 19, 2497–2506.
Gamba, C., Jones, E.R., Teasdale, M.D., Mclaughlin, R.L., Gonzalez-Fortes, G., Mattiangeli, V., Domboróczki, L., Kővári, I., Pap, I., Anders, A., Whittle, A., Dani, J., Raczky, P., Higham, T.F.G., Hofreiter, M., Bradley, D.G., Pinhasi, R., 2014. Genome flux and stasis in a five millennium transect of European prehistory. Nat. Commun. 5, 5257.
Gibbon, V.E., 2020. African ancient DNA research requires robust ethics and permission protocols. Nat. Rev. Genet. 21, 645–647.
Gigante, M., Nava, A., Paine, R.R., Fiore, I., Alhaique, F., Esposito, C.M., Sperduti, A., Bonetto, J., Cinquantaquattro, T.E., D'Agostino, B., Bondioli, L., 2021. Who was buried with Nestor's Cup? Macroscopic and microscopic analyses of the cremated remains from Tomb 168 (second half of the 8th century BCE, Pithekoussai, Ischia Island, Italy). PLoS One 16, e0257368.
Guatelli-Steinberg, D., Floyd, B.A., Dean, M.C., Reid, D.J., 2012. Enamel extension rate patterns in modern human teeth: two approaches designed to establish an integrated comparative context for fossil primates. J. Hum. Evol. 63, 475–486.
Guiry, E.J., Orchard, T.J., Needs-Howarth, S., Szpak, P., 2022. Freshwater wetland–driven variation in sulfur isotope compositions: implications for human paleodiet and ecological research. Front. Ecol. Evol. 10, 953042.
Gurfinkel, D., 1987. Comparative study of the radiocarbon dating of different bone collagen preparations. Radiocarbon 29, 45–52.
Hamilton, J., Hedges, R.E., Robinson, M., 2009. Rooting for pigfruit: pig feeding in neolithic and Iron age britain compared. Antiquity 83, 998–1011.
Hamilton, J., Thomas, R., 2012. Pannage, pulses and pigs: isotopic and zooarchaeological evidence for changing pig management practices in later Medieval England. Mediev. Archaeol. 56, 234–259.
Hardy, K., Blakeney, T., Copeland, L., Kirkham, J., Wrangham, R., Collins, M., 2009. Starch granules, dental calculus and new perspectives on ancient diet. J. Archaeol. Sci. 36, 248–255.
Harney, É., Cheronet, O., Fernandes, D.M., Sirak, K., Mah, M., Bernardos, R., Adamski, N., Broomandkhoshbacht, N., Callan, K., Lawson, A.M., Oppenheimer, J., Stewardson, K., Zalzala, F., Anders, A., Candilio, F., Constantinescu, M., Coppa, A., Ciobanu, I., Dani, J., Gallina, Z., Genchi, F., Nagy, E.G., Hajdu, T., Hellebrandt, M., Horváth, A., Király, Á., Kiss, K., Kolozsi, B., Kovács, P., Köhler, K., Lucci, M., Pap, I., Popovici, S., Raczky, P., Simalcsik, A., Szeniczey, T., Vasilyev, S., Virag, C., Rohland, N., Reich, D., Pinhasi, R., 2021. A minimally destructive protocol for DNA extraction from ancient teeth. Genome Res. 31, 472–483.
Higgins, O., Vazzana, A., Scalise, L., Riso, F., Buti, L., Conti, S., Bortolini, E., Oxilia, G., Benazzi, S., 2020. Comparing traditional and virtual approaches in the micro-excavation and analysis of cremated remains. J. Archaeol. Sci.: Reports 32, 102396.
Hillson, S., 2024. Dental Anthropology. Cambridge University Press.
Hodgkins, J., Orr, C.M., Gravel-Miguel, C., Riel-Salvatore, J., Miller, C.E., Bondioli, L., Nava, A., Lugli, F., Talamo, S., Hajdinjak, M., Cristiani, E., Romandini, M., Meyer, D., Drohobytsky, D., Kuester, F., Pothier-Bouchard, G., Buckley, M., Mancini, L., Baruffaldi, F., Silvestrini, S., Arrighi, S., Keller, H.M., Griggs, R.B., Peresani, M., Strait, D.S., Benazzi, S., Negrino, F., 2021. An infant burial from Arma Veirana in northwestern Italy provides insights into funerary practices and female personhood in early Mesolithic Europe. Sci. Rep. 11, 1–13.
