Otolith δ^{18}O and δ^{13}C evidence for stratification and fish metabolic changes in the eastern Mediterranean before the Messinian Salinity Crisis

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ABSTRACT

Stable oxygen and carbon isotopic composition of the otoliths of a pelagic and a benthic fish species are used as indicators of sea surface and bottom salinity-oxygenation conditions, as well as proxies of the fishes' metabolic response to the paleoceanographic changes in the eastern Mediterranean from 7.2 to 6.5 Ma.

The step-wise restriction of the Mediterranean–Atlantic gateway impacted the ability of the Mediterranean fishes to grow, particularly those dwelling in the sea bottom.

The most important changes identified are: 1) a negative water budget (evaporative) event at 6.82–6.81 Ma that resulted in an increase in fish metabolism and respiration, and 2) a warming event at 6.70–6.69 Ma that further intensified the environmental stress for bottom-water fish and increased their metabolic activity despite low food availability. The results provide proof that the resilience of the benthic fish population was hampered, particularly after the 6.70–6.69 Ma event, due to the increased salinity, temperature and stratification of the water column in the eastern Mediterranean as a result of the marine gateway restriction.

Keywords: Miocene, oxygen isotopes, carbon isotopes, paleoclimate, metabolic rate

INTRODUCTION

The paleoceanographic changes in the Mediterranean during the Messinian (7.25–5.33 Ma) and their impact on higher organisms offer unique insights into the resilience of marine ecosystems under extreme environmental changes. Global climate cooling (Herbert et al., 2016) and the gradual restriction of the marine gateway with the Atlantic starting at the Tortonian/Messinian boundary (Krijgsman et al., 1999; Flecker et al., 2015) led to extreme environmental conditions in the

Mediterranean, culminating in the Messinian Salinity Crisis (MSC; 5.97–5.33 Ma; Hsü et al., 1973; Krijgsman et al., 1999). The basin's restriction resulted in high-amplitude shifts in sea surface temperature (SST) and salinity (SSS), and episodic water-column stratification and dysoxia on the sea bottom, even before the MSC (e.g., Vasiliev et al., 2019; Sabino et al., 2020; Bulian et al., 2021; Kontakiotis et al., 2022). However, the impact of these events on higher organisms such as fishes remains unclear. In the Early Messinian, the presence of new endemics (Girone et al., 2010) and Paratethyan-affinity fish species (Schwarzhans et al., 2020) in the Mediterranean has been attributed to the changing paleoceanographic conditions. In this study, based on oxygen and carbon isotopic analyses of fish otoliths, we present evidence of episodic stratification of the water column in the eastern Mediterranean. We furthermore test the hypothesis that the pre-MSC amplitude of changes in SST and SSS had a negative impact on the physiological functioning and growth of marine fishes.

Otoliths are accretionary, mostly aragonitic structures in the inner ear of teleost fishes, and they are metabolically inert, making them excellent paleoceanographic archives (Campana, 1999). The oxygen isotope ratio in otoliths ($\delta^{18}O_{oto}$) is a function of temperature and the oxygen isotopic composition of ambient water, and it is not affected by somatic growth or the otolith precipitation rate, but species-specific lifestyle patterns can confound the signal (Kalish, 1991). The carbon isotopic composition of fish otoliths ($\delta^{13}C_{oto}$), on the other hand, records the isotopic signatures of the fish's diet and the dissolved inorganic carbon (DIC) in ambient water (Kalish, 1991; Solomon et al., 2006; Trueman et al., 2016; Chung et al., 2019b). $\delta^{13}C_{oto}$ is considered a proxy of the fish's metabolic rate (e.g., Solomon et al., 2006; Trueman et al., 2016; Chung et al., 2019b, 2019a), which reflects the amount of energy it uses to live and grow, and has an impact on its behavior and resilience in the face of environmental change (Gauldie, 1996; Chung et al., 2019b). Moreover, at the evolutionary level, higher metabolic rates in a population are expected to lead to higher genetic mutation rates (Trueman et al., 2016). $\delta^{13}C_{oto}$ is linked to oxygen consumption through the metabolic oxidation of dietary carbon (Chung et al., 2019a). For fish feeding on phytoplankton and zooplankton, such as the ones examined here, $\delta^{13}C_{oto}$ is positively correlated with food availability and, therefore, net primary production (Burton et al., 2011): under conditions of high (low) food availability, individual fishes with high (low) metabolic rates are more resilient. Additionally, high metabolic rate results in higher oxygen consumption, higher activity, higher respiration and greater carbon export (Chung et al., 2019b). Metabolic (respiratory) carbon is the carbon released from the respiration of food and it has a 15‰ lower $\delta^{13}C$ than the carbon isotopic composition of the dissolved inorganic carbon in the ambient water ($\delta^{13}C_{DIC}$) (Kroopnick, 1985).

