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Published

2022

Journal Title

Quaternary International

Version

Accepted Manuscript (AM)

DOI

[10.1016/j.quaint.2022.02.027](https://doi.org/10.1016/j.quaint.2022.02.027)

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Best practices for selecting samples, analyzing data, and publishing results in isotope archaeology

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Abstract

Isotopic analysis has become one of the most popular arenas of archaeological science, in part due to its versatility to uncover intriguing insights from a range of organic and inorganic archaeological materials. However, alongside an increase in popularity, the field has seen the rise of dissemination of publications that do not pass quality control, do not apply robust interpretative frameworks, or do not report data in ways that would make them amenable to critical evaluation or inclusion in large meta-analyses. This paper represents an effort to clarify some of the most pressing weaknesses and misconceptions in ‘traditional’ applications of isotopic techniques in archaeology: measurement of stable carbon, nitrogen, and oxygen isotope values of organic and inorganic materials (bulk bone collagen, bulk tooth dentine, seeds; bulk and incremental tooth enamel, molluscan shells), and strontium isotope ratio analysis of tooth enamel and cremated bone. The discussion centers on three key aspects of research:

(1) Selecting samples, with advice on building comparative baselines (or more appropriately ‘baseintervals’) and words of caution on interpreting stable carbon isotope values measured during AMS radiocarbon dating.

(2) Handling data, including tips on exploratory data analysis, graphical visualization, and statistical assessment of differences between groups; with particular reference to the Statement on p -values published by the American Statistical Association.

(3) Reporting results, with advice on using correct terminology and decimal points, calculating measurement precision and accuracy, and communicating results using effective scientific language.

The advice provided in this paper does not cover all aspects of project design and dissemination but will hopefully provide clarification within these three key areas and inspire further discussion of effective and impactful applications of isotopic techniques in archaeology.

Keywords

Isotope archaeology; Palaeodiet; Palaeoclimate; Ancient mobility; Quality control; P-values

1. Introduction

Since J. J. Thomson et al. (1921) first discussed the concept of elemental isotopes 100 years ago, isotopic analyses have gained traction in a wide array of fields including geochemistry, climatology, hydrology, ecology, plant physiology, palaeobiology, bacteriology, and archaeology (Sharp 2017). The first isotopic analyses of archaeological remains in the 1970s aimed at identifying human consumption of non-native maize in North America (Vogel and van der Merwe 1977). Not long after, Ericson (1985) proposed that strontium isotope ratios could be used to trace the geographical origins of human remains. However, it was not until foundational strides were made towards understanding tissue diagenesis and preservation of *in vivo* isotopic composition of archaeological materials (Sullivan and Krueger 1981; Lee-Thorp and Van der Merwe 1987; Masters 1987; Schoeninger et al. 1989), alongside advances in understanding biochemical mechanisms such as routing of dietary isotopes (e.g., Ambrose and Norr 1993) and incorporation of strontium into mineral bioapatite (e.g., Rokita et al. 1993), that isotope archaeology was on track to becoming one of the most popular branches of archaeological science. Today, its wide-ranging applications encompass both ‘traditional’ and rather inexpensive analyses of bulk stable isotope values (e.g., bulk bone collagen, bulk and incremental tooth enamel), as well as more

instrumentally challenging and costlier analyses (e.g., compound specific stable isotope analysis of bone collagen amino acids, high-resolution elemental mapping of tooth cross-sections).

The growth of isotope archaeology was in large part driven by a generation of women scientists including Julia Lee-Thorp, Judith Sealy, Margaret Schoeninger, Noreen Tuross, Marilyn Vogel, and M. Anne Katzenberg. Through their perseverance and adherence to high standards of scientific rigor, these scientists trained a long list of students and produced work that would come to transform the way we ask questions about the human past. Forty-five years after the birth of isotope archaeology, the core principles that characterize these researchers' work—asking meaningful questions, identifying reliable samples, building robust interpretative frameworks, and producing scientifically rigorous publications—remain the cornerstones of impactful research in this field.

This paper forms part of a Special Issue dedicated to the career and mentorship of Julia Lee-Thorp and aims to synthesize useful advice on working with isotopic data in archaeological research. As it is not possible to cover in sufficient detail all aspects of project design in one paper, we have chosen to focus on three key areas: sample selection, data processing, and data reporting. The discussion focuses on the most common applications: stable carbon, nitrogen, and oxygen isotope values of organic and inorganic materials (bulk bone collagen, bulk tooth dentine, seeds; bulk and incremental tooth enamel, molluscan shells), and strontium isotope ratio analysis of tooth enamel and cremated bone. The paper does not aim to expose errors by any particular researchers, nor does it try to generalize about trends in the depth of biochemical/geochemical training that practitioners of isotope archaeology receive. It is simply aimed at outlining where improvements can be made in the production of meaningful and interesting research.

In some parts, this paper reiterates advice presented in Roberts et al.'s (2017a) '*Calling all archaeologists: guidelines for terminology, methodology, data handling, and reporting when undertaking and reviewing stable isotope applications in archaeology*' and Szpak et al.'s (2017) '*Best practices for calibrating and reporting stable isotope measurements in archaeology*'. This paper is meant to serve as a companion to these publications (and others including Fry 2006; Bond and Hobson 2012; Sharp 2017), while providing some more recent advice on:

- Applying quality control (QC) criteria for collagen (Guiry and Szpak 2021), bioapatite (France et al. 2020) and shell (Loftus et al. 2015).
- Using both surface morphology and internal structure of seeds to identify charred plant material likely to provide reliable stable isotope measurements (Stroud et al. in prep); with further suggestions on assessment of sample preservation using elemental composition (Szpak and Chiou 2019).
- Estimating local isotopic baselines (or *baseintervals*) using species unlikely to have been affected by anthropogenic influences.
- Determining the suitability of stable carbon isotope values measured during AMS radiocarbon dating for palaeodietary and palaeoclimate research.
- Using exploratory data analysis and effective graphical visualization to guide statistical analysis and data presentation.
- Reporting statistical analyses in line with the American Statistical Association's recent Statement on *p*-values (Wasserstein and Lazar 2016; Wasserstein et al. 2019).

For a review of basic principles and research questions in isotope archaeology, please see Julia Lee-Thorp's paper *On isotopes and old bones* (2008).

2. Identifying reliable samples

A mass spectrometer will always produce a measurement, no matter the sample preservation or the technical expertise of the operator. For this reason, it is important that researchers invest time in selecting samples that are likely to have retained their *in vivo* (i.e. original) isotopic composition. In this section, we discuss considerations of sample size, sample type, sampling contexts, and preservation potential for the main materials analyzed in isotope archaeology: bones, teeth, seeds, and shells.

Equally important as selecting reliable samples is to confirm that the acquired measurements are reliable. This can be accomplished, with caveats, by assessing the degree to which the chemical composition of the archaeological samples resembles the composition of analogous modern material (Zazzo et al. 2004; Beasley et al. 2014; Loftus et al. 2015; Szpak and Chiou 2019; Guiry and Szpak 2021). Table 1 provides a summary of currently accepted or suggested quality control

(QC) benchmarks for collagen, bone bioapatite, tooth enamel bioapatite, charred seeds, and molluscan shell. It is important to recognize, however, that these criteria are not perfect, and will continue to be refined through further development (see discussion in Cheung and Szpak 2021).

It is also important to note that sample selection requires establishing a compromise between adequate sample size and ethical responsibility to preserve irreplaceable archaeological materials (Pálsdóttir et al. 2019; Squires et al. 2019). Curators are professionally obliged to safeguard archaeological collections and deny access to large sample numbers that may offer little in the way of improved resolution. To justify destructive sampling, researchers should outline reasonable expectations surrounding the isotopic variability in the local ecology and support their desired numbers of samples with statistically meaningful estimates of optimal sample size (carried out using, for example, power analyses). In addition, intentions to sample human remains must not only comply with relevant laws and regulations, but demonstrate the researchers' commitment to responsibly handle sensitive material and involve local communities in the research, particularly when it relates to descendant groups or controversial geopolitical discussions (Atalay 2010; Roberts et al. 2018; Collard et al. 2019).

