THE IMPACT OF MICROBIAL MATS AND THEIR MICROENVIRONMENTAL CONDITIONS IN EARLY DECAY OF FISH

Author(s): MIGUEL INIESTO, CELIA LAGUNA, MAXIMO FLORÍN, M. CARMEN GUERRERO, ALVARO CHICOTE, ANGELA D. BUSCALIONI, and ANA I. LÓPEZ-ARCHILLA,

Source: PALAIOS, 30(11):792-801.

Published By: Society for Sedimentary Geology

THE IMPACT OF MICROBIAL MATS AND THEIR MICROENVIRONMENTAL CONDITIONS IN EARLY DECAY OF FISH

MIGUEL INIESTO,1 CELIA LAGUNA,2 MAXIMO FLORIN,2 M. CARMEN GUERRERO,1 ALVARO CHICOTE,2
ANGELA D. BUSCALIONI,3 AND ANA I. LÓPEZ-ARCHILLA1

1Microbial Ecology Unit, Department of Ecology, Universidad Autónoma de Madrid, 28049 Madrid, Spain
2Department of Science and Technology in Agroforestry and Genetics, Universidad de Castilla-La Mancha, 13071 Ciudad Real, Spain
3Palaeontology Unit, Department of Biology, Universidad Autónoma de Madrid, 28049 Madrid, Spain

e-mail: miguel.iniesto@uam.es

ABSTRACT: This study provides experimental evidence confirming the significance of microbial mat presence in controlling the spatial and temporal development of chemical microenvironments which become established inside and outside decaying fish carcasses. Dissolved oxygen (DO) and pH profiles were monitored over 1,000 days with microelectrodes positioned inside and adjacent to carcasses. In the vicinity of fish, the DO varied from an oxygenated green upper layer to an anoxic bottom stratum and the pH was alkaline. Inside the fish, the DO and the pH were decoupled during the first week, in that anoxia remained constant and the pH decreased and became acidic. Once covered by the mat, the fish carcass turned oxic and pH returned to alkaline levels. This novel second phase occurred from day 90 to three years and would likely remain after this time. Based on the fish soft tissue quality at the end of the experiment, we discuss whether oxic-alkaline microenvironmental conditions could promote organic preservation and long-term mineralization.

INTRODUCTION

The fossilization of organisms involves changes which occur before and/or after burial (biostratinomy and diagenesis, respectively) (Fernández-López 1989). Although these changes almost invariably result in loss of soft tissue, certain physical and chemical conditions in the vicinity of the organism may enhance preservation, thereby allowing the retention of detail in fossilized soft tissue at the cellular or even molecular level (Asara et al. 2007; Schweitzer et al. 2007, 2009; McNamara et al. 2009). A key factor routinely involved in the genesis of such exceptional deposits (Konservat Lagerstätten) is what can broadly be termed “bacterial sealing” (Seilacher et al. 1985). It is commonly accepted that what can be termed Microbially Induced Sedimentary Structures (MISS) (Noffke, 2009; Noffke and Awramik 2013) enhance the likelihood of soft tissue preservation. Soft-bodied organisms and soft tissues are frequently associated with evidence for the presence of biofilms (Gall et al. 1985; Gall 1990; Seilacher 1990; Behrensmeier 2000; Briggs 2003; O’Brien et al. 2008; McNamara et al. 2009; Buscalioni and Fregenal-Martínez 2010; Pawlowska et al. 2013).

Other investigations have conducted several experiments to elucidate the relation between aspects of exceptional preservation and bacterial activity. Sagemann et al. (1999) and Grimes et al. (2001) attempted to reproduce the biogeochemical processes involved in soft tissue mineralization, and Peterson et al. (2010), Daniel and Chin (2010), Iniesto et al. (2013) and Guerrero et al. (2015) all assessed how the composition and morphology of biofilms were related to tissue preservation and, critically, retention of the integrity of carcasses.

