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**Specialist Report: Multi isotope and trace element study of
individuals from Trinity Burial Ground, Hull**

Contents

1 Introduction	p. 3
2 Amelogenin peptide analysis	p. 4
2.1 Materials and methods	p. 4
2.2. Results and interpretation	p. 5
3 Dietary reconstruction	p. 6
3.1 Carbon and nitrogen isotope analysis	p. 6
3.2 Materials and methods	p. 8
3.2.1 Bone and bulk dentine collagen	p. 8
3.2.2 Incremental dentine collagen	p. 8
3.3 Results and interpretation	p. 10
4 Assessing geographical origins	p. 21
4.1 Oxygen isotope analysis	p. 21
4.1.1 Materials and methods	p. 21
4.1.2 Results and interpretation	p. 22
4.2 Strontium isotope analysis	p. 28
4.2.1 Materials and methods	p. 28
4.2.2 Results and interpretation	p. 29
4.3 Sulphur isotope analysis	p. 32
4.3.1 Materials and methods	p. 32
4.3.2 Results and interpretation	p. 33
4.4 Lead isotope analysis	p. 36
4.4.1 Materials and methods	p. 37
4.4.2 Results and interpretation	p. 38
5 Trace element analysis	p. 43
5.1 Materials and methods	p. 43
5.2 Results and interpretation	p. 44
7 Discussion	p. 57
8 Conclusions	p. 60
 References	 p. 62
Appendix	Excel file

***Note:** The isotope background sections of this report are based on a template adjusted to suit the specifics of this particular project. Much of the wording is therefore reproduced for other projects. These sections are therefore suitable for unpublished and general reporting but may require omission or adjustment for full publication.*

Summary

This report presents the results of an isotopic study into the diet and mobility of 180 individuals from the Trinity Hull Burial Ground (TBG20). Carbon and nitrogen isotope analyses revealed that the Trinity Hull population ate a predominantly terrestrial C₃ diet with some supplementary marine and/or non-native C₄ resources which is consistent with other urban 19th century British populations. Differences in childhood carbon isotope ratios (dentine) and adulthood carbon isotope ratios (bone) show a tendency for increased consumption of marine or C₄ crops in adults, which may reflect a slight difference in diets between adults and children. Comparisons of diet between the sexes also revealed that males may have eaten more meat than females who appeared to have relied more on marine foods. However, these differences were small and not statistically significant.

Many of the individuals analysed have isotope characteristics that indicate local origins, with their strontium and lead isotope ratios consistent with a childhood spent in Yorkshire. The Trinity oxygen isotope ratios are much lower than expected, and do not fit with our current understanding of tooth enamel oxygen isotopes in archaeological British populations and therefore do not provide a good indication of childhood residency for most of the Trinity population. This type of shift in oxygen isotopes has been seen in other 19th century populations and is possibly indicative of an industrial/urban cultural or environmental influence on either the types of water consumed (well water, beer etc.) or biological fractionation processes (disease, activity, stress etc.). However, further research is required on the cultural, environmental and biological factors that influence the oxygen isotopes in tooth enamel before any conclusion can be drawn.

Despite this, three notable outliers have been identified (SK11237, SK14148 and SK21582). The first outlier, SK11237, had a very low oxygen isotope ratio indicating origins in a colder and/or more northerly country or a region of higher altitude than Britain or a continental climate. This individual also had a tooth enamel carbon isotope ratio indicative of a diet containing non-native C₄ or significant contributions from marine resources. This combination potentially suggests a childhood spent outside Britain, possibly from somewhere such as northern Scandinavia or Greenland. The second outlier, SK14148, had a high oxygen isotope ratio consistent

with a childhood spent in the western fringes of Britain, such as Cornwall, southwest Wales, or the Hebrides, or perhaps western Ireland or Atlantic coastal regions of western Europe. The third outlier is named individual [REDACTED] (SK21582), this person had lead isotope ratios too high to be from British lead sources, and more akin to significant exposure to younger, perhaps Mediterranean lead sources. This is intriguing because it is thought that [REDACTED] spent his early life in Selby, but these results strongly suggest exposure to a significant level of lead originating from outside Britain.

Trace element concentrations highlighted the high level of environmental pollution in 19th-century Hull: all but six individuals had lead burdens consistent with childhood exposure to significant amounts of lead/lead compounds. The six individuals with low lead concentrations most likely grew up in a rural location away from industrial or urban hubs where lead would be less ubiquitous within the environment. Surprisingly for an urban environment, only 10 individuals had evidence of heavy metal poisoning with elevated levels of arsenic and mercury in their tooth enamel and bone. During the 19th century mercury and arsenic were widely used in jobs (e.g. milliners, farming), as an additive in food, beauty products, dyes and various medications to treat several conditions such as tuberculosis, constipation, tooth ache, parasitic infection and the pains of childbirth. Therefore, whether contact came directly from therapeutic use, consumption, or indirectly through environmental and atmospheric pollution, some 19th-century Hullensians were likely exposed to mercury and arsenic compounds.

1. Introduction

This report presents the results of the multi-isotope and trace element analysis of Industrial Period individuals recovered from Trinity Burial Ground, Hull. One hundred and fifty-six teeth and one hundred and fifty bone samples from 180 inhumation burials were analysed for strontium, oxygen, lead, sulphur, carbon, and nitrogen isotopes and trace element concentrations to reconstruct diet and childhood mobility. The results have been interpreted and discussed in context with previously published data from contemporaneous burials.

2. Amelogenin peptide analysis

In adult human remains (≥ 18 years) morphological and metric sex estimation techniques provide accuracy levels between 80 – 95% in well preserved and relatively complete skeletons (Phenice 1969; Işcan & Derrick 1984; Buikstra & Ubelaker 1994; Walker 1995; Loth & Henneberg 1996). However human remains, especially in archaeological contexts, display varying degrees of taphonomic alterations and levels of completeness, which can often make sex estimation difficult or in some instances, impossible. In non-adult human remains (< 18 years) sex estimation techniques have shown to yield accuracy levels that ranged between 70-92% (Fazekas & Kósa 1978; Weaver 1980; Schutkowski 1993; Loth & Henneberg 1996). However, tests of these methods have not been able to reproduce these results. Therefore, sex estimation of non-adult skeletal remains is currently not possible via morphological or metric analyses (Veroni *et al.* 2010). Alternatively, DNA analysis can be used to estimate sex. However, preservation and contamination levels often make results inconclusive, and the process is destructive and costly. These factors have led to the technique being infrequently used for sex estimation of archaeological human remains. A novel technique developed by Stewart *et al.* (2017) provides a minimally destructive and robust means of estimating the biological sex of skeletal remains via acid etching of tooth enamel and subsequent identification of sexually dimorphic isomers of amelogenin peptides.

2.1. Materials and methods

Amelogenin peptide extraction was carried out for 16 deciduous teeth that were also analysed for carbon and nitrogen stable isotopes in incrementally sampled dentine, following the Stewart *et al.* (2017) method briefly summarised here: The enamel etch area was washed with 3% H₂O₂ for 30 seconds then rinsed with ultra-pure water. Enamel etching was carried out using 80 μ l 10% HCl for two minutes, the first etch was discarded and a second two minute etch performed and retained. The peptides in the etch solution were bound to C18 resin ZipTips conditioned three times with 10 μ l 100% acetonitrile and 0.1% formic acid. The peptides were eluted off the C18 resin using 4 μ l of elution buffer (60% acetonitrile/0.1% formic acid). The peptide samples were then frozen at -18°C and lyophilised.

Samples were transferred to the School of Pharmacy and Biomolecular Sciences, University of Brighton where they were dissolved in 12 μ l 0.1% trifluoroacetic acid (TFA) in water, centrifuged for 5 minutes to remove particulates and transferred to 10 μ l glass autosampler vials. 5 μ l of sample was analysed by reverse-phase nanoLC-MS with a liquid chromatograph (nanoRS U3000; Thermo Fisher Scientific) coupled to a hybrid quadrupole orbitrap mass spectrometer (Q Exactive; Thermo Fisher Scientific). The data were searched against the human proteome (UniportKB, 10/15) with MaxQuant v 1.5.1.2 using default search settings with methionine oxidation as a variable modification, unspecific digestion mode and a minimum peptide length of 6 (Stewart *et al.* 2017).

2.2. Results and interpretation

The two dimorphic amelogenin peptides (AMEL-X and AMEL-Y) clearly differentiate between males and females, as AMEL-Y is Y-chromosome specific and therefore only present in males. The results are summarised in Table 1. In eight of the individuals AMEL-Y is absent at 440.2233 m/z with only a single peak at 540.2785 m/z, indicating that these individuals are female. Two further individuals (SK 13130 and 30225), for whom separate DNA analysis identified female sex, failed to produce a peptide result. The remaining six individuals exhibited both AMEL-X and AMEL-Y isomers, indicating that these individuals are male. These results are also included in the section on incremental analysis of dentine.

Table 1. Amelogenin peptide sex estimation for non-adults sampled at Trinity Burial Ground

3. Dietary Reconstruction

3.1. Carbon and nitrogen stable isotope analysis

Isotopic analysis of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) is a well-established technique for the reconstruction of diets in past populations using archaeological teeth and bones (DeNiro & Epstein 1978; Schoeninger *et al.* 1983; Kohn 1999; Richards *et al.* 2006). Within these two tissue types, collagen is the predominant protein present and provides a rich source of averaged $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from dietary protein.

Mineral from bones or teeth may also be analysed, and – in contrast to collagen – reflect the isotopic composition of the whole diet. The composition of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from teeth and bones thus offer complementary lines of evidence for the isotopic composition of the foods consumed during the tissue's formation. This is due to the metabolism and reuse of dietary proteins acquired from the plant and animal products consumed during collagen synthesis. Therefore, once metabolic fractionation has been accounted for, these $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values can be used to garner insights into not only the types and quantities of food resources utilised by a population, but also the socioeconomic and cultural influences surrounding the dietary practices of groups as well as individuals.

The variations that arise in $\delta^{13}\text{C}$ values result from differences in ecosystems (marine vs. terrestrial) and the photosynthetic pathways (C_3 and C_4) used by plants in their manufacture of carbohydrates (Lee-Thorp 2008; Mays & Beavan 2012). As such, variations in $\delta^{13}\text{C}$ values allows differentiation between the relative contribution of C_3 or C_4 plants and the animal products based on these plants, to diet (Ambrose *et al.* 1997; Camin *et al.* 2008; Beaumont *et al.* 2013a). In temperate regions, such as Britain, plants tend to use the C_3 pathway. Therefore, isotopic evidence of dietary proteins based on non-native C_4 plants (e.g. maize, sorghum, millet) would suggest a foreign influence on diet within a British population.

Variability in $\delta^{15}\text{N}$ values reflects the balance between biological nitrogen fixation, biosphere recycling and nitrogen release (Robinson 2001). This variability facilitates the visualisation of terrestrial and marine food source input into diet as marine based food sources tend to be more enriched in ^{15}N than land-based food sources. In addition to this, $\delta^{15}\text{N}$ values also vary with trophic level due to metabolic fractionation, creating a 2–6 ‰ enrichment with every trophic level shift (Schoeninger & DeNiro 1984). This shift is most noticeable in marine food consumers as aquatic food sources have high $\delta^{15}\text{N}$ values owing to the relatively long food chains compared to those observed in terrestrial food sources (Tykot 2004). Combining the analyses of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values can facilitate the interpretation of plant, animal and marine protein contributions to past diets. This allows distinctions to be made between broad categories of food groups, such as herbivore vs. carnivore or marine vs. terrestrial input and any potentially non-local influence on diet (Ambrose & Katzenberg 2000).

