

## The what, how and why of human coprolite analysis in archaeology

Lisa-Marie Shillito\*<sup>a</sup>, John C. Blong, Eleanor J. Green<sup>b</sup>, and Eline Van Asperen<sup>a</sup>

\* Corresponding author [lisa-marie.shillito@ncl.ac.uk](mailto:lisa-marie.shillito@ncl.ac.uk)

<sup>a</sup> School of History, Classics and Archaeology, Newcastle University, NE1 7RU, UK

<sup>b</sup> 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 multiscale, multiproxy approaches can provide unique 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 of coprolite formation as well as pre and post depositional taphonomy, a goal which 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 mineralisation (Reinhard and Bryant 2008) although the exact mechanisms of preservation are not well understood. The term palaeofaeces is perhaps more accurate, though not widely used. They 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/latrine areas 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 organisation 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). 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 recognise 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” (2007). 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 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.



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

## 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 plant macrobotanical remains (Callen and Cameron 1955, Callen and Martin 1968, 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 analysed plant and animal macro and microfossils from 116 human coprolites representing seven phases of human occupation at 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 (phytoliths) from *Opuntia* and *Agave* sp. epidermal material. 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).

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). A series of studies by Bryant (1969, 1974a, 1974b, 1975), Bryant and Williams-Dean (1975), and Dean (1978) established standards for processing, analysing and interpreting pollen assemblages extracted from coprolite material which are still followed today.

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 (the study of parasites in past populations), albeit on an irregular basis, such as the studies on Maori coprolites in New Zealand (Horrocks et al. 2002). In 2012 Reinhard and Bryant suggest that the emergence of post-processual archaeology treated science with cynicism and was the reason for this decline. 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. Reinhard and Bryant 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.

The earliest studies of coprolites in Britain and Europe focus more on the contexts than coprolites themselves. 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 (Carrott et al. 1998, Osbourne 1983, McCobb et al 2003), and archaeobotanical remains attributed to faecal deposition. 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', echoing the early observations of Jones (1936).

In Europe during the early 2000s we begin to see a greater focus on human and animal waste (rather than the more general cesspit/latrine studies), particularly within geoarchaeology. The potential of coprolite deposits as a means of exploring prehistoric diet were 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. 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, Matthews 2013, Pichler et al. 2014). It is also during this period that lipid biomarker analysis began to emerge in archaeology (Evershed 2008), including applications of faecal biomarkers.

Bryant and Dean describe 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. In the following section we provide a comprehensive overview of these methods, from macro- to micro- and molecular, focusing on morphology, contents and residues respectively (Figure 2).