2.1 Bones

Sampling bones for palaeodietary studies needs to strike a balance between the desired level of detail and the minimum number of samples that will provide a 'best case scenario' for approximating the isotopic means and variability within a given group or population. The level of attainable detail is constrained by the research question and the isotope variability characterizing the relevant ecosystem. For example, projects that aim to assess the overall dietary composition and homogeneity of a given population and projects that aim to assess possible differences in dietary intake between distinct sub-groups (identified by, for example, sex, age, pathologies, or association with distinct burial types, household units or site clusters) (e.g., Müldner and Richards 2007) will require different optimal sample sizes.

Power analysis can be used to estimate the minimum number of samples required to answer a research question (Murphy et al. 2014). It can be used to perform sample size calculations for a range of reasonable standard deviations in order to determine how much the outcomes change as

a result. This information can be used to establish the optimal sample numbers that will be amenable for answering the research question while avoiding redundancy (Pechenkina et al. 2005; Stevens et al. 2006). The indices required to carry out these calculations (likely effect sizes, group differences, and measures of variability such as standard errors) can be obtained from the literature or through pilot studies. They do not have to be precise, since the goal is to obtain a general idea of the sample sizes needed to detect, for example, differences of a specified magnitude.

In their paper *Counting sheep: sample size and statistical inference in stable isotope analysis and palaeodietary reconstruction*, Pearson and Grove (2013) carried out statistical simulations to determine the optimal sampling size for a large dataset of sheep ($n=174$) whose dietary intake was influenced by a combination of anthropogenic and environmental influences. The results showed that when using at least 8 samples per group, the ‘true mean’ of the group was represented in over 90 % of the cases. When using fewer than 8 samples per group, the ‘true mean’ was often not captured. Sample sizes larger than 40 presented minimal improvement to the true mean estimation (< 0.3 %). While sample sizes between 8–40 samples may prove ideal for estimating group means, it is important to remember that estimation of group variability may require larger sample sizes (Syväranta et al. 2013). Additionally, not all samples may pass quality control assessment and may thus yield unreliable results. For this reason, we recommend sampling slightly above the optimal number of samples (per species or group).

Isotopic values of faunal remains can be used in palaeodietary reconstructions to estimate the underlying ecological ‘baseline’ values (discussed more in Section 2.6) or to investigate questions pertaining to the management and dietary patterns of the animals themselves (e.g., Cucchi et al. 2016; Nitsch et al. 2017). As with sampling human remains, faunal sampling strategies should consider the benefit of carrying out power analyses that capture estimates of possible sources and magnitudes of variability. Since it cannot be assumed that animals from spatially or chronologically distinct horizons had the same diets, it is advisable to carry out test runs to assess the variability in the isotopic values of fauna from distinct contexts or levels.

If planning to reconstruct dietary food-webs using isotope mixing models (e.g. Parnell et al. 2013; Fernandes 2016), careful consideration should be given to identifying and quantifying possible

sources of variability within the consumers, such as intra-individual variability in biological tissues and inter-individual differences in metabolic rates (Cheung and Szpak 2021; Hyland et al. 2021). Additionally, variability within the food items themselves (caused by, e.g., manuring, cooking and fermenting; Fraser et al. 2011; Royer et al. 2017) and limitations posed by biased preservation of certain food items should also inform sample selection and data interpretation. For detailed advice on sampling and using dietary mixing models, we suggest that researchers follow the best practices outlined in Cheung and Szpak (2021).

Unequal representation of distinct elements in skeletal assemblages often poses a limitation to identifying the desired Minimum Number of Individuals (MNI) from each archaeological context of interest. When skeletal assemblages are large (e.g., the faunal assemblage at Neolithic Makriyalos, northern Greece), the issue of sampling unique individuals more than once can be circumvented by selecting bones of a single element and body side for each taxon. At Makriyalos, Paul Halstead and his zooarchaeological team determined that selecting *humeri* from domestic herbivores and *radii/tibiae* from red deer would provide the largest MNI for each taxon (Vaiglova et al. 2018). At sites with smaller/less well-preserved bone assemblages (e.g., Kouphovouno, Vaiglova et al. 2020a), it may not be possible to only sample one element. In this instance, the researchers should make the best effort to minimize the possibility of sampling an individual more than once while achieving the coverage necessary to address the research question. In both scenarios, including information about the samples (element, side, element portion, and other relevant information, e.g., Balasse et al. 2013) can help demonstrate the researchers' confidence in having sampled distinct individuals.

Preservation of bones is determined by the ability of the organic and inorganic phases to survive in distinct climatic and pedogenic contexts. The organic phase is primarily composed of collagen, a protein that can remain intact in archaeological contexts up to 100,000 years old (Jones et al. 2001), unless an archaeological site is located in the tropics, or in regions with very hot/dry summers and wet winters. Because bone collagen enables measurement of both stable carbon and nitrogen isotope values, it has become the preferred sampling material for palaeodietary studies; although it primarily provides information about the protein component of consumers' diets (Sullivan and Krueger 1981; Ambrose and Norr 1993; Lee-Thorp 2008).

With its high porosity and low crystallinity, the inorganic bone mineral bioapatite can become significantly altered through long-term interaction with soil (Lee-Thorp 2008; Clementz 2012). The surface area of bone is amenable to bicarbonate adsorption (Koch et al. 1997), which can likely alter the stable carbon ($\delta^{13}\text{C}$) and oxygen ($\delta^{18}\text{O}$) isotope composition of bone apatite. Nevertheless, bone bioapatite can provide reliable stable isotopic measurements when it follows “a pathway to preservation, rather than to destruction” (Lee-Thorp and Sponheimer 2003, p. 210). This can happen in dry burial environments which favor slow removal of collagen with relatively fast increase in crystallinity, leading to million-year-old bone resembling enamel in its crystallographic features (Lee-Thorp and Sponheimer 2003). Thus, preservational context plays a role in the potential of bone mineral to retain its *in vivo* stable isotopic composition and should be carefully considered when identifying bones suitable for isotopic analysis.

Strontium, a mobile trace element in soils and sediment, can easily substitute for calcium in bone bioapatite to the point where it becomes impossible to distinguish between *in vivo* strontium and contamination from soil. Experiments have shown that bones that are calcined (i.e. heated to temperatures that turn them white) can preserve their *in vivo* strontium isotope ratios, $^{87}\text{Sr}/^{86}\text{Sr}$, (Snoeck et al. 2015), but not their $\delta^{13}\text{C}$ or $\delta^{18}\text{O}$ values (Snoeck et al. 2016). For this reason, while calcined bone may provide reliable samples for strontium isotope ratio measurements in studies of residential mobility, non-calcined bone should be avoided or used only to determine local baseline $^{87}\text{Sr}/^{86}\text{Sr}$ ratios.

2.2 Tooth enamel

Most commonly applied strategies for sampling tooth enamel include ‘bulk’ sampling (i.e. one measurement per individual) or incremental sampling (i.e. obtaining a series of samples along the axis of tooth development). Bulk enamel samples (e.g., Roberts et al. 2015) approximate the average value of all isotopic inputs over the course of tooth formation. This can provide a way to compare multi-seasonal averages between individuals. Similarly to sampling bone collagen (discussed above), power analyses can be useful for determining the optimal number of samples required to answer the research question (Pearson and Grove 2013; Syväranta et al. 2013; Cheung and Szpak 2021).

Incremental sampling produces a sequence of samples that can be used to assess sub-annual changes in isotopic inputs throughout tooth formation (Balasse 2003). The temporal resolution of these sequences varies depending on the sampling technique. Manually sampled sequences (extracting 1mm-wide samples spaced ~1mm apart, e.g., Balasse 2012; Janzen et al. 2020) provide attenuated values averaged over several months of tooth formation (Balasse 2003; Zazzo et al. 2005; Pederzani and Britton 2019). Micro-drilling with laser-ablation mechanisms (e.g., Copeland et al. 2008; Pryor et al. 2020) provide higher resolution time slices averaged over a few months, while targeted micro-drilling using histological maps of developmental features (i.e. daily growth lines) can approximate isotopic inputs with weekly resolution (e.g., Austin et al. 2016; Smith et al. 2018). Due to budgetary constraints, incremental sampling often necessitates analysis of fewer individuals than bulk sampling. For example, while Roberts et al. (2015) were able to analyze 300 individuals with bulk sampling, incremental sampling was more restrictive: Janzen et al. (2020) analyzed 60 individuals, Pryor et al. (2020) analyzed 16 individuals and Smith et al. (2018) analyzed 8 individuals. For this reason, incremental tooth enamel sampling is most amenable for answering questions of individual or group, rather than population-wide, life histories.