In this paper we describe an experiment designed to investigate the role of complex biofilms, i.e., microbial mats, in soft tissue fossilization. Unlike other experiments in which the experimental vessel was simply inoculated with some heterotrophic bacterial populations and monitored over short time periods, this investigation analyzes the processes which occurred within a microbial ecosystem as a whole over an extended time period. Microbial mats are complex benthic communities organized as millimetric multi-layered microecosystems frequently dominated by cyanobacteria. These communities have a distinct vertical structure (Cohen 1989) controlled by the extent of light penetration. Microbial mats are responsible for the biolaminated facies produced in certain lacustrine environments such as carbonate wetlands or lagoons (Fregenal-Martínez and Melendez 2000; Noffke et al. 2013). Notably, this organization of the mat creates physical and chemical gradients as the result of the activities of the living microorganisms (Wierzchos et al. 1996; Hubas et al. 2011). The various layers of the microbial mat are in constant growth. Typically, cyanobacteria, which are oxygenic photosynthetic organisms, occupy the upper layer, while several phototrophic anoxygenic bacteria reside in an intermediate layer above lower layers comprised of other anaerobic bacteria, such as sulfate-reducing species. Sediment particles, including organic remains and minerals precipitated in situ become trapped within the growing mat (Krumbein 1979). In a previous experiment in which fish were covered and buried in microbial mats, Iniesto et al. (2013) demonstrated that the inner tissues remained nearly unaltered over more than two years (27 months). The key aspects of the interaction between the carcasses and the microbial mats consist of (1) replication of the body surface (Darroch et al. 2012); (2) a decrease in the decay rate; and (3) mineral bioprecipitation within the mat (Briggs 2003; Kühl et al. 2003; Decho and Kawaguchi 2003; Dupraz and Visscher 2005; Ludwig et al. 2005; Iniesto et al. 2013). All of these processes can be initiated within a few days (Sagemann et al. 1999; Iniesto et al. 2013).

This paper concentrates on the significance of local chemical conditions within the microbial mat during the decay process of fish carcasses, specifically assessing whether these changes in environmental conditions promote preservation and enhance the likelihood of mineralization.
MATERIAL AND METHODS

Sampling and Microbial Mat Growth

Samples of microbial mats were collected from the Salada de Chiprana (Zaragoza, NE Spain), the only hypersaline and permanent inland shallow lake in Spain. It occurs in the Bajo Aragón semi-arid region and is part of the catchment area of the Ebro River. The Salada de Chiprana is fed by groundwater discharge which supplies abundant magnesium sulfate and sodium chloride. The lake has no outlet, resulting in high salinity, varying between 30 to 70%. Fluctuations in salinity are due to the changes in the volume of surface run-off which supplies the lake with abundant nutrients. The combination of this feature together with a high pH (values above 9) results in microbial mats being the prevalent communities covering the floor of the lake from the shore to a depth of 1.5 m (Guerrero et al. 1991). In their study of the physicochemical and biological features of the lake, Vidondo et al. (1993) recognized the lagoon as unique in Western Europe because of the growth of these dense microbial mats. The Salada de Chiprana mats are very compact due to the dominance of the filamentous cyanobacterium Coelosphaerium chthono-plastes (Thuret ex Gomont) (Siegesmund et al. 2008, formerly known as Microcoleus chthonoplastes). The taxa comprising the upper mat layer are Chloroflexus, Coelosphaerium, and Pseudanabaena-like. The underlying darker layer is made up of dead cyanobacteria, frustules of diatoms and anoxic microbial populations (Jonkers et al. 2003). More recently, Ludwig et al. (2005) observed how calcification of the mat occurred and revealed the importance of photosynthesis and sulfate reduction as agents driving the mineralization process.

Following collection in the field, microbial mats were grown in the lab under controlled conditions according to the protocols described by Iniesta et al. (2013). One well-developed microbial mat was grown in a glass tank illuminated by a 50W halogen lamp (Osram Decostar 46870WFL). Incident illumination approximated an intensity of 300 umols.seg-1.m-2, with a 12/12 hour dark/light regime to simulate the natural environmental conditions. A separate tank containing lake water addition, tanks were filled 24 hours prior to data collection thus representing an additional control on the substratum conditions unaffected by the presence of the carcasses.

