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Black Fish Bones in Waterlogged Deposits: The Case of the Neolithic Lake Settlement of Dispilio, Greece

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ABSTRACT: The identification of burnt bones in archaeological sites is important as it provides evidence of human-processing activities and fire-related episodes. Past zooarchaeological analyses of burnt fish and mammal bones were mostly based on macroscopic features, such as bone color and structure, and microscopic features, such as crystallinity. Such studies, however, have shown that black coloring of bones can be caused not only by burning, but also by natural mineral staining. Therefore, it is essential to develop analytical techniques for the identification of burnt bones. This paper presents preliminary results from an interdisciplinary study on the possible causes of the «black-colored bones» recovered at the Neolithic lakeside settlement of Dispilio, Greece (5500–3500 B.C.). The frequent occurrence of charcoal and burnt cultural remains in the lower layers of the deposit suggested that the first village was destroyed by fire, followed by a period of site abandonment. Nevertheless, although fish bones are often reddish/black in color in archaeological deposits, macroscopic examination of these remains suggested that less than 6% were burnt and that their coloring was caused by waterlogged depositional conditions. These observations are of great significance in reassessing the nature of the so-called «destruction level». Selected fish bones were examined through Optical and Scanning Electron Microscopy (SEM), X-ray Microanalysis (EDXA), and Infrared Spectroscopy (IR). The alterations observed on fish bone histology, mineralogy, chemistry, and crystallinity due to diagenesis and/or possible burning are presented and their correlation to the archaeological context discussed.

KEYWORDS: BURNING, GREECE, LACUSTRINE SEDIMENTS, NEOLITHIC, OXIDE STAINING, DIAGENESIS, OPTICAL MICROSCOPY, SEM, EDXA, IR SPECTROSCOPY

RESUMEN: La identificación de huesos quemados en yacimientos arqueológicos es importante por proporcionar evidencia tanto sobre actividades humanas de procesado como de episodios de incendio. Análisis zooarqueológicos previos sobre huesos quemados de peces y mamíferos se concentraron sobre todo en rasgos macroscópicos como el color y la estructura y rasgos microscópicos como la cristalización. Tales estudios, no obstante, demuestran que las coloraciones negras en los huesos pueden no sólo ser debidas a termo-alteraciones sino también a tinciones minerales. Es por ello esencial desarrollar técnicas analíticas para la identificación de huesos quemados. Este trabajo presenta los resultados preliminares de un estudio interdisciplinar sobre las posibles causas de los «huesos negros» recuperados en el asentamiento lacustre neolítico de Dispilio, Grecia (5.500-3.500 a.C.). La frecuencia de carbones y restos culturales quemados en las capas más bajas del depósito sugieren que la primera ocupación fue destruida

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por el fuego y seguida por un periodo de abandono. No obstante y aunque con frecuencia los huesos de peces son de un color negro-rojizo, el examen macroscópico de estos restos apunta a que menos del 6% se encontraban quemados y que su coloración era debida a las condiciones del depósito saturado en agua. Estas observaciones son de gran importancia para valorar la naturaleza del llamado «nivel de destrucción». Los huesos de peces seleccionados fueron examinados a través de microscopía óptica y de barrido (SEM), microanálisis radiográficos (EDXA) y espectroscopia de infrarrojos (IR). Las alteraciones observadas en la histología, mineralogía, química y cristalografía debidas a la diagénesis y/o a posibles alteraciones, se presentan y valoran en función del contexto arqueológico.

PALABRAS CLAVE: TERMOALTERACIÓN, GRECIA, SEDIMENTOS LACUSTRES, NEOLÍTICO, TINCIÓN POR ÓXIDOS, DIAGÉNESIS, MICROSCOPÍA ÓPTICA, SEM, EDXA, ESPECTROSCOPIA IR

INTRODUCTION

Identification of cooked and burnt faunal remains due to controlled or accidental fire is one of the major concerns of archaeological research (James, 1989; Asmussen, 2009). Apart from material and organic evidence of fire at an archaeological site (hearths, ash, charcoal fragments), the identification of burnt bones has commonly been based on changes in their color, crystallinity, shape, histology, as well as the presence of cracks and black charring with characteristic chemical properties. The most easily identified color used in the literature as indicative of burning, is black (Shipman *et al*., 1984). Studies have shown that, when burnt, bone color turns black due to the carbonization of the bone's organic matrix. A major change in bone mineralogy occurs only when bones are heated over 645ºC and turn white in color (calcined). At this stage, bones become highly fragmented due to lack of mechanical coherence and, as a result, they are rarely preserved at the site (Shipman *et al*., 1984; Brain, 1993; Nicholson, 1993, 1995; Stiner *et al*., 1995; Shahack-Gross *et al*., 1997).

Black coloring of skeletal remains can be caused not only by burning, but also by mineral staining, decomposition of the organic components of bone, or a combination of any of the above (Shahack-Gross *et al*., 1997; Stathopoulou *et al*., 2004; Michel *et al*., 2006; Marín Arroyo *et al*., 2008; Stathopoulou, 2008). Unlike burning, mineral staining of buried bone is a diagenetic process, which involves mainly iron (Fe) and manganese (Mn) oxides. Manganese is responsible for the black coloring of skeletal remains, while iron leads to the orange-yellow tones (Marín Arroyo *et al*., 2008). Studies have shown that possible causes of oxide staining include the following processes:

1. *Activity of Mn-, Fe-oxidizing bacteria*. The optimal conditions preferred by these bacteria are moist environments that are aerobic with near-neutral pH. The bacteria concentrate metals from their environment, most probably from water. The bones may serve as a source of nutrients for the Mn-oxidizing bacteria (Shahack-Gross *et al*., 1997; Marín Arroyo *et al*., 2008).
2. *Alterations to the degree of acidity/alkalinity (pH) and Redox potential (Eh)* of the environment, which determine the behavior of the different manganese and iron forms. An increase in pH and intensification of oxidizing conditions (associated with dry, well-aerated soils) could favor a change in Mn towards insoluble forms and the production of Fe oxides, due to the oxidation of pyrite. The presence of bicarbonate/carbonate ions and/or bacteria may enhance these processes (Hill, 1982; Vuorinen & Tuovinen, 1987; Marín Arroyo *et al*., 2008; Caldeira *et al*., 2010).

The study of bone diagenesis and thus of the origin of «black bones» is further complicated by the nature of bone itself, since bone is a heterogeneous, composite material, constructed from an intimate association of organized collagen fibres and crystals of a poorly crystallized carbonate hydroxylapatite (Posner, 1985; Person *et al*., 1995; Trueman & Martill, 2002). In all, bone diagenesis combines various processes that eventually lead to bone preservation and long-term stability through geological time and can be recognized by a num-

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ber of alterations which depend greatly on the existing local geochemical conditions (Marean, 1991; Dauphin *et al*., 1999; Hedges, 2002; Clarke, 2004; Trueman *et al*., 2004).

Although color-based identification is the most common method used in zooarchaeological research, its results have been questioned by numerous authors who, instead, have proposed the application of modern analytical techniques in order to distinguish between burning and staining. These techniques included the use of Optical Microscopy, Scanning Electron Microscopy (SEM), Transmittance Electron Microscopy (TEM), X-Ray Fluorescence (XRF), X-Ray Diffraction (XRD), and Infrared Spectroscopy (Taylor *et al*., 1995; Shahack-Gross *et al*., 1997; Koon *et al*., 2003; Stathopoulou *et al*., 2004; Michel *et al*., 2006; Enzo *et al*., 2007; Hanson & Cain, 2007; Lebon *et al*., 2008; Marín Arroyo *et al*., 2008; Piga *et al*., 2008, 2009; Stathopoulou, 2008; Chadefaux *et al*., 2009; Thompson *et al*., 2009; Reiche, 2010). A straightforward analytical protocol concerning the presence of burning in osteological material, however, still remains elusive to this day (White & Folkens, 2005; Weiner, 2010; a recent overview of the history of research and various methods in Gonçalves, 2012).

Studies on burnt fish remains

Experimental studies on thermally modified fish bones have identified a variety of changes in bone color, density, and element survival, and differential bone survival depending on pre- and post-depositional burning events (Nicholson, 1995; Steffen & Mackie, 1995; on similar experiments with human bones, Buikstra & Swegle, 1989). These approaches have highlighted various aspects of color modifications related to fish remains. Most of the investigations, however, involved intense heating of the bones that resulted in calcined remains. Richter (1986) has demonstrated that heating of fish at temperatures as low as 60ºC (mostly related to cooking) may lead to collagen loss. These alterations, however, are not macroscopically observable. As a result, the distinction between fish bones heated in low temperatures (cooked) and the appearance of unburnt but dark-colored fish bones still remains unresolved, especially within the unique taphonomic conditions of lake environments (for an overview, Schlichtherle & Wahlster, 1986; on groundwater diagenetic processes, Hedges & Millard, 1995).

In addition, the proven presence of Mn or some other metal oxide does not necessarily preclude the bones from being burnt, as they could be black due to burning and chemical staining (Shahack-Gross *et al*., 1997). Moreover, though bone burning may result from human cooking activity, we cannot disregard the possibility that it might have resulted from accidental fire (Bennett, 1999). These are some of the problems that will be discussed in this paper.

The case study:
Environmental and archaeological setting

The archaeological site of Dispilio, located on the southwest shore of Lake Orestis, Kastoria (NW Greece), is at present the sole Neolithic lake settlement that has been discovered in Greece (Figure 1). The excavations at Dispilio (University of Thessaloniki, dir. Prof. G. H. Hourmouziadis) have uncovered an area of over 1400 m^2 and a 2 m thick stratigraphic sequence of prehistoric deposits dated from the late Middle Neolithic to the Final Neolithic (ca. 5500–3500 B.C.) (Hourmouziadis, 2002). The site's initial occupation took place in dwellings built on wooden platforms in the shallow waters of the lake (phase early C, ca. 5500–5300 B.C.; Figure 2). These early deposits are currently waterlogged, resulting in a large number of well-preserved organic remains being recovered. The high incidence of charcoal and burnt remains indicates that the first village was destroyed by fire, cultural debris fell in the water, and the site was possibly abandoned for a period of time (phase late C, ca. 5300–5200 B.C.; Figure 2) (Hourmouziadis, 2002; micromorphological layer Ga, according to Karkanas *et al*., 2011: Figure 4). This destruction layer produced a broad shoaled area. In the subsequent phase (phase B, ca. 5200–5000 B.C.), the villagers settled on this mudflat terrace, and activities took place both on raised platforms in the littoral zone and on dry land.

