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STATEMENT

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ABSTRACT

In this study we show, for the first time, the absence of a vital effect in the clumped isotope carbonate (Δ_{47}) fossil ostracod signal, as well as the ability of the novel ostracod-Δ_{47} thermometer to reconstruct past hydrological conditions in complex lacustrine systems. Furthermore, through the application of Δ_{47} analyses on the ostracod species *Candona angulata* and *Cyprideis torosa* from Lake Trasimeno record (central Italy), which today precipitate their shells during the cold and the warm season respectively, we provide evidence that by combining biological (i.e., ostracod shell precipitation timing), paleontological (i.e., identification of ostracod species) and geochemical (i.e., Δ_{47}) approaches, the ostracod-Δ_{47}

thermometer can be used to accurately reconstruct past seasonality. This also implies that, despite the absence of a vital effect, not all species can be combined for Δ_{47} analyses in environments with seasonal temperature variations; rather, only those that precipitate their shells during the same season should be considered. The application of the ostracod-Δ_{47} thermometer on the Trasimeno lacustrine record gives rise to the first continental warm season paleotemperature reconstruction of the last 43 ky in central Mediterranean area. The combination of Δ_{47} and classic stable isotope ($\delta^{18}O_{ost}$) measured on ostracod shells provides the isotopic composition of the water from which the carbonate precipitated ($\delta^{18}O_{w}$) and thereby, changes in the evaporation/precipitation balance in this area. Before the Last Glacial Maximum (LGM), equivalent to the Marine Isotopic Stage 3 (MIS3, from 43 to 29ky), warm season temperatures ranged from 15 ± 1.6 °C to 22 ± 2.3 °C, being from 2 to 6 °C colder than today. Hydrological conditions during this period were similar to the present-day ones, characterized by a permanent lake and a high evaporation/precipitation ratio (E/P). The drastic decrease of the warm season temperatures (ranging from 10 ± 2.9 °C to 17 ± 3.1 °C) and of the E/P ratio during LGM and Lateglacial (MIS2, from 29 to 11.6 ky) corresponded well to the global climate cooling and low summer insolation, suggesting an amplifying role, of this last one, in the effects of the millennial scale climatic variations. At the Pleistocene/Holocene transition, both warm season temperature (25 ± 2 ºC) and the E/P ratio increased in conjunction with the summer insolation. During the early Holocene, warm season temperature (23 ± 2 ºC) closely resemble present-day values. However, cold season temperatures (12 ± 2 ºC) were approximately 4 ºC warmer than today. Notably, no hydrological differences were identified between the warm and the cold season underlying a lower seasonality contrast compared to the present, along with enhanced warm season precipitation. The good agreement between the Δ_{47} temperatures reconstructed for the last 1 ky and the temperatures presently recorded at Lake

Trasimeno (8 ºC cold and 22 ºC for warm season), confirms the accuracy of the analyses and the applicability of the ostracod-Δ_{47} thermometer to reconstruct seasonal temperature changes.

KEYWORDS

Carbonate Clumped Isotope, Freshwater ostracods, Paleotemperatures, Paleohydrology, Seasonality, Central Mediterranean.

1. INTRODUCTION

The Intergovernmental Panel on Climate Change (IPCC) considers the Mediterranean region a climate change hotspot (Ali et al., 2022). According to climate projections, the Mediterranean region is expected to warm at a rate approximately 20% higher than the global average. Additionally, it will undergo significant drying, a phenomenon not anticipated in other regions situated at the same latitude (Lionello and Scarascia, 2018; Saeger et al., 2014). The vulnerability of this area is a consequence of complex and interacting processes related to the area's landscapes, geographical location, high population density, and long history of human occupation (Ali et al., 2022).

Predictive modelling of potential future climate scenarios is key to developing strategies for adaptation and mitigation. To generate these climate projections, the forcing mechanisms driving climate variability across different temporal and geographical scales need to be better understood. Also, the modelling of forecasted changes with ancient analogues must be validated. Owning to the Mediterranean's complex topography, each region responds differently to atmospheric and marine climate dynamics (Abrantes et al., 2012). Accurate high-resolution paleoclimatic reconstructions for the different Mediterranean regions are thus required. Lake sediments are certainly among the best continental archives for developing paleoclimatic and paleoenvironmental reconstructions because they can capture rapid climate changes across regional scales (Gornitz, 2009; Cohen, 2003). Paleotemperature reconstructions have always been assumed to play an important role in understanding climatic variations; however, despite more than half a century of studies, quantitative and well-constrained temperature reconstruction remains very challenging. The complexity lies in disentangling and quantifying the effects of the various parameters that impact on the local climate and environment (e.g., temperature, hydrology, lake structure). Continental quantitative temperature records in the central Mediterranean area are very rare, mainly focussed on the last 15 ky (Heiri et al., 2015; Larocque and Finsinger, 2008; Robles et al., 2023; Samartin et al., 2017) and most of them are based on transfer functions (e.g, pollen, chironomids, and ostracods). The oldest temperature record comes from the Monticchio lake (southern Italy, Fig.1). It is based on pollen analyses, and provides air temperature of the coldest month (January) from the last ca. 102 ky (Allen et al., 1999). Marchegiano et al., (2020) applied the Mutual Ostracod Temperature Range transfer function (MOTR, Horne, 2007) to ostracod assemblages from Lake Trasimeno (central Italy, Fig.1) to reconstruct air temperature of the warmest (July) and coldest (January) months during the Late Pleistocene. However, this approach produced very large temperature ranges within which the real temperature existed (Marchegiano et al., 2020, Fig. 3).

The carbonate clumped isotope (Δ_{47}) technique (Eiler, 2007), currently mostly applied to marine carbonate fossils and sediments (de Winter et al., 2021; Henkes et al., 2013; Marchegiano and John, 2022; Meinicke et al., 2021; Peral et al., 2020), has the potential to significantly reduces the methodological uncertainties associated with lake paleotemperature measurements. This technique is based on the temperature-dependent abundance of ^{13}C-^{18}O bonds in carbonate CO_2. The increased abundance of these bonds in carbonate is associated with decreasing water temperatures, revealing the temperatures at which calcium carbonate

($CaCO_3$) precipitated. The combination of Δ_{47} and $\delta^{18}O$ provide the $\delta^{18}O_w$ and give insight on the hydrological conditions.

The ostracod-Δ_{47} thermometer is a new tool able to reconstruct lacustrine water temperatures, with an accuracy of ~ ± 2 °C (Marchegiano et al., in review). Marchegiano et al. (in review), showed the applicability of the Δ_{47} technique on ostracod shells as well as the absence of any vital effect (i.e., disequilibrium between the water isotopic signal and the one recorded by the organisms) on ostracods living at the same temperatures. Ostracods are aquatic micro-crustaceans (size from 0.3 to 5 mm) with a stable bivalve low-Mg calcite shells easily to preserve and thus ideally suited for geochemical analyses (Holmes and De Deckker, 2012). As the grow, they secrete increasingly large carapaces from the ions dissolved in the host water and pass through 8 molting stages before reaching adulthood (Turpen and Angell, 1971). The shell calcification is quick, ranging from a few hours to a few days (Börner et al., 2013), and representative of the geochemical conditions of the environment at that time (Chivas et al., 1983; Mischke et al., 2010; Pérez et al., 2011).

In this study, we generated the first ca. 43 ky continental warm season temperature record for the central Mediterranean area by applying the novel ostracod-Δ_{47} thermometer (Marchegiano et al., in review) on a ca. 8.9 m long sedimentary core from Lake Trasimeno (central Italy). The Trasimeno sedimentary core has been well analyzed in previous studies (Francke et al., 2021; Gasperini et al., 2022; Marchegiano et al., 2020, 2019, 2018) by using a multiproxy approach (i.e., micropaleontological, sedimentological and geochemical) making this record particularly suitable for the application of this novel paleothermometer.

The clumped isotope technique has already been applied on fossil ostracods to reconstruct paleoaltitude (Song et al., 2022) and paleotemperatures (Yue et al., 2022); however, no studies have ever determined whether a vital effect influences the Δ_{47} signal on fossil ostracods. To fill this gap, we performed Δ_{47} analyses on two different ostracod species coming from the same sample that lived and precipitated their shells at the same season and temperatures. Also, by combining Δ_{47} with the analyses of the classic oxygen isotopic composition of the ostracod shells ($\delta^{18}O_{ost}$), we show, for the first time, the ability of the ostracod-Δ_{47} thermometer to reconstruct $\delta^{18}O_{w}$ variations in past lacustrine environments.

