**A re-evaluation of manner of death following the Vesuvius eruption at the Roman town of Herculaneum.**

***Keywords:*** Herculaneum; Vesuvius; thermal diagenesis; bone; FTIR; collagen.

**Abstract**

Herculaneum is one of the most famous Roman settlements in the world. One of the details which remains unclear is the specific manner in which the victims died during the eruption. We address this issue here through the investigation of changes in bone apatite structure and collagen preservation using Fourier Transform Infrared Spectroscopy (FTIR) combined with collagen extraction (Craig et al., 2009). We suggest that the prolonged presence of soft tissue at the time of impact, as well as the stone chambers in which the inhabitants of Herculaneum had sought shelter, acted as a thermal buffer and thus minimised the heat-induced degradation of organic and inorganic skeletal tissues.

**Introduction**

The eruption of Mount Vesuvius on August 24, AD 79 is one of the most iconic and well-documented naturally destructive events from our known archaeological past. The exceptional preservation and quantity of the archaeological material recovered from sites such as Pompeii, Herculaneum, Oplontis and Stabiae present a resonant picture of Roman life before the eruption. The skeletal assemblage uncovered on the former seafront of Herculaneum and within ten of the twelve *fornici* (stone arched chambers; Figure 1) offers a unique snapshot of the lives and deaths of the town’s inhabitants, and has catalysed debate over the nature and manner of their deaths following recent developments in bioarchaeological analysis (Mastrolorenzo *et al.,* 2001; 2011; Petrone, 2011; Fattore *et al.*, 2012; Schmidt *et al.*, 2015). The remains have also provided a unique opportunity to examine the lethal effects of pyroclastic surges from a still-active volcano in a densely inhabited landscape (Mastrolorenzo et al. 2010, Petrone et al. 2018).

The intensity of thermal pyroclastic exposure to which the Herculaneum victims were subjected fundamentally influences our understanding of both the cause of death and the effects of such heat exposure on the nature of bone diagenesis and collagen degradation. This last point is significant as these changes may have an impact on a range of subsequent bioarchaeological analyses, particularly biomolecular (Collins *et al*., 2002; Smith *et al*., 2001).

The aim of this study is to apply newly developed analytical methods to the bone recovered from the *fornici* in order to describe the context of death. These methods have contributed to a more nuanced understanding of other cremation contexts, but have never been applied here. The results could offer an unparalleled interpretation of the impact of this natural disaster on the human bodies of ancient Romans.

**Background**

The human skeletal sample recovered from excavations at Herculaneum represents a portion of a ‘living’ population, who died trying to escape from the eruption of the Vesuvius volcano in AD 79. In total, 340 individuals have been excavated from the beach and from the adjacent *fornici* (waterfront boat chambers), where they sought shelter.

The human remains from the seafront were recovered during three main separate phases. The first was in May 1980 from the area surrounding the central stairs that link the city to the beach. The second was a year and a half later where more skeletons emerged from the southward fornici (3, 4, 5). During this phase, which ended in 1985, around 150 individuals were recovered. A second group of about 75 skeletons was recovered between 1997 and 1999 from fornici 10, 12 and 5. The third phase, which recovered about 104 individuals, occurred between 2008 and 2012 and focused on fornici 7, 8, 9, 11 and from a niche of 10.

The demographic estimates of the whole sample show a slightly unbalanced sex ratio (Males/Females=1/22) and age distribution, with a less than expected number of infants and children for an ancient population (Fattore *et al.*, 2012). This could reflect the catastrophic nature of escaping the eruption, but we should also take into account the peculiar demographic dynamics of the city as attested by the analysis of the *Album*, an inscription in marble carrying a list of names of inhabitants of Herculaneum (de Ligt and Garnsey 2012).

The most interesting aspect of these distributions concerns the sex and age composition of the individuals found on the beach compared to those found in the boat chambers (Figure 2). Adult and young adult males died primarily (although not exclusively) on the exposed beach, whilst the demographic within the *fornici*,a series of equally sized vaulted chambers adjacent to the beach, comprised mainly of adult females and juveniles/infants, most of them possibly related (Fattore *et al.*, 2012; Figures 3 and 4). This evidence gives us a glimpse into how the Herculaneum residents were reacting to the catastrophe and socially organising their escape.