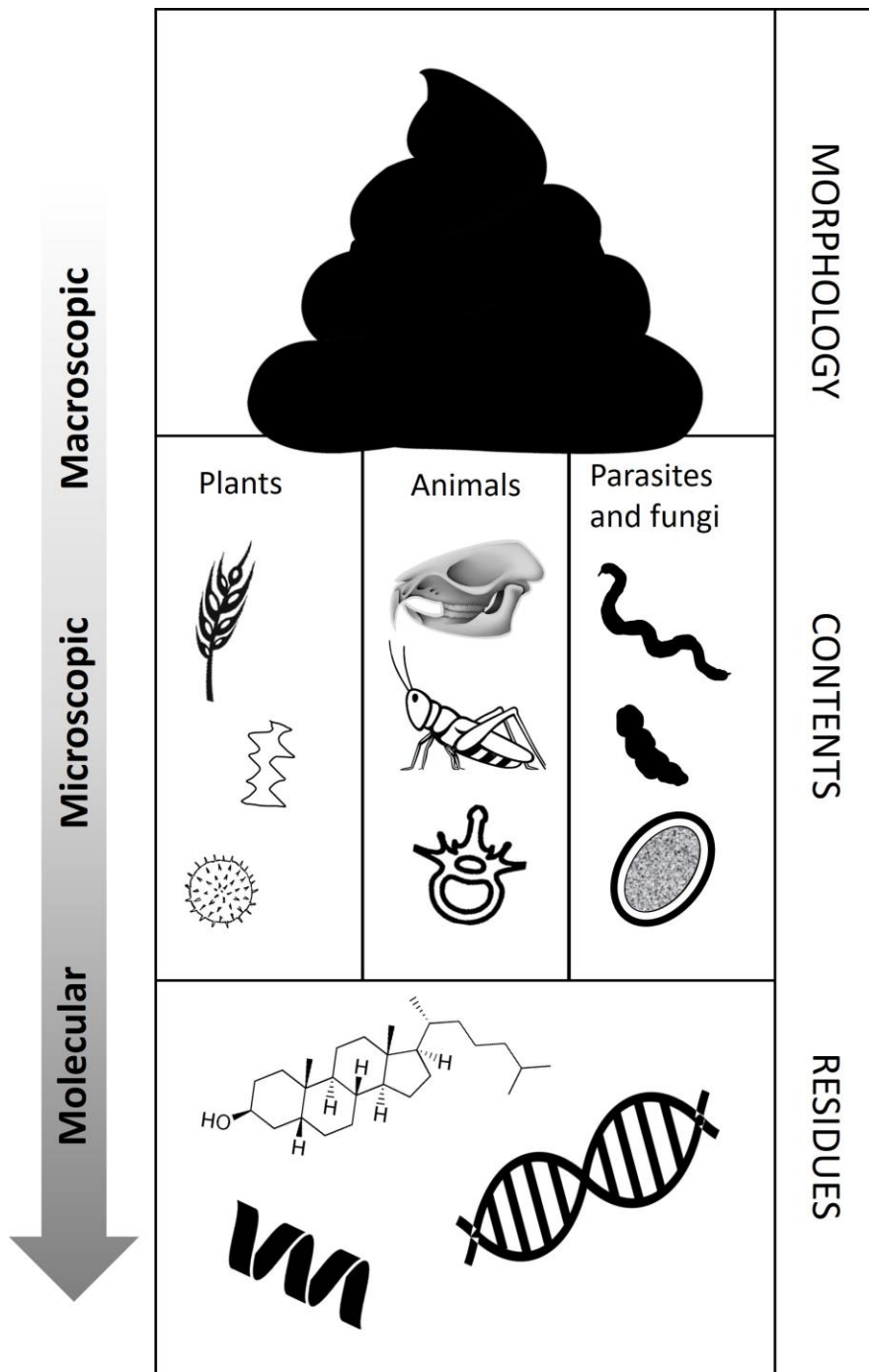


Figure 2: Components of coprolite analysis. Plants may include macrofossils such as seed and cellulose, microfossils such as pollen and phytoliths. Animals may include bones and other non-digestible parts of vertebrates and invertebrates. Parasites and fungi can include spores and eggs, as well as the 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.



### 3. Analysing coprolites: macromorphology and micromorphology

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.

#### 3.1 Morphology

In modern and palaeoecological 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. In archaeology the species that are usually of most interest are humans and associated domesticates. Linseele et al (2014) 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 Catalhoyuk (Shillito et al. 2013). However, this is not always the case. Coprolites from the Neolithic site of Durrington Walls, UK, preserved in calcareous sediments in pits, retain a typical ‘faecal’ sub rounded morphology, and were clearly identifiable as coprolites in the field (Figure 3).

