**The what, how and why of archaeological coprolite analysis**

Lisa-Marie Shillito*[a], John C. Blong[a], Eleanor J. Green[b], and Eline Van Asperen[a]

* Corresponding author lisa-marie.shillito@ncl.ac.uk

[a] School of History, Classics and Archaeology, Newcastle University, NE1 7RU, UK
[b] BioArCh, Department of Archaeology, University of York, Wentworth Way, York YO10 5DD, UK

## Abstract

Coprolites are a highly informative but still underutilized proxy for understanding past environments, palaeodiets, and ancient human health. Here we provide a critical review of the history and current state of research in human coprolite analysis encompassing, macroscopic, microscopic, and biomolecular approaches. We present new data from a number of key sites which demonstrates how new multiscalar, multiproxy approaches can provide unique archaeological insights. Coprolites should be routinely collected and examined during excavations and integrated with other archaeological and palaeoecological evidence. Future research needs to focus on better understanding coprolite formation as well as pre and post depositional taphonomy. This can be achieved through interdisciplinary collaboration between geoarchaeology and organic geochemistry.

**Keywords** palaeodiet, past diets, palaeofaeces, multiproxy, human coprolite, Quaternary, Pleistocene, Holocene

## 1. Introduction

The term coprolite, commonly used to describe archaeological faeces, originates in geology, where it is used to refer to faeces which have undergone true fossilization (Buckland 1829 p.143). In geology, the study of coprolites falls under the remit of ichnology, the study of geological traces of biological activity (Hunt et al. 2012). Archaeological samples are not true fossils, being preserved either through desiccation or partial mineralization (Reinhard and Bryant 2008) although the exact mechanisms of preservation are not well understood. In general, sites such as caves and rockshelters, which are less exposed, exhibit better preservation that more open sites (Reinhard et al. 2019).The term palaeofaeces is perhaps more accurate, though not widely used, appearing more frequently in earlier literature, such as the extensive studies of ‘paleofeces’ from Salts Cave (Watson and Yarnell 1967).

Coprolites have been found in direct proximity to habitation areas, for example in caves, mixed with other waste and discarded in midden areas, or in dedicated cesspit/latrines in settlements. Ancient human faeces can provide direct evidence of health and diet, and also insights into sanitation practices, changing perceptions of cleanliness, and social organization in the past, as well as information on the local ecology and environment (Reinhard and Bryant 2008). Whilst some of these can be addressed through other types of archaeological evidence, the information that can be obtained from faecal material is unique. Coprolites provide information on dietary patterns at a very high temporal resolution and can be used to determine specific periods of seasonal site occupancy (Bryant 1974, Vermeeren 1998, Riley 2008). Whilst Reinhard and

Bryant (1992) identify the seasonal signal as a source of dietary bias, the ability to look at resource exploitation on a seasonal basis is a huge strength of coprolites compared to other methods of dietary reconstruction. The diversity of diets at timescales reflecting human experience is an important counterpart to broad, generalized models.

Given their utility, it is unfortunate that coprolites are not routinely considered in many archaeological studies. In later periods, it could be that an abundance of other types of evidence has contributed to the seeming lack of interest in human coprolites. In some regions, such as early prehistoric North American contexts, the general absence of human skeletal remains has led to a greater focus on coprolites as evidence for human presence (Jenkins et al. 2012), the study of early diets, and as a vessel for ancient DNA (Gilbert et al. 2008), though even here it is still considered largely a niche topic (Green and Speller 2017). Coprolites are not regularly collected in the field, perhaps because they are not easily recognized. However, coprolites are no more unusual than other types of archaeological material, once an excavator is trained to recognize them, and in some contexts they can be distinctive (Figure 1). This may be an example of not seeing materials we are not expecting to see, or perhaps even materials that we do not want to see; Van der Geest (2007) suggests that the lack of study of defecation practices within anthropology is a result of our own cultural aversion to the topic, suggesting "(researchers) seem to be restrained by relatively trivial codes of decency, which stop them from openly speaking or writing about such dirty and childish matters as human defecation". This situation echoes that of William Buckland, originator of the term 'coprolite', whose colleague William Wollaston, confessed "though such matters may be instructive and therefore to a certain degree interesting, it may be as well for you and me not to have the reputation of too frequently and too minutely examining faecal products" (Wollaston, quoted in Shortland 1992 p.127).

Here we present the history and current state of research in human coprolite analysis in archaeology, encompassing macroscopic, microscopic and biomolecular approaches, with a consideration of the major research areas and questions to which coprolite analysis can contribute, and areas where further study needs to be focused. We argue that these ecofacts are a key source of information on human diet and health that cannot be obtained by other means, and should be included both where they are the only remains, and on those sites with an abundance of other proxies.

## 2. The history of coprolite analysis in archaeology

The history of coprolite analysis as an archaeological 'discipline' in the Americas has been traced back to the 1950s and 60s with a heavy focus on diet through analysis of plant macrobotanical remains (Callen and Cameron 1955, 1960; Callen and Martin 1969, Watson and Yarnell 1966), though Jones emphasized the need for comparative studies with botanical and faunal material as early as 1936. Callen (1967) for example analyzed plant and animal macro and microfossils from 116 human coprolites representing seven phases of human occupation at the Mesoamerican site of Tehuacan, Mexico. Callen's study included observations on the presence of undigested starch grains preserved in pochote (*Ceiba parvifolia*) root and cassava (*Manihot* sp.) root cells, as well as calcium oxalate druse crystals from *Opuntia* and *Agave* sp. epidermal material and other materials. Definitive methods for species identification remained elusive during this early period, with methods such as the colour and even smell of rehydrated specimens being proposed and dismissed, as it became apparent that physical methods were never 100% reliable for determining coprolite species (Bryant 1994). A series of studies by Bryant (1969, 1974a, 1974b, 1975), Bryant

and Williams-Dean (1975), and Dean (1978) established standards for processing, analyzing and interpreting pollen assemblages extracted from coprolite material which are still followed today.

Coprolite research in the US has typically focused in the arid southwest or Great Basin regions, where there is a prevalence of protected sites and cave/rockshelters which provide favourable conditions for faecal preservation via desiccation (Reinhard and Bryant 2008). Throughout the 1960s and 1970s there were several universities in the US with active research programmes in the southwest, all of which had various interdisciplinary connections between anthropology and geosciences. Much of the research during this period was undertaken in collaboration with parasitologists, and the sub-discipline of archaeoparasitology (the study of parasites in past populations), emerged during this time. Reinhard (pers comm??) notes 1969 as a landmark year, when three high profile papers were published in Science on the topic of coprolite analysis (Moore et al. 1969, Fry and Moore 1969, Hezier and Napton 1969), which consolidated this interdisciplinary approach. In the Great Basin, Robert Heizer (Hester 1982) and Jesse Jennings (Aikens 1999) were prolific field researchers. Jennings directed excavations at sites including Danger Cave andHogup Cave all of which uncovered coprolites, which researchers analysed for dietary and parasite evidence in collaboration with parasitologists (Fry 1970, 1976, 1978; Kelso 1971). Heizer excavated in the Great Basin of Nevada and directed the California Archaeological Survey at the University of California at Berkeley where he promoted the interdisciplinary analysis of coprolites, especially those from Hidden Cave and Lovelock Cave (Heizer 1967). Excavations at Dirty Shame Rockshelter in Oregon directed by Melvin Aikens produced coprolites analyzed for dietary information (Hall 1977).

The late 1960s and throughout the 1970s saw a shift of focus from the Great Basin to the Ancestral Pueblo region of the Colorado Plateau. Archaeologists Art Rohn and Don Morris, working for the National Park Service, recovered coprolites from Mug House, Mesa Verde and Antelope House, Canyon de Chelly, respectively. Cynthia Irwin-Williams (Wormington and Agogino 1994) also excavated coprolites from Salmon Ruin, and J. Richard Ambler, a protégé of Jennings, excavated coprolites from the region of Navajo Mountain and Glen Canyon. Beyond the Ancestral Pueblo region, Texas archaeologists including Harry Shafer, Vaughn Bryant, and Donny Hamilton recovered hundreds of coprolites from Hinds Cave, Baker Cave, and other rockshelters in the region (Bryant 1974b). These researchers also collaborated with parasitologists and directed research into prehistoric parasitism among Texas hunter-gatherers (Reinhard 1990).

Reinhard and Bryant (2008) note a seeming decline in the 1990s and 2000s in the US, though coprolite analysis continued in other parts of the world via archaeoparasitology, albeit on an irregular basis, such as the studies on Maori coprolites in New Zealand (Horrocks et al. 2002). In 2012 Bryant and Reinhard suggest that the emergence of post-processual archaeology treated science with cynicism and was the reason for this decline in North American university-based research. Post-processualism is an umbrella term for changes in archaeological thought, largely seen as a backlash against processualism. Processualism is somewhat similar to the quantitative revolution that occurred in geography during the same period, whereby scholars sought to create a more ‘scientific’ and rigorous discipline. Like quantitative geography, processualism itself came to be criticized by various scholars for an overly analytical focus that did not consider human agency, and for supposing that archaeological evidence could be interpreted in an entirely objective fashion. Bryant and Reinhard suggested that post-processualism emphasized subjectivity, whereas coprolite analysis is the ‘penultimate quantification in terms of archaeological science’ (2012 p 381). We would argue that neither of these statements is entirely correct, and in fact, the emphasis of post-processual theory on self-reflexivity during interpretation, is essential when considering coprolite data. Likewise, the quantification of

coprolite contents is not an exact science and is an area that still requires further research. Nevertheless, interpretations must be grounded in sound empirical data, and the shifts that happened in the late 1970s and 80s in the US led to a move away from the interdisciplinary science based approach to environmental archaeology at many institutions that had previously undertaken such work. A handful continued this type of work, notably Texas A&M University and University of Nebraska, whom still trains coprolite researchers today.

The earliest studies of coprolites in Britain and Europe focused more on the contexts than coprolites themselves, perhaps because the different nature of the archaeological sites produced these deposits, rather than the discrete coprolites that are more common in the cave sites of the Americas. Research dates to the late 1970s and 1980s, with occasional references to earlier studies. For example Warren 1911, who discusses briefly "a pint if not more" of blackberry, rose and Atriplex seeds, recovered from the pelvic region of a skeleton. Overall the volume of research never reached that seen in the US during this period. Latrines and cesspits have long been targeted by environmental archaeologists, due to the preservation of botanical and other remains (Greig 1981, Smith 2013). The waterlogged latrine deposits of the city of York, UK for example have preserved a wide range of materials including insects and archaeobotanical remains attributed to faecal deposition (Carrott et al. 1998, Osbourne 1983, McCobb et al 2003), and the Environmental Archaeology Unit of the University of York produced much of the early research on this topic (Hall and Kenward 2002). Most reports make no direct mention of 'coprolites', aside from a report on the 'Lloyds Bank Turd' (Jones 1983), a large intact specimen associated with the Viking occupation, now on permanent display at the Jorvik Museum. It is noted that whilst latrine and cesspit analyses are looking at forms of human faecal deposits, the formation processes and taphonomy of these pits can be quite different to 'discrete' coprolite deposits in non-urban contexts (Van Oosten 2013). Such deposits contain the faeces of multiple individuals, preventing an assessment of individual diet or health status. Greig (1981) noted that the potential of cesspits 'can only be fully realized when sufficiently large samples are studied by groups of environmental archaeologists in collaboration'.

In Europe during the early 2000s we begin to see a greater focus on human and animal waste (rather than the earlier more general cesspit/latrine studies), particularly within geoarchaeology. The potential of coprolite deposits as a means of exploring prehistoric diet was recognized for example by Matthews (1995), who frequently observed likely human and animal dung deposits in sediment thin sections from early urban settlements in the Middle East (Matthews et al. 1997, Matthews et al. 2014). Since then this approach has been applied to a range of sites in different geographic settings to examine health and diet (Shillito et al. 2011, Pichler et al. 2014). It is also during this period that lipid biomarker analysis began to emerge in archaeology (Evershed 1999, Evershed 2008a, 2008b), including applications of faecal biomarkers (Bull et al. 1998, Bull et al. 1999a, 1999b, Bull et al. 2000), though this technique has remained heavily focused on lipids from pottery until recently.

Bryant and Dean describe coprolite research as having a "discipline identity" crisis (2006, 62), and the lack of recognition for disciplines which bridge different fields, as coprolite investigations do. They argue that there is a difficulty in training, as coprolites contain such a variety of materials that an analyst needs expertise in several fields. We would argue that whilst individual broad ranging expertise is an advantage, coprolite analysis should be approached as a collaborative effort, as Greig (1981) proposed for cesspits. This is particularly true when combining biomolecular and physical methods, which require very different training and skillsets. In some ways coprolite analysis is a microcosm of 'multiproxy' archaeology, which has become a goal for many archaeological projects (Shillito 2017), following the recognition that multiple lines of evidence provide a more robust picture of the past, and reduce equifinality. Such a goal

can be difficult to implement, however a series of publications by Zhang and colleagues in China, where coprolite analysis has a more recent history, indicate that coprolite analysis is becoming incorporated into studies of ancient subsistence in this region from the outset. The traditional model in this region divides agriculture into the north, emphasising dry crops such as millet and the South focusing on water intensive rice, with the earliest sites in both regions around 10,000 BP (Yang et al. 2012; Zhao 1998). At the site of Tianluoshan, where the earliest rice fields have been identified (7000-6000 BP), Zhang et al.'s analysis of lipids in coprolites indicate diets dominated by plant sterols (Zhang et al. 2020a). By combining palynology, Zhang et al. have been able to identify species previously undetected in the archaeological record, including Poaceae and *Typha* sp., characterizing wetland cultivation (Zhang et al. 2020b). This both supports the model of rice cultivation, but also provides nuance, suggesting that wild resources were also very important during this period. In contrast coprolites from the sites of Yuhuicun and Houtieying, located in the transitional zone of rice and millet, are dominated by animal sterols. The combination of microscopic and biomolecular methods, incorporated with wider archaeological data, demonstrates how powerful this approach can be.

There are several methods available for coprolite identification, each with its own advantages and limitations. Once the species has been identified, the information contained within the coprolite can then be extracted and interpreted in light of the species, and context in which the deposit was found. In the following section we provide a comprehensive overview of the methods used for coprolite analysis, from macro- to micro- and molecular, focusing on morphology, contents and residues respectively (Figure 2). It should be emphasized at this point the importance of curation and retaining samples for future study in appropriate storage conditions. There are numerous anecdotal and published instances where precious samples have been poorly stored or curated, leading to loss of crucial contextual information, or even the lost of the samples (Reinhard 2017, Callaway 2019). Recently the need for standardized global access practices for ancient human remains was noted (Austin et al. 2019). Whilst coprolites have never reached the same level of popularity as skeletal human remains, it is likely that this will happen as their value becomes recognized, particularly for genetic studies, and should be subject to the same strict protocols.

## 3. Analysing coprolites: macromorphology and micromorphology

### *3.1 Morphology*

In modern and palaeocecological studies of mammal faeces the size and shape can be used as an indicator of likely species (Taglioretti et al 2014, Chame 2003). Chame (2003) discusses that diagnosis on the basis of morphometric analysis can be accurate in narrowing down the identification of taxonomic group, which can then inform subsequent analyses. Morphological criteria have been used also in palaeobiological studies to differentiate between different types of carnivores (Montserrat Sanz et al. 2016). In archaeology the species that are usually of most interest are humans and associated domesticates. Linseele et al (2013) discuss the use of morphometric methods to identify animal dung, and conclude that even in modern specimens, the variation is usually too large to be able to confidently distinguish between species. In archaeological contexts the problem is exaggerated through taphonomic processes which can alter the appearance of dung considerably from its fresh state. Reinhard and Bryant (2008) note that most coprolites they have encountered do not have a characteristic morphology, due to compression and fragmentation. The compression of coprolites under the weight of successive dumping of sediments leads to distinct faecal 'lenses' in midden deposits at Çatalhöyük (Figure 1) and other tell sites (Shillito et al. 2013b). However, this is not always the case. Coprolites from

the Neolithic site of Durrington Walls, UK for example, preserved in calcareous sediments in pits, retain a typical 'faecal' sub rounded morphology, and were clearly identifiable as coprolites in the field (Figure 3), likewise the large assemblage of coprolites that have been analysed from Paisley Caves, Oregon USA, for example (Shillito et al. in press, Blong et al. in press).

Whilst dung such as bovid and ovicaprids is usually distinct enough to be identified as 'non-human', species that are likely to co-occur with humans in the archaeological record can be easily confused on the basis of gross morphology e.g. pigs and dogs. Both species can have very variable omnivorous diets and produce coprolites that are morphologically similar to humans. In Pleistocene records, and other contexts where humans and carnivores are present together, there has been a lot of research on hyenid coprolites in particular. These are distinctly shaped and generally preserve well due the carnivorous diet of the hyena. The consumption of bone results in elevated levels of phosphorous and calcium in excrement which advances the mineralisation process (Chin 2002). Sanz et al. (2016) present descriptive criteria for distinguishing between coprolites from different carnivores, though it is noted that the size ranges considerably, and the 'morphotype 1' assigned to hyena is visually very similar to samples identified as human elsewhere. Even humans themselves have a potentially wide range of morphologies; the Bristol Stool Form Scale (Figure 4) a 7-point scale with schematic representations of the range of morphology that can be expected in human stool in clinical contexts (Lewis and Heaton 1997, Blake et al 2016). The morphologies are a result of the speed at which digested food moves through the digestive tract, as well as the type of food consumed, and the health and age of the individual. Given that human diets can be hugely variable, it is not surprising that the form of coprolites can also be varied.

Colour, translucency, and smell of reconstituted coprolite in sodium phosphate solution have been used as an estimation of species of origin: human coprolites will typically turn the solution opaque and dark brown or black in colour and will have an intense smell (Bryant 1974b; Holden 1990); carnivore coprolites typically turn the solution white, pale brown, or yellow, while herbivore coprolites turn the solution pale yellow to light brown; in both cases the solution remains translucent and there is a musty smell (Bryant 1974b, Callen and Cameron 1960; Fry 1970). Again, it is noted that this method is not conclusive; freshly excreted human faecal matter can range in colour from light to dark brown with a mixed plant/meat diet to brownish-black with a meat-centric diet, and it is assumed that this will affect rehydrated colour (Fry 1970). In addition, studies have shown that non-human coprolites can also have a black rehydrated colour (see discussion in Reinhard and Bryant 1992 and Reinhard 2017) though, studies comparing rehydration colour to other methods of determining human origin have for the most part supported the rehydrated colour guidelines established above (Reinhard 2017). Despite the limitations of morphological analysis as a species indicator, a description should always be made prior to any further analysis, given that the major of analytical methods are destructive. Jouy-Avantin et al. (2003) published a widely used standardised method for describing coprolite morphological characteristics including photographs, metric measurements, and descriptive characterisations of morphology, inclusions, hardness, colour, and taphonomic modifications.