Experiments were conducted in triplicate with the same provisions used by Iniesta et al. [2013] allowed each profile to be aligned. In all cases measurement showed that oxygen and pH conditions reached stable readings a few millimeters into the sediment; we assumed that variables remained stable below this depth. For each profile, data of each pseudo-replicate were taken every 0.5 mm. Error bars in plots show the standard deviation of pseudo-replicates. To guarantee precision and to minimize analytical method bias, means were derived from five measured values at the same depth holding the microsensor immobile. The experimental setting determined the number of points to be checked throughout of the profile, ranging from 32 (microsensor through the sediment only of the control tank) to 42 (through the fish, the microbial mat and the sediment of the man tank). Fourteen millimeters of each profile was selected to compare equivalent levels in each tanks, taking the surface of the mat or the sediment (in the case of controls) as reference. Plots do not include depth profiles lower than where the electrode detected no further variation.

The data were analyzed using the XLSTAT 2014 statistical analysis add-in tool for MS Excel. The non-parametric data was tested using the Mann-Whitney-Wilcoxon test (NF vs. IF, or sediment vs. microbial mat tank). The Kruskal-Wallis test was used to compare differences in variables with three or more groups (i.e., different times for each experimental point and each substrate). Tukey’s range test was carried out to test the significance of the pairwise comparisons. In all of the studied cases, variables were considered significantly different for p-values <0.01.

Decay of fish carcasses was checked over the course of the experiment by removing samples with their intact microbial envelope at 90 and 1,000 days after the start of the experiment. Samples were imaged using a Bruker BMT 47/40 MRI Magnetic Resonance Imaging (MRI) scanner; this non-destructive technique provides images in which the contrast differentiates between soft tissues on the basis of the differential magnetic excitation of the protons contained in water. The relative energy gained causes the more hydrated tissues to produce lighter images compared to those tissues containing less water. In addition, the microbial layer that covered every carcass was also observed by Scanning Electronic
Microscopy (SEM). Observations were carried out at the Institut de Minéralogie et de Physique des Milieux Condensés (IMPMC, Paris) using a Zeiss Ultra 55 SEM (Carl Zeiss AG, Oberkochen, Germany) equipped with a field emission gun. Images were acquired with the microscope operating at 15 kV, and a working distance of ~7.5 mm in the backscattered electron mode using the AsB detector. Samples were gold-coated prior to SEM inspection.

RESULTS

After the deposition of carcasses, an important reorganization of the structure of the mat was observed, especially within the layers made up of phototrophic bacteria, i.e., the green layer constituted by cyanobacteria and the red layer formed by anoxygenic photosynthetic bacteria. Over the course of the first two days, the mat under each fish began to turn dark green indicating an accumulation of photosynthetic pigments due to localized stimulation of cyanobacterial activity. Growth of the mat resulted in the carcass being covered completely by cyanobacteria after day 10. Subsequently, the red layer grew upwards until it reached the fish (Fig. 2A). The covering layer then developed until a thick coat of microbial mat rich in exopolymeric substances (EPS) (Fig. 2B) generated a three-dimensional sarcophagus that protected the body.

Evolution of Oxygen Profiles

Next to Fish on the Microbial Mat (Fig. 3).—Initially, the dissolved oxygen increased downwards from the surface of the water column and
reached a maximum of 570 μM just under the mat surface (Fig. 3A). Below the DO maximum, the oxygen levels began to decrease: considerable reduction occurred throughout the red layer and total anoxia was reached just below of the sediment layer's top. Two days after deposition of the fish, DO showed a substantial decrease spanning from a maximum (449 μM) close to the mat surface (less than 0.5 mm from the top) to a minimum (0 μM) just above the sediment (Fig. 3B). After seven days the DO increased (539 μM) (Fig. 3C), although it was still below its original baseline value. After three months (Fig. 3D, day 90) the DO reached its greatest observed value (632 μM) just below the surface of the mat. After three years (Fig. 3E, 1,000 days), the mat's oxygen level was low overall, highest in the overlying water, and no distinct peak in DO was observed.