Analysis of different skeletal tissues offers differing time resolution, due to differences in known age of formation or skeletal turnover rates. While bone can offer data on long-term dietary trends in the decades prior to death, with substantial time-averaging and gradual turnover, teeth are formed between antenatal life and late adolescence and do not remodel. Consequently, analysis of bulk tooth dentine can offer an averaged record of diet over the period of formation, with more precise time resolution than in bone (Richards *et al.* 2002). Incremental sampling of dentine can offer greater resolution still. Rather than producing a single data point per individual, incremental analysis of dentine creates a data timeline for individuals, allowing interpretative distinction between changes characteristic of dietary change or changes in fractionation due to maladaptive metabolic states, such as nutritional stress (Beaumont *et al.* 2018).

3.2. Materials and methods

Multi-tissue analysis was undertaken for carbon and nitrogen stable isotopes, comprising bulk analyses of bone and dentine collagen and incremental analysis of deciduous and permanent tooth dentine.

3.2.1 Bone and bulk dentine collagen

Rib bone (n=140) and bulk dentine (n=140) samples were cleaned and abraded to a depth of >100µm using a diamond tipped dental drill. Detailed information on the sample selections is available in the Excel tables (Ex2 and Ex3). Collagen extraction was carried out using the method of O'Connell *et al.* (2001), briefly summarised here: bone and dentine samples (c. 200mg) were demineralised in refrigerated 0.5M HCl for several weeks and then thoroughly washed in Millipore Alpha Q (MQ) water before gelatinisation in a pH 3 solution of HCl at 70°C for 48 hours. The samples were then filtered, frozen and freeze-dried before being weighed into tin capsules and measured in duplicate using a Thermo Scientific Delta V Advantage isotope ratio mass spectrometer in the Stable Isotope Biogeochemistry Laboratory (SIBL), Durham University. Calibration using internal reference samples (e.g., Glutamic Acid, Glycine, SPAR and Urea) and international reference standards (e.g., USGS 24, USGS 40, IAEA 600, IAEA N1, IAEA N2) determined a standard deviation of $\pm 0.1\text{‰}$

(1 σ) for collagen carbon and nitrogen isotopes. Replicate measurement of collagen samples averaged a standard deviation of $\pm 0.2\text{‰}$ (1 σ).

3.2.2 Incremental dentine collagen

Permanent (n=31) and deciduous (n=16) teeth from 34 individuals (see Tables 2 and 3) were sectioned longitudinally for incremental analysis, with further amelogenin peptide analysis (see 2.2.3) in tooth enamel being carried out on the 16 deciduous teeth for sex estimation. Collagen extraction was carried out using the method of O'Connell et al. (2001), briefly summarised here: dentine was demineralised in 0.5M HCl at 4°C for several weeks and then thoroughly washed in Millipore Alpha Q (MQ) water. Tooth dentine was then cut into 1mm increments, beginning at the root apex, using Method 2 described by Beaumont *et al.* (2013b). Dentine increments were then gelatinised in a pH 3 solution of HCl at 70°C for 48 hours. Samples were then frozen and freeze-dried before being weighed into tin capsules and measured in duplicate using a Thermo Scientific Delta V Advantage isotope ratio mass spectrometer in the Stable Isotope Biogeochemistry Laboratory (SIBL), Durham University. Calibration using internal reference samples (e.g., Glutamic Acid, Glycine, SPAR and Urea) and international reference standards (e.g., USGS 24, USGS 40, IAEA 600, IAEA N1, IAEA N2) determined a standard deviation of $\pm 0.1\text{‰}$ (1 σ) for collagen carbon and nitrogen isotopes. Replicate measurement of collagen samples averaged a standard deviation of $\pm 0.2\text{‰}$ (1 σ).

Table 2. Deciduous teeth selected for incremental dentine analysis at Trinity Burial Ground

Table 3. Permanent teeth selected for incremental dentine analysis at Trinity

3.3 Results and interpretation

The bone and bulk dentine collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data are presented in Table 4 below and in the Excel files Ex2 and Ex3. Nine bone samples and 14 dentine samples did not produce viable collagen. Two additional bulk dentine samples were excluded from further analysis, as collagen quality indicators were not within acceptable standards. Table 4 presents the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ summary data for both bone and bulk dentine, as well as $\delta^{13}\text{C}$ data for enamel apatite yielded through oxygen isotope analysis (for methods and results, see section 4.1).

Table 4. Descriptive statistics for bone and bulk dentine collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and enamel $\delta^{13}\text{C}$ in the sample from Trinity Burial Ground

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ranges at Trinity Burial Ground are varied, representing variation in dietary intake across the population and life stages. The $\delta^{13}\text{C}$ values for Trinity Hull bone collagen, representing later life, range from -20.5‰ to -18.3‰, with a mean of -19.5‰ $\pm$ 0.4 (n = 130, 1sd). This range of values at its lower end is consistent with a diet predominantly reliant on terrestrial C_3 plants (e.g., wheat or barley breads, oats, fruits, vegetables such as potatoes or legumes), dairy, and herbivorous or omnivorous animals within a C_3 food chain (Müldner & Richards 2007). However, the range is not wholly terrestrial, as $\delta^{13}\text{C}$ values greater than -20.0‰ suggest some varying contributions to dietary protein within the population from marine or non-native C_4 plant sources (Mays 1997; Montgomery *et al.* 2009). Bone collagen $\delta^{15}\text{N}$ values ranged from 9.4‰ to 15.8‰ with a mean value of 12.9‰ $\pm$ 0.8 (n = 130, 1sd). Values within this range represent significant intake of higher trophic level protein, either from omnivorous animals or aquatic food chains, which contain more levels than terrestrial food chains and therefore produce higher $\delta^{15}\text{N}$ values (Schoeninger & DeNiro 1984). Together with $\delta^{13}\text{C}$ values, the $\delta^{15}\text{N}$ data suggest – unsurprisingly for a port city – that marine and terrestrial sources of protein were both important contributors to the diets of the majority of people buried at Trinity. Bone collagen from individuals at Trinity Burial Ground was extracted from ribs, which have a rapid turnover rate relative to other skeletal elements, and in healthy adult individuals are believed to reflect time-averaged dietary inputs in approximately the last 5 years prior to death (Jørkov *et al.* 2009).

Bulk dentine values, in contrast to bone, represent diet at the time of tooth formation. For most individuals at Trinity, this would be during the formation of the third permanent molar – corresponding with late childhood through early adulthood. The $\delta^{13}\text{C}$ values for Trinity Hull bulk dentine collagen range from -21.3‰ to -18.9‰ with a mean $\delta^{13}\text{C}$ value of -19.7‰ $\pm$ 0.4 (n = 124, 1sd). When compared to bone collagen $\delta^{13}\text{C}$ data for Trinity (Fig. 1), the distribution of $\delta^{13}\text{C}$ data from bulk dentine generally has lower values. Conversely, bulk dentine $\delta^{15}\text{N}$ values did not differ appreciably from those in bone, yielding a range from 9.4‰ to 15.0‰ with a mean value of 12.8‰ $\pm$ 0.9 (n = 124, 1sd). As dentine and bone values have different formation

ages and time averaging, the differences in data range and clustering visible in Figure 1 may suggest a general difference in diet between youth and more mature adulthood. Where nitrogen ranges are broadly similar between the two tissue types, the trend towards higher carbon values in bone collagen may be indicative of an increase in consumption of marine or C₄ protein between youth and maturity in many adults at Hull. Results for enamel $\delta^{13}\text{C}$ (Fig. 2), which are reflective of the whole diet (i.e. protein, fats, and carbohydrates) during childhood or adolescence, indicate similar dietary trends to dentine collagen data, which are tied to protein source during the same period. Enamel data are consistent with a diet primarily based on C₃ terrestrial resources (range: -15.0‰ to -10.7‰; mean -13.2‰ $\pm$ 0.6, n = 156), with a possible marginal intake of marine or C₄ foods, such as maize or cane sugar. Published comparative datasets for postmedieval British enamel $\delta^{13}\text{C}$ are limited, but data at Trinity Burial Ground align well with recent mean $\delta^{13}\text{C}$ data for enamel (-13.3‰ $\pm$ 1.0, n=15) from postmedieval Manchester (Chidimuro *et al.* 2022).

More broadly for diet, bone collagen data at Hull are consistent with those from other postmedieval cities in southern Britain (Fig. 3), such as Chichester, Coventry, and London (Trickett 2006; Nitsch *et al.* 2010; Dhaliwal *et al.* 2020). Mean data for Trinity is similar to data from all sites, with $\delta^{13}\text{C}$ resembling mean values at Chichester and Coventry, while mean $\delta^{15}\text{N}$ for Hull is broadly comparable to mean values in London at Chelsea and Spitalfields.

Fig. 1: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data from Trinity Burial Ground bone and bulk dentine collagen, analytical error is shown to 2σ .

Fig. 2: Carbonate enamel and dentine collagen $\delta^{13}\text{C}$ data from Trinity Burial Ground

Figure 3. Comparative mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ bone collagen data for British urban sites contemporaneous with Trinity Burial Ground. Data for Chelsea Old Church (n=32) and Holy Trinity Coventry (n=13) is from Trickett (2006), data for Spitalfields (n=92) is from Nitsch et al. (2010), and data for St Michael's Litten, Chichester (n=40) is from Dhaliwal et al. (2020).

There are, however, some differences, which may in part be explained by geography, custom, and economy. Fish would have been eaten widely in England during the 19th century, due to long-standing religious customs around meat eating (Müldner & Richards 2005). However, consumption would potentially have been more extensive in coastal and port cities, due to ease of access. Mean $\delta^{13}\text{C}$ for Coventry, an inland city, and Chichester, a port, are both consistent with primary consumption of terrestrial protein, with some minor contributions from marine protein such as fish, shellfish, marine mammals. The similarity of mean $\delta^{13}\text{C}$ between Chichester, Coventry, and Hull, combined with higher mean $\delta^{15}\text{N}$ at Trinity Burial Ground, suggests that the proportion of dietary protein accounted for by marine foods was likely similar at the three sites, but that dietary protein at Trinity likely came from a higher trophic level, either aquatic or terrestrial. This might mean greater intake of higher trophic-level fish, such as cod, or alternately greater reliance on omnivorous meat sources, such as pork, than at the other cities. Conversely, the lower and narrower range of $\delta^{13}\text{C}$ at Trinity, relative to Chelsea or Spitalfields, is likely to represent greater relative reliance on terrestrial protein at Hull, rather than marine fish typical of the “London diet” of the 19th century (Beaumont *et al.* 2013a). These data place Trinity Burial Ground in an intermediate position for the comparative sites.

Within the population, potential sex differences in dietary intake in adulthood were explored. Summary statistics for bone collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in males and females at Trinity are presented below in Table 5 and Figure 4. Males generally had higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, with females having a wider range of $\delta^{13}\text{C}$ values, and males having a more varied range of $\delta^{15}\text{N}$ values. Individuals of unknown sex had the highest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, largely reflecting the representation of children of breastfeeding age in this group. However, differences between the sexes were not significant for mean bone $\delta^{13}\text{C}$ at $p < .05$, $t(98) = 1.8$, $p = .074$, nor for $\delta^{15}\text{N}$, $t(98) = -0.1$, $p = .918$.

Broadly, this suggests that dietary intake was very similar for adult males and females at Hull, without obvious gendered differences in custom or access.

Table 5. Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ bone collagen by sex at Trinity Burial Ground

The majority of adult data fell within a well-defined cluster. However, two outliers stand out among the data. SK10219, a young adult female, has particularly high $\delta^{13}\text{C}$ for the population, at -18.3‰, with a $\delta^{15}\text{N}$ value (12.6‰) well within the expected range for the population. Nonetheless, it is interesting to note that her high $\delta^{13}\text{C}$ value in bone is not similarly reflected in incremental dentine values covering the contemporaneous period of infancy to young adulthood (cf. Table Ex4). Buried in an earth cut grave, she had obviously defining features which would differentiate her from the rest of the population, aside from potential non-British ancestry flagged by genetic analysis. The dietary shift to a higher consumption of marine protein that she only experienced during the last few years of life could have been a choice or it may indicate she moved shortly before death to a location where such a diet was possible from her childhood home where it was not. It could also indicate a protracted sea voyage during which marine protein such as fish was consumed which she undertook shortly before she arrived and died in Hull. SK20530, a mature adult male also suggested to have potentially non-British ancestry, similarly stood out. His $\delta^{15}\text{N}$ value of 9.4‰, is unusually low for the Trinity population and in England at this time indicates a largely vegetarian diet with limited intake of animal protein. His $\delta^{13}\text{C}$ value of -19.4‰ may reflect a small contribution from C_4 plants such as maize or millet, or a low trophic level marine protein such as shellfish. For example, children buried at the Kilkenny Union Workhouse in Ireland who were eating maize as a famine-relief food have comparable $\delta^{15}\text{N}$ values (Beaumont and Montgomery 2016) as does a child buried in Whitechapel London and thought to be a recent Irish immigrant consuming a largely potato-based diet (Beaumont et al. 2013b).

