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## SAMPLE PREPARATION METHODS USED AT THE AUSTRALIAN NATIONAL UNIVERSITY RADIOCARBON FACILITY

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**ABSTRACT.** The Single Stage AMS radiocarbon facility at the Australian National University has operated for the past 14 years. This paper presents the pretreatment methods used for the major sample types dated and reflects on whether quality assurance protocols can adequately detect altered materials. The majority of fossil samples dated by the facility are from tropical and arid environments where diagenesis of both organic samples and carbonates is often severe. A large proportion of the samples submitted cannot be dated, and screening and quality assurance methods are crucial. Based on analysis of 250 measurements on bone collagen, quality assurance indicators for bone are revised. From May 2021, the laboratory has accepted dates on collagen where yield >0.5%, %C is in the range of 39.7–46.9, and the C:N ratio is between 3.00 and 3.30.

**KEYWORDS:** pretreatment, radiocarbon.

## INTRODUCTION

The Australian National University has a long history of radiocarbon dating. The first radiocarbon laboratory, based on gas proportional counting, was set up in 1965 by Henry Polach (laboratory code ANU#) and continued to operate until 2003. Between 2002 and 2014 the 14 UD Pelletron accelerator was occasionally used for radiocarbon dating (laboratory code ANUA-#). This AMS was used for radiocarbon prior to 2002, but ANUA-# numbers were not assigned. In 2007 an NEC Single Stage AMS was installed and used solely for <sup>14</sup>C analysis (laboratory code S-ANU#) (Fallon et al. 2010). This paper outlines the sample preparation methods used by the S-ANU laboratory. These are regularly reviewed and updated, and this paper describes methods used since 2011.

Samples submitted to the laboratory are derived from archaeological, forensic, palaeoenvironmental and oceanographic contexts, spanning a range of carbonates, organics and water (Figure 1). Most samples submitted to the laboratory are from regions with exceptionally poor organic preservation. Around a third of fossil samples are from Australia, a third from Southeast Asia, and a fifth from the Pacific. This paper aims to share experience obtained working on samples from challenging regions, and comments on quality assurance indicators and preservation of commonly dated materials.

## STORAGE OF SAMPLES AND DATABASING

All Australian human remains are returned to the sample submitter. Other samples are returned at the submitter's request, or where required by laboratory staff. Samples are stored in plastic bags and acid-free cardboard boxes when not returned. Aluminum foil is avoided in most cases as this degrades when exposed to high or low pH. Records are retained in paper format and in a Filemaker Pro™ database.

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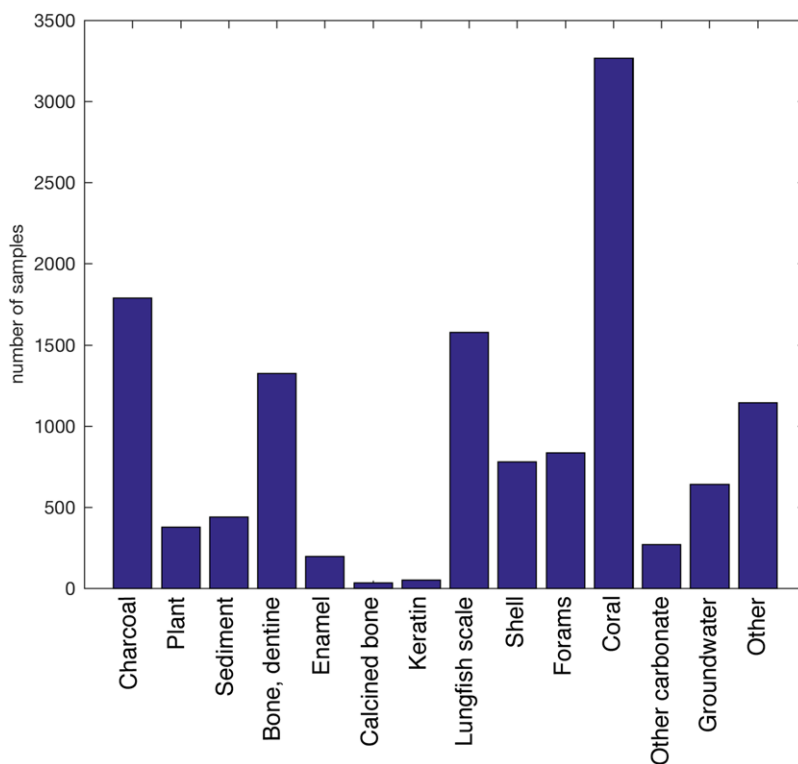


Figure 1 Materials submitted to the S-ANU laboratory since 2011. The category “other” includes laboratory tests of reagents and conservation treatments found on samples, as well some of the rarer sample types such as textiles, beeswax, paper and industrial products such as additives in the wine industry and fuels.

## GENERAL CONDITIONS

All chemicals used for chemical pretreatment are at least AR grade. Solvents are HPLC grade (Lichrosolv, DCM stabiliser amylene), and type 1 (ultrapure) water is used. In February 2021 a MilliQ™ system (Synergy UV) was replaced with a Sartorius™ Arium™ Comfort system. All glassware is cleaned using a series of 0.1% Decon™, 0.01 M HCl and ultrapure water washes (>1 hour), before combustion at 400°C/3 hours. Ezee-Filters™ are used to help remove the liquid from most samples, and cleaned by rinsing and ultrasonication (30 minutes) in ultrapure water. Samples are freeze-dried using a FreezeZone™ 2.5 freeze-drier connected to an oil-free vacuum pump.

## ISOTOPE RATIO MASS SPECTROMETRY

$\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$  are measured on a Sercon 20-22 isotope ratio mass spectrometer connected to either an ANCA GSL or a carbonate device operating in continuous flow mode. Standards are matched to the sample expected %C, %N and isotope value, and normalized using a multi-point scaling process (Paul et al. 2007). For example, collagen samples are normally measured against an in-house gelatin reference, scaled using USGS40, USGS65, and IAEA-C6, and data accuracy is assessed with IAEA-600 and USGS61. Since August 2014, samples and standards have been weighed using a Sartorius™ Cubi™

microbalance. Typical reproducibility is < 0.2‰ for δ<sup>13</sup>C and δ<sup>15</sup>N on gas from the ANCA GSL, and 0.06‰ for δ<sup>13</sup>C and 0.1‰ for δ<sup>18</sup>O on gas produced on the carbonate device.

## PHYSICAL PREPARATION

Samples are inspected, under a binocular microscope where required, and cleaned with a scalpel or handheld Dremel™ drill (bone and shell) to remove rootlets, sediment and degraded material. All attempts are made to sample single entities. Most are crushed to around 2 mm particle size using an agate pestle and mortar or a scalpel to increase surface area for cleaning, as described below. To limit the potential for cross-contamination, samples are not sieved and a range of particle sizes are present.