Inside Fish on the Microbial Mat (Fig. 4).—The oxygen profiles inside the fish (point IF) also varied over time. At days two and seven (Fig. 4B, 4C) the profiles exhibited two high peaks in the levels of oxygen: one in the water column just above the carcass (540 and 670 μM, respectively), and the other in the mat slightly below the fish (327 and 658 μM, respectively). Inside the fish the DO was zero. After three months the mat had completely covered the fish (Fig. 1D) and the maximum DO (781 μM) was 0.5 mm below the mat’s surface. The DO inside fish was lower but above zero (Fig. 4D). After 1,000 days (Fig. 4E), fish carcasses had lost half their thickness (Fig. 5C, 5D) compared to their initial volumes (Fig. 5A). Most of this reduction was achieved before day 90 and was limited after this (Fig. 5B). In this last stage (Fig. 4E) the DO profiles resembled those found at sites next to the carcasses (point NF, Fig. 3E) with low values overall that reached zero inside the fish bodies.

For each of the datasets inside (IF) and near (NF) the fish, the changes over time in the DO profile were remarkable and statistically significant (Table 1). For the IF dataset, there is no statistically significant difference between days 2 and 7; only day 90 was significantly different from all of the other days (see online Supplementary Data, Table S3). At point NF changes were more drastic and only the comparison between day 7 and day 90 was not significantly different (see online Supplementary Data, Table S1). Comparing the corresponding DO dataset for NF and IF profiles at each experimental time, only day 1,000 did not reveal significant differences (Table 2).

Control Tank (Figs. 6, 7).—In the control tank the DO remained close to anoxia throughout the water column except for the water's surface, where it was in contact with air. At the outset the DO decreased regularly from a maximum value (276 μM) at the water surface until reaching zero at a depth of 5.5 mm. Next to the fish bodies, DO values were even more limited, and reached zero 2 mm above the sediment surface after three months (Fig. 6D). Measurements taken inside the fish also revealed consistently very low values—at day two, readings ranged from the 120 μM at the fish surfaces to practically zero at the bottom stratum (Fig. 7B). On day 7, the DO was close to anoxia immediately above the bodies' surfaces (Fig. 7C) and by day 90 the bodies were entirely anoxic (Fig. 7D), although the uppermost part of the water column reached a maximum of 249 μM. DO variations over the course of time proved to be significant for both the NF and IF points (Table 1). Pair comparisons demonstrated that for both the NF and IF datasets, only day 0 was significantly different from the other experimental times (online Supplementary Data, Tables S5, S7). The DO profile comparisons inside...
and near the fish supported no significant differences respective to the experimental times (Table 2).

Comparison of Results in Experimental and Control Tanks.—For the corresponding experimental times, the DO profiles are statistically different between the mat and control tanks for both the IF and NF measurements (Table 3). In all of the above cases the DO values were lower in the control than the microbial mat tank (Figs. 3, 4, 6, 7).

Evolution of pH Profiles

Next to Fish on the Microbial Mat (Fig. 3).—The microbial mat’s initial profile was basic, with values from a pH of 10.2 just above the mat's photosynthetic surface to a pH of 8.7 in the sediment (Fig. 3A). The presence of the fish carcass resulted in a slight pH reduction which nonetheless remained basic throughout the profile (Fig. 3B, 3D). The minimum pH value observed corresponded to the sediment after 90 days (pH 8.2, Fig. 3D).