The fish remains at Dispilio

Fish remains and mammals bones were recovered in large numbers (Phoca-Cosmetatou, 2008;

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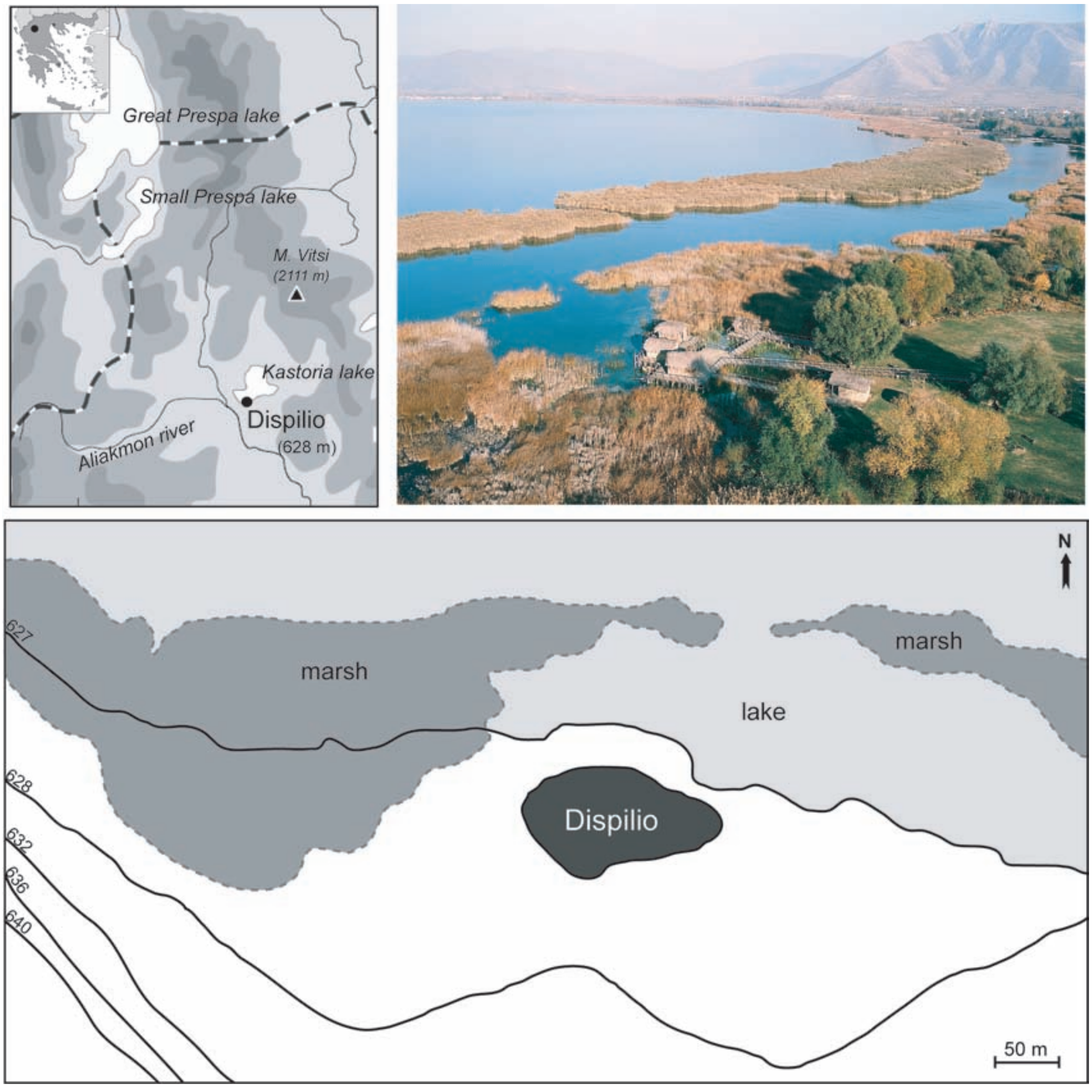

FIGURE 1

Location of Dispilio in N. Greece, modern geomorphological setting (based on Karkanas *et al*., 2011) and archaeological park with reconstruction of the Neolithic village (courtesy of: Dispilio photo archive).

Theodoropoulou, 2008). Preliminary analysis of the fish remains recorded more than 20,000 fragments so far (Theodoropoulou, 2008, and study in progress). The identified fish remains included representatives from two families of freshwater fish: Siluridae (catfish) and Cyprinidae (carps). Among the catfish, a single species was identified, *Silurus glanis*. Among the Cyprinidae, 10 species were identified, including *Cyprinus carpio*, *Abramis brama*, *Scardinius erythrophthalmus*, *Alburnus alburnus*, *Carassius carassius*, *Tinca tinca*, *Rhodeus* sp., *Leuciscus cephalus*, *Barbus* sp., and *Chondrostoma* sp. The wide diversity of freshwater fish and their recovery in large numbers testify to their importance as a regular food resource for the Neolithic inhabitants of Dispilio (Theodoropoulou, 2008 and Figure 3).

Examination of the spatial distribution of fish remains exhibited differences between the excavated areas (Figure 3). The highest concentrations

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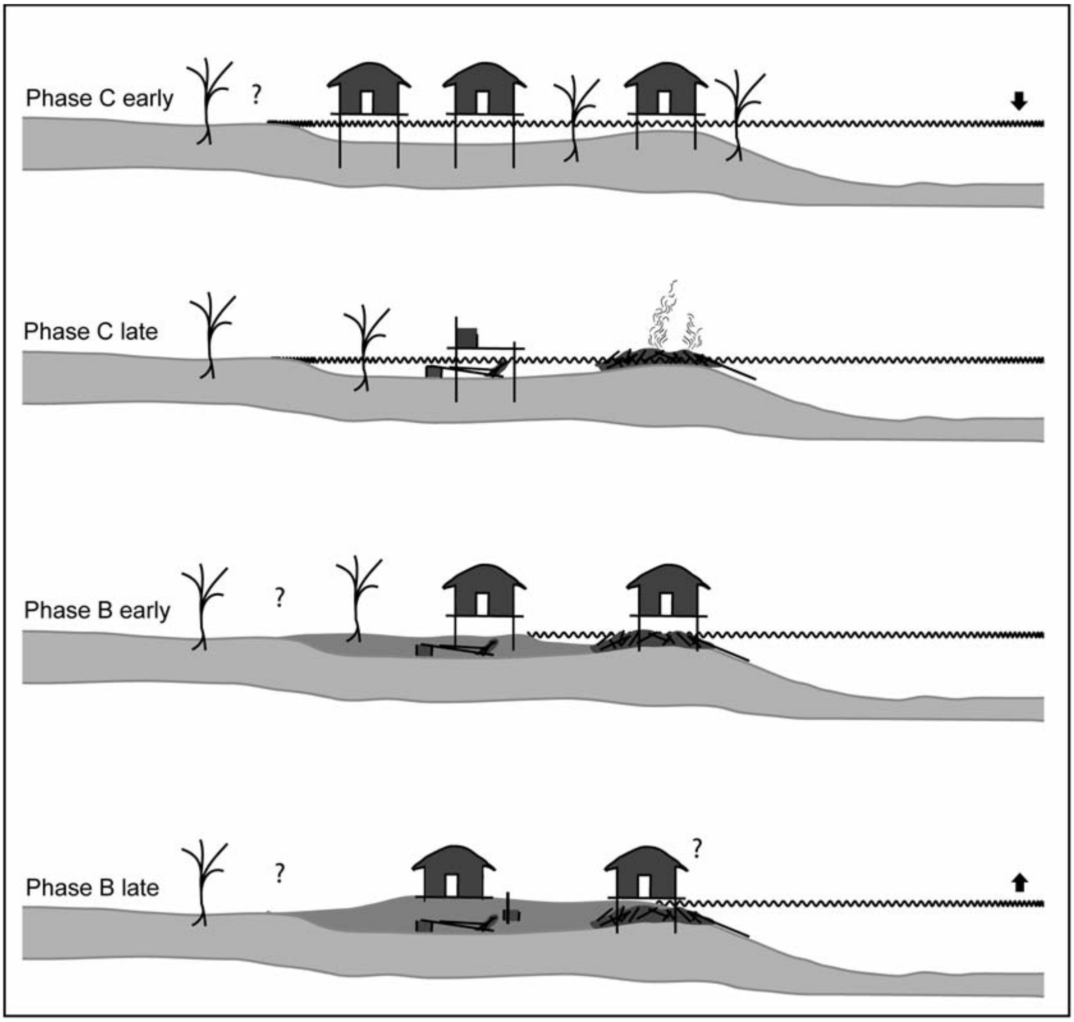

FIGURE 2
Succession of early phases of Dispilio (based on Karkanas *et al*., 2011).

of fish remains were recovered in the NW and SW parts of the East Sector. In area Δ08, a combination of cranial and post-cranial bones from catfish (*Silurus glanis*), *Cyprinus carpio*, and other cyprinids were recovered (*Abramis brama, Scardinius erythrophthalmus, Alburnus* sp., *Carassius* sp., *Tinca tinca, Rhodeus* sp., *Leuciscus cephalus, Barbus* sp., and *Chondrostoma* sp.). Study of growth incremental annuli (Theodoropoulou, 2007, 2008) showed that fish found in area Δ08 were caught during spring through autumn. Bones in this area seem to have been occasionally affected by fire, as suggested by the black-gray color affecting all sides of the bones. The fish remains recovered in area Δ04 included mainly cranial bones and thoracic vertebrae of carps and catfish. Fish from this area were caught during various fishing seasons, as seasonality studies suggest (Theodoropoulou, 2007, 2008). Interestingly, the large catfish remains systematically lacked caudal vertebrae (late phase B). This latter observation might suggest a special preparation method used for the caudal parts of fish (Theodoropoulou, 2007, 2008). Bones from this trench exhibited cut marks or were affected by trampling. In all, the spatial distribution of the fish remains at the site