MATERIAL AND METHODS

We analyzed fish otoliths at approximately every meter from the ~25-m–thick pre-MSC Messinian laminated (sapropelitic) and homogeneous marls succession of Agios Myron (Figure 1; N 35°23'35.90", E 25°12'67.91", Crete Island, Eastern Mediterranean), which spans the interval 7.2–6.5 Ma (Zachariasse et al., 2021). Two very common zooplanktivorous species were selected for analyses, the pelagic *Bregmaceros albyi* and the benthic *Lesueurigobius friesii*, based on: a) their great abundance and frequency in Neogene and Quaternary Mediterranean marine sediments, which would render their isotopes useful as paleoceanographic proxies, and b) their well-established ecology (details in the supplementary material), allowing more accurate interpretation of isotopic analyses results. Only the otoliths of adult individuals were analyzed to avoid differences in species-specific vital effects that affect isotopic ratios (Darnaude et al., 2014). The cleaning process and isotopic analyses are described in the supplement.

Carbon in otoliths comes from two sources: seawater and diet. Therefore, $\delta^{13}C_{oto}$ reflects changes in the seawater $\delta^{13}C$, which depends on pH, and in metabolically derived carbon (simply metabolic carbon; M_{oto}), which depends on the fish's diet and metabolic rate. The relative contribution of M_{oto} to $\delta^{13}C_{oto}$ can be estimated using the mass balance model of Kalish (1991): $\delta^{13}\mathrm{C}_{\mathrm{otolith}} = (\delta^{13}\mathrm{C}_{\mathrm{DIC}} + \varepsilon_{\mathrm{b}})\left(1 - \frac{M_{oto}}{100}\right) + (\delta^{13}\mathrm{C}_{\mathrm{diet}} + \varepsilon_{\mathrm{a}})\left(\frac{\mathrm{M}_{oto}}{100}\right)$, where $\delta^{13}C_{diet}$ is the isotopic ratio of the target fish species' diet items.

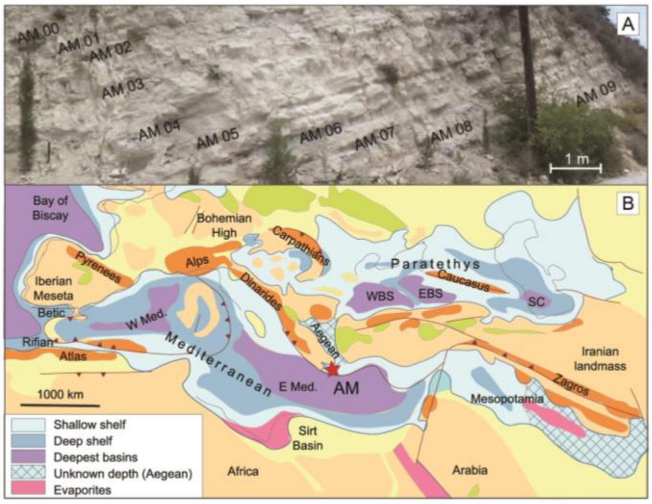

Figure 1. The Agios Myron section A) lithological alternations and sampling approach. B) paleolatitudinal location and their approximate position within the late Miocene Mediterranean basin (paleogeographic map based on Ilyina et al., 2004).

According to Sabino et al. (2022), the estimated $\delta^{13}C_{DIC}$ of stratified waters before the MSC was −3.5‰, whereas that of well-mixed waters was −0.8‰. We used the value of −0.8‰ in the calculations of $M_{B.albyi}$ and of $M_{L.friesii}$ corresponding to the carbonate and homogeneous marl levels at the base of the section, and the value of −3.5‰ in the calculation of $M_{L.friesii}$ for the laminated marl (sapropelitic) levels. We used the oxygen and carbon stable isotopic composition of foraminifera shells of the planktonic species *Globigerinoides obliquus* ($\delta^{13}C_{G.obliquus}$) (Kontakiotis et al., 2022) and the benthic species *Cibicides (pseudo)ungerianus* ($\delta^{13}C_{C.ung}$) (Zachariasse et al., 2021) as the $\delta^{13}C_{diet}$ in the case of *B. albyi* and *L. friesii*, respectively, because foraminifera were considered representative of the fishes' diets. Information on the foraminifera analyses may be found in the supplement.