Hoppe, K.A., Koch, P.L., Furutani, T.T., 2003. Assessing the preservation of biogenic strontium in fossil bones and tooth enamel. Int. J. Osteoarchaeol. 13, 20–28.
Horn, P., Hölzl, S., Storzer, D., 1994. Habitat determination on a fossil stags mandible from the site of homo-erectus heidelbergensis at mauer by use of Sr-87/Sr-86. Naturwissenschaften 81, 360–362.
Hublin, J.-J., Pääbo, S., Derevianko, A.P., Doronichev, V.B., Golovanova, L.V., Friess, M., Froment, A., Hoffmann, A., Kachache, N.E.J., Kullmer, O., Lordkipanidze, D., Moncel, M.H., Potts, R., Radovcic, J., Rak, Y.Z., Richards, M., Méndez, J.R., Rosas, A., Schmauder, M., Schmitz, R.W., Semal, P., Smith, T., Tafuri, M.A., Tattersall, I., Tournepiche, J.F., Toussaint, M., Vassiliev, S., Vialet, A., White, T., Ziegler, R., 2008. Suggested guidelines for invasive sampling of hominid remains. J. Hum. Evol. 55, 756–757.
Humphrey, L.T., 2014. Isotopic and trace element evidence of dietary transitions in early life. Ann. Hum. Biol. 41, 348–357.
Higgins, O.A., Modi, A., Cannariato, C., Diroma, M.A., Lugli, F., Ricci, S., Zaro, V., Vai, S., Vazzana, A., Romandini, M., Yu, H., Boschin, F., Magnone, L., Rossini, M., DI Domenico, G., Baruffaldi, F., Oxilia, G., Bortolini, E., Dellù, E., Moroni, A., Ronchitelli, A., Talamo, S., Müller, W., Calattini, M., Nava, A., Posth, C., Lari, M., Bondioli, L., Benazzi, S., Caramelli, D., 2024. Life history and ancestry of the late upper palaeolithic infant from Grotta delle Mura. Italy. Nat. Commun. https://doi.org/10.1038/S41467-024-51150-X, 15:8248.
Immel, A., Le Cabec, A., Bonazzi, M., Herbig, A., Temming, H., Schuenemann, V.J., Bos, K.I., Langbein, F., Harvati, K., Bridault, A., Pion, G., Julien, M.A., Krotova, O., Conard, N.J., Münzel, S.C., Drucker, D.G., Viola, B., Hublin, J.J., Tafforeau, P., Krause, J., 2016. Effect of X-ray irradiation on ancient DNA in sub-fossil bones–Guidelines for safe X-ray imaging. Sci. Rep. 6, 32969.
Joannes-Boyau, R., Adams, J.W., Austin, C., Arora, M., Moffat, I., Herries, A.I., Tonge, M. P., Benazzi, S., Evans, A.R., Kullmer, O., Wroe, S., Dosseto, A., Fiorenza, L., 2019. Elemental signatures of Australopithecus africanus teeth reveal seasonal dietary stress. Nature 572, 112–115.
Kohn, M.J., Schoeninger, M.J., Barker, W.W., 1999. Altered states: effects of diagenesis on fossil tooth chemistry. Geochem. Cosmochim. Acta 63, 2737–2747.
Kowalik, N., Anczkiewicz, R., Müller, W., Spötl, C., Bondioli, L., Nava, A., Wojtal, P., Wilczyński, J., Koziarska, M., Matyszczak, M., 2023. Revealing seasonal woolly mammoth migration with spatially-resolved trace element, Sr and O isotopic records of molar enamel. Quat. Sci. Rev. 306, 108036.