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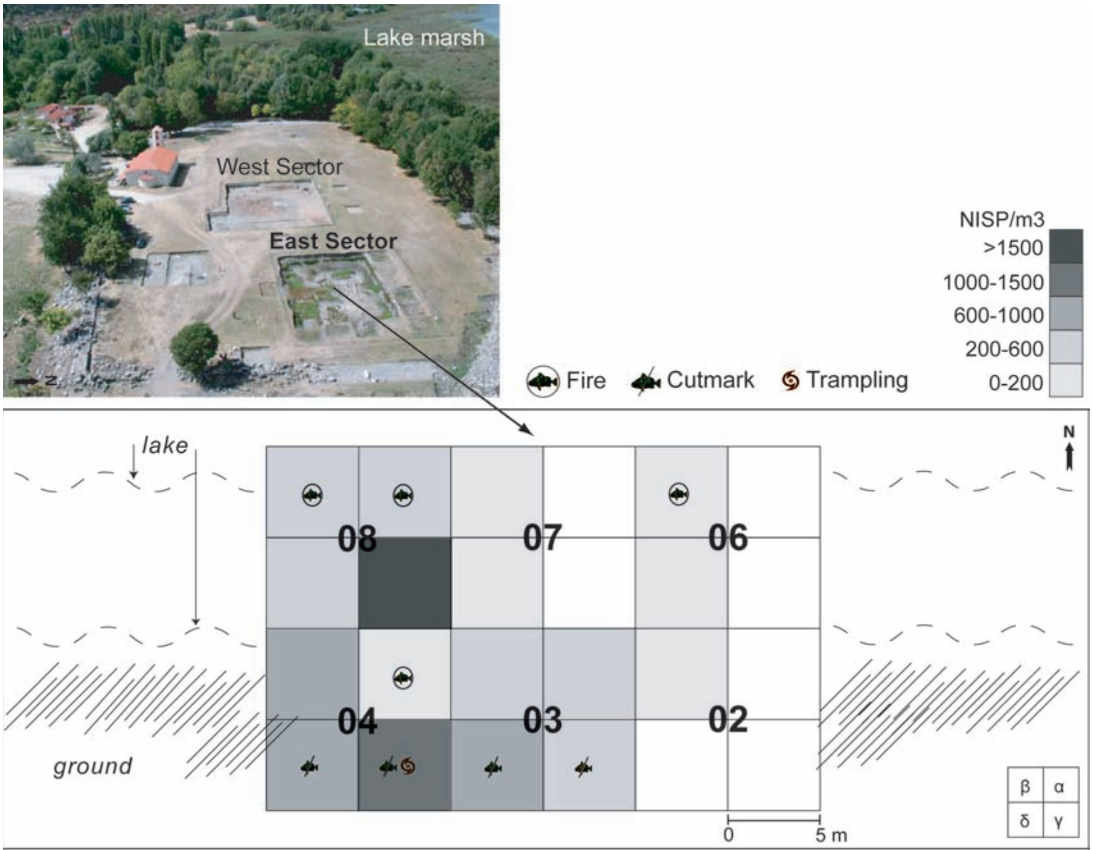

FIGURE 3

Aerial photo of the excavation area (courtesy of: Dispilio photo archive) and spatial distribution of fish remains in the East Sector.

exhibited differences between excavated areas in their relative abundance, taxonomic composition, skeletal element representation, state of preservation, and signs of burning. The observed differences might suggest different activity areas related to fish management, as follows:

- In area Δ08: cooking of catfish, carps and smaller cyprinids (probably fresh), and possibly discard of some of the bones into hearths after consumption. This conclusion is supported by the observation that most of the fish remains (65%) recovered from the lower layers (Phase C) of this trench were recorded as undoubtedly burnt. In addition, suitable cooking vessels were recovered at this part of the site.
- In area Δ04: fish were probably processed for storage. The butchering method applied included cutting and removal of caudal parts of larger carps and catfish. These parts may have been processed differently or consumed in other areas of the settlement.

Additional lines of evidence also seem to support the idea of two separate areas of activity, especially with respect to heat-related episodes. Archaeobotanical analysis has identified two distinct groups of plant remains in the NW and SW trenches (Margaritis, 2011). In the SW area, a concentration of both emmer and einkorn grains in combination with lentils were recovered and identified as, possibly, cooking refuse. In the NW area, complete ears of emmer wheat were recovered intact and were heavily affected by fire. In addition, thousands of waterlogged stored blackberry seeds and hazelnut were recovered from this area (Margaritis, 2011). This latter part of the site also produced large amounts of unburnt and macro-

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scopically burnt mammal bones, as well as large cooking vessels. Architectural evidence from these two areas followed the same pattern of two distinct zones: area Δ04 yielded fewer architectural remains than area Δ08 and might represent an open space (based on observations in the excavation diaries).

Identification of fire events and burning at Dipilio

Micromorphological analysis at Dipilio (Karkanas *et al*., 2011) demonstrated that, although there was a level with clear signs of burning (including burnt fish remains) (microfacies Ga) in late Phase C, the destruction level was identified also in a slightly higher phase in the stratigraphy (microfacies Fa). It seemed that, following the burning episode, not all the huts collapsed simultaneously. Some fell *en masse* into the water, an occurrence that would have then extinguished the fire (as evidenced by half-burnt timber pieces or burnt only on the outer surface/one side). Moreover, the preservation of charred plant material suggested that the fire would not have exceeded 450ºC and did not last very long, possibly a few hours; otherwise, no plant remains would have been recovered (Margaritis, 2011).

The collapse of construction material in this shallow environment acted as a sediment trap for finer charred material and for some unburnt seeds preserved in the waterlogged conditions (Karkanas *et al*., 2011; Margaritis, 2011). Micromorphological analysis further suggested rapid burial, restricted bioturbation, and absence of anthropogenic activities on the surface. These observations were of great significance in (re)assessing the nature of the so-called «destruction level» and the geological processes which produced it.

Aims of the study

The Neolithic settlement of Dispilio provided a suitable case study as there were multiple possible causes to explain the black color of the bones, including the destruction of the village by fire and subsequent sealing of remains in waterlogged deposits. Analysis of the faunal remains highlighted difficulties in interpreting the coloring patterns observed on the mammal and fish bones, both of which often exhibited light red to dark brown coloring affecting all sides of the bones. Some concretions and dark stains were observed on both the external sides (i.e. the external surface of some cranial elements, usually covered by a thin layer of flesh, that can be easily affected by fire) and the sides normally covered by flesh (e.g., internal surface of cranial bones or vetebral column). The latter observation ruled out the hypothesis that the dark coloration was caused by cooking. These bones were retrieved from the early occupation layers, which were considered to have been sealed by fire destruction. Moreover, macroscopic analysis of the bones has not been sufficient, in itself, for distinguishing burning from staining, given that the fish bones were recovered from a soil matrix that was very rich in charcoal and ash, and, at the same time, waterlogged and organic. The latter tend to stain bones black. Phoca-Cosmetatou (2008) suggested that the stains observed on the mammal bones could rather be due to the high concentration of charcoal in the soil than to actual burning by the destruction episode, which was not too long or involved temperatures too low for the bones to have been affected.

The present study is aimed toward a combined zooarchaeological and chemical analysis of a sample of fish bones in order to understand the characteristic black staining and coloring on the bones recovered at Dispilio and to differentiate the effects of possible pre- or post-depositional burning as opposed to effects caused by the lake's post-depositional organic environment.

MATERIALS AND METHODS

Identification of burned bones

Evidence of burning was analyzed first by using the «traditional» macroscopic observations under dry conditions that included changes in bone color, modification observed on the structural surface of the bone, and surface texture (Behrensmeyer, 1975; Shipman *et al*., 1984; Nicholson, 1993; Asmussen, 2009).

Based on observations of the fish and mammal faunal assemblage from the site, the bones were divided into seven groups (Table 1, Figure 4a):

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Fish bone sample	NISP/ SAMPLE	TAXONOMIC IDENTIFICATION	Dispilio phase	Colour	Type	Texture	Surface alterations
DispFi_01	>5	*Cyprinus carpio*	C-late (lacustrine)	dark brown to black (superficial and depth)	3	soft, mat	
DispFi_02	>5	*Cyprinus carpio, Silurus glanis*	C-late (lacustrine)	grayish brown, occasionally grayish black, (superficial and depth), colouration not homogenous	3	soft, mat	
DispFi_03	>15	*Cyprinus carpio, Silurus glanis*	C-early (lacustrine)	orange to light brown (colouration not homogeneous on the superficial surface), grayish brown to black (depth)	2	relatively soft, mat	eroded surface
DispFi_04	>3	*Cyprinus carpio*	B-early (amphibian)	dark orange to grayish brown (superficial), brown to black (depth)	3	hard, mat	
DispFi_05	>15	*Cyprinus carpio, Silurus glanis*	C-early (lacustrine)	orange to dark red (superficial and depth) with brown or greyish spots (superficial), colouration not homogenous	3	soft, mat	flaky and fractured surface
DispFi_06	1	*Silurus glanis*	B-early (amphibian)	dark red to orange with dark stains (superficial), dark brown (depth)	2	soft, shiny spots	flaky surface
DispFi_07	>50	*Cyprinus carpio, Silurus glanis*	C-early (lacustrine)	grayish black (superficial), orange (depth)	3	soft, mat	
DispFi_08	>15	*Cyprinus carpio, Silurus glanis*	C-late (lacustrine)	orange brown with grayish and blackish spots (superficial), orange red (depth)	3	soft, mat	eroded surface

TABLE 1

Macroscopical observations performed on a sample of fish remains recovered from Dispilio (109 NISP). The 'type' assignation of every sample refers to the generic definitions given above; the 'color' description is specific for each sample and thus might differ in detail from its assigned 'type' category.

– Type 1: dark red (Munsell 10R 3/2 dusky red), smooth and shiny, along the whole surface (mainly in phase C).

– Type 2: dark red to brown (Munsell 2.5YR 3/2 dusky red with 2.5YR N2.5/1 reddish black stains), smooth and shiny, flaky surface (mainly in phase C).

– Type 3: dark brown (occasionally gray-black) (Munsell 10YR 4/2 dark grayish), shiny, black stains (mainly in phase C).

– Type 4: lighter red to dark orange (Munsell 7.5YR 6/6-8 reddish yellow to strong brown), with concretions and black stains (Munsell 7.5YR 3/2 to 2.5/1 strong brown).

