Interpreting Mortuary Treatment from Histological Bone Diagenesis: A Case Study from Neolithic Çatalhöyük

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ABSTRACT This study examines the evidence for differential mortuary practices at the Neolithic site of Çatalhöyük, in Anatolia, Turkey using the histological examination of bacterial micro-bioerosion in archaeological human bone. In order to analyze bacterial micro-bioerosion, thin sections were prepared from the mid-shaft of thoracic ribs 6-8, of n=162 individuals (adults and juveniles) and analyzed using qualitative analysis of microscopic focal destructive changes with polarized light microscopy. The extent of destructive change was assessed, and the degree of preservation examined using the Oxford Histological Index. Individual, *in situ* Interment was assessed using burial and skeletal data. Results show no differences in histological bacterial micro-bioerosion between male and female burials, but histological preservation was seen to differ between juveniles and adults, findings that are interpreted as the result of differential mortuary practices at the time of death. The challenges and potential of examining recurring taphonomic signatures in bone histology and their relationship to differential burial practices are discussed.

Introduction

Çatalhöyük is a Neolithic settlement located in south-central Turkey (Fig.1) dating from roughly 7100 to 6000 cal B.C. (Bayliss et al. 2015). The site was first excavated by James Mellaart in the 1960s, then followed by a large excavation project under the direction of Ian Hodder from 1993-2017. Çatalhöyük is located in south-central Anatolia, approximately 50km from the modern city of Konya. The site is known for its architecture of stacked buildings that demonstrate meticulous dedication to repetition of reconstructing of the same building layout, over multiple sequences in time (Farid 2014; Matthews 2005; Russel et al., 2014). This dedication to tradition or social control, is also observed in the communal funerary practices, the location of paintings within houses, and the repetitive re-plastering and repainting of reliefs during the lifecycle of a building (Czeszewska, 2014).

(Figure 1 here)

Paleoenvironmental soil analysis suggests Neolithic Çatalhöyük was located on alluvial wetlands and during its occupation coincided with a period of active river alluviation, surrounding most of the area in floodwater each spring (Roberts and Rosen, 2009). Analysis of soil cores by Ayala et al., (2017), reveal four stratigraphic phases from the early Holocene onwards in which humid conditions shifted to increasingly dryer climate. The basilar layer of sediments is primarily composed of marl and sands with gravel. The lower and upper layers of sediment were dominantly silty-clays or silts and clay.

Over 700 skeletons have been excavated from the site and represent complex mortuary practices, including the retrieval and reuse of skulls and dismemberment of individuals (Hodder, 2014). Haddow et al. (2020) suggests there are a number of instances where the crania and mandibles of individuals have been removed then eventually reburied as headless primary burials at Çatalhöyük. The approximate number of primary burials where the cranium and mandible have been intentionally removed are predicted to be 15 (Pilloud et al., 2016). In addition, according to Pearson and Meskell, (2015), individuals were occasionally found to be interred with plaster encasing the skeletonized remains and or with red paint on the skull. In a recent analysis of the interment categories at Çatalhöyük, Haddow et al., (2020), six burial categories have been defined, with four main depositional interment: secondary, tertiary, primary undisturbed, and primary disturbed burials. Secondary burials are if a partial or complete skeleton has been moved by Neolithic people from a previous location and deposited in a final interment context. Tertiary burials are disarticulated, partially articulated, or isolated skeletal elements found in non-interment contexts (e.g. midden). Primary undisturbed burials are articulated or nearly complete individuals found in their original place of interment. Primary disturbed burials are in situ skeletal remains found in their original burial location and subsequently disturbed by Neolithic people. Secondary burials account for 13% of the total number of burials, tertiary burials are for 23%, the primary undisturbed burials are 39%, and primary disturbed burials are 25% of the interment observed at Çatalhöyük. Individuals are primarily buried within houses under platforms and floors, but have been also interred within building foundations, infill, benches, and middens (Boz and Hager 2013; 2014). These intramural burials placed the living both physically and symbolically with the dead (Nakamura and Meskell 2009).

Over the years, study of the human remains has been a collaborative effort which has contributed much to our understanding of mortuary practice, social structure, health, diet, and lifestyle at Çatalhöyük. There have been numerous analyses applied to the human remains with the objective to interpret significance between variations in mortuary treatments (Hillson et al., 2013; Larsen et al., 2015; Haddow, Sadvari, Knüsel, and Hadad, 2016; Haddow and Knüsel, 2017; Agarwal et al., 2017; Pilloud, Haddow, Knüsel, Larsen, 2016; Pilloud and Larsen, 2011; Boz and Hager, 2013; 2014). It remains unclear why particular individuals received differential mortuary treatments at Çatalhöyük, however the large number of isolated and partially articulated skeletal elements may be interpreted as a communal relation with the dead. This might suggest delayed burial practice was a part of the social structure of the community.

Cut marks from manual excarnation, the removal of flesh using tools, such as sharp obsidian has been observed in 2% of the sample population. These individuals display evidence of linear markings on the diaphysis of bone. It has been suggested by Russell et al. (2013), that application of obsidian tools might have lessened the need for deep cuts that could potentially leave incisions on bone. Furthermore, paintings of vultures surrounding headless bodies have been observed at Çatalhöyük during the Mellaart (1967) excavations. Pilloud et al. (2016) investigated Pre-interment vulture excarnation by as a mortuary treatment, and found the scavengers to be skilled at the removal of soft tissue from bone, often leaving surrounding ligaments and tendons fully intact. Based on the manner and consistency of skeletal element articulation at the site, it is possible that vulture excarnation was utilized at Çatalhöyük (Pilloud et al. 2016).