Boat chambers and the beach provided very different environmental conditions when the pyroclastic flow hit. Individuals within the *fornici* were far less exposed than those on the beach, and thus their manner of death may reflect these differences in conditions.

Although death by volcanic activity can leave no trace on the body (Makoye Ng’Walali *et al*., 1999), through the forensic and histological investigation of the Herculaneum victims from both contexts, Mastrolorenzo *et al.* (2001, [2010)](#_46r0co2) and [Petrone (2011, 2018)](#_3ygebqi) present the cause of death as instantaneous, thermally-induced fulminant shock. Histological observations of the skeletons from the beach and those from Oplontis exhibit bone discolouration varying from black to grey-white; the presence of both linear and polygonal microcracking; dental enamel cracks; cranial fractures as a result of thermally-induced intracranial pressure (Mastrolorenzo *et al.,* 2001), and incipient to high crystallisation of bone hydroxyapatite (Mastrolorenzo *et al.,* 2010). The lack of detectable DNA in previous studies of victims found on the beach (Mastrolorenzo *et al.,* 2010) and proteins in those in the chambers (Petrone 2018) has also provided further impetus to support the hypothesis of exposure to high pyroclastic temperatures. Further, it is also suggested that the lack of the boxer position, or pugilistic attitude in many of the victims also denotes instant vaporisation of the soft tissues upon impact in the context of Herculaneum. Absorption of thermal energy by these tissues is believed have subsequently caused a rapid decrease in aerospheric temperature within the *fornici*.

Others, however, such as Capasso (2000) predict a lower surge temperature (350-400°C) and a manner of death concordant with the gradual dehydration and contraction of the victims’ muscles and internal organs. Observations of brown not blackened bone, and the apparent lack of significant cracking in the long bones and cranial elements suggests minimal exposure to direct heat (Figure 3). Recent reanalysis of skeletal morphology by Schmidt *et al.* (2015) supports this conclusion; explicitly attributing the lack of fractures and discolouration to the presence of soft tissue. Indeed, they present examples of cranial staining, believed to demonstrate the pooling of soft tissue during decomposition. Cranial fractures are inferred to be the result of impact with debris brought into the *fornici*, and the presence of the pugilistic attitude may be attributed to soft tissue contraction and shortening – although this should be read with caution since there is little evidence to suggest that the fatal surge carried such debris along with it. Nonetheless, the inferred presence or absence of soft tissue plays a key role in the assessment of the manner of death. Evidence from other (non-skeletal) material at Herculaneum also argues for a lower temperature overall (e.g. Caricchi *et al*., 2014).

**Recent advances in the study of burned bone**

Despite their magnitude and often widespread damage, the study of the forensic and taphonomic effects of volcanic eruptions on the human body is notably absent in academic literature. Nonetheless, there has been recent growing interest in the analysis of burned and cremated human remains (see for example, Thompson, 2015a). This interest has largely focused on the development and refinement of new methodologies, particularly with regards to the analysis of bone microstructure. By using a range of analytical methods and imaging modalities, studies such as Boschin *et al.* (2015), Cascant *et al*. (2017), Ellingham *et al.* (2015; 2016) and Thompson (2015a; 2015b) have demonstrated that subtle and complex changes occur within the bone structure as a result of thermal exposure. Crucially, it has been argued that these changes can be correlated with specific burning conditions, and allow for contextual interpretation of the event itself.

With this in mind, attempts have been increasingly made to offer refined interpretations of funerary behaviour from cremated human remains (Gonçalves and Pires, 2015; Thompson, 2015a). A recent study of Roman soldiers along the Northern Frontier (Thompson *et al.,* 2016) demonstrates the potential to infer features of individual identity through the combined analysis of microscopic heat-induced changes in cremated bone, and pyre artefacts. Squires (2015) undertook a similar approach to Anglo-Saxon remains from the United Kingdom noting that “it is equally likely that the time and resources expended on the cremation process were just as significant... [as those invested in creating the grave good assemblages]” (2015; 168). Piga *et al.* (2016) combine results from the analysis of bone microstructural changes with standard osteological assessment to identify the incomplete cremation of a pregnant female from a Phoenician-Punic site in Sardinia, Italy. This was in part possible due to the identification of the physical condition of the body at the time of burning (fleshed/defleshed) in tandem with recent work in this area (Keough *et al.*, 2015); allowing for a more nuanced interpretation of the pre-burning condition of human remains.