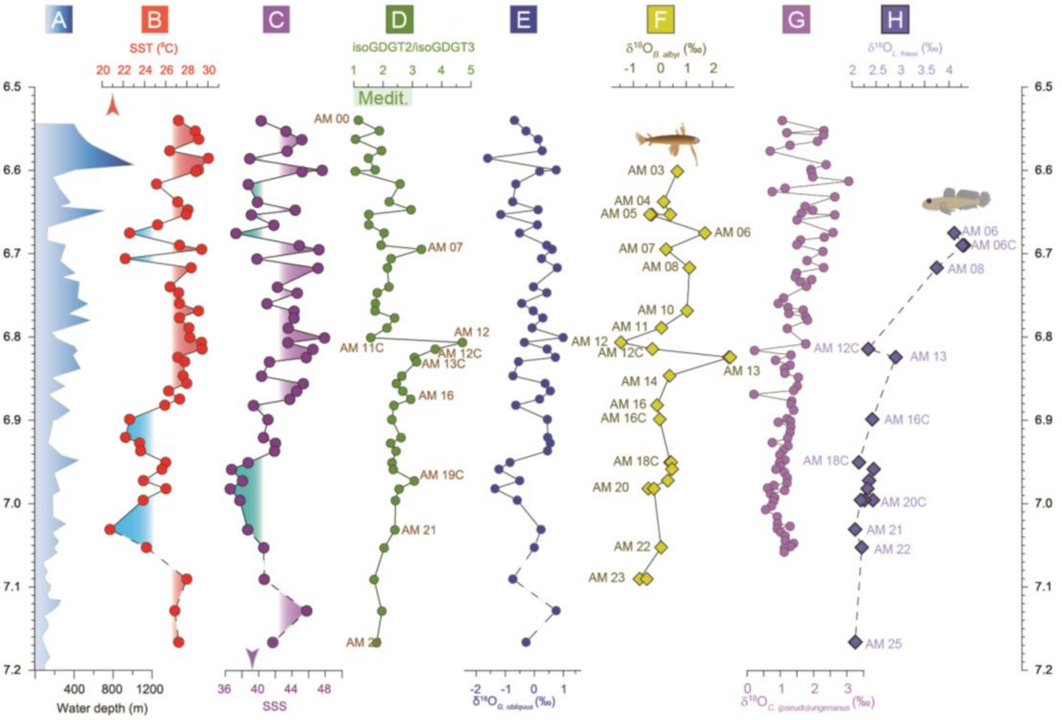

Figure 2. Agios Myron A) paleobathymetric curve (Zachariasse et al., 2021); B) sea surface temperature based on TEX^{H}_{86}, C) sea surface salinities based on SST-TEX^{H}_{86} and $\delta^{18}O_{G.obliquus}$ (Kontakiotis et al., 2022); D) Ratio of isoprenoidal glycerol dialkyl glycerol tetraethers 2 and 3 (isoGDGT2/isoGDGT3) (Butiseacă et al., 2022); E) $\delta^{18}O$ of the planktonic foraminifer *Globigerinoides obliquus* (Kontakiotis et al., 2022); F) $\delta^{18}O$ of the otoliths of the pelagic fish *Bregmaceros albyi*; G) $\delta^{18}O$ of the benthic foraminifer *Cibicides (pseudo)ungerianus* (Zachariasse et al., 2021); and H) $\delta^{18}O$ of the otoliths of the benthic fish *Lesueurigobius friesii*.

ε_b is the fractionation factor between bicarbonate and calcium carbonate, which is temperature-dependent and was determined by Emrich et al. (1970) and later modified by Grossman (1984) as: $\varepsilon_b(‰) = 12.40 - \frac{2980}{T(°K)}$, and ε_a is the fractionation factor corresponding to the transformation of carbon dioxide into calcium carbonate: $\varepsilon_a = \varepsilon_b + \varepsilon_c$, where ε_c is the fractionation factor between carbon dioxide and bicarbonate, which according to Mook et al. (1974) is also dependent on temperature: $\varepsilon_c = -\frac{9866}{T(°K)} + 24.12‰$.