It is well understood that the macroscopic appearance of whole skeletal elements may differ from the microstructural appearance of its tissues. Therefore, it is valuable to incorporate histological findings when assessing pathological, traumatic, and post-mortem diagenetic changes to the microstructure of skeletal tissues (Turner-Walker and Jans, 2008; Bell, Skinner, and Jones 1996; Jans Nielsen-Marsh, Smith, Collins, and Kars, 2004; Parker Pearson et al., 2005; Nielsen-Marsh et al., 2007; Smith et al., 2007; Hollund et al., 2012). There are three primary diagenetic pathways that cause macroscopic and microscopic alteration to bone including hydrolysis; the chemical deterioration of the bone organic component, dissolution; the chemical deterioration of the inorganic component of bone, and microbial attack; the bacterial deterioration of the tissue composite (Child, 1995; Collins et al., 2002; Hedges, 2002; Hedges & Millard, 1995; Hedges, Millard, & Pike, 1995; Henderson, 1987; Millard, Brothwell, & Pollard, 2001; Trueman & Martill, 2002; Turner-Walker, 2008). The most common of the three primary destructive changes in archaeological bone is deterioration by microbial attack (Collins et al., 2002; Nielsen-Marsh and Hedges, 2000; Hedges, 2002; Turner-Walker et al., 2002; Jans et al., 2004; Nielsen-Marsh et al., 2007; Brönnimann et al., 2018). Diagenetic change is visible in bone at a microscopic level and consists of five primary categories identifiable with normal and polarized transmitted light microscopy: type of destruction, the presence of inclusive and or infiltrated materials, the presence of micro-fissures, and the intensity of birefringence (Jans, 2005). Microbial destruction attacks both the collagen and mineral phases of the bone, impacts the pore structure, and is most often indicated by areas of remineralization originating from within the Haversian canals as they proceed to fill osteons.

Hackett (1981) characterized microbial attack to bone by its distinguishing features of size, shape, presence of a hyper-mineralized rim (ranging between 10-60µm in diameter), reorganization of the bone microstructure, and the presence of tunnel like structures. Microbial attack leaves a hypermineralized rim through the dissolution and redistribution of hydroxyapatite, creating a higher density area with respect to the surrounding bone (Dal Sasso et al., 2014). Hackett (1981) characterized four micro-focal destruction tunnel types: lamellate, budded, linear longitudinal, and Wedl tunnels. Recent research by Jans et al. (2004), Dal Sasso, Maritan, Usai, Angelini, and Artioli, (2014), and Turner-Walker (2012) indicate that while using high magnification light microscopy, the tunnels are a series of interconnected pores, often surrounded by a hypermineralized border and might suggest the vascular canalicular network of bone assists in the transportation of bacteria.

Microbial diagenesis to bone tissue microstructure has been observed in both terrestrial (Baud and Lacotte 1984; Hackett 1981; Jackes et al. 2001) and aquatic environments (Bell, Boyde, and Jones 1991; Bell et al., 1996; Davis 1997; Pesquero, Ascaso, Alcalá, and Fernández-Jalvo, 2010), and is sometimes attributed to attack by fungi (Carlile, Watkinson and Gooday, 2001; Marchiafava, Bonucci, and Ascenzi, 1974; Wedl 1864; Roux 1887; Morganthaler and Baud, 1956; Schaffer, 1894). In terrestrial interment, it is undetermined whether the microorganisms that demineralize bone are primarily endogenous and exogenous, but it is most likely the two are intertwined as the severity and type of diagenetic change is often controlled by the external environment (Grupe and Harbeck, 2019).

A number of histological methods have been developed to semi-quantify the extent of preservation of bone microstructure (Stout 1978; Garland, 1989; Hackett, 1981; Hedges, Millard, and Pike, 1995) and may contribute to better interpretation of an individual's burial history. This study is the first to histologically quantify micro-bioerosional change in relation with secondary burials; those displaying manipulation by human action, to investigate differential diagenetic expressions in the bone microstructure between biological age and sex groups, and those with pathologies as seen on the gross skeleton. Individuals with possible alteration by anthropogenic manipulation might indicate a different diagenetic expression than primary undisturbed burials, if microbial attack is endogenous in origin. Findings of the study will advance our understanding of the process of diagenetic change in archaeological human skeletal tissue, and potentially further our understanding of the social conditions that determined specific mortuary treatments to particular individuals at Çatalhöyük.

Materials

Rib sections were sampled from juvenile and adult skeletonized individuals (n=162) who were excavated between field seasons 1997 to 2010. Individuals included in this study were sampled from the following excavation areas: South Shelter (S), North Shelter (N), BACH Area, 4040 Area (4040), and the Team Poznan (TP) Area (Fig 2). The sample is comprised of 90 juveniles (aged neonate to 12 years old), 6 adolescents (12-20 years old), and 66 adult (20-50+ years old) individuals. Age groups included in the sample were neonates (n=25), infants (n=41), child (n=24), adolescents (n=6), young adult (n=12), mature adult (n=15), old adult (n=10), and adult (n=29).

(Figure 2 here)

The sample included 32 males, 31 females, 85 individuals recorded as too young to determine sex (O), and 14 individuals recorded as indeterminate (I/?). The biological sex and age of the individuals was estimated by a number of osteological team members as outlined in Hillson, Boz, and Hager (2013), and all age and sex estimated were re-assessed and/or confirmed at the time of sample collection by authors Agarwal and/or Beauchesne. Biological sex was estimated using standardized morphological features of the pelvis and skull (Buikstra and Ubelaker, 1994). In some cases, the remains did not display diagnostic elements for biological sex estimation. Sex estimation of the skull included assessment of the mastoid process, supramastoid crest, temporal line, glabella, supraorbital ridge, nuchal line, and external occipital protuberance. The mandible was assessed for robusticity of the coronoid process, ramus, gonial angle, and mental eminence. The os-coxa was evaluated using the shape of the greater sciatic notch, pubis, ventral arc, subpubic concavity and inferior ramus ridge. Age at death in adults was estimated using the Brooks-Suchey (1990) pubic symphysis stages and the Smith (1984) occlusal tooth wear (attrition) scores. Wear in the dentition was used to divide the adults into young, middle, and old age groups. The age of sub-adults was estimated using developmental indicators including measurements defined by Fazekas and Kósa (1978) of the long bones and skull, and dental development using both the Schour and Massler (1941) and Hillson (2005) methods.