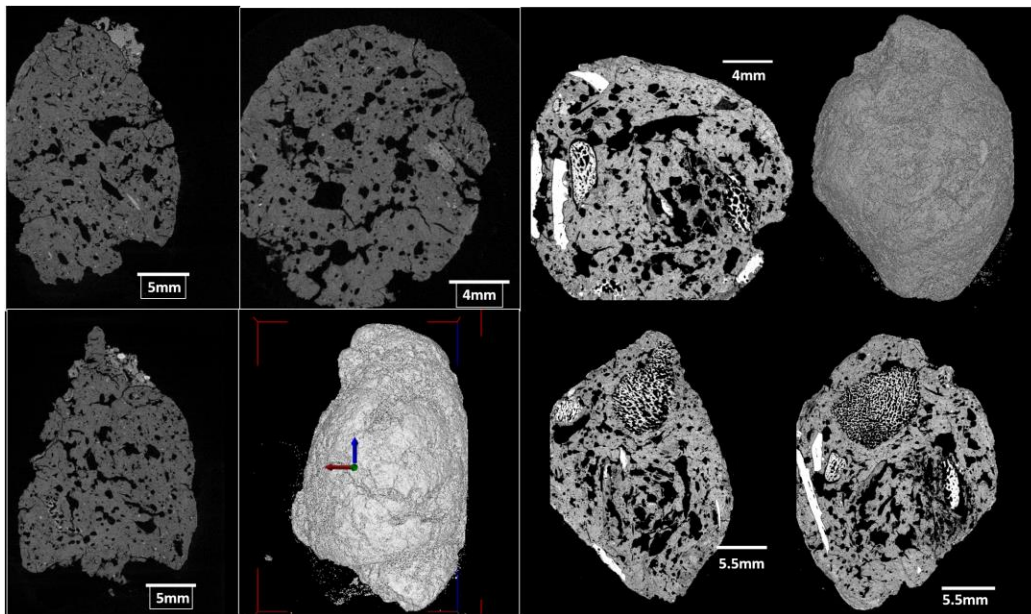


Figure 3: LEFT microCT scan of coprolite from Durrington Walls, UK showing digested bone fragments and probable plant voids; RIGHT microCT scan of coprolite from Durrington Walls, UK. Amorphous fabric with limited bone inclusions. MicroCT images taken by Zerina Johanson (British Museum).

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 of the individual. Given that human diets can be hugely variable, it is not surprising that the form of coprolites can also be varied.

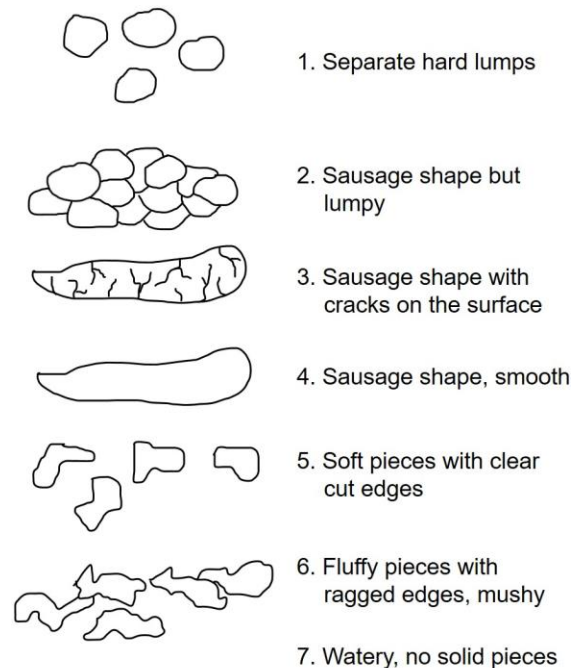


Figure 4: Bristol stool chart showing variability of human faeces morphology in modern clinical contexts

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; 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 1974, Callen 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). However, 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 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. Micromorphology can be a useful tool for ‘in situ’ analysis. For example at the early pre-pottery Neolithic site of Sheik e Abad in Iran, lenses of orange deposits were analysed 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 penning strategies (Matthews 2013, Shillito et al. 2013).

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 and courser 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 brown colour of modern faeces is due to the breakdown of bile components such as bilirubin. The reasons for the variation in coprolite colour, has not been thoroughly investigated, though it does not seem to be directly linked to species. There are some indications this could be related to preservation conditions – a comparison of coprolites in deeply buried versus more shallow deposits at Catalhoyuk 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).

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 (aDNA) (as discussed further in section \*\*). 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 sample has fibrous internal vegetation with phytoliths, external herbivore morphology/staining, which the 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 common for herbivore coprolites. They did not find evidence for calcium phosphate in the samples, which they suggest is characteristic of humans and carnivores.

Rasmussen et al. (2009) argue that micromorphology, 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.