Thus recent methodological and conceptual developments which have been applied in other archaeological contexts around the world (Thompson, 2015b) suggest that it is now possible to examine the burned remains from Herculaneum from a perspective not previously accessible.

**Collagen quality and thermal diagenesis**

In fresh, unaltered mammalian bone, collagen represents approximately 22% total weight (van Klinken, 1999). Diagenetic pressures such as heat, acidity, microbial attack and hydrology can cause collagen to be lost (Jans *et al.,* 2004; Carter and Tibbett, 2008; Milner *et al.,* 2011), and is a pervading concern in bioarchaeological studies. However, investigation suggests that collagen is remarkably robust. Sequencing of type I collagen from specimens dating to the both the Middle Pleistocene (c.780-125 Ka) and the Lower Pleistocene (c.1.5 million years ago) present compelling evidence for its longevity and survival (Buckley and Collins, 2011). It is also generally accepted that even in cases of low total abundance (yield), the structural and chemical integrity of collagen remains largely intact. Dobberstein *et al.* (2009) suggest that 99% of total collagen is lost before disruption to the amino acid, isotopic, and elemental (C:N) structure occurs, and this 1% threshold is widely used in isotopic studies as one of several key criteria with which collagen quality is determined (DeNiro, 1985; Ambrose, 1990; van Klinken, 1999).

As with bioapatite, the study of thermally-induced collagen diagenesis has become increasingly pervasive throughout archaeology and the greater scientific disciplines within the last several decades. It is now widely believed that the structural relationship between the two tissues plays a mutually advantageous role, although scholarly opinion differs with regards to which tissue (if either) is more greatly responsible for maintaining overall integrity. Some argue that collagen protects bioapatite from thermal degradation by acting as a ‘thermal shield’; reducing porosity and thus the overall mineral surface area exposed to the environment (Person *et al.,* 1996; Trueman *et al.,* 2008; Lebon *et al.,* 2010; Thompson *et al.*, 2013). Others propose that the mineral phase offers collagen protection from diagenetic alteration, and can act as fracture centres which, when split by thermal energy, expose the collagen to diagenesis (Etok *et al.,* 2007). In the context of boiling during which temperatures remain relatively low (≤100°C), Koon *et al.* (2010) and Bosch *et al.* (2011) believe that although the morphology of the collagen fibrils can be thermally altered, the association with hydroxyapatite has a stabilizing effect until temperatures reach ≥500°C (at which point collagen gelatinises).

It is likely that there exists a relatively symbiotic dependency between bone collagen and bioapatite (Dobberstein *et al.,* 2009). The nature of which is not of immediate concern to this analysis other than to note that it has been shown to occur at both the macro- and microscopic levels (Vassalo et al., 2017). Importantly, Koon *et al.* (2010) propose that collagen exhibits an ‘all or nothing’ mechanism, and that, regardless of its association with hydroxyapatite, collagen in mammalian bone will not undergo significant thermally-induced diagenetic alteration until exposed to sufficiently high temperatures (300°C - 500°C (Holden *et al.,* 1995; Munro *et al.,* 2004;Etok *et al.*, 2007; Koon *et al.,* 2010)).

**Materials and Methods**

Rib sections from 152 of the Herculaneum individuals were selected from six of the 12 *fornici* and prepared for collagen extraction using a modified Longin method (see Craig *et al.,* 2009). For comparative analysis, ribs were obtained from four individuals from the Imperial Roman necropolis at Velia (2nd Century), where previous analysis of collagen has already been carried out (Craig et al. 2009). The Velia samples were from formal fully articulated burials with no evidence of exposure to heat. Several fauna, including dogs and ovicaprids were discovered within the *fornici*, and used here for further comparison.