1.1 Study site: Lake Trasimeno

Lake Trasimeno (latitude 43º08'N, longitude 12º06'E, 258 m above sea level, Fig. 2) is an endorheic and very shallow lake (max depth of ~6 m and ~4 m in average) with a surface area of 124.3 km^2 located in central Italy (Umbria region). It has a tectonic origin and the lacustrine sedimentation started during the Middle Pleistocene (Gasperini et al., 2010). Because of its characteristics, the hydrology of the lake strictly depends on climatic and environmental variations. Lake-level fluctuations influence, at the seasonal and annual-decadal scale, the physical-chemical and biological lake properties (Ludovisi and Gaino, 2010; Marchegiano et al., 2017; Pallottini et al., 2023). The climate regime is characterized by warm-arid summers and mild-humid winters. Today, water temperatures are close to the atmospheric ones with a difference of 2 - 4 °C during the year. Temperatures are homogeneous along the entire water column due to a continuous mixing facilitated by the shallowness and the very large surface of the lake (Marchegiano et al., 2017). Previous studies (Marchegiano et al., 2018, 2019, 2020 and Francke et al., 2022) showed that, over the last ca. 47 ky, Lake Trasimeno responded rapidly to millennial scale climatic variations associated with Greenland stadial and interstadial events, according to Rasmussen et al., (2016). Low (high) lake levels corresponded to cold (warm) and dry (humid) stadial (interstadial) events. This demonstrates that Lake Trasimeno detects regional as well as global climatic signals. The living and fossil ostracod fauna of Lake Trasimeno have also been studied intensively (Marchegiano et al., 2017, 2018, 2019 and 2020), indicating a prevalence of permanent low saline lake conditions during MIS3 and the Holocene and ephemeral high salinity lake conditions during the MIS2.

Thanks to its strategical position in the central Mediterranean, its high sensitivity to climate and environmental changes and the abundance and great preservation of the ostracod fauna, Lake Trasimeno is the perfect target to test the ostracod-Δ_{47} thermometer in past sedimentary records and to reconstruct atmospheric changes in the central Mediterranean.

2. Material and Methods

2.1 Trasimeno core and its chronology

An 8.6 m long sediment core (Co1320; 43° 09.624′N, 12° 03.491′E) (Fig. 2) was retrieved at ~4.9 m water depth from a floating platform using a gravity piston corer (UWITEC®) during a sampling campaign in November 2014. The core was split lengthwise in the laboratory. While one-half was archived and stored at the University of Cologne (Germany), the other half was used for visual lithological inspection and then subsampled at 2-cm intervals for geochemical and micropalaeontological analyses (Marchegiano et al., 2018, 2019, 2020 and Francke et al., 2022).

The chronological model of the Trasimeno core Co1320 has been developed using ten radiocarbon ages (Fig. 3) and ultimately covers the last ca. 47 ky (Marchegiano et al., 2018). In this study, we updated this calibration using a Bayesian age-depth modelling calculated by means of the software rBacon 3.1 (Blaauw et al., 2018; Blaauw and Christen, 2011), and the IntCal 2020 calibration curve (Reimer, 2020) (Fig. 3). The bulk organic matter samples at 631 cm (COL3665.1.1) and 154.5 cm (COL3662.1.1) exhibit higher br/(cren+cren0) GDGT ratios, indicating a substantial contribution from soli organic matter (SOM) (Francke et al., 2022).

This implies that the incorporation of pre-aged SOM in these samples may distort the bulk ^{14}C age. Consequently, the two samples were excluded from the age-depth model. Considering the limited presence of carbonate bedrock in the catchment area and the high lake surface compared to the water volume ratio, that facilitate rapid exchange with the atmosphere, Lake Trasimeno is anticipated to have minimal reservoir and/or hardwater effects on lacustrine organic matter (Francke et al., 2022).

2.2 Carbonate classic and clumped-isotope analyses

For classic and clumped-isotope analyses, we selected a total of 16 samples (Table 1) by combining the results of the ostracod assemblage analyses (Marchegiano et al., 2018 and 2019), the derived MOTR temperature curve (Marchegiano et al., 2020) and the updated age model. These samples correspond to the main temperature and environmental variations along the entire core interval. To reach the required carbonate amount for clumped-isotope analyses (minimum 500 μg per replicate), we merged adjacent subsamples (2 cm thick) (Table 1) deposited during the same climatic event (Marchegiano et al., 2018, 2019 and 2020). Adult ostracod shells were picked and manually cleaned with a brush under the stereomicroscope to remove any sediments and other possible contaminants (i.e., organic matter). The good state of preservation (i.e., no diagenetic alteration) of the ostracod shells was checked under the scanning electron microscope (SEM) (Figure 4). Analyses for Δ_{47} and $\delta^{18}O_{ost}$ were carried out on the most abundant species *Cyprideis torosa*, *Sarcypridopsis aculeata*, *Eucypris mareotica*, *Heterocypris salina* and *Candona angulata* (Figure 4). A total of 40 to 60 valves per replicate is needed to assure sufficient material (500 - 600 μg per replicate), whereby the exact number depends on the species (i.e., size and thickness of the shells). Each sample is replicated from 5 to 15 times (300 - 1000 valves per samples in total). The Δ_{47} analyses of ostracod shells, which provide at the same time the $\delta^{18}O_{ost}$ data, were carried out at the Analytical, Environmental & Geo-Chemistry (AMGC) clumped isotope lab of the Vrije Universiteit Brussel (VUB), using a Nu Instruments Perspective-IS stable isotope ratio mass spectrometer (SIRMS) in conjunction with a Nu-Carb carbonate sample preparation system, as described in detail in De Vleeschouwer et al., (2022). The analyses were performed between March 2022 and November 2022. The carbonate standard ETH-4 was systematically measured and compared to InterCarb values (Bernasconi et al., 2021) to assess the accuracy and reproducibility. Analyses and results were monitored in the lab using the Easotope software (John and Bowen, 2016). Within the ClumpyCrunch software (Daëron, 2021), the raw measured Δ_{47} values were processed using the IUPAC Brand's isotopic parameters (Daëron et al., 2016) and converted to the ICDES 90°C scale, using the most recent values for the ETH-1, ETH-2, and ETH-3 carbonate reference materials (Bernasconi et al., 2021). Analytical and calibration uncertainties were propagated to calculate the final uncertainties on temperatures. The Δ_{47} values were converted into temperatures using the unified calibration (Anderson et al., 2021) as suggested by (Marchegiano et al., in review) and the $\delta^{18}O_w$ values were calculated from Δ_{47} and $\delta^{18}O_{ost}$ data using the formula of (Kim and O'Neil, 1997) for calcite (Table 1). Since the $\delta^{18}O_{ost}$ suffer of a species-specific offset (Von Grafenstein et al., 1999; Holmes and Chivas, 2002), to make meaningful comparisons is important to correct the $\delta^{18}O_{ost}$ by removing the species-specific offset to normalize the $\delta^{18}O_{ost}$ values with the theoretical inorganic calcite deposited at the isotopic equilibrium at the same moment in the same environment. We used the values of these offsets previously calculated and reported for *C. angulata* (2.2‰, Von Grafenstein et al., 1999) and *C. torosa* (0.8‰ Keatings et al., 2007). The vital offset of *S. aculeata* and *H. salina* have never been calculated before and without the analyses of living shells is not possible to validate these values. However, *E. mareotica* is considered to precipitate close to the isotopic equilibrium (Li and Liu, 2010) and because there is no substantial difference between the

$\delta^{18}O_{ost}$ values of *S. aculeata* and *E. mareotica* (0.009‰ in sample TRAH1-1 and TRAH1-2), we assume that this is also the case for *S. aculeata* and *H. salina* (difference of 0.18 ‰ between TRA2 and TRA2B).

