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# A quantitative study of the degradation of whale bone lipids: Implications for the preservation of fatty acids in marine sediments

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ABSTRACT

The degradation and preservation affecting the biomarker record of ancient metazoa are not fully understood. We report on a five month experiment on the fate of fatty acids (FAs) during the degradation of recent whale vertebrae (*Phocoena phocoena*). Whale bones were analysed for extractable FAs and macromolecularly bound *n*-acyl compounds. Fresh bone showed extractable FAs dominated by 16:1ω7c, 16:0, 18:1ω9c and 18:0. Calculated degradation rate constant ($k$) values showed a rapid decrease in FA concentration, with $k$ values higher for unsaturated than for saturated compounds (0.08/day for 18:1ω9c, 0.05/day for 16:0). The appearance or increased abundance of distinctive methyl branched (e.g. *i*/*ai*-15:0 and -17:0, 10Me-16:0) and hydroxy FAs (e.g. 10OH-16:0 and 10OH-18:0) were observed, providing clear evidence for the microbial degradation of bone organic matter and an input of lipids from specialised bacteria. Catalytic hydropyrolysis (HyPy) of demineralised extraction residues released up to 0.13% of the total *n*-$C_{16}$ and *n*-$C_{18}$ moieties in the degraded bones. This revealed that only a small, yet sizeable, portion of bone-derived fatty acyl units was sequestered into (proto)kerogen during the earliest stages of degradation.



## 1. Introduction

It is well known that fatty acids (FAs) may survive over geological timescales as organic remains of earlier forms of life, and their presence in ancient sediments and crude oils is well documented (Tanaka and Kuwata, 1928; Bergmann, 1963). Moreover, they are important constituents of bone marrow and numerous studies of individual vertebrate fossils have employed them as biomarkers for the evolution of animals (Das et al., 1967; Everts et al., 1968; Das and Harris, 1970; Child, 1995) or as archaeological (Evershed et al., 1995) or diagenetic tracers (Thiel et al., 2014). However, it is often difficult to assess the microbial decomposition and other taphonomic processes that affect the authenticity of the biomarker inventory of a fossil. Large lipid-rich skeletons at deep sea whale falls are, for instance, considered to support microbial communities over extended timescales (Schuller et al., 2004). To a major extent, the organic inventory of decaying bone is controlled by postmortem bacterial mat coverage and microbioerosion, which seem to negatively correlate with the bone oil content (Higgs et al., 2011). Studies of recent and fossil whale bones revealed that bacterial degradation of lipids and microbioerosion proceed from the external surfaces. Deeper penetration of seawater- or sediment-derived microorganisms into the trabecular network of the bone is initially prevented by the hydrophobic character of the lipids filling the bone marrow cavities (Collins et al., 2002; Treude et al., 2009). After initial depletion of lipids in the peripheral zone, further degradation is considered to slowly migrate inwards to the deeper collageneous bone parts (Schuller et al., 2004; Treude et al., 2009; Guilminot et al., 2013). The degradation rate is thus determined by the concentration of lipids and the size of the bones. This causes the skeletons of small or juvenile whales, with low oil content, to be decomposed more rapidly than those of adult whales (Higgs et al., 2011). However, direct observation of degradation rate and preservation potential of animal-derived FAs in the actual bone is missing.

Previous short term incubation experiments were aimed mainly at assessing the rate and mechanisms of FA degradation in sediments under oscillating redox conditions (Sun et al., 1997, 2002; Caradec et al., 2004; Lü et al., 2010). These studies used

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complex phytoplanktonic lipids in algal cells or the ubiquitous *n*-hexadecanoic acid (16:0, palmitic acid) and (9*Z*)-octadec-9-enoic acid (18:1ω9c, oleic acid) as model compounds added to marine sediment cakes. Most studies observed that the degradation rate constant (*k*) values for FAs were several times higher in oxic than in anoxic sediments (Sun et al., 1997, 2002; Caradec et al., 2004; Lü et al., 2010), although exceptions were reported (Lü et al., 2010). Likewise, the experiments showed that the degradation of the unsaturated 18:1ω9c may (Sun et al., 1997), or may not (Sun et al., 2002; Caradec et al., 2004), proceed substantially faster than for the saturated 16:0.

In this study, we have investigated the fate of FAs in the "natural laboratory" of marine whale bone to gain insight into the preservation mechanisms for individual FAs in vertebrate fossils and to better assess their utility as metazoan and/or microbial biomarkers. Our work complements a recent study (Thiel et al., 2014) where we focused on FA biomarkers in an ancient (Oligocene) whale bone and its host sediment. In that study, the ancient FAs were compared with reference bones from a freshly beached carcass of *Phocoena phocoena*, in order to better discriminate between animal-derived and microbial FAs and elucidate their diagenetic overprint. Now we have used the same recent whale bones for a five month incubation experiment in marine sediment. In addition to the early degradation and alteration of FAs, emphasis was placed on the incorporation of microbial FAs and allochthonous (sedimentary) lipids into the decaying bone. In addition, the presence of FA moieties in insoluble organic matter (OM) was analysed using catalytic hydropyrolysis (HyPy) to investigate the early sequestration, and thus preservation, of bone-derived alkyl moieties in (proto)kerogen.