### *3.2 Micromorphology*

As discussed in section 3.1, the macromorphology of coprolites can be highly variable within and between species, which translates to a high variability at the microscale. It is often possible to observe inclusions embedded within coprolites, which can give useful information on the dietary habits and precise depositional context of the faeces (Figure 5).

The visual characteristics of coprolites under the microscope, in thin section, can be used to assess likely species and give some indications of diet. Omnivores may contain both plant and bone inclusions, with the plant remains being shorter,courser and less abundant than those found in herbivores such as cattle (Bronniman et al. 2017). Coprolites in thin section range in colour from yellow to orange and brown, and even greyish. The reasons for the variation in coprolite colour, has not been thoroughly investigated, though it does not seem to be directly linked to species. In modern faeces, the typical brownish colour is due to the presence of bilirubin, excreted in bile, though as discussed in section 3.1, the exact reason for colour varaiblity within and between species is unclear. There are some indications that colour could be related to preservation conditions – a comparison of coprolites in deeply buried versus more shallow deposits at Çatalhöyük has shown that the latter have a pale brown appearance, and the former being bright orange (Shillito and Matthews 2013). Both types have been confirmed as human on the basis of sterol and bile acid profiles (Shillito et al. 2011).

Micromorphology is especially useful for 'in situ' analysis where coprolites are observed in their depositional context. For example at the early pre-pottery Neolithic site of Sheik e Abad in Iran, lenses of orange deposits were analyzed using micromorphology with high resolution sub-sampling for lipid analysis. The layers were identified as alternating animal and human dung, which has been crucial in understanding the changing use of space in the settlement over time, as well as early waste management and animal penning strategies (Matthews et al. 2013, Shillito et al. 2013a).

The use of microCT on 'thick' sections has shown potential for visualizing the contents of coprolites in three dimensions, and has potential as a non-destructive imaging technique, and to locate specific inclusions within a coprolite (Huisman et al. 2014). In Figure 3 two coprolites from Durrington Walls, UK, were scanned using microCT and found to contain very different levels of digested bone fragments. There is much potential for exploring this technique further with the view to inform sampling strategies for destructive analyses.

A high-profile example of the problems with species identification using morphology is Paisley Caves, Oregon. This rockshelter is well known as it has yielded some of the earliest evidence for human occupation in the Americas, in the form of coprolites identified as human on the basis of ancient DNA (Gilbert et al. 2008) (as discussed further in section 6.2). The identification of a particular coprolite (dated between 14,170 to 14,340 cal BP) as human received specific criticism (Goldberg et al. 2009), as it was argued that morphologically the coprolite resembled animal faeces rather than that of a human, with comparisons drawn between the Paisley sample, and modern camel. Goldberg et al. (2009) included an example of a typical 'human' coprolite from a Viking context, with a yellowish appearance and high phosphatic content. Micromorphological analysis identified that the Paisley sample has fibrous internal vegetation with phytoliths, morphology/staining consistent with a herbivore origin, which Goldberg et al. suggest is similar to herbivore reference samples. FTIR analysis indicated that the coprolite is high in silicates and organic matter, and low in minerogenic phosphates, characteristics suggested as common for herbivore coprolites rather than human or carnivore.

Rasmussen et al. (2009) argue that micromorphology and mineralogical content cannot be used to distinguish between herbivorous mammal faeces and faeces from a human with a primarily vegetative diet. They provide analysis of three coprolites recovered from the pelvic area of Nubian burials in Africa (leaving little doubt they are human) to support this claim. These coprolites had an abundance of phytoliths and do not have the FTIR signature of carbonate hydroxyl apatite suggested by Goldberg et al. 2009 to be diagnostic of a human/carnivore coprolite. They also review many coprolite studies, including several from the Great Basin, that

show the high phytolith and vegetative content possible for humans with a largely plant-based diet. The problem here is dietary differences. There is no established reference data demonstrating the morphological variability of human coprolites as a result of dietary differences. Given that these items are the remains of undigested food, it is no surprise that there may be differences between populations or even individuals with very different diets, and it is not surprising that the Paisley sample appeared different to a Viking sample

This is a problem that occurs commonly, perhaps as a result of our own biases and assumptions. Analysis of coprolites from Neolithic Turkey for example, assumed during excavation to be dog on the basis of morphology and the fact they contained relatively large fragments of bone, were determined to be human on the basis of their sterol and bile acid profiles (Shillito et al. 2011). Contents which may appear unusual or unlikely to be consumed by humans from a modern western perspective, should not be dismissed. Items such as rattlesnake fangs, fibres (McDonough 2019; Sondermann et al. 2019) and elements of rodent skeletons (e.g., vertebra, phalanges, and corpus unguis) suggesting consumption of whole rodents (Blong et al. in press) have all been found in coprolites subsequently identified as human.

## 4. Analysing coprolites: macrofossils and microfossils

The contents of coprolites can be classified broadly as plant, animal, fungal or mineral, and can range in size from the macroscopic i.e. particles that can be readily seen with the naked eye, to the microscopic, needing various levels of magnification for identification. Whilst standard light and stereo microscopy is the typical tool for analysing coprolite contents, high resolution tools including SEM have also been used (Faulkner 1991, Bryant and Williams-Dean 1975, Reinhard et al 2019). Macrofossil and microfossil analysis typically begins by subsampling half of a coprolite divided along the long axis to capture material from all food consumed while the faeces were produced in the digestive system while preserving material for future analysis (Bryant 1969, Fry 1970). Following this step, samples are rehydrated in a 0.5% solution of sodium phosphate for a minimum of 48 hours to soften and disaggregate coprolite material and allow for separation of macrofossil from microfossil material (Callen and Cameron 1960). Researchers in the past have also attempted dry processing coprolite material for macrofossils by crushing the coprolite then picking out macrofossils under a microscope (see Roust 1967), but this is more time consuming and can damage delicate macrofossils.

### *4.1 Plant and Faunal Macrofossils*

Macrofossils are the most commonly recovered and studied component of coprolites, and many early coprolite studies focused solely on macrofossil remains (see reviews in Bryant and Reinhard 2012, Hunt et al. 2012). Plant remains in coprolites typically consist of partially digested tissues, seeds and fibrous remains. Faunal remains range from tiny bone fragments, visible only when coprolites are viewed in thin section under the microscope, to hairs, feathers, scales and larger intact bones and insect fragments visible to the naked eye. Macrofossils are recovered by sieving rehydrated specimens through a standard mesh sieve or graded set of sieves (Bryant 1974a; Pearsall 2015). Macrofossils remaining on the sieve are typically gently dried for identification, although some researchers pick through macrofossils while they are wet to avoid repeated wetting and drying of material that might damage cellular material (Gorham and Bryant 2001). Macrofossils such as seeds and bone fragments (Figure 6) are often relatively straightforward to identify with some knowledge of plant and animal anatomy, identification manuals, and a good reference collection (Pearsall 2015; Reinhard and Bryant 1992). Recovery of bone from human

coprolites is very common, offering an important source of data on hunting and food preparation (Reinhard et al. 2007; Sobolik 1993). Hair, feathers, and scales recovered from coprolites can be identified to the family or genus level, and occasionally species (Day 1966, Reinhard 2000, Dove and Koch 2010). Insects remains recovered from coprolites are often highly fragmented but can retain diagnostic components enabling identification to the family, genus, or species level (Elias 2010:115).

Macrofossils are one of the most useful components of coprolites. Unlike microfossils, there are relatively few ingestion pathways and we can typically be confident they represent direct, intentional consumption (Sutton et al. 2010:52). Macrofossils typically have a higher taxonomic resolution, though are biased towards smaller bones, and less digestible materials. Macrofossils, therefore, can provide a direct link to diet and even foods consumed together in "meals" (Pearsall 2015; Riley 2012; Sutton et al. 2010:51). However, while most consumed foods leave macrofossils in coprolites, these remains are often not identifiable after breaking down in the digestive system (see section 7.2 digestive taphonomy). Bone, chitin, hair, shell, feather, tendon, and cartilage typically pass through the digestive system in recognizable form, while muscle tissue and plant material preservation can be quite variable (Wilke and Hall 1975; Holden 1994). Most ingested plant material is broken down in the digestive system, and the excreted material is a partial sample biased towards materials less affected by food processing and digestion (O'Meara 2014). Intact seeds and/or seed coats are often preserved in coprolites, but if the exterior seed coat has been compromised during food processing or digestion, then these remains may also not preserve in identifiable form (Reinhard and Bryant 1992). Plant fiber is typically not affected by the human digestive system, so this will often disproportionally dominate the plant component of a coprolite (Fry 1976). Larger bones must be processed or chewed before they can enter the digestive system, and these fragments often show signs of partial digestion such as pitting of the surface (see section *** for further discussion on the impacts of these taphonomic processes on bone).

*4.2 Plant Microfossils - pollen*

It was recognized in the 1930s that pollen grains could be well preserved in coprolite material, but it wasn't until the 1960s that this was used to provide insight into human food consumption (see review in Bryant 1974a). Pollen grains extracted from coprolites have provided important information on consumption of plant material for food and medicine, as well as information on past environments (Bryant 1974a, 9174b; Chaves and Reinhard 2003; Martin and Sharrock 1964; van Geelet al. 2008). Identifying intentional consumption of food from pollen recovered from coprolites requires knowledge of plant pollination biology (e.g., insect, wind, or self-pollinated), the expected ambient (natural) pollen rain in the environment, an understanding of how pollen moves through the human digestive system, and knowledge of past food preparation practices (Bryant 1974a; Dean 1978, 1993; Reinhard et al. 1991; Reinhard et al. 2007). Without considering these characteristics, interpretive errors can be made that lead to erroneous reconstructions of human behavior (Reinhard et al. 2007).

A primary means of determining intentional consumption is by comparing concentration and relative proportion of grains. Pollen concentration values are determined by adding a known amount of a marker, typically an exotic spore like *Lycopodium* (Benninghoff 1962; Pearsall 2015:223; Maher 1981). More recently, non-organic markers such as ceramic spheres have been used for the same purpose (Kitaba and Nakagawa 2017). High frequencies of pollen from a specific taxon in a coprolite is typically linked to intentional consumption of flowers or seeds with pollen grains still attached, or in some cases foliage of that taxa (Bryant 1974a, 1974b; Sobolik 1988; Reinhard et al. 1991; Zhang et al. 2020b). Zhang et al. (2020b) for example

identify extremely high percentages of *Typha* (cattail) pollen (> 93%) in 21 coprolites samples from the Neolithic site of Tianluoshan, in the Lower Yangtze Region of China. In comparison, concentrations in natural deposits 50m west of the settlement area are less than 10% (Ma et al. 2018). In this case the coprolite pollen can more confidently be interpreted as intentional consumption.

Pollen from anemophilous (wind-pollinated) taxa are produced in relatively high frequencies and are commonly incorporated into ambient pollen rain, so linking frequencies of pollen from anemophilous taxa to intentional consumption is more problematic (Bryant and Holloway 1983; Martin and Sharrock 1964; Reinhard et al. 1991). It is assumed that ambient pollen will be represented in relatively equal amounts across a series of samples from the same site (Reinhard et al. 1991; see also discussion of cautions with this assumption in Dean 1993). Research using a large pollen data set has demonstrated that ambient pollen is less frequently represented in coprolite pollen when large amounts of pollen-rich foods are consumed (Reinhard et al. 2006). High relative frequencies (>40%) and concentrations (<100,000 grains per gram of coprolite) of pollen from anemophilous taxa are considered to represent intentional consumption of flowers, buds, foliage, or seeds of that taxa because frequencies and concentrations higher than this are not often observed in anemophilous taxa in naturally-derived samples (Bryant 1974a; Reinhard et al. 1991). When concentrations of pollen from an anemophilous taxa reach more than 1,000,000 grains per gram then it is more assured that this represents intentional consumption of flowers, buds, foliage, or seeds of that taxa (Reinhard et al. 1991). Pollen from entomophilous (insect-pollinated) taxa present in amounts of 2 to 4% suggests intentional consumption of that taxa (Bryant 1975; Reinhard et al. 1991).

It is important to consider both relative frequency and pollen concentration for individual taxa. Pollen concentration values highlight the amount of pollen per unit of sample, highlighting variations in pollen accumulation and total pollen abundance above the mean for a specific taxon that may be masked by simply comparing relative frequencies with an arbitrary relative percent cutoff (Dean 1993; Reinhard 1993; Reinhard et al. 2006). Pollen concentration values are strongly influenced by the consumption of pollen-rich food and therefore are valuable for assessing the magnitude of pollen ingested in the diet (Reinhard et al. 2006). The most robust inferences for diet using coprolite pollen data are made through comparative statistical studies of pollen concentration values in tandem with presence of aggregate grains, particularly when assessing anemophilous taxa consumption (Chaves and Reinhard 2006; Reinhard et al. 2006). Studies have demonstrated that there can be a lot of variance in coprolite pollen concentrations (Bryant 1974a, 1974b, Sobolik 1988; Reinhard 1993); this information can be used to solidify interpretations of intentional consumption (Reinhard et al. 2006). The link between high frequencies of pollen and/or aggregates of pollen from a specific taxa and consumption of seeds or other non-flower vegetative matter of that same taxa has been demonstrated in previous studies (Reinhard 1993). Consumption of flowers is often recognized by the presence of large pollen aggregates representing pollen on the anther of a flower (Bryant 1974b). Non-floral parts of insect-pollinated taxa are not expected to have high amounts of residual pollen, so consumption of these components is not expected to be represented in the pollen record (Martin and Sharrock 1964, Bryant 1974). Pollen aggregates for both anemophilous and entomophilous taxa are also taken as evidence for intentional consumption of flowers of that taxa. The use of aggregate pollen alone to determine intentional consumption is not reliable given that pollen can be disaggregated by food processing and digestion (Reinhard 1993).

There are several caveats to the use of pollen from coprolites to reconstruct intentional plant consumption. Several studies have demonstrated that pollen distributions in human coprolites are not random, so that where you collect a sample can affect the resulting pollen data set (Beck et al.

2019; Martin and Sharrock 1964). Coprolites collected a few cm from one another in the intestinal tract of a mummy had variations between 9,000 grains/gram to 3,400 grains/gram; this variability appears to be linked to the amount of fiber consumed in the meals represented by each coprolite (Reinhard 1993). Experimental studies demonstrate that the rate that pollen exits the human digestive system can vary greatly between individuals or day to day in the same individual. Most pollen exits the digestive system 2-4 days after consumption, but lesser amounts can sometimes take up to 32 days to be fully passed from the intestine (possibly linked to pollen morphology e.g., small pollen with significant exine surface sculpturing is more likely to be caught in the intestinal folds during digestion) (Dean 1993; Kelso 1976; Sobolik 1988; Reinhard 1993; Williams-Dean 1978). However, some researchers have questioned whether modern actualistic studies are appropriate analogues for the past because high-fiber, seasonally-variable hunter-gatherer diets in the past may have caused pollen to pass through the digestive system at different rates than that observed in modern individuals (Reinhard 1993). More carefully designed experiments are needed to fully understand these processes, for example Reinhard et al. (2006) suggest harvesting commonly consumed wild plant parts to determining the number of pollen grains per gram of material to better interpret coprolite pollen concentration values.

### *4.3 Plant microfossils - Starch and phytoliths*

Phytoliths and starch are commonly analyzed together. Phytoliths are mineral deposits, usually silica, that form within and between plant cells, forming three dimensional casts of the cell morphology. Phytoliths have been observed in large quantities in animal dung (Goyleve 2012, Qiu et al. 2014, Portillo et al. 2020). Phytoltihs in human coprolites have been identified by Horrocks et al. 2002 identify spherical spinulose phytoliths tentiaviely intrpeted as nikau palm, in Maori coprolites, and Haas et al. identified miaze phytoliths in coprolites. The ability to identify phytoliths to a meaningful taxonomic level is highly variable. Some species can be identified if large enough tissue fragments are preserved, but often these preserve only as single cells, which have a poor taxonomic resolution (Shillito 2011). The presence of these in coprolites is highly dependent on the types of plant being consumed, the part of the plant that us consumed, and whether the plant produces abundant quantities of phytoliths – this in itself is an area that is not well understood (see Shillito 2013). Monocotyledonous plants that grow under conditions of high water availability, and with a silica-rich substrate, produce large quantities of phytoliths. Whilst cereals such as wheat can be identified through the distinctive morphology of the husk phytoliths, it is not clear whether these would be present in coprolites due to crop processing methods which would remove the husks prior to consumption. Calcium oxalate crystals (sometimes known as calcium oxalate phytoliths) are especially common for example in Archaic coprolites in the lower Pecos region of west Texas, due to the consumption of prickly pear and agave, which were the dietary staples in west Texas for 6,000 years (Danielson and Reinhard 1998).

Starch on the other hand is a carbohydrate molecule, composed of amylose and amylopectin molecules, which form granules whose morphology is linked to specific species. There have been limited studies of starches from coprolites. It is unclear whether these would survive the digestion system in large numbers, though they have been observed within animal faeces (Figure 5), within partially digested plant tissues. Starch grains are consumed within starch-rich plant tissues such as seeds and underground storage organs, and for humans to gain the nutritional benefits of starch the grains must be broken down during food processing and digestion (Torrence and Barton 2006). As a result of these mechanical and chemical processes many starch grains are destroyed by the time a meal is passed through the intestine and deposited as a coprolite (Samuel 2006 in Torrence and Barton 2006 book). There are situations where starch grains can be preserved in

coprolite material, typically because they are protected by plant material (e.g., plant tissue, seed coat); however, the starch assemblage recovered from coprolites likely represents a biased sample consisting of the most robust (e.g., thicker cell walls) starches (Pearsall 2015:350).

As noted above, Callen (1967) observed starch grains preserved in undigested cassava and pochote root tissue. This provides an example of starch grain preservation through the protection afforded by undigested plant tissue. Horrocks et al. (2004) identified sweet potato (*Ipomoea batatas*) starch grains in human and/or dog coprolites excavated from two prehistoric Polynesian settlements in northern New Zealand. Starch grains recovered from some of the coprolites exhibited better preservation than pollen and spores recovered from the same sample, suggesting that starch grains may preferentially preserve over other microfossils in some deposits. Starch grains have been recovered in varying amounts in Archaic-period human coprolites from the Lower Pecos canyonlands, Texas (Riley 2012), Inka-period human coprolites from the Atacama Desert in northern Chile (Vinton et al., 2009), late Archaic-period human and dog coprolites from the western coast of Peru (Haas et al. 2013), and middle to late-Holocene human coprolites from Antelope Cave in northwest Arizona (Reinhard et al. 2012). Well-preserved starch grains can be diagnostic to the species level and can provide important information on consumption of starch-bearing parts of a specific. This is particularly important in the case of underground storage organs, whose fleshy remains are typically not distinguishable to this taxonomic level (Pearsall 2015:133). However there are uncertainties over the morphological consistency of starch granules – in modern samples a range of environmental factors influence the granule morphology (Lindeboom et al. 2004, Zhao et al. 2018), and there are also concerns related to taphonomic processes (Collins and Copeland 2011).