– Type 5: lighter red to dark orange (Munsell 5YR 5/8 yellowish red to 5YR 4/4 reddish brown), eroded edges and black patches (Munsell 5YR 3/1 very dark grey).

– Type 6: yellow (mammal bones: transparent) with black stains (Munsell 2.5Y 7/4 pale yellow wih 2.5Y 2.5/1 black stains) (mainly in phase B).

– Type 7: yellow, smooth (Munsell 10YR 6/6 brownish yellow/10YR 5/6 yellowish brown to 2.5Y 6/4 light yellowish brown), not eroded (refers to the natural state of the bones).

The fish remains

Eight representative samples (109 NISP, subsequently sub-sampled) were chosen for this study (by T. Theodoropoulou) and were divided into eight groups (Groups DispFi-01 to DispFi-08) for the analyses (Figure 4b, Table 1). The samples covered the two subphases of lacustrine phase C (early-pre-destruction and late-destruction) and the early amphibian phase B (post-destruction layer; see Fig. 2). Special care was taken to include samples covering different stages of preservation and recovered from significant cultural contexts. Color types 1 to 5 characterized the majority of the material retrieved for chemical analysis. Trenches Δ04 and Δ08 were particularly targeted on the basis of their richness in mammal and fish bones, as well as the abundant evidence of anthopogenic activities and taphonomic records, as discussed above.

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FIGURE 4
a. Various types of coloured fish bone samples.

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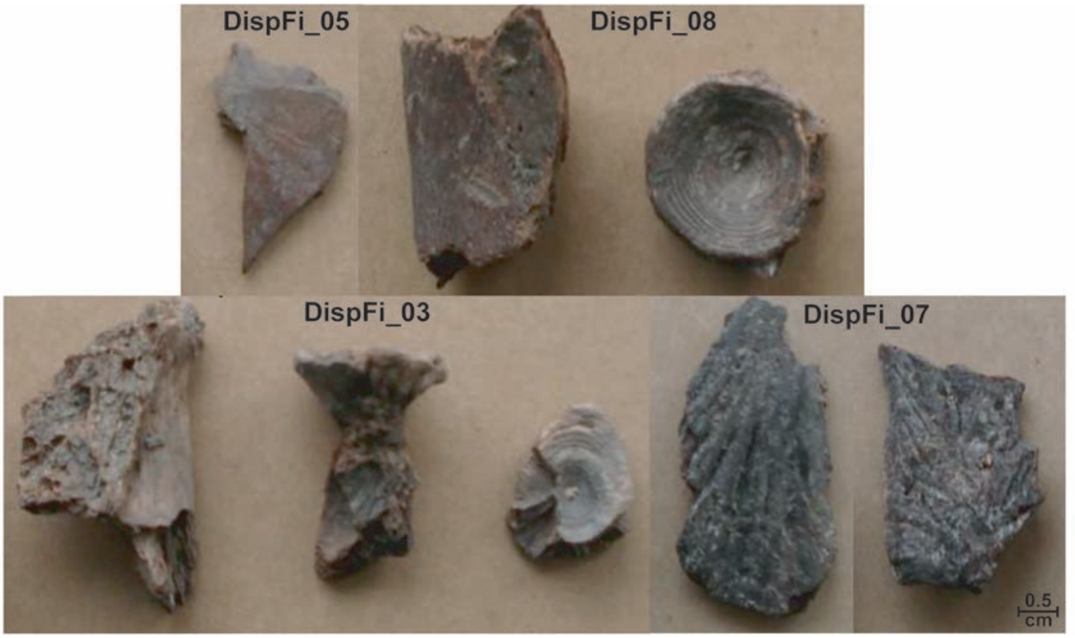

FIGURE 4
b. selection of samples chosen for analysis.

Analytical techniques

The fish remains were examined using the following analytical techniques:

POLARIZING OPTICAL MICROSCOPY: This was used to observe the state of preservation as observed from bone histology, the presence of mineral intrusions, as well as cracking caused from thermal alteration. Microbial activity can also be identified if present.

SCANNING ELECTRON MICROSCOPY (SEM): This method allows for a more detailed observation of bone histology, microbial activity, infilling of voids by secondary minerals, cracking and deformation of bone structure. For the needs of this study, a JEOL JSM-5600 Scanning Electron Microscope, connected to an OXFORD LINK™ ISIS™ 300 X-Ray microanalysis system (Energy Dispersive X-ray Microanalysis-EDX), was used.

X-RAY MICROANALYSIS (EDXA) was combined with SEM and provided qualitative and semi-quantitative chemical analyses at a wide range of magnitudes. It was used in order to trace the possible presence of Mn and Fe (which would reinforce the oxide staining theory) and provide further information on the chemical alterations of bone due to diagenesis. The use of EDXA permits identification of secondary mineral phases found within voids and cracks of the skeletal material.

INFRARED SPECTROSCOPY: We used Mid Infrared Spectroscopy – MID-IR (Attenuated Total Reflectance technique - ATR) and Near Infrared Spectroscopy – NIR. These techniques allow for the detailed identification and study of chemical components of bone mineral (CO_3^{-2}, PO_4^{-3}, OH^-, H_2O) and the identification of bone collagen or its by-products due to its modification by diagenesis or burning. This method also provides information concerning the presence of secondary phases such as metal oxides, calcite, quartz, clay minerals etc. (Stathopoulou, 2008; Stathopoulou *et al*., 2008; Thompson *et al*., 2009).

The MID-IR spectra (525–4000 cm^{-1}) were measured on a Fourier-transform instrument (Equinox 55 by Bruker Optics) equipped with a single reflection diamond ATR accessory (Durasampl IRII by SensIR). The NIR spectra of the same samples (4000–8000 cm^{-1}) were measured via a fiber optic bundle probe operating in the diffuse reflectance mode on a Fourier transform instrument (Vector 22 N by Bruker Optics). Savitzky-Golay second derivative analysis of the

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ATR and NIR spectra had been tuned in order to enhance the resolution of sharp features overlapping with broad bands (Leung *et al*., 1990).

CALCULATION OF THE CRYSTALLINITY INDEX: The crystallinity of our samples was described through the calculation of the «splitting factor» (IRSF). This was calculated according to Weiner & Bar-Yosef (1990) and, as used in Nagy *et al*. (2008), as the sum of the heights of the 605 and 565 cm^{-1} phosphate peaks, divided by the height of trough between them, at about 590 cm^{-1} (Weiner & Bar Yosef, 1990; Surovell & Stiner, 2001; Olsen *et al*., 2008). Changes to this ratio indicate a change in peak sharpness, which in turn indicates alterations in the bone apatite crystallinity. The IRSF value in modern unaltered bones is typically around 2.50-3.25. Studies have shown a correlation between changes in IRSF and burning, observed in increased IRSF values, as the crystal structure of the bone will be altered during diagenesis but also during burning. Therefore, this index has been regarded by many as a valuable tool for archaeological research (Sillen & Hoering, 1993; Person *et al*., 1996; Shahack-Gross *et al*., 1997; Surovell & Stiner, 2001; Enzo *et al*., 2007). Studies have shown, however, that it is often not discriminating enough to distinguish boiled remains (Koon *et al*., 2003).

Sample preparation

All fish bone samples (NISP=109) were documented, photographed and described macroscopically. Small quantities of each sample (NISP=8) were removed in order to be powdered for IR Spectroscopy according to Stathopoulou *et al*. (2008). In addition, small pieces were placed on special stubs to be observed by SEM. Then, the samples were embedded in epoxy resin and sectioned. Thin sections and polished samples were prepared for Optical Microscopy and SEM-EDXA, respectively. The above was done according to Stathopoulou (2008) a the Department of Historical Geology and Paleontology of the Faculty of Geology and Geoenvironment at the University of Athens.

RESULTS

Macroscopical examination of the bones

The bones sampled for this study exhibited a wide range of colors from light yellow, orange, and red to dark brown. Previous studies of the Dispilio faunal assemblages indicated that bone color and preservation could not be used as a diagnostic characteristic of burning since macroscopical examination of the inner part of the bones demonstrated that, even in cases of dark coloration of the outer surface, the inside was invariably yellow (Theodoropoulou, 2007; Phoca-Cosmetatou, 2008). This observation suggested that the bones were actually chemically stained rather than burnt. Independent work on extracting nitrogen and carbon isotopes from fish remains, however, revealed a differential pattern of macroscopical vs. geochemical features. Although light-colored fish bone samples were carefully selected on the basis of color (yellow, no stains) to ensure they were not burnt and would thus still yield collagen, the isotopic work suggested that they had undergone thermal treatment (Vika & Theodoropoulou, 2012). In all, only 1% of the fish bones and 6% of the mammal bones (of the samples studied thus far) were identified as unquestionably burnt, carbonized, or calcined (personal observations of NPC and TT).

Microscopical study

The samples of fish remains examined under a polarizing optical microscope and a SEM exhibited the following features:

a) *Color alterations*. Some bones were dominated by a single color while others appeared to exhibit a series of shades and colors, often connected to their histological features (Figures 5 and 8). The darkest colors usually occurred towards the periosteal area (Figure 5).

b) *Deposition of black-colored material* in spaces throughout the matrix, such as vascular canals, cracks, canals of secondary osteons, and canalliculi (Figures 5 and 6). This material also appeared in a linear form, following growth marks (zones, annuli, arrested growth lines-AGL) (Figures 7 and 8) or as material deposited perimetrically around the bone fragments (Figures 5 and 6). The most characteristic appearance, however, was the dendritic form, which could be observed across the bone matrix, mainly surrounding growth marks or other areas infilled with this black-colored substance (Figure 7). The latter form reinforced our

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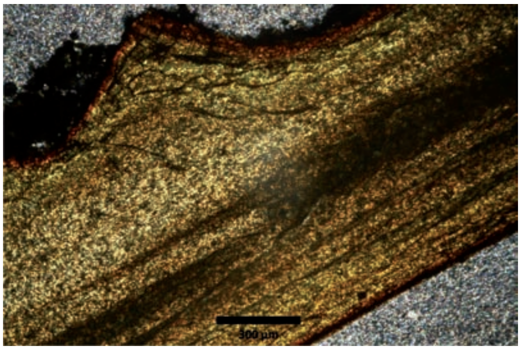

FIGURE 5

Sample DispFi-01 under the polarizing microscope. Different coloured zones within the bone with dark coloured rims and cracks across the edges.