Interment data such as burial location, in situ interment position, the association of grave goods, and indications of pathology or body parts moved were evaluated using published online Çatalhöyük archive reports (http://www.catalhoyuk.com/research). Most Interment were found within domestic buildings beneath the north and east platforms of the main room, with the exception of infants who were sometimes interred in floors of the side rooms, hearths, or ovens (Andrews, Molleson, and Boz 2005; Boz and Hager 2013). Burial preference was for individuals to be interred in the flexed position, with minor variations of extended legs or arms (Boz and Hager, 2013). Most Interment lacked burial goods and when burial goods were present, were limited in number (Boz and Hager, 2013). Burial goods were investigated on a presence or absence basis. Fiber analysis revealed adults were interred with sedge fibers and juveniles were consistently interred with a variety of plant fibers including those for funerary baskets (Rosen, 2005). Different kinds of mineral pigments have been found associated with interment including red and yellow ochre, and green and blue cinnabar in adult burials (Boz and Hager, 2013). Red ochre has been found on the skulls of adults, juveniles and infants.

As noted above, postmortem manipulation of bodies is a characteristic feature of burial at Çatalhöyük (Boz and Hager, 2014; Hodder, 2005). In a study of human remains from field seasons 1996 to 2008, Boz and Hager (2013) observed that decapitation seemed to occur in all age categories. Individuals in the sample were assessed for indicators of dismemberment (appendages dismembered or disarticulated), decapitation (head removed prior to excavation) (Table 1), and widely assessed for pathological conditions including evidence of metabolic stress and degenerative diseases (Table 2). No individuals in the sample had evidence of infectious disease. It is unclear if disarticulated individuals had their heads and limbs intentionally moved, or if it is an artifact of an addition of a new individual to a multi-burial grave. Due to this discrepancy, individuals in the sample whom were documented in the archive report as disarticulated or with their heads moved are included in assessment of post-mortem manipulation as it signifies the movement of body parts post-mortem, regardless of intentionality.

(Table 1 here)

(Table 2 here)

Methodology

Thin sections were prepared from the mid-shaft of thoracic ribs (mid-shaft rib #6-8). Sections were removed using a Buehler Isomet 1000TM precision saw and embedded in Buehler's Epothin resin. Thin sections, approximately 70-100 microns were made using a Bueller Petro ThinTM sectioning system (Agarwal, Glencross, and Beauchesne, 2013). After mounting on a glass slide, sections were examined with polarized light microscopy using a Leica MX6 dissection upright microscope, with a plain magnification factor of x0.8 and an eyepiece magnification of x10. The degree of destructive change and preservation was assessed using the Oxford Histological Index (OHI), a classification system developed by Hedges et al. (1995), so the extent of micro-bioerosion could be quantified. The OHI method was developed on thick sections of bone (Neolithic and Paleolithic) but may be applied to thin sections of tissue for detailed analysis. The OHI method has been used in both experimental forensic and biological archaeological studies (Booth and Madgwick, 2016; White et al., 2014; Booth, 2015; Madgwick, 2008; Redfern, 2008; Kontopoulos, Nystrom and White, 2016; Brönnimann et al., 2018). Each histological section was assigned a score based on the amount of microstructurally recognizable preserved bone and given a numerical value to semi-quantitate diagenetic alteration. A bone section was evaluated on a scale of zero to five to summarize the degree of post-mortem microstructural change, with zero, being less than approximately five percent of bone intact, to five, where greater than ninety-five percent is unaffected (Table 3).

(Table 3 here)

To validate statistical significance of preservation between biological sex and age groups, chi-square tests of independence were applied using R statistical analysis software (R Core Team, 2019). Comparison groups investigated included preservational differences between juvenile and adult age categories, juveniles without the presence of adolescents for comparison to the adult age categories, differences of preservation between biological males and females, differences of preservation between old age and the young adult age categories, and preservational differences between old age males and old age females. Comparative analysis was also conducted between the amount of preservation and individuals with indications of pathology or postmortem manipulation.

Results

Results of analysis show 90% of the sample was assigned a score of 0 and 1 for containing less than 15% of preserved bone microstructure (Fig. 3). 11 individuals were assigned a score of two, indicating less than 33% of bone intact. Six individuals were given a score of four or five who had 85% or more bone intact.

(Figure 3 here)

To evaluate for differences between sub-adult and adult age groups, all sub-adult age categories; neonates, infants, children, and adolescents, were combined then compared to the combined adult categories; adult, young adult, mature adult, and old age (Fig. 4). Results showed significant differences in preservation between adult and sub-adult age categories ($p = 0.002241$) indicating sub-adults to be less well preserved than adults. Only adults received OHI scores four and above (> 85% of bone intact).

Adolescence (12-20 years) is the generalized biological start of adulthood, and the period when the human skeleton goes through a series of developmental changes. At birth all primary ossification centers of the rib are present, but it is not until 12-14 years that secondary ossification centers of the rib begin to develop at the sites of articulation (Scheuer and Black, 2004). The rib is not considered fully adult until after the rib head and tubercle epiphyses develop, between the ages 17-25 (Scheuer and Black, 2004). As such, the sub-adult group was analyzed without the adolescent individuals. Statistically significant differences of preservation between adult and sub-adult age groups were maintained when the adolescent group was removed ($p = 0.001674$). Additionally, juveniles were observed to be dismembered or disarticulated less than their adult counterparts when reviewing the archive reports.

(Figure 4 here)

To check if there were differences in preservation within the adult age categories, a chi-square test of independence was conducted between the old age adult group and the young adult category but was not statistically significant (p= 0.3258). The adult age group was excluded from this analysis as it was not possible to estimate which broad age category they should be included.

To examine possible differences in microstructural preservation and burial treatments between male and female groups, chi-square tests of independence evaluated differences been males and females of all adult age groups. No statistically significant differences were observed in preservation between males and females (p = 0.6912). While no statistically significant differences were validated between males and females, four of the six individuals assigned an OHI score greater than four were male. Old age males and females were compared for differences in preservation. The chi- square test between old age males and females found no significant differences (p= 0.1266).