Fig. 4: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data from Trinity Burial Ground bone collagen by sex, analytical error is shown to 2σ

Sex differences were also considered for the earlier life data represented by bulk dentine analysis, and these are presented in Table 6 and Figure 5. In contrast to bone, some sex differences were observed. For $\delta^{13}\text{C}$, males demonstrated a wider range of values than females, who had higher minimum values and lower maximum values than males. However, differences between males and females for mean dentine $\delta^{13}\text{C}$ were not statistically significant at $p < .05$, $t(107) = 1.5$, $p = .149$. For $\delta^{15}\text{N}$, males also demonstrated a wider overall range of values, with females exhibiting a narrower and higher range. Differences in mean dentine $\delta^{15}\text{N}$ for males ($M = 12.6$, $SD = 0.9$) and females ($M = 13.0$, $SD = 0.8$), $t(107) = 2.1$, $p = .037$ were significant at $p < .05$.

Table 6. Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ dentine collagen by sex at Trinity Burial Ground

Differences in $\delta^{15}\text{N}$ values are distinguishable in Figure 5, with female $\delta^{15}\text{N}$ occupying the upper end of the range and demonstrating the highest values. Males from childhood through to early adulthood appear to have had more variability in diet than their female counterparts, with some males exhibiting $\delta^{13}\text{C}$ values below -21.0 ‰, corresponding to a wholly C_3 /terrestrial-based diet. Both male and female data display a positive correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, suggesting a spectrum of intake from a wholly C_3 terrestrial protein diet to moderate intake of marine and, possibly, C_4 resources. The lower combined $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values seen in some males (Fig. 5) may suggest a heavier reliance on terrestrial plant protein sources.

Fig. 5: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data from Trinity Burial Ground bulk dentine collagen by sex, analytical error is shown to 2σ

There is some evidence for a change in gendered associations and roles in upper class English families between the 18th and 19th centuries, with carving and serving of meat becoming a masculinised task over time, with a subsequent drop in women's consumption of meat (Pace 2020). However, among the working classes this may have owed as much to economic considerations and gendered labour divisions as to culture: Harris (2008) suggests that meat in Victorian labouring families was largely reserved for males who contributed to the family income, with less frequent intake by women and children. Fish was a more common element of the working-class diet, less expensive than meat, and available both fresh and dried, with cod, plaice, eel, mackerel, and oysters being readily available (Broomfield 2007).

Three males stand out from the main cluster of data as having either low $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values in bulk dentine (Table Ex3). SK30970 and SK12832 both had bulk dentine $\delta^{13}\text{C}$ values below the norm, at -21.3‰, with low-moderate $\delta^{15}\text{N}$ values of 11.5‰ and 11.7‰, respectively. SK12832, a middle adult, was buried prone, had multiple markers of skeletal stress, and appears to have been anatomised. SK30970, a prime adult, may have experienced interpersonal violence in his lifetime, with evidence for antemortem fractures of the ribs, face, hands, and lower extremities. SK30865, a middle adult possible male, had an unusually low bulk dentine $\delta^{15}\text{N}$ of 9.4‰ suggesting consumption of predominantly plant based protein. He was buried in a well-preserved, cheaply made coffin, and pathological data suggest poor oral health, joint disease, and trauma (including broken ribs). When considering the isotopic evidence in light of contextual and skeletal evidence, it is likely that the lower $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for this group are reflective of poorer socioeconomic status and reduced access to higher trophic level protein such as meat during childhood and adolescence.

Results of incremental dentine analysis will be reported and discussed in detail in a later supplement, and therefore are presented in summary and without extensive discussion in this report. Summary statistics for the deciduous (n=16) and permanent tooth (n=31) datasets are present in the Excel files as Table Ex4. Mean $\delta^{15}\text{N}$ in the sample was 13.4‰ $\pm$ 1.4 (range 9.4 to 16.7‰), while mean $\delta^{13}\text{C}$ was -19.5‰ $\pm$ 0.6 (range -20.7 to -18.4‰). These values, which include deciduous and permanent teeth reflecting a period from antenatal life to early adulthood, are broadly

comparable to adult bulk dentine values, with a slight offset due to breastfeeding effects in young children included in the dataset. As with bone and bulk dentine data, this range of values is consistent with a population diet predominantly based on terrestrial C₃ plant and animal foods, with some inclusion marine protein (e.g. fish, shellfish, marine birds and eggs) and possibly non-native C₄ plants.

4. Assessing Geographical Origins

4.1 Oxygen isotope analysis

Oxygen incorporated into hydroxyapatite is predominantly derived from ingested fluids, the isotopic composition of which fluctuates due to climatic and environmental variables such as temperature, rainfall, altitude and latitude (Darling & Talbot 2003). Therefore, oxygen isotopes ($\delta^{18}\text{O}$) measured in human tissues are an indirect reflection of the local meteoric water composition (Kohn 1996). Oxygen undergoes metabolic fractionation once ingested. Therefore, regression formulae must be applied to allow comparison with modern drinking water values in order to discern childhood geographical origins and palaeo-climate (Fricke *et al.* 1995; Chenery *et al.* 2012). In addition to this, $\delta^{18}\text{O}$ values can also be influenced by culturally mediated behaviour. The processing (boiling, brewing etc.) of a significant portion of an individual's drinking water before ingestion can result in higher than expected values, and as such, interpretation must be performed with caution (Camin *et al.* 2008; Daux *et al.* 2008; Brettell *et al.* 2012).

4.1.1 Materials and methods

For the enamel apatite analyses, 156 core enamel samples (c.16mg) were cleaned and abraded to a depth of >100 μm using a diamond tipped dental drill. Powdered samples were transferred to Iso Analytical for stable isotope analysis. Samples were weighed into ExetainerTM tubes and flushed with 99.995% helium. Carbonate in the samples was converted to CO₂ by adding phosphoric acid and letting the samples sit overnight for the reaction to occur. Reference materials (IA-R022, NBS-18, and IA-R066) were prepared along the same methods. CO₂ from the samples was then analysed by Continuous Flow-Isotope Ratio Mass Spectrometry (CF-IRMS). The CO₂ was sampled from the ExetainerTM tubes into a continuously flowing He stream using a double holed needle. The CO₂ was resolved on a packed column gas

chromatograph and the resultant chromatographic peak carried forward into the ion source of a Europa Scientific 20-20 IRMS where it was ionized and accelerated. Gas species of different mass were separated in a magnetic field then simultaneously measured using a Faraday cup collector array to measure the isotopomers of CO₂ at m/z 44, 45, and 46. The phosphoric acid used for digestion was prepared in accordance with Coplen *et al.* (1983) and was injected through the septum into the vials. 20% of samples were run in duplicate.

4.1.2 Results and interpretation

To assess the impact of any diagenetic alteration of the tooth enamel, the carbonate content of the samples was evaluated. Usually, low carbonate percentages indicate recrystallisation, while high carbonate percentages suggest accumulation of exogenous sedimentary carbonate from the burial environment (Koch *et al.* 1997). The tooth enamel carbonate weight percentages in the Trinity sample (mean = 5.0 ± 0.8%, 1σ) were within the expected range of 2% to 6% for mammalian tooth enamel (Xu *et al.* 2012; Skippington *et al.* 2019), indicating that the samples were not significantly altered and represent *in vivo* values.

The δ¹⁸O data for the Trinity Hull sample are summarised in Table 7 and in detail in Figure 6 and the Excel table Ex5; for consistency the δ¹⁸O values are presented as calculated V-SMOW phosphate values, except where otherwise noted. The δ¹⁸O values range from 11.2‰ to 18.4‰ with a mean value of 15.1‰ ± 0.8 (n=156, 1sd). There were no statistical differences between the sexes.

Table 7. Summary statistics for enamel δ¹³C and δ¹⁸O data at Trinity Burial Ground. Calculations for V-SMOW carbonate are taken from Sharp (2007), V-SMOW phosphate from Chenery *et al.*, (2012), and precipitation values from Daux (2008), Eq. 6., V-SMOW phosphate from Chenery *et al.*, (2012), and precipitation values from Daux (2008), Eq. 6.

However, a large proportion of the data from sampled teeth fall outside the expected δ¹⁸O range for Hull, and indeed outside of expected values for Britain (based on measured tooth enamel of archaeological remains deemed to be local; Evans *et al.* (2012)). Phosphate oxygen (δ¹⁸O_{V-SMOW}) isotope values from human tooth enamel for Britain would be expected to fall between 16.3 and 19.1‰, while drinking water values calculated from human tooth enamel for Britain are expected to fall between

-9.0 and -4.0‰. For Hull, and much of eastern coastal and central England, drinking water would be expected to range from -8.0 to -7.5‰ or a broader range of -8.5 to -7.5‰ if the “local” area was more generously expanded to include the areas west of Hull (such as Selby) which have lower documented drinking water values (Darling *et al.* 2003; Evans *et al.* 2012). Only a scant 12/156 individuals (7.7%) fall within the range of expected enamel phosphate oxygen values for humans of British origin, while for converted drinking water values, only 3/156 (1.9%) of sampled individuals have enamel data consistent with the estimated local range (or 8/156 (5.1%) if we include a more generous -8.5 to -7.5‰ regional range). When compared to phosphate oxygen isotope data from other contemporaneous urban sites in London and Coventry (Table 8), oxygen isotope data at Trinity stands out as having unusually low $\delta^{18}\text{O}$ values for the period, in terms of both range and mean values, even when accounting for differences in geography.

Table 8. Comparative phosphate $\delta^{18}\text{O}_p$ data for sites contemporaneous with Trinity

It is exceedingly unlikely that the overwhelming majority of individuals buried at Trinity originated not only outside of the local area, but outside of Britain itself. A recent study of contemporaneous 19th-century individuals from Fewston in North Yorkshire also found a lower than expected $\delta^{18}\text{O}$ range of 15.9 to 16.5‰ amongst the named Fewston-born individuals (Gowland *et al.* 2023). Therefore, other explanations must be sought for the anomalously low $\delta^{18}\text{O}$ values of the Trinity sample. Higher $\delta^{18}\text{O}$ values are, in many respects, fairly straightforward to explain, as increased evaporative processes from either natural or anthropogenic causes (such as brewing or stewing of beverages or fluid foods) will amplify fractionation in consumed liquids and produce higher human $\delta^{18}\text{O}$ values than would be expected for unmodified water intake (Brettell *et al.* 2012). However, values in a lower-than-expected range are more difficult to explain, outside of geographic mobility.

Changes to climate over time, particularly to temperature and precipitation, are one possible explanation. A climatic cold shift would lead to increased representation of ^{16}O in rainfall at higher latitudes, and therefore lower $\delta^{18}\text{O}$ values in drinking water integrated into the body pool of individuals buried at Trinity. The use-life of the cemetery falls within the well-known cooling phenomenon arising from the Little Ice

Age (15th-mid 19th centuries), associated with serial volcanic activity and affecting both summer and winter temperatures in the northern hemisphere (Wanner *et al.* 2022). Furthermore, a sequence of volcanic eruptions in the early decades of the 19th century has been identified as resulting in a period of particularly cold years in Europe (Reichen *et al.* 2022), which may have coincided with key formative periods recorded in the tooth enamel of many of the adults buried at Trinity.