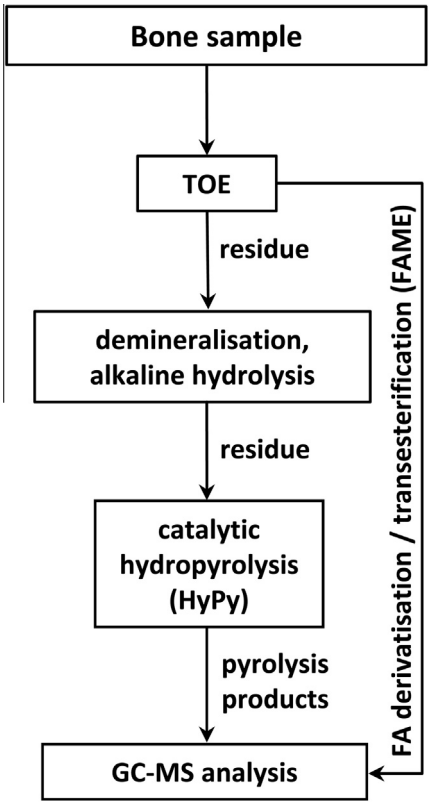


**Fig. 1.** Flow chart depicting a simplified scheme of the work-up procedure (TOE, total organic extract; FA, fatty acid; FAME, FA methyl esters).

## 2. Methods

### 2.1. Experimental set-up

Five vertebrae (diameter of vertebral body ca. 25 mm, thickness ca. 12 mm) were taken from a freshly beached carcass of a juvenile harbour porpoise (*P. phocoena*, North Sea) and incubated in two flow tanks in surface sediment from Eckernförde Bay (western Baltic Sea (54°30′N, 10°02′E). The organic-rich sediment layers were each 10 cm thick. The flow tanks (24 l capacity) were filled to the outlet with unfiltered seawater from Kiel Fjord [western Baltic Sea (54°22′4″N, 10°10′23″E), salinity ca. 15 psu]. During the five month experiment the tanks received a steady supply of ca. 250 ml/min of seawater pumped from the fjord at a depth of ca. 3 m. The installation was covered by large black plastic bags and kept at a constant temperature of 5 °C. Using a scalpel, flesh was removed from the bones before they were embedded to a shallow depth into the sediment. Monthly, one of the bones was removed and the sediment-covered vertebral body analysed for extractable FAs and macromolecularly bound *n*-acyl compounds (samples $t_1$–$t_5$). An untreated vertebra of the same specimen ($t_0$) and the host sediment were analysed as reference samples. The reference sediment was taken from the corner of a flow tank, as far as possible from the bones. The sediment was reduced, i.e. black and with a sulfidic smell. All samples were kept frozen at −20 °C until further processing.

### 2.2. Sample preparation and extraction

The flow chart (Fig. 1) gives an overview of the sample workup procedure. After scraping all visible adhering tissue off the thawed bones with a sterile scalpel, the vertebral spines were removed using solvent-rinsed garden pruning shears. One half of each vertebra body was cut into smaller pieces, freeze-dried and homogenised using a pebble mill (Retsch MM 301). The powdered sample was extracted (20 min at 40 °C, ultrasonication) with dichloromethane (DCM)/MeOH; 3:1, v/v), DCM and *n*-hexane. After volume reduction using a rotary evaporator, the total organic extract (TOE in Fig. 1) was dried under a gentle stream of $N_2$ and transesterified using trimethylchlorosilane (TMCS) in MeOH (1:9, v/v; 1.5 h at 80 °C) in a screwed vial. The resulting reaction mixture was extracted 3× using 1 ml *n*-hexane each, respectively, and the extracts were combined, yielding the extractable FA methyl ester (FAME) fraction. After removing elemental S with activated Cu, the solvent volume was carefully reduced under $N_2$, avoiding complete dryness to prevent loss of short chain FAMEs (cf. Ahmed and George, 2004). Subsequently, each FAME fraction was analysed using gas chromatography–mass spectrometry (GC–MS; Section 2.3). FAMEs were qualitatively evaluated by comparing the mass spectra and retention times with an external standard mixture (37 Component FAME Mix, Supelco). They were quantified using an internal standard, and double bond positions were determined using dimethyl disulfide (DMDS) adduction on aliquots of the FAME fractions and GC–MS analysis of the reaction products (Scribe et al., 1988).

The extraction residues intended for macromolecular analysis using HyPy were again rigorously extracted using the above solvent sequence to ensure quantitative removal of residual lipids (Thiel et al., 2014). The remaining extraction residues were freeze-dried and demineralised by dropwise addition of HCl (37%) in a constantly stirred, water-based suspension. After the reaction had subsided, the reaction mixture was neutralised by addition of DCM-extracted millipore water and dropwise addition of KOH (10%). The sample was then saponified (6% KOH/MeOH, v:v; 2 h, 70 °C, ultrasonication). The reaction mixture was extracted 3× with *n*-hexane to remove neutral lipids, acidified to pH 1 by adding HCl (37%) and extracted a further 4× with *n*-hexane to eliminate any remaining mineral-occluded soluble