The difference in the amount of preservation between individuals with body parts moved, generally yielded no difference in OHI scores from primary undisturbed burials (Fig. 5). Only one individual, Sk. 10814, an adult female whose head was displaced and found in a post-retrieval pit adjacent to the skeleton, received a score of four (>85% preservation). Her skeleton was scattered, with only few elements in articulation and she was interred with three others. Sk. 10814 is distinct from the sample recorded with indication of body parts moved and she is one of the few with well-preserved rib microstructure.

(Figure 5 here)

22 individuals within the study sample were recorded with distinct indications of secondary post-mortem manipulation (disarticulation/dismemberment). 16 individuals within the sample have had their heads moved, removed, or were headless. Eight were disarticulated or had limbs removed. There were two individuals in the sample who had their head and limbs removed (Sk. 15640 and Sk. 17412). Of the sample whose body parts were removed, nine were female, six were male, and seven were indeterminate. Post-mortem manipulation of the limbs and skull occurred in all ages of the sample, with preference in the adult category (sub-adult: n= 8; adult: n=14).

15 individuals of the sample were documented as having pathological conditions, such as degenerative joint disease and cribra orbitalia. Four of these were assigned an OHI score of four or five (>85% of bone intact), all were male. The sub-sample of individuals recorded with indicators of pathological conditions included eight males, two females, and five individuals who were indeterminate. Pathologies were primarily present in the adult sample (Adult: n=11, Sub-adult: n=4). No individuals with documented pathological indications showed evidence of dismemberment with the exception of Sk. 2056, a mature adult male with degenerative changes to the spine and hand. He was recorded with possible disarticulation of the left leg.

Bacterial micro-bioerosion in the study sample was common and consistent with bones of intact bodies that had been interred peri-mortem (Hedges 2002; Jans et al. 2004; Nielsen-Marsh et al. 2007; White et al., 2014, Booth 2015). Immediate burial protects the body from invertebrates that expedite the decomposition process and ensures maximum levels of putrefaction (Rodriguez and Bass 1983, 1985; Mann, Bass, and Meadows, 1990; Campobasso, Di Vella, and Introna, 2001; Breitmeier et al. 2005; Simmons, Cross, Adlam, and Moffatt 2010; Zhou and Bayard 2011; White and Booth 2014).Generalized bacterial destructive change was observed throughout the sample in the form of blackened remineralized osteons with the destructive change appearing to originate from the haversian canals into a hypermineralized rim. The majority of the sample demonstrated a generalized loss of recognizable mircrostructural features including lamellar structure, osteocyte lacunae, and canaliculi, and the disintegration, disaggregation and dissociation of osteons. Within the disaggregated osteons, small blackened foci were occasionally present and are interpreted as microfocal destructive tunnels, although differentiation of tunnel type was not assessed. In addition, the presence of externally derived inclusions were observed lying within Haversian canals, osteocyte lacunae and canaliculi. There was a noticeable reduction in birefringence. The decrease in birefringence is assumed to be linked to the deterioration of collagen and/or the changes in the orientation of hydroxyapatite crystals from diagenesis (Schoeninger et al., 1989). Cracked osteons were few and there was no visible external cracking observed on the periosteal surface.

Discussion

There are two primary hypotheses on the causality of bacterial micro-bioerosion to bone tissue microstructure. First, that the tissue alteration is commenced by soil micro-organisms in the burial environment, post-skeletonization (Piepenbrink, 1986 and Piepenbrink, 1989; Marchiafava et al., 1974; Hackett, 1981; Hanson and Buikstra, 1987; Yoshino et al., 1991; Grupe and Dreses-Werringloer, 1993). Self-destructive enzymes are released following autolysis, opening the soft tissues, and, dependent on the chemistry and biochemistry of the surrounding soil macro- and microflora, microorganisms gain access to penetrate bone (Child, 1995).

The second hypothesis suggests bacterial micro-bioerosion in bone is produced by endogenous transmigration of gut bacteria into the post-mortem vasculature to the internal cortical structures of bone (Bell et al., 1996, Jans et al., 2004, Guarino, Angelini, Vollono, and Orefice, 2006; Nielsen-Marsh et. al, 2007; Hollund et al., 2012; White and Booth, 2014). Within the first few days after death, the decay of an organism's immune system and mucosal membranes facilitate putrefaction and the transmigration of endogenous bacteria (Polson and Goe, 1985; Janaway, 1987; Gill-King, 1997). Differing post-mortem treatments, such as rapid extraneous soft tissue loss, can expose the skeleton to diverse levels of putrefaction, reducing the autolytic decomposition experienced by bone and amount of bacterial micro-bioerosion (Bell et al., 1996; Jans et al., 2004; Parker Pearson et al., 2005; Hollund et al., 2012; White et al., 2014; Nielsen-Marsh et al. 2007). Findings from an experimental study of buried neonate and juvenile pig carcasses reveal bone microstructure in neonatal carcasses to be absent from micro-bioerosional change, whereas the older carcasses showed intensive micro-bioerosional diagenesis (White et al., 2014). This is likely related to the development of the gut microbiome developed at birth (Mackie, Sghir, and Gaskins, 1999; White et al., 2014).

Significant differences in microstructural preservation were observed between the adult and sub-adult samples and there are a number of possible reasons these differences are being observed. First, it is probable differential interment treatments of juvenile remains influenced the progression of micro-bioerosion. According to Boz and Hager (2013; 2014), both infants and neonates had the most variability of interment location compared to adults. Neonates were found interred in less common areas, where few adults have been found, such as side-rooms, foundational layers of buildings, and near ovens. No adults have been found in side-rooms or building foundation layers. Their analysis revealed the majority of the side room burials were neonates (83%) and a high proportion of juveniles in foundation layers (73%) (Boz and Hager, 2014). External areas such as middens have yielded several neonates and few adults. Neonates were frequently found interred in baskets or containers and not disturbed post-interment evidenced by the lack of commingled remains. Analyses of headless skeletons excavated at Çatalhöyük show no evidence for taking the skulls of neonatal or preterm skeletons (Boz and Hager, 2014).