Analysis of coprolites from Neolithic Turkey for example, assumed 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 perspective, should not be dismissed. Items such as rattlesnake fangs, fibres (McDonough 2019, Sondermann et al 2019) and whole rodent carcasses (Blong et al. forthcoming) 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*

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). 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 Bryan 2001; Hollaway 1981 cited in Riley 2010:189). Macrofossils such as seeds and bone fragments are often relatively straightforward to identify with knowledge of plant and animal anatomy, identification manuals, and a good reference collection. Hair, feathers, and scales recovered from coprolites can be identified to the family or genus level, and occasionally species.

#### 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). 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, and an understanding of how pollen moves through the human digestive system (Bryant 1974a; Dean 1978, 1993; Reinhard et al. 1991). 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 taxa 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).

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 Hollaway 1983; Martin and Sharrock 1964; Reinhard et al. 1991). 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).

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). Pollen from anemophilous taxa present in amounts higher than the mean sample might indicate intentional consumption of flowers, buds, foliage, or seeds of that taxa (Dean 1993; 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 taxa that may be masked by simply comparing relative frequencies with an arbitrary relative percent cutoff (Dean 1993; Reinhard 1993). 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 1974?). Non-floral parts of insect-pollinated taxa are not expected to have high amounts of residual pollen, so consumption of these components are 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.

There are several caveats to the use of pollen from coprolites to reconstruct intentional plant consumption. Studies have demonstrated that there can be a lot of variance in coprolite pollen concentrations (Bryant 1974a, 1974b, Sobolik 1988; Reinhard 1993). 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). This suggests that comparing concentrations alone across a series of coprolites maybe problematic (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). 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).

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

Phytoliths and starch are commonly analysed together. Phytoliths are mineral deposits, usually silica, that form within and between plant cells, forming three dimensional casts of the cell morphology. Calcium oxalate phytoliths are especially common in Archaic coprolites in the lower Pecos region of west Texas. The vast majority are derived from 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. 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. Phytoliths have been observed in large quantities in animal dung and possible human faeces. The ability to identify these 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).