Kubat, J., Nava, A., Bondioli, L., Dean, M.C., Zanolli, C., Bourgon, N., Bacon, A.-M., Demeter, F., Peripoli, B., Albert, R., Lüdecke, T., Hertler, C., Mahoney, P., Kullmer, O., Schrenk, F., Müller, W., 2023. Dietary strategies of pleistocene *Pongo* sp. and *Homo erectus* on Java (Indonesia). Nat. Ecol. Evol. 7, 279–289.
Kuzminsky, S.C., Gardiner, M.S., 2012. Three-dimensional laser scanning: potential uses for museum conservation and scientific research. J. Archaeol. Sci. 39, 2744–2751.
Krzemińska, E., Sołtysiak, A., Czupyt, Z.J., 2017. Reconstructing seasonality using $\delta^{18}O$ in incremental layers of human enamel: a test of the analytical protocol developed for SHRIMP IIe/MC ion microprobe. Geol. Q. 61, 370–383. https://doi.org/10.7306/gq.1354.
Lamb, A.L., Chenery, C.A., Madgwick, R., Evans, J.A., 2023. Wet feet: developing sulfur isotope provenance methods to identify wetland inhabitants. R. Soc. Open Sci. 10, 230391.
Lauria, G., Sineo, L., Ficarra, S., 2022. A detailed method for creating digital 3D models of human crania: an example of close-range photogrammetry based on the use of Structure-from-Motion (SfM) in virtual anthropology. Archaeol. Anthropol. Sci. 14, 42.
Le Cabec, A., Tang, N., Tafforeau, P., 2015. Accessing developmental information of fossil hominin teeth using new synchrotron microtomography-based visualization techniques of dental surfaces and interfaces. PLoS One 10, e0123019.
Lee-Thorp, J.A., 2008. On isotopes and old bones. Archaeometry 50, 925–950.
Lightfoot, E., O'Connell, T.C., 2016. On the use of biomineral oxygen isotope data to identify human migrants in the archaeological record: intra-sample variation, statistical methods and geographical considerations. PLoS One 11, e0153850.
Little, E.A., Schoeninger, M.J., 1995. The late woodland diet on nantucket island and the problem of maize in coastal new england. Am. Antiq. 60, 351–368.
Lugli, F., Cipriani, A., Arnaud, J., Arzarello, M., Peretto, C., Benazzi, S., 2017. Suspected limited mobility of a Middle Pleistocene woman from Southern Italy: strontium isotopes of a human deciduous tooth. Sci. Rep. 7, 1–8.
Lugli, F., Cipriani, A., Capecchi, G., Ricci, S., Boschin, F., Boscato, P., Iacumin, P., Badino, F., Mannino, M.A., Talamo, S., Richards, M.P., Benazzi, S., Ronchitelli, A., 2019a. Strontium and stable isotope evidence of human mobility strategies across the Last Glacial Maximum in southern Italy. Nat. Ecol. Evol. 3 (6), 905–911.
Lugli, F., Di Rocco, G., Vazzana, A., Genovese, F., Pinetti, D., Cilli, E., Carile, M.C., Silvestrini, S., Gabanini, G., Arrighi, S., Buti, L., Bortolini, E., Cipriani, A., Figus, C., Marciani, G., Oxilia, G., Romandini, M., Sorrentino, R., Sola, M., Benazzi, S., 2019b. Enamel peptides reveal the sex of the Late Antique "Lovers of Modena". Sci. Rep. 9, 1–8.
Lugli, F., Figus, C., Silvestrini, S., Costa, V., Bortolini, E., Conti, S., Peripoli, B., Nava, A., Sperduti, A., Lamanna, L., Bondioli, L., Benazzi, S., 2020. Sex-related morbidity and mortality in non-adult individuals from the Early Medieval site of Valdaro (Italy): the contribution of dental enamel peptide analysis. J. Archaeol. Sci.: Reports 34, 102625.
Lugli, F., Nava, A., Sorrentino, R., Vazzana, A., Bortolini, E., Oxilia, G., Silvestrini, S., Nannini, N., Bondioli, L., Fewlass, H., Talamo, S., Bard, E., Mancini, L., Müller, W., Romandini, M., Benazzi, S., 2022. Tracing the mobility of a Late Epigravettian (~ 13 ka) male infant from Grotte di Pradis (Northeastern Italian Prealps) at high-temporal resolution. Sci. Rep. 12, 1–13.