## SOLVENT WASHING

Where samples have visible, or suspected glue or conservation treatments, a series of solvent washes is applied to suit the contaminant present (Brock et al. 2010; Dee et al. 2011; O'Regan et al. 2019). These may not fully remove the conservation treatment (Brock et al. 2018), and conserved materials are avoided where possible. In September 2019 the laboratory changed from using chloroform to dichloromethane for safety reasons. The glue or conservation treatment is rarely known, and the most common solvent wash applied is acetone, methanol and dichloromethane (1 hour each, replacing if becoming colored). Acetone and methanol can be heated to 40°C and/or ultrasonicated where the sample is robust. This series of washes is also used on young (< about 500 years) wood to remove resins. Young (< about 500 years) or very well preserved (visual inspection) bone is defatted in a 2:1 mixture of dichloromethane and methanol (1 hour, room temperature, replacing the solution until no color is removed) (Folch et al. 1957). This method is more effective at removing low levels of lipids (<2%) than the Bligh and Dyer method (Iverson et al. 2001).

## PLANT MATERIAL

### Charred Plant Material

2–20 mg of charcoal is cleaned under a bifocal microscope to remove sediments and soft or vitreous degraded material and cut or crushed to 1–2 mm diameter fragments. Most samples are pretreated using an acid-base-acid (ABA) procedure consisting of HCl, NaOH and HCl washes (Table 1). After each treatment samples are rinsed at least 3 times in ultrapure water, or until the water remains colorless.

Large (>50 mg) charcoal samples thought to be more than 30 ka are subjected to ABOx-SC (Bird et al. 1999; Wood et al. 2016b). Between 50–100 mg charcoal is prepared as described for the ABA procedure, and subjected to treatment with 6M HCl (1 hour, room temperature), 2M NaOH (30 minutes, room temperature, solution replaced until it remains colorless) and a solution of 0.1M K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> in 2M H<sub>2</sub>SO<sub>4</sub> (60°C, 20 hours, screw top tube). After each treatment the charcoal is rinsed at least 3 times in ultrapure water, or until the water remains colorless. Rather than a stepped combustion described by (Bird et al. 1999), charcoal is precombusted at 600°C for 2 hours and the CO<sub>2</sub> generated is discarded. To do this, 10–20 mg of cleaned charcoal is placed in a quartz tube sealed with a quartz wool plug and evacuated to <3×10<sup>-3</sup> Torr on a graphitization rig as described below, and 730 Torr (1 atmosphere) ultra high purity (>99.995) O<sub>2</sub> is added. Any charcoal remaining is recovered from the quartz tube.

Table 1 Pretreatment conditions in pretreatment of burnt and unburnt plant material. Between each treatment, the sample is washed at least 3 times in ultrapure water, or until the water remains colorless. RT refers to room temperature.

Code	Protocol	Sample type	HCl	NaOH	HCl	Additional steps
14C001A	Routine ABA	Charcoal <30 kBP Delicate plant material Sediment	1M/ 70°C/30 min	1M/ 70°C/1hr Replace until colorless	1M/ 70°C/ 30min	
14C001B	Cotton	Cotton Linen	1M/ 70°C/30 min	0.2M/ RT – 70 °C/ 1hr	1M/ 70°C/ 30min	
14C001D	Gentle ABA	Beeswax Resins Delicate cotton	0.1M/ RT/30 min	0.1M/ RT/ 2hr Replace until colorless. Can be heated to 70°C if necessary.	0.1M/ RT/ 30min	
14C001E	Holocellulose	Plant material <30 kBP	1M/ 70°C/30 min	1M/ 70°C/1hr Replace until colorless	1M/ 70°C/ 30min	Follow ABA with chlorite bleach: 1:1 mixture of 1M HCl and 1M NaClO <sub>2</sub> / 1hr/ 70°C.
14C001F	Alpha cellulose	Plant material >30 kBP, where possible	1M/ 70°C/30 min	1M/ 70°C/1hr Replace until colorless	1M/ 70°C/ 30min	Precede ABA with chlorite bleach: 1:1 mixture of 1M HCl and 1M NaClO <sub>2</sub> / 1hr/ 70°C and then overnight/ RT
14C001G	Base soluble	Sediment	1M/ 70°C/30 min	1M/ 70°C/1hr Replace until colorless	1M/ 70°C/ 30min	Collect the NaOH and subsequent MilliQ solutions, centrifuge to remove particu- lates and acidify with 12M HCl at 70°C to precipitate humic acids.

When samples >30 ka are dated, both ABA and ABOx-SC are undertaken on several charcoal fragments from a given site where possible. Contaminants are assumed insignificant if results are indistinguishable, and the remaining samples are pretreated with ABA. Where different, remaining samples are treated with ABOx-SC.

Charcoal ABA yields range considerably (Figure 2a). Typically, at least 50% of a charcoal sample will survive the ABA pretreatment protocol, but in 20% of cases more than 90% of a charcoal sample was lost. In tropical locations, preservation can be minimal in relatively young sites, especially where sediments have a high pH (e.g. Loc Giang; Piper et al. 2017). Charcoal quality is assessed by %C, measured volumetrically while collecting CO<sub>2</sub> for graphitization. %C of charcoal is dependent on a range of factors, including species and temperature of pyrolysis, with %C typically ranging between 50 and 85 %C (Ascough et al. 2011, Braadbaart and Poole 2008). %C of fossil charcoal can decrease through diagenesis via oxidation (Ascough et al. 2011), and because fine sediment can accumulate within vessels and be concentrated if degraded charcoal is dissolved in pretreatment (Rebollo et al. 2011). It is therefore unsurprising that %C is not related to % yield (Figure 2b). While oxidation is not always related to the presence of exogenous carbon (Ascough et al. 2011), sediment particles can introduce carbon of very different age to the charcoal (Rebollo et al. 2011). Therefore, the S-ANU laboratory will normally only date charcoal containing more than 50% C. 7% of charcoal graphitized between 2011 and 2021 did not meet this criterion. Where charcoal with < 50% C is dated, the laboratory provides a warning to the sample submitter.

### **Unburnt Plant Material**

Plant materials are physically cleaned and inspected as described for charcoal, before being crushed or cut to ca. 1 mm pieces, or in the case of wood, into thin shavings. Samples are treated using one of several acid-base-acid procedures based on sample age and fragility (assessed visually) (Table 1). %C is used as a quality assurance measure. When outside of 40–50% C, a note is attached to the date report to indicate that the accuracy of the date may be low.

### **SEDIMENT**

Sediment samples are only dated after close consultation with the sample submitter, and only from palaeoenvironmental contexts where no other potential samples are present. In most cases two ages are obtained (e.g., ABA residue 14C001A and base soluble humics 14C001G; Table 1) to assess the age difference of different sediment components, and thus aid interpretation.