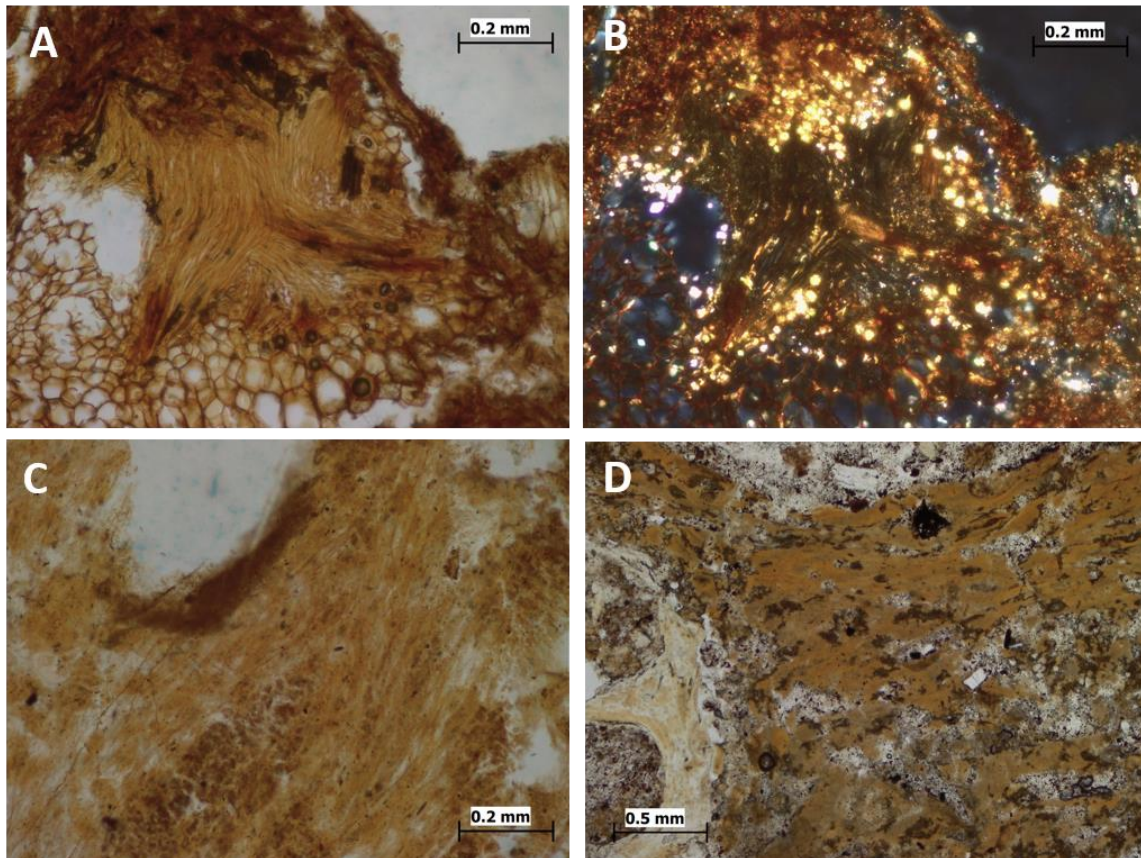


Figure 5: micromorphology of A an ovicaprid pellet from Paisley Caves, showing large plant tissue inclusions in PPL (left) and XPL (right). Under XPL starch granules are visible within the plant tissue. C omnivore coprolite from Paisley caves 1 (thin section 3.4). D omnivore/human coprolite from Catalhoyuk,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

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 2010), Ikna-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

Fungal spores in 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, Batillo (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

Intestinal parasites in coprolites have been identified dating from early prehistory to Medieval periods (e.g. Satiaga et al. Ledger et al. 2019a, in press, 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 has been more standardized across research groups, and is 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).

Archaeological parasitology, pioneered in the 1950s and 1960s, 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). 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 summarised 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.

A ‘palaeoepidemiological’ approach, focusing 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).

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. forthcoming), and other items may be ingested through craft activities, for example preparation of cordage. Inclusions entrapped in dental calculus, have been linked to craft activities in medieval populations (Radini et al. 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.

## 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

Ancient genomics has seen numerous advancements for a field only a few decades old, largely attributed to technological revolutions, namely next-generation sequencing, which has significantly reduced labour and costs (Hofreiter et al. 2015). The applications of aDNA analysis have proliferated in recent years, the most common archaeological substrates for DNA extracts are hard tissues - teeth and bone. However, more recently, there have been attempts to extract DNA from a wider range of archaeological material, including coprolites (Green and Speller 2017). Preserved within a coprolite matrix are DNA molecules which relate to the depositing individual, their diet and associated microorganisms. Previously, DNA from coprolites has been accessed using targeted approaches (e.g. PCR, 16S rRNA and metabarcoding). However, DNA derived from a range of sources is best accessed via a non-targeted (shotgun) metagenomic approach, we see this technique as future of coprolite aDNA studies.

### 6.2.1 Depositing species

Using non-target sequencing approaches, it is possible to determine the depositing species, by looking at the number of mapping reads and the damage pattern of that DNA. Furthermore, using recent bioinformatics advancements (Skoglund et al. 2013) it is possible to ascertain the biological sex of the depositing individual when high quality DNA is present.

Contamination and authentication both require a very strict procedure in aDNA research (Poinar and Cooper 2000). One of the more high-profile examples of aDNA from coprolites is that of Paisley Caves, Oregon (discussed in section \*\*). Six coprolites from the site were subject to aDNA analysis and identified as human: the DNA corresponded to Native American haplogroups A2 and B2 (Gilbert et al. 2008). However, Poinar et al. (2009) brand the radiocarbon dating of the oldest sample as “unreliable” and question the dating of the recovered coprolites - an important factor as the coprolites represented the earliest evidence of the peopling of North America at that time. Additionally, Poinar et al. judged the mitochondrial DNA (mtDNA) conclusions made by Gilbert et al. (2008) to be “inconclusive” due to the presence of contaminating sequences (human and canid) and inconsistencies in findings. Gilbert et al. (2009) responded to criticisms presented in Poinar et al. (2009) and importantly concede that coprolites from Paisley Caves may have been contaminated by other concurrent prehistoric human and canid occupants of the cave. They concede that it was impossible to unequivocally demonstrate that DNA leaching did not contaminate the coprolites, but they feel that it is unlikely that this is the case. The findings of Gilbert et al. (2008) were later corroborated by further work by the same group after additional analyses were undertaken on a larger number of coprolite samples from the Paisley Caves site Jenkins et al. (2012). This study included additional verification steps, including the analysis of associated sedimentary DNA, verification of the findings from an independent laboratory group and a more thorough investigation of DNA leaching. After this study a total of 65 coprolites from Paisley Caves have been analysed for ancient DNA and the identification of ‘human’ as the depositing species supports the original conclusions of Gilbert et al. (2008). A small number of samples have a mix of canid and human DNA which remains unexplained.