A major consideration of sample selection is the likelihood that a tooth preserves a complete annual sequence representative of the full range of variability in seasonal isotopic inputs. Partial sequences from highly worn teeth may represent incomplete annual cycles, and the absence of ‘real’ maximum or minimum values may preclude the ability to determine which seasons are represented. For example, of the 32 teeth drilled from Byzantine sites in the Negev desert (Vaiglova et al. 2020b), 10 teeth provided incomplete sequences, which are shown in Supplementary Material 2. Without being able to determine which seasons are represented, inclusion of these partial sequences in the analyses would lead to skewed results (e.g., over-representation of summer values and under-representation of winter values). To overcome this problem, only teeth with full annual sequences should be included in inter-teeth comparisons. If it is possible to determine which seasons are represented (e.g., by identifying a clear sinusoidal ‘peak’ or ‘trough’; also shown in Supplementary Material 2), partial sequences may be used to compare seasonal values. Analyzing teeth that complete mineralization over several annual cycles (e.g., horse teeth, Hoppe et al. 2004: Table 2) may also provide skewed results if different specimen

provide different numbers of annual cycles due to variability in tooth wear. To overcome this issue, fixed lengths of teeth crowns (e.g., 3cm for horse teeth, Britton et al. 2019) can be drilled to keep the temporal resolution as constant as possible.

The choice of tooth is constrained by the timing of tooth mineralization and the ability of different teeth to answer the research question. Unlike bones, which remodel during the lifetime of individuals, the elemental composition of teeth remains generally constant once formation is complete (Pederzani and Britton 2019). The precise timing of tooth development is primarily determined by species and tooth type. Earlier forming teeth (e.g., first molars, M1) can provide information about initial life patterns such as cessation of nursing, while later forming teeth (e.g., third molars, M3) record dietary inputs in later years (Weinreb 1964; Balasse et al. 2001; Hillson 2005). The duration of mineralization also determines if the tooth will record full annual sequences or not. For example, due to their shorter lengths and faster mineralization schedules, pig molars do not record full annual sequences and are thus not the tooth of choice for studying pig seasonal dietary and mobility patterns. These objectives can instead be accomplished by incrementally sampling their longer-forming incisors (Frémondeau et al. 2012; Zhang et al. 2021).

Unlike bone apatite, tooth enamel is relatively robust to diagenesis (Zazzo et al. 2004; Lee-Thorp 2008). Its higher crystallinity largely prevents exogenous trace elements like strontium from being incorporated deep inside the mineral structure (Macfadden and Cerling 1996; Snoeck et al. 2015). More recent research, however, has revealed that tooth enamel is not always as structurally robust as we routinely assume (Wood et al. 2021; Weber et al. 2021). Quality criteria outlined in Table 1 can help assess the integrity of tooth enamel for isotopic analysis prior to analysis of the results. For high-resolution analyses that employ instruments such as a Laser Ablation Inductively Coupled Plasma Mass Spectrometer (e.g., Sponheimer et al. 2006), or Secondary High Resolution Ion Microprobe (e.g., Aubert et al. 2012), elemental mapping of trace elements like uranium can be used to identify portions of enamel that may have undergone diagenetic alteration. This information can help target regions that are most likely to provide *in vivo* composition.

Depending on the questions, researchers working with tooth enamel may choose to analyze either $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of structural carbonate or $\delta^{18}\text{O}$ values of enamel phosphate. Enamel

phosphate is more resistant to diagenetic alteration (Zazzo et al. 2004), and is the preferred material for addressing questions of palaeoclimate. On the other hand, matching $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values of structural carbonate makes these samples amenable to addressing questions of both seasonal mobility and dietary intake (Makarewicz and Pederzani 2017; Pederzani and Britton 2019).

2.3 Charred plant remains

Sample selection for plant isotope analysis is also constrained by both the research question and the depositional history of the assemblage. Seeds from primary *in situ* contexts (e.g., storage deposits) can provide information about growing conditions on single years or locations (e.g., Styring et al. 2016). Seeds derived from secondary contexts necessarily provide an averaged indicator of cultivation conditions that can span multiple years or locations (e.g., Vaiglova et al. 2020a). Seed samples can either be obtained in bulk (i.e. several seeds from an individual archaeological context) or as individual single-seed samples, and the choice depends on the research aims. Single seed analysis can be useful for assessing inter-crop variability within sampling contexts, and to avoid the possibility of averaging crop growing conditions across different cultivation events. However, this analysis may not be feasible in cases where the amount of nitrogen (% N) of small samples is too low for reliable analysis of stable nitrogen isotope values ($\delta^{15}\text{N}$) (Szpak and Chiou 2019).

Bulk sampling is effective if the seeds derive from primary contexts where it can be assumed that the crops represented were cultivated in the same year; and this only applies to species of annual plants. It can also be an appropriate sampling strategy if the assessment of inter-plant variability is not crucial for answering the research question. Experimental research on modern crops from a single growing season and treatment condition suggests that analysis of homogenized bulk samples of ~10 cereal grains register variability of $\pm 0.5\text{‰}$ in $\delta^{13}\text{C}$ values and $\pm 1.0\text{‰}$ in $\delta^{15}\text{N}$ values, while analysis of smaller numbers of grains (i.e., < 5) show larger variability (Nitsch et al. 2015). Therefore, to assess the variability between growing conditions represented in single *in situ* deposits or storage contexts, we recommend that researchers aim to obtain several (ideally more than 3) bulk samples per context.

The most common way seeds are preserved in the archaeological record is via charring, although uncharred or lightly toasted seeds have been found in rare waterlogged, frozen, or desiccated contexts. Outside of these rare preservation conditions, seeds need to be exposed to temperatures above ~215 °C to become highly resistant to microbial attack and survive long-term burial. Seeds charred above ~400 °C become highly distorted and unamenable to species identification (Charles et al. 2015). Because of their highly porous structure, charred material can absorb high amounts of strontium from soil and reliable application of $^{87}\text{Sr}/^{86}\text{Sr}$ ratio analysis for provenancing archaeological plant material is currently highly problematic (Styring et al. 2019).

For analysis of stable carbon and nitrogen isotope values, selection of charred seeds requires the ability to assess the effects that charring has had on the *in vivo* isotopic composition of the material. Experimental studies have shown that temperature, and to a lesser degree duration, alter both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of cereal grains and pulse seeds (Nitsch et al. 2015; Stroud et al. in prep). These experiments have also shown that the isotopic values of seeds charred between 215–300 °C are altered in ways that can be modelled and corrected (Nitsch et al. 2015; Stroud et al. in prep). Ascertaining the temperature at which seeds have been charred requires visual inspection of both the surface morphology and the internal seed structure (cf Charles et al. 2015, Stroud et al. in prep). Assessment of the internal matrix is crucial, as recent experiments have shown that even cereal grains whose surface morphological characteristics suggest that they are ‘ideally’ preserved can be completely void on the inside (Stroud et al. in prep). Because the preservation potential of cereal grains can vary within and between archaeological contexts, plant material should be assessed on a grain-by-grain basis to ensure that only grains that are sufficiently preserved are measured and interpreted (Stroud et al. in prep). To demonstrate confidence in sample reliability, we suggest that researchers publishing plant stable isotope results provide access (in supplementary materials or online databases) to photographs of each analyzed seed that is included in the analysis.