### **HUMAN AND FAUNAL REMAINS**

#### **Bone and Dentine—Fossil**

Where bones are visibly degraded or preservation is expected to be poor, collagen preservation is assessed with %N (Brock et al. 2012). The bone surface is removed with a drill, and 2–5 mg of bone is taken for measurement by EA-IRMS. More than one %N sample is taken from a bone/skeleton where preservation is expected to be variable, for example from dentine and bone in a mandible, from different skeletal elements in an inhumation, or across a large bone (Calo et al. 2015; Jacob et al. 2018). All bones containing > 0.7% N are pretreated. Bones containing more than 0.2% N are pretreated after consultation with the sample submitter on the understanding

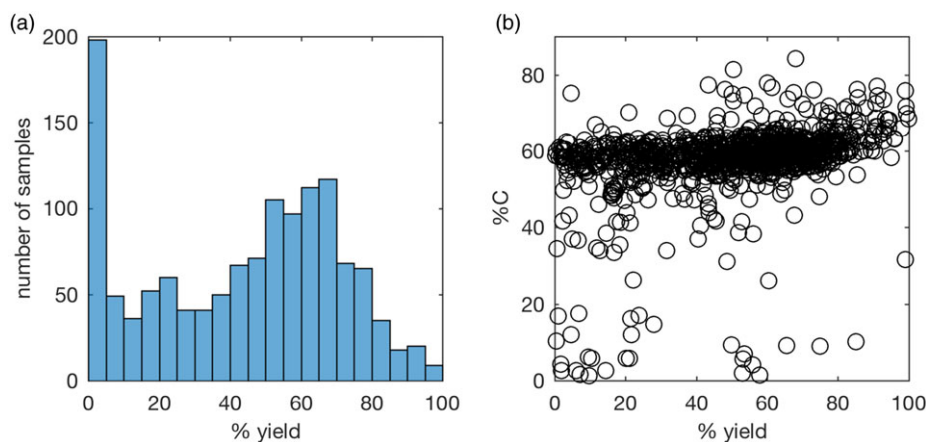


Figure 2 Success of the ABA pretreatment applied to charcoal since 2011 (a) % yield (b) comparison of %C and % yield.

that the likelihood of producing sufficient collagen is <70%. Samples with <0.2% N, the theoretical N content required to yield 1% collagen, are not pretreated.

To radiocarbon date bone, the surface and soft degraded material is removed with a drill. Samples are either ground with a hand pestle and mortar or crushed in a bone press to ca. 1 mm pieces, or drilled. Collagen yields are higher when ground as previously observed (Fewlass et al. 2019), and crushing is favored unless damage needs to be minimized, for example when samples are culturally or morphologically significant. When ground,  $6.8 \pm 1.2\text{wt.}\%$  collagen ( $n=7$ ) is extracted from the Zaglik bone standard, and when drilled  $3.1 \pm 1.7\%$  ( $n=9$ ) is extracted (starting weight >400 mg).

Collagen is extracted and purified using an ultrafiltration method, with conditions based on (Brock et al. 2010; Ramsey et al. 2004). Bone is demineralized using four treatments of ca. 20 mL 0.5M HCl. The first two are at room temperature for 1–2 hours, the third overnight at 5°C and the fourth for 1–2 hours at room temperature. Samples are then submerged in ca. 20 mL 0.1M NaOH for 30 minutes to remove humics, replacing where the solution is colored, and finally 0.5M HCl at room temperature for 1 hour. After each treatment, the sample is washed three times in ultrapure water. A centrifuge and glass pipettes are used to aid removal of the supernatant. The crude collagen is gelatinized in 10 mL 0.001M HCl (pH3) at 70°C for 20 hours in 35-mL screw top tubes in a Binder™ oven, before filtration with an Ezee-Filter™ to remove larger insoluble contaminants. Gelatin is ultrafiltered using Vivaspin™ VS15 Turbo 30kDa MWCO ultrafilters, which have a polyethersulphone membrane, until <1 mL solution remains in the ultrafilter, and the >30kDa fraction is collected and freeze-dried. Ultrafilters work on a molecular scale, with complex electrostatic interactions between the molecules in solution, and the solution and membrane. Although some small proteins are removed, the process produces a sample with a range of proteins, including collagen, of different sizes (Brock et al. 2013a).

Ultrafilters are cleaned prior to use by centrifuging ca. 15 mL ultrapure water twice (around 10 minutes each), 1-hour ultrasonication in ultrapure water, followed by centrifuging ca. 15 mL ultrapure water a further three times to remove the humectant glycerol. To monitor possible

fluctuations in age, one sample of glycerol per ultrafilter batch is collected from the washing process (Brock et al. 2013b). Glycerol has been modern in all batches of ultrafilter used by the facility. A bone specific background is subtracted (Wood et al. 2010; Fallon et al. in prep).

Collagen preservation is assessed with collagen yield (wt.%), %C and C:N ratio (van Klinken 1999). Various cut-off values have been used by different radiocarbon laboratories, and between the radiocarbon and stable isotope fields. To date, the ANU laboratory has accepted samples containing more than 1 wt.% collagen, 30% C, and having a C:N between 2.9 and 3.4, adapted from the protocol used at ORAU (Brock et al. 2010), derived from van Klinken (1999). However, measurements for both %C and C:N over the last 7 years (Figure 3b, c) are more consistent than those published by van Klinken (1999). This is most likely due to the use of ultrafiltration as well as improvements in IRMS and use of a micro-balance when weighing samples, and implies the acceptance criteria need to be re-evaluated.

For samples containing >1% collagen, and excluding a group of Egyptian mummies expected to be affected by resins (Wasef et al. 2015), %C averages  $43.3 \pm 1.2$  and C:N  $3.15 \pm 0.05$  ( $n=250$ ). From May 2021 the laboratory has accepted samples within 3 standard deviations of the average, i.e., 39.7–46.9% C and C:N ratio of 3.00–3.30. Although the C:N ratio is a relatively insensitive indicator for contaminants, it is often 3.4 or higher when dates on ultrafiltered collagen are younger than dates on hydroxyproline, justifying this tighter criteria (Marom et al. 2012; Devières et al. 2021; Spindler et al. 2021). Although %C of samples with low collagen yields are more likely to be lower than better preserved bone, most fall within the range of bone where >1% collagen was obtained. Therefore, samples containing 0.5–1% collagen will be dated where EA-IRMS values are acceptable, substantially extending the number of datable samples (Figure 3a).

$\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are also measured, but their use as indicators for preservation is somewhat limited within Australia and South East Asia as there is considerable geographic variability due to variation in plant composition and potential evapotranspiration (Fraser 2005; Murphy 2006) and, in many regions, minimal baseline data for most species.

### **Bone and Dentine—Forensic**

A simplified collagen extraction is undertaken for very recent (post-1950) samples as little contamination is expected, and the effect of young contaminants is minimal. A small (100–200 mg) sample of bone/dentine is defatted as described above, and collagen extracted by demineralization in HCl, gelatinization and Ezee filtration, as described for ancient samples.

### **Cremated Bone**

Bone burnt or charred at low temperature cannot be radiocarbon dated as the protein is damaged. However, when heated to more than 600°C so that the bone is grey or white, bone apatite recrystallizes into larger crystals that are more stable and less prone to diagenesis or contamination than the mineral phase of unburnt bone, and can be dated (Lanting et al. 2001). The carbonate in cremated or calcined bone is a mixture of endogenous carbon from the animal/human and carbonate from the fuel and thus can have an inbuilt age (Hüls et al. 2010; van Strydonck et al. 2010; Snoeck et al. 2016).



