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A search for biomarker evidence of an intermittent deep-sea hypersaline anoxic basin in the eastern Mediterranean during the Early Pliocene

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ABSTRACT

Deep-sea hypersaline anoxic basins (DHABs) are extreme environments harbouring unique microbial assemblages. They have, however, rarely been identified in the geological record. Here, we investigate the potential use of organic biomarkers to detect former DHAB presence in the eastern Mediterranean during the Early Pliocene. Our findings suggest challenges in identifying DHABs in the geological record using biomarker records; this is due to pelagic organic matter overprinting the DHAB signals.

1. Introduction

Deep water hypersaline anoxic basins (DHABs) are present along the Mediterranean Ridge of the eastern Mediterranean Sea (Camerlenghi, 1990; Cita, 1991). DHABs originate from the dissolution of evaporitic deposits laid down during the Messinian salinity crisis, which on exposure to seawater can lead to dense brines flowing down the seafloor topography and accumulating in depressions (Camerlenghi, 1990). DHABs are some of the most extreme and hostile environments on Earth, but despite their harsh nature, these environments can sustain diverse microbial communities, particularly at the brine-seawater interface (BSI), which typically demonstrates a steep gradient of salinity, temperature, density, O₂ and pH (Daffonchio et al., 2006). Crucially, the diverse and unique microbes inhabiting these basins may offer insight into early conditions on Earth and assist with the detection of extraterrestrial life (i.e. Fisher et al., 2021). Although DHABs are well studied in the modern, little is known about their occurrence in the past, in part due to the limited tools for their identification in the palaeorecord.

Microbial biomarkers may aid identification of past DHABs. Enhanced activity of certain archaea within the BSI of modern DHABs (Daffonchio et al., 2006) indicates that archaeal lipids may be suitable DHAB biomarkers in the palaeorecord. Extended archaeol (C₂₅-C₂₀ *sn*-2 archaeol; Ext-AR), for example, is considered indicative of the halophilic archaeal class Halobacteria (Vandier et al., 2021). The structure of Ext-AR is thought to help reduce membrane permeability, enabling these microbes to survive in salinities >50 psu (Vandier et al., 2021). In the fossil record, Ext-AR has the potential to provide insight into past

halophilic microbial communities and their hypersaline environments (Birgel et al., 2014; Natalicchio et al., 2017).

Here, we examined a unique Early Pliocene record from the Lido Rossello (LR) outcrop in southwest Sicily which features six finely laminated layers. These enigmatic layers are absent in time-equivalent sediments of nearby (<2 km) Punta di Maiata (PM). LR's laminated layers exhibit enhanced preservation of total organic carbon (TOC), biogenic barium and opaline skeletons, and an absence of benthic organisms, indicating anoxic conditions (Cutmore et al., 2023). Furthermore, a slump level below the first laminated interval points to a physical depression at LR, and in combination with the inorganic geochemical records, an intermittently present DHAB has tentatively been suggested at this site during the Early Pliocene (Cutmore et al., 2023). Here, we analysed archaeal membrane lipids in the two upmost finely laminated layers from LR, L5 (4.665–4.657 Ma) and L6 (4.634–4.631 Ma), and compared this with the time-equivalent sediments from PM to explore whether archaeal lipids can be used to detect DHABs in the geological record.

2. Methods

Lipids were extracted from sediment samples (LR, n = 75; PM, n = 35) using a modified Bligh and Dyer extraction method and sample work up as previously described by Bale et al. (2021). Extracts were separated with a reverse phase method on an ultra-high-performance liquid chromatograph (Agilent 1290 Infinity I) and analyzed on a Q Exactive Orbitrap MS (ThermoFisher Scientific) using settings as previously

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described by Bale et al. (2021). Lipid biomarkers are reported as peak area per gram of TOC (Cutmore et al., 2023). Normalizing biomarker abundances to TOC compensates for different degrees of oxygen exposure and the dilution or concentration of organic matter by inorganic sediment.

