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Reconstructing paleoclimate in central Italy since Late Pleistocene: The Lake Trasimeno ostracod record

THÉSE

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pour obtenir le grade de Docteur dès Sciences,
mention Sciences de la Terre et de l'environments

par

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de

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Table of Contents

Summary
Résumé

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Summary

During the last decades, the evolution of future climate has been among one of the principal topics in environmental research. Palaeoclimate studies revealed that understanding past climate changes and their impact worldwide is fundamental for modelling future climatic scenarios. Lacustrine sediments represent one of the best natural archives to generate high-resolution palaeoenvironmental reconstructions. The use of different proxies allows estimating several environmental features (i.e. salinity, trophic state, chemical water composition) that are directly related to climatic parameters such as temperature, precipitation and seasonality.

An 8.59 m long sedimentary core was retrieved from Lake Trasimeno (central Italy, 43°08’N; 12°06’E) in order to reconstruct the climate history of central Italy throughout the last ca. 47,000 cal yr BP. The hydrology of this endorheic lake strongly depends on local climate (i.e. precipitation/evaporation ratio) providing thus a unique continuous palaeoclimatic and palaeoenvironmental lacustrine record. Its position, in the center of the Mediterranean region, makes it a relevant site to constrain the geographical climatic variability of this area.

The analyses of this Lake Trasimeno sedimentary core allowed us reconstructing past climatic and hydrological changes using ostracod assemblages as the main proxy. To attain a realistic palaeoenvironmental interpretation a detailed knowledge of modern ostracodes is fundamental to be able to use them as analogues. The sampling of the living ostracod fauna and the prevailing physico-chemical parameters of the waters where they live allowed us identifying different ecological niches. These results have been essential to obtain a robust palaeoclimatic reconstruction of the site.

In the last 47,000 years Lake Trasimeno’s ostracod fauna pointed out several lake level changes. A comparison with other proxies in the same core and the correlation with the oxygen isotope curve from Greenland (NGRIP) allowed to interpret them as climatically driven changes (i.e. humid and arid periods). In particular, during the interstadial phase (ca. 47,000 – 33,000 cal yr BP) of Marine Isotopic Stage (MIS) 3 relatively more humid conditions, occasionally interrupted by short dry periods,

were indicated by the prevalence of the *C. torosa* association (i.e., permanent lake). An aridification trend was recognized during MIS2 (ca. 33,000 – 10,400 cal yr BP), during which *S. aculeata* and *S. aculeata* - *E. mareotica* association were present (i.e., shallow/temporary waterbody). At the Pleistocene-Holocene transition an increase in moisture is shown by the dominance of the torosa-angulata association. At ca. 9,000 cal yr BP the lake reached the highest water level with a total absence of ostracods. Hence, the increasing rainfall responsible for this high lake level is delayed by ca. 2,700 years in comparison with the NGRIP record. Humid conditions continued to ca. 4,200 cal yr BP when the reappearance of ostracods (*angulata-torosa* and *stevensoni* associations) after almost 4,800 years of absence suggests the setting of dryer conditions that continue until present day.

A successfully application of the Mutual Ostracod Temperature Range method to the Lake Trasimeno ostracod record between 44,000 and 9,000 cal yr BP allowed reconstructing palaeotemperatures. It demonstrated for the first time the ability of the method to yield a continuous record of rapid climatic change.

The analyses of historical documentation revealed that despite the high population of the catchment, human impact on the lake hydrology has been minimal. Thus, the observed variations in ostracod associations appear to be mostly driven by climate.

The analyses of Lake Trasimeno sedimentary core allowed us to improve the existing knowledge about the evolution of climatically-forced environmental conditions for the last 47,000 cal yr BP. The comparison with other climatic records indicates that the lake is sensitive to global climatic patterns. Furthermore, these results are challenging existing views about latitudinal moisture distribution in the Italian peninsula and, thus, contribute to constrain the outcome of climate models for the Mediterranean area.

Résumé

Au cours des dernières décennies, l’évolution du climat futur représente un des sujets principaux dans le domaine de la recherche environnementale. Plusieurs études paléo-climatiques ont révélé que la compréhension des changements climatiques passés et de leur impact général est fondamentale afin de modéliser les possibles scénarios climatiques futurs. Les sédiments lacustres représentent une des meilleures archives naturelles pour les reconstructions paléo-environnementales à haute résolution. De plus, l'utilisation de différents proxys permet d'évaluer une multitude de caractéristiques environnementales (tels que la salinité, état trophique, la composition chimique d'eau) qui sont directement liés aux paramètres climatiques comme la température, la précipitation et la saisonnalité.

Une carotte sédimentaire longue de 8.59 m a été échantillonnée dans le Lac Trasimène (Italie centrale, 43°08'N; 12°06'E) afin de reconstruire l'histoire climatique de l'Italie centrale dans les derniers approx. 47,000 ans cal BP. L'hydrologie de ce lac endoréique dépend fortement du climat local (c'est-à-dire du ratio précipitation/évaporation) fournissant ainsi un enregistrement lacustre paléo-climatique et paléo-environnemental très unique. Sa position, au centre de la région méditerranéenne, en fait de plus un site adéquat pour restreindre géographiquement la variabilité climatique de cette zone.

Les analyses faites sur cette carotte sédimentaire provenant du Lac Trasimène nous ont permis de reconstruire les changements climatiques et hydrologiques passés utilisant des assemblages d’ostracodes comme principaux proxys. Afin de réaliser une interprétation paléo-environnementale réaliste, une connaissance détaillée des ostracodes moderne (vivants actuellement dans le lac) est fondamentale pour pouvoir au final les utiliser comme des analogues. Les données d’échantillonnage des ostracodes vivants, intégrées aux paramètres physicochimiques des eaux où ces derniers vivent nous ont permis d’identifier plusieurs niches écologiques distinctes. Ces résultats ont été essentiels pour l’élaboration d’une reconstruction paléo-climatique robuste du site.

La population d’ostracodes dans le Lac Trasimène a indiqué, pour les 47,000 dernières années, plusieurs changements de niveau du lac. Une comparaison avec d'autres proxys dans la même carotte et la corrélation avec la courbe d'isotope d'oxygène du Groenland (NGRIP) ont permis d’interpréter ladite population comme étant clairement influencée par les changements climatiquement (c'est-à-dire périodes humides et arides). En particulier, pendant la phase interglaciaire (approx. 47,000 - 33,000 cal BP) de la « Marine Isotopic Stage (MIS) 3 », des conditions relativement plus humides bien qu’épisodiquement interrompues par de courtes périodes sèches, ont été indiquées par la présence de l’asocciation *C. torosa* (c'est-à-dire, présence d’un lac permanent). Une certaine période d’aridification a été reconnue pendant le MIS2 (approx. 33,000 - 10,400 cal BP), et pendant laquelle les associations *S. aculeata* et *S. aculeata-E. mareotica* démontrent d’avoir été présentes (c'est-à-dire, lac peu profond/ provisoire). À la transition Pléistocène-Holocène, une augmentation de l'humidité est démontrée par la prédominance de l'association *torosa-angulata*. Ainsi, aux environs de 9,000 ans cal BP, le lac a atteint son niveau d’eau le plus haut, corrélé avec une absence totale d'ostracodes. De ce fait, l’augmentation des précipitations responsables de ce haut niveau du lac est retardée d’approx. 2,700 ans en comparaison avec les valeurs du NGRIP. Des conditions humides ont continué pour approx. 4,200 cal BP jusqu’à la réapparition de population d’ostracodes (associations *angulata-torosa* et *stevensoni*) après presque 4,800 ans d'absence suggérant la mise en place de conditions de sécheresse plus prononcées qui continuent de nos jours.

Une application fructueuse de la méthode de « Mutual Ostracod Temperature Range » sur la chronique d’ostracodes du Lac Trasimène entre 44,000 et 9,000 cal BP a permis de reconstruire les paléo-températures. Cette application a démontré pour la première fois la capacité de la méthode à livrer un enregistrement de rapides changements climatiques permanents.

Les analyses de documentation historique ont révélé que malgré une haute population dans le bassin versant du lac, l'impact humain sur son hydrologie reste minimal. Ainsi, les variations observées entre les associations d’ostracodes semblent être surtout dues au climat.

Les analyses réalisées sur la carotte sédimentaire provenant du Lac Trasimène nous ont permis d'améliorer l’état actuel de connaissance sur l'évolution des conditions environnementales directement influencées par le climat pour le 47,000 dernières années cal BP. La comparaison avec d'autres enregistrements climatiques indique que le lac est sensible aux facteurs climatiques globaux. Par ailleurs, ces résultats contredisent certaines vues sur la distribution des valeurs d’humidité aux latitudes de la péninsule italienne et, ainsi, contribuent à une connaissance plus précise sur les modèles climatiques pour la région méditerranéenne.

CHAPTER 1

Introduction

In the last decades, the growing awareness that human activity is substantially affecting Earth's climate entailing major societal consequences has triggered a wide number of investigations. These studies aim to improve our knowledge about different aspects of climatology. The urgency to visualize possible future scenarios as well as plausible mitigation strategies has been one of the goals of climate modelers. However, these models need to be validated by "real" proxy data. Geologists have known for many years that climate has constantly shifted throughout Earth history producing often-dramatic changes. The resulting changes in both biotic and abiotic spheres have been recorded in several natural archives (e.g., ice cores, tree rings, lacustrine and oceanic sediments; Cronin, 1999). The reconstruction of past climate dynamics is thus critical to unravel the forcing-mechanisms behind its variability as well as to validate the modeling of forecasted changes. Palaeoclimatology (the study of past climates) is a research field in continuous evolution as a result of the constant advancements in technology that allowed, among others, to increase the resolution of the investigated time windows. The latter is fundamental when studying the most recent record in order to separate and evaluate the anthropogenic impact on natural systems (Gornitz, 2009). The proxy data used in palaeoclimatology can be biological (e.g., fossil remains), geochemical (e.g., stable isotopes, elemental composition) and petrophysical (e.g., magnetic susceptibility, density). However, the analyses of a single proxy can be biased giving a partial view of the environmental conditions that have changed it. The combination of different environmental indicators (i.e., multiproxy approach) provides independent evidence for a common observation, ultimately leading to produce more robust palaeo-reconstructions (Battarbee et al., 2004; Bradley, 1999).

1.1 Lacustrine sediments as natural archives

Proxy data can be extracted from several natural archives. Lake sediments are certainly among the best archives in the continental realm to produce palaeoclimatic and palaeoenvironmental reconstructions (Cohen, 2003). In comparison with marine environments lacustrine basins have a relatively shorter life and thus, are less influenced by major geological events while their smaller size makes them more sensible to changes in the catchment even to those resulting from the weakest climatic variations. Moreover, because they contain many different proxies (e.g., paleontological, sedimentological, geochemical), lacustrine records can provide accurate and robust environmental reconstructions compared to other continental archives such as tree rings or speleothems (Gornitz, 2009). Lacustrine sediments contain a wide variety of information (e.g., salinity, PH, water chemical composition, trophic state) that are the result of the prevailing environmental conditions (e.g., temperature, precipitation/evaporation regime).

The formation and type of lacustrine basins are very often primarily influenced by regional climate. In general under cold and arid intervals, the scarcely vegetated catchment area and the prevalence of physical weathering facilitate the transport of clastic sediments into the lake (Gornitz, 2009). The amount of this transport is directly function of the runoff intensity. During comparatively warmer and humid conditions, the increasing vegetation cover reduces the availability and the transport of detrital material. Conversely, the dominance of chemical weathering promotes the release and the transport of nutrients into the lake (Gornitz, 2009). The direct consequence of this augmentation in nutrient availability is the eutropication of the system along with the primary productivity leading to an overall increase in the formation of organic-rich sediments. Additionally, there is a larger contribution of allochthonous organic components due to the abundant vegetation cover and higher runoff linked to enhance humid conditions.

The excellent preservation of proxies coupled to an accurate age model, thus makes lacustrine archive invaluable in providing very high resolution time scale paleoclimatic and paleoenvironmental information.

1.2 Lake Trasimeno, central Mediterranean region

Climate has been the focus of many studies in the Mediterranean region because of the substantial socio-economical and environmental impacts that former changes in climate have had and will potentially have in future. The vulnerability of this area is due to the complexity and variety of processes that affected it as a consequence of its morphology and geographical location. Due to its morphological heterogeneity each region within the Mediterranean realm reacts differently to both atmospheric and marine circulations (Abrantes et al., 2012). Moreover, its location between subtropical and mid-latitude climate regimes subdivide the area in two main regions characterized by strong differences in seasonality and precipitation patterns (Lionello, 2012). The northern region is mainly influenced by the North Atlantic Oscillation (NAO) and by other regional features (i.e., Scandinavian pattern, East Atlantic and East Atlantic/northern Russia patterns). Conversely, the descending branch of the Hadley cell mostly affects the southern portion of the region during the whole year, as well as the Asian monsoon in summer and El Niño Southern Oscillation (ENSO) teleconnection that is mainly affecting rainfall distribution (Alpert et al., 2006; Mariotti et al., 2002). Overall, these different forcing mechanisms result in the occurrence of several climate types within the Mediterranean region (i.e., differences in precipitation/evaporation regime, moisture distribution and temperature; Fig. 1) and thus challenging the climate modeling of the region as well as the forecasting of future climatic scenarios.

Accurate high-resolution palaeoclimatic reconstructions for the different regions are thus required to fully comprehend the interaction of the prevailing climate forcing mechanisms and to constrain their variability.

Due to its central position in the Mediterranean region Lake Trasimeno (43°09’N; 12°06’E, Perugia, Italy) provides an ideal site to investigate former changes in climate. Moreover, the very shallow (today maximum depth is 6 m and 4 m in average) and endorheic nature of the lake makes it very sensitive even to rapid and/or short climatic variations.

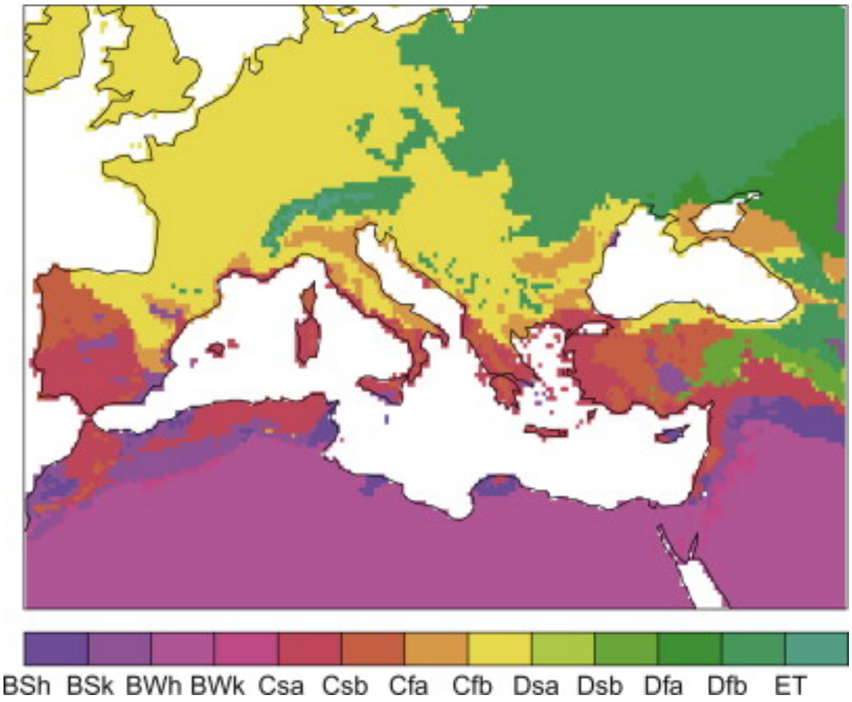

Figure 1: Köppen climate types in the Mediterranean region: subtropical steppe (BSh), midlatitude steppe (BSk), subtropical desert (BWh), midlatitude desert (BWk), Mediterranean climate with hot/warm summer (Csa/b), humid subtropical with no dry season (Cfa), maritime temperate (Cfb), humid continental with hot/warm summer (Dfa/b), continental with dry hot/warm summer (Dsa/b), and tundra (ET), (from Lionello, 2012).

In contrast to other central Italian lakes such as Bolsena, Vico and Bracciano, which are volcanic craters, the onset and evolution of Lake Trasimeno has been mostly ruled and driven by the Northern Apennine tectonics. Surprisingly, the tectonic evolution of the lake has not been intensely investigated yet (Gasperini et al., 2010). However, one important outcome of the existing studies is that the lake basin has been originated and maintained by extensional tectonics since the Early Pliocene (Gasperini et al., 2010). The catchment area of Lake Trasimeno covers about 376 km^2 enclosing high mountains in the northern, eastern and southern parts, and a flat zone towards the west. The mountains contain Oligocene to Miocene formations enclosing turbidites with marine sandstones, claystones and some marly claystones (Burzigotti et al., 2003). Instead, the flat western part is mostly composed of Pliocene to Holocene lacustrine and fluvial deposits (Burzigotti et al., 2003; Gasperini et al., 2010).

Lake level fluctuations have been caused not only by variations in the evaporation/ precipitation ratio but also by several human interventions to regulate them. The latter have been always recorded in historical documents as shown in the results of several anthropological investigations (Burzigotti et al., 2003; Ludovisi and Gaino, 2010). The combination of anthropological and limnogeological datasets is quite unique in the Mediterranean region and fundamental in any attempt to disentangle the impact of climate and human activities in the area. Moreover, recent studies have shown that both climatic- and human-induced lake level fluctuations have played a major role controlling of the physic-chemical behavior of this waterbody (i.g., salinity, alkalinity, trophic state; Ludovisi and Gaino, 2010; Marchegiano et al., 2017). More detailed information about Lake Trasimeno can be found in the following chapters.

The University of Cologne (Germany) in collaboration with the National Research Centre of Pisa (Italy) and the University of Geneva (Switzerland) joined efforts to produce the first high-resolution paleoclimatic and paleoenvironmental record of Lake Trasimeno. In September and November 2014 a coring campaign was carried out using a gravity piston corer (UWITEC®) set in a floating platform. An 859 cm long sedimentary core with a diameter of ~6.3 cm was retrieved in Lake Trasimeno (Co1320; 43° 09.624’N, 12° 03.491’E) (Fig. 2) at ~4.9 m of water depth. The coring site was chosen based on a previous seismic study (Gasperini et al., 2010) highlighting this area as one of the most appropriated to retrieve a continuous and undisturbed sedimentary record. Several proxies have been used to study this core including whole core petrophysical data such as magnetic susceptibility and density measurements along with lithological observations; geochemical data such as elemental analyses using high resolution X-ray fluorescence (XRF); micropaleontological remains in particular ostracods (the main goal of this thesis); stable isotopes on carbonates ($\delta^{18}O$ and $\delta^{13}C$) as well as organic and inorganic geochemistry (TIC and TOC).

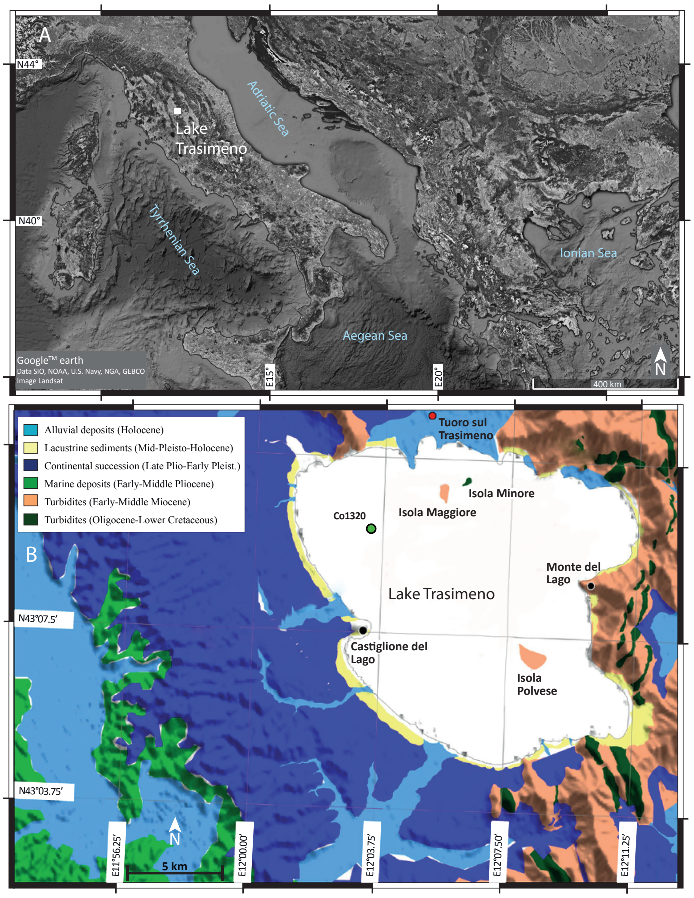

Figure 2: Map of Lake Trasimeno showing the location of Co1320 sedimentary core (from Francke et al., in review)

1.3 Ostracods as a paleoenvironmental proxy

Ostracods are tiny bivalve aquatic crustaceans (mostly 0.3-5 mm long). They constitute the most complete fossil record of the phylum Arthropoda since the Ordovician to the present, with an estimate number of fossil and living species of ca. 65,000 (Ikeya et al., 2005). Ostracods are widely distributed in all kinds of marine and continental water bodies, from very shallow to abyssal depths, in flowing and calm waters, but also in seasonally intermitted and underground waters. Two main subclasses are at present recognized: the Myodocopa and Podocopa (Siveter, 2008; Williams et al., 2008). All freshwater ostracods belong to the Podocopa subclass and Podocopida order (Horne et al., 2012). In particular, the species identified in this core belong to the Darwinuloidea, Cyproidea and Cytheroidea superfamilies that are all benthic.

Most ostracods secrete a low-Mg calcite carapace formed by two valves dorsally articulated, which are easily preserved in the fossil record. They grow by successive moults (eight larval stages), each composing a new carapace usually with a shape and ornament that can be rather different to that of their adult stage (allometric growth). The possible sexual dimorphism, sometimes very accentuated, can be completely observed only in the last moult (Meisch, 2000). The hermetic closure of the carapace mainly serves as a protection from predation but also, in some species, as a protection from the short-term desiccation of the habitat, allowing them to adapt and survive in temporary/ephemeral waterbodies (Meisch, 2000). The surface ornamentation it is generally constant within species but, in some cases the presence/absence of some ornament elements (e.g. nodes and spines) can be strongly influenced by environmental factors (e.g. salinity and temperature) representing an invaluable tool for paleoenvironmental reconstructions (Carbonel and Hoibian, 1988; Frenzel et al., 2010; Keen, 1982; Kühl, 1980).

Ostracods can reproduce both sexually and parthenogenetically, depending sometimes on ecological factors such as temperature. Several nutritional modes (e.g. filter and deposit-feeding) have been developed depending on the species. They mainly feed on algae, organic detritus, bacteria, plant materials, small living or dead animals but also on bottom sediments (Meisch, 2000).

Ostracods have high sensibility to the variation of several ecological parameters such as salinity, temperature, water level, oxygen saturation, chemical composition and water energy (Baltanás et al., 1990; Keatings et al., 2010; Marquez et al., 2016), making them very useful to reconstruct past environmental changes, biostratigraphy, archaeology and water pollution (Jiřiček, 1985; Mazzini et al., 2015; Ruiz et al., 2013). In this work we use ostracode remains from Lake Trasimeno to reconstruct paleoclimatic and paleoenvironmental changes since the Late Pleistocene to the present.

Because the societal impact of climatic- and anthropogenic-induced environmental changes it is important to identify any organisms that can provide a record of such changes in the past. The easy and frequent fossilization of ostracode carapaces, their great species diversity, their fast reproduction and their abundance in almost every aquatic environment make them one of the most effective paleoenvironmental indicators (Horne et al., 2012 and references therein).

Their distribution in different environments is influenced by the temporal and physico-chemical stability of the ecosystem (Smith and Horne, 2002). The observed preferences of each species for certain environmental conditions allow using them in (paleo) environmental studies. Even if some species present a very large ecological tolerance, some others are almost limited to certain habitats with specific and peculiar environmental conditions (Meisch, 2000). These species, thus, became very useful as proxies in the already mentioned (paleo) environmental studies.

In freshwater environments and more specifically in shallow lakes, ostracods can provide information about the influence of climate on the paleohydrological regime of the area. For example, they can be used to reconstruct the runoff, the moisture regime and the precipitation/evaporation balance. Furthermore, they can be valuable indicators to detect internal changes of the lake system such as salinity, trophic state, lake level, temperature and ion–composition, which are strictly linked to regional and global climate (Rodriguez-Lazaro and Ruiz-Muñoz, 2012). A general requirement is the knowledge of the ecological tolerance of the fossil species found in a particular environment. Thus, assuming the validity of the uniformitarian principle (i.e. the existence of a strong ecological relationship between living organisms and their fossil counterparts), having a modern analogue becomes extremely important to achieve a reliable paleoecological reconstruction. In the last decades the development of consistent ecological training sets, in which living ostracods have been related to the prevailing physico-chemical parameters of the water, allowed to develop several transfer functions (e.g. Horne, 2007; Mezquita et al., 2005; Mischke et al., 2007; Viehberg, 2006; Viehberg and Mesquita-Joanes, 2012). This approach adds a quantitative value to paleoenvironmental and paleoclimatic reconstructions, providing a new venue on the use of ostracods as proxy.

In this study, a qualitative climate- and human-induced paleoenvironmental reconstruction has been inferred by the high-resolution analysis of ostracod assemblages throughout a sedimentary core of Lake Trasimeno (Chapter 3 and 5). Moreover, the application of the Mutual Ostracod Temperature Range (MOTR) method (Horne, 2007) allowed the estimation of paleotemperatures throughout time (Chapter 4)

1.4 Aim of this work and thesis outline

The aim of this study is to provide a high-resolution climatic and hydrological reconstruction since the Late Pleistocene using ostracod assemblages. The endorheic and shallow Lake Trasimeno in central Italy is an outstanding example of a highly sensitive system to regional environmental changes because it is solely dependent on the prevailing meteorological conditions. However, given the complexity of paleoclimate signals, a multiproxy approach (i.e. micropaleontological, geochemical, sedimentological and archaeological) was critical to properly interpret such record. This approach further allowed correlating the response of Lake Trasimeno with other global indicators of climate change.

The thesis has been organized as follows: The first introductory **Chapter 1** summarizes the geographical and climatic settings of the area. It also introduces the use of lacustrine sediments and in particular of ostracod assemblages as paleoenvironmental proxies. The subsequent chapters focus on more specific and targeted questions. Each of the following chapters corresponds to a scientific manuscript published, submitted or in preparation to be submitted to peer-review journals.

Chapter 2 Ecology and distribution of living ostracod assemblages in a shallow endorheic lake: the example of the Lake Trasimeno (Umbria, central Italy). This chapter_focuses on the relationship between modern ostracod assemblages and their living environment. The presence of different species were related to the dominant physico-chemical parameters of the water column such as temperature, dissolved oxygen content, conductivity, total dissolved solids, depth, pH, concentration of major anions and cations and alkalinity, as well as the different types of substratum and macrophytes coverage. This study provides a starting point to evaluate both past and present climatically- and anthropogenically-induced changes of this fragile environment. This chapter has been already published as an article in the *Journal of Limnology*.

Chapter 3 Arid and humid phases in central Italy during the Late Pleistocene revealed by the Lake Trasimeno ostracod record. This chapter illustrates the use of ostracod assemblages, lithology and TOC to reconstruct successive lake level and salinity fluctuations. The good correlation of these lake level changes with the Greenland Interstadial (warm and humid) and Greenland Stadial/Heinrich (cold and arid) events highlights the global impact of these climatic changes. This chapter corresponds to an article already published in *Palaeogeography, Palaeoclimatology, Palaeoecology.*

Chapter 4 Rapid Late Pleistocene climatic changes reconstructed from a lacustrine ostracod record (Lake Trasimeno, Umbria, central Italy). This chapter illustrates the successful application of the Mutual Ostracod Temperature Ranges (MOTR) method. Sub-chapter 4.1 shows the calibration of *Eucypris inflata*. This chapter will be part of an article that will be soon submitted to *Quaternary Science Reviews*.

Chapter 5 Lake Trasimeno (central Italy): A record of society, environment and climatic interactions during the Holocene. This chapter describes the relationship between ostracod assemblages, TOC, lithology and the climatically-driven lake level changes. Moreover, a detailed analysis of existing archeological and historical documents allowed separating anthropological from climatic signals. This chapter will be soon submitted as an article to *The Holocene*.

Chapter 6 Coeval Mediterranean rainfall and global marine circulation patterns during the Last Glacial summarizes the results of a multiproxy reconstruction of the lake level fluctuations at lake Trasimeno linked to the precipitation regime in central Italy. This chapter is an article presently in review in *Nature scientific Reports.*
Finally, the conclusion and outlook chapter abridges the different aspects discussed in the thesis as well as potential prospective and follow-up research directions.

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CHAPTER 2

Ecology and distribution of living ostracod assemblages in a shallow endorheic lake: The example of Lake Trasimeno (Umbria, central Italy)*

Ostracod assemblages from Lake Trasimeno (Umbria, central Italy), the largest endorheic lake in Italy, were investigated relating their species distribution and ecology to modern physical, chemical and biological parameters. Nineteen living species were collected in the lake (*Darwinula stevensoni, Candona (Candona) candida, Candona (Neglecandona) angulata, Fabaeformis- candona fabaeformis, Pseudocandona marchica, Cypria ophtalmica, Ilyocypris gibba, I. salebrosa, I. getica, Cypridopsis vidua, Eucypris virens, Trajancypris clavata, Herpetocypris helenae, Heterocypris salina, H. incongruens, Isocypris beauchampi, Cyprideis torosa, Limnocythere inopinata,* and *L. stationis*). All the identified species belong to the fresh-water Italian ostracod fauna but *Cyprideis torosa* is documented in an oligohaline athalassic lacustrine environment in Italy for the first time. The occurrence of *Ilyocypris salebrosa* represents the southernmost record in Italy and the westernmost in Eurasia. The recovery of *Limnocythere stationis* represents the westernmost record in Eurasia. The distribution of the different ostracods recovered in Lake Trasimeno is linked to the dominant physical and chemical parameters for each ecological niche. Physical and chemical data along with substratum type, grain-size and presence of aquatic macrophytes have been related to different ostracods using a multivariate analyses approach (NMDS, CCA, Spearman’s rank correlation test).

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These results allow to differentiate several ecological niches within the lake and indicate that the main parameters affecting the ostracod assemblages are the aquatic macrophyte coverage, the Total Organic Carbon (TOC) and, to a lesser extent, temperature and type of substrate. *Cyprideis torosa* and *Candona* (*Neglecandona*) *angulata* have been recovered both in the distal part of the lake and in the lakeshore area. In both cases they are associated with scarce or absent aquatic macrophytes and low amounts of TOC. The alternate dominance of these two species in the distal deeper assemblages seems to be mainly linked with the bottom oxygen availability, being *C. (N.) angulata* dominant in the most oxygen-depleted sediments and *C. torosa* dominant in higher oxygen conditions. Along the lakeshore area they are often discovered together with other prevailing species, such as *Cypridopsis vidua* that is common in very shallow to shallow (20-140 cm) sites with high TOC content, abundant macrophytes and algae, and *Limnocythere inopinata*, which dominates slightly deeper areas (around 150-210 cm) where the previous species are almost absent. The Spearman's rank correlation test showed significant positive correlation between some ostracods and macrophyte species.

2.1 Introduction

Among benthic invertebrates, ostracods (tiny bivalve crustaceans) are particularly sensitive to environmental changes, being strongly dependent on several physical and chemical factors such as size, depth, energy level, and turbidity of the waterbody as well as temperature, dissolved oxygen content, water chemistry, food supply and substratum nature (including aquatic vegetation) (Rodriguez-Lazaro and Ruiz-Muñoz, 2012). The ability of several cypridoidean species to disperse passively in continental waters, either as adults and/or juveniles through survival in torpid stage and/or as eggs through diapause, leads them to easily populate or re-populate aquatic ecosystems (Mesquita-Joanes et al., 2012). Such characteristics make them a very useful tool for the reconstruction of environmental changes through time, since their calcite shells are easily fossilised and preserved in the sediments. The knowledge of the autoecology and synecology of ostracod species is particularly important when these organisms are used as proxies to investigate the present and past environmental vulnerability (Viehberg and Mesquita-Joanes, 2012).

Endorheic lakes, in particular, are known to be vulnerable ecosystems through time because their hydrological budget is mostly ruled by evaporation due to the absence of a surficial drainage output. As a result, endorheic lakes are very sensitive to changes in air temperature and precipitation and thus they deserve special attention in the on-going debate about the possible effects of climate change and human impact on biodiversity and environmental preservation. Lake Trasimeno (Umbria, central Italy) is the largest endorheic basin in Italy. During the last 130 years the basin has been affected by climate changes, especially rainfall and air temperature variations, together with increasing human impact causing relevant lake level oscillations (Ludovisi and Gaino, 2010). These events caused periodical economic crises and the progressive depletion of the aquatic biocoenosis (particularly of the planktonic, macrobenthos, macrophyte and fish communities) (Martinelli, 2012). Such dramatic events are not uncommon in endorheic lakes, as documented, among others, by the historical changes undergone by the large lakes Chad, Aral and Neusiedl, located at different latitudes and under different climate conditions. Lake Chad (semi-arid Sahel region, Africa) was affected by a dramatic desiccation event driven by droughts occurred over the last 50 years, which caused its shrinkage from 25,000 km^2 to only 1350 km^2 (Helfert and Holz, 1985; Lauwaet et al., 2012). Lake Aral (central Asian desert) underwent repeated lake level oscillations over the past 10-15 kyr, linked to natural climate changes, that culminated into the environmental disaster that is still affecting the lake since 1960’s, due to the irresponsible deviation for irrigation purposes of its two main inflow rivers (Micklin, 2007; Shukla, 2015). The consequences of the Lake Aral shrinking affected dramatically not only the ecological balance of the region and the lake itself (high salinity increase of the waterbody from 10 to 35‰; Boomer et al., 1996), but also the health of local population that now uses high saline water for irrigation (causing an important decrease of the agricultural output) and as drinking water (Ataniyazova, 2003). Lake Neusiedl (Austrian-Hungarian border), the largest shallow closed-basin in central Europe, completely dried up between 1866 and 1871 following more than ten temporary desiccation episodes since its formation 13,000 years ago (Soja et al., 2013). The study on Lake Neusiedl ostracods has shown that past changes in the lake level induced the reduction of the *Phragmites* belt causing a decrease in the ostracod fauna biodiversity (Löffler, 1990) and economic damages to tourism and fishery activities (Soja et al., 2013; Gallinaro et al., 2014).