Inside Fish on the Microbial Mat (Fig. 4).—The high alkalinity observed before the fish carcasses were placed into the tank (Fig. 4A) contrasted with several pH readings obtained subsequently from inside the fish. The greatest fluctuations in pH occurred during the first week as the profiles became progressively more acidic (Fig. 4B, 4C). The pH values on the second day were close to neutral inside the fish carcasses (pH 7.4) and slightly acid (pH 6.4) in the sediment. On day seven the pH profile was more variable; the pH had decreased further inside the fish (pH 6.9) and its minimum value (pH 6.1) was recorded in the red layer, below which the pH began to slowly rise with depth (Fig. 4C). After 90 days, the pH values increased, reaching their highest value in the superficial mat and then decreasing through the carcass and down into the sediment, after which the values are constant with depth (Fig. 4D).

Statistical analysis revealed the differences between the profiles at different experimental times are significant for both the NF (Table 1) and IF (Table 1) datasets (see online Supplementary Data, Tables S2 and S4 for comparison by pairs). Comparison between the NF and IF measurements (Table 2) disclosed that differences were significant except for day 2 (p-value 0.140).

Control Tank (Figs. 6, 7).—The variation in the pH profiles of the IF measurements over time were not statistically significant; those for the NF data were (Table 1). Pairwise comparisons indicated that the differences between experimental times were significant within the case of both the IF and NF (except for days 2 and 7, see online Supplementary Data, Tables S6 and S8). Profile variations of points NF and IF at different times were significant except for day 7 (Table 2), which was slightly more acidic near the fish.

Comparison of Corresponding Results in Experimental and Control Tanks.—The differences between the control and mat tanks at locations near fish were consistently significant (except for day 2; Table 3); when significantly different, the values recorded in the case of the microbial mat were more basic. Inside of the fish, the profiles were not significantly different at either days 2 and 7 (Table 3). On day 90, the pH of the mat returned to a basic condition and was significantly different from the control (Table 3).
Experiments in taphonomy are necessary in order to reproduce the original conditions in which the early stages of fossilization took place, and thereby to better understand the conditions that lead to exceptional preservation. We describe here the chemical conditions generated on a millimetric scale by microbial mats after the deposition of fish carcasses.

Decomposition of the fish was influenced by the microbial mat in two key steps, which were absent in the control tank. Stage one occurred during the first week. Drops in DO next to the fish could either have been the result of the inhibition of photosynthetic activity or an increase in the respiration rate of the microorganisms involved in the aerobic decomposition of the carcasses sufficient to mask the on-going production of oxygen. Inhibition of photosynthesis seems the less likely scenario as the mat became activated nearby the carcasses and the mat growth rate increased quickly, trapping fish bodies entirely. In addition, the pH next to the carcasses remained basic, which is a direct consequence of the increase of photosynthetic activity. Furthermore, the mat's activity would have been stimulated by the release of organic and inorganic nutrients into the environment from decomposition of the body. Inside the fish, this first phase was characterized by anoxia, and despite the extensive DO variation, the pH did not display any relevant acidification trend until after day 7. The DO underwent a rapid and significant reduction caused by depletion of oxygen by heterotrophic aerobic respiration and the pH profile changed from basic to slightly acidic. Under these conditions, the microorganisms responsible for decomposition could either ferment or use anaerobic respiration pathways, leading to a pH decrease in response to the release of acid compounds. Throughout these first days, decomposition processes were active as demonstrated by the noticeable loss of thickness of the body (see Fig. 5D; Iniesto et al. 2013).

Most importantly, the variations in pH and DO are decoupled during the first stage both temporally and spatially. The interior of the fish is

---

**DISCUSSION**

**TABLE 1.**—Statistical analysis using the Kruskal-Wallis test to compare differences in DO and pH between the experimental times under each experimental condition (mat, control, NF, or IF). Tukey's range test to check significance of pairwise comparisons furnished in online Supplementary data. Differences are considered significant (in bold) at a p-value < 0.01; df = degrees of freedom; F = Snedecor distribution.