Luhmann, T., Robson, S., Kyle, S., Harley, I.A., 2006. Close Range Photogrammetry: Principles, Techniques and Applications. Whittles.
Madgwick, R., Esposito, C., Lamb, A., 2023. Farming and feasting during the Bronze Age–Iron Age transition in Britain (ca. 900–500 bce): multi-isotope evidence for societal change. Front. Environ. Archaeol. 2, 122158.
Madgwick, R., Mulville, J., Evans, J., 2012. Investigating diagenesis and the suitability of porcine enamel for strontium ($^{87}Sr/^{86}Sr$) isotope analysis. J. Anal. Atomic Spectrom. 27, 733–742.
Mahoney, P., 2012. Incremental enamel development in modern human deciduous anterior teeth. Am. J. Phys. Anthropol. 147, 637–651.
Mahoney, P., 2015. Dental fast track: prenatal enamel growth, incisor eruption, and weaning in human infants. Am. J. Phys. Anthropol. 156, 407–421.
Mahoney, P., Mcfarlane, G., Smith, B.H., Miszkiewicz, J.J., Cerrito, P., Liversidge, H., Mancini, L., Dreossi, D., Veneziano, A., Bernardini, F., Cristiani, E., Behie, A., Coppa, A., Bondioli, L., Frayer, D., Radovčić, D., Nava, A., 2021. Growth of neanderthal infants from krapina (120–130 ka), Croatia. Proceedings of the Royal Society B 288, 20212079.
Makarewicz, C., Marom, N., Bar-Oz, G., 2017. Ensure equal access to ancient DNA. Nature 548, 158.
Marchewka, J., Skrzat, J., Wrobel, A., 2014. Analysis of the enamel hypoplasia using micro-CT scanner versus classical method. Anthropol. Anzeiger 71, 391–402.

Mcarthur, J.M., Howarth, R., Bailey, T., 2001. Strontium isotope stratigraphy: LOWESS version 3: best fit to the marine Sr-isotope curve for 0–509 Ma and accompanying look-up table for deriving numerical age. J. Geol. 109, 155–170.
Melton, N., Montgomery, J., Knüsel, C., 2013. Gristhorpe Man: a Life and Death in the Bronze Age. Oxbow.
Messana, C., Tornero, C., Madgwick, R., Lamb, A., Evans, J., Colominas, L., 2023. Between valleys, plateaus, and mountains: unveiling livestock altitudinal mobility in the Iron Age Iberian Peninsula (3rd c. BC) through a multi-isotope approach. Front. Environ. Archaeol. 2, 1245725.
Michel, V., Ildefonse, P., Morin, G., 1996. Assessment of archaeological bone and dentine preservation from lazaret cave (middle pleistocene) in France. Palaeogeogr. Palaeoclimatol. Palaeoecol. 126, 109–119.
Micheletti, N., Chandler, J., Lane, S., 2015. Investigating the geomorphological potential of freely available and accessible Structure-from-Motion photogrammetry using a smartphone. Earth Surf. Process. Landforms 40, 473–486.
Montgomery, J., 2010. Passports from the past: investigating human dispersals using strontium isotope analysis of tooth enamel. Ann. Hum. Biol. 37, 325–346.
Montgomery, J., Evans, J.A., Horstwood, M.S.A., 2010. Evidence for long-term averaging of strontium in bovine enamel using TIMS and LA-MC-ICP-MS strontium isotope intra-molar profiles. Environ. Archaeol. 15, 32–42.
Müller, W., Anczkiewicz, R., 2016. Accuracy of laser-ablation (LA)-MC-ICPMS Sr isotope analysis of (bio) apatite–a problem reassessed. J. Anal. Atomic Spectrom. 31, 259–269.
Müller, W., Fricke, H., Halliday, A.N., Mcculloch, M.T., Wartho, J.A., 2003. Origin and migration of the alpine iceman. Science 302, 862–866.