At present no data are available about Pleistocene changes in water level, despite Lake Trasimeno was supposed to have been settled during the Early Pleistocene (Gasperini et al., 2010). The results of on-going investigations on these older sediments will be useful to disentangle the role of natural and human-induced climatic changes on the evolution of the lake system. The main aim of the present research is to investigate the different living ostracod assemblages and their relation with the main physical, chemical and biological parameters, in order to use these results to reconstruct past environmental and climate variations and their impact on the lake. Additionally, these data improve the knowledge of the living ostracod fauna of Lake Trasimeno, up to now only investigated by von Hartmann (1964), as well as that of the still poorly known ostracod population of the Italian lakes.

2.2 Methods

2.2.1 Study area

Lake Trasimeno (Perugia, Umbria - 43°08’N; 12°06’E, lake bottom altitude 251.57 m a.s.l.) is the largest lacustrine system of central Italy and the fourth largest Italian lake in size (~120 km^2). It is a shallow (average depth 4.7 m, maximum depth 6.3 m) endorheic lacustrine basin with few small tributaries. Since 1960, the Rigo Maggiore, Tresa, Moiana and Maranzano ditches were deviated into the artificial Anguillara Channel (Taticchi, 1992). This latter, together with the natural Paganico and Pescia ditches, represents the lake surficial inflows. No natural outflow exists, only an artificial channel built in 1898 near S. Feliciano village spills the Trasimeno waters into the Tiber River through the Caina creek (Fig. 1). There is no consensus concerning the groundwater input to the lake. Whilst Tiberi (1980) excludes any contribution, ARPA Umbria (2005) suggests that ~2.5% of the total water budget is coming from subsurface waters. Its closed condition makes the hydrological balance of Lake Trasimeno strongly affected by climate variations (precipitation vs evapotranspiration) (Ludovisi and Gaino, 2010; Dragoni et al., 2012). Climate modulates strong lake level variations and at least three negative fluctuations were recorded during the last ninety years (Dragoni et al., 2012). The last low stand occurred between 1988 and 2012, when the lake level was 1.87 m below the 'hydrometric zero' (257.33 m a.s.l.; refer to http://www.biondiriccardo.it/LAGO/elaborations.htm) (Dragoni et al., 2012).

The shallow character of the lake allows a complete mixing of the water column throughout the year (polymictic lake), producing a continuous homogenization of the physical and chemical parameters such as temperature, conductivity, dissolved oxygen and pH along the water column. Lake Trasimeno waters are rather well oxygenated (surface DO varies between 5.8 and 12.6 mg L^{-1}; bottom DO 6.6-12.1 mg L^{-1}) with pH values ranging between 8 and 8.5 and summer maxima around 10, high Cl and Na contents (maximum values of 8.98 and 8.57 m^2 L^{-1}, respectively) and mesotrophic to eutrophic conditions (Taticchi, 1992; Ludovisi and Gaino, 2010; Charavgis et al., 2012). These parameters change seasonally and/or over longer periods: for example, in the last 40 years, conductivity has varied from around 750 in the 70's to 1700 µS cm^{-1} at the beginning of this century (Ludovisi and Gaino, 2010), corresponding to possible variations of TDS of around 1 g $L^{-1}$of magnitude. Chloride concentration has largely varied too, from 3.63 to 8.98 m^2 L^{-1}as a result of lake level dropping (Ludovisi and Gaino, 2010). Along the southern coast of the lake, the bottom sediments are mainly clay and silty-clay whereas sands with variably content of clay and silt dominate along the northern shores (Charavgis et al., 2012). Mineralogical analyses show a great abundance of smectite (Charavgis et al., 2012), a mineral that usually adsorbs heavy metals and organic matter (Charavgis et al., 2012). According to Morgantini and Peruzzi (2012), the sediments have a content of organic matter (OM) up to 4%, mainly of algal origin as shown by the TOC/N_{tot} ratio ranging between 4 and 8.

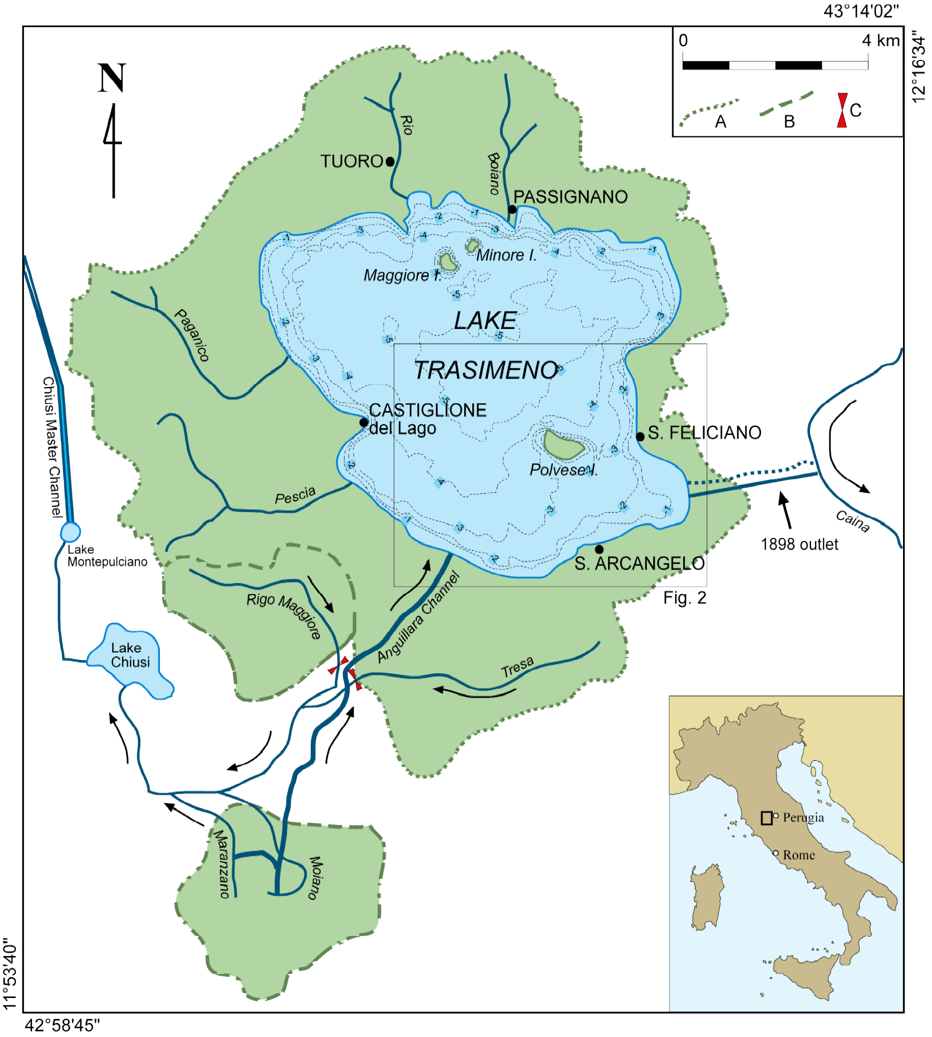

Figure 1: Geographic location of the Trasimeno Lake. A, Natural catchment area; B, artificially-joined basins; C, sluice gates of the artificially-joined channels. Redrawn and modified from Fig. 4 (page 73) and Fig. 2 (page 125) in A. Martinelli (ed.), Tutela ambientale del Lago Trasimeno. Libri Arpa Umbria, Perugia, 2012 (with permission).

Recent studies on environmental conservation of Lake Trasimeno have shown that the lake is a relevant site for plant communities characterized by a good biodiversity level (Venanzoni et al., 2006). Aquatic macrophytes cover around 25% of the lake surface, particularly in the coastal zones, while the central pelagic area is devoid of aquatic vegetation due to the water turbidity and increasing water depth preventing its development (Havens et al., 2009). However, historical data indicate that the distribution of the hydrophyte and helophyte vegetation has greatly fluctuated depending on the lake level (Havens et al., 2009). During the low lake level of the so-called 1944-1960 'crisis', the entire lake area was populated by aquatic macrophytes belonging to genera Potamogeton, Myriophyllum and Chara, whilst riverbed communities dominated by Phragmites australis expanded in the lakeshore area. On the contrary, during the lake level high stand of the 1960s, the aquatic vegetation was reduced ~30% (Havens et al., 2009) and riverbed communities were restricted to the emerged areas. Due to human impact and increased agricultural activities, a decrease in the macrophyte biodiversity was noticed between 1960s and 2007 (Charavgis et al., 2012). Phytoplankton blooms mainly composed by dinoflagellates, diatoms and cyanobacteria, this latter group dominating since 1992, underlines the rather high trophic level of the lake (Havens et al., 2009; Elia et al., 2012).

2.2.2 Sampling and data collection

During July 2014, a sampling campaign was carried out along the southern and south-eastern shores of Lake Trasimeno and on a N-S transect starting approximately from the middle of the lake (2.5 km north of Island Polvese) towards the coast in front of the Anguillara Channel (Fig. 2). This area is of particular interest because it includes both the inflow and outflow channels, a relatively high anthropogenic pressure on the lakeshores (villages and cultivated fields and hills), and encloses the deepest part of the lake. In the field, the sample sites were roughly divided in two sets: sites deeper than 300 cm, distant from the lakeshore and characterised by few macrophytes, and sites shallower than 300 cm, apparently characterised by a quite variable range of bottom sediment and more or less abundant macrophytes.

Forty samples from the uppermost sediments (4 cm) were collected by scuba-diving using a rectangular hand-net (28 cm x 14 cm, 120 µm mesh size) (Tab. 1). One surface water sample was collected among free-floating macrophytes with a sampling bottle. In the field, samples were preserved in 75% ethanol and stained with Rose Bengal for 36-50 h, to differentiate living (or recently died) from sub-Recent ostracods (Walton, 1952; Bernhard et al., 2006). They were washed in the laboratory with tab water through a 125 µm-mesh sieve, dried and observed under a stereomicroscope for ostracod identification on the base of the shell features of adult specimens. Gonzalez Mozo et al. (1996), Meisch (2000) and Fuhrmann (2012) were followed for taxonomic identification.

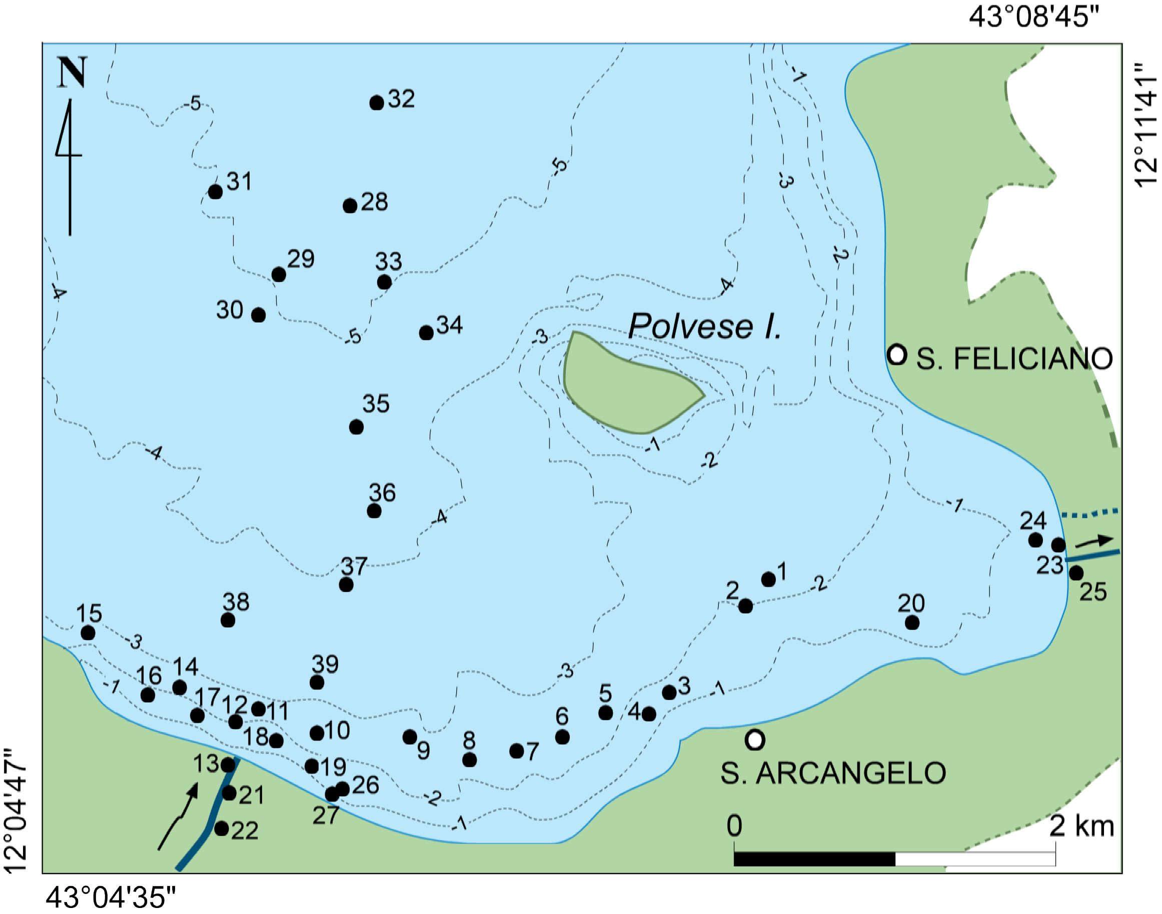

Figure 2: Geographical location of sampling sites

While Rose Bengal staining is widely adopted in foraminifer studies, the method is not as commonly used by ostracodologists and sometimes its results have been questioned (Browers et al., 2000). Our results in the ostracode samples of Lake Trasimeno show that the staining is not reliable to discriminate between living and sub-Recent specimens. In the present study, we have considered as living (or recently died) specimens, stained or not stained, those with preserved appendages or setae on the shell surface. On this basis, the density of each species (expressed by individuals per 1 dm^2) was counted after identification.

All macrophytes were listed at each ostracod sampling station and their coverage was further estimated, as percentages, in the field. Different collection methods were used: floating masses of filamentous macroalgae were collected by means of a 25 µm-mesh plankton net; epilithic algae by scraping submerged stones; vascular plants by direct observation in situ or collecting samples using a grappling iron. All macroalgae were fixed in formalin (4% was the approximate final concentration).

Taxonomical determination and nomenclature were based on specific algae (John et al., 2002) and vascular plants literature (Pignatti, 1982; Conti et al., 2005).

During the sampling, physical and chemical parameters of the lake water such as temperature (T, °C), dissolved oxygen content (DO, mg L^{-1}), conductivity (C, µS cm^{-1}), Total Dissolved Solids (TDS, mg L^{-1}) and pH were measured, using a Hydrolab Minisonde 4a multi-parameter instrument. Water depth (D, cm) was measured with a graduated rope. The concentration of major anions, cations and alkalinity (HCO_3^{1-} + CO_3^{2-}) were measured on two water samples collected near the bottom at sites 1 and 28, following standard methods by LabService s.r.l (Anguillara Sabazia, RM, Italy).

A visual estimation of the grain size and other components, such as plant remains and mollusk shell fragments, was made to get qualitative analyses of the substrate. The presence of abundant plant detritus (from few millimeters to few centimeters in size), persistent even after a H_2O_2 treatment, prevented us to perform standard grain size analyses based on the weight of the different size fractions. Laser grain size analyses particularly adapted for the finest fraction were not performed due to the abundance of particles greater than 0.7 mm.

Rock-Eval Pyrolysis has been used for characterizing the amount and nature of the organic matter of the bottom sediment. This method provides a good estimation of total organic carbon (TOC) and its quality based on the hydrogen and oxygen indices (Tissot and Welte, 1984; Peters, 1986; Meyers and Lallies-Vergès, 1999). It was initially used to study the hydrocarbon potential of petroleum source rocks (Espitalié et al., 1985) and nowadays it is also used in limnogeological studies (Ariztegui et al., 2001; Steinmann et al., 2003). A small amount of lake bottom sediments (ca. 0.45 - 0.55 mg) was pyrolysed

sample	latitude N	longitude E	D (cm)	T (°C)	pH	C (μs/cm)	DO%	DO (mg/l)	TDS (g/l)	bottom grain-size	Notes
PAN 1	43°05'20.1"	12°9'04.2"	240	25.33	7.95	1353	63.1	4.88	0.86	CA	in front of the S. Arcangelo Ichtyogenic Centre; no bottom vegetation
PAN 2	43°05'19.6"	12°08'53.3"	206	25.33	7.95	1353	63.1	4.88	0.87	SS	vegetated bottom
PAN 3	43°05'15.4"	12°08'31.8"	173	25.33	7.95	1353	63.1	4.88	0.87	SSg	vegetated bottom
PAN 4	43°05'10.8"	12°08'31.2"	140	25.09	7.87	1362	64.5	5.20	0.87	SSg	*Phragmites* fragments
PAN 5	43°05'12.2"	12°08'16.2"	230	25.1	7.76	1340	35.1	2.69	0.87	FSvd	vegetated bottom
PAN 6	43°05'04.8"	12°08'00.1"	250	24.98	8.30	1360	56.3	4.78	0.87	FSs	vegetated bottom
PAN 7	43°04'54.5	12°07'27.2"	250	25.06	8.30	1359	60.5	4.75	0.87	SSg	vegetated bottom
PAN 8	43°04'53.7"	12°07'02.6"	240	25.09	7.38	1319	45.2	3.87	0.84	FSs	no bottom vegetation
PAN 9	43°05'03.0"	12°06'32.0"	270	25.01	8.30	1357	67.8	4.91	0.87	FSs	vegetated bottom
PAN 10	43°05'11.8"	12°05'58.5"	240	25.15	8.29	1362	55.2	4.67	0.87	SS	no bottom vegetation
PAN 11	43°05'20.1"	12°05'46.6"	240	25	8.21	1362	57.1	4.60	0.87	FSg	in front of the Anguillara Channel; no bottom vegetation
PAN 12	43°05'20.1	12°05'46.6"	200	25	8.21	1362	57.1	4.60	0.87	FSg	in front of the Anguillara Channel; vegetated bottom
PAN 13	43°05'11.9"	12°05'36.9"	200	24.40	7.58	1245	30.9	2.90	0.80	FSvd	pond on the left bank of the Anguillara Channel
PAN 14	43°05'24.9"	12°05'25.4"	210	24.95	8.43	1361	56.0	4.63	0.87	SS	no bottom vegetation
PAN 15	43°5'35.0"	12°05'6.5"	250	24.8	8.15	1359	57	4.56	0.87	FSs	no bottom vegetation
PAN 16	43°05'24.3"	12°05'21.3"	180	24.94	8.17	1365	53.1	4.34	0.88	CA	no bottom vegetation
PAN 17	43°05'18.6"	12°05'33.8"	160	25	8.05	1364	59	4.65	0.87	SS	no bottom vegetation
PAN 18	40°05'10.9"	12°05'48.0"	150	25.14	8.10	1364	63	4.90	0.87	SSg	no bottom vegetation
PAN 19	43°05'04.9"	12°06'04.7"	120	24.20	8.15	1389	58.6	4.93	0.89	SSg	no bottom vegetation
PAN 20	43°05'28"	12°09'52.9"	130	23.72	7.91	1370	58.8	4.91	0.88	FSg	in front of Passo Serpaio dock; vegetated bottom
PAN 21	43°05'07.7	12°05'32.2"	20	23.62	7.31	1123	55.8	4.88	0.72	FSvd	along the right artificial bank of the Anguillara Channel; nearby edge with *Phragmitetum australis*
PAN 22	43°05'06.3"	12°05'31.8"	20	22.88	6.97	1338	43.3	3.68	0.86	FSvd	small channel on the right of the Anguillara Channel; nearby edge with *Phragmitetum australis*
PAN 23sur	43°06'23.3"	12°11'15.9"	surficial sample	22.26	6.87	1406	44.3	3.37	0.90		surface sample among free floating macrophytes along the artificial outflow channel (Oasi La Valle)
PAN 23 bot	43°06'23.3"	12°11'15.9"	80	22.26	6.87	1406	40	3.37	0.90	FSvd	bottom sample along the artificial outflow channel (Oasi La Valle)
PAN 24	43°06'18.4"	12°11'02.9"	50	24.20	7.46	1356	33.9	2.79	0.87	FSvd	nearby the dock of Oasi La Valle
PAN 25	43°06'16.2"	12°11'15.0"	20	25.19	7.41	1442	60	4.60	0.92	FSvd	small pond at the entrance of Oasi La Valle; *Phragmitetum australis* and hygrophilic macrophites
PAN 26	43°05'01.3"	12°06'08.1"	60	24	7.27	1397	52	4.28	0.89	FSvd	Panicale harbour; *Phragmitetum australis*
PAN 27	43°05'01.6"	12°06'07.9"	30	24.21	7.37	1387	53.5	4.52	0.89	FSvd	pond at the Panicale harbour; *Phragmitetum australis*
PAN 28	43°07'51.6"	12°06'13.4"	510	23.68	7.72	1231	45.8	3.55	0.80	CA	no bottom vegetation
PAN 29	43°07'37.2"	12°05'46.8"	500	23.65	8.31	1351	90.1	6.80	0.86	CA	no bottom vegetation
PAN 30	43°07'29.9"	12°05'41.0"	490	23.66	8.33	1348	81.7	7.15	0.86	SS	no bottom vegetation
PAN 31	43°08'00.0"	12°05'31.4"	500	23.63	8.27	1349	60.8	5.28	0.86	SS	no bottom vegetation
PAN 32	43°08'43.1"	12°6'24.0"	520	23.77	8.28	1347	66.7	5.46	0.86	SS	no bottom vegetation
PAN 33	43°07'35.5"	12°06'24.4"	500	23.74	8.35	1352	62.9	5.17	0.86	CA	no bottom vegetation
PAN 34	43°07'02.2"	12°06'47.5"	480	23.58	8.49	1343	75.9	6.5	0.86	FSs	no bottom vegetatiom; abundant *Dreissena* shells
PAN 35	43°6'38.3"	12°6'37.2"	450	23.66	8.48	1349	65.9	5.45	0.86	SS	no bottom vegetation
PAN 36	43°06'16.7"	12°06'20.4"	430	23.51	8.66	1351	70	5.88	0.86	SS	no bottom vegetation
PAN 37	43°05'58.3"	12°6'00.3"	390	23.41	8.35	1353	70.1	5.42	0.87	SS	no bottom vegetation
PAN 38	43°05'45.2"	12°05'33.0"	350	23.16	8.15	1359	57.4	4.99	0.87	SS	no bottom vegetation
PAN 39	43°05'27.4"	12°05'56.0"	310	22.98	8.5	1360	54	4.39	0.87	FSvd	no bottom vegetation

Table 1: D, water depth (cm); T, bottom water temperature (°C); pH, bottom water pH; C, bottom water conductibility (µs cm^{-1}); DO%, percentage of dissolved oxygen in bottom water; DO, dissolved oxygen in bottom water (mg L^{-1}); TDS, total dissolved solutes (mg L^{-1}); CS, clayey silts with minute plant remains and mollusc shell fragments; SS, silty sands with vegetal remains and mollusc shell fragments or juvenile specimens; SSg, silty sands with vegetal remains, mollusc shell fragments or juvenile specimens and gravels; FSvd, fine sands with plant detritus and few mollusc shells; FSs, fine sands with abundant mollusc shells and scarce plant detritus; FSg, fine sands with mollusc shells, vegetal detritus and gravels.

(starts isothermally at 300°C and after heated to 650°C) and completely oxidized (starts isothermally at 400°C and heated up to 850°C). Following the alternative Rock-Eval method of Steinmann et al. (2003) for organic matter from recent lake sediments, four peaks were measured during the organic carbon decomposition: S1 (during the 300°C isotherm), S2a (between 300- 400°C, peaking at ca. 365°C), S2b (above 400°C, peaking at ca. 465°C) and S3 (CO2 released above 400°C). The total amount of residual and pyrolysed organic carbon corresponds to TOC. The S2/TOC ratio or Hydrogen index (HI) and the S3/TOC or Oxygen index (OI) represent the H/C and the O/C ratios of the organic matter, respectively (Espitalié et al., 1985). These two parameters allow separating different sources of organic matter (Ariztegui et al., 2001; Steinmann et al., 2003).

samples	Cl^-	Alk	SO_4^{--}	Ca^{++}	Mg^{++}	K^+	Na^+
PAN 1	5.78	1.88	0.94	0.6	2.96	0.13	0.81
PAN 28	6.52	1.79	0.92	1.42	1.86	0.18	0.84

Table 2: Water chemical analyses (m^2 L); Alk, total alkalinity

2.2.3. Statistical analyses

The collected data were processed through multivariate analyses using different algorithms: Non Metric Dimensional Scaling (NMDS), Canonical Correspondence Analysis (CCA), and the Spearman's rank correlation test using the PAST software, ver. 2.17b (Hammer et al., 2001). Three matrices have been used to perform multivariate analyses, using as respondents the sample sites and as variables the density (percentage of individuals dm^{-2}) of each taxa in each sample, including a minimum 2% density. In the first matrix the physical and chemical parameters measured at each sampling station (depth, TDS, T, DO, pH, TOC), bottom grain size (GR) and macrophytes (mac) were added to the variables. This matrix was used to run the NMDS using Euclidean distance in Q- mode (samples) and the Canonical Correspondence Analysis (CCA) in R-mode (species). The variables of the second matrix data include the ostracod densities and the frequencies of each identified macrophyte species. The variables of the third matrix data are the ostracod densities and the different type of substratum. The Correlation test using Spearman's rank index was applied to these matrices, to identify a relation among the different data sets.

2.3 Results

2.3.1. Physical and chemical parameters of the water

During summer 2014 and for the first time since 1988, Lake Trasimeno held a water level above the hydrometric zero. Indeed, the lake level was +12 cm, while in the previous years it was -41 cm (2013) and -133 cm (2012) with a maximum drawdown of -142 cm recorded in 2008 (data from Club Velico Trasimeno, http://www.clubvelicotrasi- meno.it/livellolago.aspx). This high lake level was linked to particularly high rainfall conditions which affected the Lake Trasimeno hydrographic basin during 2014, corresponding to a annual rainfall of 940 mm (Perugia Province, unpublished data) in comparison with the mean annual rainfall of 700-800 mm year^{-1} for the 1963-2014 period (Valigi et al., 2016).

The physical and chemical parameters measured at each sampling site are reported in Tab. 1. Bottom water temperatures display small variations, being the differences between the distal (deepest) area and the lakeshore zone not greater than 3°C, ranging from 22 to 25°C. Conductivity and TDS are rather homogeneous, 1123 to 1442 µS cm^{-1}, and 0.7 to 0.9 g L^{-1}, respectively; pH values are more variable, from 6.9 to 8.7, as well as the dissolved oxygen content at the bottom that varies from 2.69 to 7.15 mg L^{-1}. According to the results reported in Tab. 2 the water of Lake Trasimeno can be defined as chlorine, sulphate-calcium and magnesium-rich. Because of the present high stand conditions of the lake, the measured chlorine concentration is lower in comparison with the values of the last twenty years (5.78 vs 6.06-8.98 m^{2}L^{-1}, respectively), although the conductivity values are only slightly lower (1231-1442 vs 1440 µS cm^{-1}during 2005) (Ludovisi and Gaino, 2010).

2.3.2. Bottom sediment features

Sediment texture along with the amount and type of organic matter (OM) has been investigated in all sampling sites. They contain fine sediments (clays, silts, fine sands) with some coarser elements such as plant detritus, shells (or shell fragments) and gravels. Six different kind of substrates were distinguished based on visual observations of the bottom sediments: CS, clayey silts with few plant remains and mollusk shell fragments; SS, silty sands with vegetal remains and mollusk shell fragments or juvenile specimens; SSg, silty sands with vegetal remains, mollusk shell fragments or juvenile specimens and gravels; FSvd, fine sands with vegetal detritus and few mollusk shells; FSs, fine sands with abundant mollusk shells and scarce plant detritus; FSg, fine sands with mollusk shells, vegetal detritus and gravels (Tab. 1).

CS and SS substrates are mainly located in the center of the lake, in the deeper zone, except for samples 10, 14, and 17; substrates SSg and FSg are typical of lakeshore areas; FSvd is found in vegetated lakeshore zones; and FSs is recognizable mainly around depths of 240-270 cm except for the deep site 34 (480 cm). TOC ranges from 0.36 to 10.21% of sample weight, but most of the samples varied between 1.90 to 2.40%. OI ranges between 127 and 329 mg CO_2 g^{-1} and HI between 144 and 744 mg Hc g^{-1}. Using a Van Krevelen-type plot it is possible to separate different types of organic matter and therefore their sources (Fig. 3a). Three types of organic matter are identified (Meyers and Lallies-Vergès, 1999): Type I (microbial biomass or waxy coatings of land plants), Type II (algae) and Type III (woody plant matter). HI decreases whereas OI increases from Type I to III. As shown in Figs. 3a and 3b, almost all samples from Lake Trasimeno are grouped around Type II and Type III, i.e., the organic carbon present in the bottom sediments derives from both algae and terrestrial plants. Some exceptional samples, PAN 14 and PAN 9, show relatively higher values indicating higher algal content and plotting close to the Type I field.

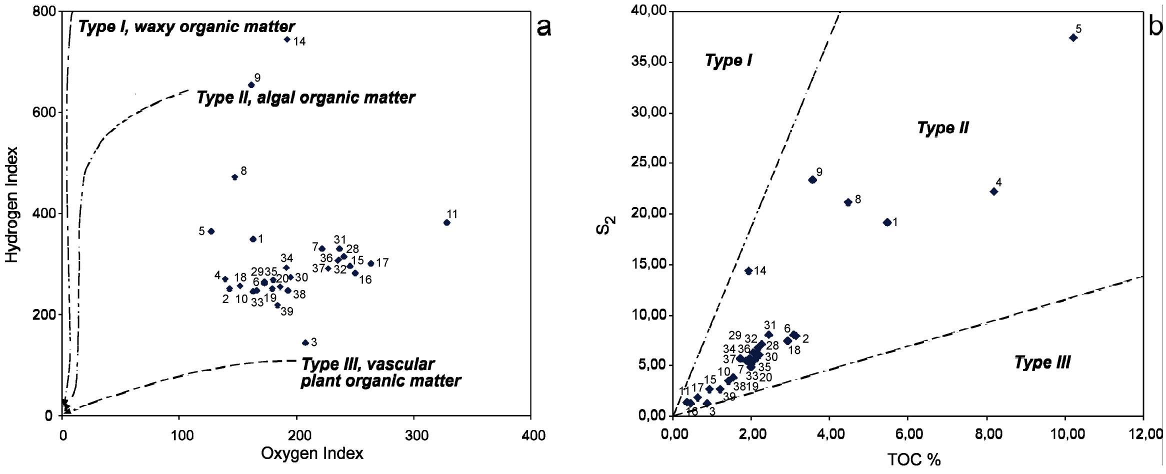

Figure 3: a) Van Krevelen-type diagram for Lake Trasimeno sedimentary organic matter; dots represent samples and dashed lines trace the maturity paths for organic matter types. b) S2 *vs* TOC diagram. Dashed lines define the boundaries of organic matter types.

2.3.3. Ostracod assemblages

Thirty-eight out of the forty-one collected samples contained living ostracod assemblages. The composition and density of the ostracod coenoses recorded during our campaign corresponds to high-stand conditions (+ 12 cm above the hydrometric zero). On the whole, nineteen species were recognized (Figs. 4 and 5). *Darwinula stevensoni* (Brady and Robertson, 1870), *Candona* (*Candona*) *candida* (O.F. Müller, 1776), *Candona* (*Neglecandona*) *angulata* G.W. Müller, 1900, *Fabaeformiscandona fabaeformis* (Fischer, 1850), *Pseudocandona marchica* (Hartwig, 1899), *Cypria ophtalmica* (Jurine, 1820), *Ilyocypris gibba* (Ramdohr, 1808), *I. salebrosa* Stepanaitys, 1959, *I. getica* Masi, 1905, *Cypridopsis vidua* (O.F. Müller, 1776), *Eucypris virens* (Jurine, 1820), *Trajancypris clavata* (Baird, 1838), *Herpetocypris helenae* G.W. Müller, 1908, *Heterocypris salina* (Brady, 1868), *Heterocypris incongruens* (Ramdohr, 1808), *Isocypris beauchampi* (Paris, 1920), *Cyprideis torosa* (Jones, 1850), *Limnocythere inopinata* (Baird, 1843), and *Limnocythere stationis* Vavra, 1891 were represented by living individuals. Moreover, only loose valves of *Potamocypris variegata* (Brady and Norman, 1889) and *Fabaeformiscandona harmsworthi* (Scott, 1899) were recovered, thus these species are not considered as living in the lake. Several carapaces and valves of *H. helenae* were collected in the lake. The valve outline is slightly different from the typical outline of the species illustrated by Gonzalez Mozo *et al*. (1996) and Fuhrmann (2012), with the dorsal margin slightly inclined forward rather than horizontal. Conversely, the features visible on the inner margin are comparable. Masi (1909), describing the valves of '*Cypria intermedia* var. *latialis*' from the surroundings of Rome and Sicily, remarked that the dorsal border was faintly inclined forwards. Masi's variety is considered synonym of *H. helenae* by Pieri *et al*. (2015). Thus, we consider this slightly different feature to fall within the morphological variability of *H. helenae*. Except 10 samples in which only loose valves or very few carapaces were recovered (samples 1, 13, 14, 16, 19, 20, 21, 21bis, 22, 27), all the other samples displayed abundant carapaces and valves (from 50 to 500 specimens including instars). *C. torosa, C. vidua, C.* (*N.*) *angulata,* and *L. inopinata* are the most abundant species. They are represented by several adult carapaces (271, 87, 78, and 53, respectively) and hundreds of loose valves. Except for the asexual *C. vidua*, *L. inopinata* is represented only by female specimens whereas the other two species were represented both by males and females with the following sex female/male ratios (calculated on the recovered carapaces): *C. torosa* 6:5 and *C.* (*N.*) *angulata* 3:1.

These dominant species have been recovered in the bottom samples of the lake with densities reaching maximum values of 25.0 (*C. torosa*), 12.2 (*C. vidua*), 6.3 (*C*. (*N*.) *angulata*), and 36.8 (*L. inopinata*) individuals dm^{-2}. The remaining species occur only in few samples. Carapaces, adult loose valves and juveniles were recovered except for *I. beauchampi*, *T. clavata* and *E. virens* that were represented by only 1-3 valves.