In brief, samples were cleaned using a sterile scalpel and crushed to pieces of approximately 2-5mm in length. 60mg was weighed into a vial and demineralised in 0.6M HCl, gelatinised and lyophilized. Extracted collagen was weighed and percentage weight calculated for each sample. Collagen yield is commonly expressed as a percentage of the bone’s overall mass. Full details of the method can be seen in Martyn *et al*. (2018). Remaining cleaned bone samples were crushed into a granular powder using a pestle and mortar for analysis by Fourier Transform Infrared Spectroscopy - Attenuated Total Reflectance (FTIR-ATR; see Thompson *et al.,* 2013; 2016 for a detailed protocol). The scans were performed using a Nicolet 5700 FTIR Spectrometer controlled by OMNIC 7.3 software. Before each bone sample was analysed the diamond stage was cleaned with propanol and background spectra were collected. Three replicate measurements were taken for each sample and mean averages were used in subsequent analysis. The spectra were then recorded between 2000 cm-1 and 400 cm-1, at a resolution of 4 cm-1. The following crystallinity measures proposed by Thompson *et al.* (2013) were used in this study:

Crystallinity Index (CI) = (565 cm-1 + 605 cm-1) / 595 cm-1
Carbon / Phosphate ratio (C/P) = 1415 cm-1 / 1035 cm-1
Carbonate / Phosphate ratio (CO/P) = 1650 cm-1 / 1035 cm-1
Carbonate / Carbonate ratio (CO/CO3) = 1650 cm-1 / 1415 cm-1
Carbonate / Phosphate ratio (CO3/P) = 900 cm-1 / 1035 cm-1
Phosphate High Temperature (PHT) = 625 cm-1/610 cm-1
Line width = the full width at half maximum of the phosphate peak at 1035 cm-1

As has been noted previously (Thompson *et al*., 2013; 2016) the numbers in these measures relate to positions on the FTIR spectra and the ratios themselves examine different aspects of heat-induced change as influenced by burning conditions. Therefore, use of all seven indices allows for the analysis of a range of possible burning intensities: C/P, CO/CO3, CO3/P, CO/P and line-width best describe low temperature burning; CI and line-width work best for describing middle intensity, and; PHT and C/P high intensity burning events.

**Results**

**Collagen Yield**

Of the 152 individuals analysed, only twelve (8%) exhibit percentage collagen yields which fall below the 1% threshold indicative of structural and chemical deterioration (DeNiro, 1985; Ambrose, 1990; van Klinken, 1999; Dobberstein *et al.,* 2009). Of these 12 individuals, 7 are identified as infant or juvenile. 10 were deposited in *fornice* 9 and 2 in *fornice* 8. Table 1 presents the average collagen yields of the individuals in the six fornici studied.

A 2-sided independent Kruskall-Wallis test was applied to all 152 samples to identify statistical significance between percentage collagen yield and *fornici*. The test showed that the distribution in collagen yields is significantly different between the chambers(H= 56.157, P= 0.000; Table 1). The highest average percentage yield is exhibited by the individuals from *fornice* 10 (average yield = 13.3%, mean rank 103.11) which also sheltered the greatest number of victims (43) and had the highest estimated total body mass, based on the numbers of infants, juveniles, men and women present (Fig 1). The lowest average percentage yield is exhibited by *fornice* 7 (average yield = 3.8%; mean rank = 40.68) which correlates with the lowest population of victims and the lowest total body mass. This trend is visible across the *fornici* populations, seeing a decrease in average percentage yield as the total population within each *fornice* also decrease (Table 1; Figure 5). An exception to this trend is presented in *fornice* 9 which exhibits the second largest population (37) but fifth lowest average (average yield = 4.1%; mean rank = 39.58).

In addition, δ13C and δ15N data obtained for 81 of the 152 samples were checked for C:N ratios which fall within the acceptable ranges indicative of diagenetically unaltered collagen. These ranges are 2.9-3.6 and 3.1-3.5 as proposed by DeNiro (1985) and van Klinken (1999), respectively. All samples fall within these ranges, suggesting that their structure and chemical quality are not compromised by diagenetic interference.