Müller, W., Lugli, F., McCormack, J., Evans, D., Anczkiewicz, R., Bondioli, L., Nava, A., 2024. Human life histories. In: Anbar, A.D., Weis, D. (Eds.), Treatise on Geochemistry, 3rd ed. Elsevier, Oxford, 281-328.
Müller, W., Nava, A., Evans, D., Rossi, P.F., Alt, K.W., Bondioli, L., 2019. Enamel mineralization and compositional time-resolution in human teeth evaluated via histologically-defined LA-ICPMS profiles. Geochem. Cosmochim. Acta 255, 105–126.
Nava, A., 2024. Understanding the maternal-infant nexus from dental histology and high-resolution compositional biogeochemistry: implications for bioarchaeological research. In: Bulletins et mémoires de la Société d'Anthropologie de Paris, vol. 36. BMSAP.
Nava, A., Bondioli, L., Coppa, A., Dean, M.C., Rossi, P.F., Zanolli, C., 2017a. New regression formula to estimate the prenatal crown formation time of human deciduous central incisors derived from a roman imperial sample (Velia, Salerno, I-II cent. CE). PLoS One 12, e0180104.
Nava, A., Coppa, A., Coppola, D., Mancini, L., Dreossi, D., Zanini, F., Bernardini, F., Tuniz, C., Bondioli, L., 2017b. Virtual histological assessment of the prenatal life history and age at death of the Upper Paleolithic fetus from Ostuni (Italy). Sci. Rep. 7, 9427.
Nava, A., Fiorin, E., Zupancich, A., Carra, M., Ottoni, C., DI Carlo, G., Vozza, I., Brugnoletti, O., Alhaique, F., Cremonesi, R.G., Coppa, A., Bondioli, L., Borić, D., Cristiani, E., 2021. Multipronged dental analyses reveal dietary differences in last foragers and first farmers at Grotta Continenza, central Italy (15,500–7000 BP). Sci. Rep. 11, 1–14.
Nava, A., Frayer, D.W., Bondioli, L., 2019. Longitudinal analysis of the microscopic dental enamel defects of children in the Imperial Roman community of Portus Romae (necropolis of Isola Sacra, 2nd to 4th century CE, Italy). J. Archaeol. Sci.: Reports 23, 406–415.
Nava, A., Lugli, F., Lemmers, S., Cerrito, P., Mahoney, P., Bondioli, L., Müller, W., 2024. Reading children's teeth to reconstruct life history and the evolution of human cooperation and cognition: the role of dental enamel microstructure and chemistry. Neurosci. Biobehav. Rev. 163, 105745.
Nava, A., Lugli, F., Romandini, M., Badino, F., Evans, D., Helbling, A.H., Oxilia, G., Arrighi, S., Bortolini, E., Delpiano, D., Duches, R., Figus, C., Livraghi, A., Marciani, G., Silvestrini, S., Cipriani, A., Giovanardi, T., Pini, R., Tuniz, C., Bernardini, F., Dori, I., Coppa, A., Cristiani, E., Dean, C., Bondioli, L., Peresani, M., Muller, W., Benazzi, S., 2020. Early life of neanderthals. Proc. Natl. Acad. Sci. U. S. A. 117, 28719–28726.
Nava, A., Mahoney, P., Bondioli, L., Coppa, A., Cristiani, E., Fattore, L., Mcfarlane, G., Dreossi, D., Mancini, L., 2022. Virtual histology of archaeological human deciduous prenatal enamel through synchrotron X-ray computed microtomography images. J. Synchrotron Radiat. 29 (1), 347-253.
Nehlich, O., 2015. The application of sulphur isotope analyses in archaeological research: a review. Earth Sci. Rev. 142, 1–17.
Nehlich, O., Fuller, B.T., Jay, M., Mora, A., Nicholson, R.A., Smith, C.I., Richards, M.P., 2011. Application of sulphur isotope ratios to examine weaning patterns and freshwater fish consumption in Roman Oxfordshire, UK. Geochem. Cosmochim. Acta 75, 4963–4977.
Nielsen-Marsh, C.M., Hedges, R.E., 2000. Patterns of diagenesis in bone I: the effects of site environments. J. Archaeol. Sci. 27, 1139–1150.