The isotopic and morphological changes caused by charring are currently only understood for a relatively small number of cereals: emmer (*Triticum dicoccum* (Schrank) Schübl.), einkorn (*Triticum monococcum* L.), hulled barley (*Hordeum vulgare var distichum* L.), bread wheat (*Triticum aestivum* L.), spelt (*Triticum spelta* L.), oat (*Avena sativa* L.), rye (*Secale cereale* L.), maize (*Zea Mays* L.), and one millet species: pearl millet (*Pennisetum glaucum* L.); and two pulse

species: lentil (*Lens culinaris* Medik.) and pea (*Pisum sativum* L.) (Nitsch et al. 2015; Charles et al. 2015; Styring et al. 2019; Hart and Feranec 2020; Stroud et al. in prep). For this reason, before further charring experiments are carried out, caution should be taken when interpreting isotopic data derived from species for which the effect of charring is unknown.

2.4 Molluscan shell and eggshell

Shells of aquatic and terrestrial molluscs preserve valuable short-term and incremental records of palaeoenvironmental parameters such as water temperature and C₃/C₄ plant abundance (Goodfriend 1992; Johnson et al. 1998; Andrus 2011). However, aspects of the animal's life history, habitat, and physiology may incorporate systematic biases and offsets into the preserved environmental record (Schöne 2008; Ivany 2012). An animal typically slows down or speeds up shell production under particular environmental conditions (e.g., temperature, food availability) or physiological circumstances (e.g., reproductive cycle, recovery from predatory attacks), resulting in those periods becoming either over- or underrepresented in their internal record. Additionally, an animal's behavior may change over the course of its life. For example, many marine molluscs will move to higher or lower regions within the intertidal zone as they grow larger (and thus record a changing temperature range across their shell), or their growth rate will slow down with age (Schöne 2008). As these factors are unique to each species, it is important to carefully consider the ecology and biology of the given species. A robust study of shells from modern and well-characterized environments can help to assess the sampling resolution required to attain the desired temporal resolution of the temperature record (e.g., daily, monthly, annual), as well as to evaluate any systematic biases (e.g., seasonal, ontogenetic). It can also provide information on any inter-individual variation caused by differences in life histories and microhabitats, which should be taken into consideration when deciding how to apportion samples within individuals or entire assemblages (e.g., Galimberti et al. 2017).

In addition to validating the usefulness of particular species for stable isotope studies, shell samples need to be assessed for preservation (Leng and Lewis 2014). Carbonate shells are prone to dissolution and recrystallisation in many depositional settings where water is present in the sediments. Unfortunately, the process of recrystallisation may overwrite the original environmental signal recorded in their $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values, and these shells should be regarded

as unsuitable for stable isotope analysis (Brand 1989). Additionally, cooking practices may have also altered the shell structure, such that burned or heated shells must likewise be avoided (Milano et al. 2016). For these reasons, it is necessary to evaluate the crystal microstructure of individual shells, especially where preservation varies considerably across a site.

Visual examination of sectioned shells under a microscope can quickly reveal changes to shell structure, with recrystallized shell looking amorphous and ‘flaky’, rather than highly crystalline. Many aquatic species and almost all terrestrial species precipitate part or all of their shell in the aragonitic polymorph of carbonate, as opposed to calcite (Lowenstam 1954; Goodfriend 1992). As recrystallized shell is always calcitic, and the two polymorphs are simple to distinguish with a range of analytical chemistry methods (i.e. XRD, Raman spectroscopy, chemical staining), assessment of the presence of aragonite and calcite offers a convenient diagenetic check during sample selection. The methods, however, vary considerably in terms of how destructive and/or quantitative they are. FTIR (Fourier Transform Infrared Spectrometry) with an ATR (Attenuated Total Reflectance) attachment offers a suitable balance of analytical speed and simplicity, damage to the sample, and polymorph quantification (Loftus et al. 2015). This enables each powdered sample to be assessed for diagenesis prior to stable isotope analysis.

2.5 Samples obtained during AMS radiocarbon dating

In radiocarbon dating laboratories, $\delta^{13}\text{C}$ values are measured alongside radiocarbon determinations for several reasons: 1) to correct for reservoir effects which may have shifted the measured age away from the true age (e.g., marine reservoir effect, Knipper et al. 2020), 2) to correct for fractionation to the $^{14}\text{C}:^{12}\text{C}$ ratio that occurred during the graphitization stage of sample preparation, and 3) to systematically normalize all radiocarbon measurements to $\delta^{13}\text{C} = -25.0\text{‰}$, even if they have not been influenced by reservoir or graphitization fractionation effects (Stuiver and Polach 1977; Reimer et al. 2004). These measurements are performed either directly on the Accelerator Mass Spectrometer (AMS) or by directing a portion of the CO_2 gas from the AMS to the Isotope Ratio Mass Spectrometer (IRMS). However, these measurements are often not usable for palaeodietary/palaeoclimatic interpretations, or for identifying small differences between samples (e.g., $< 0.2\text{‰}$) for three reasons (personal communication: Richard Staff, Scottish

Universities Environmental Research Centre; David Chivall and Peter Ditchfield, Oxford Radiocarbon Accelerator Unit).

First, the graphitization stage during sample preparation induces fractionation, which essentially alters the $^{13}\text{C}:^{12}\text{C}$ ratio by an indeterminate amount. Secondly, much larger samples are injected into the AMS than would be suitable for IRMS and this can push the IRMS to its limit, potentially introducing error. Third, the AMS $\delta^{13}\text{C}$ values are often normalized using a one-point rather than two- or multi-point calibration, which leads to lower measurement accuracy. At the Oxford Radiocarbon Accelerator Unit, the uncertainties attached to AMS $\delta^{13}\text{C}$ measurements are not calculated, but the system flags measurements only when the $\delta^{13}\text{C}$ value obtained during combustion for graphitization and the $\delta^{13}\text{C}$ value obtained by the AMS differ by $> 5.0\%$. For this reason, when clients request accompanying $\delta^{13}\text{C}$ values, separate aliquots of the samples are prepared for analysis using an IRMS, where the uncertainty is often better than 0.5% . Altogether, stable carbon isotope values produced via AMS dating are not the same as those obtained on an IRMS. Researchers should thus only use AMS $\delta^{13}\text{C}$ values for palaeodietary/palaeoclimatic research when their accuracy and precision can be determined and when the measurement error fits within the expected uncertainty for answering the research question.

2.6 Final word on sampling: disentangling natural from anthropogenic factors

The extent to which isotopic measurements of archaeological materials can shed light on questions of archaeological interest is constrained by our ability to isolate the effects of human behavior from the effects of environmental factors that may have simultaneously influenced the chemical composition of the sample material. For interpreting strontium isotope ratios, comparative maps of biologically available strontium can be built by modelling the spatial distributions of $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of modern plants, surface water, snail shells, insects (Evans et al. 2010; Snoeck et al. 2020; Wang et al. 2021; Holt et al. 2021). For interpretation of archaeological stable carbon and nitrogen isotope values, the ecological background can be approximated through measurement of specimens with low likelihood of impact from anthropogenic factors (e.g., wild species, unmanaged landscapes). However, the identification of non-anthropogenically influenced samples is fraught with many challenges.

Firstly, rather than focusing on estimating average *baseline*, we should instead focus on approximating a range (or ‘*baseinterval*’) of local background values. *Baseintervals* will be able to capture both the natural variability within the given ecology and the measurement error accompanying the isotopic measurement (e.g., Stroud et al. 2021). Secondly, *baseintervals* should not be thought of as static. Since isotope ecologies are determined by a range of dynamic factors including precipitation patterns, soil dynamics, and distribution of local vegetation and faunal species, they may have changed on annual, decadal, centennial, or millennial scales (Gannes et al. 1997). These changes would have caused reverberating effects through the food chain and influenced the isotopic composition of food sources (primary and secondary producers) as well as consumers in distinct trophic levels. Measurements of reference samples that are as contemporary as possible to the samples of interest are therefore key for demonstrating that observed patterns in isotopic data are most likely to be the result of changes in human behavior rather than shifts in the underlying ecology.