3. Results

3.1 Classic and clumped-isotopes

The Δ_{47} values of the ostracod samples range from 0.6485 to 0.5941 ‰ with a SE from 0.009 to 0.015 ‰ (Table 1). Values of the $\delta^{18}O_{ost}$ and $\delta^{13}C_{ost}$ vary from -0.45 ‰ to 3.20 ‰ and from -3.47 ‰ to 3.99 ‰ (SE of 0.1 ‰ on average). The number of replicated measurements carried out per sample was determined based on the availability of sample material. The large number of replicates per specimen ensures the robustness of the results. The repeatability along the 8 sessions of the standards used to standardize the results and the ostracod samples was 33.2 ppm and 31.4 ppm, respectively. The repeatability of the $\delta^{18}O_{ost}$ and $\delta^{13}C_{ost}$ was 39.0 ppm and 17.8 ppm, respectively. The ETH4 standard, used to assure and control the quality of the analyses, presents a Δ_{47} value of 0.4460 ‰ and a standard error (SE) of 0.0059 for 67 replicates. The difference between our measured value of ETH4 and the official one from Bernasconi et al., (2021) (ETH2- Δ_{47} value of 0.4505 ± 0.0015) is negligible (Δ_{47} of 0.0045) and falls within the calculated SE. This confirms both the accuracy and precision of the Δ_{47} analyses presented in this study. The box plots for Δ_{47} temperatures (Figure 6) are made including all replicate measurements per each sample (see Table 1 in supplementary material) and show median temperatures of 17 ºC during the MIS3, of 12 ºC during MIS2 and of 23 ºC during the Holocene. The larger probability density in MIS3 and the Holocene is due to the internal higher replicate variability in the measured samples. This differs from the analytical uncertainties reported for each Δ_{47}-temperature data that is, instead, determined using the "pooled" standardization method outlined by Daëron (2021) that considers the reproducibility constraints obtained from both standard and sample analyses.

4. Discussion

4.1 Species-specific effect on the ostracod-Δ_{47} paleothermometer

In the first application of the ostracod-Δ_{47} thermometer on living ostracod that precipitated their shells at known temperatures, Marchegiano et al. (in review) demonstrated the absence of a vital offset at genus and species level. In this study, to confirm the absence of a species-specific effect also in the fossil record, we analyzed the Δ_{47} of two different ostracod species coming from the same sediment layers that, thus, precipitated their shells at the same time, season and in the same temperature and environment. The absence of a consistent offset, within the analytical uncertainty, between *S. aculeata* (TRA-2) and *H. salina* (TRA2b) (Δ_{47} 0.002 ± 0.01‰, Tab.1) and between *S. aculeata* (TRAH2-1) and *E. mareotica* (TRAH2-2) (Δ_{47} 0.0007 ± 0.01‰ Tab.1) confirms the absence of the vital offset in fossil ostracod shells at genus and species level. This finding provides a further confirmation that the ostracod-Δ_{47} thermometer can be applied independently to the ostracod species and throughout the geological time.

Although there is no vital offset in the ostracod-Δ_{47} signal, it is important to know the timing of precipitation of ostracod shells to provide accurate paleotemperature reconstructions. As a consequence, not all species can be combined together but only the ones that precipitate their shells during the same season. Thus, by combining the Δ_{47} technique, with paleontological and biological knowledge of the ostracod fauna, the ostracod-Δ_{47} paleothermometer has the potential to reconstruct past temperatures and seasonality variations.

4.2 Ostracod-Δ_{47} temperature reconstruction

To correctly use ostracod shells as a geochemical tool, it is necessary to understand their ecological preferences and life history (i.e., timing of shell precipitation). The species used in this study are widespread and quite well known.

Cyprideis torosa (Fig. 4) is a species that can live at a wide range of salinities (Griffith and Holmes, 2000) in permanent water conditions (Meisch, 2000) and was found living today in the freshwater Lake Trasimeno (Marchegiano et al., 2017). This species seems to produce two generations per year with adulthood calcification in spring and autumn (Heip, 1976 and Roberts et al., 2020). Today, the water temperature of Lake Trasimeno ranges from 11 ºC (April) to 23 °C (June) during spring and from 16 °C (October) to 8 °C (December) in autumn (World Lake database 2023). Δ_{47} analyses made on recent *C. torosa* shells (TRAR2) yield temperatures of 22.5 ± 2.3 °C. It therefore seems that, at Lake Trasimeno there is a prevalence of spring generations or that shells continue to calcify also during the summer. In this study, we consider this species as indicative of late-spring/early-autumn (warm season) temperatures (today May-October water mean T are 21.4°C). In the Trasimeno record, *C. torosa* is very abundant during the relatively warm and humid MIS 3 (from ca. 46 to 34 cal ky BP), and the Holocene, indicating a permanent lake with low saline water (Marchegiano et al., 2018 and 2019). The *C. torosa* Δ_{47} temperature during MIS 3 ranges from 15 ± 1.6 °C to 22 ± 2.3 °C and of 23.2 ± 2.5 °C in the Early Holocene (Table 2).

Sarcypridopsis aculeata and *Eucypris mareotica* (Fig. 4) are often found together and indicate ephemeral-shallow lake conditions and high salinity conditions (Martin-Puertas et al., 2008). They do not live in Lake Trasimeno today (Marchegiano et al., 2017) and disappeared from the fossil record at the Pleistocene-Holocene transition suggesting a change from prevalent ephemeral to permanent lake conditions (Marchegiano et al., 2018 and 2019). *S. aculeata* develops up to three summer generations per year (time of shells calcification) and adults very rarely survive in the colder months (Meish, 2000). *E. mareotica* hatches between late June and early July and reaches the adults stage in late July-early August (Li and Liu, 2010). Today, the water temperature of Lake Trasimeno during summer ranges from 26°C (August) to 21ºC (September) (World Lake database 2023) (Table 2). Δ_{47} analyses on recent *S. aculeata* and *E. mareotica*, to confirm their precipitation season in Lake Trasimeno, are not possible because these species are not part of the living fauna today. However, if we assume that during the entire Holocene temperatures were similar to today, the Δ_{47} analyses made on Early Holocene *S. aculeata* shells (TRA2, temperature of 24.9 ± 2°C), could confirm that *S. aculeata* precipitates its shell during the summer season at Lake Trasimeno (today mean water T are of 24°C). The seasonal attribution can also be extended to *E. mareotica* since no significant difference in temperature is observed between samples TRAH2-1 (*S. aculeata)* and TRAH2-2 (*E. mareotica*) (13 and 13.2 ± 3 °C respectively) that come from the same sediment layers. In the Trasimeno record, *S. aculeata* and *E. mareotica* are the most abundant species at the end of the MIS 3 and during the cold and dry MIS 2 (from ca. 34'000 to 10'300) indicating shallow-ephemeral lake conditions and higher salinities (Marchegiano et al., 2018). The Δ_{47} temperatures derived from *S. aculeata* shells during MIS2 range from 10 ± 2.9 °C to 17 ± 3.1 °C and it is of 24.9 ± 2°C at the Pleistocene-Holocene transition (Table 2).

Heterocypris salina (Fig. 4) is a cosmopolitan species often found together with *S. aculeata* and *C. torosa* but it does not tolerate ephemeral conditions. This species lives today in Lake Trasimeno (Marchegiano et al., 2017). It has 2 to 3 summer generations (time of shells calcification) and lives for 45 days (Meish, 2000). At the Pleistocene-Holocene transition, samples TRA 2 and TRA 2B, *S. aculeata* and *H. salina* were measured and provided a very close temperature (24.9 ± 2 °C and 24.3 ± 2.2 °C respectively) confirming that they both precipitate during the summer season (Fig. 5 and Table 2).