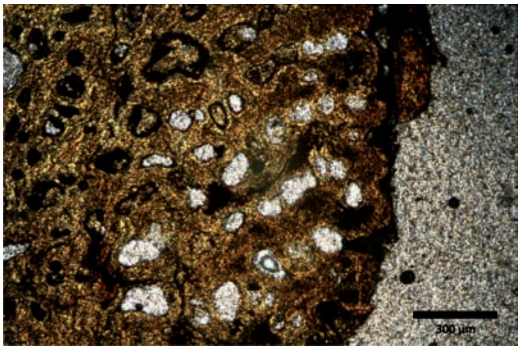

FIGURE 6

Sample DispFi-02 under the polarizing microscope. Canals of vascular bone, infilled with calcite and black coloured deposits.

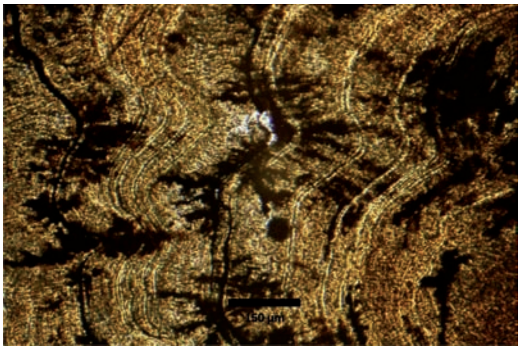

FIGURE 7

Sample DispFi-03 under the polarizing microscope. Dendritic form of black coloured deposits, which spread out from infilled cracks parallel to bone growth lines.

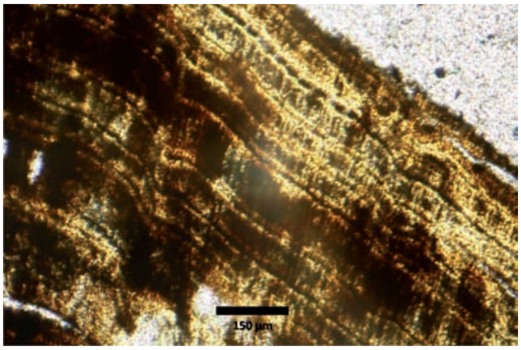

FIGURE 8

Sample DispFi-05 under the polarizing microscope. Growth lines within bone, of different colours. Black coloured deposits, which spread out across the bone, resembling "opaque" infiltrations due to carbon deposition.

suspicion that all the described depositions were due to oxide staining. This was verified through chemical analyses, as discussed below. It was not possible to exclude the possibility of carbon deposition as well, as we had phases that resembled «opaque» infiltrations (Hanson & Cain, 2007; Figure 8).

c) *Cracking and deformation of bone structure.* Micro-cracking was present in all samples and observed in all directions and in different magnitudes. The cracks were parallel and vertical to the bone edges (Figures 5, 14, 18, and 19), often leading to a network of cracks that moved throughout the bone (Figures 11, 13, 19, and 20). Some moved concentrically around the medullar cavity or seemed to follow growth lines (Figures 5, 14, 15, 18, and 19). In vascular bone, they often developed around the canals or even the secondary osteons (Figure 20). In some areas, they were accompanied by simultaneous shrinkage. In some cases, this led to the creation of small fissures, vertical to the larger cracks (Figures 11, 12, 19, and 20). Criss-cross cracks, which characterize high intensity burning, were not observed. Cracks were usually infilled with either calcite or the previously described black-colored phase (Figure 5).

d) *No significant alteration of histology*. The characteristic structural features of fish bone were well preserved in all samples. Areas of vascular and avascular bone were observed (Figures 6, 9, 10, and 20). Secondary bone and primary and secondary vascular canals

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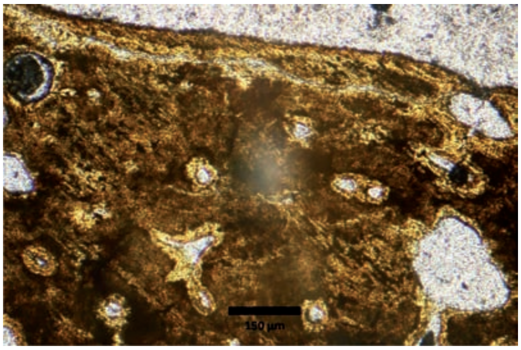

FIGURE 9

Sample DispFi-05 under the polarizing microscope. Well preserved vascular area of bone, where we may observe the formation of secondary osteons.

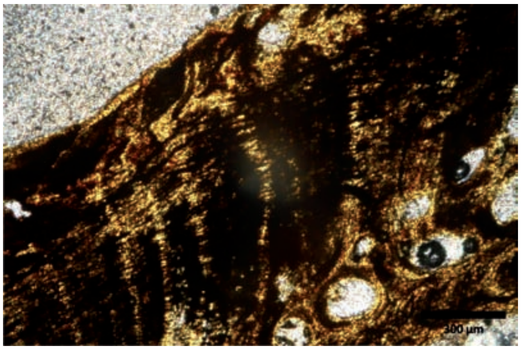

FIGURE 10

Sample DispFi-07 under the polarizing microscope. Linear forms that could be attributed to Sharpey's fibers are observed. Below this, secondary bone is present with well preserved vascular canals.

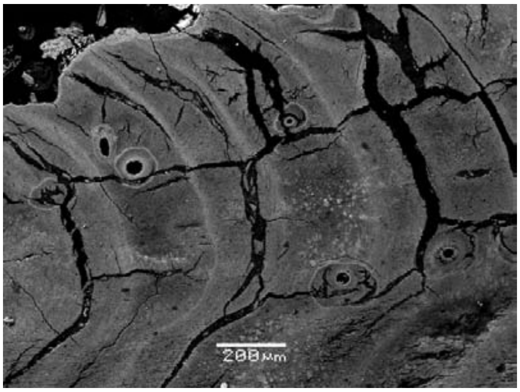

FIGURE 11

Sample DispFi-05 under the SEM (BSE mode). Secondary osteons within the bone matrix. One may also observe areas rich in Fe and Mn, such as the rims around the osteons and the spots present on the right part of the picture. Cracks develop a network across the bone.

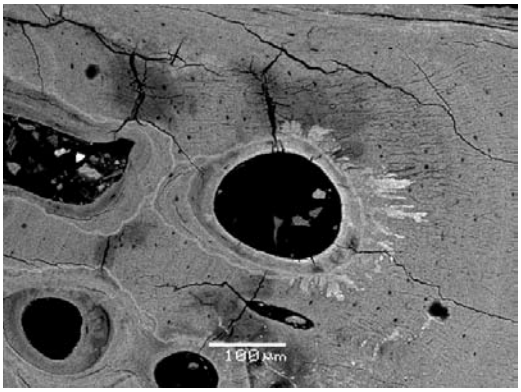

FIGURE 12

Sample DispFi-03 under the SEM (BSE mode). Secondary osteons within the bone matrix. One may also observe areas rich in Fe and Mn, such as the rims around the osteons and vascular canal, and the vein typed occurrence surrounding the osteon. Cracks are observed between bone voids and seem to have developed a network of vertical fissures. Lacunae is also visible.

were distinguishable, as well as were secondary osteons (in some cases) (Figures 11-13, 20). Lacunae and canaliculli were observed in avascular areas, where the osteocytes once lay (Figure 15). Zones, annuli, and AGL (Figures 5, 7, and 8) appeared to vary in color, either due to oxide staining or to hypermineralization (Figures 14 and 15). They often part vascular from avascular bone (Figures 14 and 18), primary from secondary bone (Figure 12). Reversal lines between successive bone deposits were observed (Figure 12), as well as linear forms that could be attributed to Sharpey's fibers (Figure 10). Under the SEM, the typical appearance of a «healthy» bone at larger magnitudes was visible (Figure 16); the only exceptions were three fragments (from subgroups DispFi-05, -07 and -08) where the beginning of crystal fusion might have been caused by thermal alteration of the bone apatite (Figure 17).

e) *No microbial activity.* It is important to point out the total absence of structural destruction due to microbial activity.

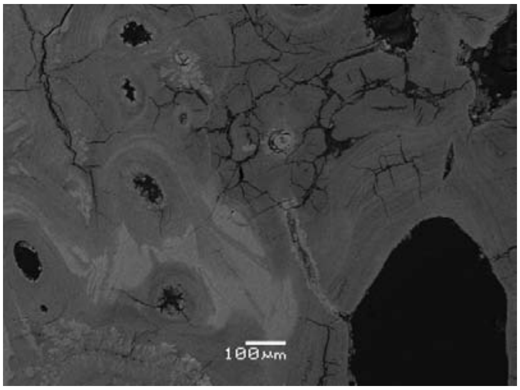

FIGURE 13

Sample DispFi-02 under the SEM (BSE mode). Secondary osteons within the bone matrix. One may also observe areas rich in Fe and Mn, such as the light coloured areas between and around the osteons, and the vein typed occurrences. Cracks are observed and seem to have developed a network across the bone.

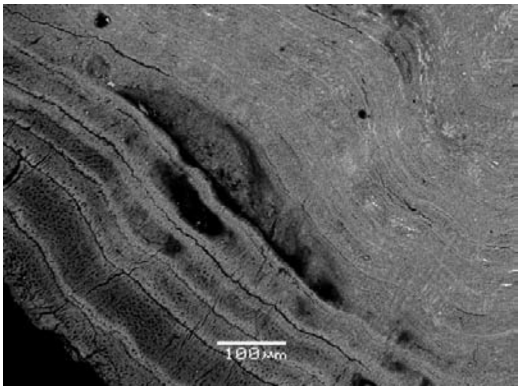

FIGURE 14

Sample DispFi-03 under the SEM (BSE mode). Vascular and avascular areas within bone matrix. In both, one may observe growth marks, sometimes parted by parallel cracks. The zones of vascular bone are distinguished by light coloured linear areas and cracks. No chemical alterations were traced in these areas and so we attribute the difference in colour, to the hypermineralization of the growth lines.