Thirdly, multiple plant and animal species may be necessary to capture the isotopic variability of specific vegetation communities, ungulate dietary niches, or human food production systems (Codron et al. 2011, 2013). This is where questions may arise regarding whether or not the comparative species may have been impacted by anthropogenic factors, especially in archaeological contexts where intensive agriculture or landscape management was pursued. For example, wild herbivores may have raided crop fields, and their bone collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values would thus reflect the consumption of both natural forage and plants that may have been influenced by manuring, irrigation or crop selection (e.g., Gillis et al. 2020). Human activities such as woodland clearance may have forced wild herbivores to graze in open environments with higher plant $\delta^{13}\text{C}$ values compared to those in closed–canopy woodlands (Bonafini et al. 2013). Additionally, weeds may have grown in arable cultivation plots while nut or fruit trees may have been planted on the edges of managed fields, where they may have been similarly influenced by human activities that impact the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ composition of soil.

To identify faunal species most appropriate for building reference *baseintervals*, it is important to consider the specific dietary adaptations (e.g., browsing vs. grazing) and physiological requirements (e.g., obligatory vs. non-obligatory drinking) of the available species. Additional

considerations include questions of population size, forage availability, and predation pressure, as these may have influenced dietary niche size and composition and caused directional isotopic changes to wild animal populations over time (Codron et al. 2011).

Identifying comparative plant species needs to take into account both the likelihood that they grew in managed soils, as well as possible isotopic variability between distinct plant parts and growing seasons (Tieszen 1991; Fraser et al. 2011; Cernusak et al. 2013). Although the directional effects of certain crop management systems (e.g. application of farmyard manure or other organic waste, small or large-scale systems of water management) have been demonstrated experimentally (Bogaard et al. 2007; Fraser et al. 2011; Wallace et al. 2013; Flohr et al. 2019; Jones et al. 2021), the magnitude of the effects would be challenging to estimate in environments where experiments have not been carried out. For example, using the manuring offset of crops that were grown in Britain, Germany, and Romania (Fraser et al. 2011) to estimate the degree of manure application on crops grown in South Africa would be inappropriate, because the two environments are not directly comparable due to differing climate, soil types, and preservation potential of archaeological materials.

With these caveats in mind, sampling for comparative baseintervals should aim to capture, to the best of a researcher's ability, the full range of isotopic variability within a given ecosystem. Interpretations of measured archaeological values should then be constrained by predictions surrounding the extent to which the measured species may have been influenced by natural and anthropogenic factors (Roberts et al. 2017b; Knipper et al. 2020; Gillis et al. 2021). Additional lines of evidence, such as pollen records, patterns of agro-pastoral intensification and extensification (Styring et al. 2017; Price et al. 2020; Marston 2021), human landscape modification (Rull et al. 2021), and local-scale environmental change (Roffet-Salque et al. 2018) can furthermore be used to clarify the mechanisms that may have influenced the isotope composition of archaeological flora, fauna, and human remains.

3. Using exploratory data analysis to guide formal analysis

Exploratory data analysis is an important part of any analysis and should be carried out prior to formal analysis through inspection of graphical representation of the data. Simple univariate plots

such as histograms, boxplots, and violin plots can shed light on the behavior of individual variables, while scatterplots can elucidate the connections between them. Exploratory data analysis should not be a rote exercise, but should instead involve a thoughtful investigation of the data. Many books have been written about the principles of good data visualization (e.g., Cleveland 1994; Wilkinson 2005) and packages such as `ggplot2` in R (Wickham 2016) provide a variety of flexible tools for effectively exploring data.

3.1 Suggestions for effective data visualization

Simple aesthetic adjustments and choices can have a large impact on the readability of figures. One does not need to be proficient with coding nor to use programs like R and Python to produce graphs that are aesthetically pleasing and easily readable. Here we outline some of the most relevant advice for visualization of isotopic data; referenced examples are reproduced in Supplementary Material (SM) 1.

(a) Plot the data in ways appropriate for addressing the research question. If the objective is to assess how much variation there is between individual data points, standard deviation is most effective for showing the spread of the data. If the objective is to assess how good the estimation of the group mean is, standard error is more appropriate. Boxplots, either individual or plotted side by side to show group comparisons, can highlight important and interesting patterns in datasets. For archaeological data which may be non-normally distributed (bimodal, skewed), violin plots may be more informative than boxplots (e.g., Cavazzuti et al. 2019; figure reproduced in SM 1.A). Bivariate scatterplots (e.g., Levin et al. 2011; reproduced in SM 1.B), especially when enhanced by scatterplot smoothers (such as loess curves, e.g., Wierzbowski et al. 2017; reproduced in SM 1.C), are an effective way of exploring the relationships among variables. The idea can, with care, be extended to rotating three-dimensional plots that may reveal relationships that are not evident from collections of bivariate plots alone (see, for example, Wainer 2007, pp. 126–127).

Crucially, the most appropriate method of visualization will depend on the relationships being explored. For example, with intra-tooth sequences of isotopic values, one can look at the *shape* of the sequences to gather information about seasonal variability in dietary inputs (e.g., Vaiglova et al. 2018; reproduced in SM 1.D), the *intra-tooth variability* to assess the magnitude of variation in

dietary inputs between different individuals (e.g., Vaiglova et al. 2020b; reproduced in SM 1.E), or the *position* of different parameters (such as the maximum $\delta^{18}\text{O}$ value) on the tooth axis (e.g., Balasse et al. 2021; reproduced in SM 1.F). Figures can thus be used appropriately to highlight these relationships of interest. See Weissgerber et al. (2019) for advice and examples of matching visualization to research questions.

(b) Do not use randomly assigned combinations of symbols and colors. Looking at the spread of data to see which species, groups, or individuals overlap allows researchers to choose symbols based on what will make the data points most easily discernible. The size of plotting symbols can often be controlled (made smaller or larger), while color can be shaded and made more or less transparent. This can be used to the researcher's advantage, yielding more flexibility beyond the software defaults. For example, in the dataset presented in Vaiglova et al. (2021; reproduced in SM 1.G), many human datapoints overlapped with those of dogs and pigs. Thus, the authors chose to use different colors, rather than symbols, to represent these three species. Where less overlap was visible (between humans and cattle/sheep/goats), different symbols were used. Altogether, only 5 colors were used to represent 18 species, and the color-symbol combinations were used in ways that led to least overlap between similar symbols.

(c) Keep graphs as simple as possible. Overcrowding figures with too many overlapping groups may obscure actual patterns of interest from being discernible. For example, if individuals from distinct age or sex categories have meaningfully different isotopic values, it is useful to display them separately. However, if their values are not meaningfully different, they can be combined into fewer groups to prevent the reader's eye from trying to detect patterns where they do not exist. Where a large number of units are chosen to be displayed, an effective strategy is to group them into clusters that share the same symbol, and that are further differentiated with different colors (e.g., Knipper et al. 2017; reproduced in SM 1.H)

(d) Make graphs readable to people with color vision deficiency. Millions of people around the world suffer from color vision deficiency (CVD), with prevalence for just one type of deficiency (red-green) reported to be between 4–8% in men and ~0.4% in women (Birch 2012). To make publication figures as readable as possible to the wider audience, we suggest that researchers use

CVD-friendly palettes to prepare their graphical images, and run them through a simulator (e.g., <https://www.color-blindness.com/coblis-color-blindness-simulator/>) to check that they are compatible with different types of deficiencies. If CVD-friendly palettes are not used, using black borders around symbols (as opposed to colored borders) makes the colors more discernible. The combination of green and red is most commonly challenging, but different shades of purples and oranges can work well with the full range of color deficiencies. Vaiglova et al. (2021; reproduced in SM 1.G) used the IBM palette to make patterns in a scatter plot of $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$ values visible on a yellow-shaded background. If combinations like green and red are used, the groups can be distinguished using different symbols (e.g., Levin et al. 2011; reproduced in SM 1.B), but this only works with small numbers of groups.