**Crystalline Structure**

Figure 6 presents the combined CI and C/P data which is an effective way of highlighting burning intensity. Comparative baseline C/P and CI values for bones exposed to low, medium and high intensity burning are presented, collected in accordance with the same methodology (see Thompson et al., 2016 for method and summary data).

Crystallinity index (CI) values ranged between 2.30 and 4.33 which corresponds with low Intensity burning events. This range has been verified through calibration with controlled experimental studies, but also other work on archaeological remains (Figure 6). The other crystallinity measures also support this temperature range, with the C/P and CI values coinciding with those exhibited by the Low Intensity experimental samples (see Supplementary Data).

**Discussion**

Overall, good collagen preservation and crystallinity values which fall within the range indicative of no to low-intensity burning events, suggest that the Herculaneum victims within the *fornici* were not exposed to significantly high temperatures at time of death. This means that either estimates for the flow temperature are too high, or that other mechanisms had an effect in buffering the victims’ skeletons from exposure to the full thermal energy of the pyroclastic surge. Several paleomagnetic analyses undertaken at Herculaneum and contemporaneous sites estimate the temperature of the pyroclastic event to have been c. 350°C-400°C (Kent *et al.*, 1981; Capasso, 2000; Zanella *et al.*, 2000; Cioni *et al.,* 2004), although Mastrolorenzo et al. calculated 480°C just outside the chambers (2001). Others, such as Caricchi *et al.* (2014) and Leone *et al.* (2016) have undertaken work investigating the exposure of mortars, plasters and wood to the pyroclastic temperatures at different parts of the site. Their estimates (240°C -370°C) are concordant with previous work and may suggest an even lower exposure temperature, assuming that these temperatures could indeed apply to the beach and *fornici* areas, as well as within the town itself. Recent additional work by Giordano *et al*. (2018) examined the influence of building structure on the temperatures of the pyroclastic flows, and concluded that internal thermal disequilibrium was apparent. This means that temperatures could be 100°C lower when walls or building material was mixed up in the flow, when compared to locations where there was no presence of building material. Regardless, in either situation the highest temperature noted was 440°C (Giordana *et al*., 2018).

As such, an alternative explanation for why the victims at Herculaneum were exposed to temperatures significantly lower than the pyroclastic movement must be explored. Drawing upon various lines of evidence, one suggestion is the presence of soft tissue at the time of, and following, impact. The data show correlation between greater collagen and apatite preservation, and the number of individuals within each *fornice*. This suggests that density played an important role in the preservation of these substrates. In accordance with the Flory-Rehner theory, van der Sman (2007) proposes that a higher density of soft tissue holds the potential for greater thermal protection of bone. When tissue is heated, a temperature gradient is formed due to a delay in the transfer of energy from the exposed outer surface to the inner portions. Indeed, if the period of exposure is sufficiently short, such internal temperatures may not be experienced at all. At Herculaneum, the heating of the cadavers through pyroclastic exposure is likely to have caused the outer tissues (dermis, muscles, tendons) to swell. The movement of water into the central portion of the cadaver (i.e. the thoracic area; interfaces with long bones in extremities; the pelvic region) may have served to bake the skeleton as opposed to burn it.

Such a theory is supported in a small way by the occasional presence of the pugilistic attitude in some individuals; a position which is caused by dehydration and contraction of the muscles and other soft tissues of the body (Bohnert *et al.,* 1998; Keough *et al.,* 2015; Ubelaker, 2015) and thus suggests that not all victims vaporised. These results are particularly interesting considering similar studies on bones from other small enclosed chambers show considerable variation in collagen levels and high levels of crystallisation, and thus seem greatly influenced by local environmental conditions (Salesse *et al*., 2014). It has been argued previously that once burned, bones display limited subsequent diagenetic change (Snoeck *et al*., 2014). Correlation between collagen yield/CI and *fornice* population sizes/total body mass may thus demonstrate this relationship between density and tissue thermal buffering. Controlled cremation studies show that it takes between 40 minutes and two hours at temperatures in excess of 650°C (average 800-1,000°C) to fully destroy cadaveric soft tissue (Bohnert *et al.,* 1998; Gerling *et al.,* 2001; Schwark *et al.,* 2011). Even when subjected to temperatures in excess of 1,000°C, human tissue will not simply vaporise, as proposed by Mastrolorenzo *et al.,* (2001;2010) and Petrone (2011; 2018). Evidence suggests that it is often the case with fire victims that the side or portion of the body upon which they lie during burning remains relatively less degraded that areas undergoing direct exposure (Gerling *et al.,* 2001). Contrary to the majority of experimental investigations which burn single cadavers at a time, the Herculaneum victims died together in close proximity, with victims’ bodies overlapping in some cases.