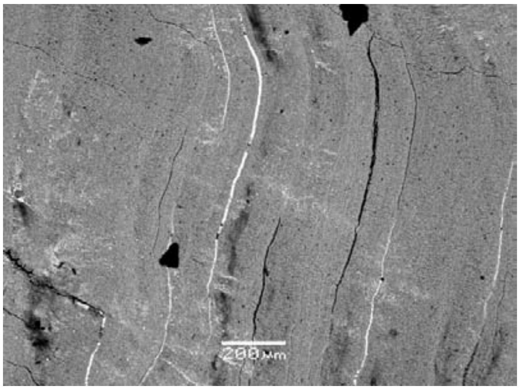

FIGURE 15

Sample DispFi-03 under the SEM (BSE mode). Growth lines within bone, with infilled cracks moving parallel to them. The light coloured material consists of Fe, Mn oxides.

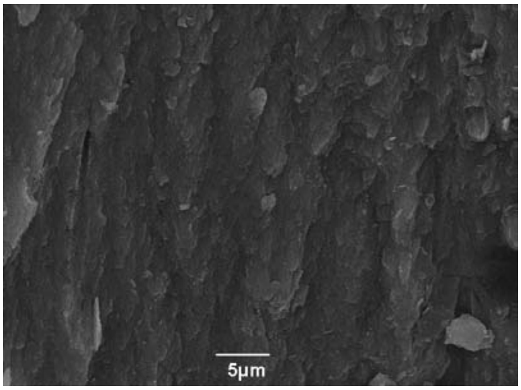

FIGURE 16

Sample DispFi-04 under the SEM (stub sample). Typical image of "healthy" bone.

Chemical Analyses

The following observations were based on the spot chemical analyses:

a) *Chemistry of bone mineral*. The sampled bones exhibited a variety of chemical elements within their mineral phase. Apart from Ca and P that are the basic components of bone apatite, amounts of Fe (0.5–7%) and Mg (0.7–1.2%) were observed in all samples. In several samples Mn (0.3–1.2%) was also detected. All samples contained traces of some of the elements Na, K, Si, Al, and S that are also characteristic of fresh bone, but in smaller quantities. The enrichment of bone with these elements indicated substitutions that correlated to the regional geochemical burial conditions. The Ca/P ratio, which indicates diagenetic alterations of either Ca or P, varies between 1.6 and 2.4. The Mg/Ca ratio, which describes the substitution of Ca by Mg

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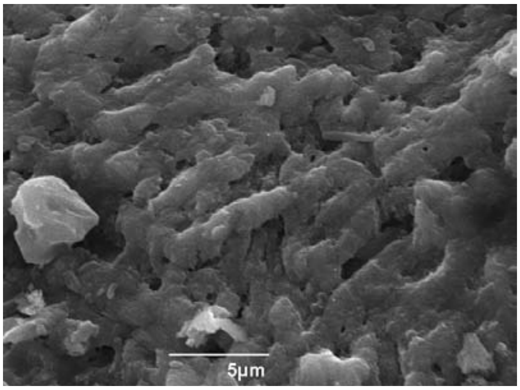

FIGURE 17

Sample DispFi-05 under the SEM (stub sample). Images of bone that might have been caused by thermal alteration of the bone apatite, causing the beginning of crystal fusion.

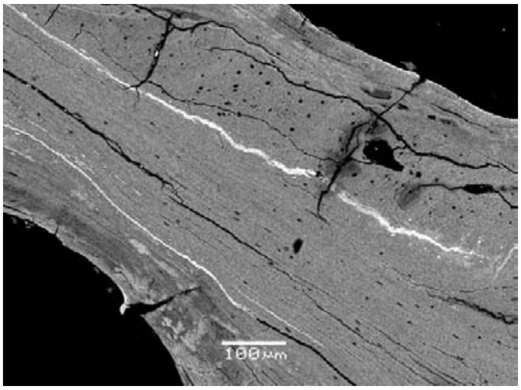

FIGURE 18

Sample DispFi-03 under the SEM (BSE mode). Vascular and avascular areas within bone matrix. We can distinguish them by parallel cracks, which are often infilled by Fe, Mn oxides. Vertical cracks are also present.

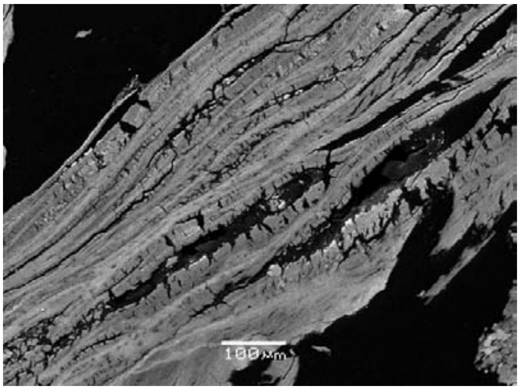

FIGURE 19

Sample DispFi-05 under the SEM (BSE mode). Dense parallel network of cracks along bone surface. The cracks seem to have secondarily become the starting point for smaller, vertical fissures.

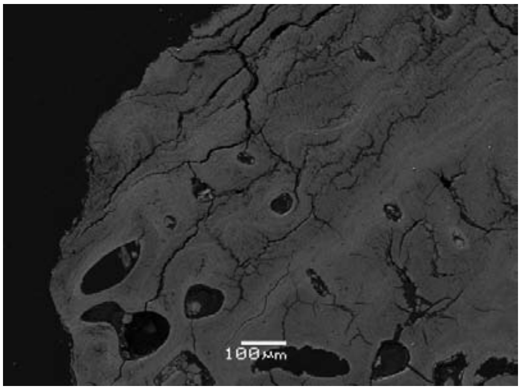

FIGURE 20

Sample DispFi-02 under the SEM (BSE mode). Vascular bone with secondary osteons and evident growth marks. A network of cracks has developed, sometimes moving around the osteons.

varies between 0.03 and 0.07. Using the backscattered electron mode (BSE) of the SEM, differentiations in the brightness of various areas could be observed, which we characterized as either «dark» or «light» colored (Figure 14). This may have been due either to differentiations in composition or to variations in the degree of mineralization. In the first case, the darker areas corresponded to the chemical composition described previously in this paragraph, while the lighter colored areas, to chemistry alterations due to chemical elements, such as Mn, Fe, etc. Analytically, light-colored areas could be seen across the bone matrix of all studied samples; they appeared as spots (Figure 21), veins (Figure 12), irregular shaped areas, zones, and thin linear forms (Figure 11). They were also found surrounding bone voids such as vascular canals (Figure 20) or secondary osteons (Figures 13 and 20). Their chemical analyses confirmed that the bone apatite in these areas was particularly rich in Fe and/or Mn. They mainly contained, except for Ca and P, Fe (3–16%), Mn (0.4–17%), Mg (1–1.5%) Ba (0–1.5%) and

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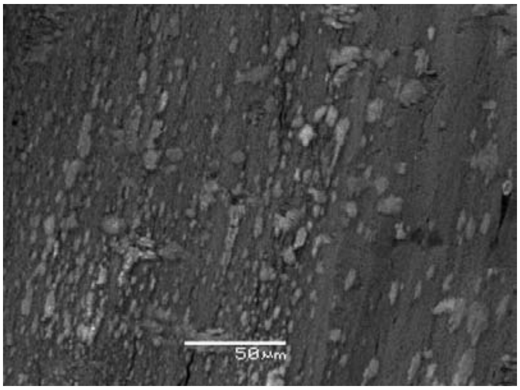

FIGURE 21

Sample DispFi-05 under the SEM (BSE mode). Light coloured spots within bone containing Fe, Mn.

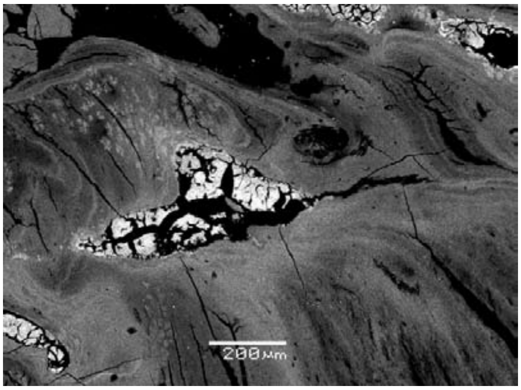

FIGURE 22

Sample DispFi-03 under the SEM (BSE mode). Fe, Mn oxides within vascular canal.

traces of Na, Si, Al, K. The Ca/P and Mg/Ca ratios varied between 1.6–2.4 and 0.04–0.07, respectively. In some samples, the dark-colored rims observed were due to the smaller amount of Fe compared to that in the inner bone. Still, the possibility of a combination of causes, alterations in chemistry and alterations in mineral density due to burning, cannot be ruled out.

b) *Infilling by secondary minerals.* The chemical analyses of many light colored areas proved that they were the result of either the infilling of cracks (Figure 18) and voids of the bone matrix (e.g., vascular canals; secondary osteon channels; canaliculi and lacunae along growth lines; Figures 15 and 18), and/or the deposition of mineral phases superficially. In the first case, grains of mineral phases, such as calcite ($CaCO_3$ often rich in Mn and Ba, Mg), quartz (SiO_2), clay minerals, and various other silicates containing elements such as Mg, Fe, Ca, K, Na, Ba, Ti, Mn, Cr were reported. Oxides of Fe and Mn were also present (Figure 22) and contained, except for Fe (3–45%) and Mn (26–40%), amounts of Ba (0–3.5%) and traces of Si, Cr, Ni, Ti, Mg, Al, K, S. Various micro-fossils were also found within the above material, both calcitic and siliceous in composition. Small fragments of bone and other phosphoric organisms were also observed. In the second case, crystals and crystal aggregates covered large areas along bone surfaces and consisted mainly of Mn oxides (Figure 23).

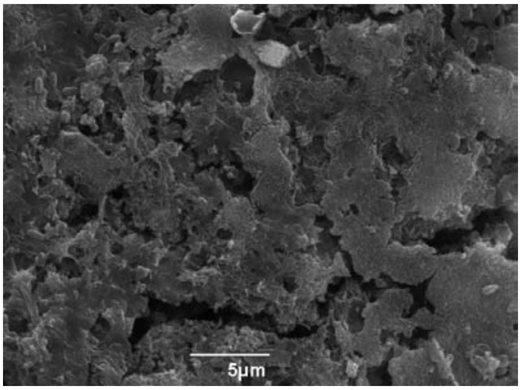

FIGURE 23

Sample DispFi-02 under the SEM (stub sample). Crystals and crystal aggregates covering large areas along bone surfaces and consisting mainly of Mn oxides.