Metabolic disease is an additional factor that may produce low $\delta^{18}\text{O}$ values, as disturbances to metabolism may impact on isotopic fractionation. In particular, anaemias have been shown to reduce $\delta^{18}\text{O}$ values, relative to healthy controls, in both archaeological remains and experimental animal studies (Reitsema & Crews 2011; Carroll *et al.* 2018). The heavy burden of metabolic disease present in the skeletal population at Trinity, including anaemias, prompts serious consideration of pathology as a contributor to the unusually low oxygen isotope values at this site. However, the unusual range of $\delta^{18}\text{O}$ values at Trinity are likely multifactorial in cause, arising from a combination of climatic, pathological, or other factors currently unidentified. Nineteenth century sites comparable in isotopic analytical scope to Trinity are lacking, and further research is needed to characterise relationships between climate, metabolism, and isotopic norms for 19th century British populations.

Due to the anomalous shift in the population baseline away from published ranges, it is not advisable to use conventional map-based methods to identify non-locals in the population. Consequently, in order to create a site-sensitive measure of local origin, a limit of two standard deviations from the mean (13.5-16.7‰) was used to define the local range (Fig. 6). While the majority of $\delta^{18}\text{O}$ data is predictably constrained within one standard deviation from the mean, a coherent data cluster is continuous to two standard deviations. Several individuals fall outside of this two SD range (Table 9). Three of these, SK11298, SK11378, and SK21710, may be of local origin, as they are only just beyond the two standard deviation upper boundary, are reasonably continuous with the main cluster of data, and are not appreciably different in value from individuals whose data falls within the boundary. Furthermore, in the case of SK11298, the tooth sampled is a deciduous second molar from a young child, and the elevated $\delta^{18}\text{O}$ values observed are likely to relate to

breastfeeding during the period of tooth formation, which is known to result from fractionation in human milk, an infant's primary water source (Smith *et al.* 2022).

Fig. 6: $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values from the Trinity Burial Ground individuals. The dashed boxes indicate the expected local isotope range at Hull, based on 1σ (black) and 2σ (grey) from the mean.

Table 9: Enamel $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ data outliers at Trinity Hull

Two other individuals are quite distinct from the broader population. SK11237, a middle adult male, has a structural carbonate $\delta^{13}\text{C}$ value that at -10.7‰ is high for the Hull population, suggesting his diet contained C_4 or marine food sources, alongside an exceedingly low $\delta^{18}\text{O}$ value. The $\delta^{18}\text{O}$ value is low enough to suggest an Arctic-type climate, being similar to values observed in human enamel for Inuit Greenlanders (Fricke *et al.* 1995; Daux *et al.* 2008). However, as the larger population's absolute range is shifted lower than expected, it may also be that this individual's values are artificially low and specific geographic origin inferences should be approached with caution. Nonetheless, this combination of isotope data may point to origins at high latitude far to the north of Britain, in which case the high $\delta^{13}\text{C}$ will reflect some marine protein consumption. Alternately, if the high $\delta^{13}\text{C}$ derives from consumption of C_4 plants such as millet or maize, the low $\delta^{18}\text{O}$ may be indicating origins to the east in continental Eurasia, or possibly to the west in the Americas, where such crops can grow. A third, less likely, possibility is that SK11237 originated at high altitude in a region such as the Alps, where consumption of C_4 crops such as sorghum and millet has been recorded (Paladin *et al.* 2020, Fiorin *et al.* 2023). Regardless, based on oxygen data it is unlikely that this individual spent his youth in Hull, or indeed in the local region.

In contrast, the enamel $\delta^{18}\text{O}$ value of SK14148 – a middle adult possible male – was a standout at the high end of the scale, clearly distinct from the main population. This individual had a drinking water value equivalent to -5.4‰ (phosphate $\delta^{18}\text{O}$ value of 18.4‰), falling well outside the expected local drinking water value of -8 to -7.5‰ (Darling *et al.* 2003; Evans *et al.* 2012). As previously discussed, $\delta^{18}\text{O}$ values may be increased through cultural modification of drinking water or liquid foods. However, in this particular instance, due to the shift of the general population towards a lower absolute $\delta^{18}\text{O}$ range, the higher values are striking. Taken at face value, such $\delta^{18}\text{O}$ values are consistent with a childhood spent in the western fringes of Britain, such as Cornwall, southwest Wales, or the Hebrides, or perhaps western Ireland or Atlantic coastal regions of western Europe (Darling *et al.* 2003). Due to the anomalous nature of $\delta^{18}\text{O}$ at Trinity Burial Ground, these data need to be interpreted in concert

with other mobility indicators to achieve any confidence in suggested places of origin.

4.2 Strontium isotope analysis

The isotope analysis of strontium ($^{87}\text{Sr}/^{86}\text{Sr}$) can provide information on the movements of past populations by identifying individuals who have different isotopic compositions to the geographic area in which they were found (Evans *et al.* 2012). Strontium isotopes can provide a direct link between an individual and their geographic origin as they are ultimately derived from the local geology. The strontium isotope ($^{87}\text{Sr}/^{86}\text{Sr}$) characteristics of plants and local animals in different regions vary depending on the relative contributions of strontium from different underlying rocks and the presence or absence of superficial drift deposits (Bentley 2006). Strontium is released into the environment through weathering and dissolution processes into the overlying soils, plants, and animals (Bentley 2006). The strontium isotope ($^{87}\text{Sr}/^{86}\text{Sr}$) characteristics of plants and local animals in different regions vary depending on the relative contributions of strontium from different underlying rocks and the presence or absence of superficial drift deposits (Bentley 2006). Strontium is released into the environment through weathering and dissolution processes into the overlying soils, plants, and animals (Bentley 2006). Strontium becomes incorporated into the hydroxyapatite lattice of human bone through ingestion of food and water (Montgomery 2010). Assuming that the majority of a population's food and drink is sourced locally, the strontium isotope composition in human skeletal material should then reflect the bioavailable strontium isotope ratios in their region of origin (Montgomery *et al.* 2007; Montgomery 2010).

4.2.1 Materials and methods

One hundred and fifty-seven core enamel samples (c.5-25 mg) were prepared for strontium isotope analysis using column chemistry methods outlined in Font *et al.* (2008) at the Arthur Holmes Isotope Geology Laboratory (AHIGL), Durham University. In brief, samples were digested overnight in 3M HNO_3 on a hotplate at 100°C before being loaded onto cleaned and preconditioned columns containing Eichrom strontium-specific resin. A purified Sr fraction was eluted from the column in $400\mu\text{L H}_2\text{O}$ and acidified with 15.5M HNO_3 to yield a 3% HNO_3 solution.

Following Sr purification, the size of the ^{86}Sr beam was tested for each sample to derive a dilution factor so that each sample yielded a beam size of approximately 30V ^{88}Sr to match the intensity of the isotopic reference material, NBS987. Samples were aspirated using an ESI PFA-50 nebuliser coupled to a Glass Expansion Cinnabar micro-cyclonic spray chamber. Sr isotopes were measured using a static multi-collection routine with each measurement comprising a single block of 50 cycles with an integration time of 4s per cycle (total analysis time ~3.5mins). Instrumental mass bias was corrected for using an $^{88}\text{Sr}/^{86}\text{Sr}$ ratio of 8.375209 (the reciprocal of the more commonly used $^{86}\text{Sr}/^{88}\text{Sr}$ ratio of 0.1194) and an exponential law. Corrections for isobaric interferences from Rb and Kr on ^{87}Sr and ^{86}Sr were performed using ^{85}Rb and ^{83}Kr as the monitor masses but were insignificant. In all samples the ^{85}Rb intensity was < 1mV with an $^{85}\text{Rb}/^{88}\text{Sr}$ ratio of < 0.0003 (average 0.0001). ^{83}Kr was between 0.32 and 0.39mV in all samples. Samples were analysed during two analytical sessions during which the average $^{87}\text{Sr}/^{86}\text{Sr}$ ratio and reproducibility for the international isotope reference material NBS987 was 0.710248 ± 0.000018 (2σ ; $n=11$) for the first session and 0.710255 ± 0.000017 (2σ ; $n=11$) for the second session. Two samples were run in both sessions to ensure reproducibility, producing values that were within error of each other. Maximum error based on internal precision of individual analysis and analytical reproducibility of the reference material is considered to be 0.000013 (2σ). Sr isotope data for samples is normalised to an 'accepted' value for NBS987 of 0.71024.

4.2.2 Results and interpretation

The Trinity Hull strontium isotope data are summarised in Table 10 and presented in Figure 7 and 8 alongside comparative data from the Medieval site Hull Magistrates Court (Roberts *et al.* 2013) and 19th century Fewston, North Yorkshire (Gowland *et al.*, 2023). Detailed results are found in the Excel table Ex6. There is currently no published strontium isotope data from 19th century burials in Hull. However, as strontium is predominantly derived from bedrock, there is unlikely to be any changes to regional strontium isotope ratios over archaeological timescales although there may well be changes in from where and from how far food is procured by a growing city such as Hull particularly with the arrival of the railway. Nonetheless, data from any archaeological human samples recovered from the region make useful

comparators. The strontium isotope ratios from the Trinity Hull population range from 0.708577 to 0.713487 (mean = 0.709514 ± 0.000708), which is similar to data obtained from other Hull populations (Roberts *et al.* 2013), and within the range expected for Britain (Evans *et al.* 2010).

Table 10: Summary of the strontium isotope ratios from the Trinity Hull tooth enamel

Hull is situated on a bedrock of Cretaceous Chalk overlain by Jurassic clays, limestone, and sandstones (Gaunt *et al.* 1992). The average bioavailable strontium isotope range for regions with this type of geology has been estimated from plants to lie between 0.7074 and 0.7097 (Evans *et al.* 2010). It is expected that people who source most of their food and drink from within this region would have strontium isotope ratios close to this range. As can be seen in Figure 7, most of the Trinity Hull individuals do have strontium isotope ratios consistent with a childhood spent in Hull. Although the strontium isotope ratios observed in these Trinity Hull individuals are common in burials from England, they are also common in regions across Europe, and therefore do not provide a conclusive indication of local origins. Additionally, there are 33 individuals (22 male, 9 female and 2 indeterminate), with strontium isotope ratios above this expected range. Although more males than females have strontium isotope values outside the expected range for Hull, a Kruskal-Wallis H test showed that there was no significant difference in strontium isotope ratios between the sexes ($H(2) = 1.0232$, $p = 0.31176$).

Despite these individuals plotting outside the expected strontium isotope range for Hull, they plot closely with the Medieval population from Hull Magistrates Court. However, higher biosphere strontium isotope ratios are found elsewhere in Yorkshire on the older Permo-Triassic and Carboniferous rocks to the north and east. For example, a recent study at Fewston in North Yorkshire of contemporaneous 19th-century individuals found a strontium isotope range of 0.7102 to 0.7115 amongst named individuals originating on the Carboniferous sandstone bedrock at Fewston (Gowland *et al.* 2023) which would encompass the majority of individuals in the Trinity Hull population who are not local to Hull. However, childhood origins outside of Yorkshire cannot be excluded. There is one clear outlier (SK14782) with a strontium isotope ratio notably higher than the rest of the Trinity Hull population.

SK14782 exhibits a tooth enamel strontium isotope ratio of 0.71349 which is too high to originate from within Yorkshire. High strontium isotope ratios such as these are more commonly found in regions with granitic or older Palaeozoic geology, such as western and northern England, Wales, and Scotland. For example, such a value is commonly found amongst individuals from the Devonian Old Red sandstone of Hereford (Evans et al. 2012).

Figure 7: Trinity Hull strontium and oxygen isotope data alongside regional comparative data (Roberts et al., 2012). The horizontal dotted lines represent the bioavailable strontium isotope range for Hull (Evans et al., 2018). The grey box represents the local oxygen range defined as 2sd from the Trinity Hull population mean. The analytical error for $^{87}\text{Sr}/^{86}\text{Sr}$ is within the symbol.