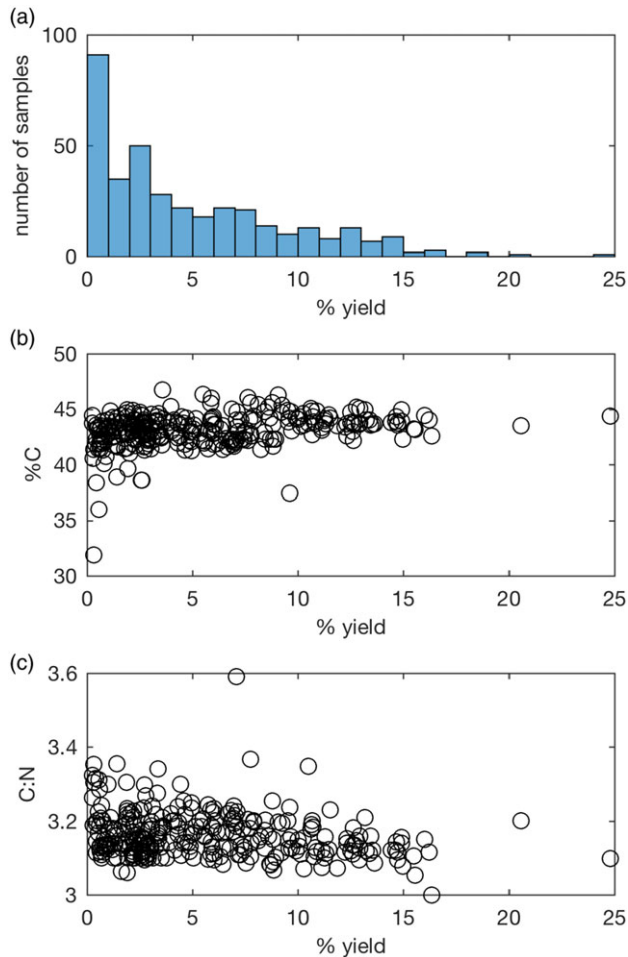


Figure 3 Collagen quality indicators of samples pretreated since August 2014: (a) % collagen yield, (b) %C, (c) C:N.

After thorough removal of the surface with a drill and grinding to  $<1$  mm, around 400 mg cremated bone is reacted with 1 M acetic acid (1 mL/ 50 mg bone) for 20 hours in a Vacutainer™ evacuated to  $<1 \times 10^{-2}$  Torr and rinsed 5 times with ultrapure water. This treatment removes 5–10% of the bone.

To assess whether the sample is calcined, 1–1.5 mg bone is ground with 200–300 mg KBr and pressed into a pellet under vacuum for FTIR analysis. The average of 100 transmission spectra are recorded on a Bruker Tensor 27 between  $-400$  and  $-4000$   $\text{cm}^{-1}$ , at a resolution of  $4$   $\text{cm}^{-1}$ . The infra-red splitting factor (IRSF) provides an indication of crystal order, with values above 4 indicative of partial recrystallization and values around 7 indicative of complete recrystallization (Olsen et al. 2008; Stiner et al. 1995). It is calculated from the sum of the absorption bands at  $603$   $\text{cm}^{-1}$  and  $565$   $\text{cm}^{-1}$  divided by the distance from the baseline to the valley between them ( $\text{CI} = \text{B}_{605} + \text{B}_{565} / \text{V}_{590}$ ) (Weiner and Bar-Yosef 1990). The carbonate:phosphate ratio ( $\text{C/P} = \text{B}_{1415} / \text{B}_{1035}$ ) of modern bone is approximately 0.23 (Garvie-Lok et al. 2004) and decreases when calcined. Currently, the facility dates samples with an



IRSF above 5 and C/P below 0.2 as calcined bone. However, it is imperative that these values are published alongside the radiocarbon dates as it is possible that their interpretation will change in the future, with the caveat that these indices are not always comparable between laboratories, or between FTIR methods.

No method is yet available to assess whether cremated bone is affected by exogenous contamination. Although Rose et al. (2019) suggest that contamination is minimal when just 5% of the bone is removed during pretreatment and Zazzo et al. (2013) have accurately dated Pleistocene-aged calcined bone, van Strydonck et al. (2009) have found some cremated bones provide erroneously young ages, suggesting that contamination may occur. To our knowledge, no work has yet assessed the preservation of calcined bone within tropical environments and dates on calcined antler (IRSF 6.9) from Con Co Ngur, Vietnam, produced an age that was younger than its context by more than 500 calibrated years (Oxenham et al. 2018).

### **Tooth Enamel**

Chemical pretreatment of tooth enamel is currently in development and is not yet able to always produce accurate radiocarbon ages, particularly beyond the late Holocene. However, it is offered by the facility as a method of producing direct minimum age estimates on skeletal remains that are not possible to date in any other way and/or where a specific chronological question is posed (e.g., whether a burial may be intrusive even though no grave cut is visible).

Where a tooth is large enough, enamel is currently dated with two methods (Wood et al. 2016a), and the age difference used as an indicator for degree of alteration. The surface and dentine is removed with a drill, and the enamel fractured along all existing cracks to allow soft material in cracks to be removed with the drill. After inspection under a binocular microscope to ensure all dentine and visibly recrystallized areas are removed, dust is removed by ultrasonication in ultrapure water. A sample of at least 200 mg is crushed in a pestle and mortar under ultrapure water. Samples are ground for at least 15 minutes to ensure no large pieces remain. The sample is then split, and an aliquot of at least 120 mg is mechanically ground in a McCrone™ microniser, using agate beads, for 30 minutes in 5-minute intervals separated by 2-minute rests to ensure the sample does not heat up. The handground and micronized samples are then reacted with 1M acetic acid (1 mL/50 mg enamel) for 20 hours in a Vacutainer™. This is evacuated to  $<1 \times 10^{-2}$  Torr before the addition of acid, and again after 1 hour of reaction. The cleaned enamel is rinsed 5 times with ultrapure water, freeze-dried and stored under vacuum until graphitization.

This process removes around 30% of the enamel weight. The microniser is gentler than ball mills, and preferentially fractures the enamel along grain boundaries, where substantial amounts of carbonate contaminants may be located. This means that while the acid primarily attacks the prism surfaces of the handground enamel, it attacks the crystallite surfaces (and crystallite core) in the micronized sample, often leading to an increase in age.

As with cremated bone, it is not yet possible to identify exogenous carbonates within tooth enamel.  $>0.8\%$  C and unexpected  $\delta^{13}\text{C}$  values may indicate severe contamination (Wood et al. 2021), and so IRMS is undertaken on the cleaned enamel. FTIR, a technique routinely used to assess preservation in the fields of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  (Miller et al. 2018), is

also undertaken although it is not yet possible to identify which indices provide the best indication of exogenous carbonate contamination. Calcite has never been observed in the tooth enamel dated, and brushite formed during acidification is rare.