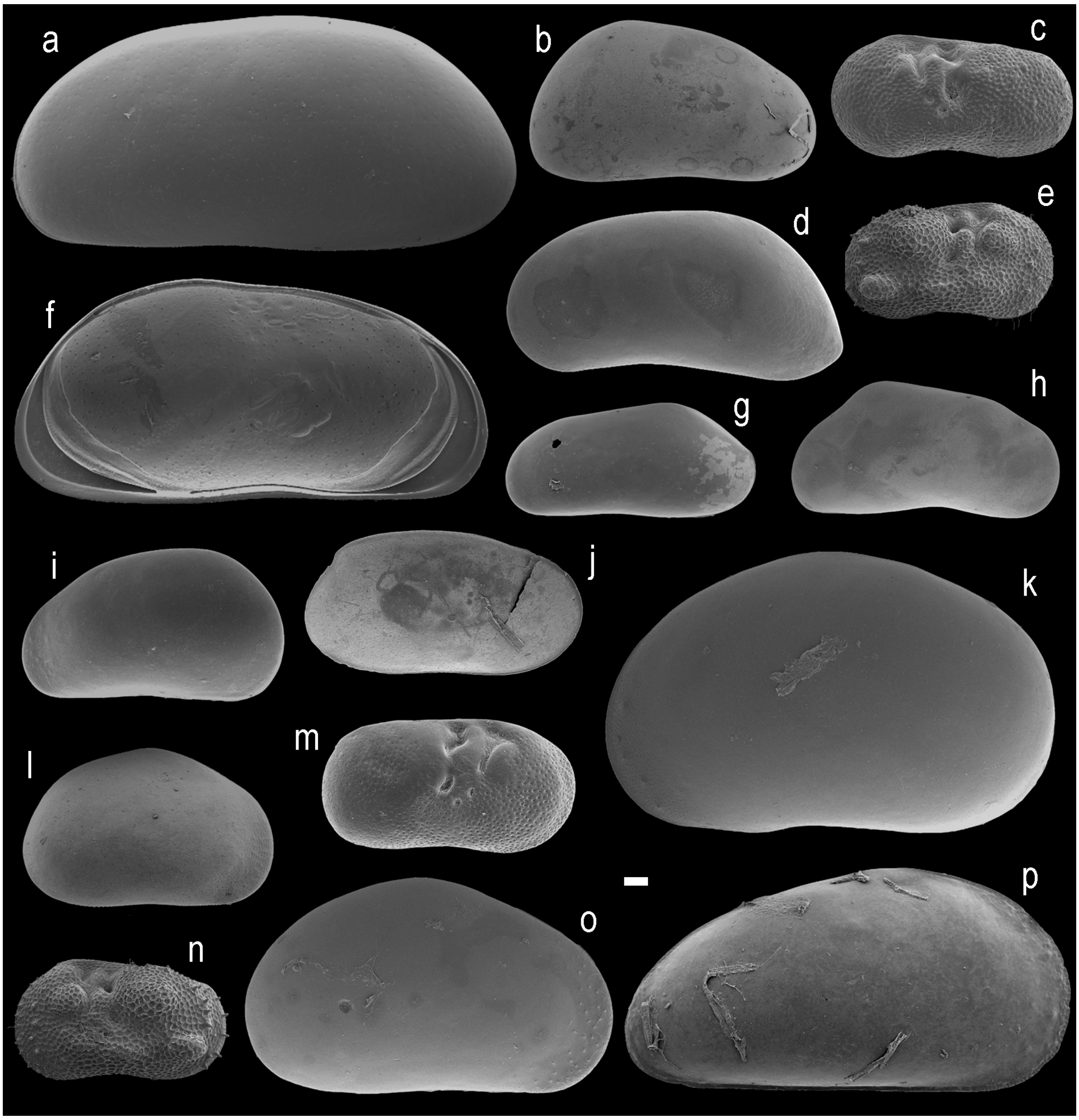

Figure 4: SEM photos of the living ostracods from Lake Trasimeno. a. *Herpetocypris helenae*, female LV, sample PAN 11; b. *Candona* (*Candona*) *candida*, female RV, sample PAN 23bot; c. *Ilyocypris gibba*, female LV, sample PAN 6; d. *Candona* (*Neglecandona*) *angulata*, female LV, sample PAN 38; e. *Ilyocypris salebrosa*, female RV, sample PAN 11; f. *Herpetocypris helenae*, LV, inner view, sample PAN 11; g. *Fabaeformiscandona fabaeformis*, female LV, sample PAN 3; h. *Fabaeformiscandona harmsworthi*, male (?) RV, sample PAN 24; i. *Pseudocandona marchica*, female LV, sample PAN 39; j. *Isocypris beauchampi*, female RV, sample 35, slightly damaged anteriorly and posteriorly; k. *Eucypris virens*, female LV, sample PAN 39; l. *Heterocypris salina*, female RV, sample PAN 11; m. *Ilyocypris getica*, female RV, sample PAN 9; n. *Ilyocypris salebrosa*, female LV, sample PAN 11; o. *Heterocypris incongruens*, female RV, sample PAN 11; p. *Trajancypris clavata*, female RV, sample PAN 11. LV, left valve; RV, right valve; C, carapace. White bar corresponds to 0.1 mm.

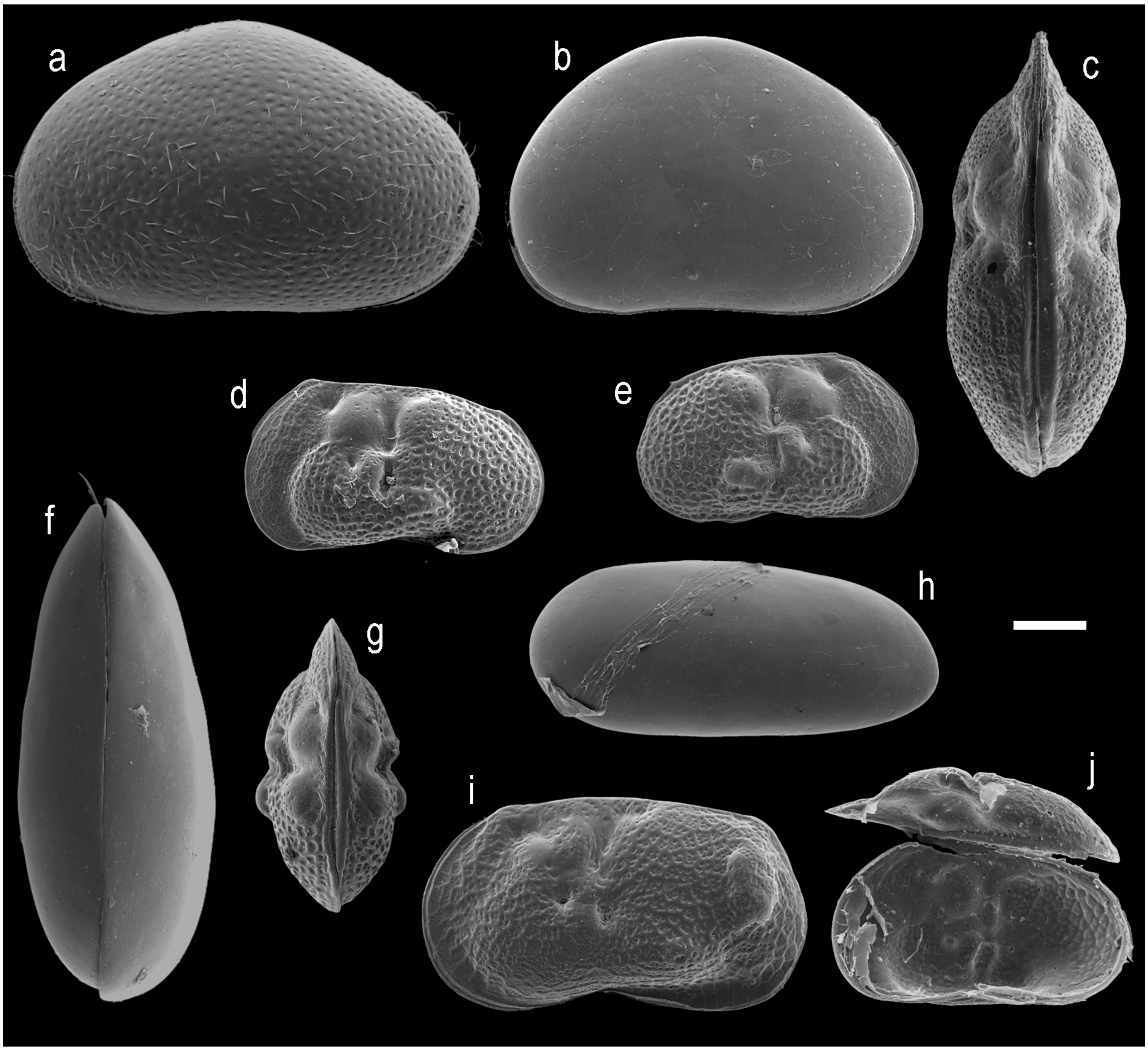

Figure 5: SEM photos of the living ostracods from Lake Trasimeno. a. *Cypridopsis vidua*, female LV, sample PAN 13; b. female *Cypria ophtalmica*, RV, sample PAN 23bot; c. *Limnocythere inopinata*, female C, dorsal view, sample PAN 2; d. *Limnocythere stationis*, female LV, sample PAN 5; e. female *Limnocythere stationis*, RV, sample PAN 5; f. *Darwinula stevensoni*, female C, dorsal view, sample PAN 3; g. *Limnocythere stationis*, female C, dorsal view, PAN 5; h. *Darwinula stevensoni*, female RV, sample PAN 6; i. *Limnocythere in- opinata*, female LV, sample PAN 10; j. *Limnocythere stationis*, open female C with appendage remains, sample PAN 6. Abbreviations as in Fig. 6. White bar corresponds to 0.1 mm.

2.3.4. Macrophytes assemblages

Macrophytes were identified in twenty-one out of the forty-one sampling stations. Species richness ranges from one to nine different taxa. The absence of macrophytes is in correspondence with the deepest stations in the central zone of the lake and with turbid waters. A total of 17 macrophyte taxa (ten flowering plants, seven algae) were recorded, among which the most common were *Phragmites australis* (Cavanilles) Trinius ex Steudel, *Najas marina* Linnaeus*, Ceratophyllum demersum* Linnaeus*, Potamogeton perfoliatus* Linnaeus and *Vallisneria spiralis* Linnaeus*.* The first three species were also the most abundant locally. Communities characterized by *P. australis* were prevalent along and strictly close to the banks, while those dominated by *N. marina* or *C. demersum* were the more widespread in the lake.

Ostracods were found in all sampling stations with aquatic vegetation. They were either in the substrate among the vegetation or, less often, attached to it.

2.3.5. Ostracod communities, macrophytes and substrate

The NMDS plot (Fig. 6), related to the first matrix, shows the distribution of the sampling sites depending on their physical and chemical, sedimentological and biological parameters. The dots in the Shepard plot appear strongly aligned; the very low value of stress (0.056) and the consequent very high nonlinear fit (R2=0.997) confirm the rejection of the null model (R2=0), where all the observations ‘fall in the same point’.

The sample distribution in the plot illustrates the different ecological niches recognized in the study area. Samples characterised by depths below 300 cm are all grouped in the upper portion of the diagram. They share pH values between 7.7 and 8.7, absence of macrophytes, fine substrate, and ostracod assemblages (*C.* (*N.*) *angulata*-*C. torosa* assemblage) and display a rather wide range of by DO values (3.55-6.80 mg L^{-1}). Conversely, samples with depth above 300 cm are more scattered in he diagram, showing a plurality of niches. Some samples located in the upper part of the plot (6,14,17,19 on the left and 7, 8, 9, 11, 15 on the right) are devoid or contain scarce macrophytes. They are separated in two different groups, due to the different dominance of ostracod species, with *C.* (*N.*) *angulata* and *C. torosa* prevailing in the groups on the left and on the right, respectively. Other shallow samples, in the lower part of the plot, are strongly influenced by TOC and macrophytes abundance (5, 4 and 20), with samples 4 and 20 characterized by the dominance of *C. vidua* and sample 5 by *C.* (*N.*) *angulata*. Additional parameters influencing this distribution are oxygen depletion and substrate features that enclose silty sands, plant detritus, shells and gravels.

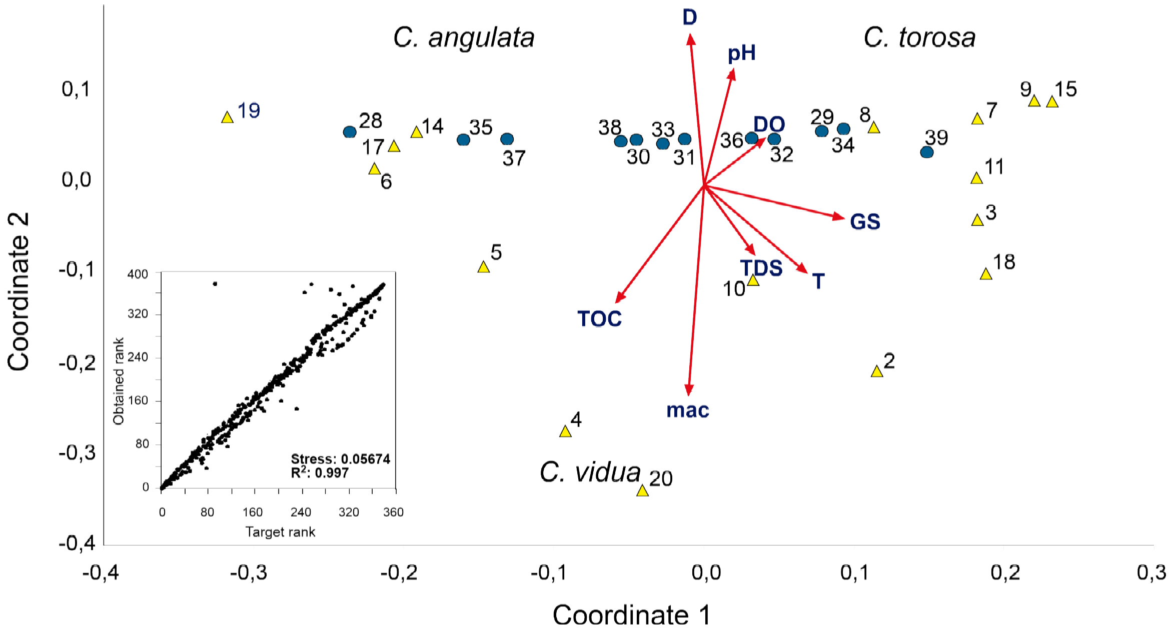

Figure 6: Non-Metric Multidimensional Scaling (NMDS) ordination plot for sampling sites (circle, deep samples; triangles, lakeshore samples), abiotic parameters (D, water depth; DO, dissolved oxygen in bottom water; GS, bottom grain size; pH, bottom water pH; T, bottom water temperature; TDS, total dissolved solutes; TOC, total organic carbon), and macrophytes (mac) (Euclidean similarity meas- ure). The Shepard plot is shown on the left lower corner. The dominant species of each sample group are also reported in the plot.

The Canonical Correspondence Analysis plot (Fig. 7), also related to the first matrix, shows the relationship between physical and chemical parameters and ostracod species. Axe 1 represents 61.22% of the total variance. A Monte Carlo permutation test (999 permutations) allowed for rejecting ($P<0.01$) the null hypothesis of non-significant gradients on the first axis (ter Braak and Verdonshot, 1995). The first axis separates the species on the base of the macrophytes occurrence (regression coefficient 0.78) and TOC (regression coefficient 0.50) and, to a lesser extent, depth (regression coefficient -0.48) and pH (-0.41) gradients. *C. vidua*, *D. stevensoni* and *C. ophtalmica* correlate positively with macrophytes and TOC whereas they correlate negatively with depth and pH. *H. helenae*. *H. salina* and *H. incongruens* display a strong correlation to depth and pH.

The Spearman’s rank values with P<0.001 show that *P. australis* is the macrophyte species with the highest correlation with ostracods displaying a moderate positive correlation with *H. helenae* (rho = 0.51121) and *C. vidua* (rho= 0.55081) and a moderate negative correlation with *C. torosa* (rho = -0.5423). Moderate positive correlations are also observed between *C. demersum* and *L. inopinata* (rho = 0.50615). Finally, a very strong positive correlation (rho = 1) has been identified between *Potamogeton natans* Linnaeus and *L. stationis*. For the substratum type, the Spearman’s rank values with P<0.001 show that *H. salina* strongly correlates with fine sands containing plant detritus, shells and gravels (rho = 0.66009).

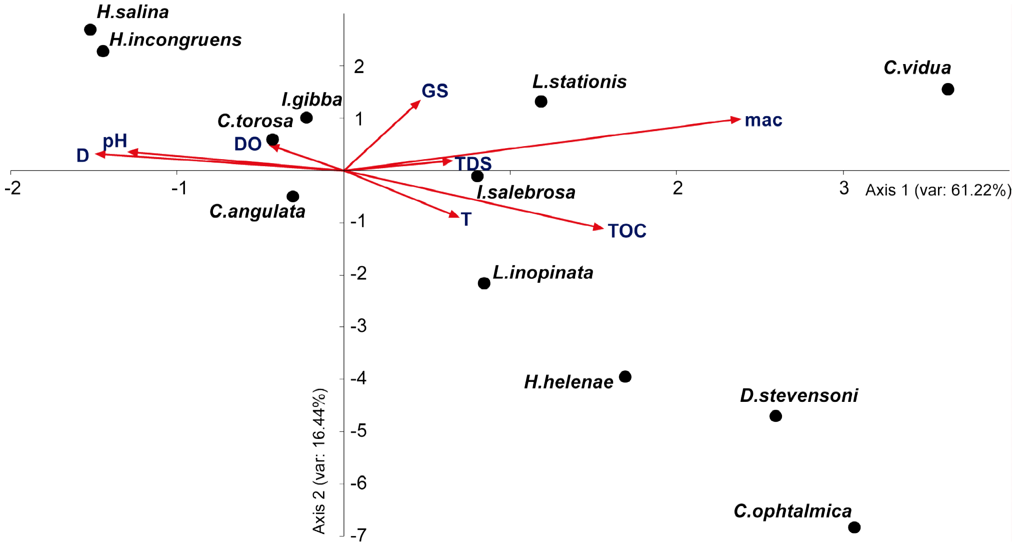

Figure 7: Canonical Correspondence Analysis (CCA) ordination plot for ostracods, abiotic parameters (D, water depth; DO, dissolved oxygen in bottom water; GS, bottom grain size; pH, bottom water pH; T, bottom water temperature; TDS, total dissolved solids; TOC, total organic carbon), and macrophytes (mac) in the space defined by the first two canonical axes (77.66% eigenvalue).

2.4 Discussion

Living ostracod assemblages have been seldom investigated in Italian lakes. Some information on ostracods are reported for Lake Maggiore (Fox, 1965), Ragogna (Colizza *et al.*, 1987), Mantova (Melis *et al.*, 1995, 1996; Salvi and Degrassi, 1995), Bracciano (Mastrantuono, 1995), and Martignano (Mastrantuono and Mancinelli, 2003). Additionally, Pieri *et al*. (2015) reported ostracods from some small mountain lakes in Piedmont, the Dolomites (northern Italy) and the central Apennines (Abruzzo Region). However, only few of these studies analyse the presence of ostracods in their environment, linking their assemblages with the dominant physical, chemical and bi- ological conditions. Moreover, the relationship between ostracods, macrophytes and the substrate is poorly investigated in Italian freshwater systems (Mazzini *et al*., 2014b) despite the definition of such links could be extremely useful to identify former ecological niches and habitat (Matsuda *et al*., 2015).

Little is known about the ostracods of Lake Trasimeno. Von Hartmann (1964) pointed out the presence of living *C. torosa* and, more generally, the occurrence of ostracods has been signaled in several articles dealing with the diet of fishes inhabiting the lake and crucial for the local economy (Mantilacci *et al*., 1990). Meisch (1985) recovered few valves of *P. variegata* from an irrigation channel near Lake Trasimeno.

The 19 species collected among the living meiobenthos of Lake Trasimeno are widely recorded in other Italian freshwater or brackish sites (Pieri *et al*., 2015) but three of them (*C. torosa*, *I. salebrosa*, *L. stationis*) deserve an in-depth discussion.

C. torosa is widespread in Eurasia and Africa (Wouters, 2002) in marine-derived brackish waters of marginal environments or water bodies influenced by the sea. However, Van Harten (1990) defined the species as anomalohaline due to its ability to adapt also to athalassic waters. In fact, records of this species in athalassic waters were reported by Klie (1938) from Germany (Gruber See, Bavaria; Trammer See, Plön), and thermal waters in Iceland, while Mezquita *et al*. (1999) recorded it in freshwater-oligohaline springs in eastern Spain. The identification of *C. torosa* in Lake Trasimeno further confirms this statement. Neale (1988) and Griffiths and Holmes (2000) reported a very wide salinity range 0.4 -150 g L^{-1} for *C. torosa*, with specimens displaying rather large size and developing noded shells below the osmoregulation threshold of 8 g L^{-1} (Van Harten, 1996, 2000; Keyser and Aladin, 2004; Keyser, 2005; Boomer and Frenzel, 2011; Frenzel *et al*., 2012). As already observed by von Hartmann (1964), in our samples *C. torosa* shells from Lake

Trasimeno are mostly noded (69%), but on some valves nodes are barely visible or totally absent. Following Sandberg's notation (1964), in our specimens we typically observed well-developed nodes 1, 2 and 3, whereas nodes 2a, 5 and 6 are often visible, and nodes 4 and 7 are very rarely present (Fig. 8). Nodes are also present on instars, starting from the A-3 moult. Concerning the size, on average the specimens of Lake Trasimeno are slightly larger than those reported by Decima (1964) for a marginal marine population of *C. torosa* recovered at Forte dei Marmi (Tuscany, Italy) (average length of the female right valve 1.03 mm *vs* 0.92 mm and average length of the male left valve 1.17 mm *vs* 1.00 mm, respectively).

I. salebrosa in Lake Trasimeno is the southernmost appearance of this species in Italy. Mazzini *et al*. (2014b) and Pieri *et al*. (2015) reported it only from northern Italy (Lombardia and Emilia-Romagna regions). More generally, Italy is the Eurasian westernmost country in which this species occurs. Up to now it has been described from lakes Biwa and Okinawa in Japan (Schornikov, 2004; Smith *et al*., 2011), lakes Bosten and Taihu in China (Mischke, 2001; Mischke and Schudack, 2001, shells only; Yu *et al*., 2005), in Korea (Lee *et al*., 2000), in India as *Ilyocypris shawneetownensis* (Bhatia and Singh, 1971; Mischke, 2001), and in Thrace Turkey (Özuluğ, 2005). Smith *et al*. (2011) consider *Pelocypris alatobulbosa* Delorme, 1970 from Canada synonim of *I. salebrosa* and suspects its presence also in Serbia, quoted by Karan-Žnidaršič and Petrov (2007) under the name *Ilyocypris decipiens*.

Living specimens of *L. stationis* were previously found in Italy only at Cà Nuova rice-field (Lombardia, northern Italy; Moroni and McKenzie, 2007). Because this species has not been previously reported in the Italian fossil record, these authors considered it as part of the 'foreign guest' contingent that was introduced by man in Italy with the trial strains of seed rice or other cereals coming from abroad and/or in dust associated with trade goods. However, recently *L. stationis* has been recovered in the fossil record of the Panicarola borehole (south Lake Trasimeno onshore), possibly aged Early-Middle Pleis- tocene (Marchegiano, *in preparation*) and from Holocene boreholes drilled in Gorgo Basso and Lake Preola (Trapani, Sicily) (Curry *et al*., 2013) thus, although rare, this species must be considered part of the Italian ostracod fauna. Outside Italy, the species has been reported in Eurasia, particularly in France (Paris, 1920), Austria (Löffler, 1971), Germany (Wohlgemuth, 1914; Fuhrmann and Pietrzeniuk, 1990; Fuhrmann, 2012), Macedonia (Petkovski, 1964), Finland (Meisch, 2000), Russia (Schornikov, 2004), Turkey (Altınsaçlı, 2001), Israel [Mischke *et al*., 2012 (shells only)], Jordania [Mischke *et al*., 2012 (shells only)], Yemen [Mohammed *et al*. (2014) (shells only)], Sudan (Martens, 1984), China (Schornikov, 2004), Japan (Smith and Janz, 2009), Thailand (Savate- nalinton, 2014), and Korea (Lee *et al*., 2000).

C. torosa and *C.* (*N.*) *angulata* are the most frequent species in the lake. These halophilic species are mainly found in marginal marine environments (Meisch, 2000; Fuhrmann, 2012). Their frequency and abundance in Lake Trasimeno could be explained by the chemistry of the lake waters, which are rich in chlorine and sodium (Charavgis *et al*., 2012). According to Ludovisi and Gaino (2010) this peculiar chemical composition is due to the geology of the catchment area and the watershed flowing in the lake that is rich in these ions as a consequence of weathering processes or wastewater discharges. The groundwater hydrogeochemistry of the Trasimeno area shows that the aquifers linked to alluvial and Plio-Pleistocene sediments are mainly alkaline-earth bicarbonate or chloride alkaline sulphate in nature. Analogously, the deep aquifers settled in Miocene turbidites are enriched in chloride and alkaline-earth bicarbonate due to the dissolution of chlorides or the incongruent dissolution of aluminosilicates (plagioclase and K-feldspar) (ARPA Umbria, 2005). Tiberi (1980) excluded a significant exchange between ground- water and lake waters but ARPA Umbria (2005) estimated a groundwater contribution of ca. 2.43-3.64 Mm^3 $year^{-1}$. The latter would represent around 2.5% of the total water supplied to the water body by rainfall and surficial runoff and would be enough to drive the chemical composition of the lake.

C. torosa and *C.* (*N.*) *angulata* have been recovered both in the distal part of the lake and in the lakeshore area. As shown in the NMDS and CCA plots (Figs. 6 and 7) in both cases these species are associated with scarce or absent macrophytes and low TOC values. The alternate dominance of these two species in the distal deeper assemblages seems to be linked mainly to bottom water oxygen availability: *C.* (*N.*)

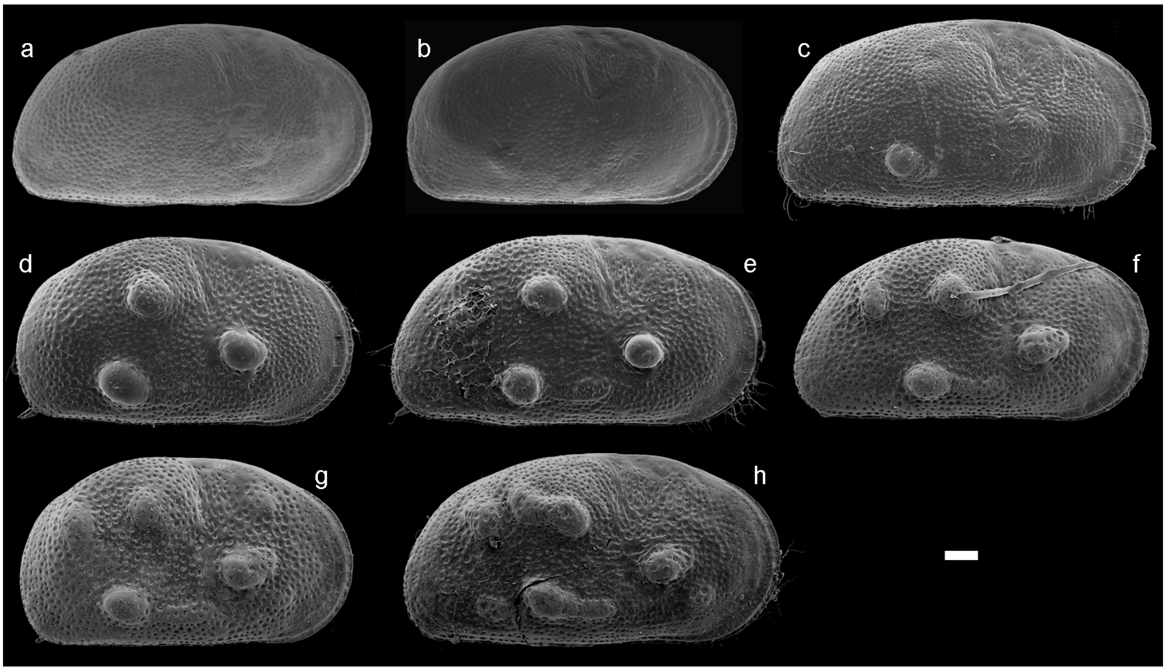

Figure 8: SEM photos of Cyprideis torosa from Lake Trasimeno. a. Male RV, un-noded; b. Female RV, un-noded; c. male RV, node 2; d. female RV, nodes 1, 2, 3; e. male RV, nodes 1, 2, 2a, 3; f. male RV, nodes 1, 2, 2a, 3, 6; g. female RV, nodes 1, 2, 2a, 3, 4, 6; h. male RV, nodes 1, 2, 2a, 3, 5, 6, 7. Abbreviations as in Fig. 6. White bar corresponds to 0.1 mm.

angulata dominates in the most oxygen-depleted sediments (3.55-5.45 mg L^{-1}) while *C. torosa* becomes dominant with higher oxygen values (5.46-6.80 mg L^{-1}). The former is rather surprising although the tolerance range for dissolved oxygen of *C.* (*N.*) *angulata* is not known (Ruiz *et al*., 2013). Indeed, previous work shows that this species generally occurs in oxygenated waters from 9.20 to 22.8 mg L^{-1} (Martin *et al*., 1993; Akdemir, 2008; Özuluğ, 2012; Altınsaçlı, 2014). On the contrary, *C. torosa* is known for its ability to survive also in depleted oxygen environments until hypoxia (Jahn *et al*., 1996; Mesquita-Joanes *et al*., 2012).

Although *C. torosa* and *C.* (*N.*) *angulata* sometimes are the dominant species also in samples from shallow lakeshore areas, they are often accompanied by other species such as *C. vidua* that become frequent in very shallow to shallow (20-140 cm) sites with high TOC, macrophytes and algae (samples in the low left quarter of NMDS plot, Fig. 6). It is known that *C. vidua* is a phytophilic species that prefers abundant plant coverage containing macrophytes as well as algae (Characeae) (Meisch, 2000). In

Lake Trasimeno this species correlates strongly with the presence of vegetation (Figs. 6 and 7) and we found only this species (mainly instars) among the roots of free-floating plants as *S. polyrrhiza*, *L. minor* and *L. trisulca*, while, at the bottom of the same site, adults of this species are accompanied by *C. ophtalmica* and *C.* (*C.*) *candida*. The same has been recognized by Kiss (2007) at Lake Fehér (Hungary). It is particularly interesting to confirm the strict relation between some species and free-floating macrophytes, already recognized by Kiss (2007) and Mazzini *et al*. (2014a). *L. inopinata* dominates areas with low presence of *C. torosa* and *C.* (*N.*) *angulata*, slightly deeper (around 150-210 cm) (samples in the low right quarter of the NMDS plot, Fig. 6). It seems to prefer less vegetate bottoms. Despite the ability of this species to adapt to several environmental conditions (Meisch, 2000), in Lake Trasimeno it shows a moderate correlation to T (Fig. 7) suggesting that it could be a polythermophilic species.

The rest of the ostracod species occur in few samples and represents a subordinate component of the ostracod assemblage, except for *H. helenae*, *I. salebrosa*, and *H. in- congruens* that could be locally dominant. The first species represents up to 67% of the ostracod fauna in a very shallow (20 cm) lakeshore pond densely populated by *P. aus- tralis.* It is accompanied by *I. gibba* (20%) and *I. salebrosa* (13%). *I. salebrosa* reaches 57% of the ostracod community (accompanied by *H. salina* and *H. incongruens*) in a 200 cm deep pond lateral to the inflow of the Anguillara Channel, rich in algae (pennate and centric diatoms, *Cladophora glomerata* Linnaeus, *Oedogonium* sp. and *Spyrogyra* sp.) and with moderate *P. australis* coverage. *H. incongruens* reaches 25% of an assemblage dominated by *C. vidua* (75%) along the cemented banks of the Anguillara Channel, nearby its inflow in the lake, in very shallow conditions (20 cm) and rather coarse bottom with macrophyte allochtonous remains and pebbles. The ecology of *H. helenae* and *H. incongruens* is rather well known (Meisch, 2000; Fuhrmann, 2012) and their presence in the described environments does not record any additional autoecological data. Conversely, *I. salebrosa* ecology is relatively unknown. In Lake Biwa (Japan) it has been collected at water depths ranging from 5.5 to 16 m (Smith *et al*., 2011), while in Turkish lakes in the Thracia region occurs at rather high temperatures (24.6-30°C), pH (8.0-8.8) and, at least at Lake Gala, it was found with *V. spiralis* and *Myrophyllum spica- tum* Linnaeus (Özuluğ, 2005). In Lake Trasimeno *I. salebrosa* has been collected at shallower depths (0.2 to 2.5 m), slightly lower pH (7.6-8.3), and comparable temperatures (24.4-25.3°C). Furthermore, it seems that it tolerates rather low oxygen content (DO 2.9-5.2 mg L^{-1}), and oligohaline waters (TDS 0.80-0.87 g L^{-1}). Only in one sample it is associated with *P. australis* and in another samples with abundant diatoms.

The results of the multivariate analyses indicate that the central and southern areas of Lake Trasimeno enclose several ecological niches. The main parameters affecting the ostracod assemblages are the macrophyte coverage, TOC and, to a lesser extent, depth and pH. The changes in macrophyte covers are clearly influenced by sediment characteristics, as well as hydrodinamism and underwater irradiance (Van Duin *et al*., 2001). In Trasimeno, the deepest part of the lake has a dominant clay substrate and a complete absence of macrophytes, they colonize lakeshore areas encompassing coarser sediments. *Phragmites australis* communities dominate the southwestern coast, nearby the Anguillara Channel, while aquatic macrophytes (*Potamogeton* spp., *V. spiralis*, *Najas* spp. and *C. demersum*) are frequent along the southeastern coast.

The TOC values obtained by Rock-Eval Pyrolysis mirrors the macrophyte distribution, being higher along the coast (except for the most western sector) and decreasing towards the distal part of the lake. This is in agreement with the TOC distribution previously reported by Morgantini and Peruzzi (2012) indicating the highest TOC values in the southeastern shoreline and the lowest contents in the southwestern area. Intermediate values (around 2%) were recorded in the central (deepest) part of the lake. The differences recorded on the TOC distribution could be linked to multiple, complex processes, also occurring outside the lake. For instance, land use changes can affect the input of nutrients in the inflowing rivers and thus can lead to changes in lake productivity that subsequently alter the TOC burial in the sediment (Sifeddine *et al*., 2011). The linkage between macrophyte distribution and TOC is also shown by the HI and OI that clearly suggest a mixed source of allochtonous (plant material such as vascular plants) and autochtonous organic matter (algal type such as phytoplancton). Based on TOC/Ntot ratios, Morgantini and Peruzzi (2012) ascribed an algal origin to this sedimentary organic matter. Most likely, these differences in interpretation are due to the use of different analytical methods. As suggested by Meyers (1997) TOC/N_{tot} ratios can be affected by the presence of clay-rich sediments ('matrix effect') that adsorb ammonium ions (NH^{4+}) causing a decrease of this ratio. The latter is coherent with the high OI values obtained by Rock-Eval and explain the mixed character of the OM as shown in the Van Krevelen plot of Fig. 3.