3. Results and discussion

We detected a range of archaeal lipids in both LR and PM outcrops. The identified isoprenoidal glycerol dialkyl glycerol tetraethers (isoGDGTs) include: GDGT-0, -1, -2, -3 and -4, and crenarchaeol, monohexose crenarchaeol, and crenarchaeol isomer (combined as 'crenarchaeol'). Of these, GDGT-0 and crenarchaeol were dominant (Fig. 1b&c). The identified isoprenoidal dialkyl glycerol diethers (DGDs) include: archaeol and monohexose archaeol (combined as 'AR'; Fig. 1d); *sn*-2 and *sn*-3 extended archaeol, C₂₅-C₂₀ monohexose *sn*-2 archaeol, and C₂₀-C₂₅ monohexose *sn*-3 archaeol (combined and described as 'Ext-AR'; Fig. 1e); C₂₅-C₂₅ di-extended archaeol (di-Ext-AR; Fig. 1f); *sn*-2 and *sn*-3 hydroxyarchaeol (combined as 'OH-AR'; Fig. 1g). The intact polar lipids detected, i.e. those with a monohexose head group, are likely primarily of fossil origin and not sourced from in situ sedimentary production (Schouten et al., 2010; Lengger et al., 2012), hence why they were combined with their corresponding core lipids.

A similar pattern can be observed in the LR and PM TOC, isoGDGT (crenarchaeol and GDGT-0) and OH-AR records: low values coincide with northern hemisphere (NH) summer insolation minima, while high values coincide with NH summer insolation maxima (Fig. 1i). The finely laminated L5 and L6 intervals are an exception, whereby significant peaks in TOC, isoGDGT and OH-AR abundance do not occur in the time equivalent PM succession. In contrast, AR and Ext-AR both decline in the laminated layers and remain low in PM. Di-Ext-AR abundance is low throughout both LR and PM records, with the exception of a significant peak in upper L5 which continues until 4.65 Ma.

Ext-AR, a biomarker of hypersaline environments (Birgel et al., 2014; Natalicchio et al., 2017), would be expected to increase in abundance in the finely laminated intervals as inorganic proxies indicate the presence of a DHAB at that time (Cutmore et al., 2023). Instead, Ext-AR abundance is consistently elevated throughout the LR record compared to PM, and actually decreases in the laminated intervals. This phenomenon

may be an artefact of increased preservation of pelagic OM during these periods, which likely serves as the primary source of TOC, consequently diluting the benthic signal. The evidence for this lies in the peak of isoGDGTs observed in the laminated layers, which likely originate from pelagic Thaumarchaeota (Zeng et al., 2019; Sinninghe Damsté et al., 2002; Pitcher et al., 2011). It is unlikely that the isoGDGT peaks are the result of enhanced in-situ sedimentary production since these lipids are thought to degrade rapidly (Lengger et al., 2012). Indeed, when not normalized for TOC, Ext-AR abundance per gram of dry sediment peaks significantly in the finely laminated layers (Fig. 1h), though this too may be an increased preservation effect. The higher Ext-AR abundance in the LR intervals between the laminated layers compared to time-equivalent PM samples may indicate sustained enhanced salinity in the LR sediments and sediment-bottom water interface (SWI) despite the DHAB being absent. The AR aligns with the Ext-AR record indicating that AR may be sourced from archaea inhabiting the DHAB during L5 and L6, as well as the sediments/SWI at LR in the intervening periods, with these environments supporting enhanced diversity and abundance of archaea (Daffonchio et al., 2006).

Di-Ext-AR present in L5 may be a more robust DHAB indicator as it does not appear to be diluted by preserved pelagic OM. This biomarker is known to be produced by specific archaea, including certain halophilic archaea (De Rosa et al., 1983) and the methanogen *Methanomassiliicoccus luminyensis* (Becker et al., 2016). This biomarker peak persists after the laminated layer, indicating continued survival of these microbes in the sediments/SWI once the brine pool disappeared. Unlike Ext-AR, di-Ext-AR is not detected in such high abundance in other intervening periods or in L6, indicating possible temporal microbial community variability of this intermittent DHAB. This may potentially have been driven by chemical differences: the L6 layer, for example, does not contain any siliceous organisms or the high concentrations of opal that were found in the L5 layer (Cutmore et al., 2023).