O'Connell, T.C., Kneale, C.J., Tasevska, N., Kuhnle, G.G., 2012. The diet-body offset in human nitrogen isotopic values: a controlled dietary study. Am. J. Phys. Anthropol. 149, 426–434.
O'Regan, H.J., Lamb, A.L., Wilkinson, D.M., 2016. The missing mushrooms: searching for fungi in ancient human dietary analysis. J. Archaeol. Sci. 75, 139–143.
Ottoni, C., Borić, D., Cheronet, O., Sparacello, V., Dori, I., Coppa, A., Antonović, D., Vujević, D., Price, T.D., Pinhasi, R., Cristiani, E., 2021. Tracking the transition to agriculture in Southern Europe through ancient DNA analysis of dental calculus. Proc. Natl. Acad. Sci. USA 118, e2102116118.
Pálsdóttir, A.H., Bläuer, A., Rannamäe, E., Boessenkool, S., Hallsson, J.H., 2019. Not a limitless resource: ethics and guidelines for destructive sampling of archaeofaunal remains. R. Soc. Open Sci. 6, 191059.
Parsley, R., Lawson, M., Pojeta JR, J., 2018. A practical and historical perspective of the how and why of whitening fossil specimens and casts as a precursor to their photography. Fossil Imprint 74, 237–244.
Pederzani, S., Britton, K., 2019. Oxygen isotopes in bioarchaeology: principles and applications, challenges and opportunities. Earth Sci. Rev. 188, 77–107.
Peripoli, B., Gigante, M., Mahoney, P., Mcfarlane, G., Coppa, A., Lugli, F., Lauria, G., Bondioli, L., Sconzo, P., Sineo, L., Nava, A., 2023. Exploring prenatal and neonatal life history through dental histology in infants from the Phoenician necropolis of Motya (7th–6th century BCE). J. Archaeol. Sci.: Reports 49, 104024.
Porr, M., Alt, K., 2006. The burial of bad dürrenberg, Central Germany: osteopathology and osteoarchaeology of a late mesolithic shaman's grave. Int. J. Osteoarchaeol. 16, 395–406.
Prendergast, M.E., Sawchuk, E., 2018. Boots on the ground in Africa's ancient DNA 'revolution': archaeological perspectives on ethics and best practices. Antiquity 92, 803–815.
Price, T.D., Burton, J.H., Bentley, R.A., 2002. The characterization of biologically available strontium isotope ratios for the study of prehistoric migration. Archaeometry 44, 117–135.
Quagliariello, A., Modi, A., Innocenti, G., Zaro, V., Conati Barbaro, C., Ronchitelli, A., Boschin, F., Cavazzuti, C., Dellù, E., Radina, F., Sperduti, A., Bondioli, L., Ricci, S., Lognoli, M., Belcastro, M.G., Mariotti, V., Caramelli, D., Mariotti Lippi, M., Cristiani, E., Martino, M.E., Muntoni, I.M., Lari, M., 2022. Ancient oral microbiomes support gradual Neolithic dietary shifts towards agriculture. Nat. Commun. 13, 6927.
Radini, A., Nikita, E., Buckley, S., Copeland, L., Hardy, K., 2017. Beyond food: the multiple pathways for inclusion of materials into ancient dental calculus. Am. J. Phys. Anthropol. 162, 71–83.
Radini, A., Nikita, E., 2023. Beyond dirty teeth: integrating dental calculus studies with osteoarchaeological parameters. Quat. Int. 653, 3–18.
Reid, D.J., Beynon, A.D., Ramirez Rozzi, F., 1998. Histological reconstruction of dental development in four individuals from a medieval site in Picardie, France. J. Hum. Evol. 35, 463–477.
Reynard, L.M., Tuross, N., 2015. The known, the unknown and the unknowable: weaning times from archaeological bones using nitrogen isotope ratios. J. Archaeol. Sci. 53, 618–625.
Reynaga, D.K.M., Munizzi, J.S., Mcmillan, R., Millaire, J.-F., Longstaffe, F.J., 2023. Effects of consolidants and their removal by polar solvents on the stable isotope compositions of bone. Quat. Int. 660, 31–41.