### **Keratin**

A range of keratin samples are dated, including wool, nail, hair and horn. 5–20 mg is physically cleaned. The surface is drilled from horn, and a sample taken with the drill. Clean strands of hair and sections of woolen textile are picked under a binocular microscope. Both dirt (which can look like a stain) and tissue need to be separated from nails, and are easier to distinguish after soaking the nail in ultrapure water (overnight, 5°C). Using tweezers and a curved scalpel, dirt, tissue, and a thin surface layer are removed from both surfaces of the nail under a binocular microscope. The nail is kept wet by repeatedly dipping in ultrapure water. Where possible, the laboratory favors radiocarbon dating of finger nails to reduce potential contamination from hair dye and cleaning products (de la Torre et al. 2014).

Pretreatment was modified from O'Connell and Hedges (1999) and Santos et al. (2015) and adopted in 2020. Forensic samples of hair, nail and horn are de-fatted in a mixture of dichloromethane and methanol (2:1) (2 hours, room temperature), replacing the solution after 1 hour and ultrasonicated for 15 minutes (hair) or 30 minutes (nail). After rinsing in ultrapure water, samples are subjected to an ABA pretreatment involving 0.1M HCl (30 minutes), 0.1M NaOH (1 hour) and 0.1M HCl (30 minutes) at room temperature, rinsing with ultrapure water after each treatment. Ancient samples are treated in a similar way, but without the de-fatting step. If hair is visibly dirty, the NaOH treatment and following water rinses are undertaken in an ultrasonication bath as the alkali opens the cuticle scales allowing trapped particulates to be freed more readily.

Prior to 2020, forensic keratin samples were de-fatted in the same way as forensic bone and treated with an ABA protocol consisting of 1M HCl (30 min), 0.1M NaOH (1 hour, replaced until colorless), and 1M HCl (30 min), heating to 70°C where possible and rinsing three times in ultrapure water after each treatment.

### **Other Protein**

Other proteinaceous samples are treated on a case-by-case basis. For example, primarily collagenous lungfish scales taken from living or recently deceased animals are not pretreated (e.g. Fallon et al. 2016), and where a small amount of carbonate is present, e.g. in coral tissue, a dilute acid (e.g. 0.5M HCl, overnight, 5°C) is used (e.g. Komugabe-Dixson et al. 2016).

### **CARBONATE: SHELL, FORAMS, AND CORAL**

The dirty and degraded surface of shell and coral, as well as other carbonates such as otoliths and celtis seeds, is removed with a drill until a dense and translucent material is left. At times this will involve removing more than half the thickness of a shell. Where an aragonite nacre is present, the nacre is separated and lightly cleaned with a drill. 5–10 mg shell is immersed in sufficient 0.1M HCl (80°C) to remove 10–50% weight, rinsed in ultrapure water and dried in a hotblock. The sample is not crushed so that the surface is preferentially leached. Foraminifera and ostracods are picked and cleaned of sediment by the sample submitter

and leached in 0.1 M HCl to remove 10–20% weight. Modern (post-1950) coral is not pretreated.

Preservation of carbonate is assessed using a combination of visual observation, Feigl's stain and powder x-ray diffraction (XRD). In many, though not all (Webb et al. 2007), cases carbonate is recrystallized as calcite. Therefore, for calcite samples such as oyster shell, visual inspection is the only indicator of preservation and shells are not dated where there is any doubt about sample integrity. Prior to 2021, visibly degraded shells would occasionally be dated and a warning issued to the sample submitter. Since 2021, samples have not been dated where dense and translucent material with a uniform pearly sheen cannot be found, or in the case of oyster or nacre, when the layers are not easily separable.

If the sample is aragonite, carbonate is screened with Feigl's stain (Feigl 1937), and if >30 ka, XRD. Feigl solution is prepared by dissolving 7.2 g MnSO<sub>4</sub>.H<sub>2</sub>O per 100 mL boiling ultrapure water and adding 1g AgSO<sub>4</sub>. After cooling, the solution is passed through a baked glass fiber filter, and enough water is added to return the solution to 100 mL. 1 to 2 drops of 10% NaOH are added while stirring. After 1–2 hours, the solution is filtered a second time to remove a black precipitate, and the solution is stored in a dark glass bottle. The dry acid leached shell is covered in Feigl solution at room temperature, and after 10–20 minutes the shell is rinsed in ultrapure water and inspected under a binocular microscope. Pieces entirely covered in a black stain are selected for dating, and a scalpel is used to remove as much of the stain as possible. Where results of staining are inconclusive, results are interpreted in light of visual inspection. The texture of the shell appears crucial to the success of the staining procedure, and we have noticed the Feigl staining of nacre in particular is unreliable, perhaps due to high concentrations of protein between the carbonate crystals (Jacob et al. 2008).

XRD is undertaken on aragonite samples that are > 30 ka. Before 2018, carbonate was ground under acetone, dried and passed through a 0.11 mm mesh sieve onto a quartz low background holder. XRD was undertaken using a Siemens D501 X-ray diffractometer operating at 40kV and 40mA using Cu K $\alpha$  radiation, and a step size of 0.05° and speed of 0.3 minutes per degree between 25–50° 2 $\theta$ . Siroquant V3 was used to quantify the calcite content with a detection limit of around 0.3wt.% calcite for a 50 mg sample. Since 2018, the sample has been hand ground in acetone, and suspended on a quartz holder and dried. XRD is carried out with a Malvern Panalytical Empyrean Series 3 which is equipped with Bragg-Brentano divergent beam optic (BBHD) and a PIXcel<sup>3D</sup> detector (1D scanning mode, 3.347° active length), using CoK $\alpha$  radiation. Samples are analyzed over a range of 20–58° 2 $\theta$ , with step width of 0.0131303° 2 $\theta$  and a total dwell time of 200 s/step. Phase identification is carried out with the software *DiffraPlus* Eva 10 and the ICDD PDF-2 database and phase quantification is performed with HighScore Plus 4.8. The detection limit is about 0.1wt.% calcite.

### Dissolved Inorganic Carbon

Dissolved inorganic carbon (DIC) from water is dated using a method adapted from Bragança et al. (2021). A range of waters has been dated, including both seawater and groundwater. Approximately 50 mL of water is loaded into 100 mL glass containers with crimp style septa seals in a glove bag under a N<sub>2</sub> or He environment. The samples are then acidified with 0.5 mL 85% H<sub>3</sub>PO<sub>4</sub> with a Hamilton Gastight™ syringe and needle (Terumo™ 0.45×13 mm) for 24 hours at room temperature to react. CO<sub>2</sub> is cryogenically collected

over 10 minutes with water removed using dry ice and ethanol. A second 50 mL aliquot is prepared if carbon content is low. Prior to this we precipitated  $\text{SrCO}_3$  from groundwater DIC. In brief, we added 1–2 g of  $\text{SrCl}_2$  per L of groundwater. Several pellets of NaOH were used to increase pH and speed up the precipitation. The sample was centrifuged, water removed and  $\text{SrCO}_3$  was rinsed 3–5 times with ultrapure water, centrifuging between each rinse, before freeze-drying.