<table>
<thead>
<tr>
<th>Comparison between the experimental times of the differences in:</th>
<th>df</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO near the fish in microbial tank</td>
<td>88</td>
<td>4.340</td>
<td>0.000</td>
</tr>
<tr>
<td>pH near the fish in microbial tank</td>
<td>88</td>
<td>2.294</td>
<td>0.000</td>
</tr>
<tr>
<td>DO near the fish in control tank</td>
<td>48</td>
<td>6.337</td>
<td>0.000</td>
</tr>
<tr>
<td>pH near the fish in control tank</td>
<td>48</td>
<td>3.668</td>
<td>0.000</td>
</tr>
<tr>
<td>DO inside the fish in microbial tank</td>
<td>118</td>
<td>94.895</td>
<td>0.000</td>
</tr>
<tr>
<td>pH inside the fish in microbial tank</td>
<td>82</td>
<td>50.112</td>
<td>0.000</td>
</tr>
<tr>
<td>DO inside the fish in control tank</td>
<td>64</td>
<td>5.939</td>
<td>0.000</td>
</tr>
<tr>
<td>pH inside the fish in control tank</td>
<td>62</td>
<td>1.121</td>
<td>0.311</td>
</tr>
</tbody>
</table>

FIG. 5.—Fish carcass thickness loss. A) Day 2 fish appearance at the moment of measurement. B, C) Volume loss analyzed by RMI (coronal view). On day 90, the fish was 4 mm thick (B) whereas on day 1,000 the fish thickness was reduced to 3 mm (C). Arrows show the measured thickness, 1 = fish tail, 2 = swim bladder (still preserved after 1,000 days. D) Diagram presents the progressive volume loss of fish carcasses decaying in the microbial mat. The plot also includes data derived from Iniesto et al. (2013).
TABLE 2.—Statistical analysis using the Mann-Whitney Wilcoxon test to compare differences in DO and pH between the two points analyzed (NF and IF) at each experimental time for the microbial mat and the control sediment. Differences are considered significant (in bold) at a p-value < 0.01; df = degrees of freedom; F = Snedecor distribution.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO in mat tanks at day 2</td>
<td>22</td>
<td>18.104</td>
<td>0.000</td>
</tr>
<tr>
<td>DO in mat tanks at day 7</td>
<td>22</td>
<td>24.200</td>
<td>0.000</td>
</tr>
<tr>
<td>DO in mat tanks at day 90</td>
<td>22</td>
<td>15.263</td>
<td>0.000</td>
</tr>
<tr>
<td>pH in mat tanks at day 2</td>
<td>22</td>
<td>1.437</td>
<td>0.140</td>
</tr>
<tr>
<td>pH in mat tanks at day 7</td>
<td>22</td>
<td>7.133</td>
<td>0.000</td>
</tr>
<tr>
<td>pH in mat tanks at day 90</td>
<td>22</td>
<td>33.988</td>
<td>0.000</td>
</tr>
<tr>
<td>DO in control tanks at day 2</td>
<td>16</td>
<td>1.174</td>
<td>0.329</td>
</tr>
<tr>
<td>DO in control tanks at day 7</td>
<td>16</td>
<td>1.318</td>
<td>0.234</td>
</tr>
<tr>
<td>DO in control tanks at day 90</td>
<td>16</td>
<td>2.186</td>
<td>0.023</td>
</tr>
<tr>
<td>pH in control tanks at day 2</td>
<td>16</td>
<td>0.360</td>
<td>0.984</td>
</tr>
<tr>
<td>pH in control tanks at day 7</td>
<td>16</td>
<td>5.537</td>
<td>0.000</td>
</tr>
<tr>
<td>pH in control tanks at day 90</td>
<td>16</td>
<td>0.879</td>
<td>0.396</td>
</tr>
</tbody>
</table>

The results presented here investigate a more complex system compared to previous decay experiments (e.g., inoculation with a heterotrophic bacterial veil, such as Sagemann et al. [1999]) by incorporating the effect of an actual microbial mat (i.e., with photosynthetic autotrophic conditions suggest differential preservation between the upper and lower carcass surfaces.