Richards, M.P., Mannino, M.A., Jaouen, K., Dozio, A., Hublin, J.-J., Peresani, M., 2021. Strontium isotope evidence for Neanderthal and modern human mobility at the upper and middle palaeolithic site of Fumane Cave (Italy). PLoS One 16, e0254848.
Roberts, P., 2022. Isotope analysis in archaeology grand challenge. Frontiers in Environmental Archaeology 1.
Sabel, N., Johansson, C., Kühnisch, J., Robertson, A., Steiniger, F., Norén, J.G., Klingberg, G., Nietzsche, S., 2008. Neonatal lines in the enamel of primary teeth—a morphological and scanning electron microscopic investigation. Arch. Oral Biol. 53, 954–963.
Sabin, S., Fellows Yates, J.A.. Dental Calculus Field-Sampling Protocol (Sabin version) v2. protocols.io. https://dx.doi.org/10.17504/protocols.io.7vrhn56.
Santos, G.M., Southon, J.R., Griffin, S., Beaupre, S.R., Druffel, E.R.M., 2007. Ultra small-mass AMS ^{14}C sample preparation and analyses at KCCAMS/UCI Facility. Nucl. Instrum. Methods Phys. Res. Sect. B Beam Interact. Mater. Atoms 259, 293–302.
Scaggion, C., Castelli, S., Usai, D., Artioli, G., 2022. 3D digital dental models' accuracy for anthropological study: comparing close-range photogrammetry to μ-CT scanning. Digit. Appl. Archaeol. Cult. Herit. 27, e00245.
Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675.
Schoeninger, M.J., Deniro, M.J., 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochem. Cosmochim. Acta 48, 625–639.
Scorrer, J., Faillace, K.E., Hildred, A., Nederbragt, A.J., Andersen, M.B., Millet, M.-A., Lamb, A.L., Madgwick, R., 2021. Diversity aboard a Tudor warship: investigating the origins of the Mary Rose crew using multi-isotope analysis. R. Soc. Open Sci. 8, 202106.
Silvester, C.M., Hillson, S., 2020. A critical assessment of the potential for Structure-from-Motionphotogrammetry to produce high fidelity 3Ddental models. Am. J. Phys. Anthropol. 173, 381–392.
Sirak, K.A., Sedig, J.W., 2019. Balancing analytical goals and anthropological stewardship in the midst of the paleogenomics revolution. World Archaeol. 51, 560–573.
Smith, T.M., Arora, M., Austin, C., Ávila, J.N., Duval, M., Lim, T.T., Piper, P.J., Vaiglova, P., De Vos, J., Williams, I.S., Zhao, J., Green, D.R., 2024. Oxygen isotopes in orangutan teeth reveal recent and ancient climate variation. Elife 12, RP90217.
Smith, T.M., Austin, C., Green, D.R., Joannes-Boyau, R., Bailey, S., Dumitriu, D., Fallon, S., Grün, R., James, H.F., Moncel Williams, I.S., Wood, R., Arora, M.,, M.-H., 2018. Wintertime stress, nursing, and lead exposure in Neanderthal children. Sci. Adv. 4, eaau9483.
Smith, T.M., Cook, L., Dirks, W., Green, D.R., Austin, C., 2021. Teeth reveal juvenile diet, health and neurotoxicant exposure retrospectively: what biological rhythms and chemical records tell us. Bioessays 43, 2000298.
Smith, T.M., Reid, D.J., Sirianni, J.E., 2006. The accuracy of histological assessments of dental development and age at death. J. Anat. 208, 125–138.
Smith, T.M., Tafforeau, P., Reid, D.J., Pouech, J., Lazzari, V., Zermeno, J.P., Guatelli-Steinberg, D., Olejniczak, A.J., Hoffman, A., Radovčić, J., Makaremi, M., Toussaint, M., Stringer, C., Hublin, J.J., 2010. Dental evidence for ontogenetic

differences between modern humans and Neanderthals. Proc. Natl. Acad. Sci. U.S.A. 107, 20923–20928.