Infrared Spectroscopy

In Figure 24 (top), we show the absorption spectra of a representative fossilized fish bone from Dispilio over the NIR (left) and ATR (right) spectral ranges. Eight frequency ranges (also noted in the absorption spectra) are detailed in the lower part of Figure 24, where the spectra of all fish bone samples are shown in the 2nd derivative mode, in comparison to two fresh bone references (ART-1, a relatively fresh bovine bone and the NIST Standard Reference material 1486, which consisted of bone meal; data from Stathopoulou, 2008). These ranges corresponded to a) the ν_3 modes of the carbonate radicals, b) the ν_1 and ν_3 modes of PO_4^{3-}, c)

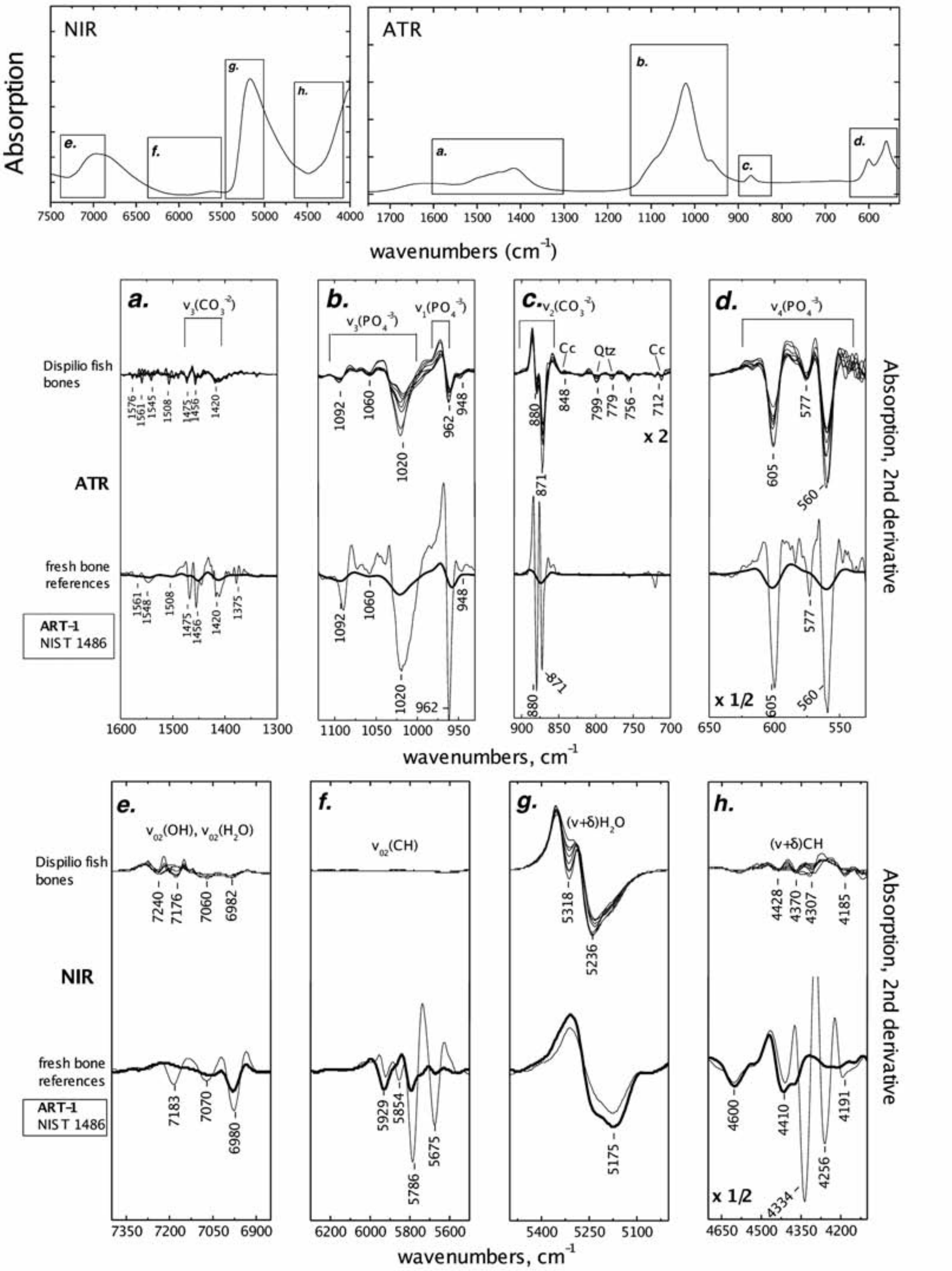

FIGURE 24

Selected frequency ranges and their generalized assignments for all fish bone samples, in comparison with two fresh bone references (ART-1, a relatively fresh bovine bone, noted with the thick line and NIST Standard Reference material 1486, which consists of bone meal). These ranges are shown in the 2nd derivative formalism (bottom), which allows for the enhancement of the resolution of sharp features overlapping with broad bands, according to Stathopoulou et al. 2008. Representative NIR and ATR absorption spectra of one of the fish bone samples (DispFi-01) are also given (top) for comparison. Details are given in the main text.

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the ν_2 modes of the carbonate radicals, d) the ν_4 modes of the PO_4^{3-} units, e) the stretching overtone of the OH^- and H_2O, f) the stretching overtone of the CH, g) the stretching-bending combination mode of H_2O species, and h) the stretching-bending combination mode of CH. The high resolution of the infrared data, enhanced further by the derivative analysis, allowed for the following observations.

Numerous peaks were observed in the 1300–1600 cm^{-1} range (circa 1475, 1508, 1545, 1561 and 1576 cm^{-1}), apart from the main peaks at around 1415 cm^{-1} and 1450 cm^{-1}, that corresponded to the ν_3 modes of the carbonate radicals, and specifically type-B carbonates (substitution for phosphate species). These peaks may be attributed to type –A carbonates (substitution for OH^-). It is possible for organic components to also produce peaks in this range (Montgelard, 1997; Shahack-Gross, 1997), especially in the case of the fresh bones.

In the area of the ν_1 and ν_3 modes of PO_4^{3-}, our samples exhibited the typical bands for hydroxylapatite (circa 962 cm^{-1} and 1017–1020 cm^{-1}). The band at 1092 cm^{-1}, which was often observed in bone samples, appeared quite weak in the specific samples.

From the known ν_2 modes of CO_3^{2-} (circa 880 cm^{-1} and 870 cm^{-1}), attributed to substitutions for OH^- (type –A carbonates) and PO_4^{3-} (type-B carbonates) in the apatite structure, respectively, the second exhibited higher intensities, while our samples seemed to lack the mode at 865cm^{-1} often present in archaeological bone. This latter peak increased with bone crystallinity; relatively fresh bone usually lacks it (Stathopoulou *et al*. 2008). Calcite in our samples produced a characteristic infrared peak at 712 cm^{-1}, which correlated to a peak at 848 cm^{-1}. The presence of calcite was known to also contribute to the intensity of the 870 cm^{-1} component. Quartz, on the other hand, produced a characteristic doublet of infrared peaks at 799 cm^{-1} and 779 cm^{-1}. These secondary minerals were not observed in samples DispFi-05, -06, -07 and -08.

In addition to the well-known ν_4 modes of PO_4^{3-} units in hydroxylapatite (split at ca. 560 and 600 cm^{-1}), a frequency component at about 575 cm^{-1} appeared, when using second derivative analysis.

In some cases, the peaks at 577 cm^{-1}, 865 cm^{-1}, 962 cm^{-1} and 1092 cm^{-1} have been proven to be connected and have been attributed to the effect of diagenesis (Stathopoulou *et al*., 2008).

In the 6900–7400 cm^{-1} range, a very weak «shoulder» was observed at 6982 cm^{-1} that was due to the overtone of the fundamental OH^- mode. The mode at about 7240 cm^{-1}, which had been correlated to the band at 5318 cm^{-1}, was attributed to a surface water species (Stathopoulou *et al*., 2008), which did not seem to appear in fresh bone. The peak at 7060 cm^{-1} was attributed to clay mineral phases, such as kaolinite (Stathopoulou, 2008).

Organic remains were not traced in the 5600–6200 cm^{-1} range, when both reference samples produced peaks in this area. On the contrary, organic components could be traced in the 4100–4700 cm^{-1} range (4185, 4307, 4370 and 4428 cm^{-1}), but once again different to those present in the fresh bone samples. These differentiations in the vibrational signature of the organic component of bone may be attributed to the different status of these remains, due to their decomposition during diagenesis.

The crystallinity index (Splitting Factor; SF) has been used in studies of «black bones» to discriminate between burnt and unburnt material, based on the increase in the size of the apatite crystal that occurs during thermal alteration of the bone through burning (Shipman *et al*., 1984; Nielsen-Marsh & Hedges, 1999; Stiner *et al*., 2001; Munro *et al*., 2007; Nagy *et al*., 2008). We must point out that the increase in the SF is a process that occurs even during ontogenesis as bone matures, is enhanced naturally after death during diagenesis, but can also be accelerated by burning.

Previous work of ours has shown that this index was not always discriminative and did not correlate well to other crystallinity indices, calculated by other techniques (Stathopoulou, 2008; Stathopoulou *et al*., 2008). One of the reasons for this seems to be that the peak at 577cm^{-1}, which appears within the trough used for its calculation, has never been taken under consideration. Despite shortcomings of past works (Shemesh, 1990; Weiner & Bar-Yosef, 1990; Lee-Thorp & Sponheimer, 2003; Lebon *et al*., 2008; Thompson *et al*., 2009) that did not consider the peak at 577cm^{-1} when calculating the IRSF, we proceeded with the calculation of the Splitting Factor (Table 2) in order to allow comparison with other authors' previously published data. According to this, the values obtained for the splitting factor in this study (Table 2) were between 3.6 and 4.0 (with one exception at 3.4).

Sample	DispFi-01	DispFi-02	DispFi-03	DispFi-04	DispFi-05	DispFi-06	DispFi-07	DispFi-08
SF	3.6	3.4	3.8	3.7	4	3.9	3.9	3.9

TABLE 2

Splitting Factor values calculated for the fish bones used for this study.