### *4.3 Microfossils – fungal*

Whilst fungal spores in animal dung have been studied extensively (e.g. Perrotti and Van Asperen 2018, van Asperen et al. 2020), fungal spores in human coprolites are understudied. Besides edible mushrooms, many fungi are used medicinally. Other fungi may have been ingested accidentally together with foodstuffs, including plant pathogens. Whilst most edible mushrooms are not encountered as macrofossils in coprolites, their spores could potentially survive passage through the digestive tract. Many of these spores are very small (<10μm in diameter), and many are not identifiable to species level. However, some of these spores could provide significant information about diet and health. For example, Battillo (2018; see also Reinhard 2006) found spores from the corn smut *Ustilago maydis* in human coprolites from Turkey Pen Ruin rockshelter, Utah. The consumption of this fungus was deliberate, and perhaps encouraged as a dietary supplement which counteracts some of the nutritional deficiencies that are associated with a maize-dominated diet. The cultivation of the fungus, to the detriment of the corn, and its consumption for medicinal reasons and as a delicacy is still prevalent among Pueblo groups and does not lead to any adverse effects (Dahl 2009).

## 5. Non-dietary inclusions: parasites and inorganic material

The majority of categories discussed so far are items consumed as food, and some which may have been ingested for medicinal purposes. Other items occur as a result of gastro-intestinal infection, and injection of inedible items. Non-food pathways to consumption is something that needs to be considered in future studies. Several items identified in coprolites from Paisley Caves for example have ethnographic analogues that suggest consumption for medicinal purposes, such

as sagebrush leaves (Blong et al. in press), and other items may be ingested through craft activities, for example preparation of cordage. Inclusions of minerals and pigments entrapped in dental calculus, have been linked to craft activities in medieval populations (Radini et al. 2016, 2019), and Radini et al. (2017) discuss the range of potential depositional pathways through which microdebris can enter the mouth. This in turn could translate to these items entering the digestive system. Other items that have been observed in coprolites include diatoms, which may enter the digestive tract for example through drinking water where these organisms live (Figures 5 and 7).

Intestinal parasites in coprolites have been identified dating from early prehistory to Medieval periods (e.g. Bouchet 1995, Florenzano et al. 2012, Ledger et al. 2019a, 2019b, Jones 1983), with extensive work on Roman cesspits and latrines (e.g. Greig 1981; Mitchell 2017; Williams et al. 2017). Parasites are organisms that spend at least part of their lifecycle within the bodies of individuals of a different species, which is called a host, and in general negatively affect the fitness and sometimes even the survival of the hosts. They are found in coprolites in the form of eggs or larvae (Dittmar 2009). Intestinal parasites are common in humans where sanitary conditions are poor and include helminths and intestinal protozoa. Compared to microfossil studies from coprolites, the approach to quantifying parasites was initially more standardized across research groups, based largely on knowledge of parasite life-cycles along with archaeological data. Furthermore, infection with protozoa can now be shown using specific enzyme-linked immunosorbent assays and molecular biology techniques (Araújo et al. 2015). There were standardized procedures producing reliable and comparable data sets across regions. However this changed by 2003 as new, non-quantitative approaches emerged (Bain 2001, Araujo 2012, Reinhard 2017).

A detailed history of 'archaeoparasitology' in the Americas is given in Reinhard and Araújo (2012), who identify three phases: an exploratory phase (1910 to 1974), a population phase (1976–1987), and synthesis of archaeology and parasitology from 1987 onwards. Archaeological parasitology, in its early phase , initially sought to identify the origin and movement of parasites through time and space using presence/absence studies (Jones 1982, 1985, Camacho et al. 2018, Camacho and Reinhard 2020). Certain parasites are specific to particular host species, and presence of human-specific helminths, such as *Enterobius vermicularis* (Reinhard et al. 2016), or egg size can enable the identification of human coprolites. Some of the earliest studies focus on simply the identification of various parasites within human faeces, for example early evidence of pinworm infection (e.g. Fry and Moore 1969; Moore et al. 1969), which not only indicates a definitive human origin for the coprolite, but also suggests high population density (Reinhard et al. 2016). Such studies can also account for the origins of parasitic infections. For example, the nematode *Ascaris lumbricoides* parasitizes humans, whilst its close relative *A. suum* parasitizes pigs. An early date for *A. lumbricoides* eggs (Giuffra et al. 2000) suggests that the parasite jumped from humans to pigs after domestication. In contrast, *Taenia* spp. are present in human coprolites from areas where beef and pork are consumed, but absent from pre-Columbian New World sites, suggesting consumption of these foodstuffs caused the first infections with this parasite (Gonçalves et al. 2003).

Findings of parasites that have several hosts can indicate consumption of food sources with a parasite load, or contact with intermediate hosts such as livestock or fish (Gonçalves et al. 2003). Such parasites need not have infected the human in whose faeces they are found; they may simply have been ingested with the food and passed (false parasites; Reinhard 2017). For example, the presence of *Diphyllobothrium latum* and *D. pacificum* in human coprolites reflect consumption of their intermediate hosts, fresh water and marine fish respectively (Gonçalves et al. 2003).

The approaches summarized above are known as pathoecology: the study of how parasites were transmitted within and between populations (Martinson et al. 2003; Reinhard and Bryant 2008; Reinhard and Araújo 2014). Pathoecology encompasses human factors such as archaeological reconstructions of living conditions, sanitation and hygiene, along with biological factors including the presence of particular pathogens, their reservoirs and hosts. Physical factors can also be involved. The conceptual basis of pathoecology has been attributed to Russian zoologist Yevgeny Pavlovsky, who has also been credited with coining the term palaeoparasitology' (Slepchenko and Reinhard 2018). Slepchenko and Reinhard's review of Pavlovsky's work and other palaeoparasitology research from Russia illustrates the significant contribution that this work has made to the broader field.

A 'palaeoepidemiological' approach, focuses on the statistical analysis of parasite data and the quantification of 'eggs per gram' (Camacho et al. 2018). This has enabled the assessment of overdispersion, the well-documented phenomenon that in a population, a small number of hosts carry large numbers of parasites, whilst the rest of the population carry no or few parasites (Camacho et al. 2018). This phenomenon influences both individual fitness as well as population stability. Larger sample sizes from as wide a range of contexts are needed for prevalence and palaeoepidemiological studies to avoid sampling the same individual multiple times (Reinhard 2017). This is a problem especially among hunter-gatherers, where prevalence tends to be lower than among agricultural populations, and sites are more ephemeral (Reinhard 1988; Araújo et al. 2015; Reinhard et al. 2016; Camacho et al. 2018).

## 6. Biomolecular approaches

There are several early instances of biochemical analyses of coprolites, but Wilke and Hall (1975) note that at the time this was not a well progressed area, although they suggested it would "receive further attention in future studies" (p.3), a forecast now shown to be correct. Fry (1970, 1976) conducted a study of 146 coprolites from early through late Holocene occupations at Danger Cave, Hogup Cave, and Glen Canyon in Utah. Fry's study looked at plant and faunal content, but also micronutrients and an early application of "lipid class gas chromatographic analysis", along with various other analyses typically carried out on modern faecal material in medical laboratories (e.g., guaiac test for blood, ICTO test for bilirubin). Fry's seminal study provided evidence of seasonal occupation of some sites and a broad-based subsistence strategy focused largely on consumption of local plant resources that remained remarkably homogenous over 10,000 years of occupation. Fry's study served as a benchmark for future multiproxy coprolite studies in the region.

Since the earlier phases of coprolite research, archaeological science as a whole has seen fundamental shifts in the types methodologies and technologies that are available. The field of biomolecular archaeology, encompassing DNA, lipids and proteins, has grown at a fast pace. Ancient 'lipidomics' and genomics are now at the forefront of the field and ancient proteomics is a rapidly emerging technique. The term biomarker as used in archaeology and geoscience refers to any biological molecule, the presence of which indicates a specific living organism. In archaeological applications it has been used to refer to aDNA, proteins and lipids, all of which are organic molecules, but with very different chemistries. Lipid molecules have poor solubility in water, and are unlikely to be moved from the site of deposition. Proteins on the other hand are composed of amino acids which vary greatly in their solubility. DNA is a polar molecule, and modern DNA is highly soluble in water. Additionally, DNA degradation is complex and results in characteristic post-mortem damage (Lindahl 1993), similarly damage patterns can be observed in

ancient proteins (Hendy et al. 2018). Whilst these changes in primary structure can hinder downstream analysis, they are useful features for authenticating truly ancient biomolecules.

### *6.2 DNA*

For a field only a few decades old, ancient genomics has seen remarkable advances. This progress can be largely attributed to technological revolutions (i.e. next-generation sequencing and high-performance computing) which have significantly reduced costs associated with DNA sequencing and provided a suite of new bioinformatic tools for data analysis (Hofreiter et al. 2015). Although hard biological tissues are the most common archaeological substrates selected for ancient DNA analysis, aDNA workflows have been optimised for a wide range of archaeological material (Green and Speller 2017). Coprolites are an abundant source of ancient DNA deriving from varied sources; DNA molecules from the depositing individual, their diet and associated microorganisms are potentially preserved within a coprolite matrix. Previously, DNA from coprolites has been accessed using targeted approaches (e.g. PCR, 16S rRNA and metabarcoding). However, when analysing a substrate which contains DNA from a range of sources, such as a coprolite, a non-targeted metagenomic approach, coupled with strict contamination control and authentication, may be most suitable to fully characterise the genetic make-up of these unique biological archives. We suspect metagenomics approaches will be most commonly implemented in future coprolite aDNA investigations. The recovery of aDNA, not just from the depositing individual, but from that individual's living environment, means coprolites can provide a truly rare glimpse of everyday life in the past.

#### *6.2.1 Depositing species*

As discussed in section 3, identifying the depositing species is the first question for coprolite investigations. As subsequent morphological inspections should have been completed, targeted aDNA analysis (i.e. using primers to target the DNA of candidate species) is recommended if this is the only question of interest. For a more comprehensive investigation, metagenomic approaches can also be used to determine the depositing species (Hagan et al. 2020) and to ascertain the biological sex of the depositing individual (Skoglund et al. 2013). Using metagenomic data, Hagan et al. demonstrate that the microbial community also distinguishes between a human or a dog host (2020).

The host origin of fourteen coprolites from the lower levels of Paisley Caves, Oregon became the subject of controversy in 2008. When targeted mitochondrial DNA (mtDNA) analysis identified the samples as human (six with Native American haplogroups A2 or B2), three of these coprolites became the earliest evidence of the peopling of North America (Gilbert et al. 2008). However, Poinar et al. (2009) deemed the mtDNA identifications inconclusive, in part, due to the presence of putative contaminating sequences (e.g. canid DNA) and insufficient evidence that the human DNA had not moved through the sediment from higher levels (DNA leaching). In response, Gilbert et al. (2009) concede that it was impossible to unequivocally demonstrate that DNA leaching derived from concurrent prehistoric human and canid occupants of the cave had not contaminated the coprolites from the lower levels, but the group felt it was unlikely that this was the case. To investigate the potential of leaching, Jenkins et al. (2012) included the analysis of associated sedimentary samples alongside 65 coprolites. The identification of 25 coprolites as human supports the original conclusions of Gilbert et al. (2008) (although a small number of samples have a mix of canid and human DNA which remains unexplained).

The movement of DNA from excretions such as urine and feces through cave sediments in New Zealand has been documented. Despite there being around 350 years between Moa (extinct large, flightless birds) and Ovis sp. (sheep) occupying New Zealand (Holdaway and Jacomb 2000), DNA from both species was recovered from the same cave strata (Haile et al. 2007). Furthermore, the quantity of Ovis DNA decreased with sediment depth, suggesting the non-native sheep DNA had leached through to the older levels, in this case via urine percolation (Haile et al. 2007). Haile et al. (2007) suggested that sediment must be flushed with excretion for that organism's DNA to be detected. DNA leaching appears to occur to different degrees depending on the sediment conditions (Hebsgaard et al. 2009). A contemporary study in zoo enclosures confirmed that soil structure impacts the extent of DNA leaching alongside population density (Andersen et al. 2012). These studies demonstrate we still know relatively little about the mechanism of DNA leaching and —as open systems— it is likely that coprolites will be similarly affected if leaching occurs.

Discrepancies between host identifications are yet another reminder of the importance of adhering to field standards when working with ancient DNA (Poinar 2003). It is imperative to use stringent authenticity criteria when assessing findings, both bioinformatically (e.g. tools to assess characteristic features of damaged DNA (Jónsson et al. 2013; Malaspinas et al. 2014; Weiß et al. 2015)) as well as by applying critical logic to assess identifications (Gilbert et al. 2005). Whilst authentication is important in all aDNA investigations, the authenticity of metagenomic data generated from coprolites is paramount, firstly as this is a relatively new sub-discipline and secondly, as a wide range of species DNA are retrieved from these substrates. Currently, identification of species from DNA sequences relies on comparisons to publicly available databases (e.g. NCBI (Federhen 2012)). Whilst these records are extensive, they do have limitations, including contamination of reference genomes (Merchant et al. 2014; Lu and Salzberg 2018), omissions of uncharacterised species (especially microbial taxa), and a lack of contemporary reference genomes for archaeological investigations (see Breitwieser et al. 2017 for a comprehensive review). These limitations make the criteria for good practice laid out in the early days of aDNA work pertinent for coprolite investigations and any species identified by a small number of sequences must be interpreted with caution.

*6.2.2 Diet*

Ancient DNA analysis has been applied to coprolites to investigate dietary resources, initially through the PCR amplification of single markers and more recently through high-throughput sequencing techniques. The majority of dietary investigations have been undertaken on animal coprolites and demonstrate the potential to retrieve high-resolution dietary data which can be linked to subsistence and/or provisioning (Poinar et al. 1998; Hofreiter et al. 2000; Wood et al. 2008; Wood et al. 2012; Wood et al. 2016). Surprisingly, very few studies have attempted similar reconstructions using human material. Poinar et al. (2001) demonstrated the potential for specific dietary reconstruction by identifying 12 different plant and animal species in a targeted PCR investigation of Native American coprolites from Hinds Cave. More recently, attempts have been made to reconstruct dietary resources available to medieval European (Appelt et al. 2014a) and pre-Columbian (Rivera-Perez et al. 2015) cultures using Eukaryotic viral genetic material recovered via a non-targeted, high throughput approach. There are limitations to ancient viromics, particularly relating to the survival of genetic material and species identification, and considering zoonotic potential, the presence of a Eukaryotic virus does not necessarily mean the viral host was consumed. A more fruitful method to access dietary DNA from coprolites is via confident species assignment from deep sequencing metagenomic data.

*6.2.3 Ancestral gut microbiomes*

In modern medicine, investigations into the microorganisms that occupy the digestive tract - collectively known as the gut microbiota - have excelled, due to the recognition that the profile of microbes living in us have immense implications for human health, mood (Schmidt 2015) and behaviour (Johnson and Foster 2018). There are two substrates in the archaeological record which preserve evidence of ancestral microbiomes: dental calculus and coprolites (Warinner et al. 2015). Clinically, the microbes present in faeces are taken to represent the gut microbiota (Peterson et al. 2009); archaeologically, coprolites are taken to represent faeces and therefore at least some of the surviving bacteria are thought to be derived from the guts of past people. It is surmised that the move towards modern lifestyles (urban, modern healthcare, homogenised annual diet, processed foods etc.) have significantly altered the microorganisms in the gut (Segata 2015). Furthermore, declines in microbial diversity correspond with a lifestyle gradient: hunter-gatherer groups exhibit the most diverse microbiomes, followed by traditional agricultural communities, and least diverse are highly-urbanised, post-industrial microbiomes (Jha et al. 2018).

The microbial profiles of traditional communities differ to those leading modern lifestyles in three main ways: 1) increased species diversity; 2) the presence of characteristic species; 3) differences in the relative abundance of phyla (Davenport et al. 2017). It is hypothesised that these characteristics reflect gut signatures of the past (Sonnenburg and Sonnenburg 2019), and thus coprolites provide an opportunity to test these hypotheses. To date, over 30 microbial genetic investigations of archaeological faecal samples have been published (Luciani et al. 2006; Tito et al. 2008; Tito et al. 2012; Santiago-Rodriguez et al. 2013; Appelt et al. 2014b; Cano et al. 2014; Tett et al. 2019; Hagan et al. 2020), which support the hypothesis that gut flora of traditional communities reflect its ancestral state. These studies have primarily focused on prehistoric American contexts (Americas: 30, Europe: 1). Notably, coprolite samples recovered from the Rio Zape Valley (Durango, Mexico) appear to have preserved optimally for aDNA investigation as four papers —including the landmark ancient gut microbiome paper by Tito et al (2008)— have successfully characterised gut microbiomes this site (Tito et al. 2008; Tito et al. 2012; Tett et al. 2019; Hagan et al. 2020). Nevertheless, ancient gut microbiomes do not preserve equally well in all archaeological contexts. This issue is discussed in greater depth below (see ‘6.2.3.2 Community Signature’).

Pucu et al. 2019. Compare the results of micro and macrofossil content analysis with previously published microbiome data on the same coprolites, and demonstrate that the former can provide useful information that helps interpret the results from microbiome analysis. Hagan et al. (2019) suggest that microbes can also be used as a species indictor and distinguished between human and dog on the basis of bacterial sequences. A recent paper by Borry et al (2020). uses a combination of host and microbial DNA to infer the host source of fecal material. The use of DNA for confirming species has been problematic for various reasons discussed above, not least because amplification methods can enhance trace levels of dog DNA in humans that have consumed dogs, or in dogs that have consumed human faeces. By using shotgun metagenomics Borry et al. looked at the gut microbiome, which differs between mammal species, in addition to the host DNA, and used a bioinformatics approach to predict faecal source based on the taxonomic composition of the microbiome. Interestingly, a comparison between the samples Borry et al. analyse, and a previously published dataset on the parasites from the same samples, indicates that the parasites are good predictors of whether a sample has a dog or human origin (Jiminez et al. 2012).

6.2.3.1 Treponema and Prevotella as indicators of pre-industrial microbiomes

Whilst a community assessment of microbial flora can provide insight into the relative abundance and presence/absence of taxa, there are problems identifying and authenticating ancient microbial species (Warinner et al. 2017). Thus, taking a more streamlined approach can be beneficial whilst species identification and their roles in gut ecologies are being investigated. One group of microbes of interest are Treponemes; bacterial species found in the human intestines (Smibert 1981). Treponema sp. are not typically observed in the guts of industrialised communities (Angelakis et al. 2019), but are detected in modern hunter-gatherer communities (Schnorr et al. 2014), traditional horticulturalists (Gomez et al. 2016) and non-human primates (Nishida and Ochman 2019). In these aforementioned groups, the identification of Treponema sp. improve nutrient extraction from fibrous foods (De Filippo et al. 2010) broadening dietary choices. Treponema have also been identified in coprolites (Tito et al. 2012), suggesting that the loss of this species is linked to modern lifestyles.