Squires, K., Booth, T., Roberts, C., 2019. The ethics of sampling human skeletal remains for destructive analyses. In: Squires, K., Errickson, D., Márquez-Grant, N. (Eds.), Ethical Approaches to Human Remains. Springer, pp. 265–297.
Squires, K., Roberts, C.A., Márquez-Grant, N., 2022. Ethical considerations and publishing in human bioarcheology. Am. J. Biol. Anthropol. 177, 615–619.
Stantis, C., Schaefer, B.J., Correia, M.A., Alaica, A.K., Huffer, D., Plomp, E., Di Giusto, M., Chidimuro, B., Rose, A.K., Nayak, A., Kendall, E.J., 2024. Ethics and applications of isotope analysis in archaeology. Am. J. Biol. Anthropol. 24992.
Stewart, N.A., Gerlach, R.F., Gowland, R.L., Gron, K.J., Montgomery, J., 2017. Sex determination of human remains from peptides in tooth enamel. Proc. Natl. Acad. Sci. USA 114, 13649–13654.
Tafforeau, P., Smith, T.M., 2008. Nondestructive imaging of hominoid dental microstructure using phase contrast X-ray synchrotron microtomography. J. Hum. Evol. 54, 272–278.
Tafforeau, P., Zermeno, J.P., Smith, T.M., 2012. Tracking cellular-level enamel growth and structure in 4D with synchrotron imaging. J. Hum. Evol. 62, 424–428.
Trentacoste, A., Lightfoot, E., Le Roux, P., Buckley, M., Kansa, S., Esposito, C., Gleba, M., 2020. Heading for the hills? A multi-isotope study of sheep management in first-millennium BC Italy. J. Archaeol. Sci.: Reports 29, 102036.
Trickett, M.A., Budd, P., Montgomery, J., Evans, J., 2003. An assessment of solubility profiling as a decontamination procedure for the Sr-87/Sr-86 analysis of archaeological human skeletal tissue. Appl. Geochem. 18, 653–658.
Ungar, P.S., 2017. Evolution's bite: A story of teeth, diet, and human origins. Princeton University Press.
Vazzana, A., Higgins, O., Oxilia, G., Lugli, F., Silvestrini, S., Nava, A., Bondioli, L., Bortolini, E., Di Domenico, G., Bernardini, F., Tuniz, C., Mancini, L., Bettuzzi, M., Morigi, M.P., Piperno, M., Collina, C., Romandini, M., Benazzi, S., 2022. High-accuracy methodology for the integrative restoration of archaeological teeth by using reverse engineering techniques and rapid prototyping. J. Archaeol. Sci. 44, 103511.
Wagner, J.K., Colwell, C., Claw, K.G., Stone, A.C., Bolnick, D.A., Hawks, J., Brothers, K. B., Garrison, N.A., 2020. Fostering responsible research on ancient DNA. Am. J. Hum. Genet. 107, 183–195.
White, T.D., Black, M.T., Folkens, P.A., 2012. Human Osteology. Elsevier Academic Press.
Witzel, C., Kierdorf, U., Schultz, M., Kierdorf, H., 2008. Insights from the inside: histological analysis of abnormal enamel microstructure associated with hypoplastic enamel defects in human teeth. Am. J. Phys. Anthropol. 136, 400–414.
Wright, L.E., Schwarcz, H.P., 1998. Stable carbon and oxygen isotopes in human tooth enamel: identifying breastfeeding and weaning in prehistory. Am. J. Phys. Anthropol. 106, 1–18.
Yeung, A., 2019. The'As Low as Reasonably Achievable'(ALARA) principle: a brief historical overview and a bibliometric analysis of the most cited publications. Radioprotection 54 (2), 103–109.
Ziesemer, K.A., Ramos-Madrigal, J., Mann, A.E., Brandt, B.W., Sankaranarayanan, K., Ozga, A.T., Hoogland, M., Hofman, C.A., Salazar-García, D.C., Frohlich, B., Milner, G.R., Stone, A.C., Aldenderfer, M., Lewis Jr., C.M., Hofman, C.L., Warinner, C., Schroeder, H., 2019. The efficacy of whole human genome capture on ancient dental calculus and dentin. Am. J. Phys. Anthropol. 168, 496–509.