Differences in microstructural preservation between sub-adult and adult age groups could also be attributed to variations in mineral densities of bone between juvenile and adult remains. Bone mineral content in children tends to regress at the beginning of the post-natal period and develop with age (Guy, Masset, and Baud, 1997; Saunders and Hoppa, 1993). In a comparative study of human and animal bones by Robinson et al., (2003), human rib tissues were observed to have greater porosity measurements than the animal tissues, with the exception of immature domestic animal bone which exhibited higher porosity than their adult counterparts. It is possible the increase of diagenesis in the juvenile remains is related to differences in pore network micro-architecture (Bala et al., 2016). In a study by Lefèvre et al. (2019), significant differences between bone mineralization, crystallinity, and carbonation were observed between fibulas of adult and juvenile cortical bone samples with these properties inferior in juvenile tissues. Demineralization during diagenesis disrupts the protein mineral, rendering the collagen susceptible to hydrolysis, leading to a general increase in porosity and average pore size. Due to the delicate fragility and small size of sub-adult tissues it is likely their microstructure may be more susceptible to diagenesis and taphonomic weathering (Von Endt and Ortner, 1984; Zapata, Pèrez-Sirvent, Martínez-Sánchez, and Tovar, 2006; Hanson et al., 1987). Additionally, the difference in preservation between old age adults and the younger adult categories can be associate to findings of age related bone loss in ribs as endosteal resorption exceeds periosteal formation (Sedlin, 1964; Pirok et al., 1966; Pavon et al., 2010) and decrease in intracortical porosity (Agnew and Stout, 2012).

Our results indicate a lack of statistically significant differences in the preservation of bone tissue between males and females. This may be supported by previous findings that post-mortem manipulation in both sexes, is an interment feature of the site (Boz and Hager, 2014; Hodder 2005). The small sample size of old age adults, comprised of five males and five females, is a limitation to the study. It is possible no statistically significant differences were observed between these groups because the sample size was too small and lacked statistical power. There is the potential that differences in preservation between males and females of the old age category may become more distinct with a larger sample. All old age males and females were assigned scores that ranged between zero to two. The young adult and mature adult categories tended to be better preserved than the old adult group. All individuals assigned a score of 4 or above (>85% bone preserved) were within the younger adult age categories.

The majority (n=156) of the sample was poorly preserved and assigned an OHI score of two or less; (<33% preservation). Only six individuals in the sample received an OHI score four or five (>85% preservation). Four of these individuals had the presence of pathological conditions and one had their head removed. A sixth individual (Sk. 1424) who was assigned an OHI score of four had the presence of black staining and fibers on the skeleton. It is possible the poor preservation of the sample can be attributed to the fragile nature of rib elements and is a limitation to the study. The rib may be more sensitive to diagenetic processes due to lower levels of calcium and phosphorus than other, more robust, long bones like the femur (Lambert, Vlasak, Thometz, and Buikstra 1982). Archaeological micro-bioerosional studies may favor the utilization of robust long bones such as the femur, due to their thick cortex, high cortical content, survival rate in the archaeological record, and ability for DNA and isotope sampling. However, in the interest of conservation and the preservation of materials, destructive histological analysis of long bones is not ideal. The histological study of ribs is useful, as it requires minimal tissue sampling and specimen preparation. Furthermore, the examination of ribs facilitates age assessment using histological protocols (Stout and Paine, 1992; Stout, Dietze, Işcan, and Loth, 1994; Stout, 1986; Stout and Lueck, 1995; Stout and Teitelbaum, 1976), as well as more detailed microstructural analyses of metabolic health (Agnew and Stout, 2012; Agarwal and Stout, 2003).

With the knowledge that individuals in this study were subject to variable early post-mortem treatments (Mellart, 1964; Haddow and Knüsel, 2017; Pilloud et al., 2016; Boz and Hager, 2013; 2014), the skeletal microstructure would be expected to have diverse levels of bacterial attack if an individual was not interred immediately after death. The six individuals with the best preservation, relative to the rest of the sample, were documented with indications of pathological conditions or had body parts moved with the exception of Sk. 1424.

There were no significant preservational differences observed in the bone microstructure of individuals displaying by pathology or dismemberment. Only five individuals of the sample had intact bone microstructure. Four of the six individuals assigned a score of four or greater were documented with the presence of pathological conditions including congenital or neuromechanical abnormalities of the skeleton, inflammatory bone deposits, and degenerative diseases (Sk. 10813, Sk. 8410, Sk. 2056, and Sk. 2886). Sk. 10813 was a large male interred with burial goods and had the presence of a bony growth at bregma, received an OHI score of five (Fig. 6). The three remaining individuals documented as having a pathological condition and received a score of four. Sk. 8410, a mature adult male with degenerative joint disease of the spine, pelvis and hands, was interred with four others. Sk. 2056, a mature adult male was recorded with the left leg possibly disarticulated, osteophyte growth, eburnation and degeneration on cervical vertebrae (C3-C4), and degenerative changes on the hamate and metacarpals indicating hand injury. He was interred above a female (Sk. 2058). Sk. 2886, a young adult male, had the presence of fractures to multiple bones of the upper thorax and lateral incisor, and Scheuermann's asymmetry of dorsal vertebrae. It is possible the presence of pathological conditions acted as a safeguard against bacterial micro-diagenesis in these individuals. Archaeological research of pagetic bone tissue microstructure showed extensive regions were unaffected by bacterial micro-bioerosion (Bell and Jones, 1991) and chronic inflammatory diseases and bone forming pathologies, such as treponemal disease, tend to stimulate osteoblastic action increasing preservation in archaeological bone. Additionally, it has been observed that when mineralization is impaired by pathological attack, the density of bone microstructure is impacted (Boyde, Hendel, Hendel, Maconnachie, & Jones, 1990; Boyde, Maconnachie, Reid, Delling, & Mundy, 1986; Boyde & Jones, 1983). As there was no distinct signature in dismembered, pathological, or primary undisturbed burials of the sample, additional research is necessary to define the causative agents of bacterial micro-bioerosion.