Both laminated layers show a peak in OH-AR abundance, a diether lipid found in methanotrophic archaea, which perform anaerobic oxidation of methane (AOM; Schubert et al., 2006; Naeher et al., 2014), and methanogenic archaea (Koga et al., 1998; Pancost et al., 2001). Methanogenesis is an important process within the BSI of modern DHABs (Borin et al., 2009), therefore the enhanced presence of OH-AR in the laminated layers may indicate the presence of methanogenic

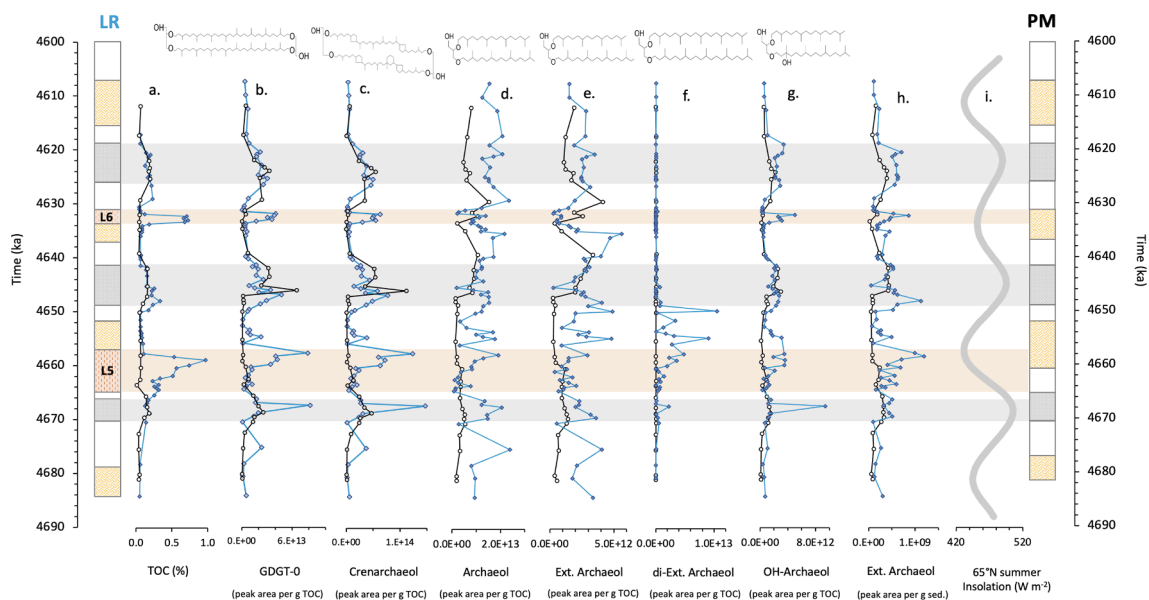


Fig. 1. LR (blue) and PM (black) records of: a. TOC (%); b. GDGT-0 (peak area per g TOC); c. crenarchaeol; d. AR; e. Ext-AR; f. di-Ext-AR; g. OH-AR; h. Ext-AR (peak area per g sediment); and i. summer insolation at 65°N (Berger and Loutre, 1991). Bands show laminated layers present only in the LR record (orange) and periods of maximum precession (grey). Grey line shows summer insolation at 65°N (Berger and Loutre, 1991). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

archaea. However, AOM has been shown to occur in sediments after the extrusion of methane-rich fluids (Pancost et al., 2000); therefore, the enhanced presence of OH-AR may indicate AOM archaea in methane-rich sediments underlying the brine pool. Indeed, it may have been the episodic extrusion of methane-rich fluids that were responsible for the upward migration of saline deposits from underlying rock which consequently led to brine formation (MEDINAUT/MEDINETH SSP, 2000). The lower abundance of the OH-AR biomarkers in the intervals between the laminated layers may indicate reduced AOM due to the lower emissions of methane-rich fluids which led to the absence of the brine pool.

4. Conclusions

Our findings indicate the challenge of unambiguously identifying DHABs in the geological record using archaeal lipid biomarkers. This arises from the strong overprint of the DHAB signal by enhanced preservation of pelagic OM due to the anoxic conditions of the DHAB. It appears that sediment/SWI hypersalinity persisted in the intervals between the presence of the DHAB. Consequently, Ext-AR may be a useful indicator of hypersaline sediments/SWI when the anoxic basin is absent and not overprinting the DHAB signal by enhanced preservation of pelagic OM.

CRedit authorship contribution statement

A. Cutmore: Writing – review & editing, Writing – original draft, Visualization, Project administration, Investigation, Formal analysis, Data curation. **N. Bale:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Conceptualization. **L. Lourens:** Writing – review & editing, Supervision, Conceptualization. **S. Schouten:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Stefan Schouten reports financial support was provided by Netherlands Earth System Science Centre. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data generated for this study are archived and publicly available via the Mendeley Data repository online (Cutmore et al., 2024; <https://doi.org/10.17632/kp8988bs5h.1>).

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