The second phase corresponds to day 90 when substantial changes were observed in the areas surrounding the fish and the fish interior: a DO recovery exceeding its initial value (maximum reached above the fish) in conjunction with a pH return to the original basic condition. After the initial period of decay, what remains of the carcass is more difficult to decay and therefore decomposition proceeds slower, as implied by the decrease in the rate at which the thickness of the specimens decreased (Fig. 5D). The decrease in the rate of microbial consumption of oxygen was balanced by the rate of oxygen production by photosynthesis and resulted in the oxygenation inside of the fish. During this second phase, the oxic environment would drastically limit the anaerobic decomposers’ activities in the upper layers of the mat. As a result of the slower local release of acids and the increase in photosynthesis, the pH would rise. At this point in the presence of the microbial mat, preservation mechanisms would mainly depend on high pH values and oxic conditions, driven mainly by the activity of cyanobacteria. In the course of the experiment, the system loses activity and dynamism as nutrients are depleted, resulting in lower oxygen production and consequent mat stabilization.

The results presented here investigate a more complex system compared to previous decay experiments (e.g., inoculation with a heterotrophic bacterial veil, such as Sagemann et al. [1999]) by incorporating the effect of an actual microbial mat (i.e., with photosynthetic autotrophic

![Fig. 6.—Dissolved oxygen (DO) and pH profiles recorded at point NF (next to fish) in the control sediment throughout experiment. A) Before carcass deposition. B) At 2 days. C) At 7 days. D) At 90 days. Initially DO level decrease regularly from the water column surface until attaining anoxia at the sediment surface. The DO concentration remained close to anoxia except for the water surface in contact with air all the way through the experiment. Notice that on day 90 the DO level further decreased becoming zero at 2 mm above of the sediment’s surface. Bars represent standard deviation.](image-url)
aerobic and anaerobic microorganism communities). Furthermore, the long duration of the experiment (3 years) revealed a novel phase of the process which follows the anaerobic conditions previously described during decay (Briggs and Kear 1994; Raff et al. 2008). Although it is accepted that authigenesis and soft-tissue preservation should occur in anoxia and low pH (Sagemann et al. 1999), according to our results the process seems to be influenced by different chemical conditions in the presence of mats. In particular, the interior of the fish placed in the control tank remained anoxic throughout the experiment and the pH was significantly lower than in the mat tank. In spite of this, controls decomposed substantially. The decay of fish covered by mats was slowed down to such an extent that even some soft structures (skin, muscles and tendons) were retained over a longer period of time (see Figs. 2A, 5; Iniesto et al. 2013). Therefore, major differences in preservation occurred during the second phase, when DO and pH conditions are driven by the oxygenic phototrophic microorganisms, and the system as a whole is oxic with a basic pH value.

The role played by microbial communities in inducing mineralization in microbial mats (bioprecipitation) is complex. Some microbial activities of the mats, such as aerobic (Visscher et al. 1998) or anaerobic (Vasconcelos et al. 2006) photosynthesis, sulfate reduction, or even aerobic carboxylic acid degradation can lead to a pH increase and carbonate precipitation (Dupraz and Visscher 2005). The organic EPS matrix entails a two-fold role being equally involved in carbonate formation and degradation, and its role varies depending on environment-specific physicochemical and biological features (Dupraz et al. 2009). This matrix can work as a cation chelator, removing Ca from the environment as a consequence (Dupraz and Visscher 2005). However, when the EPS matrix undergoes biological degradation, the Ca$^{2+}$ is released, favoring carbonate precipitation (Dupraz and Visscher 2005), with the matrix acting as a template that drives crystal nucleation (Costerton et al. 1995). As a result of the
localized CaCO₃ precipitation within mats, lithification of layers can occur (Decho et al. 2005). Our results elucidate some of the relationship between microenvironmental changes and bioprecipitation process. The inside of the fish remains anoxic from the outset; two days after the fish was deposited on the mat, anoxia is accompanied by a pH decrease throughout the entire profile. By contrast, outside the fish DO decreases (most probably as a result of heterotrophic activity but the pH remains basic (pH ~ 9). The growth of heterotrophic microorganisms may be limited by nutrient availability as fish scales are difficult to degrade and contain little N. When this occurs, heterotrophic bacteria can degrade the EPS matrix made up mainly of lipids and carbohydrates (Sand and Gehrke 2006) in order to obtain needed nutrients. Oxygenic photosynthesis is on-going but masked by oxygen consumption due to aerobic respiration and it explains the high pH outside of the fish. The addition of a source of Ca²⁺ (e.g., via the degradation of the EPS matrix) would create the necessary conditions for carbonate precipitation as suggested by Iniesto et al. (2013), who observed a Ca-enriched film on a fish decayed for 15 days.