Candona angulata (Fig. 4) prefers slightly salty permanent waters and it is often found together with *H. salina* and *C. torosa.* This species today lives in the deepest part of Lake Trasimeno in association with *C. torosa* (Marchegiano et al., 2017). *C. angulata* is a winter species that lives from November to March and precipitates its shell from November to February (Meish, 2000). Today, the winter water temperatures at Lake Trasimeno range from 12°C (November) to 7ºC (February) (World Lake database 2023). In the Trasimeno record, *C. angulata* is present almost during the entire interval, but it is found at such small percentages that Δ_{47} analyses could not be conducted. It is, instead, very abundant in the Holocene and in this study was used to reconstruct seasonal variations during this time period. The Δ_{47} analyses made on *C. angulata* (TRAR2) gave temperatures of 8 ± 2.9 °C in recent shells and of 11.8 ± 2.1 °C in the Early Holocene (TRAHOL1) (Fig. 5 and Table 2). On the same samples *C. torosa,* a late-spring/early-autumn species, recorded temperatures of 22.5 ± 2.3 °C and 23.2 ± 2.5 °C respectively (Fig. 5). The good correspondence with the today mean water temperatures of Lake Trasimeno (8°C during November-February and of 21.4°C during May-October) confirms the accuracy of the analyses, the applicability of the ostracod-Δ_{47} on fossil shells and its ability in reconstruct paleotemperatures, even at seasonal scale.

4.3. Paleoclimate reconstruction

In this study, all Δ_{47} temperatures measured on *C. torosa*, *S. aculeata, E. mareotica,* and *H. salina* shells are considered as warm season temperature (from late spring to early autumn). Instead, Δ_{47} temperatures from *C. angulata* are considered as cold season temperatures (from November to March).

Warm season water temperatures based on ostracod-Δ_{47} fall into the July minimum and maximum mutual ostracod air temperature range (MOTR) reconstructed for this interval by Marchegiano et al. (2020), within the uncertainties, and correspond well to the peak (higher T) and minima (lower T) of the MOTR curves (Fig. 5). This suggests a close connection between air and water temperature variations in Lake Trasimeno and a prompt reaction of the lake system to climatic changes. The LATEMIS3 (Table 1) is the only sample, including the Δ_{47} uncertainties, that falls outside the MOTR (Fig. 5). This could be explained by a lack of (or reduced) response of the ostracod fauna (i.e., change in the ostracod assemblages) to the fast environmental change at the onset of this cold event (probably associated to H3).

Colder and warmer periods at Lake Trasimeno are certainly linked to the millennial-scale climatic variations (warm-humid interstadial GI, cold-arid stadial GS and coldest and driest Heinrich events), as confirmed by prior studies (Marchegiano et al., 2018, 2020 and Francke et al., 2022). These millennial-scale climatic events are likely associated to reduction (cold events) and enhancing (warm events) of the Atlantic Meridional Overturning Circulation (AMOC) plus a displacement of cooling conditions throughout the central Mediterranean area during the GS and Heinrich events (Francke et al., 2022). However, because of chronological limitations, it is not possible to identify exactly which events have been recorded.

The *C. torosa*-Δ_{47} temperatures during MIS3 ranges from 15 ± 1.6 °C to 22 ± 2.3 °C, with a median temperature of 17 °C, indicating that warm season temperatures were from 2 to 6 °C lower than today (Fig. 6 and Table 2). During the same interval, air temperatures of the coldest month based on pollen from south Italy (Lake Monticchio) are around -8°C, reaching -16 ºC in the coldest events (Allen et al., 1999). Meanwhile, marine annual surface temperatures based on alkenones for the Alboran Sea range from 10 to 16ºC (Cacho et al., 1999) (Fig. 6). In this interval, the coldest event (15 ± 1.6 ºC) at Lake Trasimenno could be tentatively associated to the Heinrich events (H4). Likewise, the warmest events (20 ± 2.1 and 22 ± 2.3 ºC) are likely linked to the GIs (10 and 8) and the event at ca. 18 ± 1.7 °C to is probably associated with the GS (12).

At the transition between MIS 3 and MIS 2, which corresponds to the onset of full glacial conditions, *S. aculeata*-Δ_{47} warm season temperature drastically decrease to 10 ± 2.9 ºC and then slowly increase up to 15 ± 3 ºC at the end of MIS 2. The median warm season temperature for the MIS2 is 12°C (from 12 to 7 ºC colder than today warm season mean temperatures) (Fig. 6). The substantial decrease in temperature observed at Lake Trasimeno is only marginally reflected in the marine surface mean annual temperatures at the Alboran Sea and it is nearly absent in the Monticchio winter temperature, which remains around -8ºC. This divergence in behavior, between the three records may be attributed to a diverse climate response and/or the influence of regional parameters. However, differences between the air temperature of the coldest month (pollen from Lake Monticchio, southern Italy) and warm season water temperatures (Δ_{47} – ostracod from Lake Trasimeno, central Italy) can potentially be explained by the reduced summer insolation experienced during MIS2 (Fig. 6). The coldest temperatures in this interval could be tentatively associated to the Heinrich's events. If we consider that the last cold event of this interval was the Younger Dryas, summer temperatures at Lake Trasimeno (15 ± 3 ºC) are very comparable to the pollen summer temperature at Lake Matese (southern Italy, Robles et al., 2023) and the chironomid summer temperatures at Lake Piccolo di Avigliana (northern Italy, Larocque and Finsinger, 2008) both recording a temperature of 16 ºC. The brGDGT mean annual temperatures of Lake Matese, in the same period, is instead 12 ºC (Robles et al., 2023) (Fig. 6).

At the Pleistocene - Holocene transition (i.e., the onset of the interglacial), warmer season temperatures increased in 10 ºC (temperatures of 25 ± 2 ºC, 3 ºC higher than the warmer season temperatures today, see Table 2) with Holocene median temperatures of 23°C (Fig. 6). Because of the very shallow/ephemeral conditions of Lake Trasimeno during MIS 2 and the Pleistocene

– Holocene transition (Marchegiano et al., 2018 and 2019), a possible overestimation of lake water temperatures from 2 to 4 ºC (i.e., the modern temperature difference between lake water and atmosphere at Lake Trasimeno today) need to be considered as temperatures could be closer to the atmospheric ones.

During the Early Holocene, the warm season temperatures closely resemble those of the present day. However, the cold season temperature (12 ± 2.1 ºC) is approximately 4ºC warmer than those of today (8ºC), indicating a reduced seasonality (Fig. 6). This decrease in seasonality during the Early Holocene aligns well with previous findings in hydrological records from central-southern Italy (Magny et al., 2012 and Marchegiano et al., 2019). The temperature record based on Δ_{47} measurements from Lake Trasimeno provides the first confirmation of a lower seasonality, also in temperatures, than today for central-southern Italy.

Latest Holocene (last ca. 1 ky) Δ_{47} temperatures are the same as today at Lake Trasimeno (8ºC winter and 22ºC for late spring/early autumn).

4.4 Paleohydrology reconstruction

Because of the endoreic and shallow nature of Lake Trasimeno, its water geochemistry is strictly dependent on the climatic variations and particularly, on the E/P (Frondini et al., 2019; Ludovisi and Gaino, 2010). This was confirmed by the comparison between recent $\delta^{18}O$ and $\delta^{2}H$ lake water values and the local evaporation line (LEL) (Frondini et al., 2019) and by the moderate positive correlation (R^2= 0.24) between past $\delta^{13}C$ and $\delta^{18}O$ in bulk carbonates data indicating an exchange between lake water and atmosphere due to evaporation (Francke et al., 2022). The oxygen isotopic composition of precipitation ($\delta^{18}O_p$) at Lake Trasimeno today varies between -9.9‰ in January and -1.9‰ in July (Waterisotopes Database, 2017) as a consequence of decreasing summer precipitation and increasing temperatures. The differences between $\delta^{18}O_p$ and $\delta^{18}O_w$ today is between ca. 9 and 11 ‰ during winter and ca. 3 and 6 ‰ during summer, indicating a large loss of light isotope ($\delta^{16}O$) through evaporation. However, the small difference of 2 ‰ between today $\delta^{18}O_w$ summer (ca. 3.8 to 1.2 ‰) and $\delta^{18}O_w$ winter (-1 to 1 ‰) (monitored values from Frondini et al., 2019) also suggests a minor contribution of summer precipitation to the annual $\delta^{18}O_w$ budget.

There is a good agreement between the $\delta^{18}O_w$ values reconstructed from ostracod shells and the $\delta^{18}O$ curve on bulk carbonate from Francke et al., (2022) suggesting a co-variance of these two isotopic signals (Fig. 5). However, the slight difference in the absolute values between $\delta^{18}O$ of calcite (measured on bulk carbonate) and $\delta^{18}O_w$ of the water (measured on ostracods) could be due to both the isotopic fractionation between $\delta^{18}O$ of the water and the one recorded in the carbonate as well as a difference in timing of carbonate precipitation between ostracod (few hours and related to the calcification season) and bulk carbonate (annual average).