Tett et al. 2019 have focused on the investigation of one gut microbe, Prevotella copri, identifying four distinct clades. Additionally P. copri was significantly more prevalent and diverse in "non-Westernised" (defined in Tett et al. (2019)) compared to Westernised populations. The analysis of pre-colonial South American coprolites from Rio Zape reflect the non-Westernised prevalence and diversity of P. copri (Tett et al. 2019). The Prevotellaceae family have been previously identified in coprolites from the same site (Tito et al. 2012; Hagen et al. 2020) and from a single Medieval coprolite from Namur, Belgium (Appelt et al. 2014b). Investigations into Treponema and Prevotella sp. suggest that these genera are diminished in the industrialised world.

6.2.3.2 The recovery of coprolite metagenomic signatures

The microbial content of coprolites is not only made up of authentic gut microbes, but microorganisms that infiltrate the matrix from the depositional environment. Microbial signatures from coprolites have been shown to be more similar to one another than to a modern microbiome (Tito et al. 2008). These microbial similarities and differences could reflect diachronic and dietary factors, but likely also indicate that the degradation process influences the microbial profile. It is important to note that coprolites are open systems, and that microbiota can infiltrate the coprolites from the surrounding depositional environment. Whilst the microbial signatures of some well-preserved coprolites clustered with the faecal rather than sedimentary group (Tito et al. 2008), it is likely that environmental contamination will have some effect, even in ideal environments such as cool, arid cave contexts. Furthermore, a subsequent study by the same research group demonstrated that the extent to which coprolites are affected by soil microbes varies hugely between samples (Tito et al. 2012). Thus, coprolite microbiomes may cluster more closely together due to the common presence of environmental microbes, which will of course be absent from modern faecal signatures.

Environmental contamination of coprolites can be mitigated to an extent by a careful sampling strategy. As the outer surface of a coprolite is in direct contact with the surrounding sediment, more of the exogenous, environmental species are identified here compared to the coprolite core (Cano et al. 2014). As such, coprolite subsamples should be taken from the core and homogenised in sterile conditions (Wood and Wilmshurst 2016). Other methodological research has demonstrated that the use of the Human Microbiome Project protocol (utilising the MoBio PowerSoil DNA extraction kit) yields less DNA than methods optimised for aDNA extractions (Hagan et al. 2020). Whilst these findings are unsurprising, Hagan et al. helpfully note that the clogging of silica columns during DNA purification steps is common when working with coprolites. To alleviate clogging, they recommend splitting the lysates equally between two silica columns (Hagan et al. 2020). Importantly, Hagan et al. recommends guidance for the

standardisation of coprolite DNA analysis and demonstrates the suitability of comparing modern microbial profiles to ancient ones. Nevertheless, until optimised bioinformatic tools are developed to more effectively differentiate between the endogenous host microbiome and exogenous environmental microbes, studies attempting to characterise the microbial diversity or relative abundance of phyla within ancient guts, must proceed with extreme caution.

As demonstrated, coprolites offer huge potential for the future study of the evolution of the human gut microbiome in the future. The ability to pinpoint specific timepoints will enable researchers to link particular lifestyles, or subsistence strategies with compositional changes in the gut microbiome. At this time, we are yet to see the true implications of newly emerged techniques and deep metagenomic Illumina sequencing. The generation of a high number of reads using a non-targeted approach will not only improve representative species diversity in the sample but importantly improve our ability to authenticate dietary and gut derived DNA due to higher coverage of these species. We are convinced the future aDNA investigations of coprolites will add interesting and complementary results to traditional archaeological findings (regarding diet) and other bioarchaeological techniques applicable to coprolite analysis (as discussed throughout this review).

### *6.3 Lipids*

Lipids are part of all living organisms and are a highly complex and diverse group of molecules. An advantage of lipids compared to other biomolecules is that they are relatively stable, and can persist with minimal alteration, or break down to a stable byproduct. All lipids are composed of a combination of glycerol and fatty acids and can be subdivided into various groups according to their structure.

#### *6.3.1 Faecal lipid biomarkers*

Faecal sterols form in the gut through bacterial conversion of dietary sterols and give an indication of whether the subject had an omnivorous, carnivorous or herbivorous diet, depending on the proportion of animal and plant derived sterols in the faeces. Bile acid profiles are unique to particular genera and species, with humans having a dominance of deoxycholic acid and lithocholic acid, whereas goat for example has been distinguished by chenodeoxycholic acid, and pig by hyodeoxycholic (Prost et al. 2017, Harrault et al. 2019). Together sterol and bile acid molecules have been used to identify sources of faecal pollution in water from modern-day sewage pollution and agricultural runoff (Grimault et al. 1990, Shah et al. 2007). This method was modified by Bull (1999a) to account for the diagenetic transformation of coprostanol in archaeological contexts. There has been an increasing use of faecal biomarkers in archaeological investigations whereby a combined characterisation and quantification of both sterols and bile acids has, enabled for example the identification of an ancient sewage culvert and insights in the agricultural practices of the Minoans (Bull et al., 1999b; Bull et al., 2002), identifying use of space in early settlement sites (Baeten et al., 2012, Shillito et al., 2011a). Lipid biomarkers can be analyzed from bulk sediments where faecal deposition is suspected, and from discrete coprolites. A notable example of the former is the investigation of anthropogenic dark earths or terra preta, the product of anthropogenic additions of organic matter, including faecal waste, to natural ferrosols (Glaser and Birk 2012).

One of the debated Paisley samples discussed in section 3.2 and 6.2 has also been subject to lipid analysis (Sistiaga et al. 2014), which indicated a sterol profile consistent with what is presented as

an animal origin. However the sterols by themselves only give an indication of the major dietary profile (i.e. meat or plant dominant), and must be used in conjunction with bile acids separating humans from other omnivores and herbivores (Bull et al. 2002). Given the likely diets of the Great Basin population, the fact that meat may only have been a minor dietary component of these early populations (Reinard et al., 2012), there is no reason why a sample that has abundant plant residues could not be human (Shillito et al. 2018), particularly given the corroborated aDNA results (Jenkins et al. 2012).

Hormone analysis has not yet been used to any extent, aside from a few early studies. Sobolik et al. (1996) undertook an analysis of diet and steroid composition of 12 coprolites, using a combination of chromotagphic and radioimmunoassay techniques to measure testeosterone and estradiol levels. This analysis indicated that ratios were better than absolute concentrations for distinguishing sex, due to steroid degradation. In experimental samples the ratio was higher in males, as might be expected, ranging from 3 – 118, whereas female values ranged from 0.2 – 7. Menstrual cycle impacted values in females, with the follicular phase having overlap with lower male values. Rhode (2003) applied liquid chromatographic analysis of faecal steroids (estrogen, testosterone, and progesterone) extracted from a series of 13 coprolite recovered in middle to late Holocene occupations at Hidden Cave, Nevada. Rhode's research utilized relative abundance of estrogen and testosterone to identify probable sex of individuals defecating in the cave, finding that all of the samples had ratios in the typical range of females, although some samples were at the higher end that overlapped with the low end of male ratios.

Hormones offer a lot of potential for identifying links between dietary patterns and biological sex, which could used to explore questions of child care practices and sexual division of labor in past societies. However, the relationship between biological sex and the levels of hormones in faeces is complex even in modern studies, and much further work is needed to understand for example the relationship between diet and oestrogen levels in faeces (Lewis et al. 1997), also taking into account post-depositional changes.

### *6.3.2 Lipids as dietary indicators*

Lipids have been used primarily as a species indicator but there is potential to assess a wider range of faecal lipids and other organic molecules as dietary indicators. Lipid analysis of Neanderthal faecal matter indicated high coprostanol with lower levels of plant derived sterols, suggesting that Neanderthal diet consisted primarily of meat (Sistiaga et al. 2014). This study provided that oldest known case of faecal matter preserved at an archaeological site and highlights the usefulness of biomarkers for reconstructing dietary patterns into the Pleistocene. A study of faecal deposits from an early Neolithic site in Iran identified C28 long chain fatty acids alongside the faecal sterols (Shillito et al. 2013a). The same samples were also analyzed for phytoliths and were found to contain large jigsaw multicell phytoliths which have been associated with leaves. Together this offers tantalizing evidence of the consumption of waxy leaves and warrants further investigation (Shillito and Elliott 2013).

There is a range of literature looking at the impacts of diet on sterol profiles in modern subjects. Westrate et al. (1999) for example conducted experiments on adult human males and females, showing that a high intake of vegetable oil phytosterol esters increased the amount of neutral sterols that were excreted. Cuevos Terra et al (2017) analyzed the faecal sterols of clinical trial subjects on normal and plant enriched diets and found that the plant enriched diets led to greater phytosterols in faeces, but also higher levels of cholesterol excretion, suggesting that plant sterol intake blocks the biotransformation of cholesterol. Individuals with a normal diet had a higher

conversion of cholesterol, whilst those on the plant enriched diets showed less conversion of cholesterol to coprostanol – thought to be due to the plant sterol interaction with gut microbes. The conversion of plant sterols varied significantly between subjects.

These molecules have an advantage in that they are less mobile than DNA, but it is unclear if the reference profiles currently used are applicable to all archaeological populations. Given the link between gut microbes and the formation of faecal sterols, further work is also needed on how a changing gut microbiome impacts the faecal sterol profile. For example one study
One study reported that a quarter of North Americans studied exhibited little to no conversion of cholesterol to coprostanol (Wilkins et al. 1974). This may potentially result in false negatives when differentiating human coprolites from carnivores, and may explain the lack of agreement between mtDNA and faecal biomarker results when these two methodologies are compared (Shillito et al. in press).

### *6.4 Protein analysis*

Ancient proteomics is emerging as an essential new tool in bioarchaeology (Cappellini et al. 2014; Hendy, Welker, et al. 2018), but has yet to be applied extensively to coprolites. Proteins have been shown to survive within a coprolite matrix using cytochemical staining (Santiago-Rodriguez et al. 2013) however the coprolite proteome (i.e., the full suite of proteins preserved within the substrate) has yet to be characterised. Whilst proteins lack the taxonomic precision of DNA, they are frequently tissue specific, offering a range of applications complementary to aDNA. It is expected that proteins relating to the depositing individual's biological system (particularly digestive proteins), their diet and their gut microbiome may all be recovered from coprolites. Host proteins could be used to identify disease states (e.g. Jersie-Christensen et al. 2018) while protein characterisation may provide higher resolution insights into dietary components and tissues (e.g. Hendy et al. 2018). For example, while genetic analysis of a coprolite may identify fragments of cattle DNA, only proteomic analysis can distinguish the source of those DNA sequences - were people eating beef or drinking cow's milk? The investigation of the functional gut microbiome through protein analysis is becoming more common in clinical studies (e.g. Lee et al. 2017; Lai et al. 2019). Thus, investigations into the proteins expressed by gut microbiota will identify not just which species are present (dead, alive or dormant) but what they are doing.

## 7. Coprolites for the future - Beyond material categories to broader questions

The greatest advance in the archaeological study of ancient subsistence and past lifeways has been the shift to multiproxy approaches, whereby different types of archaeological evidence are considered alongside each other rather than in isolation (Twiss et al. 2009, Shillito 2017). The coprolite is a self-contained multi-proxy 'package' of information that can provide information on health and diet, from the information contained within them, and through the archaeological context in which they are found. There is still much potential to go beyond simply the identification of material within coprolites, and consider how coprolites, and their patterns of deposition, fit into the bigger picture of social organization. An excellent example of these potentials can be seen in recent work in China, where researchers are beginning to incorporate multiproxy analysis of coprolites into broader research on the origins of agriculture in the Neolithic, as discussed in section 2. This type of study should serve as a benchmark for how this type of research can be carried out, and integrated with other lines of dietary evidence such as

pottery residue analysis (Shoda et al. 2018). The high resolution of coprolites and the individual snapshot they provide is also unique, and provides a means of looking at seasonal variability of diet. Most other methods of palaeodietary reconstruction look at broad patterns and shifts, or lifetime information.

In modern contexts, there is an abundance of anthropological and geographic research on faecal pollution and its socio-cultural implications, and there is much potential to engage with this research in understanding how past societies viewed faecal and other types of waste. In modern contexts, faeces typically attract responses of disgust in many societies, and are immediately removed from sight (Van Der Geest 2007, Mariwah and Drangert 2011, Jewitt 2011), though this varies. The concept of dirt and boundaries between 'good' and 'bad' are culturally constructed (Douglas 1966), and attitudes to faeces vary between cultures, with some finding both human and animal faeces to be taboo or unclean. This is clearly not the case in many archaeological contexts where they can be found directly adjacent to habitation and activity areas, and in unusual contexts, for example at Çatalhöyük there is evidence that animal scats were spread on the chest of individuals in burials (Jenkins 2012).

A difficulty lies in how best to approach these materials, which methods to use, and how to apply them. There is currently no consensus on which analytical methods should be used, or how to present data, making comparative studies extremely difficult. With all of the recent advances in types of analyses that can be conducted on coprolites, the ideal scenario would be that researchers approach coprolite sampling with a clear methodology that will not inhibit any particular type of analysis from being conducted. Wood and Wilmshurst (2016) provide a detailed description of coprolite sampling methods with a particular focus on avoiding contamination for sensitive multiproxy analyses like DNA and palynology. In the following section we will discuss some possibilities for advancing a standardized methodology, and also more basic experimental and taphonomic research that needs to be conducted so that coprolite analysis can be better integrated into archaeological research.

### *7.1 Methodological considerations*

There are a number of methodological issues related to quantifying the amount of material recovered from coprolites. There are debates over pollen quantification, and variable opinions on the number of exotic marker spores that should be added to achieve the most precise ratio of exotic to fossil pollen and therefore most precise concentration values. Maher (1981) suggests that for a 0.95 percent confidence limit the ratio of fossil pollen to exotic marker to fossil pollen should be 1:1, but that analysts can use a 2:1 ratio to maintain a good level of precision for concentration estimated while reducing counting effort. If the ratio of fossil to marker exceeds 2:1 then there is increasing change that the concentration values will not be accurate (Maher 1981). Clearly it is important to get the ratio of fossil right but accomplishing this requires some information about the expected number of fossil pollen grains in a sample in order to achieve this ratio. For sediment core samples this is typically done by experimenting with a few samples, however, coprolites represent discrete and limited samples, so it is often not possible to experiment in this way to get the best fossil to marker ratio. Concentrations reported in the literature ranging from those devoid of pollen (e.g. Bryant 1975 on coprolites from Coahuila, Mexico) to 100,000 to several million grains per gram of coprolite (e.g. Dean 1993; Kelso and Solomon 2006; Reinhard et al. 2006; Reinhard et al. 1991). Variability can also be significant within a single site, for example Sobolik (1988) reported concentration values of 6,438,455 to < 1,000 grains per gram of coprolite material from coprolites recovered from the same latrine feature at Baker Cave, Texas. For coprolites with fossil pollen concentrations in the millions it

might be necessary to add 500,000 or 3,500,000 Lycopodium tablets per gram of coprolite to achieve the desired 2:1 ratio. Lycopodium marker spores come in tablets with anywhere from 8,000 to 20,000 marker spores per tablet, so it could take up to 175 tablets tablets per gram of sample to achieve the desired ratio. At the current rate of $0.80 per tablet this is not a cost-effective approach.

There is a similar long-standing debate over the best way to assess dietary input through analysis of macrofossils. Previous studies have attempted to quantify coprolite macroremains using count, weight, or volume (Pearsall 2015:398), for example comparing volume of a class of food item (e.g., plant fiber) across coprolites to determine relative input, or noting presence/absence of food items to assess how commonly they were included in the diet (Pearsall 2015:398). A problem with this is that human produce feces with variable weight/volume depending on many factors including diet, age, stature of individual. These variable samples of human diet are then impacted by taphonomic processes over time before they are recovered from an archaeological site, resulting in a set of samples without uniform weight/volume. It is common for there to be a significant range of weight/volume for a series of coprolites recovered from a site (Blong et al. in press Battillo 2019). This makes it difficult to compare absolute quantities of materials from one coprolite to the next.

This can be worked around to an extent by presenting quantitative data as a percentage of the total to make it more comparable (Faulkner 1991; Fry 1976). Faulkner's (1991) study highlights issues with quantitative data. Faulkner's study assesses relative input of wild versus cultivated foods based on percent weight of all macrofossils obtained from eight coprolites. However, 39% of the dietary weigh attributed to wild foods comes from a single coprolite with a large quantity of hickory shell. Quantitative weight data is affected by differences in the densities of materials recovered from the coprolites, for example stone, and to a lesser extent bone, are denser, while plant fiber is less dense. Weight percent stone for a coprolite containing one small pebble would outweigh the weight percent plant fiber in that coprolite, even if the majority of the coprolite volume consisted of plant fiber. Often remains such as seeds and insects have very little mass and are noted as trace amounts. Quantitative count data is similarly skewed; counts of masticated bone fragments from a single bone would appear higher than a few seeds suggesting this made up a greater proportion of the coprolite material, but what does this tell us about the proportion of animal versus plant inputs into the diet?

Other methods have included presence/absence aka ubiquity method (Popper 1988) and the percent subjective method (Colyer and Osborne 1965, Sobolik 1991). The latter uses a visual estimation of the percent total volume of each constituent, which are then placed into pre determined categories with a range of error. It has been suggested this introduces the least amount of biases (Popper 1988). Some studies have used a visual assessment of macro contents presented using ordinal scale percent of total volume (Bryant 1974b; Cowan 1978; Yarnell 1969). This approach has been criticized because it can potentially "overestimate highly visible dietary items and underestimate some of the less-visible items like small seed fragments" (Faulkner 1991).

Another issue is that it is often not possible to cleanly separate some materials and as a result the quantitative data is not reliable. An example of this is hair and plant fiber. These common coprolite constituents are typically closely intertwined (Figure 6) and it is not possible to cleanly separate them from one another (Blong et al. in press, Sonderman et al. 2019). The purpose of food processing and digestion is to break food down as much as possible so that it can be digested more easily to recover as much nutritional content as possible. As a result the material that is passed through in a coprolite has often been severely impacted by mechanical and chemical processes that have reduced it to tiny fragments. This results in a very fine fraction of material

that is too large to pass through the commonly used 180 or 250 micron screen mesh, but too fine to accurately separate into constituents. This material is often classified as residual (e.g., Faulkner 1991). Faulkner (1991) attempted to present qualitative macrofossil data but was only able to separate out and weigh 70% of the total weight of macrofossils from a series of eight coprolites, leaving 30% of the total weight of the eight coprolites as unsorted "residual" material. There is potential here to further analyse this 'unidentifiable' material using biomolecular methods.