(Figure 6 here)

Conclusion

The objective of this study was to compare preservational differences between groups demarcated by biological sex and age and between primary undisturbed burials and individuals with indications of dismemberment, decapitation or pathological condition. Our findings reveal differential preservation rates between adult and juvenile age groups with less bone microstructure intact in the juvenile remains. Investigation between individuals with indication of body parts removed or pathological condition did not find differential preservation when compared to the primary undisturbed interment. These findings may assist our interpretation of the lifeways at Çatalhöyük and of the post-mortem burial history of an individual in a terrestrial environment. Further research into the mechanisms driving bacterial micro-bioerosion is needed to confirm the source of bacterial micro-bioerosion.

In histological studies such as this one, it is important to develop an understanding of whether observed differences in bone microstructure can be attributed to differences in mortuary treatments, and if the integrity of bone microstructure is influenced by age or pathology. A limitation and future research objective is the further analysis of the whole sample, through use of a more robust specimen, such as the femur. It is possible the poor preservation observed in the rib is due to the fragility of the bones low mineral content and high porosity, therefore, ribs may not fully represent the degree of post-mortem change of an individual due to their delicate cortical structure. Further research of the rib sections in this sample, have the potential for additional analysis such as the identification of specific inclusive materials that have penetrated into the bone microstructure using scanning electron microscopy, application of X-ray computed micro-tomography to estimate percentage of remineralization, mercury intrusion porosimetry to quantify the amount of microporosity, and application of the Cracking Index to quantify cracked osteons to provide a more robust review of diagenesis at Çatalhöyük.

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Figure 1. Google earth image showing geographic location of Çatalhöyük.

Figure 2. Illustration of Çatalhöyük East and West mounds and excavation locations (AncientLocations.net)

Table 1. Individuals Interred with Indications of Dismemberment and Decapitation

Caption: Burial data of individuals sampled for histological assessment collected from the Çatalhöyük archive reports including indication of dismemberment, decapitation (skull indicates both cranium and mandible), pathology, biological age and sex, burial position and deposition, and excavation area. Recording of NA for burial position and burial deposition indicates when this information was not documented in the Çatalhöyük archive report or project database.

Table 2. Individuals Interred with Indications of Pathology

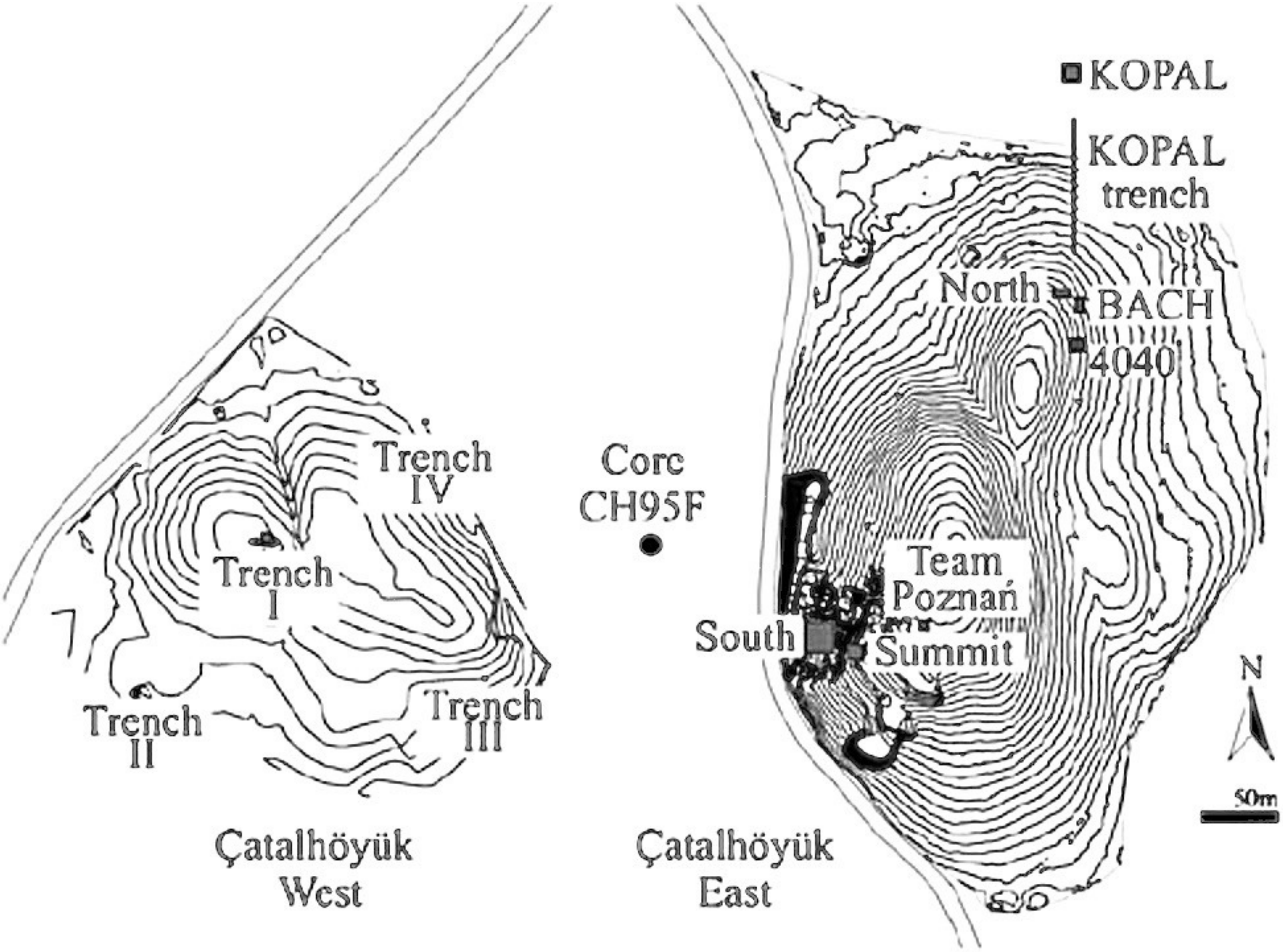
Table 3. Oxford Histological Index modified from Millard (1993) and Hedges et al. (1995).