During the mild and more humid MIS 3, $\delta^{18}O_w$ warm season values range from 2.0 ± 0.2 to 1.0 ± 0.2 ‰, within the range of present-day variability. Lower values correspond to colder temperatures (18 ± 1.7 °C) and higher values to warmer ones (ca. 22 ± 2.3 °C) suggesting colder-dryer and warmer-humid periods. Although ostracod assemblages indicate permanent lake conditions, they also suggest that the variations in precipitation and/or evaporation amount are very low.

During the cold and arid MIS2, $\delta^{18}O_w$ warm season values range from 1.5 ± 0.3 to 0 ± 0.3 ‰ (Fig. 5). Considering that several studies suggest a decrease in precipitation during MIS2 in the central Mediterranean area (e.g., Allen et al., 1999; Follieri et al., 1988), and that ostracod assemblages at Lake Trasimeno also indicate low lake level/ephemeral conditions (Marchegiano et al., 2018), the lighter values during MIS2 compared to MIS3 could be due to a decrease of evaporation as consequence of lower summer insolation (Figure 5).

At the Pleistocene – Holocene transition, $\delta^{18}O_w$ warmer season values are the highest of the entire interval ($\delta^{18}O_w$ 2.7 ± 0.2 ‰) probably due to an increase in evaporation (water enriched of heavier $\delta^{18}O$) caused by high temperatures and highest summer insolation (Figure 5). Ostracod assemblages still indicate low lake level/ephemeral conditions (Marchegiano et al., 2018) suggesting conditions more arid than today, as also recorded by pollen archives from southern and central Italy (Magny et al., 2012).

In the Early Holocene, $\delta^{18}O_w$ decrease and warm and cold season values are very close ($\delta^{18}O_w$ 1± 0.2 and 0.9 ± 0.2 ‰) (Figure 5). Ostracod assemblages indicate an increase of lake level (Marchegiano et al., 2019), these values suggest lower E/P ratios (and thus high humidity) during both seasons, indicating a low seasonality contrast in the hydrology. However, seasonal temperatures are instead very different (11.8 ± 2.1 and 23.2 ± 2.5 °C). The same paleohydrological behavior has been observed in Lake Pergusa and Lake Preola (southern Italy) (Magny et al., 2012) as well as in eastern Mediterranean (Kolodny et al., 2005; Marino et al., 2009; Develle et al., 2010). Conversely, records from northern Italy and western Mediterranean recorded a higher seasonality contrast with drier summers during and after ca. 9800 cal a BP (Finsinger et al., 2010 and Peyron et al., 2011). This study also confirms that, during the Early Holocene, climatic conditions in central Italy were similar to those ones in southern Italy and, thus, that the climatic limit between northern and southern climatic zone in Italy, previously settled at 40° latitude by Magny et al. (2012), needs to be moved to 43° latitude, as previously suggested by Marchegiano et al. (2019).

In the latest part of the Holocene, the reconstructed $\delta^{18}O_w$ values (Fig. 5) are very similar to today ones and indicate a difference of 2‰ between seasons suggesting high winter and low summer precipitation as typical of present-day central Mediterranean climate. This larger contrast of precipitation amount between seasons seems to have started in central – southern Italy around ca. 4.5 cal ky BP (Magny et al., 2012). Similar seasonal patterns in precipitations have been also observed in the western Mediterranean (García-Alix et al., 2021; Toney et al., 2020)

5. CONCLUSIONS

- The analyses made on recent samples (last ca. 1ky) confirm the applicability of the ostracod-clumped isotope thermometer to reconstruct paleotemperatures, seasonality and hydrological conditions.
- The absence of a vital effect at genus and species level is confirmed also in the signal of fossil Δ_{47}-ostracod shells signal.
- Despite the absence of a vital effect, in environments with seasonal temperature variations, not all species can be combined and measured together, instead only those that precipitate their shell during the same season.
- The MOTR technique is a very useful high-resolution proxy for identifying the larger temperature variations to select the samples for further Δ_{47} analyses.
- The application of the ostracod-Δ_{47} thermometer on the Trasimeno lacustrine core (central Italy), provides the first continental warm season paleotemperature and hydrological conditions for the last 43 ky in the central Mediterranean area.
- Warm season temperatures were heavily impacted by millennial scale climatic events.
- Insolation conditions in the central Mediterranean area enhanced the effect of the millennial scale climate variability, by increasing warm season temperature differences between warmer/high insolation MIS 3 (median temperatures of 17 °C) and colder/lower insolation MIS2 (median temperatures of 12 °C). This trend is also observed in warm season hydrological conditions with high evaporation during MIS3 and low ones in the MIS2.

- During the Early Holocene warmer than today cold season temperatures are observed and similar hydrological conditions between warm and cold season underlying a lower seasonality contrast than today and enhanced warm season precipitation.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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DATA AVAILABILITY

Data are available in the supplementary material section during the review process, and they will be successively deposited in a data repository, most probably EarthChem.

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FIGURE AND TABLE CAPTIONS

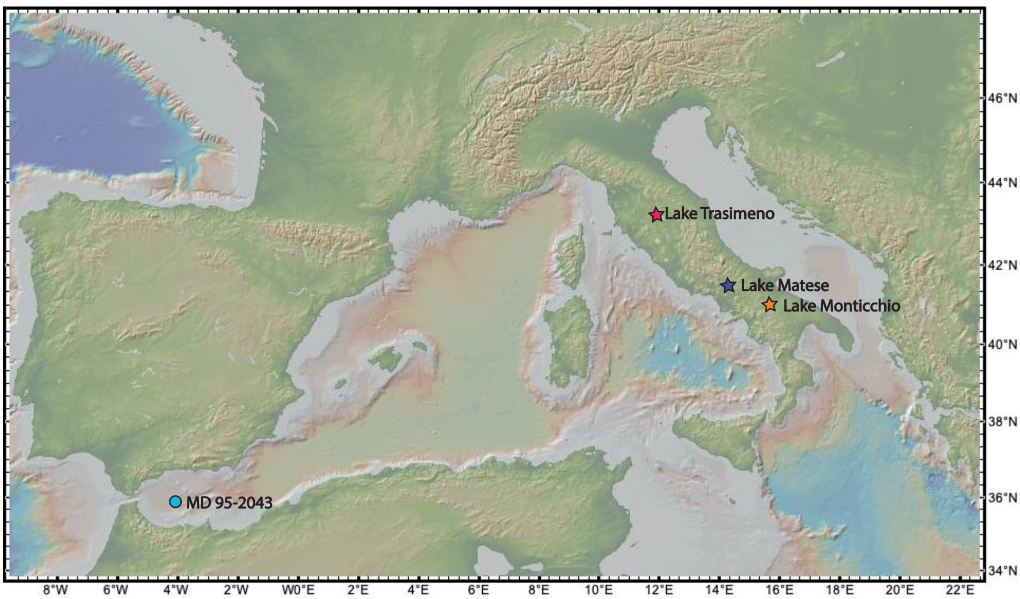


Fig. 1: Location of the records discussed in this study. Marine record MD 95-2043 from Alboran Sea (Cacho et al., 1999). Lacustrine records from Lake Trasimeno (this study), Lake Matese (Robles et al., 2023) and Lake Monticchio (Allen et al., 1999)