2.5 Conclusion

The living ostracod fauna of the shallow endorheic Lake Trasimeno has been investigated for the first time. Within the 19 species identified, several assemblages have been recognized, related to different ecological niches distinguished on the basis of the physical and chemical water parameters as well as different type of substratum and macrophytes coverage, confirming once more the strong ties between ostracods and their host environment. Although no endemic taxa have been identified in the lake, the occurrence of the three species *C. torosa*, *I. salebrosa*, and *L. stationis* addresses several issues related to biodiversity and (palaeo)-limnology. In particular, the occurrence of *C. torosa,* living in an inland lake with low salinity waters, highlights the need of a cautious palaeoenvironmental interpretation when this species occurs in older records. A careful observation of the shell morphology must be carried out especially when this is the dominant species. The occurrence of *I. salebrosa* expands the distribution area of the species to central Italy, with consequences on its meaning within the Mutual Ostracod Temperature Range applications (Horne, 2007). *L. stationis* has been considered for a long time an alien species, disseminated by passive transport in the rice fields of Northern Italy. As a matter of fact, the occurrence of fossil representatives in Umbria and Sicily and the occurrence of this species living in present Lake Trasimeno, could tell a completely different story, linked to loss of habitats and lack of specific studies more than to freshwater invasions as previously thought.

The knowledge of the present-day ostracod population of Lake Trasimeno represents a further *tessera* in the mosaic of the distribution of ostracods in Italian inland waters. It provides a starting point to evaluate both past and present climate- and anthropogenically-induced changes of this fragile environment.

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CHAPTER 3

Arid and humid phases in central Italy during the Late Pleistocene revealed by the Lake Trasimeno ostracod record*

A multiproxy approach in a sediment core from Lake Trasimeno has been used to reconstruct the climate history of central Italy during the Late Pleistocene to Early Holocene period (ca. 47,000–9,000 cal yr B.P.). Ostracod assemblages and sedimentological data (lithology and carbonate content) have been used to infer past hydrological changes in the area. Ostracods were analyzed throughout the core using diversity indexes and multivariate statistic analyses (Cluster and PCA). Three main associations linked to lake level and salinity variations were recognized: 1) the *C. torosa* association, indicating permanent lacustrine conditions with high lake levels and low salinities; 2) the *S. aculeata* association, linked to very shallow/temporary waters with higher salinity conditions; and 3) the *S. aculeata* - *E. mareotica* association pointing to temporary water conditions and the highest salinities. Furthermore, the presence of *C. fuscata* and *L. blankenbergensis* during wide parts of the Late Pleistocene indicates temperatures lower than present days. Alternations of these three ostracod associations compares well with the oxygen isotope curve from Greenland (NGRIP) and are thus interpreted as climatically driven. At the Holocene transition (Termination 1), the ostracod associations indicate a delay in the increasing warming and humidity with respect to the NGRIP temperature record.

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3.1 Introduction

During the last decades, future climate evolution has been among one of the most discussed and debated societal issues and therefore a principal goal of environmental research. Palaeoclimate studies revealed that understanding past climate changes and their worldwide impact is fundamental for modeling future climate (Gornitz, 2009). However, climate variability depends on various internal and external feedback mechanisms, which make it difficult to interpret and to study.

The development of reliable proxies (e.g., physical, chemical, biological and isotopic) is thus one of the challenges in climate research that must be addressed. Moreover, given the complexity of palaeoclimate signals, multiproxy approaches are imperative to disentangle them (Allen et al., 1999; Ariztegui et al., 2008; Battarbee et al., 2004; Bradley, 1999; Francke et al., 2013).

Regional palaeoclimate and palaeoenvironmental studies provide critical pieces to the puzzle of past global climate changes and they are also key for understanding local feedback mechanism of the different systems (Solomon, 2007). The Mediterranean region is often referred to as one of the most important areas in the Northern Hemisphere climate system for this kind of studies. Given its mid-latitude position as well as its semi-enclosed basin setting, it is very sensitive to climate variations from both high and low latitudes and therefore represents an excellent natural laboratory to investigate global climate changes (Kennett, 1982; Watts et al., 1996; Allen et al., 1999; Cacho et al., 2001; Magny et al., 2006). In particular, the diverse and complex environments of the Italian peninsula make it a unique place for studying the reaction of aquatic systems to global climate change. Despite the high abundance of palaeoclimatological studies during the Late Pleistocene, several periods of climate instability in Europe and particularly in Italy are still poorly understood (Allen et al., 1999; Chondrogianni et al., 2004). The investigation of high-resolution and independently dated paleoclimate records provides an excellent opportunity to fulfill this lack of knowledge (Regattieri et al., 2017).

Lacustrine sedimentary records represent outstanding archives of multiproxy information (Cohen, 2003) making lakes the ideal site for palaeoenvironmental and paleoclimate reconstructions (Ariztegui et al., 2008; Fritz, 2008). In particular endorheic and shallow lakes are highly sensitive to local environmental changes, as they strictly depend on local meteorological conditions (Helfert and Holz, 1985; Lauwaet et al., 2012; Soja et al., 2013). One ideal example of such lakes is the endorheic and very shallow (maximum 6 m depth) Lake Trasimeno (central Italy) containing ostracod-rich sediments that are analyzed in detail in this study.

Non-marine ostracods are microscopic benthic crustaceans that produce low-Mg calcite shells and are present in most aquatic environments (Frenzel and Boomer, 2005; Holmes, 2002; Horne et al., 2002). Their distribution, assemblage composition, and abundance are strongly dependent on several ecological factors such as temperature, water chemistry composition (such as salinity and calcite saturation) and other biotic and abiotic parameters (Baltanás et al., 1990; Keatings et al., 2010; Marquez et al., 2016). Moreover, the ability of several cypridoidean species to disperse passively in continental waters, either as adults and/or juveniles through survival in torpid stage and/or as eggs through diapause, allows them to easily populate or re-populate the aquatic ecosystems (Mesquita-Joanes et al., 2001). Their often-excellent preservation, abundance, and sensitiveness to environmental changes make them an invaluable proxy to detect palaeoenvironmental and paleoclimate changes in shallow lakes as shown by Löffler (1990) for the Neusiedlersee (Austria), Sohar and Meidla (2000) for lakes Elistvere and Ermistu (Estonia), and Mazzini et al. (2015, 2016) for Lake Shkodra (Albania) among others. Furthermore, they play a fundamental role in reconstructing the past conditions of freshwater environments, as they are frequently the only calcareous organisms preserved in the fossil record.

The last 50,000 cal yr B.P. have been characterized by high-frequency climate variations (Abrantes et al., 2012). The millennial-scale climate variability, consisting in cycles of warmer (interstadial) and colder (stadial) periods has been recognized all over the world (Voelker, 2002). In this article, we show that the ostracod fauna of Lake Trasimeno responded to and registered these events by changing assemblages providing a unique possibility to link regional with global climate changes.

The main goal of this study is to provide a high-resolution multiproxy record for the Late Pleistocene-Holocene transition that will improve the existing palaeoenvironmental reconstructions in central Italy. It also highlights the use of ostracod assemblages as a valuable proxy, attesting the importance of regional studies for understanding abrupt climatic changes. It further provides a plausible analogue for future variations of the lacustrine system driven by the ongoing changes in climate.

3.2 Study area

Lake Trasimeno (43°09'N; 12°06'E) is located in central Italy and is the largest lake of its peninsular territory with a surface area of 124 km^2 (Fig. 1). Despite its large areal extension, the lake is very shallow (maximum depth of ~6.3 m) and its bathymetry very smooth (Ludovisi et al., 2005). At present, it is an eutrophic lake with phosphorus being the limiting nutrient (Ludovisi and Poletti, 2003). The principal feature of the lake is that its hydrology depends strictly on local climate undergoing strong lake level variations. They are the result of changes in the precipitation/evaporation ratio as a result of the relatively small extension of the lake watershed (396 km^2) and the lack of significant permanent tributaries. This combination has caused dramatic floods and droughts throughout the last centuries and human interventions were made to control the lake level since Roman times (Ludovisi and Gaino, 2010). More recently, an enlargement of the catchment basin was done in 1957-62 to solve droughts problems (Fig. 1).

At least three distinct lake-level lowstands were recorded during the last ninety years, the most recent one took place between 1988 and 2012 when the lake level was 1.87 below the “hydrometric zero” (257.33 m a.s.l.; Dragoni et al., 2012). Significant rainfall in 2014 (940 mm yr^{-1}) (Perugia province, internal report) caused an increase in the lake level for the first time in the last ninety years that reached 12 cm above the “hydrometric zero” (data from Club Velico Trasimeno, http://www.clubvelicotrasimeno.it/livellolago.aspx)

The shallow character of the lake allows a complete daily vertical water mixing and no clear thermal stratification is attained even during hot summers. Physical and chemical parameters (i.e., pH, conductivity, alkalinity, ionic concentration and dissolved oxygen) are spatially homogeneous, showing no substantial differences between the shallower and deeper part (see Marchegiano et al., 2017 for more details). Periodic changes of physical and chemical parameters are strictly correlated with lake level variations. Due to its endorheic nature, the variations in the volume of the lake water mass are translated into changes in salinity and water chemistry. Today, a Total Dissolved Solid (TDS) of around 0.8‰ characterizes the lake waters (Marchegiano et al., 2017). However, the general lake level drop that occurred from the 70's to the beginning of the 21st century caused a significant increase in the conductivity from 750 to 1700 µS cm^{-1}s, roughly corresponding to around 1‰ TDS increase (Ludovisi and Gaino, 2010). Additionally, during this period the chloride concentration rose from 3.63 to 8.98 meq L^{-1} (Ludovisi and Gaino, 2010) and both parameters decreased during the 2014 highstand (Marchegiano et al., 2017). Similarly, alkalinity seems to be correlated to salt content and ionic concentration,

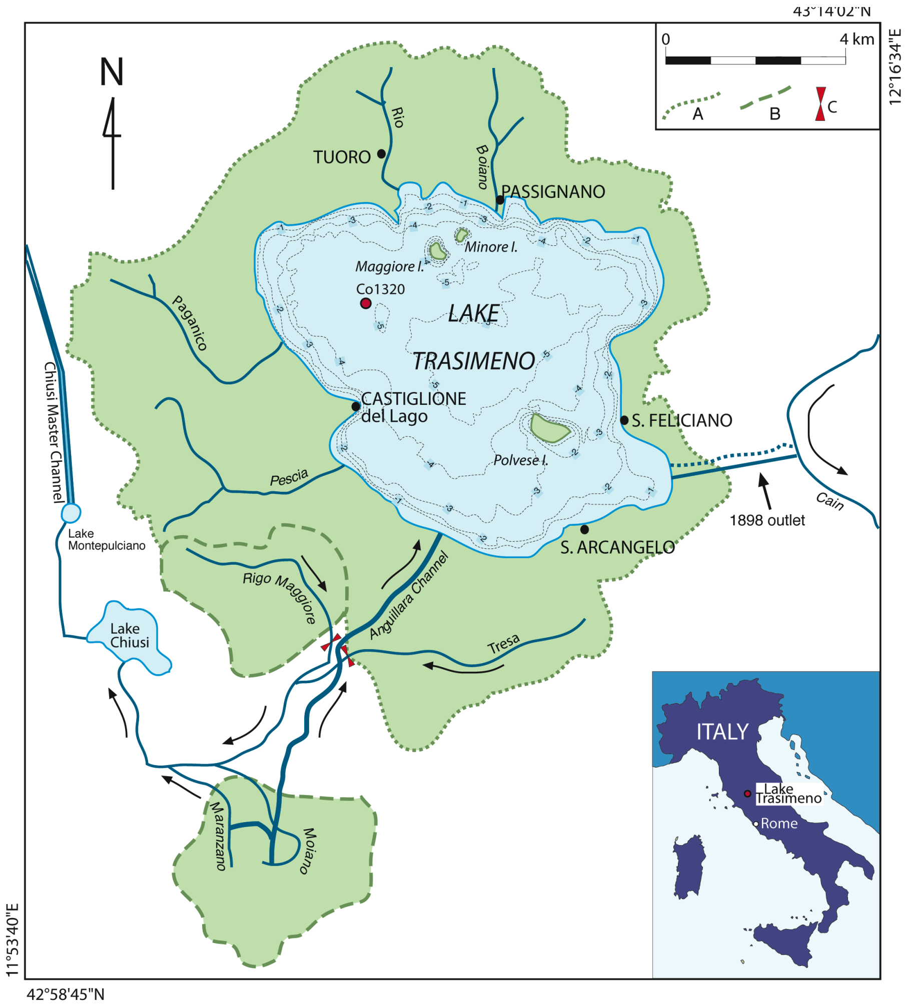


Figure 1: Map of Lake Trasimeno showing the location of core Co1320. Legend: A. natural catchment area; B. artificially-joined basins; C. sluice gates of the artificially-joined channels (modified from Marchegiano et al., 2017).

and thus, increases during the dry phases (Ludovisi and Gaino, 2010 and Marchegiano et al., 2017).

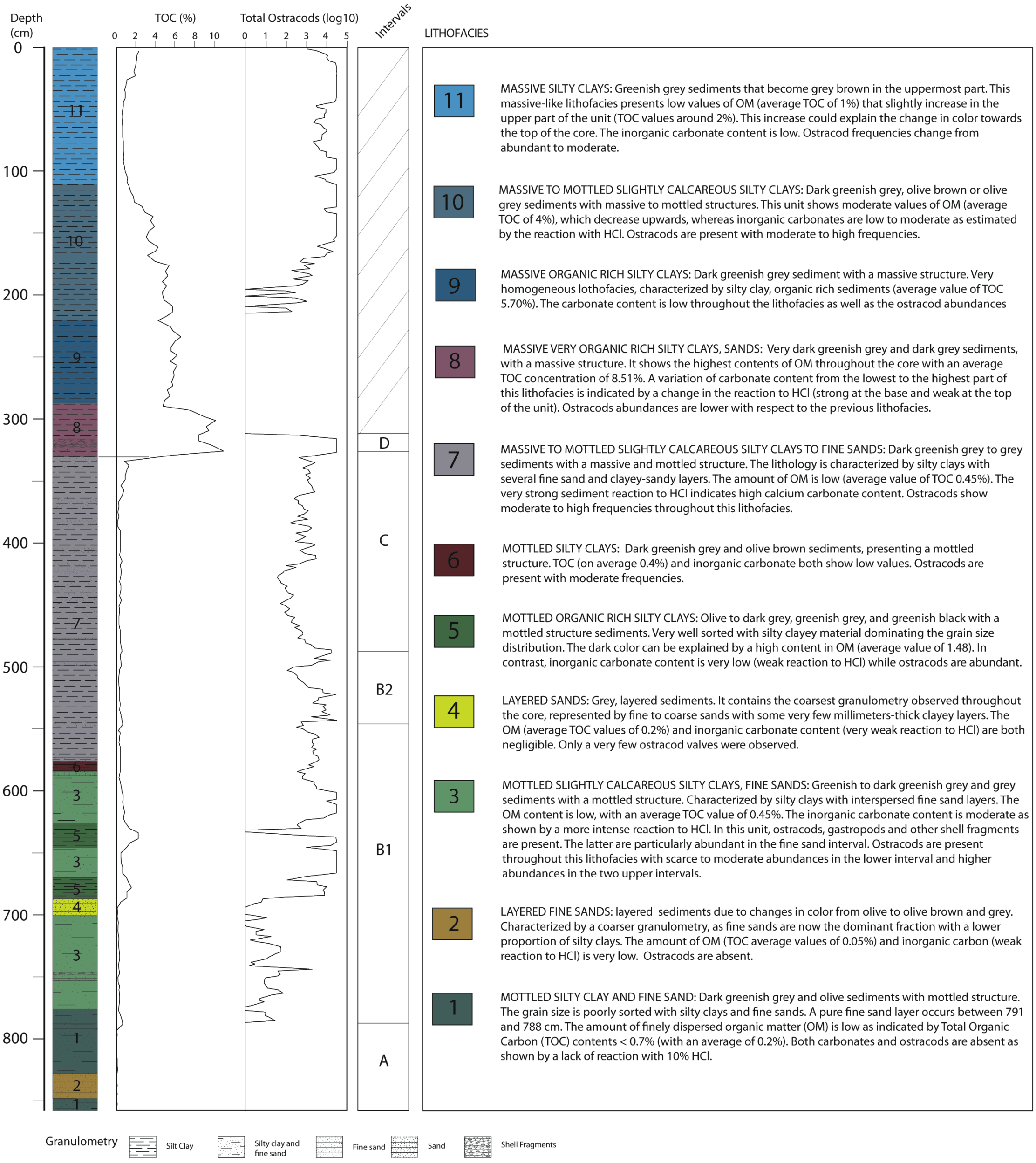
3.3 Material and methods

During two sampling campaigns in September and November 2014, an 859 cm 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®). The 3 m long cores with a diameter of ~6.3 cm were cut into 1 m long sections in the field and shipped to the University of Cologne (Germany). In the laboratory, the cores were split lengthwise and one half was preserved as archive while the other half was used for visual core inspection and high resolution X-Ray fluorescence (XRF) core scanning. Subsequently, the working half was sub-sampled at 2 cm resolution. Each 2 cm slice was split into two parts for geochemical and stable isotope analyses, and for micropaleontological investigations, respectively. Samples for geochemical analyses were freeze-dried, homogenized, and ground to <63 µm. An aliquot of 10 mg was dispersed in 10 ml DI water for analyses of the total organic carbon (TOC) content using a DIMATOC 100 carbon analyzer (Dimatec Corp., Germany).

The age model is based on radiocarbon ages derived from terrestrial plant remains, charcoal and bulk sedimentary organic matter measured at the Cologne AMS facility, Germany (see table in Fig. 3). Sample pre-treatment and graphitization followed the guidelines described in more detail by Rethemeyer et al., (2012). The software package clam2.2 (Blaauw, 2010) and the IntCAL13 calibration curve (Reimer et al., 2013) were used for age-depth modeling and for the calibration of the radiocarbon ages into calendar years before present (cal a BP).

The subsamples for micropaleontological analyses (428 in total) were treated with 5% H_2O_2 for 1 hour to remove the organic matter. Subsequently, the samples were sieved with 125 µm-sieve mesh using deionized water, and, finally, oven-dried at 40C°. In each sample, ~300 juvenile and adult ostracod valves were manually picked using a fine brush and deionized water under the stereomicroscope. Each dry sediment fraction where the ostracod were picked was weighted and the ostracod abundances was normalized to 1g of dry sieved sediment. The species were identified following

Gonzalez Mozo et al. (1996), Meisch (2000), Fuhrmann (2012) and Mazzini et al. (2014). Ostracod microslides are stored at the Department of Earth Sciences of the University of Geneva (Switzerland).



11 MASSIVE SILTY CLAYS: Greenish grey sediments that become grey brown in the uppermost part. This massive-like lithofacies presents low values of OM (average TOC of 1%) that slightly increase in the upper part of the unit (TOC values around 2%). This increase could explain the change in color towards the top of the core. The inorganic carbonate content is low. Ostracod frequencies change from abundant to moderate.

10 MASSIVE TO MOTTLED SLIGHTLY CALCAREOUS SILTY CLAYS: Dark greenish grey, olive brown or olive grey sediments with massive to mottled structures. This unit shows moderate values of OM (average TOC of 4%), which decrease upwards, whereas inorganic carbonates are low to moderate as estimated by the reaction with HCl. Ostracods are present with moderate to high frequencies.

9 MASSIVE ORGANIC RICH SILTY CLAYS: Dark greenish grey sediment with a massive structure. Very homogeneous lothofacies, characterized by silty clay, organic rich sediments (average value of TOC 5.70%). The carbonate content is low throughout the lithofacies as well as the ostracod abundances

8 MASSIVE VERY ORGANIC RICH SILTY CLAYS, SANDS: Very dark greenish grey and dark grey sediments, with a massive structure. It shows the highest contents of OM throughout the core with an average TOC concentration of 8.51%. A variation of carbonate content from the lowest to the highest part of this lithofacies is indicated by a change in the reaction to HCl (strong at the base and weak at the top of the unit). Ostracods abundances are lower with respect to the previous lithofacies.

7 MASSIVE TO MOTTLED SLIGHTLY CALCAREOUS SILTY CLAYS TO FINE SANDS: Dark greenish grey to grey sediments with a massive and mottled structure. The lithology is characterized by silty clays with several fine sand and clayey-sandy layers. The amount of OM is low (average value of TOC 0.45%). The very strong sediment reaction to HCl indicates high calcium carbonate content. Ostracods show moderate to high frequencies throughout this lithofacies.

6 MOTTLED SILTY CLAYS: Dark greenish grey and olive brown sediments, presenting a mottled structure. TOC (on average 0.4%) and inorganic carbonate both show low values. Ostracods are present with moderate frequencies.

5 MOTTLED ORGANIC RICH SILTY CLAYS: Olive to dark grey, greenish grey, and greenish black with a mottled structure sediments. Very well sorted with silty clayey material dominating the grain size distribution. The dark color can be explained by a high content in OM (average value of 1.48). In contrast, inorganic carbonate content is very low (weak reaction to HCl) while ostracods are abundant.

4 LAYERED SANDS: Grey, layered sediments. It contains the coarsest granulometry observed throughout the core, represented by fine to coarse sands with some very few millimeters-thick clayey layers. The OM (average TOC values of 0.2%) and inorganic carbonate content (very weak reaction to HCl) are both negligible. Only a very few ostracod valves were observed.

3 MOTTLED SLIGHTLY CALCAREOUS SILTY CLAYS, FINE SANDS: Greenish to dark greenish grey and grey sediments with a mottled structure. Characterized by silty clays with interspersed fine sand layers. The OM content is low, with an average TOC value of 0.45%. The inorganic carbonate content is moderate as shown by a more intense reaction to HCl. In this unit, ostracods, gastropods and other shell fragments are present. The latter are particularly abundant in the fine sand interval. Ostracods are present throughout this lithofacies with scarce to moderate abundances in the lower interval and higher abundances in the two upper intervals.

2 LAYERED FINE SANDS: layered sediments due to changes in color from olive to olive brown and grey. Characterized by a coarser granulometry, as fine sands are now the dominant fraction with a lower proportion of silty clays. The amount of OM (TOC average values of 0.05%) and inorganic carbon (weak reaction to HCl) is very low. Ostracods are absent.

1 MOTTLED SILTY CLAY AND FINE SAND: Dark greenish grey and olive sediments with mottled structure. The grain size is poorly sorted with silty clays and fine sands. A pure fine sand layer occurs between 791 and 788 cm. The amount of finely dispersed organic matter (OM) is low as indicated by Total Organic Carbon (TOC) contents < 0.7% (with an average of 0.2%). Both carbonates and ostracods are absent as shown by a lack of reaction with 10% HCl.

Figure 2: Lithology, Total Organic Carbon (TOC) and ostracod abundance (log(10) n° valves/1g of dry sediment) of the core Co1320. Intervals from A to D are referred to the paleoclimatic and paleoenvironmental reconstruction described in the text (Discussion). The uppermost part of the core (striped portion) will be described in a forthcoming paper. Lithofacies were subdivided based on the visual core description, the organic matter (OM) and the calcium carbonate content (reaction with 10% HCl).

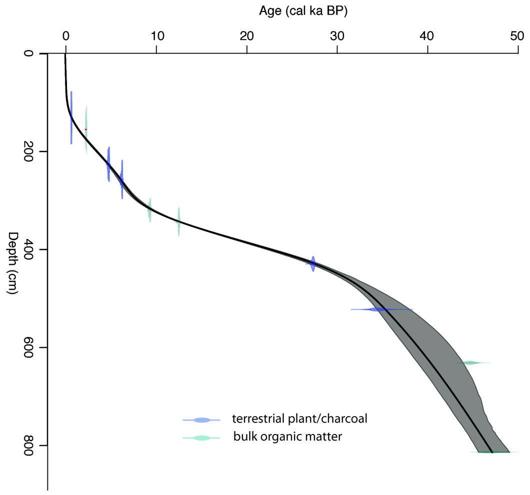
A matrix with the abundance of all the ostracod species in each sample was used to calculate the diversity indexes in order to obtain a detailed characterization of the palaeocommunities, namely abundance, species richness, Shannon index and equitability.

Multivariate analyses were performed using a matrix with the ostracod percentage abundances, excluding taxa presenting a relative percentage below 2% in the sample. In this paper, two different statistical analyses were performed: the unconstrained cluster analysis (Chord distance measure and the un-weighted pair group method using arithmetic average-UPGMA) in Q- and R-modes and principal component analyses (PCA) using the PAST software, ver. 2.17b (Hammer et al., 2001). Cluster analysis dendrograms, whose suitability was checked through the cophenetic correlation coefficient (c) (Sokal and Rohlf, 1962; Mouchet et al., 2008), were used to determine ostracod species associations and sample groups based on their ostracod community composition. A principal component analysis (PCA) was performed to identify the main components related to the variations in species composition and to obtain the ordination of samples presenting ecological similarity. To test its accuracy bootstrap analyses was used to estimate the variances of factor loadings (Chatterjee, 1984).

3.4 Results

3.4.1 Chronology

A robust chronological model has been developed using ten radiocarbon ages (Fig.3). Four dates are derived from terrestrial plant material, one from charcoal and five from bulk organic matter samples, respectively. Whereas the terrestrial plant material and the charcoal samples provide reliable ages, the five bulk organic matter samples can be affected by reservoir and/or hardwater effects, or by the incorporation of old soil organic matter from the catchment. Plus, due to the lack of old limestones in the catchment of the lake, the absence of stratification even during hot summers, and the high lake surface to water volume ratio, reservoir and/or hard water effects are likely negligible. The calculated age model implies that core Co1320 covers a time interval between 47,100 cal yr B.P. and present day.



AMS Lab ID	Core depth (cm)	Material	C weight (µg)	F ^{14}C	^{14}C age (a BP)	Calibrated age (cal a BP)
COL3171.1.1	130.5	errestrial plant	988	0.918	685 ± 35	625 ± 65
COL3662.1.1	154.5	bulk organic matter	995	0.75464	2,260 ± 40	2,250 ± 100
COL3172.1.1	225.5	charcoal	1000	0.593	4,190 ± 40	4,730 ± 120
COL3173.1.1	256.5	terrestrial plant	575	0.51	5,410 ± 50	6,160 ± 140
COL3663.1.1	319	bulk organic carbon	999	0.35637	8,290 ± 50	9,280 ± 155
COL3664.1.1	343	bulk organic carbon	993	0.26943	10,550 ± 55	12,500 ± 120
COL3174.1.1	429	terrestrial plant	791	0.057	23,000 ± 100	27,700 ± 720
COL3175.1.1	522	terrestrial plant	320	0.023	30,300 ± 810	34,900 ± 1600
COL3665.1.1	631	bulk organic carbon	999	0.00599	41,100 ± 620	44,900 ± 880
COL3666.1.1	827	bulk organic carbon	992	0.00436	43,700 ± 830	47,200 ± 1,800

Figure 3: Age model of core Co1320. Refer to the main text for more details about the nature of the dated samples as well as the interpolation of the data points.

3.4.2 Ostracod analyses

In spite of the relative inhomogeneity of the lithology of core Co1320, ostracod abundance and assemblages changed throughout time. A total of 428 samples comprising 99,900 ostracod valves have been used to produce a palaeoenvironmental reconstruction. Ostracods were found with communities composed of adults and juveniles in 333 out of 428 samples, while 95 samples were barren.

In this contribution, we analyze and discuss in detail the Late Pleistocene portion of the Trasimeno sedimentary core and the transition to the Early Holocene within a time window between 47,000 to 9,100 cal. yr B.P. The study of the Holocene portion of the core is still on going and will be the object of a forthcoming article.

A total of 19 ostracod species referable to 15 genera have been recognized (Figs. 4 and 5): *Darwinula stevensoni* (Brady & Robertson, 1870)*, Candona* (*Candona*) *candida* (O.F. Müller, 1776)*, Candona* (*Neglecandona*) *angulata* G.W. Müller, 1900*, Candona* (*Neglecandona*) *neglecta* Sars, 1887*, Ilyocypris bradyi* Sars, 1890*, Eucypris mareotica* (Fisher, 1855)*, Trajancypris serrata* (G.W. Müller, 1900)*, Herpetocypris helenae* G.W. Müller, 1908*,* (only fragments), *Heterocypris incongruens* (Ramdohr, 1808)*, Heterocypris salina* (Brady, 1868)*, Cypridopsis vidua* (O.F. Müller, 1776), *Plesiocypridopsis newtoni* (Brady & Robertson, 1870)*, Sarscypridopsis aculeata* (Costa, 1847)*, Potamocypris paludum* (Gauthier, 1939), *Limnocythere blankenbergensis*

Diebel, 1968, *Limnocythere inopinata* (Baird, 1843)*, Cyprideis torosa* (Jones, 1850), *Cytheromorpha fuscata* (Brady, 1869) and *Amnicythere* sp. *Amnicythere* was only identified to the genus level and could represent a new species.

All the recovered species are distributed in the core with different abundances generating diverse ostracod assemblages (Fig. 6). Among all the species, the most represented is *C. torosa*, recovered throughout the entire core with higher percentages in the lower and in the uppermost portions. *S. aculeata* and *E. mareotica* are scarce in the lower portion of the core but abundant to very abundant in the upper part. *C. fuscata* shows a limited presence in the lower part. *C. angulata, D. stevensoni* and *L. inopinata* display notable occurrences only in the uppermost part of the core where they are accompanied by *C. vidua*, *H. helenae* and *C.* (*C.*) *candida.*

The community indexes (abundance, richness, equitability, and Shannon-Wiener diversity indexes) are reported in Figure 7. In the lower portion of the sedimentary core (from 859 to 633 cm) ostracods are absent (from 859 to 787cm) or, conversely, make very abundant assemblages but with a low number of species (richness values from 1 to 7), often with the absolute dominance of only one species (*C. torosa*). As a consequence, the equitability and diversity indexes display very variable values including the lowest values of the whole sediment core pointing to fluctuating populations. Further upwards (from 632 to 414 cm), ostracod abundances are high until 488 cm and then decrease abruptly, the number of species increases (richness values from 4 to 12) as well as the equitability and diversity indexes, which reach the maximum values typical of different size populations with different specializations. In the uppermost part (from 414 to 315 cm), frequencies are very low up to 329 cm and then abruptly increase, whereas the others indexes values show remarkable fluctuations with low values particularly from 405 to 403 cm.

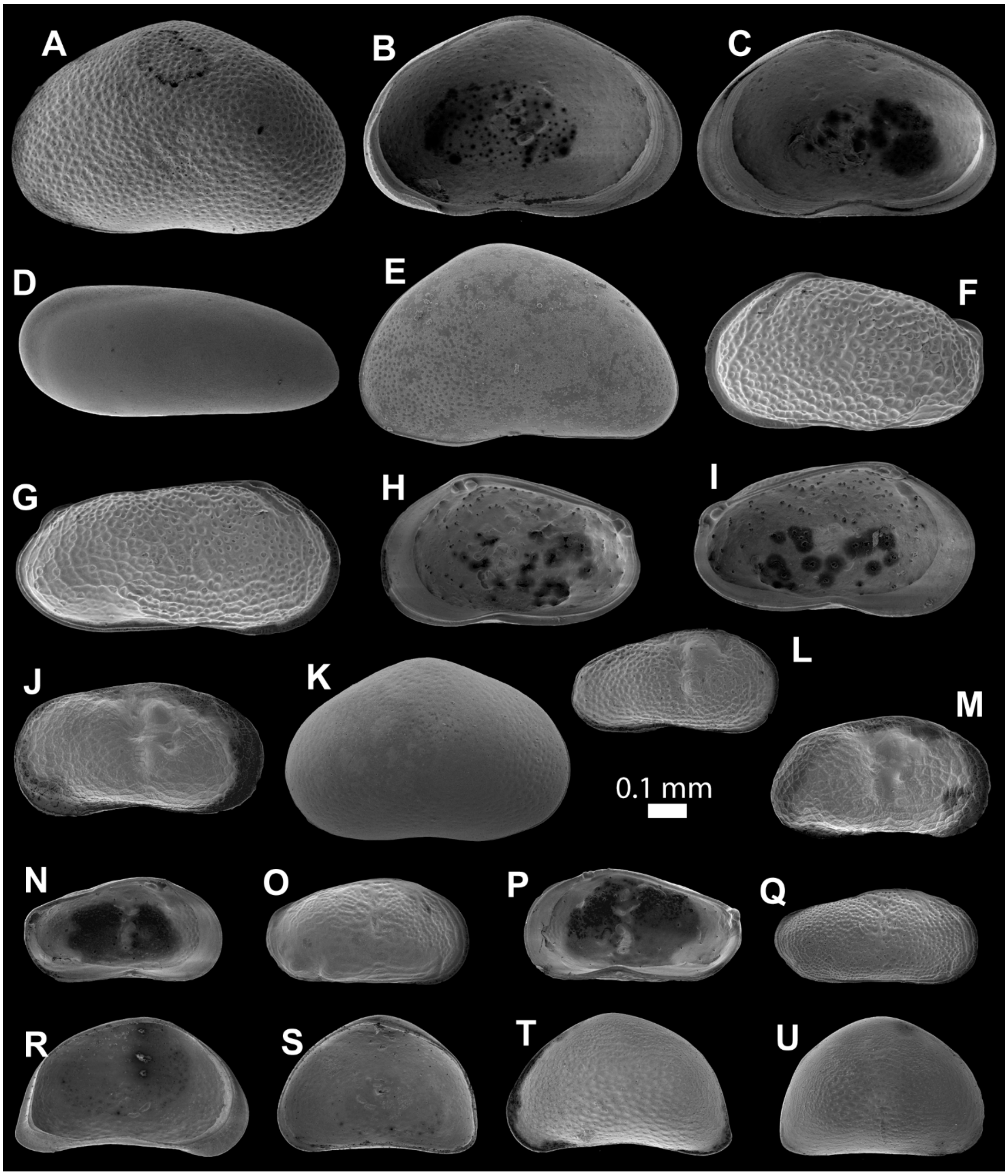


Figure 4: SEM microphotographs from Lake Trasimeno fossil ostracods. A. *S. aculeata*, left valve in lateral view (sample 378); B. *S. aculeata*, left valve in inner view (sample 378); C. *S. aculeata*, right valve in inner view (sample 378); D. *D. stevensoni*, right valve in lateral view (sample 392); E. *P. newtoni*, left valve in lateral view (sample 331); F. *C. fuscata*, female left valve in lateral view (sample 364); G. *C. fuscata*, male right valve in lateral view (sample 364); H. *C. fuscata*, female right valve in inner view (sample 364); I. *C. fuscata*, female left valve in inner view (sample 364); J. *L. inopinata*, female right valve in lateral view (sample 421); K. *C. vidua*, right valve in lateral view (sample 249); L. *L. blankenbergensis*, female right valve in lateral view (sample 402); M. *Limnocythere* sp., female right valve in lateral view (sample 402); N. *Amnicythere* sp., female left valve in inner view (sample 356); O. *Amnicythere* sp., female right valve in lateral view (sample 356); P. *Amnicythere* sp., female right valve in inner view (sample 361); Q. *Amnicythere* sp., male right valve in lateral view (sample 375). R. *P. paludum*, left valve in inner view (sample 385); S. *P. paludum*, right valve in inner view (sample 385); T. *P. paludum*, left valve in lateral view (sample 404); U. *P. paludum*, right valve in lateral view (sample 404).