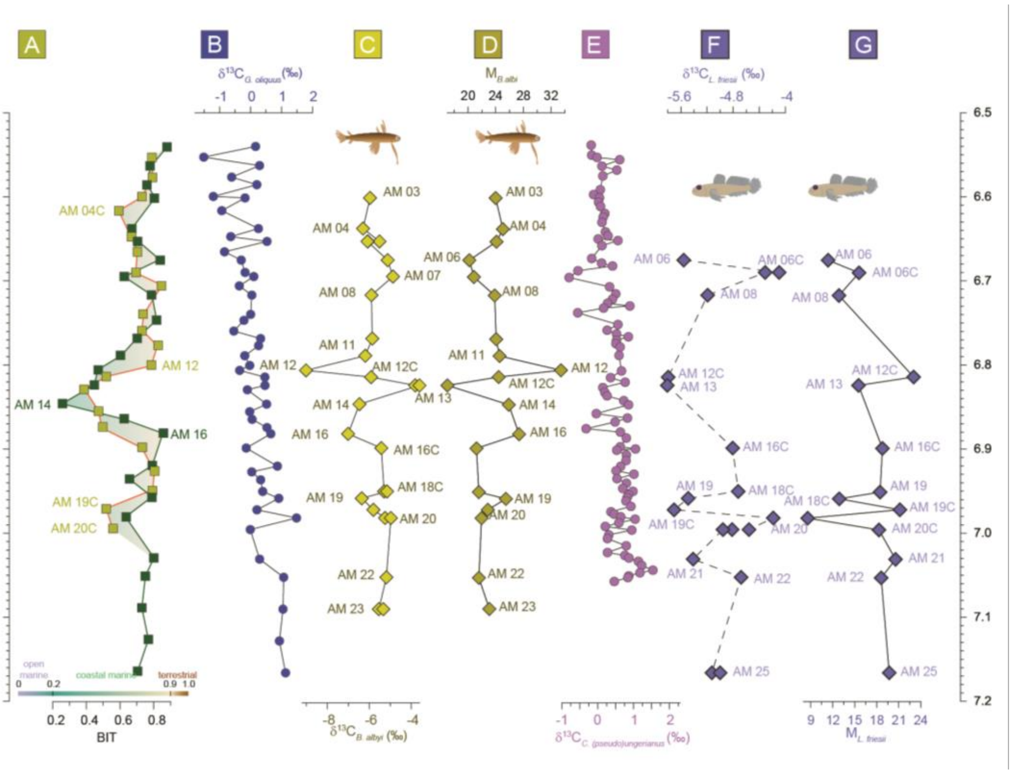

Figure 3. Agios Myron A) Branched and Isoprenoid Tetraethers (BIT) index (Butiseacă et al., 2022); B) $\delta^{13}C$ of the planktonic foraminifer *Globigerinoides obliquus*; C) $\delta^{13}C$ of the otoliths of the pelagic fish *Bregmaceros albyi*; D) metabolic carbon of *B. albyi*; E) $\delta^{13}C$ of the benthic foraminifer *Cibicides (pseudo)ungerianus* (Zachariasse et al., 2021); F) $\delta^{13}C$ of the otoliths of the benthic fish *Lesueurigobius friesii*; and G) metabolic carbon of *L. friesii*.

The results of he otolith analyses were interpreted considering: a) the $\delta^{18}O$ and $\delta^{13}C$ of *G. obliquus* (present study and Kontakiotis et al., 2022) and *C. (pseudo)ungerianus*

(Zachariasse et al., 2021); b) paleodepth estimates based on foraminifera (Zachariasse et al., 2021); c) Tetra Ether Index (TEX^{H}_{86})-derived SST and TEX_{86}^{H}-$\delta^{18}O_{G.obliquus}$-based SSS estimates (Kontakiotis et al., 2022); d) the ratio of isoprenoidal glycerol dialkyl glycerol tetraethers 2 and 3 (isoGDGT2/isoGDGT3) (Kontakiotis et al., 2022), which increases with isoGDGTs input from subsurface-dwelling archaea (Schouten et al., 2002); and e) BIT (branched and isoprenoid tetraether) index values (Butiseacă et al., 2022).

RESULTS AND DISCUSSION

Surface and bottom-water conditions

The oxygen stable isotopic ratios of the demersal fish *L. friesii* ($\delta^{18}O_{L.friesii}$) and benthic foraminifer ($\delta^{18}O_{C.ung}$) have more positive values than those of the pelagic fish *B. albyi* ($\delta^{18}O_{B.albyi}$) and planktonic foraminifera ($\delta^{18}O_{G.obliquus}$) (Figures 2E–H, S1), as expected since the former occupy the bottom of the water column. $\delta^{18}O_{B.albyi}$ follows the general pattern of $\delta^{18}O_{G.obliquus}$.