### **Combustion, Acidification, and Graphitization**

Organic samples, normally containing 0.25–1 mg C, are weighed into an Ag cup (Sercon™ SC1081) and placed into a quartz tube (Infusil™ silicaware 4×6×200 mm) with 30 mg CuO (Sercon SC0003). An Ag cup is used rather than the typical Ag wire to avoid powdered samples or collagen from adhering to the quartz tube wall and hindering sealing. Before use, Ag and CuO are ultrasonicated in methanol (repeated until no dust is released), and baked (900°C, 3 hours), and quartz tubes are baked (900°C, 3 hours). Samples are evacuated to  $<3 \times 10^{-3}$  Torr and sealed prior to combustion in a muffle furnace (900°C, 6 hours). CuO is the major source of carbon contamination in the combustion/graphitization process, and in addition to testing each batch for both young and old carbon, efforts have been made to reduce the quantity of CuO. Prior to 29/10/2019, 60 mg CuO was used. This was reduced to 20 mg (29/10/2019–12/12/2019). While sufficient to combust the samples, samples and standards sometimes failed to graphitize presumably because gaseous contaminants such as  $\text{SO}_2$  were not removed with the reduced Cu. 30 mg CuO appears sufficient for the majority of samples.

Carbonates, including  $\text{SrCO}_3$  from DIC, tooth enamel and cremated bone are placed in a 10 mL Vacutainer™ vial and evacuated to  $<3 \times 10^{-3}$  Torr. 0.3–0.4 mL 85%  $\text{H}_3\text{PO}_4$  is added to carbonates with a Hamilton Gastight™ syringe and needle (Terumo™ 0.45×13 mm). 1 mL is used for enamel and cremated bone. The reaction is left at 80°C until complete.

$\text{CO}_2$  is cryogenically collected and purified on a vacuum line built with Swagelok™ Ultratorr, using a dry ice-ethanol trap.  $\text{CO}_2$  is collected in a reaction rig fitted with Kimax™ culture tubes (6×50 mm) for graphitization with ultra high purity  $\text{H}_2$  over an Fe catalyst (560°C, 6 hours or until reaction is complete). Fe is produced from 3.0–3.3 mg  $\text{Fe}_2\text{O}_3$  (Aldrich™ 529311) by reaction with  $\text{H}_2$  (1000 Torr, 400°C, 90 minutes). Granular magnesium perchlorate (Alfa Aesar™ 11626) is used to remove water generated during the graphitization process (Santos et al. 2004).

Our standard reactors have a volume of ca. 3.6 cm<sup>3</sup> and are used to produce samples between 0.25–1 mg C. Samples between 0.15 mg–0.25 mg C are dated using a small reaction vessel, and matched in size to primary and secondary standards. The small reaction vessels were machined in the Research School of Earth Sciences mechanical workshop and are combined with shortened, pre-baked borosilicate tubes to hold  $\text{Mg}(\text{ClO}_4)_2$ . These modifications result in decreased the reactor volume to  $\sim 2.1$  cm<sup>3</sup>. Samples below 0.15 mg are only dated in specific cases, for example, where the sample is very young and larger samples cannot be recovered. In these cases graphitization temperature is reduced to 450°C (Strzpek 2014).

Samples containing large amounts of gaseous contaminants, such as sediments, cremated bone and tooth enamel, require an extra cleaning step to remove  $\text{SO}_2$ . These samples are converted to  $\text{CO}_2$ , either by acidification with phosphoric acid (cremated bone and enamel) or combustion (organics), as described above. The  $\text{CO}_2$  is cryogenically purified as described

Table 2 Secondary pretreatment standards routinely used in the ANU radiocarbon facility.

Lab name	Context details	Material	Age
Coal	Research School of Earth Sciences, ANU	Coal	>50 ka
Kauri	Renton Road, near Auckland Airport (Alan Hogg) (Marra et al. 2006)	Wood	MIS7
VIRI L	VIRI sample L (Scott et al. 2010)	Wood	Consensus age: $75.719 \pm 0.0395$ pMC
Zaglik	Coelodonta antiquitatis, Coel-09-1, Zaglik, Irkutsk, Russia (Alexander Shchetnikov)	Bone	Sediments dating 70–150 ka
Latton	Early form of Mammuthus primigenius or a separate species Mammuthus cf. trogontherii, Latton, UK (Katharine Scott) (Lewis et al. 2006)	Bone	Macrofossils suggest an interglacial climate and/or MIS7. This mammoth type is found only in MIS7 and earlier deposits.
Vergulde	Cow, Vergulde Draek shipwreck, Australia (Jeremy Green, Western Australian Museum)	Bone	Wrecked AD 1656
Batavia	Cow, Batavia shipwreck, Australia (Jeremy Green, Western Australian Museum)	Bone	Wrecked AD 1629
Crem bone sample 3	Wapse 2 (Philip Naysmith) (Naysmith et al. 2007)	Cremated bone	Average age: $2790 \pm 52$ BP
Dunragit, A–E	Dunragit 1999 227, UK. These are multiple bones from the same burial, not a single bone (Philip Naysmith) (Hamilton 2015)	Cremated bone	SUERC-36378 $4125 \pm 30$ BP
Belfast Cellulose	From Chrono Centre, Queens University Belfast	Wood cellulose	Age $4510 \pm 10$
TIRI/FIRI Turbidite	TIRI K/FIRI C turbidite sample (Scott et al. 2004)	Turbidite	TIRI $18,155 \pm 34$ BP FIRI $17,176 \pm 11$ BP
Marble	IAEA-C1	Marble	>50 ka

for graphitization but is collected in a quartz tube containing CuO wire and Ag foil. This is sealed and heated to 900°C for 6 hours, and the cleaned CO<sub>2</sub> is then cryogenically collected and purified for a second time, before graphitization. This extra step adds a small amount of laboratory contamination, and an extra background correction has been calculated (Wood et al. 2021).

### Data Quality: Standards and Duplicates

Approximately 1 in 20 samples is duplicated from sampling to AMS measurement, to assess whether quoted errors are appropriate. A range of pretreatment-specific standards are processed (Table 2). Where possible, samples are matched to the material and age of the unknowns. Where no standard is available, coal for organics or marble for inorganics is normally treated and dated. Data reproducibility is explored in Fallon et al. (in prep).