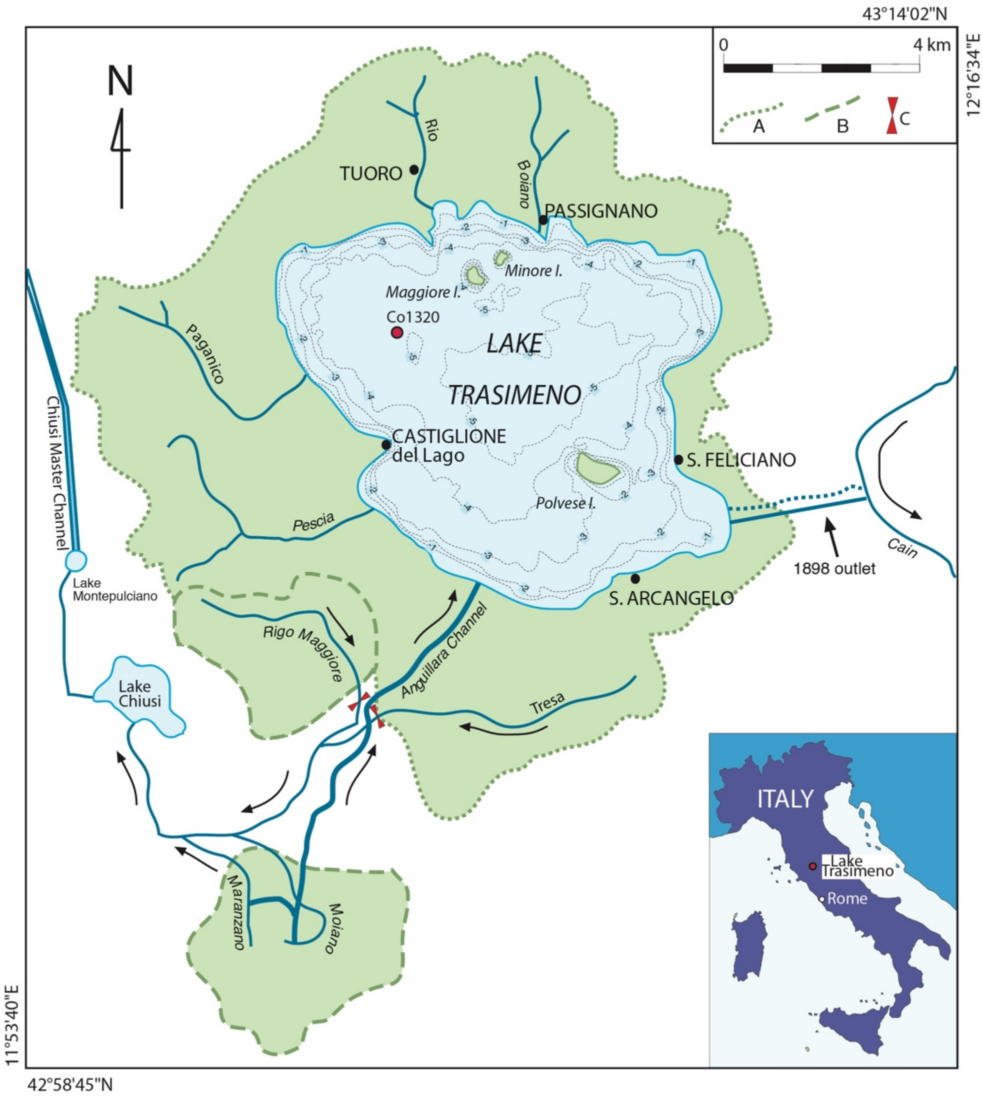
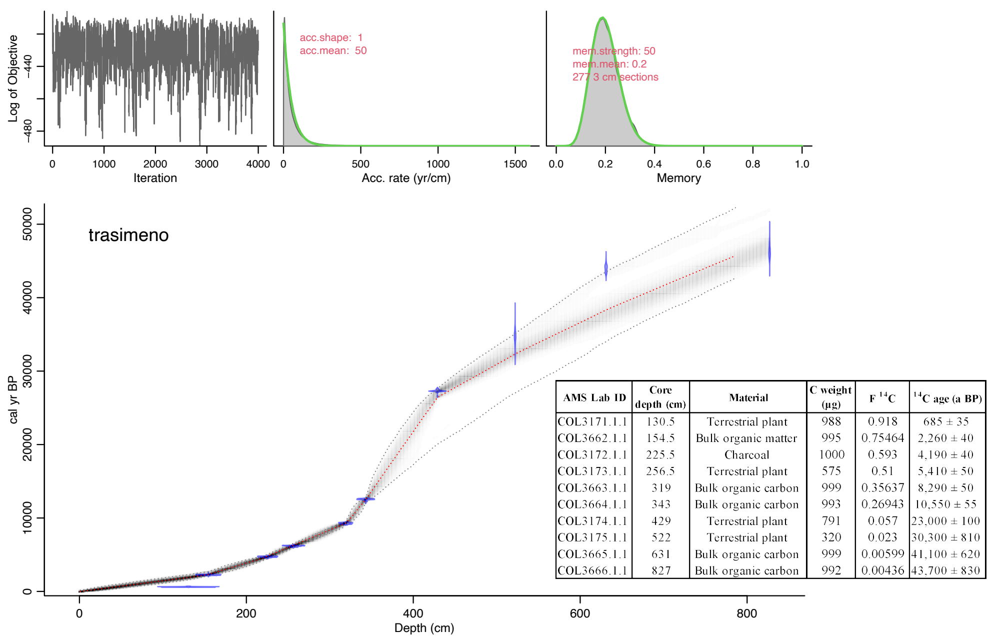


Fig. 2: Lake Trasimeno and core location, modified from Marchegiano et al. (2018).



AMS Lab ID	Core depth (cm)	Material	C weight (µg)	F ^{14}C	^{14}C age (a BP)
COL3171.1.1	130.5	Terrestrial plant	988	0.918	685 ± 35
COL3662.1.1	154.5	Bulk organic matter	995	0.75464	2,260 ± 40
COL3172.1.1	225.5	Charcoal	1000	0.593	4,190 ± 40
COL3173.1.1	256.5	Terrestrial plant	575	0.51	5,410 ± 50
COL3663.1.1	319	Bulk organic carbon	999	0.35637	8,290 ± 50
COL3664.1.1	343	Bulk organic carbon	993	0.26943	10,550 ± 55
COL3174.1.1	429	Terrestrial plant	791	0.057	23,000 ± 100
COL3175.1.1	522	Terrestrial plant	320	0.023	30,300 ± 810
COL3665.1.1	631	Bulk organic carbon	999	0.00599	41,100 ± 620
COL3666.1.1	827	Bulk organic carbon	992	0.00436	43,700 ± 830

Fig. 3: Chronology of the Trasimeno core. The chronology is based on radiocarbon ages from Marchegiano et al., (2018) modelled with Bayesian statistics using Bacon 3.1 0 by Blaauw & Christen (2011) and IntCal 2020 (Reimer et al. 2020).