(e) Use legends to your advantage. Many researchers prefer to spell out the graphical parameters of figures using caption descriptions such as “Samples from Area A are shown with closed diamonds (\blacklozenge , solid regression line), samples from Area B are shown with open diamonds (\blacklozenge , dashed regression line)”. We argue that it is considerably easier for readers to discern the aesthetic assignments of graphs by looking at legends rather than reading long descriptions in captions. Vaiglova et al. (2020b; reproduced in SM 1.E) provide an example of how nested legends can be used to describe the aesthetic assignments of several variables (species, chronological period, site, material measured) for separate panels, and for all panels combined.

4. Formal analysis: do not focus on statistical significances

For decades, statisticians and researchers in many fields of science have decried the rote application of null hypothesis testing, specifically the aspect of it that compares a computed p -value to an arbitrary threshold (often 0.05 or 0.1) and declares the result “statistically significant” or not. The p -value itself is prone to many misconceptions (see for example Goodman 2008; Greenland et al. 2013), while statistical significance is often confused with practical significance (i.e., those results that are of scientific importance). This way of thinking has become entrenched to the point that publication can hinge on whether or not results achieve statistical significance, in turn opening the door to various questionable research practices such as p -hacking (Simmons et al. 2011; Head et al. 2015).

In 2016, the American Statistical Association (ASA) responded to these worrying and widespread misuses of statistical testing by issuing a ‘Statement on p -values’ (Wasserstein and Lazar, 2016). The statement provides important clarifications on what p -values do and do not measure, what they do and do not represent, and what they should and should not be used for. The statement includes six principles; commentaries from the drafters of the statement (not all of whom agreed with the final version) are available as online supplements. Subsequently, in 2019, *The American Statistician* published a Special Issue on moving to a world beyond ‘ $p < 0.05$ ’ with articles espousing a range of views on the controversies now swirling around this most common of statistical tools. The lead editorial to the Special Issue (Wasserstein et al. 2019) called for the abandonment of strict thresholding of statistical quantities (such as the p -value) as well as of the use of the term “statistically significant.”

Drawing on these debates, this section aims to provide some of the most pressing clarifications on the uses and misuses of statistical significance testing in isotope archaeology. It outlines advice on using the most common statistical test applied to isotopic data: comparing meaningful differences between groups (e.g., human groups distinguished by age, sex, social status, type of burial, or between different plant or animal species). While we do not wish to advocate for the abolishment of p -values, we strongly reiterate the statistical community’s advice against 1) using the term ‘statistically significant difference’ (and related practices such as using boldface or asterisks in a table to highlight specific groups) and 2) employing ‘significance thresholds’ (such as 0.05 and 0.1), which can inaccurately distinguish between p -values of 0.051 and 0.049 (values that are, in fact, not meaningfully different).

4.1 How do we test for meaningful differences between groups?

In a study investigating ancient agricultural management at the Neolithic site of Kouphovouno, southern Greece, Vaiglova et al. (2020a, p. 43) assessed differences in $\delta^{13}\text{C}$ values between four main crop species (free-threshing wheat, hulled barley, einkorn and pea). The paper reported:

The data for the four main species (where $n > 1$) are normally distributed (Shapiro-Wilk test, $W = 0.95$, $p = 0.09$) and homogenous (Levene’s test, $F(3,38) = 1.12$, $p = 0.35$), so an ANOVA test was used to assess statistically significant differences between the mean $\delta^{13}\text{C}$ values of the four groups. The results indicate that there are significant differences ($F(3,38) = 25.26$, $p < 0.01$) and

a post hoc Bonferroni test reveals that the differences are between free-threshing wheat and hulled barley ($p < 0.01$), free-threshing wheat and pea ($p < 0.01$) and einkorn and pea ($p = 0.004$).

While this example does conform to many current conventions on statistical reporting, it is incomplete in important ways. For example, the actual means and their standard errors are missing. Instead, the authors provide only test statistics and (sometimes thresholded) p -values. This leaves the reader unable to judge if the differences among the groups are of scientific interest and deserving of further exploration; or what magnitude of difference would even be meaningful in this context. Tests for normality and homogeneity (especially the former) are popular in many fields. Strictly speaking, they may not be necessary, since the t -test and analysis of variance are relatively robust to minor departures from assumptions under mild conditions (Miller 1986). Furthermore, even if preplanned as part of the analysis, these tests would still need to be included in any corrections for multiple testing (to preserve overall Type I error control), thereby detracting from the comparisons of main interest. And, of course, each decision made on the basis of a hypothesis test is an opportunity for additional errors.

Rather than focusing on the test statistics and their p -values, along with accompanying statements about statistical significance, a more appropriate approach would be to provide plots showing the data distribution in each group along with the point estimates (i.e. mean differences) and their associated confidence intervals (CI). Instead of focusing on whether or not these intervals include zero, the researchers should define and focus on the range of “compatible” values by stating, for example, what differences would be meaningful to interpret. In this way, a richer engagement with the data can be presented, replacing the dry and arbitrary assessments of statistical significance, or lack thereof.

Following this advice, a more accurate summary of the analysis of the differences in $\delta^{13}\text{C}$ values between the crops at Kouphovouno is as follows:

Figure 1 shows the distribution of the $\delta^{13}\text{C}$ values of the four main crops in the assemblage, where mean $\delta^{13}\text{C}$ values are $-24.3 \pm 0.6 \text{ ‰}$ ($n=15$) for barley; $-22.7 \pm 0.5 \text{ ‰}$ ($n=17$) for free-threshing wheat; $-23.6 \pm 0.8 \text{ ‰}$ ($n=6$) for einkorn; $-25.0 \pm 0.9 \text{ ‰}$ ($n=7$) for pea. Small sample sizes make it difficult to assess normality, however, the crops exhibit somewhat similar levels of variability. A

one-way analysis of variance was conducted to compare the $\delta^{13}\text{C}$ values of the four crops; here, $F(3,38)=25.26$, with a p -value of 0.002, indicates that there are differences in the mean $\delta^{13}\text{C}$ values between the crop species.

To explore these differences further, Tukey's HSD intervals were computed to control for post-hoc multiple comparisons. Results are shown in Figure 2 and Table 2. Pea appears to have lower values than free-threshing wheat, einkorn, and hulled barley. However, inspection of the confidence intervals in the comparison with hulled barley indicates that slightly larger values are also compatible with the collected data and the small number of observations in einkorn make it hard to draw conclusions. Hulled barley has lower values than free-threshing wheat and einkorn, though again the confidence intervals in the latter indicate that slightly larger values are also compatible with the collected data. Finally, free-threshing wheat has higher values than einkorn, although with the small number of observations of these groups, it is hard to be definitive.

*Focusing on isotopically meaningful differences—which we define as > 1.0 ‰ in this context—and keeping in mind the small sample sizes, the distinctions between the two types of wheat (free-threshing wheat and einkorn) and peas can be explained by peas' higher demand for water during the seed-filling period. As wheat and barley grown under the same watering conditions exhibit physiologically-determined differences (with barley having $\delta^{13}\text{C}$ values *c.* 1–2 ‰ lower), the measured difference of -1.6 ‰ (CI: $-2.3, -0.8$) is compatible with the two crops growing under the same watering regime.*

4.2 Further comments on reporting meaningful differences: decimal places and effect sizes

When reporting p -values and other values such as means or test statistics, the question often arises as to how precisely those results should be reported. For p -values, two or three digits after the decimal are usually sufficient, hence 0.049 is preferred to 0.049326. Little important knowledge is gained by the extra trailing digits. On the contrary, unnecessary digits may impart a false sense of precision. Likewise, there is very little to be learned by reporting a p -value of 10^{-16} , or other similarly small numbers, which are in essence artifacts of machine precision. For averages and test statistics, a similar logic applies, and we should refrain from implying more precision than is supported by the data themselves. Ehrenberg (1981) advocates rounding to “two effective digits” to balance the lightening in cognitive load and information loss. In addition, meaningful isotopic differences may not necessarily lie at the highest level of measurement precision, and sample

homogeneity needs to be taken into account when deciding how many decimal places to report and interpret (see more in Section 5.3).