There is a significant positive $\delta^{18}O_{B.albyi}$ shift at 6.82 Ma (Figures 2F), followed by a negative one 6.81 Ma, which are mimicking the change in the Archaea community (isoGDGT2/isoGDGT3 index). Considering that $\delta^{18}O_{L.friesii}$ does not show a similar positive shift (Figures 2H, S1), the $\delta^{18}O_{B.albyi}$ pattern reflects a short-lived event of remarkable negative water budget at 6.82 Ma (Butiseacă et al., 2022), and $\delta^{18}O_{B.albyi}$ stabilizes already at 6.81 Ma. $\delta^{18}O_{L.friesii}$ shows the same shifts (Figure 2H), but with a smaller amplitude. In contrast to the surface-water organisms (*B. albyi* and *G. obliquus*), $\delta^{18}O_{L.friesii}$ and $\delta^{18}O_{C.ung}$ increase afterwards, from 6.81 Ma until 6.5 Ma (Figure 2G, H), which suggests that the event at 6.82 Ma triggered persistent stratification and increased bottom-water salinity. This interpretation is in line with the increase in abundance of Neogloboquadrinids (Zachariasse et al., 2021) that are indicators of high nutrient availability in a cold, stratified water column (Sierro et al., 2003), as well as with the presence of high-salinity–tolerant benthic foraminifera and the Mn/Al depletion at this level, suggesting decreased bottom-water oxygenation throughout the Mediterranean after a gateway restriction step at 6.8 Ma (Kouwenhoven et al., 2003; Sierro et al., 2003; Lyu et al., 2022).

In contrast, the positive shift of $\delta^{18}O_{B.albyi}$ at 6.68 Ma is accompanied by an analogous shift of $\delta^{18}O_{L.friesii}$ (Figures 2F, 2H, S1), reflecting similar change in both surface and bottom waters, most likely in temperature (~4.7 °C; Figure 2B) rather than salinity (Kontakiotis et al., 2022).

Fishes metabolic responses

The carbon stable isotopic ratios of the fish otoliths ($\delta^{13}C_{B.albyi}$ and $\delta^{13}C_{L.friesii}$; Figure 3C, F) are more negative than those of the foraminifera ($\delta^{13}C_{G.obliquus}$ and $\delta^{13}C_{C.ung}$), because $\delta^{13}C_{diet}$ is more negative than $\delta^{13}C_{DIC}$. The calculated $M_{B.albyi}$ is 17.03–33.44%, while $M_{L.friesii}$ is 8.61–23.02%. In modern marine fish, known M_{oto} values range from 7% to 43% (e.g., Kalish, 1991; Weidman and Millner, 2000; Høie et al., 2003; Solomon et al., 2006; Chung et al., 2019a). $M_{L.friesii}$ is lower than $M_{B.albyi}$, because *L. friesii* is a demersal fish and much less active than the pelagic *B. albyi* (Sherwood and Rose, 2003).

Theoretically, an increase in seawater temperature accelerates the fish physicochemical processes, leading to higher metabolic demands, respiration and food consumption, which results in greater M_{oto} and lower $\delta^{13}C_{oto}$ (Trueman et al., 2016). However, $\delta^{13}C_{oto}$ may be positively or negatively correlated, or not correlated at all with temperature depending on the species, oceanographic conditions and setting (Kalish, 1991; Martino et al., 2019). $\delta^{13}C_{B.albyi}$ is in phase with $\delta^{18}O_{B.albyi}$, but

$\delta^{13}C_{L.friesii}$ and $\delta^{18}O_{L.friesii}$ show different patterns (Figures 2, 3, S1). From 7.2 to 6.9 Ma, $\delta^{13}C_{L.friesii}$ is positively associated with the SST-TEX^{H}_{86} (Kontakiotis et al., 2022), as for example in the cold peak at 7.03 Ma (Figures 2B, 3F), meaning that these fish adapted well to the SST change (Figure 4A). However, from 6.8 Ma onwards, the $\delta^{13}C_{L.friesii}$ and SST-TEX^{H}_{86} diverge, probably due to the strong stratification of the Mediterranean water column after this time and the increasing salinities in the bottom water. After 6.8 Ma, when the high sea bottom salinity is coupled to SST increases, the benthic fish metabolic demands increase as well (Figure 3, Table S1).