DISCUSSION

Identification of thermally modified bones from archaeological sites and of their heat temperature has lately received much attention in the literature, mainly when concerning human and mammal remains (see previous section «Studies on burnt fish remains»); fish, however, have received less attention. The study of the fish remains recovered from the waterlogged site of Dipilio presented an exceptional case study for evaluating our ability to differentiate between thermally modified bones, post-depositional burning of the bones, and chemically stained bones deposited in lacustrine sediments. The Dipilio fish remains were dark in color, an observation that could have led to a biased conclusion of burnt bones, especially since the archaeological material exhibited evidence of a destruction layer caused by burning.

This study emphasized the significance of combining different analytical methods, both macroscopical and microscopical (Optical microscopy, SEM, EDXA, IR Spectroscopy), for the identification of the taphonomic history of bones especially regarding thermal modification, both before and after deposition.

The use of thin sections showed that the dark coloration that dominated the bone surface was also found in the inner layers of all bones sampled, although it may have appeared in lighter shades. This suggested that, whatever the cause of the fish bone coloration, it affected the entire bone. Based on this observation, both long-term burning and mineral staining could have been responsible for the dark coloration. Long-term burning was, however, unlikely. The micromophological data suggested a quick episode of burning as the burned huts collapsed quickly into the water of the lake (Karkanas *et al*., 2011). This hypothesis was supported by the recovery of well- preserved charred seeds sealed in this depositional phase, which would not have survived high temperatures or long heating (Margaritis, 2011).

Burning is known to microscopically affect bone histology and produce extended types of micro-cracking, depending on the temperature of the fire (Shipman *et al*., 1984; Nicholson, 1993; Hanson *et al*., 2007). Microscopical analysis of the sampled fish remains showed that the bones were well preserved (Figures 9 and 16) (similar to the histological section of a fresh bone). On the other hand, all studied samples exhibited micro-cracking, occasionally quite intense, which led to the development of networks (Figures 11-13). Secondary osteons and vascular canals were often surrounded by cracks, while, in many cases, they were accompanied by simultaneous shrinkage. This often led to the creation of small fissures, vertical to the larger cracks. The described phenomena could imply the effect of heat, on either whole or part of the material, without excluding the possible diagenetic origin of cracking. Cracks that have been reported in the literature as attributed to the effect of intense fire are mainly the «criss-cross» cracks that appear along the edges of bone, found around and within osteons of lamellar bone. The samples studied here did not exhibit the typical appearances of such cracks, a fact which could suggest either a fire of lower intensity or a burning episode of shorter duration, had it occurred.

Three bone fragments from the late C phase in trench Δ04 (sub-groups DispFi-05, -07 and -08), however, exhibited areas of altered bone matrix, which resembled the effects of thermal alteration on the apatite of bone by means of crystal fusion (Figure 17). These occurrences could imply exposure to high temperatures, possibly above 650ºC. The episodes that could have caused such heating are not clear and further research is needed in this direction. The micromorphological and archaeobotanical data suggested burning at temperatures

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not higher than 450°C (Karkanas *et al*., 2011; Margaritis, 2011). Consequently, if these occurrences are eventually proven to be the result of crystal fusion these fish bones could not have been affected by the same episode which produced the charred seeds. A direct and prolonged contact with intense fire (>700°C) could then be suggested.

The histological sections also demonstrated the absence of microbial destruction of the bone mineral. This is not surprising, as these organisms seem to prefer open-air environments and do not affect bones that are deposited in anaerobic aquatic environments. This observation is important for all waterlogged sites. It therefore seems likely that post-depositional staining in a suitable burial environment (moist, anaerobic, oxidizing environment with neutral to alkaline pH) would provide a more plausible explanation to the generalized black coloration, rather than long-term burning. This would conform with the known data on the geochemical conditions of the lake, considered to be a dimictic, eutrophic lake, with alkaline pH values that generally range from 5.9–8.2 in winter to 6.8–9.5 in summer (Koussouris *et al*., 1987).

Microscopically, the samples exhibited a variety of colors ranging from yellow to dark brown. The chemical analysis by EDXA demonstrated oxide staining of the bones. Differential concentrations of Fe (Iron) and Mn (Manganese) were detected in the bone matrix and the bone voids/cracks. Manganese is known to color bone black, even when present in small quantities. Iron, on the other hand, is responsible for the red-orange shades of bone. Other mineral phases were also detected in the inner structure of the bones or surrounding the skeletal remains. Many of these contained important concentrations of Fe and Mn and could have been the source of these elements in the staining process. It thus became obvious that, at some point during the depositional history of the site, the regional geochemical conditions were such that Fe and Mn oxides, as well as hydroxides, were deposited and stained the bones (see conclusions). Since the fish remains recovered from Dipilio displayed sub-superficial staining, we concluded that it occurred after deposition as well as after any possible thermal alteration by fire in the lacustrine sediments.

The fish bone samples were generally enriched in chemical elements, some in significant concentrations. The element Ca is known to be substituted by a number of elements, many of which could be detected in our samples. These substitutions, as well as P depletion in bone, could lead to an alteration in Ca/P ratios in diagenetically altered bone. Theoretically, fresh bone is characterized by a Ca/P ratio of about 2.14, while mineral apatite by a ratio of about 2.16. An increase in this ratio can be attributed to either the substitution of phosphate in apatite by other species, or the deposition of calcite within the bone. Its decrease may be attributed to the aforementioned substitutions of Ca. The ratios in the Dispilio samples varied between 1.6 and 2.4. The lower ratios can be attributed to significant substitutions of Ca by other elements, such as Mg. The total Phosphorous (TP) of the lake water has been reported to be as high as 26μg/l (Theodoropoulou, 2007), a fact that could be connected to the depletion of P and the substitution of PO_4^{-3} in bone.

Based on the phenomenology of the Infrared spectra, our fish bone material produced bands similar to those of fresh bone, as well as mineral carbonate apatites, with differentiations that depended on the degree of crystallinity of each material and possibly secondary minerals within them. Some of the peaks attributed to diagenetic alterations within the bone structure (865 $cm^{-1}$and 1092 cm^{-1}) (Stathopoulou *et al*., 2008) were either absent or weak. Apart from calcite and quartz, no metal oxides could be verified through a spectroscopic signature. On the other hand, organic remains seemed to have been preserved within the samples; differences between them did not correspond to different trenches or horizons at the site. When compared to the fresh bone reference samples, the by-products of the decomposition of the organic component of bone due to diagenesis became evident by their different spectroscopic signature. According to Lebon *et al*. (2008), the organic matter of bone (mainly collagen) is rapidly carbonized around 300°C, while, above 450°C, the remaining organic carbon is gradually removed. Therefore, we can suggest that, if burning did affect our material, it did not exceed 450°C. During heating, the apatite mineral is intensively altered. From 100°C, water is progressively removed; above 300°C, carbonate content rapidly decreases. We have no indications of such alterations in our samples. As a result, the amount of defects in the lattice decreases and leads to crystal growth in temperatures above 500°C (Lebon *et al*., 2008; Thompson *et al*., 2009). According to the values of the Splitting Factor (SF), which vary for fresh bone between 2.5 and 3.25, crystallinity in the Dispilio samples had slightly increased. This process begins naturally after death during diagenesis or burning. Lebon *et al*. (2008) stated that the SF is relatively stable up to 600ºC and then strongly increases to values up to 8-10 for samples heated over 700ºC. Thus, the SF does not allow for a clear discrimination between cooked or heated bones in the wide range of temperatures up to 650ºC. As the SF values of our samples were relatively low, it strengthens the idea that thermal alteration by fire seemed unlikely. Therefore, the small increase in the SF may, instead, be attributed to diagenesis. Still, it remains to be examined whether the bones were also affected by low temperature fire.

CONCLUSIONS

In conclusion, the «black» fish bones from Dispilio were certainly affected by oxide staining in the lacustrine sediments and owe their color to this process. Based on the results of the macroscopic, microscopic, and spectroscopic study, however, the possibility of a combination of diagenetic and thermal alteration cannot as yet be ruled out. If burning of the material occurred, it was not intense (i.e. at high temperatures) or of long duration, and possibly not direct. The soil matrix at the site, which was very rich in charcoal and ash but at the same time waterlogged and organic, may have also played a role in bone coloration, in combination with the aforementioned phenomena. Oxide staining is a very common phenomenon in sites rich in organic matter, as its decomposition produces an acidic environment that favors the deposition of metallic ions such as Mn (Shahack-Gross *et al*., 1997; Marín-Arroyo *et al*., 2008). Based on the mineral phases traced within the surrounding sediment in our polished sections, we suggest that the surrounding rocks and sediments constituted the origin of Mn and Fe in the bones. It was also possible that part of the Mn did not have a geological origin, and that an anthropogenic source should be considered, namely the accumulation of vegetable and animal organic matter. Ions of the metal could be released during its decomposition and then enter the sediments in which the bones were buried (Shahack-Gross *et al*., 1997; Marín-Arroyo *et al*., 2008). Although not conclusive at the present state of knowledge, our study has suggested that the black color of the Dispilio material probably had multiple origins.

We presented here preliminary results from an ongoing project. In the next stage, the sample of fish bones will be enlarged and combined with that of mammal bones, in order to verify the observed results gained so far. From a methodological point of view, this will involve the application of an extended methodology, including X-ray Diffraction and the Rietveld method, combined with IR Spectroscopy, following Stathopoulou *et al*. (2008). Another important aspect concerning the study of Dispilio faunal remains, which is not presented in detail here but will be studied in the future, regards the spatial distribution of black bones between the two trenches Δ4 and Δ8 and between the three phases, B, early C, and late C.

To conclude, the current preliminary study exhibited the necessity of a multidisciplinary approach for the study of colored bone material from archaeological sites. We demonstrated how different causal factors can be identified and separated. An extended dataset can offer answers on the history of a site and geochemical conditions involved and can highlight important methodological aspects related to the understanding of black-colored bones. The importance of this study in the interpretation of waterlogged cultural deposits extends beyond the confines of Greek archaeology, as observed from the increasing interest in lacustrine cultures that flourished all over Europe (Menotti, 2012).

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