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### REFERENCES

- Agerskov Rose H, Meadows J, Palstra SWL, Hamann C, Boudin M, Huels M. 2019. Radiocarbon dating cremated bone: a case study comparing laboratory methods. *Radiocarbon* 61:1581–1591.
- Ascough PL, Bird MI, Francis SM, Lebl T. 2011. Alkali extraction of archaeological and geological charcoal: evidence for diagenetic degradation and formation of humic acids. *Journal of Archaeological Science* 38:69–78.
- Bird MI, Ayliffe LK, Fifield LK, Tumej CSM, Cresswell RG, Barrows TT, David B. 1999. Radiocarbon dating of “old” charcoal using a wet oxidation, stepped-combustion procedure. *Radiocarbon* 41:127–140.
- Braadbaart F, Poole I. 2008. Morphological, chemical and physical changes during charcoalfication of wood and its relevance to archaeological contexts. *Journal of Archaeological Science* 35:2434–2445.
- Bragança D, Oliveira F, Macario K, Nunes V, Muniz M, Lamego F, Abril G, Nepomuceno A, Solís C, Rodríguez-Ceja M. 2021. Establishing water sample protocols for radiocarbon analysis at LAC-UFF, Brazil. *Radiocarbon* 63:1225–1232.
- Brock F, Dee M, Hughes A, Snoeck C, Staff R, Bronk Ramsey C. 2018. Testing the effectiveness of protocols for removal of common conservation treatments for radiocarbon dating. *Radiocarbon* 60:35–50.
- Brock F, Geoghegan V, Thomas B, Jurkschat K, Higham TFG. 2013a. Analysis of bone “collagen” extraction products for radiocarbon dating. *Radiocarbon* 55:445–463.
- Brock F, Higham T, Ditchfield P, Ramsey CB. 2010. Current pretreatment methods for AMS radiocarbon dating at the Oxford Radiocarbon Accelerator Unit (ORAU). *Radiocarbon* 52:103–112.
- Brock F, Higham T, Ramsey CB. 2013b. Comments on the use of Ezee-Filters™ and ultrafilters at ORAU. *Radiocarbon* 55:211–212.
- Brock F, Wood R, Higham TFG, Ditchfield P, Bayliss A, Ramsey CB. 2012. Reliability of nitrogen content (%N) and carbon: nitrogen atomic ratios (C:N) as indicators of collagen preservation suitable for radiocarbon dating. *Radiocarbon* 54:879–886.
- Calo A, Prasetyo B, Bellwood P, Lankton JW, Gratuze B, Pryce TO, Reinecke A, Leusch V, Schenk H, Wood R, Bawono RA, Kompiang Gede ID, Citha Yuliati NLK, Fenner J, Reepmeyer C, Castillo C, Carter AK. 2015. Sembiran and Pacung on the north coast of



- Bali: a strategic crossroads for early trans-Asiatic exchange. *Antiquity* 89:378–396.
- De la Torre HAM, Ormsby KM, Fuller BT, Santos GM. 2014. <sup>14</sup>C measurements elucidate isotopic differences between nails and hair in modern humans. *Radiocarbon* 56:53–65.
- Dee MW, Brock F, Bowles AD, Ramsey CB. 2011. Using a silica substrate to monitor the effectiveness of radiocarbon pretreatment. *Radiocarbon* 53:705–711.
- Devièse T, Abrams G, Hajdinjak M, Pirson S, De Groote I, Di Modica K, Toussaint M, Fischer V, Comeskey D, Spindler L, Meyer M, Semal P, Higham T. 2021. Reevaluating the timing of Neanderthal disappearance in northwest Europe. *Proceedings of the National Academy of Sciences* 118:e2022466118.
- Fallon S, Wood R, Esmay R. in prep. Status report of the Australian National University Radiocarbon Facility.
- Fallon SJ, Fifield LK, Chappell JM. 2010. The next chapter in radiocarbon dating at the Australian National University: status report on the single stage AMS. *Nuclear Instruments and Methods in Physics Research, Section B: Beam Interactions with Materials and Atoms*: 268:898–901.
- Fallon SJ, McDougall A, Espinoza T, Roberts DT, Brooks S, Kind P. 2016. Updated methods to age the Australian lungfish: reply to Kemp (2015). *Radiocarbon* 57:195–196.
- Feigl F. 1937. *Qualitative analysis by spot test*. New York: Nordemann Publications Company.
- Fewlass H, Tuna T, Fagault Y, Hublin JJ, Kromer B, Bard E, Talamo S. 2019. Pretreatment and gaseous radiocarbon dating of 40–100 mg archaeological bone. *Scientific Reports* 9:5342.
- Folch J, Lees M, Stanley GHS. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry* 226:497–509.
- Fraser R. 2005. A study of stable carbon, nitrogen and oxygen isotopes in modern Australian marsupial herbivores, and their relationships with environmental conditions [PhD dissertation]. Australian National University.
- Garvie-Lok SJ, Varney TL, Katzenberg MA. 2004. Preparation of bone carbonate for stable isotope analysis: the effects of treatment time and acid concentration. *Journal of Archaeological Science* 31:763–776.
- Hamilton D. 2015. Radiocarbon dating and Bayesian modelling. In: Thomas J (ed.), *A neolithic ceremonial complex in Galloway*. Oxbow: Oxford, pp. 140–145.
- Hüls CM, Erlenkeuser H, Nadeau MJ, Grootes PM, Andersen N. 2010. Experimental study on the origin of cremated bone apatite carbon. *Radiocarbon* 52:587–599.
- Iverson SJ, Lang SLC, Cooper MH. 2001. Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. *Lipids* 36:1283–1287.
- Jacob DE, Soldati AL, Wirth R, Huth J, Wehrmeister U, Hofmeister W. 2008. Nanostructure, composition and mechanisms of bivalve shell growth. *Geochimica et Cosmochimica Acta* 72:5401–5415.
- Jacob E, Querci D, Caparros M, Barroso Ruiz C, Higham T, Deviese T. 2018. Nitrogen content variation in archaeological bone and its implications for stable isotope analysis and radiocarbon dating. *Journal of Archaeological Science* 93:68–73.
- Komugabe-Dixson AF, Fallon SJ, Eggins SM, Thresher RE. 2016. Radiocarbon evidence for mid-late Holocene changes in southwest Pacific Ocean circulation. *Paleoceanography* 31: 971–985.
- Lanting JN, Aerts-Bijma AT, van der Plicht J. 2001. Dating of cremated bones. *Radiocarbon* 43: 249–254.
- Lewis SG, Maddy D, Buckingham C, Coope GR, Field MH, Keen DH, Pike AWG, Roe DA, Scaife RG, Scott K. 2006. Pleistocene fluvial sediments, palaeontology and archaeology of the upper River Thames at Latton, Wiltshire, England. *Journal of Quaternary Science* 21: 181–205.
- Marom A, McCullagh JSO, Higham TFG, Sinitsyn AA, Hedges REM. 2012. Single amino acid radiocarbon dating of Upper Paleolithic modern humans. *Proceedings of the National Academy of Sciences of the United States of America* 109:6878–6881.
- Marra MJ, Alloway BV, Newnham RM. 2006. Paleoenvironmental reconstruction of a well-preserved Stage 7 forest sequence catastrophically buried by basaltic eruptive deposits, northern New Zealand. *Quaternary Science Reviews* 25(17–18):2143–2161.
- Murphy B. 2006. *Grass, fire and kangaroos: can stable isotopes reveal Australia's past and future ecology?* [PhD dissertation]. Charles Darwin University.
- Naysmith P, Scott E, Cook G, Heinemeier J, Van der Plicht J, Van Strydonck M, Bronk Ramsey C, Grootes PM, Freeman S. 2007. A cremated bone intercomparison study. *Radiocarbon* 49(2):403–408.
- O'Connell TC, Hedges REM. 1999. Investigations into the effect of diet on modern human hair isotopic values. *American Journal of Physical Anthropology* 108:409–425.
- O'Regan G, Petchey F, Wood R, Mcalister A, Bradshaw F, Holdaway S. 2019. Dating South Island Māori rock art: Pigment and pitfalls. *Journal of Archaeological Science: Reports* 24:132–141.
- Olsen J, Heinemeier J, Bennike P, Krause C, Margrethe Hornstrup K, Thrane H. 2008. Characterisation and blind testing of