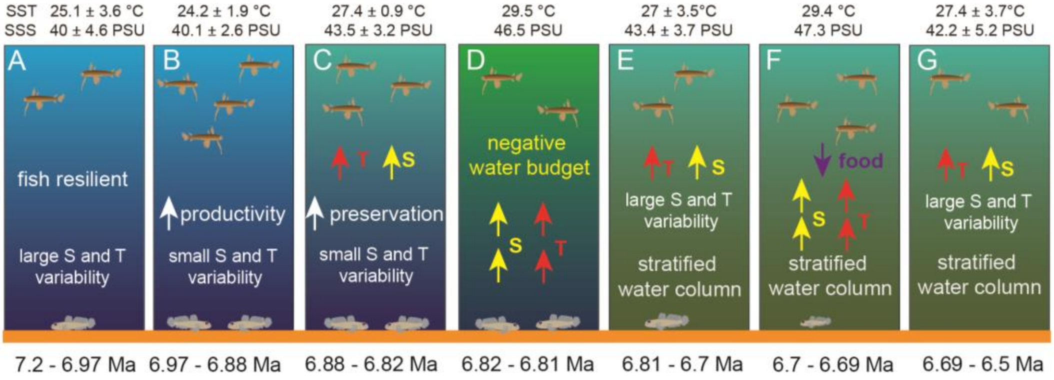

Figure 4. Schematic model of the response of surface and bottom-dwelling marine fishes to the paleoceanographic and paleoclimatic changes taking place from 7.2 to 6.5 Ma in the Eastern Mediterranean.

Negative peaks in $\delta^{13}C_{B.albyi}$ and $\delta^{13}C_{L.friesii}$ at 6.97–6.96 Ma coincide with increased relative production of organic matter from marine versus terrestrial sources indicated by BIT (Figure 3A) (Butiseacă et al., 2022) during sapropel formation, with greater preservation of organic matter, and probably greater production by higher organisms even within bottom waters (Figure 4B, C), which is not picked up by $\delta^{13}C_{G.obliquus}$ and $\delta^{13}C_{C.ung}$. However, the negative peak of $\delta^{13}C_{B.albyi}$ at 6.88–6.85 Ma (Figure 3C) is accompanied by a shift in the organic matter source, from more terrestrial (higher BIT) at 6.88 Ma, to more marine (lower BIT) at 6.85 Ma (Figure 3A). Therefore, given the lack of strong SST changes in this interval (Figure 2B), $\delta^{13}C_{B.albyi}$ seems to be driven primarily by $\delta^{13}C_{diet}$ rather than by $\delta^{13}C_{DIC}$.

The event at 6.82–6.81 Ma strongly impacts fish metabolism (Figures 3D, 3G, 4D), as seen in the negative peak of $\delta^{13}C_{B.albyi}$ and positive shifts of $M_{B.albyi}$ and $M_{L.friesii}$, which are accompanied by a BIT shift (Figure 3A) to increased relative contribution of terrestrial organic matter (Butiseacă et al., 2022) and to maximum in isoGDGT2/isoGDGT3 (Figure 2D). Together with the $\delta^{18}O$ patterns, this event points to a temporary influx of high-nutrient terrestrial material, leading to phytoplankton bloom and consequent increase in zooplankton abundance, which provides more food for the fish population both in surface and bottom waters (Figure 4D).

Finally, the positive peak of $\delta^{13}C_{B.albyi}$ and $\delta^{13}C_{L.friesii}$ at 6.70–6.69 Ma is accompanied by a negative of $M_{B.albyi}$ and a counter-intuitive positive peak of $M_{L.friesii}$ (Figures 3D,3G, 4). Normally, $\delta^{13}C_{oto}$ decreases when M_{oto} increases, since the relative contribution of $\delta^{13}C_{diet}$ to $\delta^{13}C_{oto}$ increases, and $\delta^{13}C_{diet}$ is always more negative than $\delta^{13}C_{DIC}$ (indeed $\delta^{13}C_{C.ung}$ is very low). $M_{B.albyi}$ is decreasing with increasing $\delta^{13}C_{B.albyi}$, as expected, but the benthic fish maintains high metabolism (high $M_{L.friesii}$) due to the temperature high (SST-TEX^{H}_{86} = 29.4 °C at 6.70 Ma), although food is not sufficient (high $\delta^{13}C_{L.friesii}$).

CONCLUSIONS AND OUTLOOK

In the pre-MSC Messinian, the Mediterranean fishes showed a strong response to the intense variability of temperature and salinity accompanied by increased stratification of the water column due to the restriction of the Mediterranean–Atlantic gateway.

Two major events seem to have had the strongest impact on marine fishes during the pre-evaporitic Messinian. At 6.82 Ma, excess evaporation was followed by temporary mixing and thereafter increased bottom-water salinity and stratification, which drove an increase in fish metabolism and respiration. Later on, a warm peak at 6.70–6.69 Ma imposed on the stratified water column of the Eastern Mediterranean with high bottom-water salinity. These conditions intensified the environmental stress for bottom-water fish, increased their metabolic activity despite the low food availability and negatively impacted the overall resilience of the benthic fish population.