We recommend moving forward that when feasible analysts present qualitative data, for example in the case of bones and seeds where it is often possible to present an estimated minimum number of individual specimens represented. Otherwise we propose that a qualitative assessment of percent total volume assessed by viewing the >250 micron portion of the coprolites under a dissecting microscope is an acceptable means of communicating relative presence with no loss in accuracy. A qualitative abundance scale might be preferred, because it is very difficult to get accurate counts and weights because the material in the coprolites is typically highly fragmented as a result of chewing/digestion, which leads on to the next part of this discussion.

*7.2 Taphonomy – what does a human diet look like?*

Digestive taphonomy is the "effects of any of the physical or chemical processes of the animal digestive system and accessory organs on plant or animal matter"; what is seen by the analyst is only a partial representation of what was consumed (O'Meara 2014). Unlike animal dung, which has been studied extensively by archaeologists investigating crop domestication and animal management (Charles 1998, Shahack-Gross 2011, Linseele et al 2013, Marinova et al. 2013, Elliott et al. 2015, Portillo et al. 2017, Égüez et al. 2018, Portillo et al. 2020), there has been comparatively limited analysis of human faeces in terms of understanding taphonomic processes and integration with other lines of archaeological evidence.

Some of the earliest studies of macrofossils in coprolites comprised 'experimental' studies to try and understand the impact of digestion on materials recovered. Calder (1977) carried out experiments to assess the survival of various foods expected in traditional Maori diets in New Zealand, and found that qualitatively some items left identifiable markers in faecal materials (e.g. keratinous, siliceous and cellulose material), but others such as fish scales left no trace at all. The most significant body of work seeking to understand the effects of human digestion on consumed food items has been focused on bone taphonomy (O'Meara 2014). Several actualistic studies have been conducted seeking to better understand the impacts of human digestion on fish bones (Butler and Schroeder 1998; Jones 1986, 1990; Nicholson 1993). These studies report that fish bones are typically heavily impacted by mastication, fragmentation, and chemical digestive processes to the point that most bones were either completely gone or were not taxonomically identifiable. Bones that did survive were distinctly altered (e.g., crushing, surface erosion and edge rounding from acids). Examples of digestive traces include "pitting, rounding, deformation, staining, or leaving an organic residue" (Butler and Schroeder 1998). Some bones were passed without any noticeable alteration, presumed by Jones (1986, 1990) to represent bones protected by flesh that was not disaggregated during digestion. There were notable trends in the skeletal elements that survived (e.g. vertebra more likely to survive). Nicholson (1993) reports that smaller fish bones were completely digested but bones from larger fish were also more digested than bones from medium sized fish (possibly due to mastication), and scales survived digestion (contra Calder 1977).

Nicholson (1993) reports that only 1 – 6 % of fish skeletal material from species in Clupeid family survived digestion, while Butler and Schroeder (1998) report that 26% of tui chub (Cyprinidae: *Gila bicolor*) bones survived digestion, including many bones with no traces of

digestion. This suggests differential digestive impacts depending on the species of fish. Cyprinidae bones have been documented in several coprolites recovered from the Great Basin (Blong et al. in press); this may be linked to more robust skeletal elements in Cyprinidae when compared to Clupeid species (Butler and Schroeder 1998). Nicholson notes that the results of this experiment could change depending on how fish was prepared (boiling, roasting, drying), number and condition of teeth in an individual, amount of time spent chewing, and health of the individual. Experiments on other items are rare. Crandall and Stahl (1995) conducted an experiment involving the consumption of a skinned, eviscerated, and segmented insectivore by an adult human male.The bones recovered from faeces were examined for skeletal element representation, breakage and digestive damage. The analysis found severe skeletal attrition comparable to and sometimes in excess of, the damage exhibited in microvertebrate accumulations from the scats of small mammalian carnivores.

It is clear that we don't have a good understanding of how materials in coprolites relate quantitatively to what was originally consumed, and the same is true of biomolecular methods. Repeated experiments where as many variables as possible are controlled for, would go some way towards this, and enable researchers to assess whether strict quantitative interpretation is worth pursuing. An interdisciplinary approach in collaboration with clinical research for example, offers potential as protocols and infrastructure are already established for the types of controlled dietary experiments that are needed.

*7.3 Taphonomy– preservation and post-depositional processes*

Archaeological samples have the added complication of post-depositonal processes to consider, and these also need to be better understood. The signals that we see on archaeological coprolites are not just a product of consumption and digestion, but processes that occur after the coprolite is deposited. It is difficult to establish the extent to which original signals are altered, without having extensive, reliable experimental material to consider. Taphonomy study has been a key aspect of most other archaeological specialisms. Zooarchaeology, palynology and archaeobotany have long histories of experimental taphonomic work. Organic residue analysis in pottery has also been built on a robust programme of experimental work, whereby food products have been prepared in pottery, and subjected to burial experiments (Evershed 2008). Research on faecal lipids as species indicators has also been underpinned by robust experimental work, focusing on the diagenesis and mobility of these molecules within soils (Bull et al. 1998, Bull et al. 2000). Ideally the same samples from the modern 'feeding' experiments discussed in 7.2, would then undergo burial experiments to investigate the impacts of taphonomic processes.

As with many types of archaeological materials, the conditions of the site have a impact on coprolite preservation. Recently, Reihard et al. (2019) provide an overview of the taphonomic conditions that impact the preservation potential of coprolites, dividing these into biological and sedimentological. In desert environments, the filtration of sand into coprolites can occur and become compressed into the coprolites from the surrounding sedimentary matrix. In open air sites, including near the entrance of caves, water containing minerals such as calcium can percolate into samples, resulting in the mineralization of the coprolites. Percolating water disperses biological components and undergo mineral replacement.

Biological taphonomic agents include flies, beetles mites and fungi, and are abundant especillay in ancient latrine deposits, which tend to be quite dense. In these types of latrine deposits, the faeces can remain moist for extended periods of time and communities of decomposing organisms become established. Flies, nematodes and mites are associated with poor preservation

potential for microfossils, and visual evidence of these types of organisms typically suggests poor preservation potential. Reinhard et al. (2019) suggest this can occur to the detriment of the original intestinal material, and only highly resistant biological material (ligin, silica, calcium oxalate, starch and thick cellulose structures) preserves in these types of latrines. Conversely, in mixed midden deposits containing isolated individual specimens, these tend to preserve incredibly well. Isolated coprolites dispersed within mixed midden deposits do not attract large quantities of decomposers, and if they are dessicated or otherwise buried rapidly, preservation conditions are optimal. Similar conditions to those described by Reinhard et al. (2019) for Turkey Pen Ruins in the American southwest, are also observed for example in deeply stratified midden deposits at Catalhoyuk in Turkey (Shillito et al. 2011).

At the Zape site discussed in section 6.2, researchers note an unusually isolated archaeological context within a deep cave environment, that likely contributed to the exceptional preservation of coprolites, bones and botanical remains. The 'trash' deposits where coprolites were buried were located 35 feet away from the cave entrance and separated by a rock fall. The sub-humid climate and altitude of 1800m above seal level is also a contributing factor (Tett et al. 2019). This echoes observations from other sites such as Catalhoyuk, where coprolites and other organic remains are exceptionally well preserved in deeply stratified midden deposits that were raidly buried following deposition (Shillito and Matthews 2013).

A key consideration needs to be the mechanism of the coprolite formation as it is still not clear exactly how faeces transform into a coprolite. The two identified pathways are desiccation and partial mineralisation (Reinhard and Bryant 2008). It is easier to understand how biomolecules survive within desiccated coprolites (often recovered from dry rock shelters), since the sample undergoes limited chemical changes. The mineralisation process, however, appears to be more complex. Mineralisation can be facilitated by: an excess of phosphate and calcium derived from the diet or the environment (Hollocher and Hollocher 2012), and / or bacterial mineralisation (Briggs 2003). Mineralisation is progressive: a fully mineralised sample should yield no authentic organic molecules; thus, the extent of the mineralisation is crucial for biomolecular investigations. Mineralised coprolites tend to be recovered from anaerobic, waterlogged conditions, and experimental approaches have provided some limited insights into the preservation process. For example, an experimental study which submerged hyena droppings in water for three weeks found that these conditions had little morphological impact, though, notably, the droppings became so hard that they could not be imprinted (Larkin et al. 2000). The potential of biomolecular investigations into partially mineralised samples from waterlogged conditions is yet to be explored. It is clear that the mechanisms of coprolite formation should be more deeply investigated as the preservation pathway of a specimen is inevitably going to affect the biomolecules that preserve within the matrix.

## 8. Conclusions

Inevitably given the wide range of methods now available, there will not always be enough material to carry out all different types of analysis, or to archive material for the future. In these cases the methods most suitable for the specific research questions must be selected. We argue that in archaeology species identification is the most important first step. Whether or not this is through aDNA or lipid biomarkers depends on a range of factors. Both methods are time consuming and can be expensive. Whilst DNA is more sensitive due to amplification of the signal, limited number of investigations at present means that there is still a lot of unknowns regarding taphonomy. Given the lower mobility of lipids and their higher stability, this offers an alternative method for determining species, though requires a minimum quantity of lipid to be preserved.

Coprolites inherently represent a biased data set for interpreting human diet. Food items such as flesh and vegetables will not leave any recognizable macroscopic traces in faeces, while plant fiber, seeds, bones often leave identifiable macrofossils (Hall et al. 1983; Pearsall 2015:398). The macroscopic dietary record from coprolites is generally biased towards smaller items such as hairs, seeds, and small animal bones, whereas the bones of larger mammals are typically not represented. Because of this, it is important to integrate the macroscopic coprolite record with additional lines of evidence to produce a more complete picture of human diet. Yet the information that we can get from coprolites is at a much higher resolution, and more detailed than other forms of dietary evidence in archaeology, even if it is more qualitative.

O'Meara (2014) makes the important point that whilst coprolite analysis will likely never provide a 'complete' picture of past consumption, the archaeological record as a whole is inherently incomplete, and coprolite analysis is no different in this respect from any other aspect of archaeology. Nevertheless, research would benefit from a more systematic approach to experimental work, exploring a range of dietary profiles and the factors affecting differential survival of residues and physical contents. Given human diets have varied hugely from early prehistory to present, as well as geographically, there is still a lot of work to be done on assessing the variability and range of dietary signals, both inclusions and chemical residues, and likely needs to be tailored to the region and time period under investigation.

**Acknowledgements**
Research on samples from Paisley Caves has been carried out as part of a Natural Environment Research Council Grant, reference number NE/P003001/1. EG is supported by the Arts & Humanities Research Council (grant number AH/N005015/1). Thank you to the reviewers who provided very extensive and insightful comments. We thank reviewer 1 especially for drawing our attention to the Borry et al paper and provided the tabulated data for the parasite results from these samples. Thank you to Dr. Camilla Speller for helpful suggestions on the ancient DNA and protein sections.

**References**

Addyman, P.V. (1989) The archaeology of public health at York, England, World Archaeology, 21:2, 244-264

Allen, S.D., Almond, M.J., Bell, M.G., Hollins, P., Marks, S., & Mortimore, J.L. (2002). Infrared spectroscopy of the mineralogy of coprolites from Brean Down: evidence of past human activities and animal husbandry. *Spectrochimica acta. Part A, Molecular and biomolecular spectroscopy,* 58 5, 959-65 .

Andersen K, et al. (2011) Meta-barcoding of 'dirt' DNA from soil reflects vertebrate biodiversity. *Molecular Ecology* 21, 1966–1979

Angelakis, E. et al. (2019). Treponema species enrich the gut microbiota of traditional rural populations but are absent from urban individuals. New microbes and new infections, 27, 14–21.

Appelt, S., Armougom, F., et al. (2014b). Polyphasic analysis of a middle ages coprolite microbiota, Belgium. PloS one, 9(2), e88376.

Appelt, S., Fancella, L., Le Bailly, M., Raoult, D., Drancourt, M. and Desnues, C. 2014a. Viruses in a 14th-Century Coprolite *Applied and Environmental Microbiology* 80 (9) 2648-265

Araújo, A., Reinhard, K., Ferreira, L.F. (2015). Palaeoparasitology – human parasites in ancient material. *Advances in parasitology* 90: 349-387.

Austin RM, Sholts SB, Williams L, Kistler L, Hofman CA. Opinion: To curate the molecular past, museums need a carefully considered set of best practices. Proc Natl Acad Sci U S A. 2019;116(5):1471–1474. doi:10.1073/pnas.1822038116

Baeten, Jan, Marinova, Elena, De Laet, Véronique, Degryse, Patrick, De Vos, Dirk, & Waelkens, Marc. (2012). Faecal biomarker and archaeobotanical analyses of sediments from a public latrine shed new light on ruralisation in Sagalassos, Turkey. Journal of Archaeological Science, 39(4), 1143-1159.

Battillo, J. (2018). The role of corn fungus in Basketmaker II diet: A paleonutrition perspective on early corn farming adaptations. *Journal of archaeological science: Reports* 21: 64-70. doi: 10.1016/j.jasrep.2018.07.003

Bain, A. 2001. "Archaeoentomological and Archaeoparasitological Reconstructions at Îlot Hunt (CeEt-110): New Perspectives in Historical Archaeology (1850- 1900)".

Battillo, J. 2019. Farmers who forage: interpreting paleofecal evidence of wild resource use by early corn farmers in the North American Southwest. Archaeol Anthropol Sci 11, 5999–6016 https://doi.org/10.1007/s12520-019-00944-y

Beck, C.W., Bryant, V.M. & McDonough, K.N. Evidence for non-random distribution of pollen in human coprolites. Archaeol Anthropol Sci 11, 5983–5998 (2019). https://doi.org/10.1007/s12520-019-00839-y

Benninghoff, W.S. (1962). Calculation of pollen and spores density in sediments by addition of exotic pollen in known quantities. *Pollen et Spores* 6: 332-333

Blake, M. R., Raker, J. M. and Whelan, K. (2016) Validity and reliability of the Bristol Stool Form Scale in healthy adults and patients with diarrhoea-predominant irritable bowel syndrome. *Alimentary Pharmacology and Therapeutics* 44: 693-703. doi:10.1111/apt.13746

Blong, JC., Adams, ME., Sanchez, G., Jenkins, DL., Bull ID and Shillito, L-M. in press. Investigation of Younger Dryas and early Holocene subsistence in the northern Great Basin through multiproxy analysis of coprolites from the Paisley Caves, Oregon, USA *Archaeological and Anthropological Sciences*

Borry, M, Cordova, B, Perri, A, Wibowo, MC, Honap, TP, Ko, J, Yu, J, Britton, K, Girdland Flink, L, Power, RC, Stuijts, I, Garcia, DS, Hofman, CA, Hagan, RW, Kagone, TS, Meda, N, Carabin, H, Jacobson, D, Reinhard, K, Lewis, Jr., CM, Kostic, A, Jeong, C, Herbig, A, Hubner, A & Warinner, C 2020, 'CoproID predicts the source of coprolites and paleofeces using microbiome composition and host DNA content', PeerJ, vol. 8, e9001. https://doi.org/10.1101/871533, https://doi.org/10.7717/peerj.9001, https://doi.org/10.7717/peerj.9001/supp-1

Bouchet F (1995) Recovery of helminth eggs from archeological excavations of the Grand Louvre (Paris, France) *Journal of Parasitology* 81: 785-787

Breitwieser, FP., Lu, J. and Salzberg, SL.. 2017 A review of methods and databases for metagenomic classification and assembly Briefings in Bioinformatics, 1-15

Briggs, DEG. 2003 The Role of Decay and Mineralization in the Preservation of Soft-Bodied Fossils Annual Review of Earth and Planetary Sciences 31: 275-301

Bronniman, D., Pumpin, C., Ismail-Meyer, K., Rentzel, P. and Eguez, N. (2014). Excrements of Omnivres and Carnivores. In: Nicosia, C. and Stoops, G. (eds.), *Archaeological Soil and Sediment Micromorphology*. Wiley & Sons, Hoboken, pp. 67-81.

Butler, VL and Schroeder, RA 1998 Do Digestive Processes Leave Diagnostic Traces on Fish Bones? Journal of Archaeological Science 25: 957-971

Bryant, V.M. (1969). Late full-glacial and postglacial pollen analysis of Texas sediments. Unpublished PhD Thesis, University of Texas at Austin.

Bryant, V.M. (1974a) Pollen Analysis of Prehistoric Human Feces from Mammoth Cave. In: Watson P.J. (ed.) *Archaeology of the Mammoth Cave Area*. Academic Press, Cambridge, pp. 203-249

Bryant, V.M. (1974b). Prehistoric Diet in Southwest Texas: The Coprolite Evidence. *American Antiquity* 39(3): 407-420. doi: 10.2307/279430

Bryant, V.M. and Glenna W. Dean, G.W. 2006. Archaeological coprolite science: The legacy of Eric O. Callen (1912–1970) *Palaeogeography, Palaeoclimatology, Palaeoecology* 237, Issue 1, 51-66

Bryant, V.M., Holloway, R.G. (1983). The role of palynology in archaeology. In: Schiffer, M.B. (ed.). *Advances in archaeological method and theory Vol. 6.* Academic Press, New York, pp. 191-224

Bryant, V.M. (1994). Callen's Legacy. In: Sobolik, K.D. (ed.), Paleonutrition: The Diet and Health of Prehistoric Americans, Occasional Paper No. 22, Center for Archaeological Investigations, Southern Illinois University, Carbondale, pp. 151–160.

Bryant, V.M. and Williams-Dean, G. (1975). The coprolites of man. *Scientific American* 232(1): 100-109

Bryant, V.M. and Dean, G.W. (2006). Archaeological coprolite science: The legacy of Eric O. Callen (1912–1970). *Palaeogeography, Palaeoclimatology, Palaeoecology* 237(1), 51-66. doi: 10.1016/j.palaeo.2005.11.032

Bryant, V.M. and Reinhard, K.J. (2012). Coprolites and archaeology: the missing links in understanding human health. In: Hunt, A.P., Milàn, J., Lucas, S.G., Spielmann, J.A. (eds.), *Vertebrate Coprolites*. New Mexico Museum of Natural History & Science Bulletin 57, Albuquerque, pp. 379-387

Buckland, W. (1829) Additional remarks on coprolites and fossil sepia. *Proceedings of the Geological Society of London* 11: 142-143

Bull, I. D., Simpson, I. A., van Bergen, P. F. and Evershed, R. P. 1999. Muck 'n' molecules: Organic geochemical methods for detecting ancient manuring. Antiquity 73, 86-96.

Bull, I. D., Betancourt, P. P. and Evershed, R. P. 1999. Chemical evidence supporting the existence of a structured agricultural manuring regime on Pseira Island, Crete during the Minoan Age. Aegaeum 20, 69-75.

Bull, I. D., van Bergen, P. F., Poulton, P. R. and Evershed, R. P. 1998. Organic geochemical studies of soils from the Rothamsted Classical Experiments-II, Soils from the Hoosfield Spring Barley Experiment treated with different quantities of manure. Organic Geochemistry 28, 11-26.