This demonstrates we still know relatively little about the mechanism of DNA movement through sediment (DNA leaching), and as open systems it is likely that coprolites will be affected if leaching occurs. The possibility of vertical movement of DNA in sediments has been documented in caves in New Zealand, DNA sequences from non-indigenous species (sheep) were found in the same strata as DNA from the extinct moa (Haile et al 2007). These species were separated on New Zealand by 350 years (Holdaway and Jacomb 2000), furthermore the quantity of DNA from sheep decreased with depth and, in this case, urine percolation is suspected. 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 findings are yet another reminder of the importance of adhering to field standards when working with ancient DNA (Poinar 2003) and using stringent authenticity criteria when assessing findings, both bioinformatically (e.g. tools to assess characteristic features of damaged DNA (Jónsson et al. 2013; Malaspina et al. 2014; Weiß et al. 2015)) and using critical logic to assess identifications (Gilbert et al. 2005). Whilst this is important in all aDNA investigations, the authentication 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. Identification of species from DNA sequences comes from comparisons to publicly available databases (e.g. NCBI (Federhen 2012)), whilst these records are extensive, they do have some limitations. For example, contamination of genomes (Merchant et al. 2014, Lu and Salzberg 2018), uncharacterised (especially microbial) species, and a lack of contemporary genomes for archaeological investigations (see Breitwieser et al. 2017 for a comprehensive review). This makes the criteria for good practice, laid out in the early days of aDNA work, still relevant for coprolite investigations and species identified by a small number of reads must be taken cautiously.

### 6.2.2 Diet

Ancient DNA analysis has been applied to coprolites to investigate dietary resources, initially through the testing of single markers and more recently through high-throughput sequencing techniques. The majority of dietary investigations have been undertaken on animal coprolites and show that it is possible to retrieve high-resolution dietary data which can be linked to subsistence and/or provisioning through this approach (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. demonstrated the potential for specific dietary reconstruction early, identifying 12 different plant and animal species in a targeted PCR investigation of Native American coprolites from Hinds Cave (2001). More recently (Rivera-Perez et al. 2015) reconstructed dietary resources available to pre-Columbian cultures using a non-targeted, high throughput approach.

### 6.2.3 Ancestral gut microbiomes

In modern medicine, investigations into the gut microorganisms - collectively known as the gut microbiota - have excelled, due to the realisation 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 the bacteria are representative of the microbes inhabiting the guts of past people. Coprolites exclusively provide access to this information.

It is expected 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). 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) a different relative abundance of phyla (Davenport et al. 2017). It is hypothesised that these characteristics reflect gut signatures of the past (Sonnenburg and Sonnenburg 2019). To date, there have been published microbial genetic investigations of 26 archaeological faecal samples (Luciani et al. 2006; Tito et al. 2008; Tito et al. 2012; Santiago-Rodriguez et al. 2013; Appelt et al. 2014; Cano et al. 2014). These studies have primarily focused on prehistoric American contexts (Americas: 25, Europe: 1).