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Supplementary Information

Oxygen and carbon isotopic analyses of fish otoliths and foraminifera tests

Forty-seven sediment samples taken along the Agios Myron section were wet-sieved using various mesh sizes (minimum 63 μm) and the residues were all screened for fish otoliths. From the picked specimens, the two target species, *Bregmaceros albyi* and *Lesueurigobius friesii*, were identified in 20 and 14 samples, respectively. Single otoliths from each level (and duplicates from samples AM05 and AM20C) were measured (L: length and W: width), cleaned and analyzed. We selected well-preserved otoliths, making sure that all their morphological characteristics were still present and that there were no particular coloration and/or signs of bioerosion or encrustation that may point toward extensive diagenetic alteration (Agiadi et al., 2022).

Up to five individual tests each of the shallow-dwelling planktonic foraminifer species *Globigerinoides obliquus* and the benthic foraminifer *Cibicides (pseudo)ungerianus* were picked from the 250–300 μm sieve fraction of the same forty-seven processed sediment samples, according to standard practices (Seidenkrantz et al., 2000; Elderfield et al., 2002).

The otoliths and the foraminifera specimens were sonicated in MeOH for about 10 s to remove clay particles adhering to the tests and rinsed at least five times in ultraclean water. Randomly selected specimens of foraminifera and otoliths were observed using a Jeol JSM 6360 Scanning Electron Microscopy (SEM) or a stereoscope, respectively, to confirm their good preservation state (Antonarakou et al., 2019; Agiadi et al., 2022).

Stable carbon and oxygen isotopic analyses were performed using a Thermo Scientific MAT 253 Plus mass spectrometer coupled to a GasBench II with carbonate option, in continuous flow mode, using a thermostated sample tray and a GC PAL autosampler at the Goethe Universität – Senckenberg BiK-F Joint Stable Isotope Facility in Frankfurt am Main, Germany. Analytical precision was 0.08‰ for $\delta^{18}O$ and 0.06‰ for $\delta^{13}C$.

In both cases, we analyzed replicates of 10% of the data reveal to confirm reproducibility of the results; natural sample variability was better than 0.1‰. Results are reported against the Vienna Pee Dee Belemnite (VPDB) standard using the δ notation and expressed in per mil (‰).

Ecological information on the selected fish species

Bregmaceros albyi (Sauvage, 1880) is one of the most common extinct fish species in the Neogene and Quaternary Mediterranean, found both as articulated skeletons and otoliths in great abundances (Landini and Sorbini, 2005; Agiadi and Karakitsios, 2012; Agiadi et al., 2013). *Bregmaceros* spp. are small pelagic fishes with 14 extant species distributed in the Atlantic, Indian and Pacific Oceans and mostly occupying the euphotic zone (Froese and Pauly, 2022). Even though *Bregmaceros* sp. is still present in the Eastern Mediterranean until the Late Pleistocene (Cornée et al., 2019), *B. nectabanus* Whitley, 1941 is the only representative currently inhabiting the basin, and it is considered a non-indigenous species (Agiadi and Albano, 2020).

Bregmaceros spp. are pelagic species occupying usually the upper part of the water column, although *B. nectabanus* is known to regularly enter anoxic waters (Froese and Pauly, 2022). They feed on zooplanktonic invertebrates, particularly copepods, and some species also on phytoplankton.

Lesueurigobius friesii (Malm, 1874) is a benthic, subtropical marine fish living at depths between 10–130 m of the East Atlantic and the Mediterranean and feeding mainly on polychaetes, small crustaceans and mollusks (Froese and Pauly, 2022). It has a preferred temperature today between 7.2 and 18.4 °C, with a mean of 10.4 °C (Kaschner et al., 2016). Its presence in the Mediterranean fossil record goes back to the late Tortonian (Agiadi et al., 2017) and it is common in shallow-waters until the present (Agiadi et al., 2019; Agiadi and Albano, 2020). As a benthic species, *L. friesii* is site-attached, and therefore its isotopic composition is expected to closely reflect the local conditions on the sea floor (Mirasole et al., 2017).