- radiocarbon dating of cremated bone. *Journal of Archaeological Science* 35:791–800.
- Oxenham MF, Trinh HH, Willis A, Jones RK, Domett K, Castillo C, Wood R, Bellwood P, Tromp M, Kells A, Piper P, Pham ST, Matsumura H, Buckley H. 2018. Between foraging and farming: strategic responses to the Holocene Thermal Maximum in Southeast Asia. *Antiquity* 92:940–957.
- Paul D, Skrzypek G, Fórizs I. 2007. Normalization of measured stable isotopic compositions to isotope reference scales – a review. *Rapid Communications in Mass Spectrometry* 21:3006–3014.
- Piper PJ, Nguyen KTK, Tran TKQ, Wood R, Cobo Castillo C, Weisskopf A, Campos F, Dang NK, Sarjeant C, Mijares ASB, Oxenham M, Bellwood P. 2017. The Neolithic settlement of Loc Giang on the Vam Co Dong River, southern Vietnam and its broader regional context. *Archaeological Research in Asia* 10: 32–47.
- Ramsey CB, Higham T, Bowles A, Hedges R. 2004. Improvements to the pretreatment of bone at Oxford. *Radiocarbon* 46:155–163.
- Rebollo NR, Weiner S, Brock F, Meignen L, Goldberg P, Belfer-Cohen A, Bar-Yosef O, Boaretto E. 2011. New radiocarbon dating of the transition from the Middle to the Upper Paleolithic in Kebara Cave, Israel. *Journal of Archaeological Science* 38:2424–2433.
- Santos GM, De La Torre HAM, Boudin M, Bonafini M, Saverwyns S. 2015. Improved radiocarbon analyses of modern human hair to determine the year-of-death by cross-flow nanofiltered amino acids: common contaminants, implications for isotopic analysis, and recommendations. *Rapid Communications in Mass Spectrometry* 29:1765–1773.
- Santos GM, Southon JR, Druffel-Rodriguez KC, Griffin S, Mazon M. 2004. Magnesium perchlorate as an alternative water trap in AMS graphite sample preparation: a report on sample preparation at KCCAMS at the University of California, Irvine. *Radiocarbon* 46:165–173.
- Scott EM, Bryant C, Carmi I, Cook G, Gulliksen S, Harkness D, Heinemeier J, McGee E, Naysmith P, Possnert G, van der Plicht H, van Strydonck M. 2004. Precision and accuracy in applied  $^{14}\text{C}$  dating: some findings from the Fourth International Radiocarbon Inter-comparison. *Journal of Archaeological Science* 31(9):1209–1213.
- Scott E, Cook G, Naysmith P. 2010. The Fifth International Radiocarbon Intercomparison (VIRI): An assessment of laboratory performance in Stage 3. *Radiocarbon* 52(3):859–865.
- Snoeck C, Staff RA, Brock F. 2016. A reassessment of the routine pretreatment protocol for radiocarbon dating cremated bones. *Radiocarbon* 58:1–8.
- Spindler L, Comeskey D, Chabai V, Uthmeier T, Buckley M, Deviese T, Higham T. 2021. Dating the last Middle Palaeolithic of the Crimean Peninsula: New hydroxyproline AMS dates from the site of Kabazi II. *Journal of Human Evolution*, 156:102996.
- Stiner MC, Kuhn SL, Weiner S, Bar-Yosef O. 1995. Differential burning, recrystallization, and fragmentation of archaeological bone. *Journal of Archaeological Science* 22:223–237.
- Strzypek KM. 2014. Deep-sea coral amino acid archives of marine exosystem function in east and southeast Australian waters [PhD dissertation]. Australian National University.
- Van Klinken GJ. 1999. Bone collagen quality indicators for palaeodietary and radiocarbon measurements. *Journal of Archaeological Science* 26:687–695.
- Van Strydonck M, Boudin M, De Mulder G. 2009.  $^{14}\text{C}$  dating of cremated bones: The issue of sample contamination. *Radiocarbon* 51:553–568.
- Van Strydonck M, Boudin M, De Mulder G. 2010. The carbon origin of structural carbonate in bone apatite of cremated bones. *Radiocarbon* 52:578–586.
- Ventresca Miller A, Fernandes R, Janzen A, Nayak A, Swift J, Zech J, Boivin N, Roberts P. 2018. Sampling and pretreatment of tooth enamel carbonate for stable carbon and oxygen isotope analysis. *JoVE* e58002.
- Wasef S, Wood R, El Merghani S, Ikram S, Curtis C, Holland B, Willerslev E, Millar CD, Lambert DM. 2015. Radiocarbon dating of Sacred Ibis mummies from ancient Egypt. *Journal of Archaeological Science: Reports* 4:355–361.
- Webb GE, Price GJ, Northdurft LD, Deer L, Rintoul L. 2007. Cryptic meteoric diagenesis in freshwater bivalves: Implications for radiocarbon dating. *Geology* 35:803–806.
- Weiner S, Bar-Yosef O. 1990. States of preservation of bones from prehistoric sites in the Near East: a survey. *Journal of Archaeological Science* 17:187–196.
- Wood R, Duval M, Mai Huong NT, Tuan NA, Bacon AM, Demeter F, Durringer P, Oxenham M, Piper P. 2016a. The effect of grain size on carbonate contaminant removal from tooth enamel: towards an improved pretreatment for radiocarbon dating. *Quaternary Geochronology* 36:174–187.
- Wood R, Fleury ABC, Fallon S, Nguyen TMH, Nguyen AT. 2021. Do weak or strong acids remove carbonate contamination from ancient tooth enamel more effectively? The effect of acid pretreatment on radiocarbon and  $\delta^{13}\text{C}$  analyses. *Radiocarbon* 63:935–952.

- Wood R, Jacobs Z, Vannieuwenhuysse D, Balme J, O'Connor S, Whitau R. 2016b. Towards an accurate and precise chronology for the colonization of Australia: The example of Riwi, Kimberley, Western Australia. *PLoS ONE* 11.
- Wood RE, Bronk Ramsey C, Higham TFG. 2010. Refining background corrections for radiocarbon dating of bone collagen at ORAU. *Radiocarbon* 52:600–611.
- Zazzo A, Lebon M, Chiotti L, Comby C, Delqué-Količ E, Nespoulet R, Reich I. 2013. Can we use calcined bones for radiocarbon dating the Paleolithic? *Radiocarbon* 55: 1409–1421.