The microbial content of coprolites is not only made up of authentic gut microbes, but microorganisms that infiltrate the matrix from the environment. The first, and still one of the only, shotgun comparisons of microbial signatures from coprolites examined showed the archaeological samples were more similar to one another than they were to the living gut microbiome (Tito et al. 2008). Whilst the researchers discuss human and animal contamination, there is less focus on environmental microbial signatures. The profile of the coprolites is compared to soil and said to be more closely aligned to a faecal signature, but it is unlikely that environmental contamination will have had *no* effect. A later study by the same group showed that coprolites are affected by soil contamination to a varying extent (Tito et al. 2012). Samples may cluster more closely together due to the presence of environmental microbes, which will of course be absent from modern faecal signatures. As the field advances, and more metagenomic studies are published, bioinformatic standards will become commonplace: any unauthentic (i.e.

environmental microbial contaminant) DNA will be removed by bioinformatic filtering before analysis of similarities are undertaken.

A Procrustes PCA analysis comparing the microbial identifications of the outer surface to the core found a significant difference between the two regions (Cano et al. 2014). The outer surface of a coprolite is in direct contact with the surrounding sediment, meaning more contaminating, environmental species are identified here compared to the coprolite core (Cano et al. 2014). In fact it is now recommended practise for subsampling of coprolites for any multiproxy analysis to occur in sterile conditions, as to not rule out aDNA analyses, and for material to be taken from the core and homogenised (Wood and Wilmshurst 2016). Coprolites, paired with the advent of powerful sequencing platforms, 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 derived and ancient gut microbes 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), other techniques used to investigate coprolites (regarding, depositing species and diet) and otherwise irretrievable information relating to the ancestral gut microbiome.

### 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 biomarkers

Steroids are a lipid group with an unusual structure – their backbone consists of four carbon rings. In faeces, the sterol profile varies according to the dietary profile (herbivore, omnivore, carnivore) and are the result of digestive processes, whilst the bile acid profile is distinct between different species. Together these molecules have been used to identify sources of faecal pollution in water from modern-day sewage pollution and agricultural runoff (Bull et al., 2002). This method was modified by Bull to account for the taphonomic processes in archaeological samples. Bull's method adjusted the level of \*\*\*

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. Bile acid profiles are unique to particular species, with humans having a dominance of deoxycholic acid and lithocholic acid. 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 practises of the



Minoans (Bull et al., 1999; Bull et al., 2002), identifying use of space in early settlement sites (Baeten et al., 2012, Shillito et al., 2011a) Lipid biomarkers can be analysed 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 ferrasols. (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 chromatographic and radioimmunoassay techniques to measure testosterone 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 (2001) applied liquid chromatographic analysis of fecal 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 be 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 fecal 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 the oldest known case of fecal matter preserved at an archaeological site and highlights the usefulness of biomarkers for reconstructing dietary patterns into the Pleistocene. A study of fecal deposits from an early Neolithic site in Iran identified C28 long chain fatty acids alongside the faecal sterols. The same samples were also analysed 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 Elliot 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) analysed the faecal sterols of clinical trial subjects on normal and plant enriched diets and found that the plant enriched diets led to greater PS 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.

#### *6.4 Protein analysis*

Ancient proteomics is an emerging archaeological discipline (Cappellini et al. 2014; Hendy et al. 2018). Proteins have been shown to survive within a coprolite matrix using cytochemical staining (Santiago-Rodriguez et al. 2013) however proteins from coprolites have not yet been characterised. Like DNA recovery, it is expected that proteins relating to the depositing individual's biological system, their diet and their associated species will be recovered. Whilst proteins are not as taxonomically specific as DNA, they are tissue specific, which offers a range of applications, complementary to aDNA. For host investigations, ancient protein analysis has the potential to identify disease states (e.g. Jersie-Christensen et al. 2018). For dietary investigations, this means it would be possible to achieve a higher resolution of dietary components (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? For investigation into ancestral microbiomes, 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).