It is good habit to report effect sizes or other meaningful summaries of the data, with their accompanying degrees of freedom, in addition to test statistics. These should have measures of uncertainty attached to them (e.g., standard errors or standard deviations as appropriate). If the data have been transformed for analytical purposes, it can be helpful to bring the results back to the original scale for interpretation. As in the rewritten example above, it is advisable to discuss the effect sizes and the uncertainty in the context of the original data and the scientific questions, rather than simply stating differences to be ‘statistically significant’.

5. Writing scientifically rigorous publications

5.1 Avoiding common misuses of terminology

To ensure that isotope research is easily comprehensible to practitioners working across varying subfields of isotope geochemistry, it is important that the data be disseminated using widely accepted conventions and standardized definitions of terms. Coplen (2011) presents the guidelines for reporting isotope ratio measurements established by the Commission on Isotopic Abundances and Atomic Weights of the International Union of Pure and Applied Chemistry and Roberts et al. (2017a) provides examples of the most common misuses of the terminology in archaeological research. Here we summarize some of the most misused conventions:

1. Isotope ‘delta’ notation (e.g., $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$) is defined as the *relative difference of isotope ratios* between samples and international standards. It is in effect a ‘ratio of a ratio’ and should by convention be referred to as a ‘value’ (and not a ‘ratio’, ‘signature’, ‘composition’ or ‘fingerprint’). Notation that expresses the relative abundance of specific isotopes (e.g., $^{87}\text{Sr}/^{86}\text{Sr}$) should be referred to as a ‘ratio’ (and not a ‘value’, ‘signature’ or ‘fingerprint’).
2. The words ‘enriched’/‘higher’ and ‘lower’/‘depleted’ are not interchangeable. Stable isotope *values* (e.g., $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$) and *ratios* (e.g., $^{87}\text{Sr}/^{86}\text{Sr}$) can be higher or lower, but they cannot be enriched or depleted. Enrichment and depletion describe the abundance of specific isotopes themselves (e.g., ^{13}C , ^{15}N , ^{18}O) and need to specify which samples are

being compared to one another, e.g., “Sample A is enriched in ^{15}N compared to Sample B”.

3. ‰ is permil (parts per thousand), and not equivalent to ppm (parts per million).
4. The terms ‘isotope’ and ‘isotopic’ have distinct uses. ‘Isotope’ is used when the word is modified (e.g., “The stable carbon isotope value”) while ‘isotopic’ is used when it stands alone (e.g., “The isotopic composition” or “isotopic variability”) (Sharp 2017).
5. Negative values should be denoted with an en (–) dash and not a hyphen (-) (Coplen 2011).
6. The ‘delta’ symbol in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ should be italicized as per the convention of denoting quantity symbols (Coplen 2011).

5.2 Transparency in measurement calibration and error estimation are indispensable for establishing data reliability

Two of the biggest inconsistencies in isotope archaeology are the frequency with which publications report how raw isotope values were converted to calibrated δ -(delta) values (even though the choice of calibration method can have a significant impact on the accuracy of the results; Pollard et al. 2011; Szpak et al. 2017) and the level of detail provided in assessing measurement reliability. Precision and accuracy are two distinct indices of measurement error, and both are important for establishing data reliability. Accuracy refers to the ‘trueness’ of the measurements (i.e. how close they are to the true values of the samples), while precision captures the reproducibility of the measurements (i.e. whether repeated measurements on one instrument produce the same results). Yet, most publications in isotope archaeology do not report these two indices, nor do they specify what calibration methods (e.g., one-point, two-point, multi-point calibration) were used to calculate the δ -values. In a review of 487 archaeological publications presenting original isotopic data, Szpak et al. (2017) found that 83% of the studies provided no information about how the δ -values were calibrated, 95% of the studies did not mention accuracy and only 4% of the studies distinguished between accuracy and precision. The authors concluded that “Consequently, it was usually impossible to assess whether or not the value that was reported was a specific marker of *precision*, or some broader catch-all marker of analytical error” (Szpak et al. 2017, p. 612).

It is necessary to move away from the current reporting of measurement error that provides only relatively uninformative statements such as “Measurement uncertainty for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was $< 0.1 \text{ ‰}$ ”. To accurately calculate measurement precision and accuracy, both *calibration standards* and *check standards* should be used consistently in every analytical session and publications should report measurement uncertainty for particular runs, rather than ‘rolling averages’ for a given laboratory (Szpak et al. 2017). This becomes particularly important as archaeological isotope research moves towards larger meta-analyses, where distinctly different measurement errors might render smaller datasets incomparable. Based on recommendations from the Joint Committee for Guides in Metrology (2008) and a series of other studies (p.614), Szpak et al. (2017, p. 614) present a series of best practices for calibrating raw isotope values and calculating measurement uncertainty. They provide a user-friendly Microsoft Excel tool that can calculate precision, accuracy, and a pooled standard deviation of the two for particular analytical sessions, and offer an example paragraph and Online Supplement File showing how to fully report measurement error for a given project.

5.3 The number of decimal places reported should be consistent with both measurement uncertainty and sample homogeneity

Stable isotope values of unknown samples can only be as precise as the reference materials analyzed alongside them. Thus, if the measurement precision is reported as $\delta^{13}\text{C} = 0.1 \text{ ‰}$, sample $\delta^{13}\text{C}$ values should only be reported to one decimal place (Bond and Hobson 2012). However, many archaeological materials do not present the same level of internal homogeneity as the reference materials. For example, in a study of inter- and intra-bone variability (assessed through measurement of multiple skeletal elements from animals representing a wide variety terrestrial, marine, and freshwater species), the mean intra-bone variability was $0.63 \pm 0.06 \text{ ‰}$ and the mean inter-individual isotopic variation was $1.45 \pm 1.15 \text{ ‰}$ (Hyland et al. 2021). Thus, even though precise $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements can be obtained to two decimal places, the second decimal place may not provide any meaningful information. Authors should thus make sure that the reported values are consistent across a publication and that the reported error does not give a false impression about the interpretability of the data. In addition, elemental compositions (%C, %N) should be reported to one decimal place, while C:N ratios should be reported to two decimal places (see discussion in Guiry and Szpak 2021).

Strontium isotope measurements can be precise up to 5 or 6 decimal places (depending on the instrumentation) but in a study investigating intra-individual variation in $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in human tooth enamel, Knudson et al. (2016) found that differences between individual measurements ranged from below detection level to 0.00015. They thus concluded that meaningful archaeological interpretations about palaeomobility using $^{87}\text{Sr}/^{86}\text{Sr}$ ratios can be made at the third or fourth decimal place.

5.4 Writing with clarity and brevity

“A scientific article that presents all of the data and all of the scientific discourse that the author intended to present is not necessarily a successful article. It only becomes one when most of the people who read it can perceive accurately and quickly what the author really meant.” (Lindsay 2011, p. 64). Reading papers that are not written clearly and precisely is laborious and time consuming, and when pressed for time, we often end up not being able to read these papers in detail. This can result in papers that present important and interesting research not receiving the attention and credit they deserve.

Writing clearly and precisely is not necessarily a question of language proficiency. In his book *Scientific Writing = Thinking in Words*, David Lindsay (2011, p. 12) writes:

“Science has a language of its own that has nothing to do with the scientist’s native tongue. It is the language of logic in which reasoned arguments are developed from well-presented evidence and lead to sound and consistent conclusions. That language is the same regardless of the origin and preferred tongue of the person who writes it and good scientific writing depends primarily on expressing the science precisely and clearly. Subsequent editing by a native speaker to tidy up English expressions and comply with modern vernacular is relatively easy and the article will be a good one.”

Numerous authors have written in detail on how to produce high-quality scientific writing (notably Alley 1996; O’Connor 2002; Gustavii 2016). Here we summarize advice from Lindsay (2011) on

how to build a logical structure for a scientific paper, how to achieve clarity and flow by presenting the relevant information in the right order, and how to make simple but powerful stylistic adjustments to enable the reader to grasp the meaning quickly and accurately. It is beyond the scope of this paper to summarize all of the key advice. In a plea for scientific practitioners—both those who are comfortable with writing, and those who find writing challenging—to consult this and other resources, we point out three main pieces of advice.