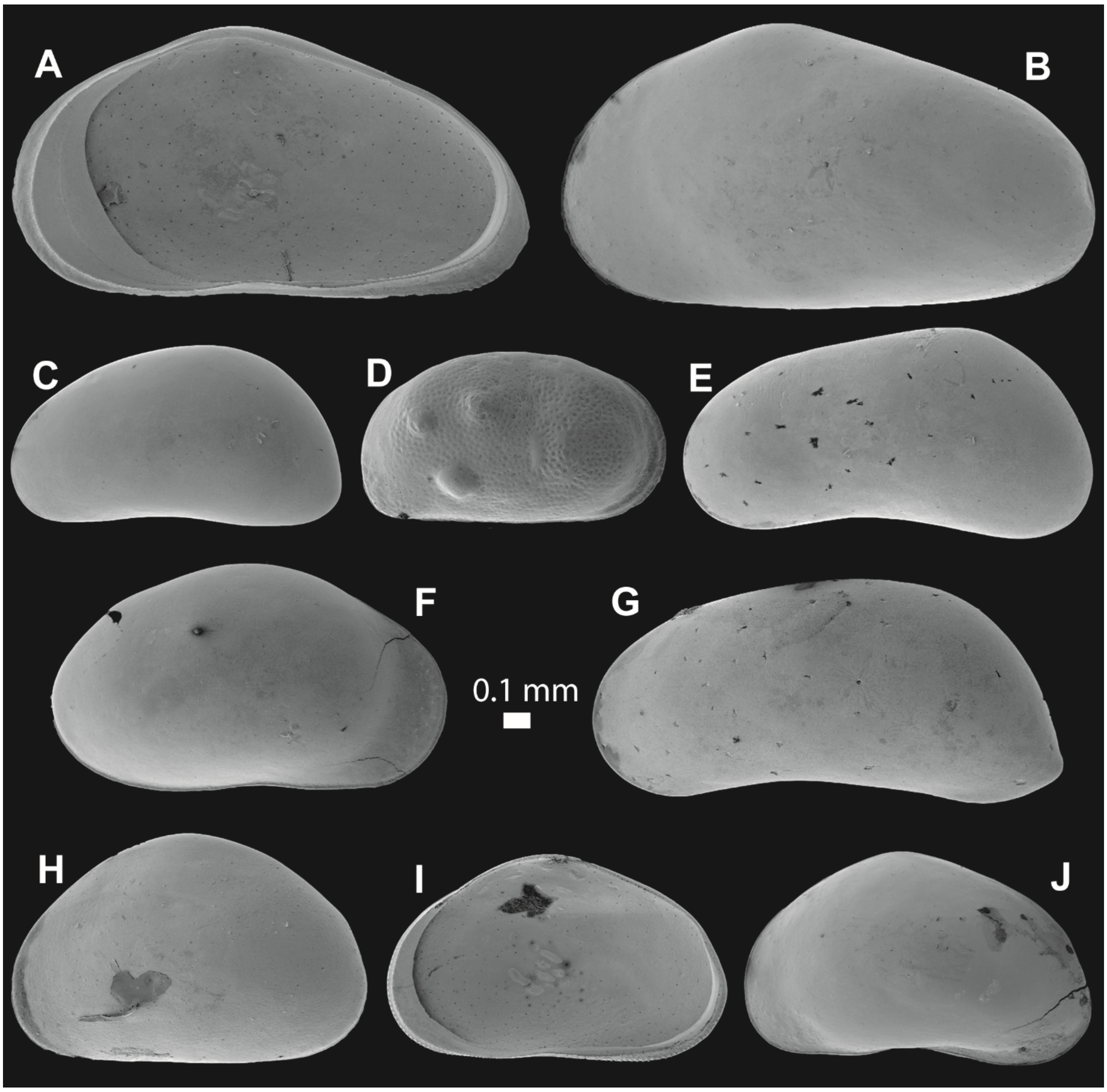


Figure 5: SEM microphotographs from Lake Trasimeno fossil ostracods. A. *T. serrata*, right valve in inner view (sample 385); B. *T. serrata*, left valve in lateral view (sample 385); C. *C.* (*C.*) *candida*, female left valve in lateral view (sample 385); D. *C. torosa*, female right valve in lateral view (sample 412); E. *C.* (*N.*) *neglecta*, left female valve in lateral view (sample 404); F. *H. incongruens*, right valve in lateral view (sample 596); G. *C.* (*N.*) *angulata*, left female valve in lateral view (sample 414); H. *H. salina*, left valve in lateral view (sample 421); I. *H. salina*, right valve in inner view (sample 421); J. *E. mareotica*, left valve in lateral view (sample 423).

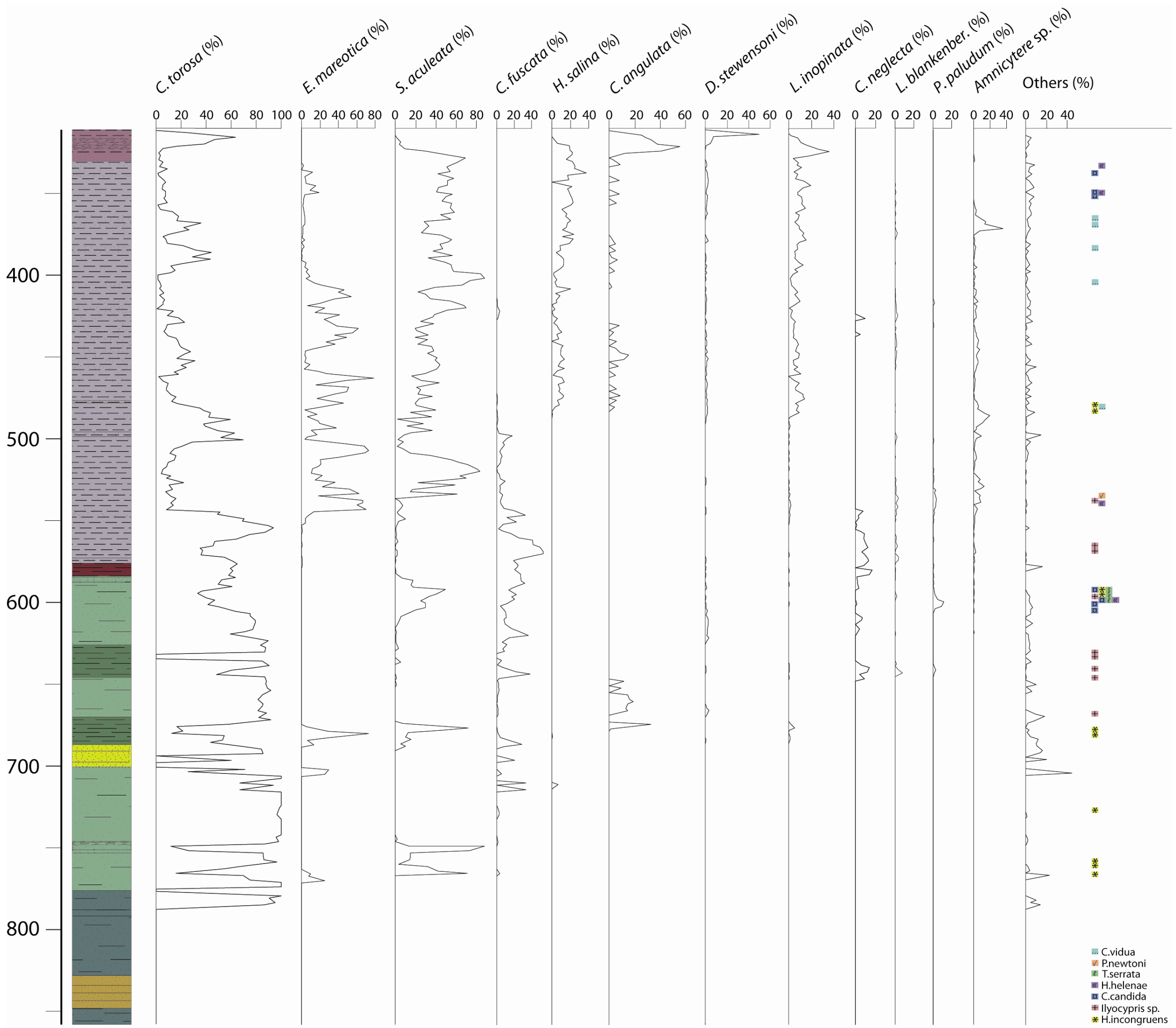


Figure 6: Percentage abundances (valves/g) of the ostracod species recovered in the core Co1320. "Others" includes the percentages of Candoninae juveniles indet., *Ilyocypris* sp. juveniles, and the accessory species which were represented by adult and juveniles individuals but were recovered only in a few samples.

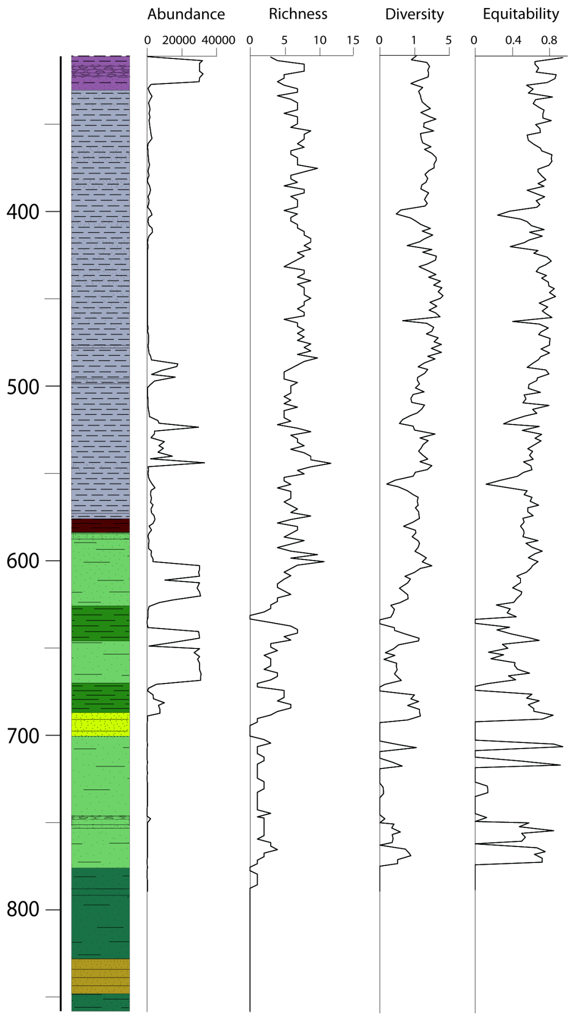


Figure 7: Ostracod community indexes: [abundance (valves/g), richness (S=number of species), Shannon index ($H=\sum P_i \ln P_i$, where the summation is calculated from species i=1 to species i=S, $P_i=n_i/n$, and n is the total number of specimens of each i species), and equitability (E=H/lnS where H is the Shannon index and S the number of species)] calculated along the core Co1320.

3.4.3 Multivariate analyses applied to the ostracod assemblages

Figure 8 reports the dendrogram in R-mode of the cluster analyses (cophenetic correlation coefficient = 0.93) in which, at a high distance values >1.2, three clusters are discriminated: cluster A, including the species *S. aculeata, H. salina, L. inopinata, E. mareotica, Amnicythere* sp., *D. stevensoni* and *C.* (*N.*) *angulata;* cluster B, with *C.* (*N.*) *neglecta, C. fuscata, C. torosa,* and *L. blankenbergensis;* cluster C, including only *P. paludum.* On the whole, the ostracod association represented by cluster B was mainly recovered in the lower part of the sediment core while species of cluster A (particularly *E. mareotica* and *S. aculeata*) dominated the ostracod assemblages of the middle part of the core until 330 cm. *P. paludum* was rare and was recovered scattered in the sediment core associated with species of both clusters A and B.

The dendrogram in Q-mode (cophenetic correlation coefficient = 0.91) is reported in Figure 9. At a high distance value >0.9 it shows the separation of six clusters. Cluster 1 includes 83 samples. The dominant species is *S. aculeata* which is present in 100% of samples with a weighted average percentage of 50.1 (weighted average percentage (w.a.%)= $\sum(y_i \times n_i)/N$, where y_i is the % value of the species, n_i the number of samples belonging to the association in which this percentage is present and N the total number of samples belonging to the association). *S. aculeata* is followed by *C. torosa* (w.a. % 18.3), *H. salina* (w.a.% 9.3), *E. mareotica* (w.a.% 6.6) and *L. inopinata* (w.a.% 6.1) (Table 1). *Amnicythere* sp., *C. fuscata* and *C.* (*N.*) *angulata* are also present with very low w.a.%. Cluster 2 groups 37 samples in which the most represented species are *E. mareotica,* recovered in all the samples with a w.a.% of 48.1, and *S. aculeata,* collected in 97.3% of the samples with a w.a.% of 21.6% (Table 1). *C. torosa* is always present but with low frequencies (w.a.% 13.0). *H. salina L. inopinata*, *Amnicythere* sp.,

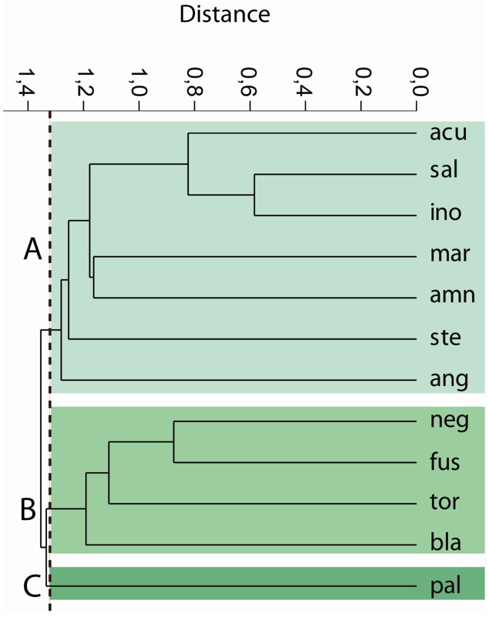


Figure 8: Dendrogram resulting from the cluster analysis in R-mode (species) using the UPGMA method and the Chord distance applied to the Late Pleistocene-early Holocene samples from the sediment core Co1320. The dotted line indicates the distance level of separation of the three clusters. Legend: **acu**. *Sarscypridopsis aculeata*; **amn**. *Amnicythere* sp.; **ang**. *Candona* (*Neglecandona*) *angulata*; **fri**. *Limnocythere blankenbergensis*; **fus**. *Cytheromorpha fuscata*; **inc**. *Heterocypris incongruens*; **ino**. *Limnocythere inopinata*; ***mar***. *Eucypris mareotica*; **neg**. *Candona* (*Neglecandona*) *neglecta*; **pal**. *Potamocypris puludum*; **sal**. *Heterocypris salina*; **ste**. *Darwinula stevensoni*; **tor**. *Cyprideis torosa*.

Cluster Q-mode	Association	Species	% Abundance	w.a.%	Presence of the species in the clusters defined in Q-mode	
					n°samples	%
CLUSTER 1	*Sarscypridopsis aculeata*	*S. aculeata*	from 12 to 89	50.07	83/83	100
		C. torosa	from 2 to 67	18.27	78/83	93.9
		H. salina	from 2 to 38	9.26	55/83	66.27
		E. mareotica	from 2 to 25	6.63	56/83	67.47
		L. inopinata	from 2 to 36	6.1	57/83	68.67
		Amnicythere sp.	from 2 to 36	2.52	31/83	37.34
		C. fuscata	from 2 to 22	1.55	16/83	19.27
		C. angulata	from 2 to 15	1.46	19/83	22.89
		P. paludum	from 2 to 11	0.35	5/83	6.02
		D. stevensoni	2	0.18	15/83	18.07
		L. blankenbergensis	from 2 to 3	0.10	4/83	4.82
		C. candida	from 2 to 3	0.06	2/83	2.40
CLUSTER 2	*Eucypris mareotica* and *Sarscypridopsis aculeata*	*E.mareotica*	from 24 to 79	48.08	37/37	100
		S.aculeata	from 2 to 46	21.65	36/37	97.30
		C.torosa	from 2 to 39	13.02	37/37	100
		H.salina	from 2 to 20	4	19/37	51.35
		L.inopinata	from 2 to 14	3.67	22/37	59.45
		Amnicythere sp.	from 2 to 13	2.7	14/37	37.84
		C.fuscata	from2 to 9	1.80	13/37	35.13
		C.angulata	from 3 to 8	1.13	7/37	18.91
		L. blankenbergensis	from 2 to 4	0.45	6/37	16.21
		C.neglecta	from 5 to 10	0.40	2/37	5.40
		P.paludum	from 2 to 3	0.38	5/37	13.51
CLUSTER 3	*H. incongruens*	*H. incongruens*	45	45	1/1	100
		C. torosa	25	25	1/1	100
		E. mareotica	25	25	1/1	100
		C. fuscata	5	5	1/1	100
CLUSTER 4	*Cyprideis torosa*	*C.torosa*	from 34 to 100	76.73	105/105	100
		C.fuscata	from 2 to 54	10	57/105	54.28
		S.aculeata	from 1 to 18	2	34/105	32.38
		C.neglecta	from 2 to 17	2	28/105	26.67
		E.mareotica	from 3 to 29	2	14/105	13.33
		C.angulata	from 8 to 38	2	12/105	11.42
		Amnicythere sp.	from 2 to 19	0.39	6/105	5.71
		D.stevensoni	from 2 to 7	0.32	10/105	9.52
		L. blankenbergensis	from 2 to 9	0.23	7/105	6.67
		H.incongruens	25	0.23	1/105	0.95
		L.inopinata	from 2 to 11	0.2	3/105	2.85
		P.paludum	2	0.02	1/105	0.95
		H.salina	from 2 to 6	0.07	2/105	1.90
CLUSTER 5	*C. angulata*	*C.angulata*	from 41 to 55	48	2/2	100
		L. inopinata	from 15 to 26	20.5	2/2	100
		H. salina	from 6 to 19	12.5	2/2	100
		C. torosa	from 5 to 16	10.5	2/2	100
		S. aculeata	from 4 to 9	6.5	2/2	100
		D. stevensoni	1	0.5	1/2	50
CLUSTER 6	*D. stevensoni*	*D. stevensoni*	50	50	1/1	100
		C. torosa	25	25	1/1	100
		C. angulata	25	25	1/1	100

Table 1: For each ostracod association, its taxonomical composition, the percentage abundance of each species in the cluster samples, their corresponding weighted average (w.a%), and the percentage of the cluster samples in which the species is present are reported.

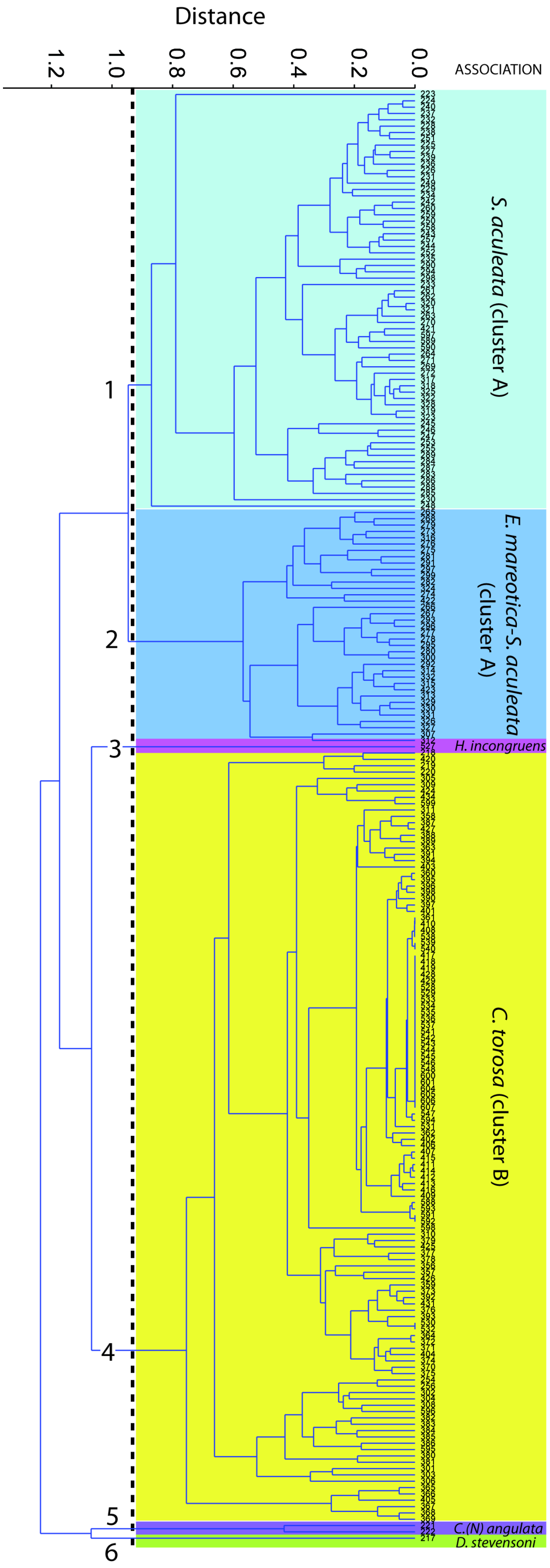


Figure 9: Dendrogram resulting from the cluster analysis in Q-mode (samples) using the UPGMA method and the Chord distance applied to the Late Pleistocene-early Holocene samples from the sediment core Co1320. The dotted line indicates the distance level of the separation of the four clusters.

C. fuscata, and *C.* (*N.*) *angulata* represent the accompanying species with very low w.a.% between 4 and 2. Clusters 1 and 2 are mainly represented by ostracod species belonging to cluster A (R-mode) of Figure 8. Cluster 3 includes only one sample at 706-704 cm in which the dominant species is *H. incongruens* (45% of the assemblage). This species is rare along the sediment core and, when present, generally displays frequencies <1%. Cluster 4 includes 105 samples in which the main ostracod species belong to cluster B (R-mode) of Figure 8. In this cluster, *C. torosa* is present as the absolute dominant species in 100% of the samples with a w.a.% 76.7 (Table 1). In 54.3% of the samples it is associated with *C. fuscata* that shows a 10.0 w.a.%. Other subordinated species are *S. aculeata, C.* (*N.*) *neglecta, E. mareotica,* and *C.* (*N.*) *angulata* with a very low w.a.% around 2. Cluster 5 includes two samples dominated by *C. (N.) angulata* (w.a.% 48) accompanied by subordinated *L. inopinata, H. salina* and *C. torosa*. Finally, cluster 6 includes only one sample dominated by *D. stevensoni* (w.a.% 50) accompanied by *C. torosa* and *C. (N.) angulata*.

The principal component analyses (PCA) algorithm was also applied. Bootstrapping was carried out with 1000 bootstraps replicates. In the screeplot, 95% bootstrapped confidence intervals are given for each Eigenvalues and the broken stick values are also reported (Fig. 10A). Only the first two components are above the broken stick values, although the broken stick is inside the 95% confidence intervals for the component 2. Component 1 explains 69,4% of variance and component 2 16,3%.

The principal component loadings > of ±0.5 were considered for the PCA interpretation (Fig. 11). Analyzing the PCA biplot (Fig. 11), component 1 is positively correlated with *C. torosa* (loading 0.82) and negatively correlated with *S. aculeata* (loading –0.51), while the component 2 is positively correlated with *E. mareotica* (loading 0.78) and negatively correlated with *S. aculeata* (loading –0.59). The other species display loading values comprised between ±0.5 (Fig. 11).

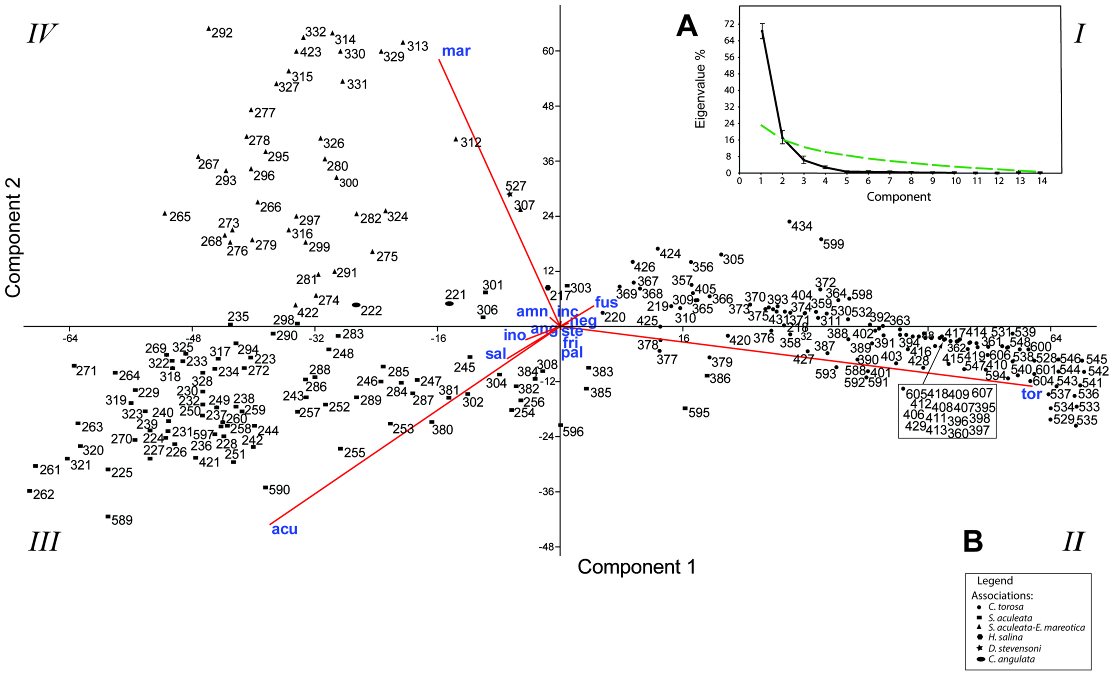


Figure 10: PCA biplot resulting from the multivariate analysis applied to samples from the sediment core Co1320. A. Screeplot for the PCA analysis. Solid black line: Eigenvalues curve; dashed grey (green in the on line version) curve: broken stick curve. 95% bootstrapped confidence intervals are given for each Eigenvalues. B. PCA biplot. Legend as in Figure 8.

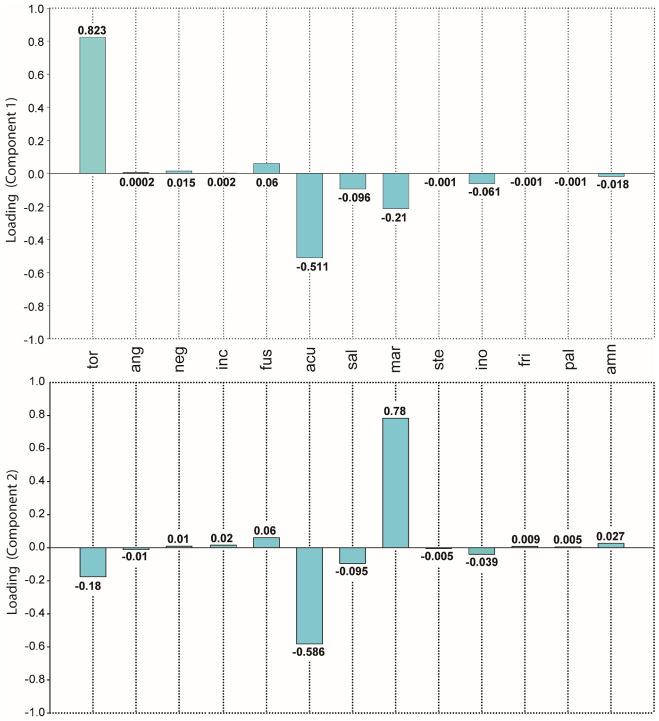


Figure 11: Loadings for Component 1 (permanent/temporary waterbody) and Component 2 (salinity) of the PCA analysis reported in Figure 10. Legend as in Figure 8.

3.5 Discussion

The results of PCA has been interpreted taking into account the ecological preferences of *C. torosa, S. aculeata* and *E. mareotica* which were the only three species whose loading values for component 1 and component 2 were above ±0.5. Their ecological preferences reported in the literature indicate that: *C. torosa* is characteristic of brackish (salinity 0.4 to more than 100‰), shallow permanent waters in marginal marine and athalassic environments (see *inter alia* Meisch, 2000; Rueda Sevilla et al.,2006; Cabral et al., 2017; De Deckker and Lord, 2017; Marchegiano et al., 2017; Pint and Frenzel, 2017); *S. aculeata* inhabits permanent and temporary small freshwater (rarely) to slightly saline (up to 17‰) water bodies such as coastal ponds influenced by marine water, rivers and lakes (see *inter alia* De Deckker, 1981; Meisch, 2000; Rueda Sevilla et al., 2006); *E. mareotica* is typical of temporary saline lakes (from 5 up to 110‰) with sodium chloride waters, although it can live in permanent water bodies as well, where it is generally rare (see *inter alia* De Deckker, 1981; Baltanás et al., 1990; Mezquita et al., 2005)

Consequently, it is possible to infer that the component 1 of the PCA analysis could represent the permanent/temporary character of the waterbody, whereas the component 2 could describe the salinity. Thus, the biplot in Figure 10B suggests that samples located in quadrants I and II were deposited in a permanent shallow and slightly brackish waterbody with low salinity variations, whereas samples located in quadrants III and IV were deposited mainly in very shallow to temporary waterbodies characterized by low (quadrant III) and high (quadrant IV) salinities, respectively.

The results from the cluster analyses, interpreted along with the PCA, were plotted against the age model of core Co1320 to show the alternation of different ostracod associations through time (Fig. 12). Based on the autoecology of the ostracod assemblages of the dominant species (as shown by the loading factors of the Principal Component Analysis), four palaeoenvironmental intervals (A, B, C, D) have been recognized (Fig. 12), which in turn can be linked to the NGRIP (North Greenland Ice Project, temperature record, NGRIP-members, 2004).

Interval A is a short interval, located at the bottom of the core (859-787 cm, 47,100-45,500 cal yr B.P.), is barren of ostracods and the corresponding sediments (lithofacies 1 and 2) display the absence or very low amount of carbonate (Fig. 2). As limestones are absent in Lake Trasimeno's catchment, an intense runoff and a high lake level could have diluted the carbonate concentration in the lake water preventing the ostracods from secreting their calcium carbonate shell. Low ion concentrations associated with high lake levels, humid conditions, and a low E/P ratio have been confirmed by historical limnological data (Ludovisi and Gaino, 2010). This would imply that humid conditions prevailed between 47,100 and 45,400 cal yr B.P., a time period that could tentatively be correlated with the GI-12 of the NGRIP ice core record (Fig. 12). Humid conditions in the Mediterranean region during GI intervals (Allen et al., 1999) are consistent with the lithological data from Lake Trasimeno.

In Interval B, from 787 to 490 cm (45,500-33,500 cal yr B.P.), the recovered ostracods pertain mainly to the *C. torosa* association (sub-Interval B1, 45,500-36,500 cal yr B.P.), thus indicating the presence of a permanent, slightly saline lake. At the top of the section, *C. torosa* association was replaced by the *S. aculeata* and *E. mareotica* - *S. aculeata* associations (sub-Interval B2 36,500-33,500 cal yr B.P.). The common presence of *C. fuscata* and *L. blankenbergensis* in most of interval B indicates palaeotemperatures lower than today, as these species are not living in Italy at present days. *C. fuscata* has only been recorded in permanent lentic and lotic brackish environments in Finland, Poland and Germany (Hagerman, 1967; Olenska and Sywula, 1988; Savolainen and Valtonen, 1983; Sywula, 1966, 1971; Usskilat, 1975) whereas *L. blankenbergensis* was found in Europe as fossil only in the Last Glacial-early Holocene deposits of Germany (Diebel, 1968; Griffiths, 1995; Günther, 1987; Pint et al., 2012).

Sub-Interval B1 does not appear to be completely homogeneous. The *C. torosa* association is replaced by the *S. aculeata* association from 44,800-44,600 cal yr B.P., 44,300-44,200 cal yr B.P., 42,200 cal yr B.P., and 39,300-38,700 cal yr B.P., suggesting a significant decrease of the lake level and temporary environments. Furthermore, between 42,300 and 42,200 cal yr B.P. the presence of few samples characterized by the *E. mareotica-S. aculeata* association indicates high salinities and temporary waters. Conversely, from ca. 40,500 to 36,500 cal yr B.P. the presence of *C. neglecta* could indicate more diluted waters.

Sub-Interval B2 represents the beginning of the consistent lake level drop, pointed out by the presence of *S. aculeata* and *E. mareotica* that prefer very shallow (down to few decimeters, Liu et al., 2009; Mischke et al., 2010) to temporary (Baltanás et al., 1990; Mezquita et al., 2005; Martin-Puertas et al., 2008) water bodies. Only at 502-498 cm (34,250-34,000 cal yr B.P.) and at 490 cm (33,500 cal yr B.P.) these species are replaced again by the *C. torosa* association indicating a short interval of permanent lake. In the whole interval B, the ostracod community is mainly characterized by abundant but low diversity assemblages. During high lake levels (*C. torosa* association), *C. torosa* is very abundant and predominant among the other few accompanying species (low equitability values), indicating a particularly favorable habitat for its development. Nowadays, similar conditions have been found in the central part of Lake Trasimeno at water depths between 3 and 5 m. Here, ostracod assemblages are represented only by *C. torosa* and *C. angulata* (Marchegiano et al., 2017). The high abundance values of the ostracod assemblages could be explained by the relatively high nutrient availability due to more humid conditions with enhanced OM and nutrient supply from the catchment, which is also confirmed in some horizons by higher TOC values.

These lake level and salinity variations in interval B can be correlated with the global climate changes derived from the NGRIP curve (NGRIP-members, 2004). The entire B interval corresponds to the relatively cold and arid Marine Isotopic Stage (MIS) 3, a long mild period in which at least six Greenland Interstadials (GI) are included from GI-11 to GI-6. In particular, GI-11, GI-10, GI-9, GI-8 and GI-6 are evidenced by the *C. torosa* association. During these warmer and more humid interstadial phases, Lake Trasimeno had a permanent waterbody (*C. torosa* association with the scarce presence of the cold species *C. fuscata* and *L. blankenbergensis*). Moreover, the presence of *C. neglecta* during the GI-8 and the beginning of GI-9 could suggest even more diluted water and thus higher lake levels. Conversely, the occurrence of *E. mareotica* – *S. aculeata* and *S. aculeata* associations and the corresponding decrease of the lake level could be correlated with the colder and more arid Heinrich event 4 (H4) and with the progressively more arid conditions recorded in the sub-Interval B2, corresponding to the last phases of MIS 3.