Bull, I. D., van Bergen, P. F., Nott, C. J., Poulton, P. R. and Evershed, R. P. 2000. Organic geochemical studies of soils from the Rothamsted Classical Experiments-V. The fate of lipids in different long-term experiments. Organic Geochemistry 31, 389-408.

Calder, AM. (1977) Survival Properties of Organic Residues through the Human Digestive Tract *Journal of Archaeological Science* 4, 141-151

Callaway E. 2019. Lost Denisovan bone reveals surprisingly human-like finger
Photos of missing fossil show these ancient hominins had slimmer digits than their Neanderthal relatives. Nature. https://www.nature.com/articles/d41586-019-02647-9

Callen, E.O. (1967). Analysis of the Tehuacan coprolites. In: Byers, D.S. (ed.) *The prehistory of the Tehuacan Valley, Vol. 1 Environment and Subsistence*. Austin, University of Texas Press, pp. 261-289

Callen, E.O., and Cameron, T.W.M. (1955). The Diet and Parasites of Prehistoric Huaca Prieta Indians as Determined by Dried Coprolites. *Proceedings of the Royal Society of Canada* 5: 51-52.

Callen, E.O., and Cameron, T.W.M. (1960). A prehistoric diet revealed in coprolites. *New Scientist* 8(190): 39-40.

Callen, E.O., and Martin, P.S. (1969). Plant Remains in Some Coprolites from Utah. *American Antiquity* 34: 329- 331.

Cano, R.J., Rivera-Perez, J., Toranzos, G.A., Santiago-Rodriguez, T.M., Narganes-Storde, Y., Chanlatte-Baik, L., García-Roldán, E., Bunkley-Williams, L., Massey, S.E. (2014). Paleomicrobiology: revealing fecal microbiomes of ancient indigenous cultures. *PLoS ONE* 9(9): e106833. doi: 10.1371/journal.pone.0106833

Camacho, M., Iñiguez, A.M., Reinhard, K.J. (2018) Taphonomic considerations on pinworm prevalence in three Ancestral Puebloan latrines. *Journal of Archaeological Science: Reports* 20, 791-798

Camacho M, Araújo A, Morrow JJ, Reinhard KJ (2018) Recovering Parasites from Mummies and Coprolites: Establishing an Epidemiological Approach. Parasites & Vectors. 11:248, https://doi.org/10.1186/s13071-018-2729-4.

Camacho, M., Reinhard, K.J. Pinworm research in the Southwest USA: five decades of methodological and theoretical development and the epidemiological approach. Archaeol Anthropol Sci 12, 63 (2020). https://doi.org/10.1007/s12520-019-00994-2

Cappellini, E., Collins, MJ and Gilbert, MT. 2014 Biochemistry. Unlocking ancient protein palimpsests Science 343(6177):1320-2

Carrott, J., Hall, A. R., Hughes, P., Jaques, D., Johnstone, C., Kenward, H., and Worthy, D., 1998, An assessment of biological remains from excavations at St Saviourgate, York (site code: 1995.434), *Reports from the Environmental Archaeology Unit, York*, 98/14

Chame, M. (2003). Terrestrial mammal feces: a morphometric summary and description. *Memórias do Instituto Oswaldo Cruz* 98 Suppl. I. doi: 10.1590/S0074-02762003000900014

Charles, Michael (1998) Fodder from dung: the recognition and interpretation of dung-derived plant material from archaeological sites. *Environmental Archaeology* 1: 111-122

Chaves SAM, Reinhard KJ (2006) Critical Analysis of Prehistoric Evidence of Medicinal Plant Use, Pi7auí, Brazil. Journal of Palaeogeography, Palaeoclimatology, and Palaeoecology 237:110-118.]

Chin, K. (2002). Analyses of coprolites produced by various carnivorous vertebrates. *The Paleontological Society Papers Vol. 8 The fossil record of predation*, pp. 43-50. doi: 10.1017/S1089332600001042

Collins, MJ. And Copeland, L. 2011. Ancient starch: Cooked or just old? *Proceedings of the National Academy of Sciences* 108 (22) E145; DOI: 10.1073/pnas.1103241108

Cooper A, Poinar HN. 2000. Ancient DNA: do it right or not at all. Science 289 (5482):1139

Crandall, B. D. and Stahl, P. W., (1995). Human digestive effects on a micromammalian skeleton. J Archaeol Sci 22: 789-797

Cuevas-Tena, M., Alegría, A. & Lagarda, M.J. (2017) Determination of Fecal Sterols Following a Diet with and without Plant Sterols *Lipids* 52: 871. https://doi.org/10.1007/s11745-017-4286-6

Cummings LS, Yost C, Puseman K, Hill DV, Varney RA (2007) Microscopic and Chemical Evaluation of Three Coprolites from the Paisley 5 Mile Point Caves, Oregon. Paleo Research Institute Technical Report 07-91. Paleo Research Institute, Inc., Golden, Colorado

Dahl, K. (2009). Corn soot woman's timeless lesson: eat your smut. *Etnobiología* 7: 94–99.

Danielson, D.R., Reinhard, K.J. (1998). Human dental microwear caused by calcium oxalate phytoliths in prehistoric diet of the lower Pecos region, Texas. *American journal of physical anthropology* 107(3): 297-304. doi: 10.1002/(SICI)1096-8644(199811)107:3<297::AID-AJPA6>3.0.CO;2-M

Day, M.G. (1966), Identification of hair and feather remains in the gut and faeces of stoats and weasels. Journal of Zoology, 148: 201-217. doi:10.1111/j.1469-7998.1966.tb02948.x

Davenport, E.R., Sanders, J.G., Song, S.J., Amato, K.R., Clark, A.G., Knight, R. (2017). The human microbiome in evolution. *BMC biology* 15: 127. doi: 10.1186/s12915-017-0454-7

De Filippo, C. et al. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proceedings of the National Academy of Sciences of the United States of America, 107(33), 14691–14696.

Dean, G. (1993). Use of pollen concentrations in coprolite analysis: an archaeobotanical viewpoint with a comment to Reinhard et al. (1991). *Journal of ethnobiology* 13(1): 102-114

Dittmar, K (2009) Old Parasites for a New World: The Future of Paleoparasitological Research. A Review. Journal of Parasitology Vol. 95, No. 2, pp. 365-371.

Douglas, M. (1966) *Purity and danger: An Analysis of Concepts of Pollution and Taboo*

Dove, C. and Koch, S. 2010. Microscopy of Feathers: A Practical Guide for Forensic Feather Identification *Journal of the American Society of Trace Evidence Examiners* 1: 15-61

Égüez, N., A. Zerboni, and S. Biagetti (2018) Microstratigraphic analysis on a modern central Saharan pastoral campsite. Ovicaprine pellets and stabling floors as ethnographic and archaeological referential data *Quaternary International* 483: 180-193.

Elias, S. (2010). Advances in Quaternary entomology (1st ed., Developments in Quaternary Science; 12). Amsterdam; Boston; London: Elsevier.

Elliott, S., Bendrey, R., Whitlam, J., Aziz, K.R. and Evans, J. (2015) Preliminary ethnoarchaeological research on modern animal husbandry in Bestansur, Iraqi Kurdistan: Integrating animal, plant and environmental data. *Environmental Archaeology* 20:283-303.

Evershed, R.P. (2008a) Organic residue analysis in archaeology: the archaeological biomarker revolution *Archaeometry* 50: 895-924

Evershed, R.P (2008b). Experimental Approaches to the Interpretation of Absorbed Organic Residues in Archaeological Ceramics. *World Archaeology* 40(1), 26-47

Evershed, R.P 1999. Lipids as carriers of anthropogenic signals from prehistory. Philosophical Transactions of the Royal Society B: Biological Sciences 354(1379):19–31

Faulkner, C.T. (1991). Prehistoric diet and parasitic infection in Tennessee: Evidence from the analysis of desiccated human paleofeces. *American antiquity* 56(4): 687-700. doi: 10.2307/281546

Federhen, S. (2012). The NCBI Taxonomy database. *Nucleic acids research* 40(D1): D136-D143. doi: 10.1093/nar/gkr1178

Florenzano, A. , Mercuri, A. M., Pederzoli, A. , Torri, P. , Bosi, G. , Olmi, L. , Rinaldi, R. and Bandini Mazzanti, M. (2012), The Significance of Intestinal Parasite Remains in Pollen Samples from Medieval Pits in the Piazza Garibaldi of Parma, Emilia Romagna, Northern Italy. *Geoarchaeology* 27: 34-47.

Fry GF, Moore JG. 1969. Enterobius vermicularis: 10,000-year-old human infection. *Science.* 166 (Suppl. 3913):1620

Fry, G. (1970). Preliminary analysis of the Hogup Cave coprolites. In: Aikens, C.M. (ed.), *Hogup Cave*. University of Utah Antropological Papers 93, University of Utah Press, Salt Lake City, pp. 247-250

Fry, G.F. (1976). *Analysis of Prehistoric Coprolites From Utah*. University of Utah Anthropological Papers 97. Salt Lake City, University of Utah Press.

Fry GF (1978) Prehistoric diet at Danger Cave, Utah, as determined by the analysis of coprolites. University of Utah Anthropological Papers 99, Salt Lake City

Gilbert, M.T.P., Bandelt, H.-J., Hofreiter, M., Barnes, I. (2005). Assessing ancient DNA studies. *Trends in ecology and evolution* 20(10): 541-544. doi: 10.1016/j.tree.2005.07.005

Gilbert, M.T.P., Jenkins, D.L., Götherstrom, A., Naveran, N., Sanchez, J.J., Hofreiter, M., Thomsen, P.F., Binladen, J., Higham, T.F.G., Yohe, R.M., Parr, R., Cummings, L.S., Willerslev, E. (2008). DNA from Pre-Clovis Human Coprolites in Oregon, North America. *Science* 320(5877): 786-789

Gilbert, M.T.P., Jenkins, D.L., Higham, T.F.G., Rasmussen, M., Malmström, H. Svensson, E.M., Sanchez, J.J., Cummings, L.S., Yohe, R.M., Hofreiter, M., Götherstrom, A., Willerslev, E. (2009). Response to comment by Poinar et al., on "DNA from Pre-Clovis Human Coprolites in Oregon, North America". *Science* 325(5937): 148

Giuffra, E., Kijas, J.M.H., Amarger, V., Carlborg, Ö., Jeon, J.T., Andersson, L. (2000). The origin of the domestic pig: independent domestication and subsequent introgression. *Genetics* 154: 1785-1791.

Glaser, B., Birk, J.J. (2012). State of the scientific knowledge on properties and genesis of Anthropogenic Dark Earths in Central Amazonia (*terra preta de Índio*). *Geochimica et cosmochimica acta* 82: 39-51. doi: 10.1016/j.gca.2010.11.029

Goldberg, P., Berna, F., Macphail, R.I. (2009). Comment on "DNA from Pre-Clovis human coprolites in Oregon, North America". *Science* 325: 148-c. doi: 10.1126/science.1167531

Gomez, A. et al. (2016). Gut Microbiome of Coexisting BaAka Pygmies and Bantu Reflects Gradients of Traditional Subsistence Patterns. Cell reports, 14(9), 2142–2153.

Gonçalves, M.L.C., Araújo, A., Ferreira, L.F. (2003). Human intestinal parasites in the past: new findings and a review. *Memórias do Instituto Oswaldo Cruz* 98(Suppl. 1): 103-118

Gorham LD, Bryant VM. 2001. Pollen, phytoliths, and other microscopic plant remains in underwater archaeology. The International Journal of Nautical Archaeology. 30:282–298

Green, E.J. and Speller, C.F. (2017). Novel Substrates as Sources of Ancient DNA: Prospects and Hurdles. *Genes* 8(7): 180. doi: 10.3390/genes8070180

Grieg J. (1981) The investigation of a medieval barrel latrine from Worcester. Journal of Archaeological Science 8, 265–282

Grimalt, J.O., Fernandez, P, Bayona, JM. & J. Albaices. 1990. Assessment of faecal sterols and ketones as indicators of urban sewage inputs to coastal waters, Environmental Science and Technology 24: 357-63

Green, Eleanor Joan and Speller, Camilla Filomena (2017) Novel substrates as sources of ancient DNA: prospects and hurdles. Genes. pp.1-26

Haas, J., Creamer, W., Mesíam L.H., Goldstein, D., Reinhard, K., Rodríguez, C.V. (2013). Evidence for maize (*Zea mays*) in the Late Archaic (3000-1800 B.C.) in the Norte Chico region of Peru. *Proceedings of the National Academy of Sciences* 110(13): 4945-4949. doi: 10.1073/pnas.1219425110

Hagan RW, Hofman CA, Hübner A, Reinhard K, … (2019) Comparison of extraction methods for recovering ancient microbial DNA from paleofeces. American Journal of Physical Anthropology https://doi.org/10.1002/ajpa.23978

Hall, H.J., 1977. A paleoscatalogical study of diet and disease at Dirty Shame Rockshelter, southeast Oregon. Tebiwa. Miscellaneous Papers of the Idaho State University Museum of Natural History 8, 1–14.

Hall AR, Jones AKG, Kenward HK 1983. Cereal Bran and Human Faecal Remains from Archaeological Deposits - Some Preliminary Observations. In B Proudfoot (ed) *Site, Environment and Economy*, BAR International Series 173, Oxford, p. 85-104

Hall, AR and Kenward, HK. 2002. University of York Environmental Archaeology Unit digital archive https://archaeologydataservice.ac.uk/archives/view/EAU/

Harrault L, Milek K, Jardé E, Jeanneau L, Derrien M, et al. (2019) Faecal biomarkers can distinguish specific mammalian species in modern and past environments. PLOS ONE 14(2): e0211119. https://doi.org/10.1371/journal.pone.0211119

Haile, J., Holdaway, R., Oliver, K., Bunce, M., Gilbert, M.T.P., Nielsen, R., Munch, K., Ho, S.Y.W., Shapiro, B., Willerslev, E. (2007). Ancient DNA Chronology within Sediment Deposits: Are Paleobiological Reconstructions Possible and Is DNA Leaching a Factor? *Molecular Biology and Evolution* 24(4): 982–989, https://doi.org/10.1093/molbev/msm016

Hebsgaard, M.B., Gilbert, M.T.P., Arneborg, J., Heyn, P. (2009), 'The Farm Beneath the Sand' – an archaeological case study on ancient 'dirt'. *Antiquity* 83(320): 430-444. doi: 10.1017/S0003598X00098537

Heizer, R. F. (1967). Analysis of human coprolites from a dry Nevada cave. Papers on Great Basin Archaeology, Reports of the University of California Survey 70, Berkeley, CA

Heizer, RF. And Napton, LK. 1969. Biological and Cultural Evidence from Prehistoric Human Coprolites *Science* 165 (3893) 563-568.

Hendy, J., Welker, F., Demarchi, B., Speller, C., Warinner, C., Collins, M.J. (2018). A guide to ancient protein studies. *Nature ecology & evolution* 2: 791-799

Hendy, J., Colonese, A.C., et al. (2018). Ancient proteins from ceramic vessels at Çatalhöyük West reveal the hidden cuisine of early farmers. Nature communications, 9(1), 4064.
Hofreiter, M., Poinar, H.N., Spaulding, W.G., Bauer, K., Martin, P.S., Possnert, G., Pääbo, S. (2000). A molecular analysis of ground sloth diet through the last glaciation. *Molecular ecology* 9(12): 1975-1984. doi: 10.1046/j.1365-294X.2000.01106.x

Hofreiter, M., Paijmans, J.L.A., Goodchild, H., Speller, C.F., Barlow, A., Fortes, G.G., Thomas, J.A., Ludwig, A., Collins, M.J. (2015). The future of ancient DNA: Technical advances and conceptual shifts. *Bioessays* 37(3): 284-293.

Holdaway, R.N., Jacomb, C. (2000). Rapid extinction of the moas (Aves: Dinornithiformes): Model, test, and implications. *Science* 287(5461): 2250-2254. doi: 10.1126/science.287.5461.2250

Holden, T. (1994). Dietary evidence from the intestinal contents of ancient humans with particular reference to desiccated remains from northern Chile. In: Hather, Jon G. (ed.) Tropical Archaeobotany. Applications and New Developments. Routledge, London, pp. 65-85.

Hollocher, K. and Hollocher, T.C. (2012). Early process in the fossilization of terrestrial feces to coprolites, and microstrucuture preservation. In A. P. Hunt et al., (Eds.). Vertebrate Coprolites: Bulletin 57. New Mexico: New Mexico Museum of Natural History and Science, pp. 93–98.

Horrocks, M., Irwin, G., Jones, M., Sutton, D. (2004). Starch grains and xylem cells of sweet potato (*Ipomoea batatas*) and bracken (*Pteridium esculentum*) in archaeological deposits from northern New Zealand. *Journal of archaeological science* 31(3): 251-258. doi: 10.1016/S0305-4403(03)00018-9

Huisman, D.J., Ngan-Tillard, D., Tensen, M.A., Laarman, F.J., Raemaekers, D.C.M. (2014). A question of scales: studying Neolithic subsistence using micro CT scanning of midden deposits. *Journal of Archaeological Science* 49: 585-594

Hunt, A.P., Lucas, S.G., Milàn, J., Spielmann, J.A. (2012). Vertebrate Coprolite Studies: Status and Prospectus. In: Hunt, A.P., Milàn, J., Lucas, S.G., Spielmann, J.A. (eds.), *Vertebrate Coprolites*. New Mexico Museum of Natural History & Science Bulletin 57, Albuquerque, pp. 5-24

Jenkins, DL, Davis, LG, Stafford, TW, Campos, PF, Hockett, B, Jones, GT, Cummings, LS, Yost, C, Connolly, TJ, Yohe, RM, Gibbons, SC, Raghavan, M, Rasmussen, M, Paijmans, JLA, Hofreiter, M, Kemp, BM, Barta, JL, Monroe, C, Gilbert, MTP & Willerslev, E 2012, 'Clovis Age Western Stemmed Projectile Points and Human Coprolites at the Paisley Caves', *Science* vol. 337, no. 6091, pp. 223-228

Jenkins E (2012) Mice, scats and burials: unusual concentrations of microfauna found in human burials at the Neolithic site of Çatalhöyük, central Anatolia. J Soc Archaeol 12:380–403

Jersie-Christensen, R.R., Lanigan, L.T., Lyon, D., Mackie, M., Belstrøm,D., Kelstrup, C.D., Fotakis, AK., Willerslev, E., Lynnerup, N., Jensen, LJ., Capellinni, E. Olsen, JV. 2018. Quantitative metaproteomics of medieval dental calculus reveals individual oral health status. Nat Commun 9, 4744

Jewitt, S. 2011. Geographies of shit: Spatial and temporal variations in attitudes towards human waste Progress in Human Geography 35(5) 608–626

Jha, A.R. et al. (2018). Gut microbiome transition across a lifestyle gradient in Himalaya. PLoS biology, 16(11), e2005396.