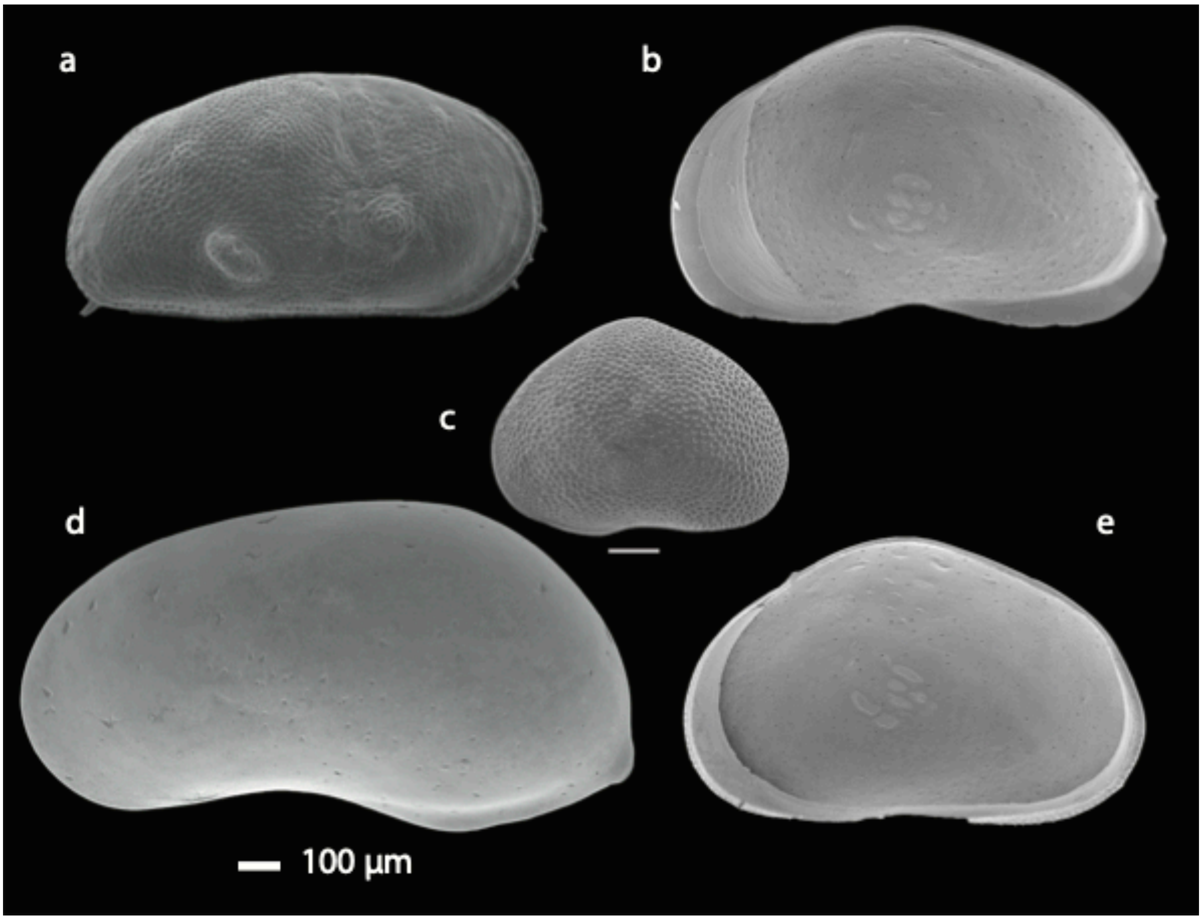


Fig. 4: Ostracod SEM pictures, made at the University of Roma Tre, obtained from samples of the Lago Trasimeno Co1320 core, a. *Cyprideis torosa*, b, *Eucypris mareotica*, c, *Sarcypridopsis aculeata*, d, *Candona angulata* and e, *Heterocypris salina.*

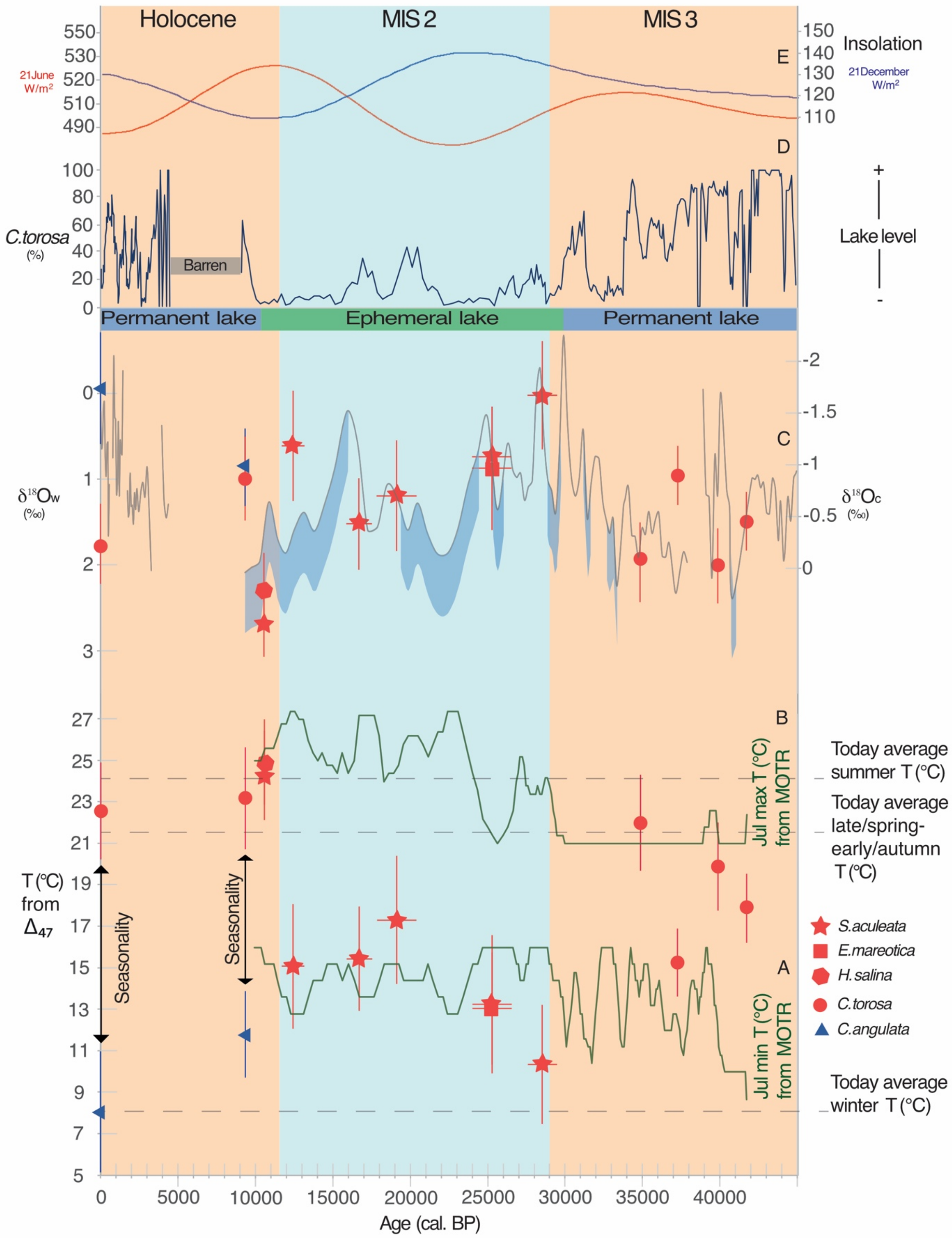


Fig. 5: Clumped isotope temperature and reconstructed $\delta^{18}O_w$ (this study) from the most abundant species *Cyprideis torosa*, *Eucypris mareotica*, *Sarcypridopsis aculeata*, *Candona*

angulata and *Heterocypris salina* (red and blue symbols: red symbols, warm season species; blue symbols, cold season species). Mutual Ostracod Temperature Range (MOTR, from Marchegiano et al., 2020) A (Jul min) and B (Jul max) curves, bulk carbonate $\delta^{18}O$ data of Lago Trasimeno from Francke et al. (2022) (C black line,), the uncertainty in certain carbonate samples (blue shade) is due to the mixed composition of aragonite/calcite and the subsequent application of different correction factors (+0.6 for the aragonite, Tarutani et al., 1969) (Francke et al., 2022); $\delta^{18}O_w$ reconstructed from ostracod shells (red and blue symbols: red symbols, warm season species; blue symbols, cold season species), *C. torosa* ostracod abundance used to reconstruct lake level variations (D curve) (Marchegiano et al., 2018) and summer and winter insolation (E curve) (Laskar et al., 2004). Present mean water winter, late spring/early autumn and summer temperature are indicated.