The tooth enamel strontium concentrations from the Trinity Hull individuals range from 26 to 258 ppm (mean = 96.0 ± 43.2 ppm), all these values except for those of two female individuals, SK20157 and SK20419, are within the strontium concentration range expected for British archaeological populations (Evans et al. 2012). Individuals SK20157 and SK20419 had exceedingly high strontium concentrations of 251 ppm and 258 ppm respectively. Although these are outside the range expected in archaeological population from England which tend not to exceed ~ 150 ppm, modern British studies have recorded tooth enamel concentrations as high as 262 ppm (Brown et al. 2004). Strontium concentrations in humans can be influenced by several different factors such as calcium intake, geology, atmospheric deposition (dust, sea spray, precipitation etc.), anthropogenic influences (e.g., fertilizer) and trophic level (Burton & Wright 1995; Montgomery 2002; Maurer et al. 2012; Lazzerini et al. 2021). Therefore, the unusually high strontium concentrations observed in these two individuals may indicate a predominantly plant-based diet, low dietary calcium intake or residency in a region with unusual geology. However, archaeological humans from Scotland do have comparably high strontium concentrations, particularly those from Atlantic-facing islands such as the Western and Northern Isles (Montgomery 2010). Here, these high strontium concentrations are coupled with a strontium isotope ratio that hovers around the seawater value of ~0.7092 and this is the case for these two individuals plus nine others at Hull (SK11919, SK14676, SK14856, SK15374, SK20759, SK21405, SK30229, SK30855, SK30977) who form a long tail to the right on Figure 8. In Britain, such a combination

of strontium compositions is strongly indicative of humans who cling to the coast in a seaboard or island population dominated by sea-splash, seaspray, marine shell sand or use seaweed as fertilizer, food, or fodder (Montgomery 2010). There are other populations beyond the shores of Scotland who may fit such a profile: for example, similarly high values have been observed in Roman populations from France and Spain (Moore 2019).

Figure 8: Trinity Hull strontium ratio and concentration data alongside comparative coastal population data (Montgomery and Evans 2003; Toolis 2008). The horizontal dotted lines represent the bioavailable strontium isotope range for Hull (Evans et al., 2018). The analytical error for $^{87}\text{Sr}/^{86}\text{Sr}$ is within the symbol.

4.3 Sulphur isotope analysis

The utility of the isotopic analysis of sulphur ($\delta^{34}\text{S}$) is based on the principle that the $\delta^{34}\text{S}$ value in any given location will be distinctive to that local area (Nehlich 2015). These $\delta^{34}\text{S}$ values may remain uniform over large geographic areas, or they may vary significantly over smaller distances (Krouse 1991). Each area's $\delta^{34}\text{S}$ profile ultimately derives from the various sulphur contributions from that locale's atmosphere, hydrosphere, lithosphere, and biosphere (Peterson & Fry 1987). The bioavailable sulphur of a region, with its distinctive $\delta^{34}\text{S}$ value, is ultimately taken up by plants and used to synthesize amino acids which are, in turn, consumed by animals higher up in the food chain and incorporated into their bodily tissues, including their bone collagen. Studies suggest that there is little meaningful fractionation of sulphur as it ascends the food chain (Richards *et al.* 2003; McCutchan Jr *et al.* 2003). Therefore, the $\delta^{34}\text{S}$ values of an individual's tooth and bone collagen act as a record of the environment where that individual sourced their food. Whilst there is still much we do not know about sulphur systematics in humans and whether biospheres are susceptible to industrial atmospheric pollution via processes such as acid rain and coal burning, this data may be useful in gaining insight into issues such as mobility and can be especially helpful in revealing 'non-local' or 'migratory' individuals when comparing the $\delta^{34}\text{S}$ values of a large number of contemporaneous individuals from a site.

4.3.1 Materials and methods

Rib bone samples from 15 individuals were cleaned and abraded to a depth of >100 μm using a tungsten carbide dental drill. Collagen extraction was carried out using the O'Connell et al. (2001) method, briefly summarised here: bone samples (c. 400 mg) were demineralised in refrigerated 0.5 M HCl for several weeks and then thoroughly washed in Millipore Alpha Q (MQ) water before gelatinisation in a pH 3 solution of HCl at 70°C for 48 hours. The samples were then filtered, frozen and freeze-dried before being weighed into tin capsules using a tungsten activator. Stable isotope measurements were performed in the Stable Isotope Biogeochemistry Laboratory (SIBL) at Durham University using a Costech Elemental Analyser (ECS 4010) coupled to a Thermo Scientific Delta V Plus Advantage isotope ratio mass spectrometer. Sulphur isotope ratios are reported in standard delta (δ) notation in per mil (‰) relative to the VCDT scale. Correcting of $\delta^{34}\text{S}$ was performed using four international standards (IAEA-S-1, IAEA-S-2, IAEA-S-3, NBS 127): this provided a linear range in $\delta^{34}\text{S}$ between -32.49 ‰ and $+22.62$ ‰. Analytical uncertainty of $\delta^{34}\text{S}$ is typically ± 0.2 ‰ for replicate analyses of the international standards. Total sulphur is determined as part of the isotopic analysis (i.e., total peak area) using an internal standard, sulphanilamide (18.6196 % sulphur).

4.3.2 Results and interpretation

The 15 $\delta^{34}\text{S}$ values from the Trinity Hull individuals ranged from -4.3 ‰ to 6.2 ‰ with a mean value of 3.3 ± 5.0 ‰ (2sd, $n=15$). This data is presented in Table 11 and Figure 9. Most of these individuals form a tight cluster between 2.4 ‰ and 4.4 ‰, with a mean value of 3.7 ± 1.2 ‰ (2sd, $n=11$). This cluster includes all four of the young children (SK11298, SK21177, SK21277, and SK22268) whose sulphur isotopes were analysed. Additionally, this cluster includes [REDACTED] who lived and worked in Hull during the period represented in his rib collagen and therefore also likely reflects the 'local' $\delta^{34}\text{S}$ value.

As all the sulphur isotope data comes from rib collagen, it reflects the last few years of the individual's life and so, with the exception of the children, it does not generally reflect childhood data (Wild *et al.* 2000). The adults who cluster in the 'local' range may be originally from Hull or may be from elsewhere but have spent several years

prior to their deaths in Hull, or in a location with similar sulphur isotope ratios. The range of this cluster is not consistent with what would be expected for the local area; due to its proximity to the coast, on a tidal estuary, Hull would be expected to have higher $\delta^{34}\text{S}$ values, i.e., over 10‰ due to the influence of oceanic sulphur (Nehlich 2015). Comparative sulphur isotope data in this region is limited, however, the majority of prehistoric (and therefore pre-industrial) Bronze and Iron Age individuals from chalk regions in east Yorkshire have $\delta^{34}\text{S}$ values above 10‰ (Parker Pearson *et al.* 2019: 508–35, digital appendices 9 & 10; Jay & Montgomery 2019). These sites are not within the coastal range and none of the individuals have evidence for the consumption of marine foods, but their location on chalk appears to explain the higher $\delta^{34}\text{S}$ values in both humans and their domestic herbivores such as cattle

Fig. 9: $\delta^{34}\text{S}$ compared to $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data from Trinity Burial Ground bulk rib collagen by sex, analytical error is shown to 2σ .

Table 11: Bone collagen $\delta^{34}\text{S}$ data from Trinity Hull

It might therefore be reasonable to suggest that individuals who were local to Hull towards the ends of their lives should have similar, if not higher, $\delta^{34}\text{S}$ values in their bone collagen. However, coal, oil, gas and sulphide ores have been measured with $\delta^{34}\text{S}$ values as low as -40‰ and high amounts of pollutants from the industrialised city may be suppressing higher oceanic $\delta^{34}\text{S}$ values in the bioavailable sulphur. While not in keeping with a priori assumptions, as with the oxygen isotope ratios presented above, the core cluster of the 11 individuals with similar $\delta^{34}\text{S}$ values, including the four children cannot reasonably be excluded from the 'local' $\delta^{34}\text{S}$ range, and should be assumed to be local. While this is not a large enough sample to consider this to be statistically significant, it is likely that these individuals spent their entire lives living locally to Hull and were not migrants to the area. It is also of note that their values are lower than the modern plants collected from inland east Yorkshire (BGS Biosphere Isotope Domain online map, Evans *et al.* 2018) which are, in turn, much lower than prehistoric and pre-industrial inhabitants of the region. As atmospheric pollution was considerably higher in the 19th century than today this could be indicating that the people in Hull were more severely impacted by industrial pollutant sulphur than either prehistoric or modern populations. More work is needed on this subject of temporal change in regional isotope profiles.

With the exception of SK 11730, the remaining three individuals which not part of the core cluster (Sk11378, Sk11730 and Sk30811) are not sufficiently different from the mean to be considered true outliers. While their absolute $\delta^{34}\text{S}$ values are too low to indicate an origin on chalk or in a coastal region, as noted with the core group if their $\delta^{34}\text{S}$ values are being altered by pollutant sulphur then it is not possible to rule out an origin in these regions, either from Hull or other similar locations. SK11730 has a much lower $\delta^{34}\text{S}$ value of -4.3‰ compared to the rest of the individuals sampled, which is not consistent with the expected $\delta^{34}\text{S}$ values for either coastal values or chalk (Nehlich 2015). Values in this range could be consistent with Jurassic clays, which are found within 50 km of Hull. Alternately, such a value would be compatible with origins in river valleys or floodplains, which may include that of the river Humber and its tributaries but there is currently no data from here to support this (Nehlich *et al.* 2011; Jay & Montgomery 2019).

4.4 Lead isotope analysis

In principle the isotope ratios of lead in pre-industrial societies reflect those of the local underlying geology (Erel *et al.* 1994; Montgomery *et al.* 2000; Komárek *et al.* 2008). Societies with metallurgical technologies however tend to exhibit elevated skeletal lead concentrations, which are accompanied by a clustering of lead isotope ratios which has been termed ‘cultural focussing’ (Montgomery *et al.* 2010). These homogenised lead isotope ratios tend to reflect the predominant lead sources available to that particular population (Montgomery *et al.* 2010; Millard *et al.* 2014). This ‘cultural focusing’ shifts skeletal lead isotope ratios away from geogenic lead congruent with geographical provenance, towards isotope ratios converging around the dominant anthropogenic ore sources utilised in a particular cultural sphere (Gulson *et al.* 1997; Kamenov & Gulson 2014). These anthropogenic skeletal lead isotope ratios are therefore indicative of socio-cultural provenance (Carlson 1996; Montgomery *et al.* 2005) and can be effective in differentiating between cultural groups within skeletal populations, as individuals exposed to foreign lead sources should stand out from those exposed to local lead, making migrants from other countries relatively easy to identify (Montgomery 2002). Furthermore, lead concentrations in tooth enamel provide a measure of childhood exposure to environmental lead pollution and can offer information beyond simply the extent of an individual’s lead burden. Being able to distinguish between geogenic and anthropogenic lead exposure in this way offers insights into the technological capabilities and/or technologies actively utilised by a particular population. It is likely that populations exhibiting predominantly geogenic lead isotope ratios do not use lead or lead products to the extent necessary for the acquisition of high, anthropogenic lead concentrations. To date studies utilising lead isotope ratios have shown that human lead burdens below 0.8 ppm in conjunction with non-ore lead isotope ratios appear to represent geogenic lead exposure and values over this are considered anthropogenic (Montgomery *et al.* 2010; Millard *et al.* 2014; Shaw *et al.* 2016; Moore *et al.* 2020).

3.4.1 Materials and methods

Tooth enamel (n=16) was cleaned prior to sampling, using a clean diamond tipped dental burr. Core enamel chips (c.20-40 mg) were removed from the teeth using a

clean diamond tipped circular saw attachment. All dentine and remaining dirt were removed using the diamond tipped burr. Tools were cleaned between each sample by being ultrasonicated in 2% Decon for five minutes and were then rinsed three times with ultrapure water.