According to Gerling *et al.*’s (2001) theory, the Herculaneum individuals would have been even less susceptible to soft-tissue damage than those observed in controlled experiments. Indeed the very nature of the thermal energy differs between the two. When exposed to fire organic matter acts as fuel. This is further exacerbated by clothing which can cause a ‘wick’ effect (DeHaan and Nurbakhsh, 2001; Christensen*,* 2002) and often accelerates the burning process. Pyroclastic surges create radiant heat which does not utilise fuel. It is generally accepted that bodies exposed to radiant heat will exhibit significantly slower consumption of soft tissue compared with bodies subjected to fire (Bohnert *et al.,* 1998; Christensen, 2002). This would be compounded by the intact walls of the *fornici* which, as Giordana *et al*’s (2018) work shows, would have slowed the process of thermal equilibrium with the external environment. Experimental work by Carroll and Smith (2018) also suggests that bodies burning within enclosed spaces exhibit less significant heat-induced changes compared to those from pyre contexts. Further, analysis by Ellingham *et al.* (2016) explicitly highlights the significant ‘buffering’ effect soft tissue can have on heat-induced changes to bone crystallinity at temperatures below 300°C, and ‘fuelling’ effect (i.e. accelerating combustion) above 800°C.

When interpreting crystallinity values it is important to note that the relationship between the CI and burning intensity is not linear. The increase in CI, for example, is slow until around 500°C is reached at which point it increases rapidly. In some cases it has been argued that increases in CI due to low intensity burning are actually the result of normal diagenesis and decomposition. Interestingly, the nearby site of Velia which suffered no such thermal incident but contains contemporaneous interred individuals, exhibits comparable values resulting from natural diagenesis (Figure 6). However, due to the detailed contextual information we have for this disaster, the known history of the site, and the presence of other heat-induced changes identified through osteological analysis, it is apparent that in this instance, the observed changes can be confidently attributed to low-intensity heat exposure, rather than natural diagenesis. As has been noted above, subsequent diagenetic change to these heat altered bones is unlikely.

**Conclusions**

Consideration of this new data combined with a synthesis of results from studies which have examined the building and artefactual material of Herculaneum, suggests that it is unlikely that the theory of instantaneous soft tissue vaporisation and displacement with ash, as proposed by Mastrolorenzo *et al.* (2001; 2010) and Petrone (2011, 2018), would have been possible under the conditions within the *fornici*. Based on the data presented as well as macro and microscopic osteological observations by Schmidt *et al.* (2015), we propose that the population density of each *fornice* directly affected collagen preservation through variability in soft-tissue ‘buffering’. The manner in which the victims of Vesuvius died at Herculaneum is tragic when approached from any perspective, but we conclude here that instant vaporisation within the *fornici* is not concordant with the observed biochemical and osteological evidence presently available to the field. Although the victims recovered on the beach were not part of our study sample, we suggest that instant vaporisation did not occur there either for some of the same reasons.

The results presented here underline the importance of conducting specific FTIR and diagenetic analyses on material from contexts which have experienced events involving significant heat. Archaeological sites need to be assessed on an individual basis taking into account the unique characteristics of the heating event. This is especially true if we consider the importance of building integrity and enclosed spaces on the temperature experienced by the victims (Carroll and Smith, 2018; Giordana *et al*., 2018). Other work at Herculaneum has demonstrated that good preservation of non-collagen organic material is also possible (Rowan, 2017), thus highlighting that organic residues can survive extremely challenging environments and analysis of these can allow for significant reinterpretations of past events.

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