### **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.

In modern contexts, there is an abundance of anthropological and geographic research on faecal pollution and its socio-cultural implications. In modern contexts, faeces typically attract responses of disgust in many societies, and are immediately removed from sight. Where we find them within and directly adjacent to activity areas. 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 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 Wilmschurst (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. As part of a study of early dietary variability in the Great Basin, we examined a number of coprolites from Paisley Caves, including subsamples of three coprolites that were previously analyzed in a parallel study (Taylor et al. 2019). Replicating analysis on subsamples of the same three coprolites has enabled us to explore methodological considerations related to appropriate size of sample, location of subsample on the coprolite, and to compare macro and microfossil analyses for varying subsample sizes taken from different parts of the same coprolite (Table 1). We observed notable differences in the proportions of taxa in subsamples analyzed in this study, and subsamples of the same coprolites analysed in Taylor et al. 2019. Our samples included coprolite material from the exterior of the sample, but it is not clear whether Taylor et al.’s samples also included exterior material. This could explain the different pollen counts, but nevertheless highlights the fact that samples collected from different parts of the same coprolite can produce different microfossil results. This confirms earlier research which has suggested that pollen grains are unequally distributed along the length of a coprolite (Martin and Sharrock 1964; Beck 2019).

Table 1. Comparison of subsamples from same coprolite. (nb Taylor et al. +400 counts, Blong +200)

Sample # (this study)	Sample weight	Pollen concentration per gram coprolite	Taxa counted (unknown counted as 1 taxa)	Sample number (Taylor et al. 2019)	Sample weight (Taylor et al. 2019)	Pollen concentration (Taylor et al. 2019)	Taxa counted (unknown counted as 1 taxa)
55	0.41 (E)	33,124	13	B7	2.86	9,900	8

56	2.45 (E)	72,988	17	B9	8.49	235,288	5
57	0.81 (E)	n/a	6	B3	2.83	19,392	10

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.

Previous studies of coprolites from Paisley Caves have produced pollen concentrations ranging from 235,000 to 9,900 per gram (Taylor et al. 2009) and 48,458 to 1244 grains per cc (Cummings et al. 2007). Our study of Paisley caves coprolites resulted in concentration values of 288,075 to 29,373 grains per gram. For many of these samples the ratio of indigenous to marker spore was well above 2:1, and reached as high as 30:1 in one sample (Blong et al. forthcoming). This means that the concentration values for some of these samples may be inaccurate, and may explain the variability in concentration values noted above. This has implications for the use of relative concentration values for taxa, and thus some error in interpreting intentional consumption based on pollen concentration values.

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 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.

forthcoming, Battillo et al. 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, Stewart 1974 ). 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 weight 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 SW Texas Coprolites; 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. AAS, Somderman 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 2014, Elliot et al. 2015, Portillo et al. 2017, Égüez et al. 2018), 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. forthcoming); 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 individual, amount of time spent chewing, health of individual.

Experiments on other items are rare. Crandall and Stahl (1995) “experiment involving the consumption of a skinned, eviscerated, and segmented insectivore by an adult human male. Bone remains from recovered faecal contents are examined for skeletal element representation, breakage and digestive damage. Detailed examination of each category suggests severe skeletal attrition which is comparable to, and at times in excess of, the damage exhibited in microvertebrate skeletal accumulations originating 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. Controlled, repeated experiments 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.

#### *Taphonomy– preservation and post-depositional processes*

Archaeological samples have the added complication of post-depositional processes to consider, and these also need to be better understood. The signals that we see in 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 'fresh' signals are altered, without having reliable modern experimental material to work with. This study of taphonomy has been a key aspect of most other archaeological specialisms. Zooarchaeology for example has a long history of experimental taphonomic work. Likewise, palynology and archaeobotany. 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).

Another key consideration is the mechanism of the coprolite formation. As stated above, it is still not clear *exactly* how faeces preserve resulting in 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 as the sample undergoes limited chemical changes. However, the mineralisation process appears to be more complex. Mineralisation can be facilitated by dietary components e.g. excess of phosphate and calcium encourage mineral precipitation, the environmental conditions of deposition can introduce constituents required via groundwater (Hollocher and Hollocher 2012) or it can be facilitated by bacteria (Briggs 2003). Coprolites, mineralised to some extent, tend to be persevered in anaerobic, waterlogged conditions. A fully mineralised sample should yield no authentic organic molecules, so the extent of the mineralisation is important for biomolecular investigations. The importance of water has already been alluded to, but an experimental study which submerged hyena droppings in water for 3 weeks, found the water had little impact on the morphology and the samples became so hard that they could not be imprinted (Larkin et al. 2000). Even so, the prospect of aDNA investigations of 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 microfossils (Hall 2000; 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.

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