Enamel was prepared for lead (Pb) isotope analysis using column chemistry methods outlined in Font et al. (2008) at the Arthur Holmes Isotope Geology Laboratory (AHIGL), Durham University. The enamel samples were digested overnight in 3M HNO₃ (nitric acid) on a hotplate at 100°C before being loaded onto cleaned and preconditioned columns containing Eichrom strontium-specific resin. 500µL of 3M HNO₃ was washed through the column to remove any enamel matrix and any additional elements. A purified Sr fraction was first collected from the column in 400mL H₂O to be analysed for Sr isotopes. A purified Pb fraction was collected from the same Eichrom Sr-specific resin column used to collect the Sr fraction, using 400µL 8M HCl. The 8M HCl Pb fraction solution was then dried down and taken up in 500µL 3% HNO₃ for on the day of analysis. Thallium (Tl) was added to each sample in order to correct Pb isotope ratios for the effects of instrumental mass bias. Each Pb sample was tested for Pb concentration and subsequently spiked with a Tl standard to achieve a Pb/Tl ratio of ~12. The ratios outlined in Baker *et al.* (2004) were used to correct the data. The samples were analysed on a Neptune Plus with a high sensitivity sample introduction system comprising an Aridus II desolvating nebuliser and the high sensitivity 'X' skimmer cone. This combination provides an order of magnitude increase in signal size to ~1000V total Pb signal per ppm of Pb in the sample aliquot. a typical Pb isotope measurement comprised a static multi-collection routine, with ²⁰⁵Tl in the axial detector, of 1 block of 50 cycles with an integration time of 4sec per cycle. Two sessions were run, one for higher concentration samples and one for lower concentration samples. The standard NBS981, diluted to 50ppb was used in the former and at a dilution of 5ppb in the later. Mean isotope ratios of the standards were recorded across both sessions of and were very similar. The mean isotope ratios were: ²⁰⁶Pb/²⁰⁴Pb = 16.941601, ²⁰⁷Pb/²⁰⁴Pb = 15.49943, ²⁰⁸Pb/²⁰⁶Pb = 0.914902, and ²⁰⁸Pb/²⁰⁶Pb = 2.167787.

4.4.2 Results and interpretation

Full lead isotope results are found in the Excel table Ex7. The 16 individuals selected for lead isotope analysis had lead concentrations ranging from 0.34 to 68.1 ppm (median = 17.3 ppm, IQR = 21.2 ppm). Apart from two individuals (SK21582 and SK30229) with exceedingly high values, the lead concentrations observed in these 16 Trinity Hull individuals are consistent with those seen in other 19th-century British populations (see Fig. 10). Elevated lead concentrations such as these are indicative of exposure to anthropogenic lead sources (≥ 0.9 ppm) and suggest an early childhood spent in a polluted environment (Montgomery *et al.* 2010; Millard *et al.* 2014). A study of lead concentrations in modern tooth enamel concluded that lead levels ranging between 2 to 10 ppm signifies high levels of exposure (Gulson & Wilson 1994). As can be seen in Fig. 10 most of the Trinity Hull individuals plot within or above this range, it is possible that these 14 individuals may have suffered the ill effects of lead poisoning (Moore *et al.*, 2021), discussed further in section 5.

It is common for the lead isotope ratios of a population to become increasingly homogenous the higher their lead concentrations become. This phenomenon is termed 'cultural focusing' (Montgomery 2002; Montgomery *et al.* 2005) and has been observed in archaeological populations across Europe (Montgomery *et al.* 2010; Shaw *et al.* 2016; Moore 2019). Generally, post-prehistoric human lead isotope ratios are culturally focused, clustering together in a narrow range reflecting the lead ore sources used by a population (Montgomery 2002; Montgomery *et al.* 2005, 2010). As can be seen in Figure 10, all the Trinity Hull individuals exhibiting anthropogenic lead concentrations cluster within the $^{207}\text{Pb}/^{206}\text{Pb}$ isotope range expected for human archaeological tooth enamel from England and Wales, i.e. native lead deriving from English and Welsh ore sources. Interestingly, two individuals sampled for lead isotopes (SK11971 and SK12832) have very low lead concentrations below 0.7 ppm. Lead concentrations this low are uncommon in 19th-century populations in England and are more commonly seen in populations preceding the Roman period. Thus, these low concentrations indicate very limited exposure to anthropogenic lead sources and suggest childhood origins in a region with very little environmental pollution (i.e., rural locations or non-industrial regions).

Fig. 10: Tooth enamel lead concentrations (ppm) against $^{207}\text{Pb}/^{206}\text{Pb}$ isotope ratios. The black dashed line indicates the threshold for anthropogenic exposure (Millard et al., 2014) and the range delineated by the red dotted lines indicates the range for high exposure (Gulson and Wilson, 1994). Comparative tooth enamel data for British burials taken from Moore et al., (2020); Shaw et al., (2016); Millard et al., (2014) and Montgomery et al., (2010). England/Wales $^{207}\text{Pb}/^{206}\text{Pb}$ range of 0.845 to 0.849 taken from Montgomery (2002).

A comparison of the Trinity Hull individuals with data from British lead ore (Haggerty et al. 1996; Rohl 1996) demonstrates how the 13 individuals with lead concentrations above 0.9 ppm cluster tightly with contemporaneous British populations within the English lead ore isotope field (see Fig. 11). For these individuals, their lead isotope ratios strongly suggest childhoods spent in England. However, it is important to note that lead ore fields tend to exhibit isotope ratios that spread over a wide range of values, meaning that there is often overlap between lead ore fields from different countries.

Of the 16 individuals analysed there were three outliers, SK11971, SK12832 and SK21582. The first two individuals, SK11971 and SK12832, plot within the expected lead ore isotope field for England but to the left of the main Trinity Hull cluster (see Fig. 11). Unlike the central cluster of Trinity Hull individuals, SK11971 and SK12832 have lead concentrations below 0.9 ppm, indicative of minimal exposure to lead pollution. Thus, the low $^{206}\text{Pb}/^{204}\text{Pb}$ lead isotope values exhibited by these two individuals represent natural, geogenic sources of lead rather than anthropogenic lead ore. While childhood origins in Britain are entirely possible for these two individuals given the greater variability in lead from bedrock and soils as opposed to lead ore, it is unusual to see someone from a 19th century population with such low lead concentrations.

The third outlier, named individual [REDACTED] (SK21582), has high $^{206}\text{Pb}/^{204}\text{Pb}$, $^{207}\text{Pb}/^{204}\text{Pb}$ and $^{208}\text{Pb}/^{204}\text{Pb}$ isotope values that plot on the very edge of the British ore field, to the right of the distinct cluster of 19th century British individuals (Figure 11). It is highly unlikely, therefore that anyone could obtain such an isotope ratio through exposure to British ore. It is possible that a geological non-ore source may exist in Britain that matches his lead isotope ratio, but it would be highly unlikely to produce the high level of lead exposure recorded in his tooth enamel. If this was not a known individual with a historically documented family history, a childhood spent

outside of Britain would be indicated by this data. One possibility is that Frank spent a significant part of his childhood abroad during tooth formation, i.e., he travelled to the lead source. Alternatively, it is also feasible that the source of the lead in his teeth came from an imported artefact or product, and he was regularly exposed to that source during enamel formation.

Fig. 11: $^{208}\text{Pb}/^{204}\text{Pb}$ and $^{207}\text{Pb}/^{204}\text{Pb}$ against $^{206}\text{Pb}/^{204}\text{Pb}$ showing how tooth enamel samples from England group with regional lead ore, the Stacey and Kramers (1975) growth curve is included for reference. Comparative tooth enamel data from Moore et al., (2020); Shaw et al., (2016); Millard et al., (2014) and Montgomery et al., (2010). British lead ore data from Rohl (1996).

General trends in European tooth enamel lead isotope ratios have been shown to relate to major orogenic events, during which lead ores are often formed (Blichert-Toft *et al.* 2016; Evans *et al.* 2018; Moore 2019). Individuals from regions with older, Hercynian, Cambrian or Precambrian orogeny (e.g., Britain, France, Germany and Scandinavia) tend to have lower $^{208}\text{Pb}/^{206}\text{Pb}$ ratios than individuals from regions with younger, Alpine orogeny (e.g., Spain, Italy and Romania) (Muechez *et al.* 2005; Moore 2019). Fig. 12 clearly demonstrates this trend with the tooth enamel samples from Italy, Lebanon, Romania and Spain plotting in a linear array above contemporaneous samples from England and France. When the Trinity Hull results are plotted alongside this data most individuals have lead isotope ratios consistent with a childhood spent in Britain. However, this highlights how unusual [REDACTED]'s lead isotope ratios are. He plots above contemporaneous British individuals, and with individuals from central and Mediterranean regions of Europe such as Italy, Romania, Turkey, and Lebanon (see Fig. 12). The low $^{207}\text{Pb}/^{206}\text{Pb}$ value alongside a high $^{208}\text{Pb}/^{206}\text{Pb}$ value suggests exposure to a younger lead source than those found in Britain and western Europe, suggesting exposure to a lead source from around the Mediterranean or eastern Europe. This lead could have been in painted objects such as toys or teething rings, lead-glazed pottery, pewter vessels, leaded glass decanters, or in a medicine, foods sweetened with lead acetate ('sugar of lead'), nipple shields or possibly present at trace levels in other metal items, e.g., silver.

Fig. 12: $^{208}\text{Pb}/^{206}\text{Pb}$ versus $^{207}\text{Pb}/^{206}\text{Pb}$ showing the relationship between human tooth enamel samples and European lead ore fields and how this relates to the orogenic age of the regions. Comparative tooth enamel data taken from Moore et al., (2020); Moore, (2019); Shaw et al., (2016); Millard et al., (2014) and Montgomery et al., (2010). Lead ore data taken from Butcher and Ponting, (2015); Marcoux et al., (2002); Baron et al., (2011) and OXALID

5. Trace Element analysis

Trace elements are minerals present in all tissues in amounts less than 1% wet weight of the material. Some of them are known to be nutritionally essential (e.g., iron, zinc, copper, manganese etc.), while others are considered to be nonessential (e.g., arsenic, lead, mercury, nickel) (Bowen 1988). There are several sources of trace elements, both natural and anthropogenic, and it is often difficult to identify a specific source of the element's origin. Natural sources include the weathering of rocks, volcanic activity, sea spray, river sediments and vegetation. Anthropogenic sources are derived from human activities such as mining and metal smelting, burning of fossil fuels, industrial operations and agricultural activities (Swaine 2000).

Many trace elements circulating throughout the body can become incorporated into actively forming regions of skeletal tissues, accumulating in bone, dentine and tooth enamel where they can stay for decades (Kohn & Moses 2013). Trace element concentrations in these skeletal tissues can be influenced by diet, geography, health, socioeconomic status and occupation (Simpson *et al.* 2021). In the case of industrial Victorian Britain, many toxic elements such as lead and arsenic were found in the everyday domestic environment and in cosmetics. Arsenic, mercury, and lead were also used medicinally, while outside of the home industrial activity also introduced high levels of arsenic and lead into the wider biosphere (Haller 1975; Whorton 2010; Frith 2013). As different skeletal tissues form at different ages and turn over at different rates (Hedges *et al.* 2007), trace element analysis of different skeletal elements can provide a record of an individual's lifetime exposure, often providing useful insights into the environments to which past populations were exposed.

5.1 Materials and methods

Individuals were chosen for analysis based on named status, pathological evidence of chronic disease (especially treponemal), or representative sampling of the overall

adult population based on age and sex. Fifteen bone (20-30 mg) and 156 tooth enamel (5-10 mg) samples were collected, cleaned, and abraded to a depth of >100 µm using a tungsten carbide dental drill. Only cortical bone samples from rib and femora were used, being far less susceptible to post-mortem contamination from the burial environment than trabecular bone (Rasmussen *et al.* 2015), and all trabecular bone was mechanically removed with a scalpel. Powdered samples were collected on foil, transferred to 1.5 mL microcentrifuge tubes and sent to the Arthur Holmes Isotope Geology Laboratory (AHIGL), Durham University.