We can conclude that between 45,400 and 33,500 cal yr B.P. Lake Trasimeno underwent different water level oscillations. Extended periods of highstands conditions (permanent lake) correspond to warmer and humid GI phases whereas short periods of lowstands correspond to the Heinrich event H4 as well as to the last part of the MIS 3 interglacial period. During these lowstand phases, the lake was very shallow and/or temporary, with more saline waters. However, the lack of evidence of significant, long-lasting desiccation events in the lithology could indicate that these temporary water conditions were rather short. Nevertheless, they were sufficient to trigger unstable environment conditions not prone for the development of *C. torosa*, since the few valves here recovered are only instars.

In interval C, from 490 to 327 cm (33,500-10,400 cal yr B.P.), the recovered ostracods pertain mainly to the *S. aculeata* association although in the lower portion (until 24,500 cal yr B.P.), they are frequently replaced by the *E. mareotica-S. aculeata* association. *C. fuscata* disappears (except for a few valves recovered at 426-424 cm), whereas *L. blankenbergensis* is scattered in the interval with low frequencies; conversely, *L. inopinata* and *H. salina* are common. Increased salinities can be especially inferred where *E. mareotica* dominates the assemblages, until 24,500 cal yr B.P., pointing to more arid conditions. Although subordinated to *S. aculeata, E. mareotica* reaches again relative high frequencies between 13,800 and 12,000 cal yr B.P. Instead, an increased in the occurrence of *L. inopinata* and *H. salina,* mainly in the upper part of the interval, confirms very shallow/temporary water conditions (Ejarque et al., 2016; Martin-Puertas et al., 2008; Van der Meeren et al., 2010), but with slightly lower salinity (De Deckker, 1981; Martin-Puertas et al., 2008). Notwithstanding the apparent homogeneity of the palaeoenvironment, two deepening episodes at 388-392 cm and 370 cm (respectively 21,200-20,500 and 17,100 cal yr B.P.) can be detected by the increased abundance of *C. torosa*, which is linked to permanent waterbodies. The very shallow/temporary waters hosted well diversified but scarcely abundant assemblages indicating the availability of different habitat types (Williams, 1943) with a low amount of nutrients probably due to the dryer conditions. This is in line with the low TOC values, suggesting a low primary productivity and limited nutrient supply from the catchment.

The ostracod record points to shallow and/or temporary water conditions corresponding to the arid climate environment attained during MIS 2. During MIS 2, the climate variability of millennial duration was reduced in comparison with the MIS 3 period (Capron et al., 2010) and the GI relatively warmer events were shorter (Helmens, 2014). In fact, according to Fletcher et al. (2010) the higher global ice volume attained during MIS 2 respect to MIS 3 translated in a weaker expression of the stadial and interstadial periods. This could explain the homogeneity of the ostracod assemblages during Interval C in which we can attempt a correlation between the high occurrence of *E. mareotica* at 482-462 cm (33,000-31,400 cal yr B.P.) and 416-410 cm (25,500-24,500 cal yr B.P.) with the H3 and H2 cold events. The time span in which *E. mareotica* increases (13,800 to 11,400 cal yr B.P.) includes the Younger Dryas Abrupt Climate Change (ACC), whereas its definitive disappearance at 334 cm (11,500 cal yr B.P.) could match the Pleistocene/Holocene transition.

In summary, the Interval C records the very shallow/temporary nature of Lake Trasimeno and underlines the high salinity of the waterbody during the cold and arid MIS 2. Furthermore, as observed in several other lacustrine records in the Mediterranean area (Fletcher et al., 2010) it is here confirmed the difficulty to identify in this time interval the millennial-scale variability because of their weak expression.

Interval D, from 327-315 cm (10,400-9,000 cal yr B.P.), is the uppermost interval of the Co1320 core. It is very short and characterized by an abrupt increase in the ostracod frequencies coupled with low diversified ostracod assemblages made mainly by *C. angulata, C. torosa* and *D. stevensoni*. *S. aculeata* and *H. salina* are present only at the very base of the Interval D, whereas in the upper part only *C. angulata, C. torosa, D. stevensoni* and *L. inopinata* are present, accompanied by subordinated *C. vidua, H. helenae* and *C.* (*C.*) *candida*.

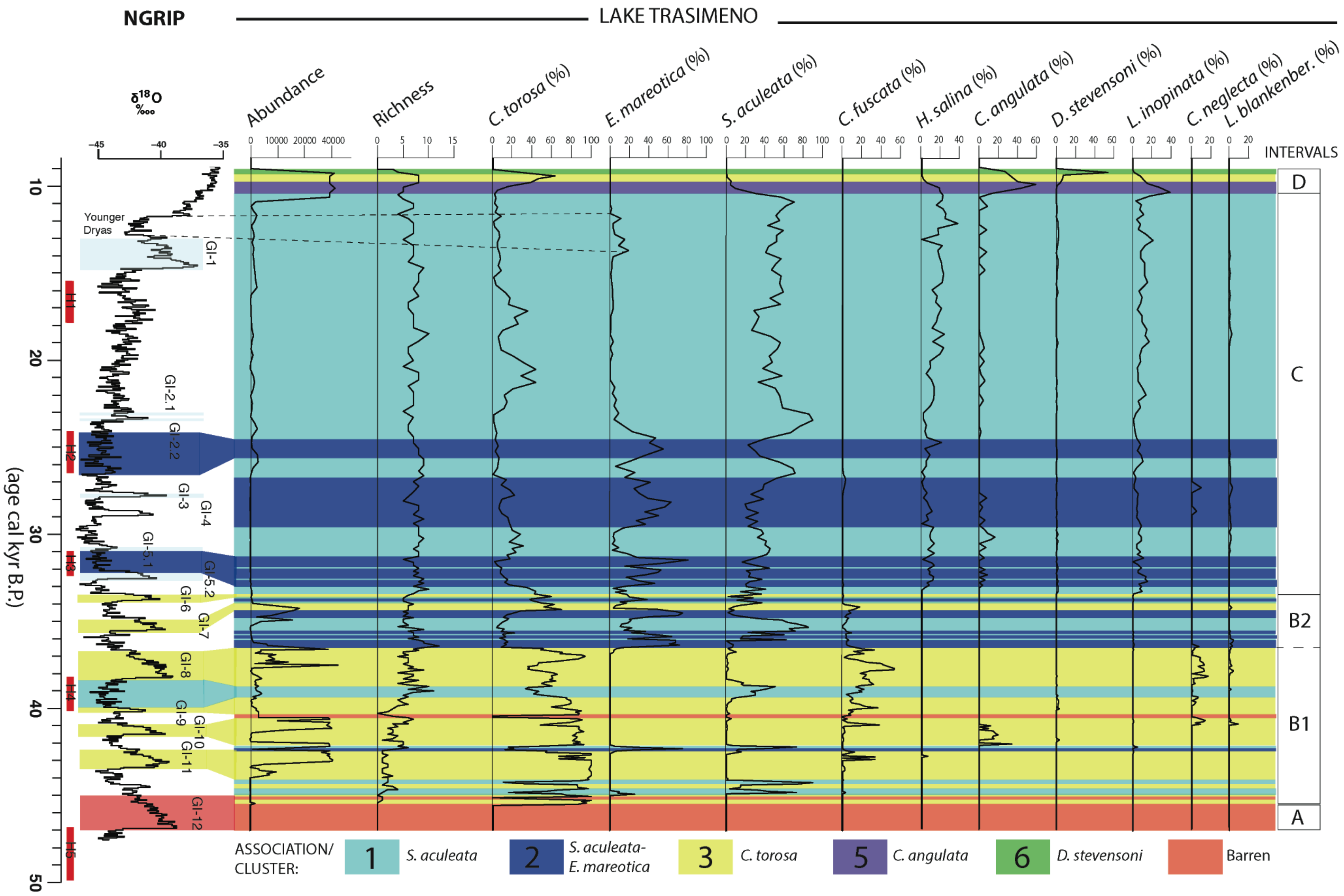


Figure 12: Palaeoenvironmental reconstruction of the sediment core Co1320 using ostracods plotted against the North Atlantic (NGRIP) (NGRIP-members, 2004) curve, and the ostracods percentage curves. Rare ostracods species (included in Others in Fig. 6) are excluded being negligible. Refer to the text for the discussion.

This latter ostracod assemblage occurs today in Lake Trasimeno at around 2.3-2.5 m water depth and 0.8‰ salinity (Marchegiano et al., 2017), and records the restoration of a permanent waterbody with low salinity. Moreover, the presence of *D. stevensoni, C. vidua* and *H. helenae* in the present lake, positively correlated with the presence of macrophytes (Marchegiano et al., 2017), could indicate a high trophic state of the lake. The highest TOC values recorded here could reflect the increase of the primary productivity in the lake and the enhanced runoff due to the high humidity and warmer conditions.

This short interval until 9,000 cal yr B.P. represents the onset of the Holocene, characterized by warmer and wetter climate conditions also recorded by various paleoclimate records in the Mediterranean region (summarized in Roberts et al., 2008).

3.5.1 Comparison to other records from the Italian peninsula

The sediments deposited in the endorheic and very shallow Lake Trasimeno are an excellent palaeoenvironmental archive. The recorded changes are driven by the millennial-climate variability occurred in the last 47,100 cal yr B.P. and ostracods have clearly reacted to them. Indeed, ostracod assemblage variations mirrored the repeated lake level fluctuations that appear to be strictly linked with the GI (humid) and GS/H (arid) events identified along the core.

However, the comparison of the ostracod associations with the NGRIP curve shows some differences in GI/GS phases duration as well as some delay in the lake system reaction to the changes in climate. For example, it is worth noting that ostracod assemblages similar to those recovered in present day Lake Trasimeno were firstly identified in the sediments that, according to the proposed age model, are aged ca. 9,000 cal yr B.P. and thus is lagging Termination 1 by ca. 2,700 years. This delayed biological response in comparison to the climate forcing is not a novelty. Such delays have been recognized both in marine and continental settings during Neogene and Quaternary times (Cosentino et al., 2012 and references therein). In Lake Trasimeno the delayed replacement of ostracod assemblages could be explained by their capability to tolerate a certain amount of environmental variations before their disappearance when facing unfavorable conditions. For example, *C. torosa* although considered a “permanent water” species can thrive also in very shallow waterbodies of less than 1 m depth (Grossi et al., 2016) and it is able to survive to short temporary desiccations through the tight closure of the valves and its brood care attitude (De Deckker, 1981; Van Harten, 1990). Similarly, *S. aculeata* as also *E. mareotica*, are tolerant species. Although they are common in temporary habitats because of their capability to produce resistant eggs, their high salinity tolerance and their very fast life-cycle (*inter alia* Rueda Sevilla et al., 2006) they can be also found in permanent shallow waters (Keatings et al., 2010; Meisch, 2000).

The Late Pleistocene humid/arid oscillations, to which the Lake Trasimeno system has clearly reacted have been also recognized in other lakes of the Mediterranean realm using different proxies such as pollen and stable isotopes (De Beaulieu et al., 2005 and references therein; Allen et al., 1999; Fletcher et al., 2010; Guilizzoni et al., 2000 and references therein). In particular, at Lago Grande di Monticchio (southern Italy), Allen et al. (1999) evidenced the strict relationship between changes in lake productivity during the GS/GI periods (being the increased nutrient availability caused by the enhanced weathering due to higher T and precipitation intervals) as well as the decrease of the woody taxa pollen during Heinrich events. Similarly, Guilizzoni et al., (2000) correlated increased productivity phases in Lake Albano (central Italy) with warmer and more humid climate phases during the Pleniglacial coupled with high lake levels and variations in ostracod assemblages. An abrupt increase of the TOC values similar to the observed in Lake Trasimeno, was also recorded in Lago Grande di Monticchio during Termination 1 (Allen et al., 1999), once more mirroring the temperature and humidity increase. Although not comparable with Lake Trasimeno, which is much shallower, Lake Albano (maximum depth 170 m) two main ostracod assemblages alternated, the *C. neglecta-Cyclocypris* sp. assemblage, characteristic of high productivity and high lake level phases and the *Ilyocypris bradyi-Potamocypris* spp. assemblage, typical of episodes of inferred low lake level and low lake productivity (Belis et al., 1999). Notwithstanding the different palaeoenvironmental setting of both lakes (shallow Lake Trasimeno, deep Lake Albano), the variations of the ostracod assemblages record similar palaeonvironmental reactions to palaeoclimate changes. For example, between ca. 28,000 and ca. 24,700 cal yr B.P., when Lake Trasimeno displayed the most drastic lake level lowering (lower portion of Interval C) with the prevalence of the *E. mareotica – S. aculeata* association, a shift from shallow to sublittoral conditions were recorded in Lake Albano (Belis et al., 1999); subsequently, around 24-23 kyr ago, the Albano lake level increased and a more stable period was recorded up to 17 kyr, with periods of further deepening. During the same interval Lake Trasimeno was monotonously characterized by the *S. aculeata* association, with the seldom presence of increasing *C. torosa* in correspondence of slightly deeper lake level.

At the Pleistocene/Holocene transition, as in Lake Trasimeno, an increase in ostracod abundance was recognized in Lago Piccolo di Avigliana (Northern Italy) by Belis et al. (2008), indicating a change in the environment with more favorable conditions for ostracods (e.g., an increase in the trophic state of the lake). However, at our site, the increase in ostracods abundance happened around 10,6 ka whereas a change to deeper water associations occurred only at ca. 9,000 cal yr B.P. Thus, the latter indicates that the observed water level increase in Lake Trasimeno is lagging ca. 2,700 years the NGRIP temperature reconstruction.

3.6 Conclusions

The sedimentary core Co1320 retrieved at Lake Trasimeno provided a continuous sediment archive covering the last ca. 47,100 cal yr B.P. This article focuses on the sediments corresponding to the late Pleistocene and the Holocene transition recognizing several of the major global climate changes occurring in this time interval. The ostracod assemblages as well as variations in their associations can be linked to climatically-forced palaeonvironmental changes during the Late Pleistocene to Early Holocene. Three main climatic phases were recognized:

The MIS 3 Interglacial phase (ca. 47,000 – 33,000 cal yr B.P., corresponding to the ostracod Intervals A, B1 and B2) is characterized by an alternation of humid and dry periods. They indicate prolonged high lake level conditions and low salinity waters (permanent lake, *C. torosa* association) during GI phases. Conversely, lowstands with more saline waters (very shallow and/or temporary lake, *S. aculeata* and *S. aculeata*

- *E. mareotica* association) characterized GS periods and Heinrich event H4.

The MIS 2 to Early Holocene period (ca. 33,000 – 10,400 cal yr B.P., corresponding to the ostracod Interval C), is characterized by a more homogeneous and prevalently cold and arid periods with shallow and/or temporary water conditions testified by the *S. aculeata* association. Short colder and dryer episodes correlate with H3 and H2 events and, probably, to the Younger Dryas ACC are shown by the increase of *E. mareotica.*

The Early Holocene interval (ca. 10,400 – 9,000_cal yr B.P., corresponding to the ostracod Interval D), pointed out warmer and wetter climate conditions. An abrupt increase in ostracods frequencies coupled with low diversified ostracod assemblages corresponding mainly to the presence of *C. angulata, C. torosa* and *D. stevensoni* indicate the restoration of a permanent waterbody with low salinity, very similar to present day conditions.

The proposed correlation between the NGRIP temperature record and the Lake Trasimeno palaeoenvironmental variations during the Late Pleistocene-Early Holocene confirms the primary role of climate on the evolution of this lake. It further endorses the great sensitivity of shallow endorheic basins to climate. Rapid variations in lake level modulated by local climate caused abrupt changes in ostracod associations, ratifying their powerful role as proxy in climate studies. Finally, our results highlight the critical role of regional studies in understanding global climatic changes. Since current climate models do not always accurately predict the prevailing conditions in continental settings, our record provides additional constraints on these models.

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CHAPTER 4

Rapid Late Pleistocene climate change reconstructed from a lacustrine ostracod record (Lake Trasimeno, Umbria, central Italy)*

In this study we present, for the first time, a detailed quantitative reconstruction of both winter (January) and summer (July) paleotemperatures from the Late Pleistocene to Holocene transition in central Italy based on ostracod assemblages. An 8.59 m long sediment core was retrieved from Lake Trasimeno (central Italy) with the aim of performing a paleoclimatic and paleoenvironmental reconstruction. Of 19 ostracod species recovered, 13 were calibrated according to their living temperature ranges, enabling us to reconstruct mean January and July temperature ranges using the Mutual Ostracod Temperature Range method. The occurrences of *Cytheromorpha fuscata* and *Limnocythere blankenbergensis* from 44,000 to 25,500 cal yr BP were particularly significant in determining colder (than present day) winter and summers, showing mean January temperatures at least 7 °C colder and mean July temperature at least 1°C in some intervals. Comparison of the MOTR-derived paleotemperature curves with the oxygen isotope curve from Greenland (NGRIP) shows a remarkable correlation of warmer Greenland Interstadial (GI) and the colder Greenland Stadial (GS)/Heinrich (H) events with clear peaks and troughs in the MOTR signals, demonstrating that a record of rapid climate change in the North Atlantic region is archived in lacustrine ostracod assemblages in central Italy.

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4.1 Introduction

Because of their often excellent preservation in sediments, abundance, and sensitivity to environmental changes, non-marine ostracods have long been considered an invaluable proxy to assess Quaternary climate changes (Horne et al., 2012). Analyses of their relationship to several climatically influenced variables, such as salinity, temperature, water depth and solute chemistry, enable the reconstruction of climate-driven variations in the hydrochemistry and hydrology of waterbodies (inter alia Horne et al., 2012; Smith et al., 2003; Viehberg and Mesquita-Joanes, 2012; Marchegiano et al., 2017a). Until recently, studies were mainly based on qualitative paleoenvironmental interpretation, although many advances have been achieved with the development of several transfer functions for non-marine ostracods (e.g. Frenzel et al., 2010; Marco-Barba, 2010; Pint et al., 2012; Viehberg and Mesquita-Joanes, 2012).

The Mutual Climate Range (MCR) method for reconstructing Quaternary paleoclimate was established using Coleoptera by Atkinson et al. (1987, 1986). Since then, the method has been applied to different groups such as mammals (Lopez-Garcia and Cuenca-Bescos, 2010; Lyons, 2003), amphibians and squamate reptiles (e.g. Blain et al., 2010, 2009), terrestrial molluscs (Moine et al., 2002), herpetofauna (Holmes et al., 2010; Sinka, 1993), plant macrofossils (Sinka and Atkinson, 1999), pollen (Pross et al., 2000; Pross and Klotz, 2002) and ostracods (Horne, 2007).

The principle of the MCR method is to determine the climatic range (usually in terms of mean monthly temperatures for winter and summer) within which a fossil assemblage of organisms could have co-existed when they were alive. It is assumed that climatic factors such as mean monthly air temperature have a significant influence on the geographical distribution of species. The calibration of species is achieved by plotting the geographic coordinates of their living occurrence records in combination with a climate dataset, so as to determine their distribution in “climate space”. For the beetle MCR method, the mean temperature of the warmest month (T_{MAX}) as well as the range between this and the mean temperature of the coldest month (T_{RANGE}) are established for each record to determine a climatic envelope for each species. When applied to fossil assemblages, the overlapping of the climatic envelopes shows the mutual temperature ranges within which those species could have co-existed. The mean temperature of the coldest month (T_{MIN}) can be determined from T_{MAX} and T_{RANGE} (Atkinson et al., 1987, 1986). In the northern hemisphere the warmest month is July and the coldest month is January. One of the most important advantages of the MCR method is that it does not consider the abundance of species but only their presence. As a result it can be used even in samples where a very small number of individuals are observed.

The first application of an MCR method to ostracod assemblages was made by Valentine (1971), who calibrated benthonic marine ostracod species from the Caroline and Virginia areas (USA) to estimate paleotemperatures in the Late Pleistocene Norfolk Formation (Sangamon Interglacial) of southeastern Virginia. Delorme et al. (1976) developed analogue MCR methods using Canadian non-marine ostracod and mollusc databases, identifying modern analogues for the fossil assemblages and then applying the MCR approach.

Horne (2007) introduced the Mutual Ostracod Temperature Range (MOTR) method. This is a non-analogue method for reconstructing paleotemperatures using freshwater / non-marine ostracod assemblages. In contrast to the method of Delorme et al. (1976), it includes all the calibrated species present in an ostracod assemblage, not just those found in a modern analogue assemblage (see Horne et al., 2012 for further discussion). Examples of the application of the MOTR method to paleoclimatic reconstruction may be found in Horne (2007), Horne et al. (2012), Holmes et al. (2010), Anadon et al. (2012), Bridgland et al. (2013), Whittaker et al. (2013), Bellucci et al. 2014, Langford et al. (2014a, 2014b, 2017), Pint et al. (2015), Cosentino et al. (2017) and Benvenuti et al. (2017).

The aim of this paper is to apply the MOTR method to infer past air temperatures using non-marine ostracod assemblages from an 8.59 m long sediment core from Lake Trasimeno, central Italy (Fig.1).

4.2 Lake Trasimeno and Co1320 sediment core

Lake Trasimeno (43°09’N; 12°06’E, Perugia, Umbria) is the largest lacustrine system of central Italy (ca. 120 km^2) (Fig.1). Its onset and evolution is related to and driven by the Northern Apennines extensional tectonics since the Early Pliocene (Gasperini et al., 2010). It is characterised by a very shallow depth (6 m maximum depth) and a smooth bathymetry (Ludovisi et al., 2005). Because of its endorheic nature, the hydrological system strictly depends on climatic variations which are mostly governed by the precipitation/evaporation ratio (Dragoni et al., 2012). Above all, the lake level and salinity variations seem to be the main consequences of the climate changes that occurred through time (Burzigotti et al., 2003; Dragoni et al., 2012; Gambini, 1995 ; Marchegiano et al., 2017a).

A paleoenvironmental reconstruction from an 8.59 m long sediment core (Co1320 Trasimeno core) has recently been the subject of a multidisciplinary investigation by Marchegiano et al. (2017a) and Francke et al. (in review). Both studies detected important changes in the Lake Trasimeno hydrology pointing out the alternation of humid and warm periods associated to high lake levels and dry and cooler periods with shallower/temporary waterbody conditions. According to the age model (Marchegiano et al., 2017a), the core encompasses the last 47 ka and the ostracods occurrence (Tab.1 list species), as well as their abundance and their ecological significance, have been analysed in detail through the Late Glacial-Holocene transition sequence (ca 47 – 9 ka) (Marchegiano et al., 2017a). This period crossed a long mild period (MIS 3), the last Pleniglacial and Tardiglacial (MIS 2) and the Holocene transition (Termination 1). The MIS 3 was characterized by high- frequency climate variations consisting of cycles of warmer (interstadial) and colder (stadial) periods (Abrantes et al., 2012). These climatic changes were firstly recognized in the ice-core record from Greenland (Grootes and Stuiver, 1997; Johnsen et al., 1992) and found thereafter in climatic records all over the world (Voelker, 2002). In central Italy, several palynological studies (summarized in Fletcher et al., 2010), revealed that the Greenland Interstadials (GI) are characterized by humid conditions, whereas dry ones prevailed during Greenland Stadials (GS).

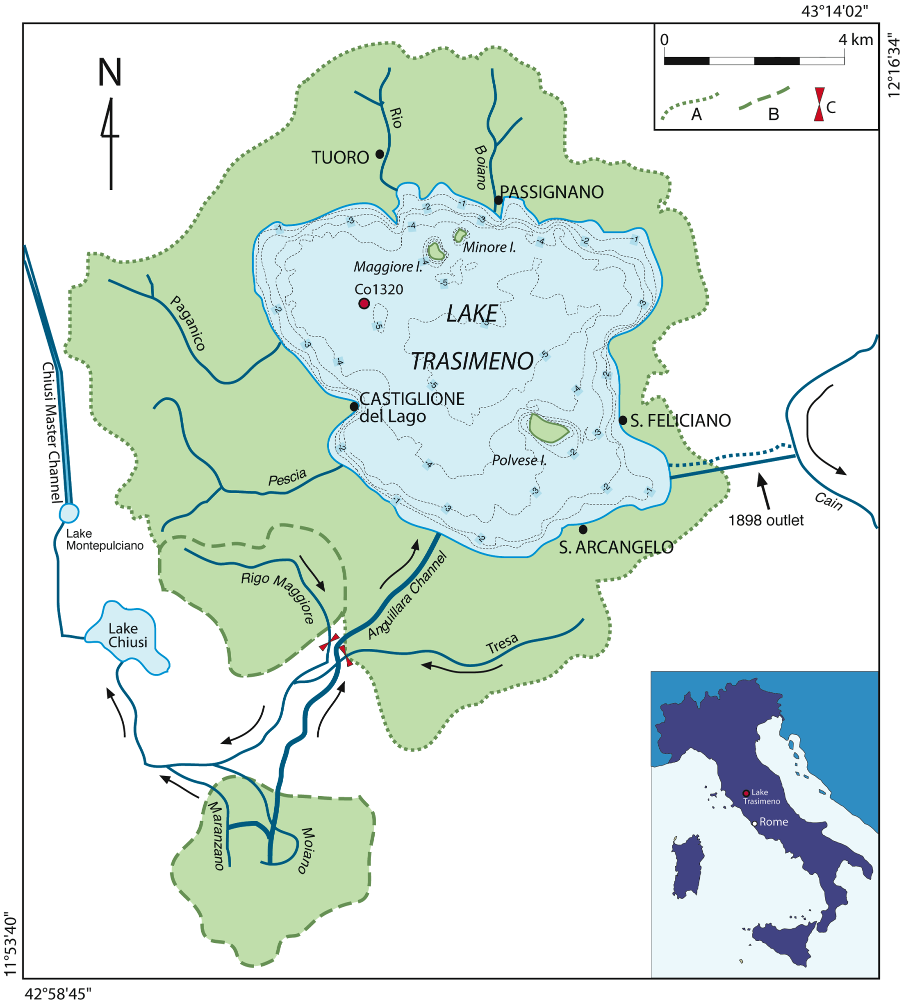

FIGURE 1: Map of Lake Trasimeno showing the location of core Co1320. Legend: A. natural catchment area; B. artificially-joined basins; C. sluice gates of the artificially-joined channels (modified from Marchegiano et al., 2017).

Marchegiano et al. (2017b) showed that the ostracod faunas recovered from the core reacted to and registered the climatic variations with important changes in their assemblages. In detail, three main associations were recognized: 1) *Cyprideis torosa* association indicating a permanent lake with high lake levels and low salinity conditions; 2) *Sarscypridopsis aculeata* association linked to very shallow/temporary waterbody and 3) *Sarscypridopsis aculeata* – *Eucypris mareotica* association indicating temporary waterbodies affected by high salinity. In particular, the high lake level conditions prevailed from ca. 44,000 to 35,700 and from ca. 10,000 to 9,200, while shallow/ephemeral lacustrine conditions existed from 35,700 to 10,000 cal yr BP.

To complement the general pattern of the environmental changes already inferred qualitatively from changes in the ostracod assemblages, a quantitative palaeoclimate reconstruction is provided here by the application of the MOTR method.

4.3 Methods

The MOTR method calibrates the temperature range of each species by mapping its living geographical distribution, using DIVA-GIS software (version 7.5) and comparing it with the WorldClim database (version 1.3) (Hijmans et al., 2001). The latter comprises interpolated global climatic parameters integrated for the period 1950 to 2000. The ostracod distributions have been taken primarily from the Non-marine Ostracod Distribution in Europe (NODE) database (Horne et al., 1998) integrated with records from other datasets, e.g. for North America (Horne et al., 2012). The reconstruction of the past mean January and July air temperature ranges for specific ostracod assemblages is made following the same principle as the MCR method (Atkinson et al., 1986, 1987), thus the overlapping of the calibrated ranges (Tab.2 calibration species range) of the species occurring in a sample gives the mutual temperature range for that interval. In Fig. 2 is shown an example of the application of the method using sample 374 of the Trasimeno sediment core.

The temperature ranges were calculated for all the samples from 7.4 to 3.1 m depth in the Co1320 Trasimeno core to obtain the paleotemperature variations during the whole sequence. The MOTR method could not be applied from 8.6 to 7.5 and from 7.2 to 7.0 m because of the absence of ostracods and/or calibrated species.

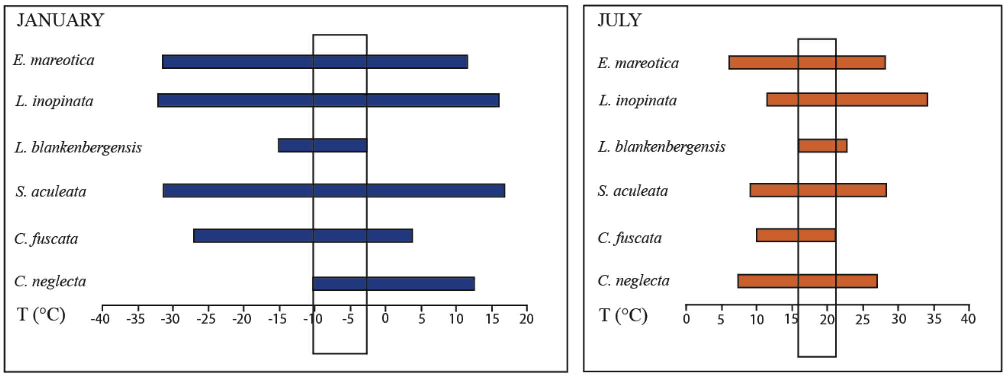

FIGURE 2: Example of the application of the MOTR method on sample 374 of the Co1320.

Of the 19 species identified by Marchegiano et al. (2017a), (Tab.1), 13 were calibrated for mean January and July air temperature ranges (Tab.2). In the case of the fossil species *Limnocythere blankenbergensis*, considered to be extinct in Europe, the calibration of the living North American species *Limnocythere friabilis* (its nearest living relative and possibly conspecific) was used (see Results and Interpretation). Since the MOTR method is developed only for freshwater species, *Cyprideis torosa,* considered as a brackish-water species, was not used. *Amnicythere* sp*,* and *Ilyocypris* sp. (for their taxonomic uncertainty), and *Trajancypris serrata*, *Potamocypris paludum* and *Herpetocypris helenae* (still not calibrated) were also excluded (see Tab. 1 for full taxonomic names of all ostracod species mentioned in the text)

Species
Amnicythere sp
Candona (*Neglecandona*) *angulata** Müller
Candona (*Candona*) *candida** Müller
Candona (*Neglecandona*) *neglecta** Sars
*Cytheromorpha fuscata** (Brady)
*Cypridopsis vidua** (Müller)
Cyprideis torosa (Jones)
*Darwinula stevensoni** (Brady & Robertson)
*Eucypris mareotica** (Fischer, 1855)
Herpetocypris helenae Müller
*Heterocypris incongruens** (Ramdohr)
*Heterocypris salina** (Brady)
Ilyocypris bradyi Sars
*Limnocythere blankenbergensis** Diebel
*Limnocythere inopinata ** (Baird)
*Plesiocypridopsis newtoni** (Brady & Robertson)
Potamocypris paludum Gauthier
*Sarscypridopsis aculeata ** (Costa)
Trajancypris serrata Alm

Table 1: ostracod species recovered in the Trasimeno sediment core

Species	July		Jan	
	Min	Max	Min	Max
Candona (*Neglecandona*) *angulata*	16	25	-5	7
Candona (*Candona*) *candida*	6	26	-40	9
Candona (*Neglecandona*) *neglecta*	7	27	-10	13
Cytheromorpha fuscata	10	21	-27	4
Cypridopsis vidua	9	34	-32	17
Darwinula stevensoni	12	30	-31	14
Eucypris mareotica	6	28	-31	12
Heterocypris incongruens	5	28	-31	18
Heterocypris salina	8	28	-31	16
Limnocythere inopinata	12	34	-32	16
Limnocythere blankenbergensis	16	23	-15	-3
Plesiocypridopsis newtoni	12	27	-31	17
Sarscypridopsis aculeata	9	28	-31	17

Table 2: calibrated ostracods species for mean January and July air temperature ranges

4.4 Results and Interpretation

4.4.1 Temperature ranges of ostracod species

Among all the species recovered in the Co1320 core, those with the narrowest calibrated temperature ranges are *L. blankenbergensis* (equivalent to *L. friabilis*), *Cytheromorpha fuscata*, *Candona angulata* and *C. neglecta* (Tab.2). However, it should be noted that even taxa with broad ranges can play a significant role in determining the mutual temperature range of an assemblage, if the palaeotemperature is in the vicinity of one end or the other of the species' calibrated range.

Limnocythere blankenbergensis is a small limnocytherid species known in Europe in the Late Glacial-early Holocene deposits of Germany (Diebel, 1968; Griffiths, 1995; Günther, 1987; Pint et al., 2012) and found for the first time in Italy in the Co1320 Trasimeno core during the cool and cold periods corresponding to the MIS 3 interstadials and MIS 2 (Marchegiano et al., 2017a), but it has never been found living in Europe. If *L. blankenbergensis* is considered to be extinct, calibration of its temperature range is not possible. However, it may be synonymous with *L. suessenbornensis*, another supposedly extinct species from the European Quaternary (Preece et al., 2007; Whittaker & Horne, 2009), and comparative studies in progress suggest that both may be synonymous with a living North American species, *Limnocythere friabilis.* Resolving this taxonomic issue is beyond the scope of this paper but, even if these species are not demonstrated to be synonymous, we can consider *L. friabilis* to be the nearest living relative of *L. blankenbergensis / suessenbornensis* and therefore make the reasonable assumption that their temperature ranges were similar. Using records from the Delorme Ostracode Autecological Database (curated by the Canadian Museum of Nature) we have determined the temperature range of living *L. friabilis* (January -15 to -3 ^{o}C, July +16 to +23 ^{o}C) and applied it to *L. blankenbergensis* in our MOTR analyses.

Cytheromorpha fuscata has not been found living in present-day Italy, but has been recorded alive in permanent lentic and lotic brackish to freshwater environments in Finland, Poland, Germany and Britain (Boomer and Horne, 1991; Hagerman, 1967; Olenska and Sywula, 1988; Savolainen and Valtonen, 1983; Sywula, 1966, 1971;

Usskilat, 1975) as well as in Canada (Neale & Delorme, 1985. Horne et al. (2012) gave its temperature range as January -27 to +4°C and July +10 to +21°C.

Candona angulata lives today in Lake Trasimeno (Marchegiano et al., 2017b) and its temperature range is January -5 to +7°C and July +16 to +25 °C (Horne et al., 2012).

Candona neglecta is part of the Italian living fauna (Pieri et al., 2009) but has not been found living in Lake Trasimeno (Marchegiano et al., 2017a). It has a wider temperature range than the previously mentioned species: January -10 to +13 °C and July +7 to +27 °C (Horne et al., 2012).