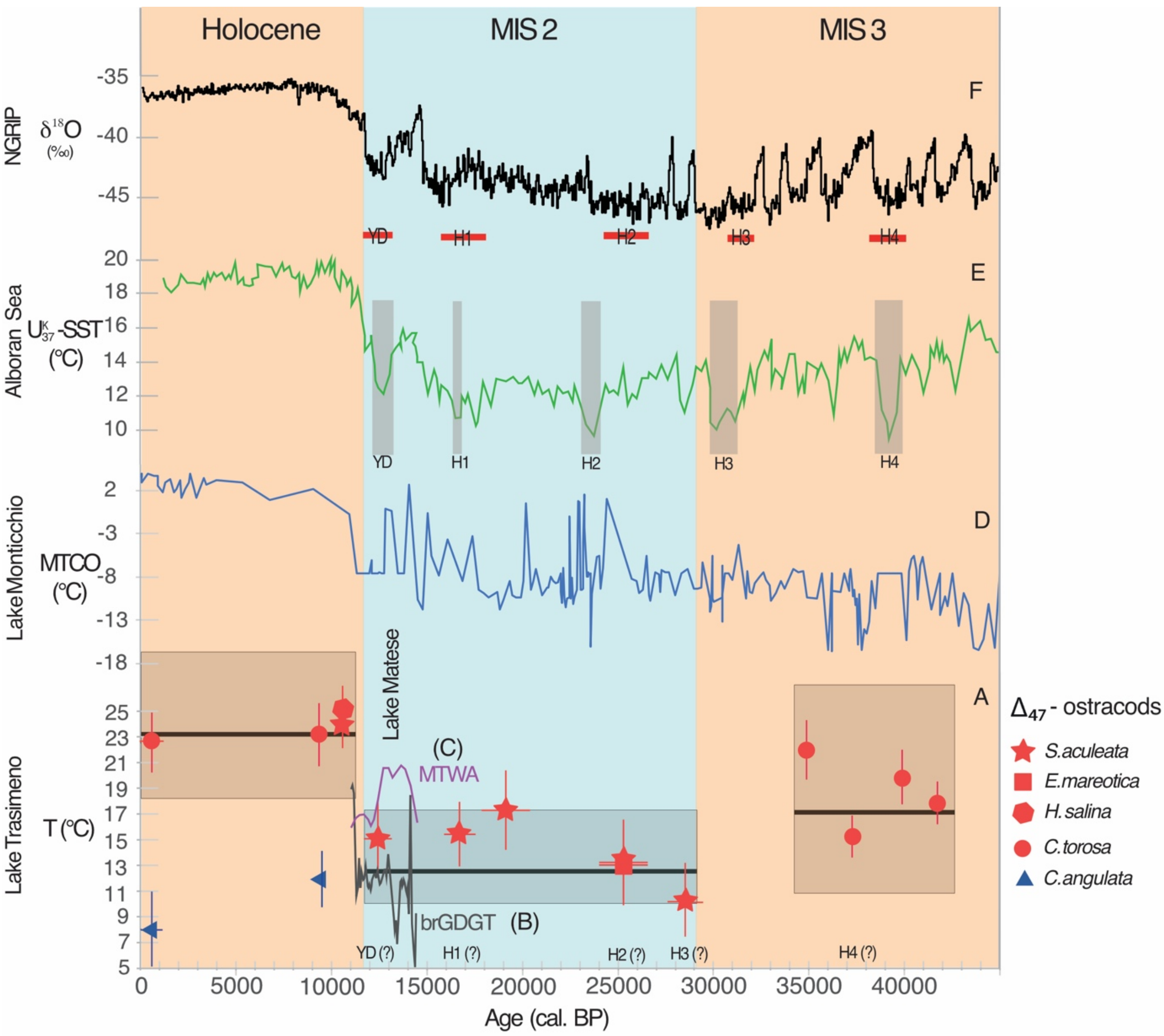


Fig. 6: Paleotemperature reconstruction at Lake Trasimeno and comparison with other Mediterranean records (A). Box plots for Δ_{47} temperatures, including all replicate measurements per each samples (see Table 1 in supplementary material), show median (black bold line), the first (25%) and third quartiles (75%, i.e. the grey box covers 50% of the probability density function) (A), brGDGT mean annual (B) and pollen reconstructed mean temperatures of the warmest month (MTWA) (C) from Lake Matese (Robles et al., 2023), pollen reconstructed mean temperatures of the coldest month (MTCO) from Lake Monticchio (D) (Allen et al., 1999) alkenones marine surface annual mean temperature from Alborean Sea (E) (Cacho et al., 1999) and the $\delta^{18}O$ from the North Greenland Ice Core Project (F) (Rasmussen et al., 2016).

Sample name	Depth (cm)	n°. replicates	Δ47 (‰)	Δ47 (‰) SE	Δ47-T	d13C (‰)	d18O (‰)	Specie	Vital offset (‰)	d18Ocorr (‰) (VPDB)	d18Ow (‰)	d18Ow (‰) SE	Average age (ky. cal BP)	Age
TRAR1	14-83	6	0.6485	0.0144	8.0	-0.49	3.13	C. angulata	-2.2	0.93	-0.0596	0.326	0.7	HOLOCENE
TRAR2	14-83	10	0.6012	0.0111	22.5	-3.17	0.42	C. torosa	-0.8	-0.38	1.7554	0.234	0.7	
TRAHOL1	316-322	9	0.5993	0.0115	23.2	-0.28	-0.45	C. torosa	-0.8	-1.25	0.9891	0.248	9	
TRAHOL2	316-322	10	0.6356	0.0111	11.8	2.17	3.2	C. angulata	-2.2	1	0.8604	0.226	9	
TRA2	328-330	12	0.5941	0.0101	24.9	3.99	0.03	S. aculeata	0	0.03	2.6674	0.202	10.5	
TRA2B	328-330	11	0.596	0.0105	24.3	3.6	-0.21	H. salina	0	-0.21	2.2886	0.215	10.5	
TRAGI1	336-348	6	0.6247	0.014	15.1	2.54	0.055	S. aculeata	0	0.055	0.6114	0.321	12	LGM-MIS2
TRAH1	363-375	8	0.6235	0.0123	15.4	3.5	0.86	S. aculeata	0	0.86	1.5216	0.267	17	
TRAGS2	375-393	6	0.6175	0.014	17.3	3.58	0.15	S. aculeata	0	0.15	1.1930	0.323	19	
TRAH2-1	413-431	7	0.6314	0.0131	13.0	3.09	0.61	S. aculeata	0	0.61	0.7368	0.290	25	
TRAH2-2	413-431	5	0.6307	0.0153	13.2	1.7	0.696	E. mareotica	0	0.696	0.8720	0.360	25	
LATEMIS3	445-477	6	0.6405	0.014	10.3	0.1	0.5	S. aculeata	0	0.5	0.0215	0.316	28	
TRA4	561-569	10	0.6029	0.0111	22.0	-1.32	0.74	C. torosa	-0.8	-0.06	1.9688	0.233	35	BLGM-MIS3
TRA5	603-616	14	0.6241	0.0094	15.2	-0.47	1.15	C. torosa	-0.8	0.35	0.9557	0.173	37	
TRA6	658-654	11	0.6094	0.0107	19.9	0.64	1.21	C. torosa	-0.8	0.41	2.0096	0.219	40	
TRA7	732-748	14	0.6157	0.0093	17.9	-3.47	1.12	C. torosa	-0.8	0.32	1.4897	0.171	42	

Table 1: Carbonate classic and clumped isotopes results. The Δ_{47}-T are calculated using the unified calibration (Anderson et al., 2021) and the $\delta^{18}O_w$ calculated from Δ_{47} and $\delta^{18}O_{ost}$ data using the formula of Kim and O'Neal (1997). BLGM: Before Last Glacial Maximum; LGM: Last Glacial Maximum.

SPECIES	CALCIFICATION SEASON	AGE	SAMPLE	Δ_{47}-T (2SE)	TODAY AVERAGE SEASON WATER-T
C. torosa	late-spring/early-autumn	HOLOCENE	TRAR2	22,5 ± 2.3	21.4
			TRAHOL1	23,1 ± 2.5	21.4
		BLGM-MIS3	TRA4	22 ± 2.3	21.4
			TRA5	15,2 ± 1.6	21.4
			TRA6	19,9 ± 2.1	21.4
			TRA7	17,9 ± 1.7	21.4
S. aculeata	summer	HOLOCENE	TRA2	24,9 ± 2	24
		LGM-MIS2	TRAGI1	15,1 ± 3	24
			TRAH1	15,4 ± 2.5	24
			TRAGS2	17,3 ± 3.1	24
			TRAH2-1	13,0 ± 2.7	24
			LATEMIS3	10,3 ± 2.9	24
E. mareotica	summer	LGM-MIS2	TRAH2-2	13,2 ± 3.3	24
H. salina	summer	HOLOCENE	TRA2B	24,3 ± 2.2	24
C. angulata	winter	HOLOCENE	TRAR1	8 ± 2.9	8
			TRAHOL2	11,8 ± 2.1	8

Table 2: Ostracod species used for Δ_{47} – T reconstruction, their shell calcification season *(C. torosa*, from Heip, 1976, Roberts et al., 2020 and consideration from this study; *S. aculeata* Meisch et al., 2000; *E. mareotica*, Li and Liu, 2010; *H. salina*, Meisch et al., 2000; *C. angulata,* Meisch et al., 2000) and present average seasonal Lake Trasimeno water temperatures (World Lake database 2023)