Jiménez et al. (2012) Zoonotic and Human Parasites of Inhabitants of Cueva de Los Muertos Chiquitos, Rio Zape Valley, Durango, México. Journal of Parasitology 98:304-309

Johnson, K.V.-A., Foster, K.R. (2018). Why does the microbiome affect behaviour? *Nature reviews microbiology* 16: 647-655

Jónsson, H. et al. (2013). mapDamage2.0: fast approximate Bayesian estimates of ancient DNA damage parameters. Bioinformatics , 29(13), 1682–1684.

Jones, V.H. (1936). The Vegetal Remains of Newt Kash Hollow Shelter. In: Webb, W.S. and Funkhouser, W.D. (eds.), *Rockshelters in Menifee County, Kentucky*. Lextington, University of Kentucky Reports in Archaeology and Anthropology 3

Jones A. 1983. A coprolite from 6–8 pavement. In: Hall AR, Kenward HK, Williams D, Greig JRA, editors. *Environment and living conditions at two Anglo-Scandinavian sites,* vol. 1983. York: Council of British Archaeology p. 225–9

Jones, A.K., 1986. Fish bone survival in the digestive systems of the pig, dog and man: some experiments. In Fish and archaeology: Studies in osteometry, taphonomy, seasonality and fishing methods (Vol. 294, pp. 53-61). British Archaeological Reports Oxford.

Jones, Andrew KG. 1982. "Human parasite remains: prospects for a quantitative approach." Environmental archaeology in the urban context. Vol. 43. London: Council for British Archaeology 66-70.

Jones, Andrew KG. "Trichurid ova in archaeological deposits: their value as indicators of ancient faeces." Palaeobiological Investigations. Reseach Design, Methods and Data Analysis, Symposia of the Association for Environmental Archaeology B. Vol. 5. 1985.

Jones, A.K., 1990. Experiments with fish bones and otoliths: implications for the reconstruction of past diet and economy. Experimentation and Reconstruction in Environmental Archaeology, pp.143-146.

Jónsson, H., Ginolhac, A., Schubert, M., Johnson, P.L.F., Orlando, L. (2013). mapDamage 2.0: fast approximate Bayesian estimates of ancient DNA damage parameters. *Bioinformatics* 29(13): 1682-1684. doi: 10.1093/bioinformatics/btt193

Jouy-Avantin, F., A. Debenath, A. Moigne, H. Moné (2003) A standardized method for the description and the study of coprolites *Journal of Archaeological Science* 30 pp. 367-372

Kelso, G.K. (1971). Hogup Cave, Utah: comparative pollen analysis of human coprolites and cave fill. Unpublished MA theses, University of Arizona.

Kelso, G.K. (1976). Absolute pollen frequencies applied to the interpretation of human activities in northern Arizona. Unpublished PhD thesis, University of Arizona

Kelso, JK and Solomon, AM 2006 Applying modern analogs to understand the pollen content of coprolites Palaeogeography, Palaeoclimatology, Palaeoecology 237: 80-91

Kitaba, I., Nakagawa, T. (2017). Black ceramic spheres as marker grains for microfossil analyses, with improved chemical, physical, and optical properties. *Quaternary international* 455: 166-169. doi: 10.1016/j.quaint.2017.08.052

Lai, L.A. et al. (2019). Metaproteomics Study of the Gut Microbiome. Methods in molecular biology , 1871, 123–132.

Larkin, NR., Alexander, J. and Lewis, MD. 2000. Using Experimental Studies of Recent Faecal Material to Examine Hyaena Coprolites from the West Runton Freshwater Bed, Norfolk, U.K. Journal of Archaeological Science 27, Pages 19-31

Lee, P.Y. et al. (2017). Metaproteomic analysis of human gut microbiota: where are we heading? Journal of biomedical science, 24(1), 36.

Lewis, S.J., Heaton, K.W. (1997). Stool form scale as a useful guide to intestinal transit time. *Scandinavian Journal of Gastroenterology* 32(9): 920–924

Lewis, S.J., Heaton, K.W., Oakey, R.E., McGarrigle, H.H.G. (1997). Lower serum oestrogen concentrations associated with faster intestinal transit. *British journal of cancer* 76: 395-400

Lindahl, T. (1993). Instability and decay of the primary structure of DNA. *Nature* 362: 709-715

Lindeboom, N., Chang, P.R. and Tyler, R.T. (2004). Analytical, Biochemical and Physicochemical Aspects of Starch Granule Size, with Emphasis on Small Granule Starches: A Review. *Starch* 56: 89-99. doi:10.1002/star.200300218

Ledger ML, Anastasiou E, Shillito L-M, Mackay H, Bull ID, Haddow SD, Knüsel C, Mitchell PD. (2019a) Parasite infection at the early farming community of Çatalhöyük. *Antiquity* 93(369), 573-587

Ledger, M.L., Grimshaw, E., Fairey, M., Whelton, H.L. (2019b). Intestinal parasites at the Late Bronze Age settlement of Must Farm, in the fens of East Anglia, UK (9th century B.C.E.). *Parasitology* 146(12): 1583-1594. doi: 10.1017/S0031182019001021

Leek, F. F. (1972). Teeth and Bread in Ancient Egypt. The Journal of Egyptian Archaeology, 58, 126–132

Lindeboom, N. , Chang, P. R. and Tyler, R. T. (2004), Analytical, Biochemical and Physicochemical Aspects of Starch Granule Size, with Emphasis on Small Granule Starches: A Review. *Starch* 56: 89-99. doi:10.1002/star.200300218

Linseele, V., Riemer, H., Baeten, J., De Vos, D., Marinova, E. & Ottoni, C. (2013). Species identification of archaeological dung remains: A critical review of potential methods. *Environmental Archaeology*, 18(1): 5-17. doi: 10.1179/1461410313Z.00000000019

Lu, J. and Salzberg, S.L. (2018). Removing contaminants from databases of draft genomes. *PLoS Computational Biology* 14(6): e1006277. doi: 10.1371/journal.pcbi.1006277

Luciani, S., Fornaciari, G., Rickards, O., Martínez Labarga, C., Rollo, F. (2006). Molecular characterization of a pre-Columbian mummy and in situ coprolite. *American journal of physical anthropology* 129(4): 620-629. doi: 10.1002/ajpa.20314

Ma C, Wang B, Liu Z et al (2018) A preliminary study on vegetation, environment and human activity of pollen records during Middle-late Holocene in Tianluoshan site, Ningshao Plain. Q Sci 38:1304–1312

Maher, L.J. (1981). Statistics for microfossil concentration measurements employing samples spiked with marker grains. *Review of Palaeobotany and Palynology* 32: 153-191

Malaspinas, A.-S. et al. (2014). bammds: a tool for assessing the ancestry of low-depth whole-genome data using multidimensional scaling (MDS). Bioinformatics , 30(20), 2962–2964.

Marinova, E., Veerle Linseele & Marlu Kühn (2013) Bioarchaeological research on animal dung – possibilities and limitations, Environmental Archaeology, 18:1, 1-3

Mariwah, S. and Drangert, J-O. 2011. Community perceptions of human excreta as fertilizer in peri-urban agriculture in Ghana Waste Management & Research 29(8) 815–822

Martin, P.S. and Sharrock, F.W. (1964). Pollen analysis of prehistoric human faeces: a new approach to ethnobotany. *American Antiquity* 30: 168-80

Martinson, E., Reinhard, K.J., Buikstra, J.E., De la Cruz, K.D. (2003). Pathoecology of Chiribaya parasitism. *Memorias do Instituto Oswaldo Cruz* 98 (Suppl. 1): 195–205.

Martin, PS. And Sharrock, FW. 1964. Pollen analysis of prehistoric human faeces: a new approach to Ethnobotany *American Antiquity* 30: 168-80.

Matthews, W. (1995). Micromorphological characterization and interpretation of occupation deposits and microstratigraphic sequences at Abu Salabikh, Iraq. In: Barham, A.J. and Macphail, R.I. (eds.), Archaeological Sediments and Soils: Analysis, Interpretation and Management. London, Institute of Archaeology, pp. 41-76

Matthews, W., French, C.A.I., Lawrence, T., Cutler, D.F., Jones, M.K., 1997. Micro-stratigraphic traces of site formation processes and human activities. World Archaeol. 29, 281-308.

Matthews W, Shillito L-M, Elliot S, Bull ID, Williams J. 2014. Neolithic lifeways: microstratigraphic traces within houses, animal pens and settlements. In: Whittle, A; Bickle, P, ed. Early Farmers: The View from Archaeology and Science. British Academy and OUP, pp.251-279

McCobb, L.M.E., Briggs, D.E.G., Hall, A.R., Kenward, H.K. (2003). The Preservation of Invertebrates in 16th-Century Cesspits at St Saviourgate, York. *Archaeometry* 46(1): 157-169. doi: 10.1111/j.1475-4754.2004.00150.x

McDonough, K.N. 2019. Middle Holocene menus: dietary reconstruction from coprolites at the Connley Caves, Oregon, USA. Archaeol Anthropol Sci https://doi.org/10.1007/s12520-019-00828-1

Merchant S, Wood DE, Salzberg SL. 2014. Unexpected cross-species contamination in genome sequencing projects. PeerJ 2:e675 https://doi.org/10.7717/peerj.675

Mitchell, P.D. (2017). Human parasites in the Roman world: health consequences of conquering an empire. *Parasitology* 144, Special Issue 1: 48-58.

Montserrat Sanz, Joan Daura, Natalia Égüez, Jean-Philip Brugal (2016) Not only hyenids: A multi-scale analysis of Upper Pleistocene carnivore coprolites in Cova del Coll Verdaguer (NE Iberian Peninsula) *Palaeogeography, Palaeoclimatology, Palaeoecology* 443, pp.249 – 262

Moore, J.G., Fry, G.F., Englert, E. Jr. (1969). Thorny-headed worm infection in North American prehistoric man. *Science* 163 (Suppl. 3873): 1324–1325.

Nicholson, R. A. (1993). An investigation into the effects on fish bone of passage through the human gut: some experiments and comparisons with archaeological material. Circaea 10: 38–51

Nishida, A.H. and Ochman, H. (2019). A great-ape view of the gut microbiome. Nature reviews. Genetics, 20(4), 195–206.
O'Meara, D (2014). Ruminating on the Past: A History of Digestive Taphonomy in Experimental Archaeology. In Experiments Past: Histories of Experimental Archaeology. Reeves Flores, Jodi & Paardekooper, Roeland Sidestone Press. 131-145

Osbourne, PJ. 1983 An insect fauna from a modern cesspit and its comparison with probable cesspit assemblages from archaeological sites *Journal of Archaeological Science* 10: 453-463

Perrotti, A. and Van Asperen, E. 2019. Dung fungi as a proxy for megaherbivores: opportunities and limitations for archaeological applications

Pearsall, D.M. (2015). *Paleoethnobotany, A Handbook of Procedures*. Routledge, New York.

Peterson J, Garges S, Giovanni M, McInnes P, Wang L, Schloss JA, Bonazzi V, McEwen JE, Wetterstrand KA, Deal C, et al. 2009. NIH HMP Working Group The NIH Human Microbiome Project. Genome Res.19:2317–2323
Pichler, S.L., Pümpin, C., Brönnimann, D. and Rentzel, P. (2014). Life in the proto-urban style: the identification of parasite eggs in micromorphological thin sections from the Basel-Gasfabrik Late Iron Age settlement, Switzerland. *Journal of Archaeological Science* 43: 55-65

Poinar, H.N., Hofreiter, M., Spaulding, W.G., Martin, P.S., Stankiewicz, A., Bland, H., Evershed, R.P., Possnert, G., Pääbo, S. (1998). Molecular coproscopy: dung and diet of the extinct ground sloth *Nothrotheriops shastensis*. *Science* 281(5375): 402-406. doi: 10.1126/science.281.5375.402

Poinar, H.N., Kuch, M., Sobolik, K.D., Barnes, I., Stankiewicz, A.B., Kuder, T., Spaulding, W.G., Bryant, V.M., Cooper, A., Pääbo, S. (2001). A molecular analysis of dietary diversity for three archaic Native Americans. *Proceedings of the National Academy of Sciences* 98(8): 4317-4322. doi: 10.1073/pnas.061014798

Poinar, H., Fiedel, S., King, C.E., Devault, A.M., Bos, K., Kuch, M., Debruyne, R. (2009). Comment on "DNA from Pre-Clovis human coprolites in Oregon, North America". *Science* 325(5937): 148. doi: 10.1126/science.1168182

Poinar, H.N. (2003). The top 10 list: criteria of authenticity for DNA from ancient and forensic samples. International congress series, 1239, 575–579.

Popper, Virginia S. 1988 Selecting Quantitative Measurements in Paleoethnobotany. In: Current Paleoethnobotanv: Analytical Methods and Cultural Interpretations of Archaeological Plant Remains. Christine A. Hastorf and Virginia S. Popper (eds.), University of Chicago Press, Chicago, pp. 53-71.

Portillo, M., M.C. Belarte, J. Ramon, N. Kallala, J. Sanmartí, and R.M. Albert (2017) An ethnoarchaeological study of livestock dung fuels from cooking installations in northern Tunisia. *Quaternary International* 431: 131-144.

Portillo, M., García-Suárez, A. & Matthews, W. 2020. Livestock faecal indicators for animal management, penning, foddering and dung use in early agricultural built environments in the Konya Plain, Central Anatolia. Archaeol Anthropol Sci 12, 40.

Prost K, Birk JJ, Lehndorff E, Gerlach R, Amelung W. Steroid Biomarkers Revisited - Improved Source Identification of Faecal Remains in Archaeological Soil Material. PLoS One. 2017;12(1):e0164882. Published 2017 Jan 6. doi:10.1371/journal.pone.0164882

Pucu, E., Russ, J. & Reinhard, K. Diet analysis reveals pre-historic meals among the Loma San Gabriel at La Cueva de Los Muertos Chiquitos, Rio Zape, Mexico (600–800 CE). Archaeol Anthropol Sci 12, 25 (2020). https://doi.org/10.1007/s12520-019-00950

Radini, A, Tromp, M, Beach, A, Tong, E, Speller, CF, McCormick, M, Dudgeon, J, Collins, MJ, Rühli, F, Kröger, R & Warinner, C (2019). Medieval women's early involvement in manuscript production suggested by lapis lazuli identification in dental calculus. Science Advances, 5(1), eaau7126.

Radini R, Nikita E, Shillito L-M. (2016) Human Dental Calculus and a Medieval Urban Environment. In: Jervis, B; Broderick, L; Grau-Sologestoa, I, ed. *Objects, Environment, and Everyday Life in Medieval Europe*. Turnhout: Brepols pp.297-313.

A Radini, E Nikita, S Buckley, L Copeland, K Hardy. (2017). Beyond food: The multiple pathways for inclusion of materials into ancient dental calculus. *American journal of physical anthropology* 162: 71-83

Rasmussen, Morten; Cummings, Linda Scott; Gilbert, M. Thomas P.; Bryant, Vaughn; Smith, Colin; Jenkins, Dennis L.; Willerslev, Eske. 2009. Response to Comment by Goldberg et al. on "DNA from Pre-Clovis Human Coprolites in Oregon, North America" Science, Volume 325, Issue 5937, pp. 148

Reinhard KJ, Araujo A (2012) Synthesizing archaeology with parasitology in paleopathology. In (Buikstra J & Roberts C) A Global History of Paleopathology. Oxford: Oxford University Press, 751-764

Reinhard, K., & Bryant, V. (1992). Coprolite Analysis: A Biological Perspective on Archaeology. *Archaeological Method and Theory*, 4, 245-288.

Reinhard, K. and Bryant, V.M. (2008). Pathoecology and the Future of Coprolite Studies in Bioarchaeology, in Ann L.W. Stodder, ed., Reanalysis and Reinterpretation in Southwestern Bioarcheology. *Arizona State University Anthropological Research Papers* No. 59, 2008

Reinhard, K.J. (1988). The cultural ecology of prehistoric parasitism on the Colorado Plateau as evidenced by coprology. *American journal of physical anthropology* 77: 355-366.

Reinhard, K. (1993). The utility of pollen concentration in coprolite analysis: expanding upon Dean's comments. *Journal of ethnobiology* 13(1): 114-128

Reinhard, K.J. (2006). A coprological view of ancestral Pueblo cannibalism: Debate over a single fecal fossil offers a cautionary tale of the interplay between science and culture. *American Scientist* 94: 254-261.

Reinhard, K. (2017). Reestablishing rigor in archaeological parasitology. *International journal of paleopathology* 19: 124-134. doi: 10.1016/j.ijpp.2017.06.002

Reinhard KJ, Araújo A, Ferreira LF, Herrmann B (1986) Recovery of parasite remains from coprolites and latrines: aspects of paleoparasitological technique. HOMO—Journal of Comparative Human Biology 37:217-239.

Reinhard, K., Hamilton, D.L., Hevly, R.H. (1991). Use of pollen concentration in paleopharmacology: coprolite evidence of medicinal plants. *Journal of ethnobiology* 11(1): 117-132

Reinhard, K. and Bryant, V. (1992). Coprolite Analysis: A Biological Perspective on Archaeology. *Archaeological Method and Theory* 4: 245-288

Reinhard, K., Ambler, J., & Szuter, C. (2007). Hunter‐gatherer use of small animal food resources: Coprolite evidence. International Journal of Osteoarchaeology, 17(4), 416-428.

Reinhard, K., Bryant, V., & Vinton, S. (2007). Comment on reinterpreting the pollen data from Dos Cabezas. International Journal of Osteoarchaeology, 17(5), 531-541.

Reinhard, K. and Bryant, V.M. (2008). Pathoecology and the Future of Coprolite Studies in Bioarchaeology. In Stodder, A.L.W. (ed.), *Reanalysis and Reinterpretation in Southwestern Bioarcheology*. Arizona State University Anthropological Research Papers No. 59

Reinhard, K.J., Johnson, K.L., LeRoy-Toren, S., Wieseman, K., Teixeira-Santos, I. and Vieira, M. (2012). Understanding the Pathoecological Relationship between Ancient Diet and Modern Diabetes through Coprolite Analysis: A Case Example from Antelope Cave, Mojave County, Arizona. *Current Anthropology* 53(4): 506-512

Reinhard, K.J., Araújo, A. (2014). Prehistoric earth oven facilities and the pathoecology of Chagas disease in the Lower Pecos Canyonlands. *Journal of Archaeological Science* 53: 227–34.

Reinhard, K.J., Araújo, A.J., Morrow, J.J. (2016). Temporal and spatial distribution of *Enterobius vermicularis* (Nematoda: Oxyuridae) in the prehistoric Americas. *Korean journal of parasitology* 54: 591-603

Reinhard, K., Camacho, M., Geyer, B., Hayek, S., Horn, C., Otterson, K., Russ, J. (2019). Imaging coprolite taphonomy and preservation. *Archaeological and anthropological sciences* 11: 6017-6035

Reinhard KJ, Edwards SK, Damon, TR, Meier DK (2006) Pollen Concentration Analysis of Ancestral Pueblo Dietary Variation. Journal of Palaeogeography, Palaeoclimatology, and Palaeoecology 237:92-109.