Sarscypridopsis aculeata, *Heterocypris salina*, *Limnocythere inopinata* and *Eucypris mareotica* have wide temperature ranges (Tab.2). In the Lake Trasimeno paleoenvironmental reconstruction (Marchegiano et al., 2017a), these four species were interpreted as indicators of mainly temporary/ephemeral lacustrine conditions, as suggested by their capacity to survive dry intervals in a torpid stage and/or as resting eggs. Moreover the first two of these four species are typically found in brackish environments (De Deckker, 1981; Margalef, 1956; Mezquita et al., 1999) and the others are tolerant of brackish water; *E. mareotica* is able to inhabit both fresh and highly saline waters up to 110 per mil (Aladin et al., 2008; Löffler, 1990).

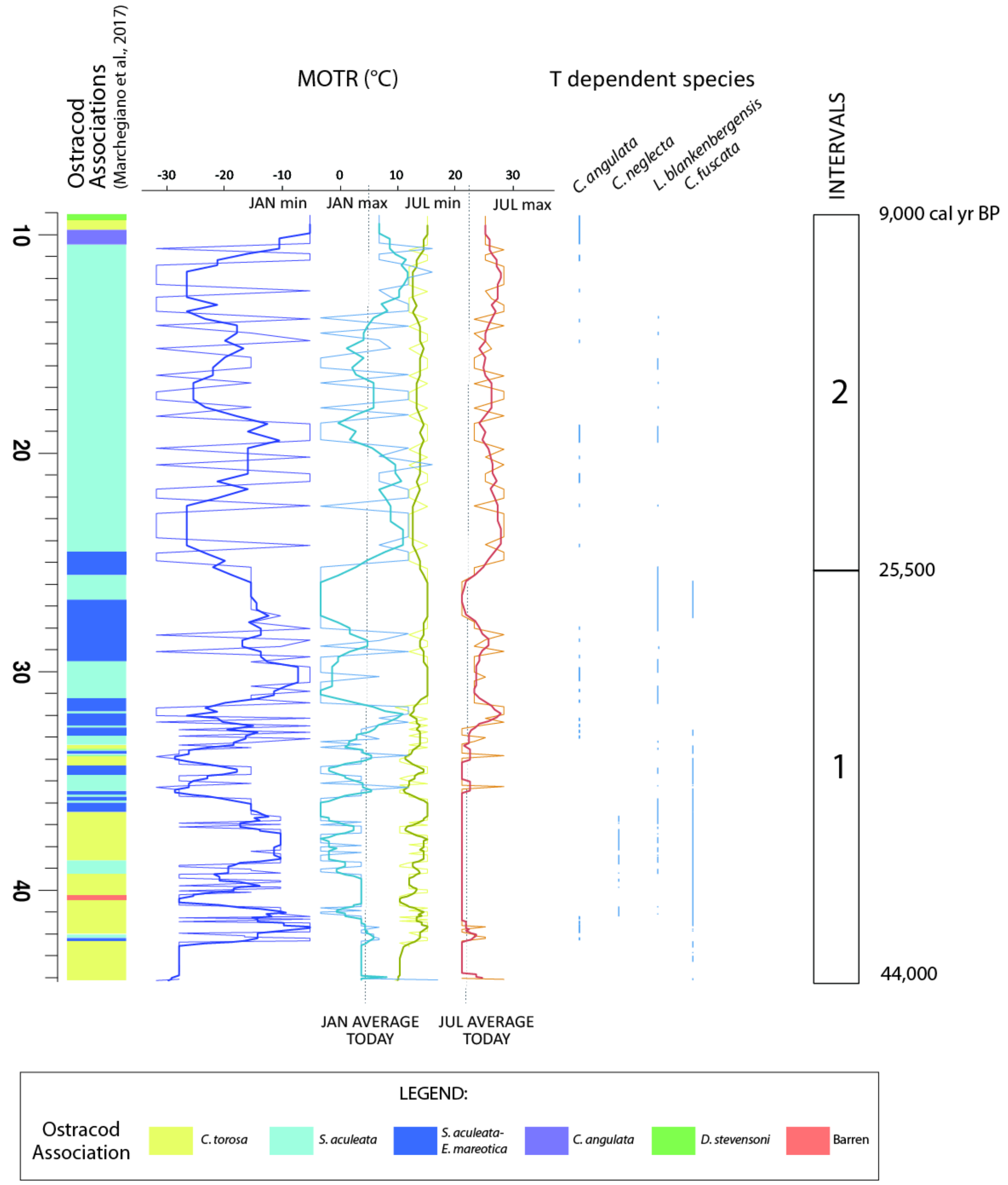

FIGURE 3: MOTR application along the Co1320 sediment core. From the right, the ostracod associations identified by Marchegiano et al., the four MOTR-derived curves, the main temperature dependent species significant in determining the MOTR and the interval separation (refer to the main text).

4.4.2 MOTR application

The temperature reconstruction using the MOTR method on ostracod assemblages from the Co1320 core is shown in Fig. 3. The minima curves of the MOTR reconstructed ranges for January and July correspond well with each other (despite their amplitudes being quite different) in terms of “colder” and “warmer” intervals, as do the maxima curves for January and July. Like other MCR methods, the MOTR method reconstructs a range of temperatures; the actual temperatures lay somewhere between the maxima and minima of the reconstructed ranges, but it is not possible to attribute greater probability to, e.g. the mean value of the two extremes. To reduce the “noise” of single-sample extremes and variation we applied 5-point moving averages, and the resulting smoothed signals are described and discussed below. Then, while it is impossible to determine precisely where, within the reconstructed ranges, the actual temperatures lay, some constraints may at least be discussed. For example the curve for January maxima can be taken to represent a coldness threshold (i.e. it was at least as cold as this) to compare with present-day mean January air temperature. This shows that there were periods, particularly in the lower half of the core (44,000 to 25,500 cal yr BP), when mean temperatures were at least a few degrees colder than today. These are mainly determined by the occurrences of *Cytheromorpha fuscata* and *Limnocythere blankenbergensis* which were particularly significant in determining colder winter and summers, with mean January temperature at least 7 °C colder and mean July temperature at least 1°C cooler, in some intervals. . Furthermore, the January minima curve shows a good “wiggle match” with a pollen-derived mean January air temperature reconstruction from Lake Monticchio (Lago Grande di Monticchio, approx. 375 km south-east of Lake Trasimeno; Allen et al., 1999) in terms of “colder” and “warmer” intervals, but the Lake Trasimeno curve has higher amplitudes and the actual temperatures indicated are almost certainly too cold (Fig. 4). If the Lake Trasimeno signal were to be tuned to the Lake Monticchio signal, by adjusting the former to within the limits of -15 and 0 indicated by the latter, the resulting lower-amplitude curve would fit within the January MOTR minima and maxima, consistent with the rule that the actual temperatures lay somewhere between the extremes of the MOTR ranges. In contrast, the January maxima curve shows little similarity with the Lago Grande di Monticchio mean January signal. This not unexpected; we consider it likely that during cold climate stages in Europe it is the minima (representing the northern limits of species’ ranges), rather than the maxima (representing southern limits of ranges), that will reflect the actual temperatures most closely, because the species are living close to their northern distributional limits. The July and January maxima curves, moreover, often show higher-than-present-day temperatures, particularly during Interval 2 (Fig. 3) which includes the Pleniglacial, and this is simply implausible. The Lake Trasimeno January minima curve also shows remarkable matches with features of the NGRIP Greenland ice core oxygen isotope record (NGRIP members, 2004) (Fig. 4). The most obvious match is the correspondence with the Younger Dryas stadial and the preceding Greenland Interstadial 1 (GI), but Greenland Interstadials 2 – 10 can also be matched, with only relatively minor adjustments in age that could be explicable in terms of varying depositional rates in Lake Trasimeno.

4.5 Discussion

In Fig. 3 the MOTR-derived curves are compared with the ostracod assemblages recognized by Marchegiano et al. (2017a) throughout the core. Interval 1 (ca. 44,000-25,500 cal yr BP) corresponds to the long mild period of MIS 3 and to the beginning of MIS 2 (Pleniglacial). During this period *C. fuscata* and *L. blankenbergensis*, both of which are thought to require permanent waters (e.g. Sywula, 1988; Curry and Baker, 2000), are significant components of the ostracod assemblages

In contrast the upper part of the sediment core (Interval 2), is characterised by prolonged very dry conditions that caused a strong reduction of the lake waterbody to possible temporary pools (Marchegiano et al., 2017a). In this interval, the persistence of *S. aculeata* (a species tolerant of brackish water and with desiccation-resistant eggs, adapted to life in ephemeral waterbodies) is significant, while *C. fuscata* is completely absent and *L. blankenbergensis* occurs only sporadically.

Despite differences in amplitude, the peaks and troughs of the MOTR January and July minima curves show remarkable matches with features of the NGRIP record (NGRIP members, 2004) (Fig. 4). These qualitative correspondences demonstrate for the first time the capability of the MOTR method to reconstruct rapid climate changes. They also confirm the Italian record of rapid environmental changes correlated with Greenland Interstadial events that was previously obtained from Lake Monticchio by Allen et al. (1999) using pollen-based reconstruction methods. Although the Mutual Ostracod Temperature Range method has frequently been applied to infer past climatic conditions, this is the first time it has been used successfully to produce continuous paleotemperature curves over a long time interval. Anadon et al. (2012) used the MOTR method to reconstruct curves for discrete, discontinuous, mid-Pleistocene to Holocene core intervals from Valle di Castiglione near Rome, Italy, but chose inappropriately to use mean values of the reconstructed ranges (rather than maxima and minima as used herein). The MOTR method is capable of reconstructing only ranges within which the real temperature existed, and considering mean values of the maxima and minima to be more likely to represent the actual temperatures is regarded as unjustified and inadvisable (Horne and Mezquita, 2008; Horne et al., 2012).

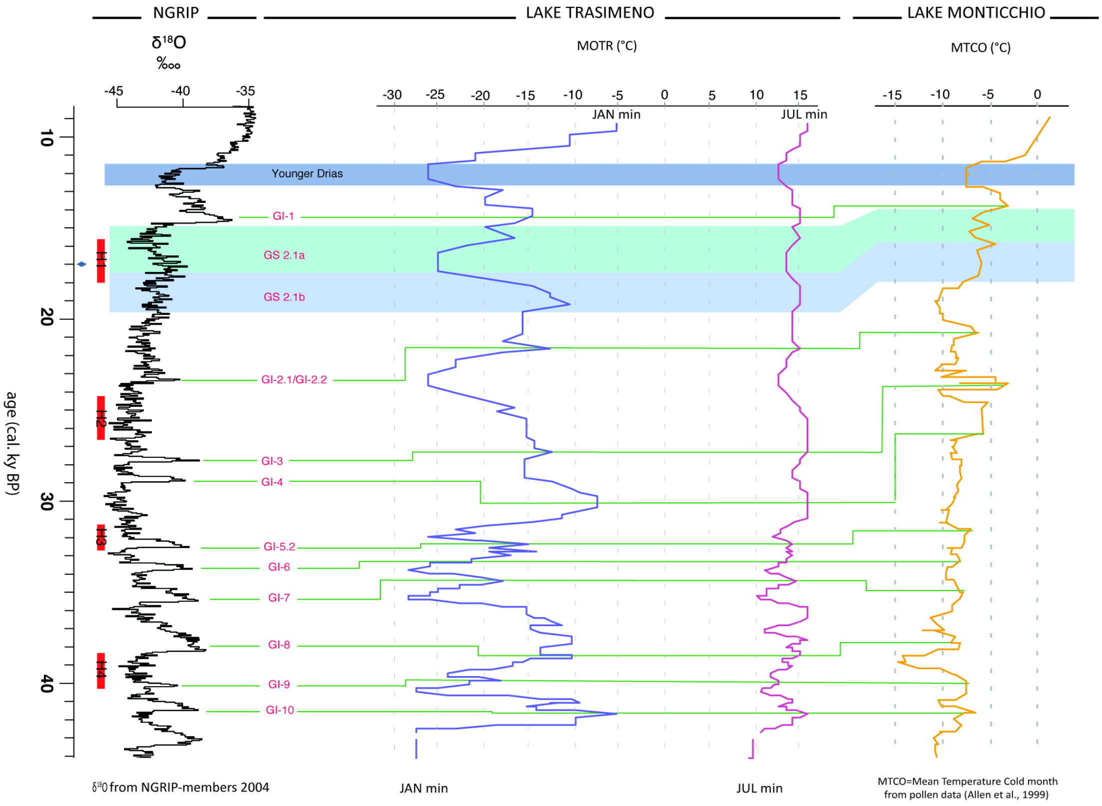

FIGURE 4: Comparison between Jan and July T_{MIN}, the NGRIP curve and the Lake Monticchio MTCO curve.

4.6 Conclusions

The application of the MOTR method to the Lake Trasimeno ostracod record between 44,000 to 9,000 cal yr BP demonstrates for the first time the ability of the method to yield a continuous record of rapid climate change. Correlation of MOTR curves with the Younger Dryas and Greenland interstadials GI 1-10 confirms a southern European record of rapid environmental change during this interval, previously determined from pollen-based methods in another Italian lake.

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CHAPTER 4.1

Temperature range calibration of the species *Eucypris mareotica* (Fischer, 1855)

4.1.1 Introduction

The Mutual Ostracod Temperature Range (MOTR) method (Horne, 2007) is a non-analogue method for reconstructing palaeotemperature using freshwater ostracods assemblages. Only the species still living today, of which is possible to know their climatic distribution, can be used. Each species is calibrated in terms of their mean July and January air temperature ranges (T), and the mutual temperature range of all the species recovered in a fossil assemblage, representing the T interval in which they could have co-existed, provides the palaeotemperature reconstruction for that time interval. Examples of the application of this method to palaeoclimatic reconstruction may be found in Horne (2007), Horne et al. (2012), Holmes et al. (2010), Bridgland et al. (2013), Whittaker et al. (2013), (Langford et al., 2014a, 2014b) and Marchegiano et al. (in prep).

The aim of this work is the calibration of the species *Eucypris inflata* (Sars, 1903), a senior synonym of *Eucypris mareotica* (Fischer, 1855). *Eucypris inflata* (Fig.1) is a common inhabitant of European, North African, and Central Asian saline waters (Altinsaçli, 2000; Baltanás et al., 1990; De Deckker, 1981; Fischer, 1855; Gauthier, 1928; Hartmann, 1964; Li and Liu, 2010; Löffler and Danielopol, 1978; Sars, 1903; Schornikov, 1964; Shadrin and Anufriieva, 2013). It is also found as one of the main species, in association with *Cyprideis torosa, Sarcypridopsis aculeata, Limnocythere inopinata, Heterocypris salina, Cytheromorpha fuscata* and *Candona angulata,* in a Late Pleistocene sedimentary core from Trasimeno Lake (Marchegiano et al., 2017). The application of the MOTR method to the assemblages recovered from this core will be an important tool for the palaeoclimatic and palaeoenvironmental reconstruction, but since *E. inflata* has not previously been calibrated this is an essential prerequisite for such work.

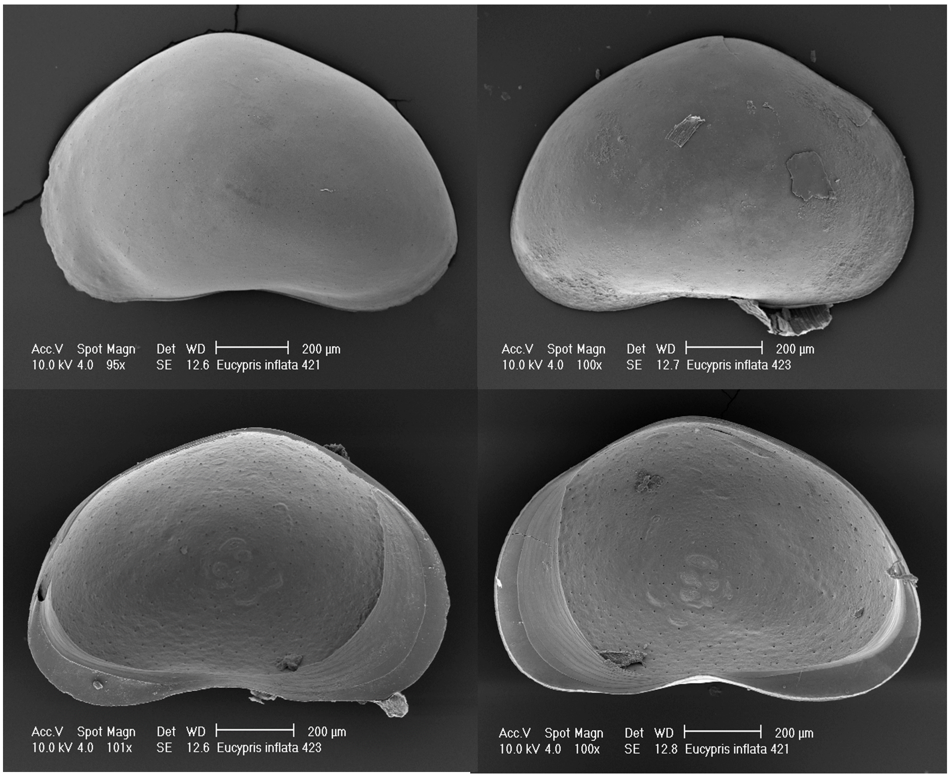

Figure 1: SEM microphotographs from Lake Trasimeno (sample 421 and 423). *Eucypris inflata* (Sars, 1903), external view left valve (LV) and right valve (RV), internal view LV and RV.

4.1.2 Methods

A dataset (Table 1) of living records of *E. inflata* was compiled from the available literature. According to "the living species calibration method" (Horne, 2007), the map to establish the modern climate distribution of the species *E. inflata* was made using DIVA-GIS (version 7.5) (Hijmans et al., 2001) where two different database were uploaded: the WorldClim database (version1.3) (Hijmans et al., 2001) containing global climatic parameters from 1950 to 2000 (Horne, 2007) and an ostracod dataset containing all living occurrences considered reliable in terms of exact identification of the species *E. inflata* and clear geographical position of the sampling area.

The temperature ranges which enclose all the living records of the species are determined by fitting WorldClim data to the mapped coordinate points of the species' distribution (Fig. 2 and 3), and are expressed in terms of the maximum and minimum values of the July and January temperature ranges.

4.2.3. Results

A total of 53 records (Tab.1) were used to define the modern climate distribution of the species. A record from Sahara region (De Deckker, 1981) could not be used because of the vague definition of the sampling recovering area.

The resulting calibration is:

mean July air temperature range (Fig. 2): +6 to + 28°C

mean January air temperature range (Fig. 3): -31 to +12°C

The limits of the July range were determined specifically by the records in Nanhongshanhu Lake (West China), (Horne et al., 2011) and Salada de tiscar, Puente-Genil , Cordoba (Spain), (Baltanás et al., 1990). For January the limits of the temperature range were determined by records in Mongolia (Van der Meeren et al., 2010) and in Sabkha d'Oran (Algerie) (Gauthier, 1928).

4.2.3.1. Distribution notes

Western Mongolia area:

This area, very sensitive to global climatic change (Shinneman et al., 2009; Van der Meeren et al., 2010), is characterized by a continental and semi-arid climate. The sampling was made during summer 2004-2005 and *E. inflata* was finding in 6 of 55 lake records in association with *Limnocythere inopinata* (Van der Meeren et al. 2010). The recovering places are characterized by high saline waters (TDS 10, 500), T average of 17.9 °C, %Ca 1.0 and Alkalinity 25.46 (Van der Meeren et al. 2010).

Table 1: Modern occurrences of *Eucypris inflata*

Country	Region	Site	locality	longdec	latdec	Reference
Greece	Athens	Vula		23.75	37.83333	Klie_1941
Turkey	Konya_Province	Lake_Musalar		32.86667	38.38333	Altinsacli_&_Griffiths_2001
Spain	Sevilla	Utrera	Zarracatin	-5.8	37.03333	Baltanas_et_al._1990
Spain	Zaragoza	Sastago	Salina_del_Camaron	0.283333	41.43333	Baltanas_et_al._1990
Spain	Ciudad_Real	Pedro_Munoz	Laguna_del_Pueblo	-2.93333	39.41667	Baltanas_et_al._1990
Spain	Cordoba	Puente-Genil	Salada_de_Tiscar	-4.81667	37.41667	Baltanas_et_al._1990
Spain	Cordoba	Puente-Genil	Salada_de_Tiscar	-4.81667	37.41667	Baltanas_et_al._1990
Spain	Zaragoza	Gallocanta	Laguna_de_Gallocanta	-2.18333	40.83333	Baltanas_et_al._1990
Spain	Cuenca	Mota_del_Cuervo	Laguna_de_Manjavacas	-2.86667	39.41667	Baltanas_et_al._1990
Spain	Sevilla	Osuna	Ballestera	-5.16667	37.36667	Baltanas_et_al._1990
Spain	Malaga	Fuente_de_Piedra	LaLaguna	-4.73333	37.1	Baltanas_et_al._1990
Spain	Ciudad_Real	Pedro_Munoz	Laguna_del_Pueblo	-2.93333	39.41667	Baltanas_et _al._1990
Spain	Zaragoza	Sastago	Salina_del_Camar⍰n	-0.28333	41.43333	Baltanas et _al._1990
Spain	Malaga		Cerero	-3.69583	37.04556	Baltanas et al._1990
Spain	Malaga		Ratosa	-3.57361	37.20778	Baltanas et _al._1990
Spain	Malaga		Salada	-3.71833	37.04556	Baltanas et al._1990
China	West	Qinghaihu		100.8358	37.00194	OMEGA_Chinese_dataset
China	West	L.Nanhongshanhu		80.06667	35.16667	OMEGA_Chinese_dataset
China	Northwest	L. (Haiding Nuur)		92.97936	35.65056	OMEGA_Chinese_dataset
China	Northwest	Kunlun mountain		93.02631	35.61542	OMEGA_Chinese_dataset
China	Northwest	Kunlun mountain		93.02758	35.61892	OMEGA_Chinese_dataset
China	Northwest	Kunlun mountain		93.03194	35.6205	OMEGA_Chinese_dataset
China	Northwest	Kunlun mountain		93.03308	35.61617	OMEGA_Chinese_dataset
China	Northwest	Kunlun mountain		93.17378	35.62978	OMEGA_Chinese_dataset
China	Northwest	Kunlun mountain		93.18017	35.61106	OMEGA_Chinese_dataset
China	Northwest	Kunlun mountain		93.18911	35.62072	OMEGA_Chinese_dataset
China	Northwest	Kunlun mountain		93.49786	35.54042	OMEGA_Chinese_dataset
China	Northwest	Kunlun mountain		93.66919	35.52119	OMEGA_Chinese_dataset
China	West	L. Donggi Cona		98.63972	35.2775	OMEGA_Chinese_dataset
Mongolia		AIRIG		93.37	48.91	Van der Meeren et al.2010
Mongolia		OIGON		96.62	49.21	Van der Meeren et al.2010
Mongolia		KHOLB2		97.14	49.02	Van der Meeren et al.2010
Mongolia		TELMEN		97.3	48.87	Van der Meeren et al.2010
Mongolia		TELMEN		97.3	48.87	Van der Meeren et al.2010
Mongolia		TSAGN1		95.28	48.94	Van der Meeren et al.2010
Mongolia		KHOIN1		91.07	49.71	Van der Meeren et al.2010
Mongolia		USHGIN		94.62	49.02	Van der Meeren et al.2010
Mongolia		KHRUSN		92.22	48.35	Van der Meeren et al.2010
Turkey		Igneada	Lake Mert	27.96096	41.8582	Altinsacli _2000
Turkey		Igneada	Lake Mert	27.9715	41.86518	Altinsacli _2001
Turkey		Igneada	Lake Hamam	27.96865	41.82626	Altinsacli _2002
Turkey		Igneada	Lake Hamam	27.96186	41.82515	Altinsacli _2003
China	Northeast	Qinghaihu	Lake Qinghai	100.1333	37	Fan Yang_ 1988
Ukraine	Southeast	Black sea	Sea of Azov	37	46	Schornikov _1964
Russia	Omsk			73.36667	54.98333	Sars_ 1903
Kazakhstan	Akmola	Astana	Lake dzher-sor(?)	71.43333	51.16667	Sars_ 1904
Kyrgyzstan			Lake Isszk-Kul	77.30713	42.36878	Bromstein_1929
Algerie			Sebkha d'Oran	-0.7723	35.54208	Gauthier _1928
Kazakhstan			Aral Sea	60	45	Aladin et ali_2008
Turkey	Aegean Sea	Gokceada island	Salt lake	25.96583	40.12833	Kilic_2000
Ukraine			Lake Bakalskoye	33.16782	45.74773	Shadrin_2013
Sahara				-	-	De Dekker_1981

China:

Lake Quinghai is a brackish water lake located on the arid, high-altitude northeastern Qinghai–Tibet Plateau in China. *E. inflata* was found here, during a summer sampling (T_{air} mean 11.4°C), in association with *L. inopinata* as the only two species present (Liu et al., 2009). The samples took at a depth range from 11.5 to 26.5 m show a salinity that vary from 14.4 to 16.5 g L^{-1}. Moreover *E. inflata* was recover in the satellite lakes of Lake Quinghai, in shallower and higher salinity waters (0.3 m depth, from 26.8 to 36.3 g L^{-1}), (Liu et al., 2009).

On July and August 2005 and June 2006 during another sampling campaign *E. inflata* have been abundantly found in Lake Quinghai, Lake Gahai and Lake Haiyanwan, in a salinity range from 4.63 to 36.62 g $L^{-1}$and PH 7.2 – 9.2 (Li et al., 2010). It is found in sodium chloride – rich, alkaline environment in association with *L. inopinata* in water with salinity from 5.93 to 26.88 g L^{-1} but it is the only one recovery in the highest salinity (14-36 g L^{-1}) (Li et al 2010).

Aral sea:

The Aral Sea was subjected at a really drastic environmental change caused by the huge withdrawals for irrigation that also caused a variation in the aquatic living fauna. In September 2007, *E. inflata* and *Cyprideis torosa* represented the only two ostracod species recovered in Small Aral (salinity about 17 g L^{-1}) and in Large Aral (salinity 100 g L^{-1}) (Aladin et al., 2008)

Turkey:

A sampling campaign was made during February, May, July and September 1999 in Lake Erikli, Hamam, Mert, Pedina and Saka (Altinsacli, 2000). *E. inflata* was found along all the year, in Lake Mert and Lake Saka. Lake Mert is an ultra-oligotrophic lagoon connected to the sea with a salinity range from 2.5 (during spring) to 6.5 per mil (during autumn) and PH from 6.9 (spring) to 8.3 (autumn). The air temperature at the time of sapling was from 10°C (winter) to 25°C (summer). In this lake *E. inflata* was found in association with *Candona neglecta*, *Heterocypris salina*, *Cyprideis torosa* and *Callistocythere diffusa*. Lake Saka is a freshwater lake (salinity from 0.22, spring, to 0.33 per mil. autumn) without any connection with the Black Sea. During

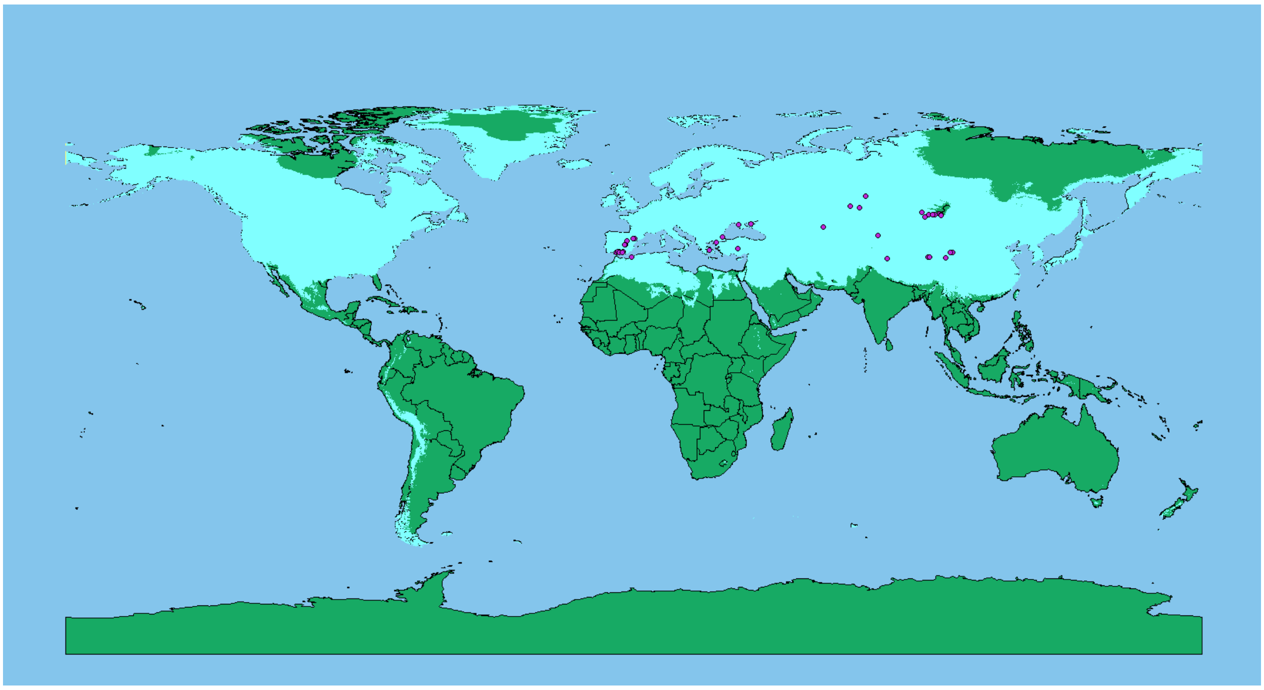

Figure 2: July mean temperature map

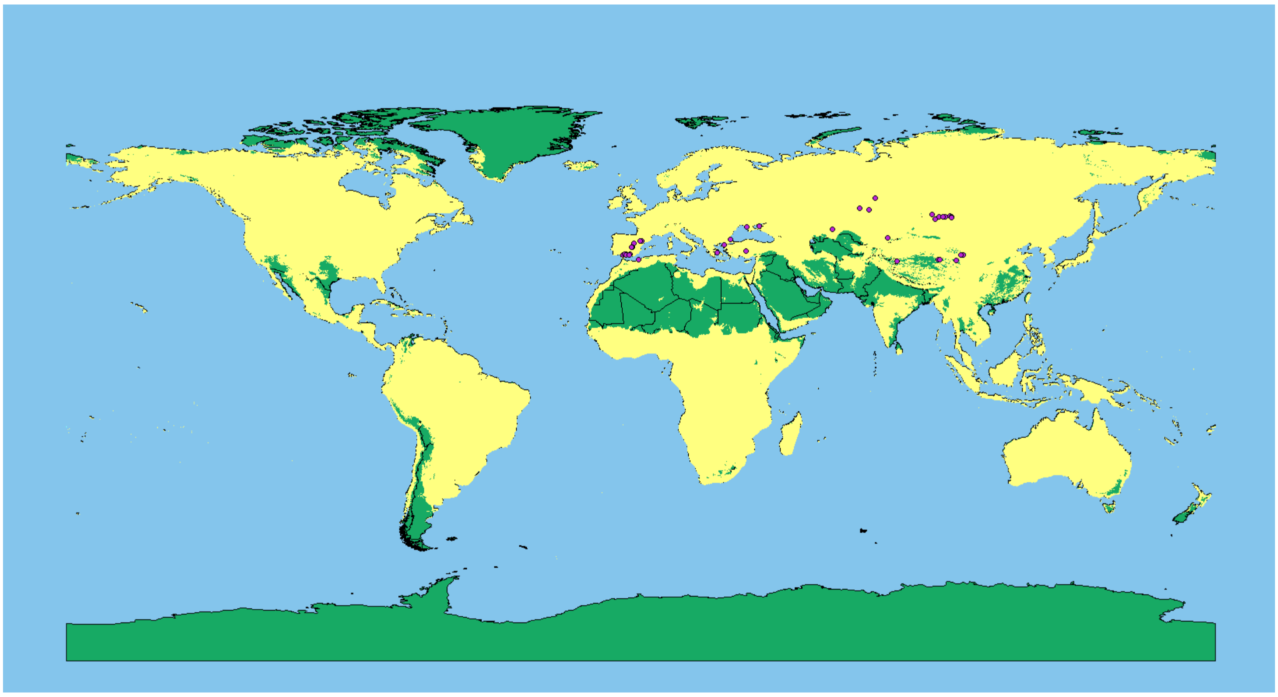

Figure 3: January mean temperature map

sampling PH slightly change during year from 7.13 in autumn to 7.91 in winter. The air temperature vary from 10°C (winter) to 25°C (summer). Ostracod assemblages were here represented by *E. inflata, Candona neglecta, Cypridopsis vidua* and *Heterocypris salina.* Kiliç et al. (2000) found living *E. inflata* during summer in Salt Lake, Gokceada Isaland in a salinity of 33.7 per mil and T of 23°C.

Ukraine:

E. inflata was found by Schornikov (1964), in the Sea of Azov, Southeast Ukraine, in salinity ranging from 2.8 to 106 per mil. Subsequently, during the period ‘2008-2012’ Shradin (2013) made a sampling campaign in Lake Bakalskoye were *E. inflata* was found as the only living ostracod in strong association with *Artemia* and Herpacticoida. It was recovered in water with a very wide salinity range (from 20 to 106 per mil.) and PH values from 7.52 to 9.7 and

Algerie:

Gautier, (1928), collected *E. inflata* in brackish water swam (Sebkha d’Oran), with a salinity range from 5 to 30 per mil.

Spain:

Baltanas et al., 1990, collected samples during winter (January or February) and spring (March or May) of 1986-1987. *E. inflata* was found in Lake Gallocanta, Lake Pueblo, Lake Salada de Tiscar, Lake Ballestera, Lake Ratosa, Lake Fuente de Piedra, Lake Salada and Lake Zarracatin as the only species present and in Lake Manjavacas and Cerero in association with *Heterocypris barbara inermis*. It is present in a salinity range from 12.9 to 80.9 per mil and strongly associated to sodium-chloride waters.

4.2.4 Conclusions

The recovery of *Eucypris inflata* in such different geographic areas, from cold temperature in Western Mongolia (Van der Meeren et al. 2010), to warm and arid area as Iberic Peninsula (Baltanas et al., 1990) suggest that its presence is not strongly dependent on temperature. In fact the temperature range inferred using the MOTR method results quite wide.