Testing for correlation between $\delta^{18}O_{oto}$, $\delta^{13}C_{oto}$ and fish size

We investigated the existence of any size-dependent patterns in the carbon and oxygen isotopic composition of the otoliths of the two species, by testing for statistical correlation between the otolith size and the $\delta^{18}O_{oto}$ and $\delta^{13}C_{oto}$ values, since otolith size is linked to fish size through species-specific functions (Edelist, 2014). We used the Spearman rank correlation coefficient and performed these analyses in R (version 4.2.1) (R Development Core Team 2022). No correlation was detected between the isotopic values and otolith length at 95% confidence level, with Spearman's ρ values of 0.13 ($p = 0.58$) for $\delta^{13}C_{B.albyi}$, 0.11 ($p = 0.70$) for $\delta^{13}C_{L.friesii}$, −0.04 ($p = 0.88$) for $\delta^{18}O_{B.albyi}$ and −0.17 ($p = 0.56$) for $\delta^{18}O_{L.friesii}$.

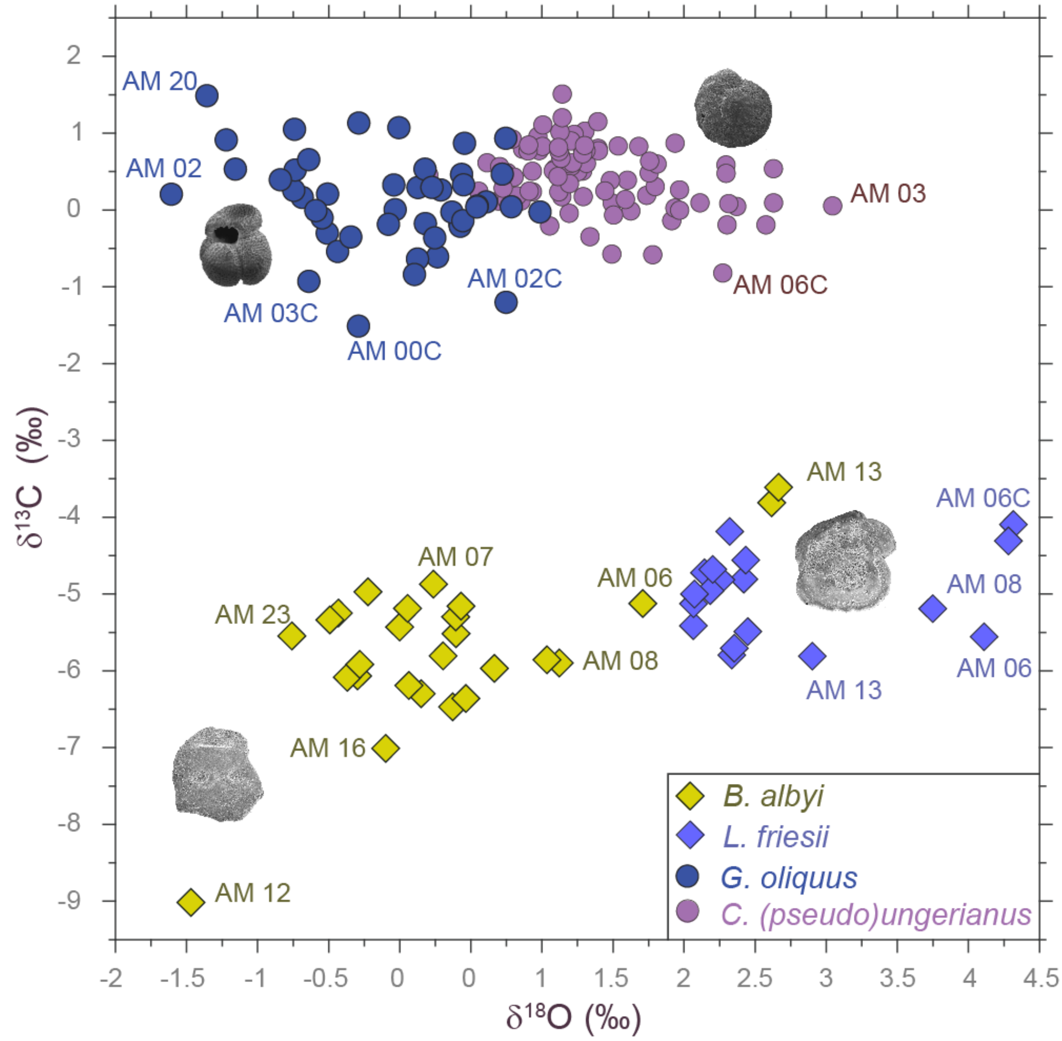

Figure S1 – δ^{18}O–δ^{13}C plot of the fish otolith and foraminifer samples analyzed from Agios Myron section. The four species clusters are clearly distinguished, and outliers suggest important biotic responses to environmental change.

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