Figure 3. 89% of the sample was assigned an OHI score of zero or one (< 15% bone intact)

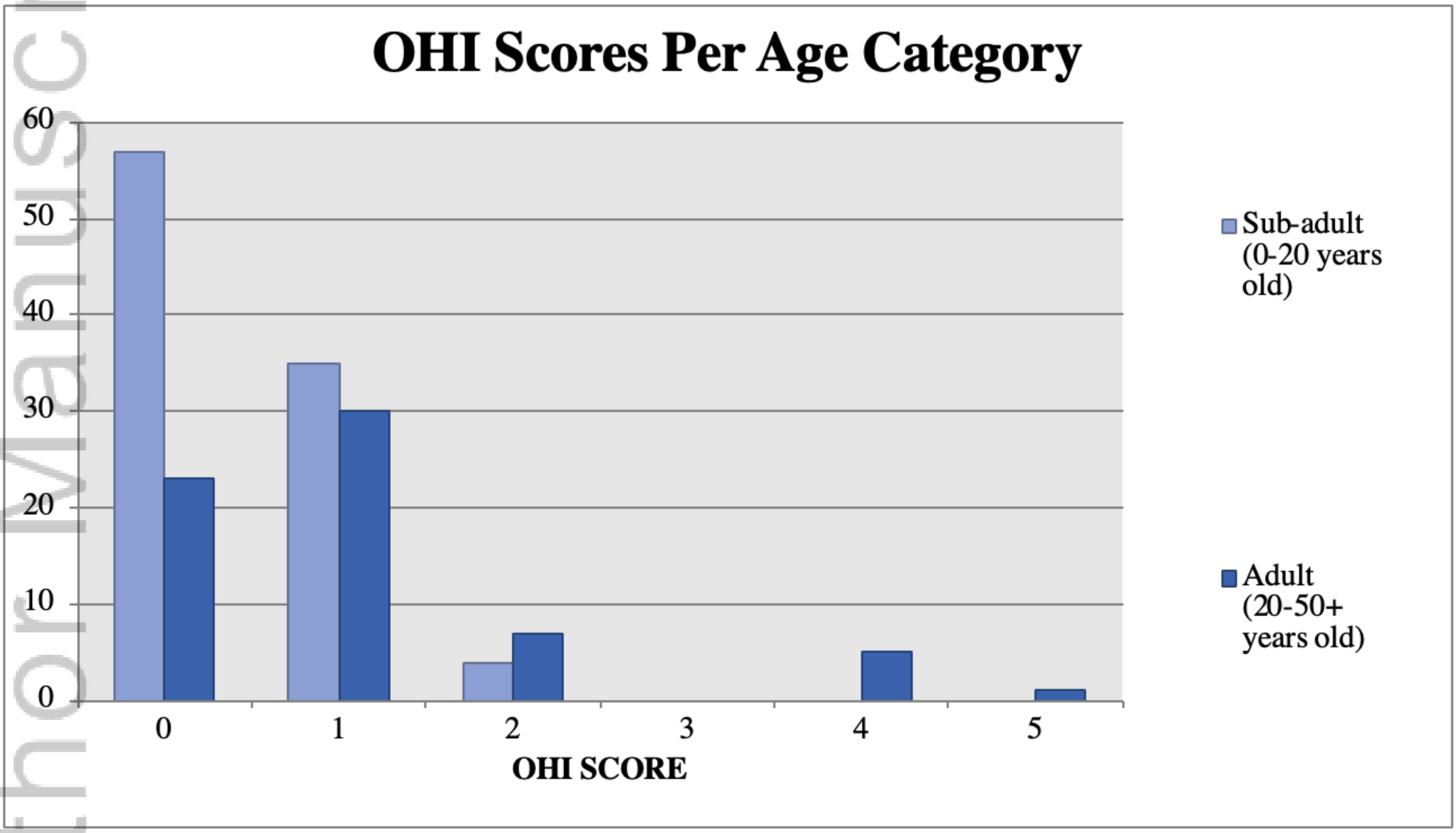
Figure 4. Frequency of OHI scores within adult and sub-adult age categories.

Figure 5. Individuals recorded with pathologies and corresponding OHI Scores

Figure 6. Top: Sk (10813) adult male assigned an OHI score of 5 (>95% of bone intact). Bottom: Sk (4593) Young adult male assigned an OHI score of 0 (<5% bone intact). Arrow indicates remineralized osteon. Haversian canal is indicated by the star.