(a) The Introduction is the “powerhouse of your article” (p.20). It orientates the reader within the wider discussion to which the authors wish to contribute new knowledge. It does not just provide the theoretical or methodological background to the study. It provides the foundation of the logical structure of the overarching argument and sets up the flow with which the Results and Discussion can be delivered effectively. To achieve these goals, writing the Introduction entails following two simple principles (p.21):

1. The hypothesis is key to the Introduction.
2. By justifying the hypothesis logically and scientifically, the writer provides just about everything necessary for readers to understand what the paper is about and why the author wrote it.

Although many writers prefer to write the Introduction last, given its central role in the logical structure of the argument, Lindsay (2011) convincingly demonstrates why it can be beneficial to start writing it first.

(b) The Introduction, Results, and Discussion work together. For the Discussion to be meaningful and convincing, the reader must be prepared (from the Introduction) to understand how the evidence gathered in this study (Results) logically feeds into the argument. To achieve their impact, results should be presented in the order of importance. Thus, rather than saving the most important for the end to create suspense, the most important finding should be presented first. To achieve this, Lindsay (2011) suggests that scientists sort their results into four categories (p.31):

1. Results that are clear and relevant to what you want to say about the hypothesis.
2. Results that allow you to say something relevant about the hypothesis but that are less convincing than the results in Category 1.

3. Results that are interesting, substantial, and worth presenting, but they don't have anything to do with the hypothesis.

4. Results that are not convincing and don't have anything to do with the hypothesis.

Once sorted, the results can be easily transformed into scaffolding for the logical argument. Category 4 results should be omitted, while Category 1–3 should be presented in exactly this order.

(c) Following the mantra that scientific writing should ‘inform, not impress’ will automatically result in avoiding some of the stumbling blocks that frequently characterize scientific writing” (p.55). This will solve two problems. Firstly, it will prevent the Discussion from becoming too long as a result of inclusion of unnecessary references and deliberations that do not lead to the conclusions being made (which, according to editors of scientific journals, are some of the most common problems of scientific manuscripts). Secondly, it will free the language from problems of clarity and brevity. Lindsay (2011) explains and provides examples for how to avoid seven main types of writing stumbling blocks: clusters of nouns; complex adjectival phrases; sentences beginning with subordinate clauses; using nouns instead of the verbs from which they are derived; use of imprecise words; use of acronyms; unfamiliar abbreviations, and symbols; footnotes, asides in parentheses, and other distractions. Examples of these stumbling blocks from isotope archaeology are discussed in Supplementary Material 3. In addition, the author lays out five steps which can be used to check the clarity of every paragraph: Is it a paragraph?; Do the sentences flow?; Are there stumbling blocks?; Can it be shortened without losing the meaning?; Does it say what you want it to say?. Due to space constraints, we are unable to provide examples for all these points of advice, but reiterate our conviction that both seasoned and up-and-coming writers will benefit from engaging with Lindsay's short book.

6. Conclusions

This paper has presented suggestions and opportunities for improvement in three areas of project design and implementation: selecting samples, processing data and disseminating results. Some of the suggestions are relatively easy to implement:

- Consider carrying out power analyses to determine optimal sample sizes
- Invest time into exploring the distribution of data prior to formal statistical analysis

- Consider issues like overcrowding and colorblind-friendly palettes when preparing graphical figures
- Do not use the term ‘statistically significant difference’ (or any paraphrased equivalents)
- Do not hinge interpretation of group differences on *p*-value thresholds
- Consider measurement uncertainty and sample type when deciding how many decimal places to report; and report this number consistently throughout a publication
- Follow the established conventions and terminology for reporting stable isotope values ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$) and isotopic ratios ($^{87}\text{Sr}/^{86}\text{Sr}$)
- Take steps to justify sample selection, preservation and integrity (e.g., when reporting analyses of plants, include a supplementary document with photographs showing the surface morphology and internal structure of all analyzed seeds; when reporting the analyses of shells, bones and teeth, provide a table with results of all chemical analyses undertaken during sample screening)
- Consider how $\delta^{13}\text{C}$ values are obtained during AMS radiocarbon dating when deciding whether to use the values for answering palaeodietary/palaeoclimatic questions

Some will require an initial investment in training, which will pay off in the future:

- Assess measurement error by calculating both precision and accuracy (using measured values of both calibration and check standards inserted into each analytical run) and provide all relevant details in supplementary materials to enable the reader to replicate the calculations independently
- Undertake targeted analyses of shell carbonate structure to determine their integrity prior to analysis
- Assess both the surface morphology and internal structure (shown in cross-sections) of charred seeds to determine their likelihood of preserving usable isotopic values

Some will require an ongoing investment in time and effort:

- Consult the evolving literature on the latest advice for quality control assessment
- Consult with professional statisticians on the design of experiments and collection of data
- Before analyzing crop species that have not been analyzed before, carry out charring experiments to determine the crops’ preservation potential

Although isotopic research in archaeology requires a group effort and not everyone involved needs to understand the intricacies of the method, time investment from all parties involved can make a difference on both the final numbers and their interpretations. When researchers who cannot run analyses on their own are given full and transparent descriptions of the laboratory protocols and workflows from the collaborating laboratories and technicians, they will be better informed about where possible sources of error can occur. When archaeologists who have not been trained in isotopic geochemistry, but who are interested in collaborating on isotopic analyses, try their hand at data calibration and other data handling, they will be better positioned to communicate the reliability of the results. When students make time to practice (or at least engage in discussions surrounding) conventions and best practices such as those suggested in this paper, they will become effective in communicating the results both verbally and in writing.

Close collaboration with professional statisticians is crucial for avoiding mistakes that have been, and are continuing to be made, by the wider scientific community. In addition, we encourage isotope practitioners to write rebuttal papers more frequently, to participate in sharing data on Open Access repositories (e.g., IsoArch, IsoMemo), and to interact with papers during the revision stage (by reading and commenting on ‘pre-prints’ online). Lastly, many people who speak to, collaborate with, or receive feedback from Julia Lee-Thorp have heard her say “Why would you want to do that?” in a variety of contexts. To follow in the footsteps of her healthy dose of skepticism, we urge researchers to keep this question in mind during all stages of research. At no point should there be a time when they cannot answer it, or find out where the answer would be.

Author contributions

Conceptualization: PV, NL; Data curation: N/A; Formal analysis: PV, NL; Funding acquisition: N/A; Investigation: N/A; Methodology: N/A; Project administration: N/A; Resources: N/A; Software: N/A; Supervision: N/A; Validation: N/A; Visualization: PV, NL; Roles/Writing - original draft: PV, NL, ES, EL, CM; Writing - review & editing: PV, NL, ES, EL, CM.

Data availability

No original data was used in this paper. Stable isotope data that was re-examined in Section 5 was drawn from Vaiglova et al. (2020) and can be found at: <https://link.springer.com/article/10.1007/s12520-019-00960-y#additional-information>.

Declaration of competing interest

The authors declare no competing interests.

Acknowledgements

We are thankful to Richard Staff, David Chivall, Peter Ditchfield, Patrick Roberts, Maura Pellegrini, Melanie Beasley, Corrie Hyland, Rick Schulting, and Melissa Ritchey for their helpful comments and suggestions.

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Captions

Figure 1 Distribution of $\delta^{13}\text{C}$ values of the four main crops at Kouphovouno. Data was originally published in Vaiglova et al. (2020a) and the statistical analysis is being re-examined here.

Figure 2 Outcome of Tukey HSD comparison between the $\delta^{13}\text{C}$ values of the four main crops at Kouphovouno. Data was originally published in Vaiglova et al. (2020a) and the statistical analysis is being re-examined here.

Table 1 Summary of quality control criteria for archaeological materials most commonly analyzed in isotopic archaeology. Method development on these criteria is an active area of research, and the reader is encouraged to check the latest literature. References present experimental studies on quality control, unless preceded by “e.g.”, in which case they present an example of an application of these criteria.

Table 2 Results of Tukey HSD comparisons between $\delta^{13}\text{C}$ values of free-threshing wheat, hulled barley, einkorn and pea. Data was originally published in Vaiglova et al. (2020a) and the statistical analysis is being re-examined here.