At the Arthur Holmes Isotope Geology Laboratory samples were digested in 1 mL of 3M HNO₃ overnight. An aliquot of each sample was the diluted to give approximately 5 mg per 10 mL (0.5 mg mL⁻¹) to keep the total dissolved solids at a sufficiently low level to reduce matrix suppression effects within the analytical instruments. Diluted samples were then analysed via ICP-MS (Thermo Scientific X-series) using an external calibration for the minor elements. The major elements Ca and P were determined via ICP emission spectroscopy (Thermo Scientific iCAP 6000) due to the higher concentration. Both instruments were optimised prior to use to maximise sensitivity and reduction of potential spectroscopic interferences. All measurements were reported in ng mg⁻¹ which is equivalent to parts per million (ppm).

5.2 Results and interpretation

Results of trace element analysis in bone are shown below in Table 12. All individuals exhibited extremely high levels of bone lead (range 19.0 to 200.1 ppm, median 52.4 ppm), and detectable levels of bone arsenic (range 0.3 to 3.7 ppm, median 0.8 ppm). These results are likely to be reflective of groundwater diagenesis or soil contamination, rather than true biogenic measures. Firstly, the ubiquity of arsenic in the sample is suspect. Secondly, while the lowest lead concentrations are consistent with biogenic origin, the highest lead concentration (200.1ppm) is not compatible with biogenic values from a time-averaged tissue, as lead concentrations of ≥140 ppm are fatal (Bellinger & Bellinger 2006). Finally, bone is known to be particularly vulnerable to diagenetic infiltration of lead and arsenic in environments where groundwater diagenesis is likely to be a factor (Pike & Richards 2002; Wilson & Pollard 2002).

While it is impossible to exclude the possibility that the mercury results are also the result of diagenesis, it is interesting to note that the results for mercury are more selective, appearing in only two samples, and may therefore be more likely to reflect true biogenic intake of mercury. One of these, SK12871, a prime aged male, also had skeletal evidence of treponemal infection – a disease commonly treated through use of mercury compounds in the 19th century. The correlation between skeletal evidence of disease and potential mercury treatment is not consistent, however, as samples for two other individuals with skeletal evidence for syphilis (SK12258 and SK14902) did not find detectable levels of mercury in bone, and the other individual with detectable levels of bone mercury (SK14148) did not exhibit any notable skeletal pathology.

Table 12: Trace element concentrations in human bone at Trinity Hull (Pb=lead, As=arsenic, Hg=mercury, nd=not detected).

For the 156 enamel samples analysed for trace element concentrations, 155 samples met quality control indicators (data for SK21709 was excluded from further analysis). Table 13 presents summary data for enamel sampled at Trinity Burial Ground, while detailed results are available in the Excel table Ex8. No mercury was detected in any of the enamel samples. Arsenic was detected in enamel from 8 individuals, which are shown in detail in Table 14.

Table 13: Descriptive statistics for trace element analysis in human enamel in the sample (n=155) from Trinity Burial Ground (Pb=lead, As=arsenic, Hg=mercury).

Lead concentrations in tooth enamel at Trinity Burial Ground range from 0.3 to 84.1 ppm (median 11.9 ppm, IQR 16.5 ppm, which is similar to median tooth enamel lead concentrations for other 19th-century urban populations in Britain (Millard *et al.* 2014).

Most individuals at Trinity have lead concentrations which indicate anthropogenic lead exposure (≥ 0.9 ppm) during the period of enamel formation, suggesting a childhood or adolescence spent in a polluted environment. Six individuals (Table 14) had tooth enamel lead concentrations below this level, indicating a youth spent in an area where pollution levels and exposure to lead were low, and this is further supported by the absence of detectable arsenic in the same enamel samples. It is therefore unlikely that these six spent their formative years in industrial and urban Hull, more likely being raised in a rural environment. As previously noted in the discussion on lead isotopes in section 4.2.2, two of these individuals (SK11971 and SK12832) have $^{206}\text{Pb}/^{204}\text{Pb}$ isotope values which are technically consistent with British origin, but such low lead concentrations are very unusual for 19th century samples and this may, therefore, be indicating origins outwith Britain. All but one of the six individuals with low lead concentrations were male, which runs counter to demographic expectations of rural-to-urban migration for the 19th century, which was typically skewed towards overrepresentation of young women who travelled to take up employment in domestic service (Davenport 2021). Any rural-originating individuals would have been at increased risk to health in their new environment through exposure to new pathogens and vectors for infectious disease, as well as typically more adverse conditions of crowding, air quality, and sanitation (Williamson 1982).

Table 14: Individuals with low tooth enamel lead concentrations at Trinity Hull (nd=not detected).

On the other end of the spectrum, the majority of the population at Trinity clearly were exposed in most cases from an early age to high levels of lead and, by extension, overall levels of pollution. Lead exposure in an urban environment such as Hull would have been pervasive, with potential sources ranging from water pipes and oil-based paints to pewter, leaded crystal, or glazed ceramic vessels, children's toys, adulterated food, as well as inhaled aerosols created by coal burning (Farmer *et al.* 1999; Millard *et al.* 2014; Montgomery *et al.* 2010). In terms of age-at-death cohorts, young children as a group had the highest consistent lead concentrations, with decreasing levels seen in older children and adolescents (Fig. 13). This

distribution is consistent with what is known regarding the inverse relationship between increasing age and decreasing absorption of ingested lead: while adults absorb only roughly 5-15% of lead intake, children may absorb up to 50% (Hursh & Suomela 1968; Alexander *et al.* 1974; Rabinowitz *et al.* 1976; Ziegler *et al.* 1978). However, a study by Moore *et al.* (2021) implicated high lead exposure with high childhood mortality in Roman populations, demonstrating the link between lead toxicity and early mortality. From adolescence to adulthood there is little evident

change in the broad representation of lead in teeth between groups. The similarity between adolescent and adult enamel lead concentrations is likely due to both similarity of exposure and age represented by analysed tissues, irrespective of age at death.

Fig. 13. Box and whisker plot of lead concentrations in human tooth enamel at Trinity Burial Ground, by age.

Differences are evident in the distribution of lead *within* adolescent and adult age groups (Fig. 14). When considering age at death, the median and distribution of lead concentrations is similar for adolescent and most adult age ranges, with a bias towards lower lead concentrations in each cohort. An exception within the adult cohorts was observed for individuals dying in young adulthood, where the median is higher than for other cohorts, and the distribution of concentrations is skewed towards higher end of the range. This may suggest more adverse childhood living conditions on average for individuals dying early in adulthood than people who survived young adulthood, dying at older ages. While all adult cohorts were weighted towards the lower end of their ranges, in middle and mature adults there were several outliers with high lead concentrations causing skewing of their respective ranges. Hence, lead concentrations were highest in individuals of brief or lengthy lifespans, suggesting that lead exposure was only one of many factors in longevity in Hull.

Correlations between lead levels and premature death in industrial period populations warrant further investigation, as lead is known to have serious and pervasive impacts on health. Lead is highly toxic, with systemic effects of lead poisoning manifesting in anaemia, immune dysfunction, kidney and liver damage, hypertension, gastrointestinal and nerve disorders, vitamin D deficiency, and infertility (Mitra *et al.* 2017; Cilliers & Retief 2019).

Figure 14: Violin plot of lead concentrations in human tooth enamel at Trinity Burial Ground by age, with detailed breakdown for adults.

Stored lead in the skeleton may also be remobilised during pregnancy and lactation, and lead passes freely via the placenta and into human milk consumed by infants (Riess & Halm 2007). In children, who are most vulnerable to absorption of ingested lead, effects may include neurological deficits, delayed skeletal development, and a failure to thrive in infants (Bithoney 1986; Kafourou *et al.* 1997; Naranjo *et al.* 2020). Symptoms of metabolic disease may be evident with blood lead levels as low as $15 \mu\text{g dL}^{-1}$ and are fatal at levels greater than $140 \mu\text{g dL}^{-1}$ (equivalent to 15 ppm and 140 ppm, respectively) (Bellinger & Bellinger 2006). Chelation therapy is clinically indicated when blood lead levels exceed $45 \mu\text{g dL}^{-1}$ (45 ppm) (Graziano 1993). Fifty-nine out of the 155 enamel samples at Trinity (38.1%) had lead concentrations exceeding 15 ppm, while 7 individuals (4.5% of the sample) had enamel lead concentrations exceeding 45 ppm.

As a result of the high prevalence of metabolic disease in the Trinity population, in particular, skeletal manifestations of vitamin D deficiency and anaemia, a link between metabolic disease and the high lead exposure common in individuals buried at Trinity was considered. Figure 15 shows the distribution of enamel lead concentrations for individuals with and without rickets at the site. There is no link between lead concentrations and rickets in adults, as the median values and distributions are similar between groups, regardless of the presence or absence of rickets. Indeed, the highest enamel lead levels are observed for individuals without rickets. For young children, however, there is a large difference in the lead concentrations between children with and without rickets. Young children with rickets have significantly higher lead concentrations than their similarly aged peers without rickets. The contrast between adults and young children may simply reflect the differences in developmental timing between sampled teeth and skeletal pathology: while the M2/M3 teeth typically sampled in adults should reflect lead exposure up to the age of ~ 12 years, deciduous teeth sampled in young children would be more reflective of lead exposure during the antenatal and early childhood period in which rickets would be expected to manifest, creating a temporal tie between pathology and toxicology. It is tempting to speculate that if similarly early-forming enamel, such as from a first permanent molar, had been analysed in adults with rickets, it might reflect similarly high lead exposure during early childhood. However, without data this must remain speculation.

Fig. 15. Box and whisker plot of tooth enamel lead concentrations by age for individuals with and without rickets (adolescents with rickets represented by median only, as $n=1$, no older children with rickets were included in the sample).

High lead exposure may also lead to anaemia, as lead toxicity interferes with heme metabolism, and pre-existing iron deficiency may create greater vulnerability to lead toxicity (Abbaspour *et al.* 2014). As previously discussed in section 4.1.2, anaemia can also alter $\delta^{18}\text{O}$ values, as impaired oxygenation of red blood cells can alter oxygen metabolism, leading to lower $\delta^{18}\text{O}$ values in tissue (Reitsema & Crews 2011; Carroll *et al.* 2018). Consideration was therefore given to whether the shift towards low $\delta^{18}\text{O}$ values might be associated with metabolic disease, linked in turn to lead exposure. However, when $\delta^{18}\text{O}$ values are plotted against lead concentrations (Fig. 16), the hypothesised link between high lead and low $\delta^{18}\text{O}$ values does not appear. While this does not undermine the link between lead exposure and metabolic sequelae, or between metabolic disease and altered $\delta^{18}\text{O}$ values, it does somewhat emphasise that interpretation of oxygen stable isotope data is complex, involving a range of unknowns in the form of geographic, cultural, and physiological factors for which we often do not have enough context in archaeological data.

Fig. 16. Relationship between lead concentrations and $\delta^{18}\text{O}$ values in human enamel at Trinity Burial Ground

While ubiquitous in industrial Britain, lead was not the only toxicological hazard to face Victorians. Arsenic was another heavy metal that was abundant in many industrial and domestic environments, with variable risks of exposure. Present in a range of medicinal preparations, many Britons of all ages were also exposed to arsenic by everyday domestic items such as textiles, book bindings and wallpapers through pigments like the infamous Scheele's Green, or even in colouring used in children's sweets (Haller 1975; Whorton 2010). In addition to the risk of acute or chronic poisoning, chronic arsenic exposure – reflected in tooth enamel – is carcinogenic, increasing the risk for a range of cancers, as well as cardiovascular disease, metabolic disease, diabetes, and neurological and hepatic dysfunction (Palma-Lara *et al.* 2020). Most individuals at Trinity did not have detectable levels of arsenic in their tooth enamel, with only a minority (8/155, or 5.1% of the sample) having evidence for arsenic exposure in their teeth (Table 15). Most of the individuals with detectable enamel arsenic died young (Fig. 17), with only two of these being adults at the time of death (SK21842 and SK30229).