OA_2930_Fig 2.JPG



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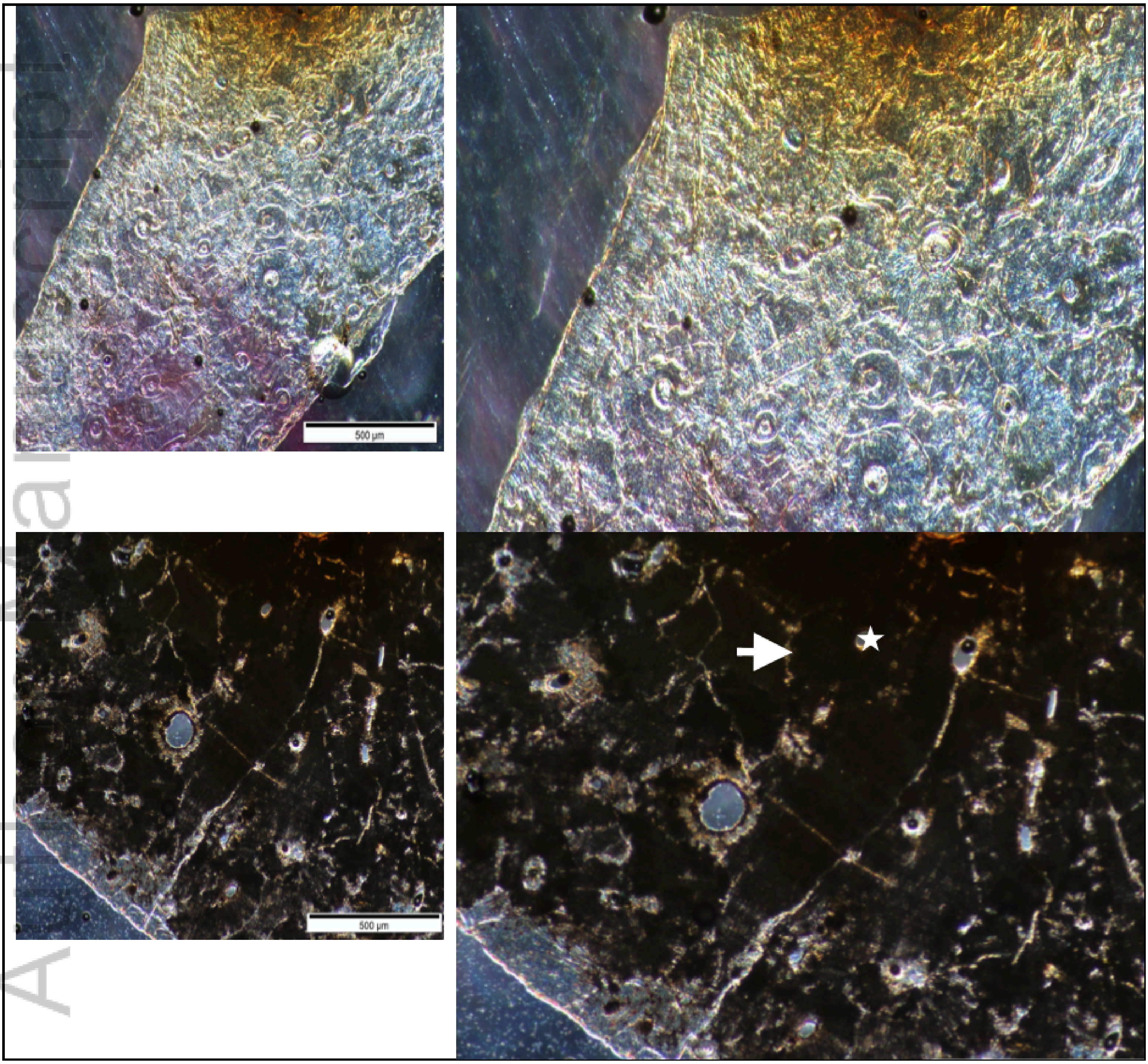
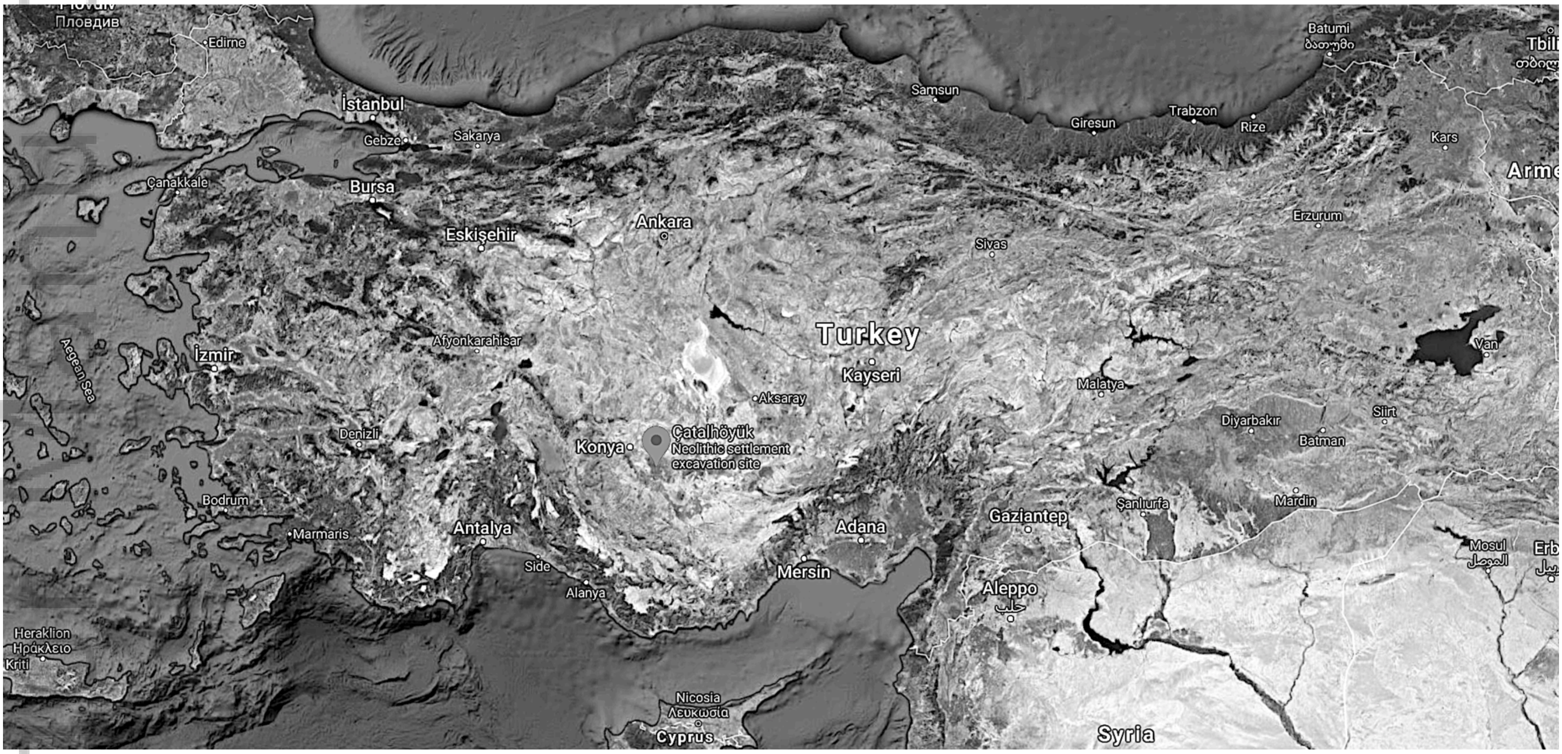


OA_2930_Fig 6.png



OA_2930_Figure 1.png