In the longer term, after being covered by microbial mats, the fish interior becomes oxic with high pH values, especially three months after deposition. These conditions during the second phase are different from the role of heterotrophic biofilms in fossilization described by Wilby et al. (1996). The taphonomic experiments conducted by Briggs et al. (1993) suggested that the shift between carbonate and phosphate is linked to pH conditions, allowing and favoring the preservation of soft-tissues. However, although Briggs and Wilby (1996) linked the occurrence of calcium phosphate to a decrease in pH, the precipitation of this mineral phase in basic environments is well known (Benzerara et al. 2004; Recillas et al. 2012). Indeed, Song et al. (2002) found that the optimal pH for calcium phosphate precipitation in experiments (at lower-moderate concentrations of phosphate, consistent with our system) ranged from 8.5 to 9 depending on the Ca/P ratio (see Song et al. 2002, figs. 2, 3). Under these high-pH conditions, the switch between phosphate and carbonate would depend on the concentration of P and the Ca/P ratio in the system, rather than on pH (Bachra et al. 1963). In addition, other minerals such as magnesium silicates (Souza-Egipsy et al. 2005; Iniesto et al. 2015) are also favored by this oxic-basic phase. Therefore, further studies remain necessary to contrast whether mineral replication of soft tissues should take place only in the course of early decay (oxic-anoxic and acid phase), or if mineralization (e.g., calcium phosphate, magnesium silicates) might occur in the oxic and basic phase of the microbial mat in the long-term, as plausible evidence suggests.

CONCLUSIONS

The experiment has confirmed that in the process of covering carcasses on their surface, microbial mats are responsible for generating complex chemical microenvironments that inhibit decomposition and increase preservation of tissues. In contrast, when the microbial mat is absent, fish exhibit active, persistent and continual decay. The mat’s role was twofold. During the first stage, DO underwent a significant reduction and the pH turned slightly acidic inside the fish. On the outside of the fish carcasses, the pH remained basic and would allow the precipitation of several mineral phases such as silicates, carbonates and phosphates, depending on the elements released by the degradation of EPS. This short-lived phase has been detected previously in experimental studies (e.g., Sagemann et al. 1999). During the second phase, the inside of the fish became oxic and the pH was basic allowing for preservation of soft inner tissues. Consequently, the microbial coverage and the subsequent protective microenvironment would be able to promote pseudomorphism and biomineralization processes, explaining the exceptional preservation of carcasses within microbial mats.

ACKNOWLEDGMENTS

This work is part of research projects CGL2013-42643P and PE-CI-0369-2755, funded respectively by the Spanish Ministry of Economy and Competitiveness (MINECO) and the government of Castilla-La Mancha. The research grant that enabled Miguel Iniesto to participate was also funded by MINECO. The SEM facility at IMPMC was supported by Region Ile de France grant SESAME 2006 1-07-593/R, INSU-CNRS, INP-CNRS, University Pierre et Marie Curie, Paris. SEM analyses performed for this study were supported by a Grant from the Foundation Simone et Cino Del Duca (PF- Karim Benzerara). MRI monitoring was realized at CAI de RMN (UCM). We are pleased to acknowledge David Moreira, whose comments and observations helped us to improve this manuscript, Berta Martin for providing statistical help, and two anonymous referees for their comments. We would also like to thanks Richard M. Fratini for the English style review of the manuscript prior to submission.

SUPPLEMENTAL MATERIAL


REFERENCES


Received 16 September 2014; accepted 25 September 2015.