Table 15: Individuals with detectable arsenic in tooth enamel

Fig. 17. Arsenic concentrations by age in tooth enamel for the sample at Trinity Burial Ground

Assessment of arsenic exposure in skeletal remains presents some difficulties, as modern standards for measuring arsenic concentration rely on blood, urine, hair or fingernails, and as such there is no agreed 'normal' range for skeletal tissues, or indeed defined threshold levels for interpretation of acute or chronic toxicity. Some researchers have suggested that skeletal values should be below 1 ppm, or, more conservatively, below 0.5 ppm (Swift *et al.* 2015). All individuals at Trinity with detectable arsenic in enamel were below this level. However, it is clear that the norm at Trinity burial ground for tooth enamel is an absence of detectable arsenic, and that any extended exposure to arsenic carries health implications.

It is interesting to note that, in addition to most individuals with arsenic being young at the time of death, all but one of these had cribrotic skeletal lesions. Cribra orbitalia is relatively unremarkable for urban sites of the 19th century as a nonspecific indicator of stress; mean crude prevalence for the postmedieval period has been reported as around 9% (Roberts & Cox 2003: 307). Nonetheless, cranial and post-cranial cribrotic lesions have been linked to malarial anaemia in wetland and estuarine environments by a range of researchers (Gowland & Western 2012; Smith-Guzmán 2015; Schats 2021; Wilson *et al.* 2023), and the Humber estuary was one of several areas of Britain where *Plasmodium vivax* was historically endemic. For instance, the death of famous local poet and member of Parliament Andrew Marvell in London in 1678 is believed to have been due to complications of malarial infection, likely contracted in Humberside (Mottram 2021). While malaria in Britain disappeared in the early 20th century, during the early and mid-19th century malaria persisted, causing significant morbidity, particularly in children (Hinde & Fairhurst 2015; Kendall & Kendall 2021), and high prevalence of 19% (CPR) for cribra orbitalia has been observed for nearby St. Peter's, Barton upon Humber (Watts 2011). Fowler's solution, a 1% potassium arsenic solution, was introduced as a treatment for malaria (or 'ague') in the early 19th century (Frith 2013; Ho & Lowenstein 2016), and it is intriguing to consider whether these individuals might have received their arsenic exposure through medicinal treatment for malaria. However, as chronic arsenic exposure itself can also result in anaemia, in linking toxicological histories and skeletal pathology it is difficult to distinguish cause and effect.

5. Discussion

The diet and mobility of 180 individuals from the Trinity Hull burial ground were investigated using a range of isotopes and trace elements, the results of which are summarised in the Excel table Ex1. Outliers identified in the previous sections, and summarised here, are also found in an Excel summary table, Ex9.

Many of the individuals analysed have isotope characteristics that indicate local origins, with their strontium and lead isotope ratios consistent with a childhood spent in East Yorkshire. The geology of Hull is reasonably varied and therefore provides a range of expected strontium isotope ratios, and most of the Trinity individuals have ratios consistent with this range. However, 33 individuals have strontium isotope ratios higher than expected, but still plotting closely with the Medieval population from Hull Magistrates Court. The majority of these could originate within Yorkshire from regions of Permo-Triassic and Carboniferous sandstones although this does not preclude them from origins elsewhere in Britain, Ireland or further afield. There is one clear outlier, SK14782, with a strontium isotope ratio too high to originate from within Yorkshire. Such values are commonly found in regions with granitic or older Palaeozoic geology, such as western and northern England, Wales and Scotland. For example, humans from the Devonian Old Red sandstone of Herefordshire have such values. For lead, the vast majority of Trinity individuals have anthropogenic lead concentrations coupled with lead isotope ratios consistent with England. There were three outliers within this dataset, individuals SK12832 and SK1197 had unusually low lead concentrations for 19th-century individuals, their lead burdens were more consistent with those seen in prehistoric populations and indicate a childhood spent in a region with very little environmental pollution. The low lead concentrations in these two individuals explain why they do not plot with the rest of the Trinity population in the centre of the English/Welsh lead ore field, as geogenic lead isotope ratios are not culturally focussed. Conversely, named individual [REDACTED] (SK21582) had a high lead concentration with an $^{206}\text{Pb}/^{204}\text{Pb}$ value too high to be from British lead sources, and suggestive of anthropogenic exposure to a younger, perhaps Mediterranean lead source. This is intriguing because it is thought that [REDACTED] spent his early life in Selby, but these results strongly suggest exposure to a significant level of lead from outside Britain. An in-depth look at

historical records pertaining to Frank's childhood and any time spent abroad might shed light on these unusual results. Exposure to an imported source may also explain this somewhat baffling result.

Oxygen and sulphur isotopes were analysed to further investigate the childhood origins of the Trinity population. Interestingly, the oxygen isotope ratios at Trinity were almost universally inconsistent with the predicted range, with most of the samples producing values much lower than expected based on hydrological models. Two potential factors involved may be climatic anomalies and/or metabolic pathology, but data is currently insufficient to draw firm conclusions. A two standard deviation cut-off was used to define a new local range, which identified two probable migrants (SK11237 and SK14148). The first individual, SK11237, had a very low oxygen isotope ratio inconsistent with Britain or Ireland and indicating origins at higher latitude, altitude or greater continentality. Taken together with the $\delta^{13}\text{C}$ data which suggests some consumption of marine or C_4 protein, the data could point to origins at high latitude far to the north of Britain in which case the high $\delta^{13}\text{C}$ will reflect some marine protein consumption or if the high $\delta^{13}\text{C}$ derives from C_4 consumption of, for example millet or maize, the low $\delta^{18}\text{O}$ may be indicating origins to the east in continental Eurasia or possibly to the west in the Americas where such crops can grow or possibly less likely, at high altitude in a region such as the Alps where consumption of C_4 crops such as sorghum and millet has been recorded. Whilst there is a clear shift to lower values in all of the individuals at Trinity, this value is so low and such a significant outlier that origins in most of northwestern Europe can still be ruled out. The second outlier, SK14148, had a high oxygen isotope ratio consistent with a childhood spent in the western fringes of Britain, such as Cornwall, southwest Wales, or the Hebrides, or perhaps western Ireland or Atlantic coastal regions of western Europe. The sulphur isotopes from the Trinity individuals do not provide much information about mobility of these individuals. The location of the site on an estuary, together with its Chalk geology would be expected to produce high sulphur isotope ratios consistent with marine environments. However, the Trinity individuals cluster around 3‰, too low for marine or Chalk bedrock influence, and most likely influenced by the highly polluted industrial environment found in Hull at the time.

Carbon and nitrogen isotope analysis of bone and dentine collagen revealed that the Trinity individuals ate a predominantly terrestrial C₃ diet with a minor supplementary C₄/marine component, which is consistent with other 19th-century British populations. However, two outliers stand out among the data. SK10219, a young adult female, with high $\delta^{13}\text{C}$ for the population, at -18.3‰. This individual was buried in an earth cut grave with no obviously defining features which would differentiate her from the rest of the population, aside from potential non-British ancestry flagged by genetic analysis. Nonetheless, it is somewhat perplexing to explain this high $\delta^{13}\text{C}$ value in bone, as she died so early in adulthood, and her incremental dentine values covering the contemporaneous period of infancy to young adulthood (cf. Table Ex4) do not similarly reflect high $\delta^{13}\text{C}$ values. The second outlier, SK20530, a mature adult male also suggested to have potentially non-British ancestry, similarly stood out, due to a $\delta^{15}\text{N}$ value of 9.4‰, which is unusually low for the Trinity population and 19th-century England more broadly suggesting a largely vegetarian diet. His $\delta^{13}\text{C}$ value of -19.4‰ suggests some consumption of either C₄ or marine proteins. Such low $\delta^{15}\text{N}$ values have been found in this time period amongst children at Kilkenny Union Workhouse who were eating a largely plant-based diet of potatoes and maize.

Potential sex differences in dietary intake were also explored. Females exhibited a less variable, and higher, range of $\delta^{15}\text{N}$ values than males, suggesting higher trophic level protein among females. Males from childhood through to early adulthood appear to have had more variability in diet than their female counterparts and the lower values in the male range, absent among females, may suggest heavier dietary reliance on terrestrial protein sources among young males, with greater consumption of marine protein in young females. However, it is important to note that these differences were not statistically significant.

Finally, trace element concentrations in human tooth enamel characterised urban Hull as a highly polluted environment, with high levels of lead being the baseline norm. Very low lead levels in the teeth of six individuals (SK11237, SK11971, SK12832, SK14782, SK30531, and SK30970) were incompatible with residence in Hull during the period of enamel formation, suggesting that these individuals originated elsewhere in rural environments. Mercury was not detected in tooth enamel, but arsenic was found at low levels in eight individuals (SK11298, SK14856, SK21277, SK21314, SK21364, SK21842, SK22268, SK30229), all but one of whom

had cribrotic pathologies. As malaria was still endemic to estuarine Hull in the 19th century, and arsenic preparations such as Fowler's solution were used medicinally for 'ague', it is possible that these individuals were exposed through pharmaceutical use of arsenic. Bone trace element was largely unsuccessful, due to the possible presence of diagenetic lead and arsenic. However, two individuals (SK12871 and SK14148) had detectable levels of bone mercury, one of whom has evidence of treponemal disease. This may be evidence of medical treatment.

6. Conclusions

- Diet for the Trinity individuals was consistent with a population eating a primarily C₃/terrestrial diet with some minor supplementary C₄/marine component.
- Bone collagen $\delta^{13}\text{C}$ values were higher than those in dentine, suggesting a shift towards increased consumption of C₄/marine foods with advancing age.
- Some sex differences were present, but these differences were not statistically significant.
- Oxygen isotope ratios are much lower than expected and may be due to either environmental/climatic influences on drinking water or biological fractionation processes (disease, activity, stress etc.). More data for the 19th century is needed.
- Strontium isotope ratios were largely consistent with the varied geology of Hull and the wider Yorkshire area with one notable exception.
- Eleven individuals had unusually high strontium concentrations for England coupled with a seawater strontium isotope ratio (SK11919, SK14676, SK14856, SK15374, SK20157, SK20419, SK20759, SK21405, SK30229, SK30855, SK30977). Such a composition is indicative of individuals from Atlantic-facing coastal or island populations, e.g. the Northern or Western Isles of Scotland.
- Sulphur isotope ratios are low and inconsistent with coastal or Chalk bedrock environments - it is likely that industrial atmospheric pollution is the cause.
- Lead isotope ratios were mostly consistent with childhood origins in England.
- Arsenic was found at low levels in eight individuals (SK11298, SK14856, SK21277, SK21314, SK21364, SK21842, SK22268, SK30229), which may

indicate medicinal exposure for ailments such as malaria, or domestic exposure.

- Two individuals (SK12871 and SK14148) had detectable levels of bone mercury, one of whom has evidence of treponemal disease. This may be evidence of medical treatment.
- The majority of individuals had been exposed to high levels of lead in childhood, with a link also being evident between high lead exposure and early death in juveniles.
- Six individuals (SK11237, SK11971, SK12832, SK14782, SK30531, and SK30970) had very low lead exposure in childhood, suggesting that these individuals grew up in rural environments.
- SK11237, had enamel carbon isotope ratios consistent with some C₄ or marine protein consumption and a very low oxygen isotope ratio indicating origins at higher altitude, latitude or continentality than Britain, Ireland and most of northwestern Europe.
- SK14148, had a high oxygen isotope ratio consistent with a childhood spent in the western fringes of Britain or coastal Atlantic Europe.
- [REDACTED]'s lead isotope ratios strongly suggest exposure to a significant level of lead from outside Britain, with values similar to younger ore sources of the Mediterranean and eastern European, e.g. in Spain, Italy, Turkey and Romania.

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