Rhode, D. E. (2003). Coprolites from Hidden Cave, Revisited: Evidence for occupation history, diet, and gender, Journal of Archaeological Science, 30, (7), 909-922

Riley, T. 2008. Diet and seasonality in the Lower Pecos: evaluating coprolite data sets with cluster analysis *Journal of Archaeological Science*, 35(10), 2726-2741

Riley, T. 2012. Assessing diet and seasonality in the Lower Pecos canyonlands: an evaluation of coprolite specimens as records of individual dietary decisions Journal of Archaeological Science 39: 145-162

Rivera-Perez, J.I., Cano, R.J., Narganes-Storde, Y., Chanlatte-Baik, L., Toranzos, G.A. (2015). Retroviral DNA sequences as a means for determining ancient diets. *PLiS ONE* 10(12): e0144951. doi: 10.1371/journal.pone.0144951

Roust, N.L. (1967). Preliminary Examination of Prehistoric Human Coprolites from Four Western Nevada Caves. *Papers in Great Basin Archaeology*, University of California Archaeological Survey Report 70, pp. 49-88

Santiago-Rodriguez, T.M., Narganes-Storde, Y.M., Chanlatte, L., Crespo-Torres, E., Toranzos, G.A., Jimenez-Flores, R., Hamrick, A., Cano, R.J. (2013). Microbial communities in pre-Columbian coprolites. *PLoS ONE* 8(6): e65191. doi: 10.1371/journal.pone.0065191

Sanz, M., Daura, J., Égüez, N., Brugal, J.-P. (2016). Not only hyenids: A multi-scale analysis of Upper Pleistocene carnivore coprolites in Cova del Coll Verdaguer (NE Iberian Peninsula). *Palaeogeography, palaeoclimatology, palaeoecology* 443: 249-262. doi: 10.1016/j.palaeo.2015.11.047

Schmidt, C. (2015). Thinking from the gut. *Nature* 518: S12-S14

Schnorr, S.L. et al. (2014). Gut microbiome of the Hadza hunter-gatherers. Nature communications, 5, 3654.

Segata, N. (2015). Gut microbiome: westernization and the disappearance of intestinal diversity. *Current biology* 15(14): R611-R613. doi: 10.1016/j.cub.2015.05.040

Shah VG, Dunstan RH, Geary PM, Coombes P, Roberts TK, von Nagy-Felsobuki E (2007) Evaluating potential applications of faecal sterols in distinguishing sources of faecal contamination from mixed faecal samples. *Water Res* 41:3691–3700

Shahack-Gross, R. (2011). Herbivorous livestock dung: formation, taphonomy, methods for identification, and archaeological significance. *Journal of Archaeological Science* Volume 38, Issue 2, Pages 205-218

Shillito, L. Grains of truth or transparent blindfolds? A review of current debates in archaeological phytolith analysis. Veget Hist Archaeobot 22, 71–82 (2013). https://doi.org/10.1007/s00334-011-0341-z

Shillito L-M, Matthews W. 2013. Geoarchaeological Investigations of Midden-Formation Processes in the Early to Late Ceramic Neolithic Levels at Çatalhöyük, Turkey ca. 8550–8370 cal BP. Geoarchaeology 28, 25 – 49

Shillito L-M, Matthews W, Bull ID, Williams J. 2013b. Biomolecular investigations of faecal biomarkers at Sheik-e Abad and Jani**.** *In:* Matthews, R; Matthews, W; Mohammadifar, Y, ed. *The Earliest Neolithic of Iran: 2008 Excavations at Sheikh-e Abad and Jani*. Oxford: Oxbow and British Institute for Persian Studies, pp.105-115

Shillito L-M, Bull ID, Matthews W, Almond MJ, Evershed RJ. 2013a. Integrated geochemical and microscopic analysis of human coprolites, animal dung and organic remains in burials. *In:* Hodder, I, ed. *Humans and Landscapes of Çatalhöyük: reports from the 2000-2008 excavations*. Cotsen Institute/BIAA, 2013, pp.75-79.

Shillito L-M, Elliott S. Phytolith indicators of plant resource use at Sheikh-e Abad and Jani. *In:* Matthews, R; Matthews, W; Mohammadifar, Y, ed. *The Earliest Neolithic of Iran: 2008 Excavations at Sheikh-e Abad and Jani*. Oxford: Oxbow and British Institute for Persian Studies, 2013, pp.185-200. In Preparation.

Shillito L-M, Bull ID, Matthews W, Almond MJ, Williams J, Evershed RP. (2011) Biomolecular and micromorphological analysis of suspected faecal deposits at Neolithic Çatalhöyük, Turkey. *Journal of Archaeological Science* 38, 1869 – 1877.

Shillito L-M, Blong J, Jenkins D, Stafford TW, Whelton H, McDonough K, Bull I. (2018) New Research at Paisley Caves: Applying New Integrated Analytical Approaches to Understanding Stratigraphy, Taphonomy and Site Formation Processes. *PaleoAmerica* 4(1), 82-86

Shillito, L-M., Whelton, H., Blong, JC., Jenkins, DL., Connolly, TJ. And Bull, ID.. in press. Pre-Clovis occupation of the Americas identified by human faecal biomarkers in coprolites from Paisley Caves, Oregon *Science Advances*

Shoda et al. 2018. Molecular and isotopic evidence for the processing of starchy plants in Early Neolithic pottery from China Sci reports 8: 1-9

Shortland, M. 1992. Plumbing the depths: on caves and the men of geology *Journal and Proceedings Royal Society of New South Wales* 125: 119-132.

Sistiaga, A., Berna, F., Laursen, R., Goldberg, P. (2014a). Steroidal Biomarker Analysis of a 14,000 Years Old Putative Human Coprolite from Paisley Cave, Oregon. *Journal of Archaeological Science* 41: 813–817. doi: 10.1016/j.jas.2013.10.016

Sistiaga, A., Mallol, C., Galván, B., Summons, R.E. (2014b). The Neanderthal meal: a new perspective using faecal biomarkers. *PloS one,* 9(6): e101045

Skoglund, P., Storå, J., Götherström, A., Jakobsson, M. (2013). Accurate sex identification of ancient human remains using DNA shotgun sequencing. *Journal of archaeological science* 40(12): 4477-4482. doi: 10.1016/j.jas.2013.07.004

Slepchenko S, Reinhard K. Paleoparasitology and pathoecology in Russia: investigations and perspectives. Int J Paleopathol 2018;22: 39-44

Slepchenko, S.M., Bugmyrin, S.V., Kozlov, A.I., Vershubskaya, G.G., & Shin, D.Y. (2019). Comparison of Helminth Infection among the Native Populations of the Arctic and Subarctic Areas in Western Siberia Throughout History: Parasitological Researches on Contemporary and the Archaeological Resources. The Korean Journal of Parasitology, 57, 607 – 612.

Smibert, R.M. (1981). The Genus Treponema. In M. P. Starr et al., (Eds.). The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria. Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 564–577.

Smith, DN. (2013). Defining an indicator package to allow identification of 'cesspits' in the archaeological record. *Journal of Archaeological Science* 40: 526-543

Sobolik, K.D. (1988). Direct Evidence for the Importance of Small Animals to Prehistoric Diets: A Review of Coprolite Studies. North American Archaeologist 14(3) 227-244.

Sobolik, K.D. (1988). The importance of pollen concentration values from coprolites: An analysis of Southwest Texas samples. *Palynology* 12(1): 201-214. doi: 10.1080/01916122.1988.9989344

Sobolik, K.D., Gremillion, K.J., Whitten, P.L., Watson, P.J. (1996). Technical note: Sex determination of prehistoric human paleofeces. *American journal of physical anthropology* 101(2): 283-290. doi: 10.1002/(SICI)1096-8644(199610)101:2<283::AID-AJPA10>3.0.CO;2-W

Sonderman, E.M., Dozier, C.A., Smith, M.F. (2019). Analysis of a coprolite from Conejo Shelter, Texas: Potential ritualistic viperous snake consumption. *Journal of Archaeological Science: Reports* 25: 85-93. https://doi.org/10.1016/j.jasrep.2019.03.032

Sonnenburg, E.D., Sonnenburg, J.L. (2019). The ancestral and industrialized gut microbiota and implications for human health. *Nature reviews microbiology* 17: 383-390

Sutton, M., Gardner, J., & Sobolik, K. (2010). Paleonutrition. University of Arizona Press, Tuscon.

Taglioretti, V., Sardella, N.H., Fugassa, M.H. (2014). Morphometric analysis of modern faeces as a tool to identify artiodactyls' coprolites. *Quaternary International* 352: 64-67

Taylor, A., Hutson, JM., Bryant, VM and Jenkins, DL. 2020. Dietary items in Early to Late Holocene human coprolites from Paisley Caves, Oregon, USA Palynology 44, - Issue 1

Tett A, Huang KD, Asnicar F, Fehlner-Peach H, Pasolli E, Karcher N, Armanini F, Manghi P, Bonham K, Zolfo M, De Filippis F, et al. (2019) The Prevotella copri complex comprises four

distinct clades that are underrepresented in Westernised populations. Cell Host & Microbe 26: 666-679. https://doi.org/10.1016/j.chom.2019.08.018

Tito, R.Y., Macmil, S., Wiley, G., Najar, F., Cleeland, L., Qu, C., Wang, P., Romagne, F., Leonard, S., Jiménez Ruiz, A., Reinhard, K., Roe, B.A., Lewis, C.M. (2008). Phylotyping and functional analysis of two ancient human microbiomes. *PLoS ONE* 3(11): e3703. doi: 10.1371/journal.pone.0003703

Tito, R.Y., Knights, D., Metcalf, J., Obregon-Tito, A.J., Cleeland, L., Najar, F., Roe, B., Reinhard, K., Sobolik, K., Belknap, S., Foster, M., Spicer, P., Knight, R., Lewis, C.M. (2012). Insights from characterizing extinct human gut microbiomes. *PLoS ONE* 7(12): e51146. doi: 10.1371/journal.pone.0051146

Torrence, R. and Barton, H. (2006). *Ancient starch research*. Walnut Creek: Left Coast Press.

Twiss, KC; Bogaard, A; Charles, M; Henecke, J; Russell, N; Martin, L; Jones, G; (2009) Plants and Animals Together Interpreting Organic Remains from Building 52 at Catalhoyuk. *Current Anthropology* , 50 (6) 885 - 895

Van Asperen, E.N., Kirby, J.R. and Shaw, H.E., 2020. Relating dung fungal spore influx rates to animal density in a temperate environment: Implications for palaeoecological studies. The Holocene, 30(2), pp.218-232.

Van Oosten, R. (2013). Cesspits and the P-P-P-P-problem: The pitfall of the Pompeii premise and the palimpsest *Quaternary International* 460: 22-29

Van der Geest, S. 2007. Not knowing about defecation. In Littlewood, R. ed. *On Knowing and Not Knowing in the Anthropology of Medicine* Left Coast Press. 75-86.

Vermeeren, C. (1998) Evidence for Seasonality from Coprolites and Recent Faeces? *Environmental Archaeology*, 3:1, 127-128

Vinton, S. D., Perry, L., Reinhard, K. J., Santoro, C. M., & Teixeira-Santos, I. (2009). Impact of empire expansion on household diet: the Inka in Northern Chile's Atacama Desert. PloS one, 4(11), e8069. https://doi.org/10.1371/journal.pone.0008069

Warren, S. Hazzledine (1911) On a Prehistoric Interment near Walton-on-Naae. *Essex Naturalist* 16: 198-208

Warinner, C., Speller, C., Collins, M.J., Lewis, C.M. (2015). Ancient human microbiomes. *Journal of Human Evolution* 79: 125-136. doi: 10.1016/j.jhevol.2014.10.016

Warinner, C. et al. (2017). A Robust Framework for Microbial Archaeology. Annual review of genomics and human genetics, 18(1). [Online]. Available at: http://www.annualreviews.org/doi/full/10.1146/annurev-genom-091416-035526.

Watson, P.J. and Yarnell, R.A. (1966). Archaeological and Paleoethnobotanical Investigations in Salts Cave, Mammoth Cave National Park, Kentucky. *American Antiquity*, 31(6): 842-849. doi: https://doi.org/10.2307/2694457

Weiß, C.L. et al. (2015). Contesting the presence of wheat in the British Isles 8,000 years ago by assessing ancient DNA authenticity from low-coverage data. eLife, 4. [Online]. DOI: 10.7554/eLife.10005.

Weststrate J.A., Ayesh, R., Bauer-Plank, C., Drewitt, P.N. (1999). Safety evaluation of phytosterol esters. Part 4. Faecal concentrations of bile acids and neutral sterols in healthy normolipidaemic volunteers consuming a controlled diet either with or without a phytosterol ester- enriched margarine. *Food and Chemical Toxicology* 37(11): 1063-1071.

Williams, F.S., Arnold-Foster, T., Yeh, H.-Y., Ledger, M.L., Baeten, J., Poblome, J., Mitchell, P.D. (2017). Intestival parasites from the 2nd-5th century AD latrine in the Roman Baths at Sagalassos (Turkey). *International journal of paleopathology* 19: 37-42.

Williams-Dean 1978. Ethnobotany and Cultural Ecology of Prehistoric Man in Southwest Texas. Unpublished PhD dissertation, Texas A&M University.

Wilke, P.J., Hall, H.J. (1975). *Analysis of ancient feces: a discussion and annotated bibliography*. Archaeological Research Facility, University of California, Berkely.

T. D. Wilkins, A. S. Hackman, Two Patterns of Neutral Steroid Conversion in the Feces of Normal North Americans. Cancer Research 34, 2550-2554 (1974).

Wood, J.R., Rawlence, N.J., Rogers, G.M., Austin, J.J., Worthy, T.H., Cooper, A. (2008). Coprolite deposits reveal the diet and ecology of the extinct New Zealand megaherbivore moa (Aves, Dinornithiformes). *Quaternary science reviews* 27(27-28): 2593-2602. doi: 10.1016/j.quascirev.2008.09.019

Wood, J.R., Crown, A., Cole, T.L., Wilmshurst, J.M. (2016). Microscopic and ancient DNA profiling of Polynesian dog (kurī) coprolites from northern New Zealand. *Journal of archaeological science: reports* 6: 496-505. doi: 10.1016/j.jasrep.2016.03.020

Wood, J.R. and Wilmhurst, J.M. (2016). A protocol for subsampling Late Quaternary coprolites for multi-proxy analysis. *Quaternary Science Reviews* 138(15): 1-5

Wood, J.R. et al. (2012). First coprolite evidence for the diet of Anomalopteryx didiformis, an extinct forest ratite from New Zealand. New Zealand journal of ecology, 36(2), 164–170.

Yang X, Wan Z, Perry L, Lu H, Wang Q, Zhao C, Li J, Xie F, Yu J, Cui T, Wang T, Li M, Ge Q. 2012. Early millet use in northern China Proc Natl Acad Sci 109(10):3726-30. doi: 10.1073/pnas.1115430109.

Yeh, H. Y., Cheng, C. J., Huang, C., Zhan, X., Wong, W. K., & Mitchell, P. D. (2019). Discovery of Eurytrema Eggs in Sediment from a Colonial Period Latrine in Taiwan. The Korean journal of parasitology, 57(6), 595–599. https://doi.org/10.3347/kjp.2019.57.6.595

Zhan, X., Yeh, H. Y., Shin, D. H., Chai, J. Y., Seo, M., & Mitchell, P. D. (2019). Differential Change in the Prevalence of the Ascaris, Trichuris and Clonorchis infection Among Past East Asian Populations. The Korean journal of parasitology, 57(6), 601–605. https://doi.org/10.3347/kjp.2019.57.6.601

Zhang, Y., Zhang, D., Wu, X. . 2020a. Pollen and lipid analysis of coprolites from Yuhuicun and Houtieying, China: Implications for human habitats and diets J Archaeol Sci Reports 29: 102135

Zhang, Y., van Geel, B., Gosling, W.D., Sun, G., Qin, L. & Wu, X. (2020b) Typha as a wetland food resource: evidence from the Tianluoshan site, Lower Yangtze Region, China. Vegetation History and Archaeobotany. DOI: 10.1007/s00334-019-00735-4

Zhang, Y., van Geel, B., Gosling, W. D., McMichael, C. N. H., Jansen, B., Absalah, S., ... & Wu, X. (2019). Local vegetation patterns of a Neolithic environment at the site of Tianluoshan, China, based on coprolite analysis. Review of Palaeobotany and Palynology,271, 104101.

Zhao, F., Jing, L., Wang, D. et al. 2018. Grain and starch granule morphology in superior and inferior kernels of maize in response to nitrogen. *Scientific Reports* 8, 6343 doi:10.1038/s41598-018-23977-0

**Figure captions:**

Figure 1: Midden deposit at Çatalhöyük showing distinctive orange coprolite lens

Figure 2: Components of coprolite analysis. Plants may include macrofossils such as seed and cellulose, microfossils such as pollen and phytoliths. Animals may include bones and other non-digestible parts of vertebrates and invertebrates. Parasites and fungi can include spores and eggs, as well as the parasite itself. The residue component may comprise lipids, proteins, DNA and other biomolecules that are both exogenous and endogenous, originating from the animal, plant and parasite components.

Figure 3: LEFT microCT scan of coprolite from Durrington Walls, UK showing digested bone fragments and probable plant voids: RIGHT microCT scan of coprolite from Durrington Walls, UK. Amorphous fabric with limited bone inclusions. MicroCT images taken by Zerina Johanson (British Museum).

Figure 4: Bristol stool chart showing variability of human faeces morphology in modern clinical contexts

Figure 5: micromorphology of A an ovicaprid pellet from Paisley Caves, showing large plant tissue inclusions in PPL (left) and XPL (right). Under XPL starch granules are visible within the plant tissue. C omnivore coprolite from Paisley caves 1 (thin section 3.4). D omnivore/human coprolite from Çatalhöyük, Turkey with digested bone inclusion. E. Omnivore coprolite from Bonucklu, Turkey, showing phytolith inclusion F. Omnivore coprolite from Boncuklu, Turkey, showing diatom inclusion

Figure 6: examples of macroscopic materials recovered from Paisley Caves coprolites. A. teeth B. rodentia bones C. fragmented digested bone D. grit/stones E. insect fragments F. *Rosa* cf. *woodsii* seeds

Figure 7: examples of microscopic materials recovered from coprolites A and B. Paisley Caves, monocotyledon epidermis phytoliths C. Paisley Caves, Apicaice and Poaceae pollen D. Paisley Caves, cf. Taenia eggs E. Durrington Walls, UK *Plantago* sp. pollen F. Paisley Caves, diatom