It has been demonstrated it can lives in both brackish (Gautier, 1928; and Liu et al., 2009) and freshwater (Altinsacli, 2000) environments indicating its ability to resist at wide salinity range (Gauthier, 1928; Shornikov, 1964; Bronstein, 1947). However it is one of the few species that can inhabit water with salinity up to 110 per mil (Loffler, 1961 and N. Aladin et al, 2008). It is found in association with *Limnocythere inopinata, Candona neglecta* and *Heterocypris salina* in salinity ranging from 0.22 to 16.5 g/l (Altinsacli, 2000, Van der Meeren et al. 2010, Liu et al., 2009 and Li et al., 2010) but only with *Cyprideis torosa* and *Heterocypris barbara inermis* in salinity up to 100 per mil (Altinsacli, 2000 and Baltanas et al., 1990). Baltanas et al., 1990, affirms in addition that it prefers high chloride content waters, suggesting that the water chemistry plays a fundamental role in the distribution of *E. inflata.*

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CHAPTER 5

Lake Trasimeno (central Italy): A record of society, environment and climate interactions during the Holocene*

The endorheic nature of Lake Trasimeno (central Italy) in combination with its central position makes it a relevant site to unravel spatial differences of the Holocene climatic variability in the Mediterranean area. Herein, we present a high-resolution study of the ostracod record of an 8.59 m long sedimentary core covering the entire Holocene period along with existing historical data, which allows us to separate anthropogenic from climatic signals. The occurrence, abundance, and vanishing of ostracod species is directly controlled by lake level variations, which are in turn related to global and regional climatic changes (i.e. moisture variations). The total organic carbon content as well as observed lithological changes provide additional information about Lake Trasimeno's conditions in the past, including the hydrologic regime and nutrient availability. Most important variations have been identified at ca. 10,000 cal yr BP, when the lacustrine basin changed from a temporary to a permanent waterbody (from *S. aculeata* to *C. angulata* association). The highest lake level and the total absence of ostracods occurs at around 9,000 cal yr BP, whereas a decreasing trend started at ca. 4,200 continuing until present day (*angulata* - *torosa* and *stevensoni* associations). The frequency of changes in the relative abundance of the main species (i.e. *C. angulata*, *C. torosa* and *D. stevensoni*) shows centennial variations. The almost unsuccessful human interventions on the lake hydrology revealed by historical documents, allow Lake Trasimeno to record an almost pristine climatic signal during most of the Holocene epoch, which is quite unusual in the highly populated Mediterranean area.

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* ***A modified version will be submitted soon to The Holocene***: Marchegiano M., Francke A., Gliozzi E., Wagner B., Ariztegui D. Lake Trasimeno (central Italy): A record of society, environment and climate interactions during the Holocene.

5.1 Introduction

Since the time of ancient societies, climate has played a fundamental role for their origin and development. The need to better understand the relationship between climate, environment reaction and human activity thus has become increasingly significant. In particular the Mediterranean region has always been considered as one of the outstanding places for palaeoclimate and palaeoenvironmental studies as it was the scenario of the development of major civilizations. Moreover, because of its transitional position between temperate mid-latitude and subtropical climates, its relatively small size and semi-enclosed nature, the basin is very sensitive even to low-amplitude climate variations as well as to anthropogenic influences (Lionello, 2012). The predicted model of future climate for this area suggests warmer and drier conditions (Giorgi and Lionello, 2008) which will have a significant impact on biodiversity, water resources and human activity. In this framework, the study of former climatic conditions is critical to model and predict possible environmental reactions to these changes. Holocene palaeoenvironmental changes are the combined results of both climate and human activity, hence, providing the opportunity to disentangle and differentiate these two signals (Aimers, 2011; Izdebski et al., 2016; O'Sullivan, 2008).

The last decades have shown a raising public awareness concerning the impact of climate on human activities and, as a result this research field became one of the most debated scientific topics. Several studies attempted to reconstruct the Holocene climatic variations in the Mediterranean area, revealing its large spatial and temporal variability (i.e. Magny et al., 2011, 2003; Roberts et al., 2012). To resolve this complexity it is important to have a larger number of high-resolution palaeoclimatic records than those available today. Former lake level reconstructions and pollen analyses from Italy (summarized in Magny et al., 2013; Peyron et al., 2017) produced key results establishing different hydrological and seasonal patterns between the northern and southern part of the central Mediterranean identifying a climatic boundary around lat. 40°N. The geographical position of Lake Trasimeno (central Italy, lat. 43°N) is thus pivotal to better constrain this boundary and to improve the possible spatial and temporal changes in the impact of climate during the Holocene.

High-resolution studies of lacustrine sediments provides exceptional archives of past climatic variability (Cohen, 2003). In particular, endorheic, shallow lakes are sensitive to local environmental changes, as their water volume frequently strictly depend on local meteorological conditions (i.e. evaporation/precipitation ratio) (Lauwaet et al., 2012; Soja et al., 2013), thus representing ideal sites to study the impact of climate (Ariztegui et al., 2008). In this study, ostracod assemblages were used as proxy to provide a detailed palaeohydrological reconstruction of the shallow, endorheic Lake Trasimeno from ca. 11, 700 cal yr BP until present days. Although Holocene ostracod studies are available from different Mediterranean sites (Anadón et al., 1994; Belis et al., 1999; Curry et al., 2016; Marco-Barba et al., 2013; Mazzini et al., 2016; Rossi et al., 2015) our study is the first in central Italy recording high-resolution climatic changes throughout the Holocene until present days. Ostracods are well known to be a useful proxy as their distribution, assemblage composition, and abundance are strongly dependent on several physicochemical factors such as temperature, water depth, salinity and other abiotic and biotic parameters (Baltanás et al., 1990; Keatings et al., 2010; Marquez et al., 2016). Nevertheless, the complexity of the climatic signals requires the use of a multiproxy approach (i.e. Ariztegui et al., 2008) hence, we have also used total organic carbon (TOC) and lithological data to support the palaeoclimatic reconstruction based on ostracods.

The main goal of this contribution is to provide a high-resolution palaeohydrological reconstruction of central Italy during the Holocene. This will be attained using a multiproxy approach to enhance the present knowledge on Mediterranean climate variability throughout time. The latter will allow an improvement of our understanding about low lacustrine systems react to on-going climatic changes. This research will also highlights the importance of ostracod records as proxies to detect climatically driven variations of inland waterbodies. The comparison of the Lake Trasimeno record to other regional archives (i.e. pollen and lacustrine sediments) will provide evidence of the local and global significance of the recorded climatic variations. Moreover, the high number of historical documentation, describing the human population at Lake Trasimeno’s shores and surroundings, allowed us to confront the proxy results with known human interventions to separate climatic from anthropogenic signals.

5.1.1. Major Holocene climate events in the Mediterranean area

Recent high-resolution records discredit the general idea of the Holocene being a stable climate epoch, revealing the existence of significant decadal to centennial climate variability superimposed on millennial variations (Abrantes et al., 2012). The beginning of the Holocene is generally represented by the so called Holocene Climatic Optimum (HO, between ca. 10, 000 and 6,000 cal yr BP) caused by strong insolation in the Northern Hemisphere (Berger, 1978) and characterised, in the extra-tropical areas, by maximum temperatures and precipitations (Gornitz, 2009). However, dryer centennial-periods at ca. 12,000, 8,200 and 5,200 cal yr BP interrupted this long-term climatic trend (Gasse, 2000). Among them, the so-called cold 8.2 ka event, which was probably due to a reduction of the Atlantic Mediterranean Overturning Circulation (AMOC) (Alley and Àgústsdóttir, 2005; Wiersma and Renssen, 2006) or to atmospheric processes (Ariztegui et al., 2000), is one of the strongest recorded events (Alley et al., 1997). At around 6,000 cal yr BP, because of the probable suppression of the monsoonal circulation system responsible for tropical rainfall (Gasse, 2000), started a trend of progressively cooler and drier conditions (Kim et al., 2004 and Marchal et al., 2002). As during the early Holocene, the middle-late Holocene was also affected by short-term secular variations. Bond et al. (2001) proposed the intensity of solar activity as the cause of these short duration climatic oscillations associating periods of less intense solar activity to colder conditions. Two time intervals have been particularly well dated and recognized worldwide during the late Holocene, the warm and dry Mediaeval Climate Anomaly (MCA; ca. 1000-1250 AD) and the cold and humid Little Ice Age (LIA; ca. 1250-1850 AD). During the latter global temperatures decreased progressively reaching a minimum around 1600 AD, which subsequently increased and have been substantially intensified during the last 150 years under the at least partial influence of anthropogenic activities (Gornitz, 2009).

However, differences in magnitude and intensity of these intervals have been identified in many regions such as in the Mediterranean Area due to local conditions. For instance remarkable differences have been observed during the early Holocene when a major increase in regional rainfall was recorded in the eastern Mediterranean suggesting an east-west partition (e.g. Guiot and Kaniewski, 2015; Kotthoff et al., 2008; Roberts et al., 2001, 2012; Magny et al., 2013). Furthermore, the western Mediterranean showed low differences in moisture between the Holocene Climatic Optimum and the 8.2 ka event, while larger differences were found in the eastern part of the basin (Abrantes et al., 2012). More in particular, dry conditions persisted in the central and eastern Mediterranean regions and northern Aegean Sea during the 8.2 event south of lat. 42°N (Ariztegui et al., 2000; Fletcher et al., 2007; Magri, 1999; Tinner et al., 2009). On the contrary, records located north of 42°N suggested an increase in precipitation (Magny et al., 2011). Furthermore, a more recent study reports that the 8.2 ka event was not really distinguishable in the central Mediterranean (Magny et al., 2013). The east-west bipolar climate in the Mediterranean region was still present during the Late Holocene (Roberts et al., 2012 and references therein). In fact, the western part (i.e. Spain and Morocco) was characterized by arid conditions during the period almost contemporaneous to the MCA whereas increasing humidity characterizes the LIA. The opposite trend is again observed in the eastern part of the basin (i.e. Anatolia, Greece and Middle East) (Abrantes et al., 2012).

Taking into account more in particular the central Mediterranean area, several palaeohydrological studies summarized in Magny et al. (2003) also indicate considerable latitudinal differences. In particular, south of ca. lat. 40°N the beginning of the Holocene was characterized by very dry conditions, as suggested by the recorded low lake levels; afterwards, from ca. 10,300 to ca 4,500 cal yr BP the climate switched towards more humid conditions followed by a dryer trend up to present days. An opposite pattern was recorded for sites north to ca. lat. 40 °N (Magny et al., 2013).

5.2 Study area

5.2.1 General features and hydrology of Lake Trasimeno

Lake Trasimeno (43°08’N; 12°06’E, lake bottom altitude 251.57 m) is a very shallow (average depth 4.7 m, maximum depth ~6.3 m), endorheic and eutrophic lake located in central Italy. It covers a surface area of 124 km^2, which makes it the fourth largest Italian lake (Fig. 1). Only very few, small tributaries drain into the lake, in particular the Rigo Maggiore, Tresa, Moiana and Maranzano, that were deviated into the artificial Anguillara Channel in 1953, and the natural Paganico and Pescia ditches. During moments of high lake levels, an artificial outflow with a threshold at 257.5 m s.l.m (Charavgis et al., 2012), merges the lake in the south-eastern part of the éake to the river Tiber via the Caina creek (Fig. 1). Because of its endorheic nature and the relative small extension of the lake watershed (396 km^2), the hydrology of the Lake Trasimeno strictly depends on the local climate, resulting in a close connection between local precipitation/evaporation ratio and lake level variations (Dragoni, 1998). This behaviour has caused dramatic floods and droughts throughout the last centuries (Dragoni, 1998). For that reason, human interventions were made in order to control the lake level since Etruscan-Roman times. More recently, an artificial enlargment of the catchment basin was done in 1957-62 aims to solve droughts problems (Fig. 1).

Complete vertical water mixing as well as no clear thermal stratification even in hot summers is observed because of the shallow character of the lake. The spatial homogeneity of physical and chemical parameters (i.e., pH, conductivity, alkalinity, ionic concentration and dissolved oxygen) shows no substantial differences between the marginal and central parts of the lake (refer to Marchegiano et al., 2017b and references therein for more details). Periodic changes of these parameters are strictly correlated with lake level variations. Due to its endorheic nature, variations in the volume of the lake water volume are in fact translated into changes in salinity and water chemistry (Ludovisi and Gaino, 2010).

The living ostracod fauna of Lake Trasimeno, studied by Marchegiano et al., (2017b), highlights *C. torosa* and *C.* (*N.*) *angulata* as the most frequent species. Today they are both present in the central part of the lake, where they were the only two occurring species, as well as in the lakeshore area. In the latter areas, they appear along with other ostracod species such as *D. stevensoni*, *L. inopinata*, *I. salebrosa*, *C. vidua*, *I. gibba*, *L. stationis*, *H. helenae*, *H. salina* and *H. incongruens,* indicating more diversified ostracod assemblages in shallower parts of the lake. Similarly, macrophytes are only observed in coastal zones while they are totally absent in the central and deeper area of the lake, probably due to the increasing water turbidity (Heavens et al., 2009 and Marchegiano et al., 2017b). This turbidity has increased since 1960 following the deviation of the Valdichiana ditches into the lake (Charavgis et al., 2012), preventing

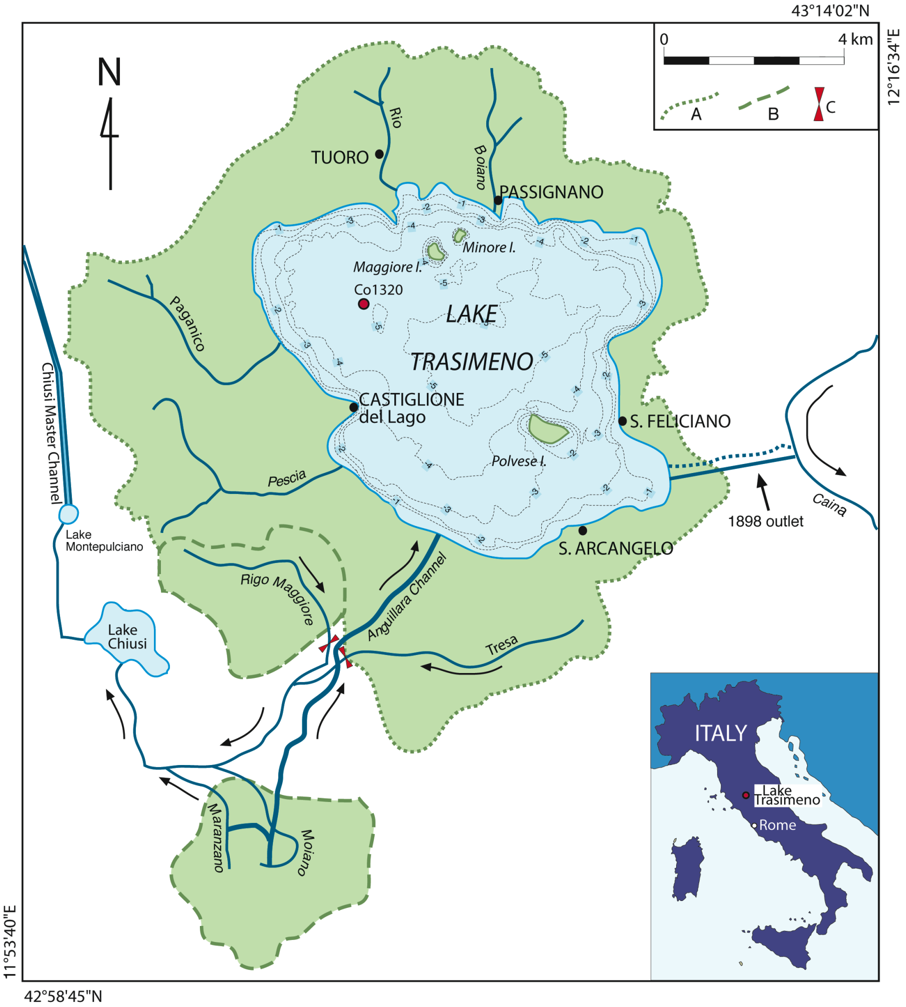


Figure 1: Map of Lake Trasimeno showing the location of core Co1320. Legend: A. natural catchment area; B. artificially-joined basins; C. sluice gates of the artificially-joined channels (from Marchegiano et al., 2017a).

the light to reach the substratum in the deepest part of the basin and thus the development of macrophytes. Historical data record changes in the distribution of the macrophytes linked to lake level variations, suggesting their reduction to ca. 30% during highstand periods (Havens et al., 2009). Moreover, due to human interventions on the lake hydrology and increasing agricultural activities, a decrease in macrophyte biodiversity was noticed between 1960s and 2007 (Charavgis et al., 2012).

The Total Organic Carbon (TOC) content in the surface sediments seems to follow the macrophytes distribution, being higher along the coast and decreasing towards the central part of the lake (Marchegiano et al., 2017b). However, the differences recorded in the TOC values could be also linked to several processes occurring outside the lake, such as allocthonous organic matter supply, changes in the nutrients input caused by the agriculture activity affecting the lake productivity, and/or decomposition after deposition (Siffedine et al., 2011).

5.2.2 Human activities in the Lake Trasimeno catchment

Traces of the first settlements in the surroundings of Lake Trasimeno are recorded since the Neolithic-Eneolithic age (7,500-7,000 cal yr BP; 5500-5000 BC) (Gambini, 1995; Moroni et al., 2015), represented by scattered pottery and lithics. Only during the late Bronze Age (ca. 1350-1000 BC) true settlements on the lakeshore of San Savino and Panicarola areas were documented by the recovery of wooden piles (De Angelis et al., 2014; Gambini, 2000; Moroni et al., 2015).

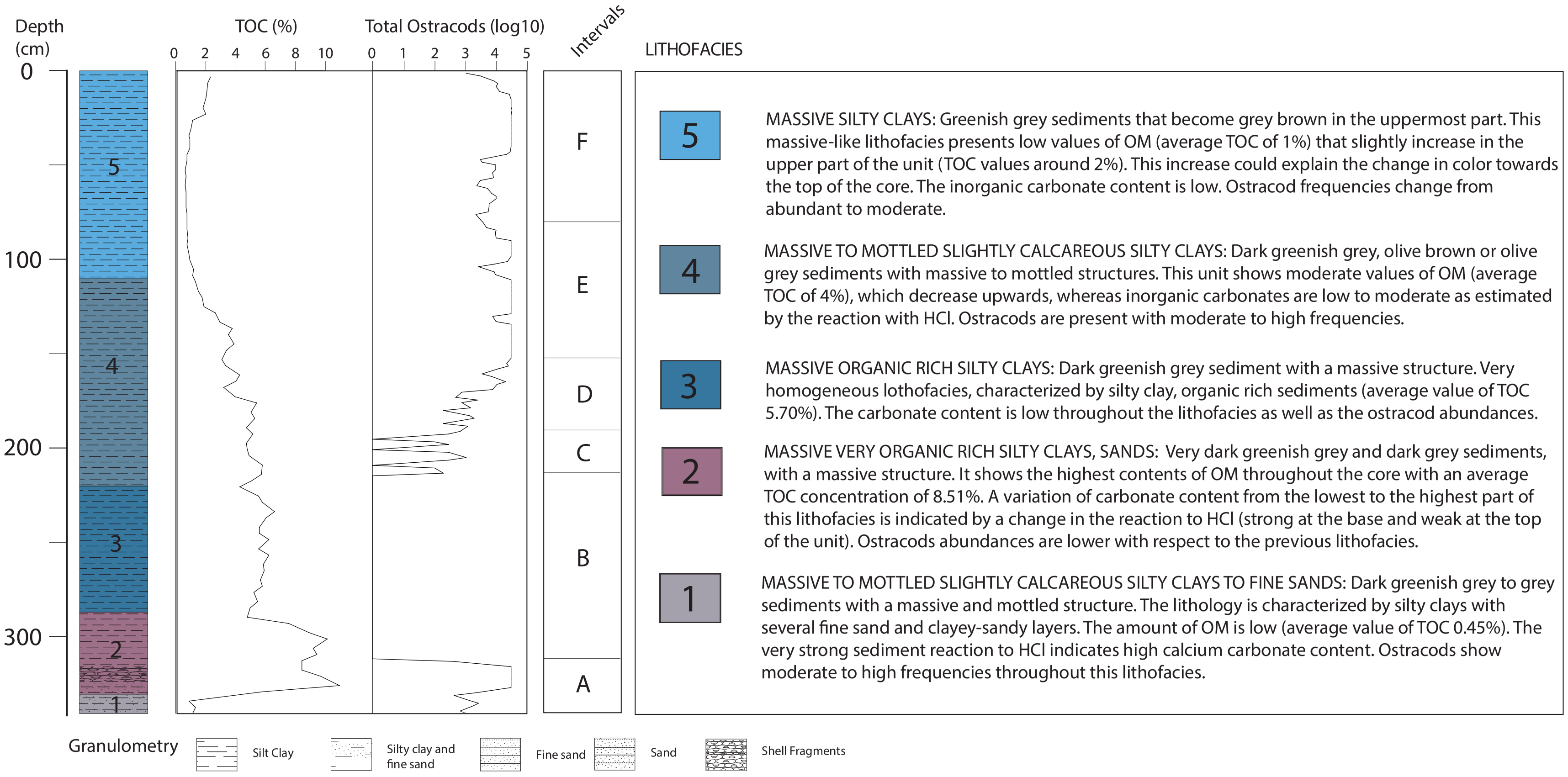
The first human interventions to regulate the hydrology of Lake Trasimeno occurred likely during Etruscan times. Remains of an ancient channel with a bottom height lower than the present day (i.e. 257,33 m s.l.m) were found during the construction of the modern artificial outlet carried out at the end of the 19th century. Information about this ancient outlet was reported by Strabo (Geography, book V-4) and Titus Livius (Ab Urbe Condita, books XX-XXV). The recovery of ancient coins with the portrait of Emperor Claudius at the archaeological site testifies that the outlet was maintained in function by the Romans, probably until the beginning of the Early Middle Ages (Gambini, 1995; 2000). Afterwards, as recorded by several historical documents, it was completely obstructed by sediments due to the lack of maintenance (Gambini, 1995). The established agricultural activities around the lakeshore were endangered, particularly by the floods that occurred in 1195 AD (Pellini, 1664), during the 15th century (Campano, 1457) as well as the ones at the end of the 16th century including catastrophic floods of the Arno and Tiber rivers (Gambini, 1995). Indeed, some human interventions were made in this period to avoid the lake inundations. The first one was the construction of a new artificial outlet in 1421-22 AD by Fortebraccio da Montone, landlord of the area, in order to keep the lake level low. This outlet was subsequently restored several times (1425 AD, 1431 AD, 1467 AD) but due to its small size, it had never the capacity to prevent major flood events (Gambini, 1995). Two tributaries of the Lake Trasimeno, the ditches Tresa and Rigo Maggiore, were deviated towards the westward Valdichiana basin in order to decrease the amount of waters reaching the lake. Unfortunately, this intervention was not successful (Gambini, 1995) as shown by the frequent inundations that occurred during the 18th and 19th centuries (1722-1732 AD; 1753 AD; 1762-1773 AD until 1821 AD; 1881 AD; 1887 AD). The most recent inundations encouraged the local authorities to intervene in order to avoid further damage on agricultural areas by flooding. Thus, the new "Pompili" artificial outlet was built in 1889 AD with a hydrometric zero at 258.42 m s.l.m., subsequently further lowered to 257.33 m s.l.m (Gambini, 1995). This intervention was efficient in limiting the highstands but did not solve the problems of the dry periods during which the lake level dramatically decreased, causing significant damages to the local fishery. In 1953 AD, in an attempt to solve this problem, the ditches Tresa, Rigo Maggiore, Moiano and Marezzano were re-directed to the lake via the artificial "Anguillara" inlet. However, as shown by the measurement of the several recent lake level lowstands, these interventions did not have the potential to solve the drought situation in the future.

5.3 Material and Methods

During two sampling campaigns in September and November 2014, an 859 cm long sedimentary core (Co1320; 43° 09.624'N, 12° 03.491'E) was retrieved at ~4.9 m water depth from a floating platform using a gravity piston corer (UWITEC®, see location in Fig. 1.). The core was split lengthwise in the laboratory. While one half was archived, the other half was used for visual lithological inspection and then subsampled for geochemical and micropaleontological analyses.

The chronology model was developed using ten radiocarbon ages. The calculated ages imply that the whole core Co1320 covers a time interval between 47,100 cal yr BP and present days (Marchegiano et al., 2017a). The ostracode record of the Late Pleistocene part of this sedimentary core (from ca. 47,100 to 9,000 cal yr BP) was already published by Marchegiano et al., in 2017a). This contribution focuses on the Holocene section of the record (from ca. 11,700 cal yr BP to the present day) (Fig. 2).

Samples for geochemical analyses, taken every 4 cm, were freeze-dried, homogenized, and grounded to <63 µm. An aliquot of 10 mg was dispersed in 10 ml DI water for analyses of the total organic carbon (TOC) content on every other sample using a DIMATOC 100 carbon analyzer (Dimatec Corp., Germany). For micropaleontological analyses, 164 samples (including 52 barren samples) were taken at a 2 cm resolution and treated with 5% H_2O_2 for one hour to remove organic matter from the sediments. Subsequently, the samples were wet-sieved to 25 µm using deionized water, and finally, oven-dried at 40C°. In each sample, 300 juvenile and adult ostracod valves were manually picked using a fine brush and deionized water under the stereomicroscope. The picked dry sediments were weighted and the abundances were normalized to 1g of dry sieved sediment. The species were identified following Meisch (2000) and Fuhrmann (2012).



MASSIVE SILTY CLAYS: Greenish grey sediments that become grey brown in the uppermost part. This massive-like lithofacies presents low values of OM (average TOC of 1%) that slightly increase in the upper part of the unit (TOC values around 2%). This increase could explain the change in color towards the top of the core. The inorganic carbonate content is low. Ostracod frequencies change from abundant to moderate.

MASSIVE TO MOTTLED SLIGHTLY CALCAREOUS SILTY CLAYS: Dark greenish grey, olive brown or olive grey sediments with massive to mottled structures. This unit shows moderate values of OM (average TOC of 4%), which decrease upwards, whereas inorganic carbonates are low to moderate as estimated by the reaction with HCl. Ostracods are present with moderate to high frequencies.

MASSIVE ORGANIC RICH SILTY CLAYS: Dark greenish grey sediment with a massive structure. Very homogeneous lothofacies, characterized by silty clay, organic rich sediments (average value of TOC 5.70%). The carbonate content is low throughout the lithofacies as well as the ostracod abundances.

MASSIVE VERY ORGANIC RICH SILTY CLAYS, SANDS: Very dark greenish grey and dark grey sediments, with a massive structure. It shows the highest contents of OM throughout the core with an average TOC concentration of 8.51%. A variation of carbonate content from the lowest to the highest part of this lithofacies is indicated by a change in the reaction to HCl (strong at the base and weak at the top of the unit). Ostracods abundances are lower with respect to the previous lithofacies.

MASSIVE TO MOTTLED SLIGHTLY CALCAREOUS SILTY CLAYS TO FINE SANDS: Dark greenish grey to grey sediments with a massive and mottled structure. The lithology is characterized by silty clays with several fine sand and clayey-sandy layers. The amount of OM is low (average value of TOC 0.45%). The very strong sediment reaction to HCl indicates high calcium carbonate content. Ostracods show moderate to high frequencies throughout this lithofacies.

Figure 2: Lithology, Total Organic Carbon (TOC) and ostracod abundance (log(10) n° individuals) of the core Co1320. Intervals from A to F are referred to the palaeoclimatic and palaeoenvironmental reconstruction described in the text (Discussion). Lithofacies were subdivided based on the visual core description, the organic matter (OM) and the calcium carbonate content (reaction with 10% HCl).

Cluster analysis in Q-mode was used to determine sample groups based on their ostracod community composition. In addition, a constrained-based cluster analysis was performed to define the sequence of the different biozones along time. The statistic calculations were performed with the PAST program (Hammer et al., 2001); version 3.14 using a matrix with the ostracod percentage abundances, excluding taxa with a relative percentage below 2% in each sample. The Bray-Curtis dissimilarity and the un-weighted pair group method using arithmetic average-UPGMA was chosen, as it yield highest cophenetic correlation coefficient (c) (Sokal and Rohlf, 1962; Mouchet et al., 2008). We also tested Morisita, Chord and Euclidean algorithms providing the same dendrograms, which confirms the correctness of the output clustering.

5.4 Results and discussion

The Holocene section of core Co1320 contains 12 ostracod species referred to 11 genera, represented by both adult and instars (Fig. 3): *Darwinula stevensoni* (Brady & Robertson, 1870), *Candona* (*Candona*) *candida* (O.F. Müller, 1776), *Candona* (*Neglecandona*) *angulata* G.W. Müller, 1900, *Ilyocypris* sp. (only instars), *Eucypris mareotica* (Fischer, 1855), *Herpetocypris helenae* G.W. Müller, 1908, *Heterocypris salina* (Brady, 1868), *Cypridopsis vidua* (O.F. Müller, 1776), *Sarscypridopsis aculeata* (Costa, 1847), *Limnocythere inopinata* (Baird, 1843), *Cyprideis torosa* (Jones, 1850) and *Amnicythere* sp. The most abundant among them are *C. torosa*, *C.* (*N.*) *angulata*, and *D. stevensoni*, while *L. inopinata* is subordinated but well distributed along the core. *S. aculeata* and *H. salina* occur only in few samples at the base of the Holocene, where *S. aculeata* is the dominant species. The other species are scattered, with low to very low abundances.

The dendrogram resulting from the cluster analysis performed in Q-mode on the ostracod percentage frequency matrix (cophenetic correlation coefficient = 0.80) is reported in Fig. 4. Five clusters are separated at a significant similarity value around 0.5.

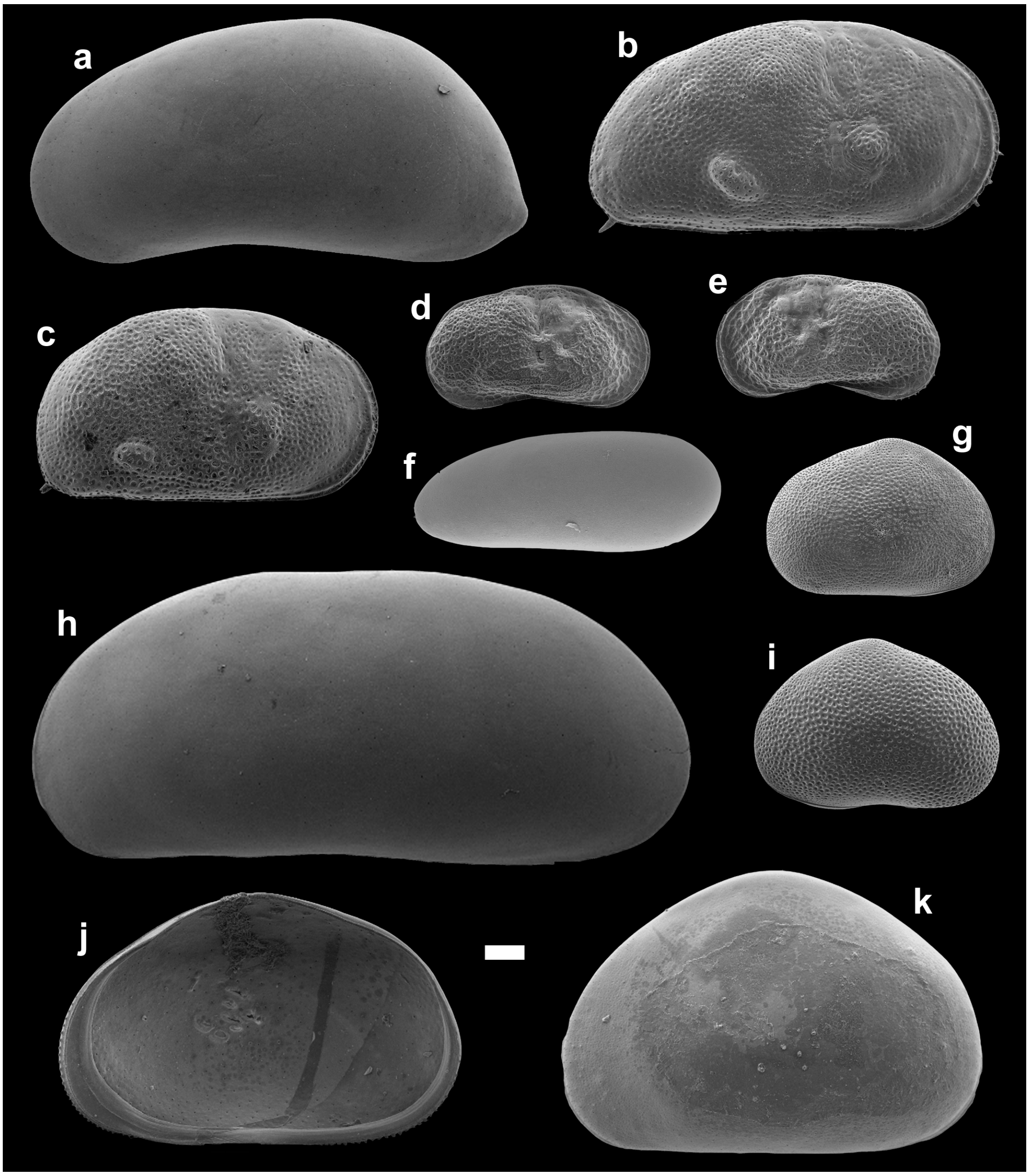


Figure 3: SEM pictures of selected ostracod valves. a. *Candona* (*Neglecandona*) *angulata*, female left valve, external view, sample 227; b. *Cyprideis torosa*, male right valve, external view, sample 220; c. *Cyprideis torosa*, female right valve, external view, sample 220; d. *Limnocythere inopinata*, female right valve, external view, sample 220; e. *Limnocythere inopinata*, female left valve, external view, sample 227; f. *Darwinula stevensoni*, female left valve, external view, sample 220; g. Sarscypridopsis *aculeata*, female right valve, external view, sample 227; h *Herpetocypris helenae*, female left valve, external view, sample 220; i. *Sarscypridopsis aculeata*, female left valve, external view, sample 227; j. *Heterocypris salina*, female right valve, inner view, sample 227; k. *Heterocypris salina*, female left valve, external view, sample 227. White bar corresponds to 0.1 mm.

Cluster 1 includes 7 samples (samples 228-222 from 338 to 324 cm) collected at the base of the Holocene portion of the sediment core. The dominant species is *S. aculeata*, accompanied by *H. salina* whose frequencies decrease towards the top of the cluster. *C.* (*N.*) *angulata* and *L. inopinata.* are also present showing an opposite trend.

Cluster 2 characterizes three samples (samples 149-148 from 216 to 212 cm and sample 141 at 199,5 cm) with the exclusive presence of *C. torosa*.

Cluster 3 groups the majority of the analysed samples (135 out of 160), in which *C. torosa*, *C.* (*N.*) *angulata* and *D. stevensoni* are the most abundant species, showing alternate dominance, while *L. inopinata* is often present although generally at lower frequencies. In particular, *C. torosa* is the dominant species of the assemblages recovered in the samples from 322 to 189,5 cm, whereas *D. stevensoni* is dominant between 189,5 and 111,5 cm and in the uppermost part of the core (16-0 cm), and *C.* (*N.*) *angulata* between 95,5 and 57,5 cm. In this latter interval, *L. inopinata* reaches its highest frequencies (10-25%).

Cluster 4 includes 7 samples (sample 221 at 324 cm and samples 82-77 from 83,5 to 71,5 cm) whose ostracod assemblages are absolutely dominated by *C.* (*N.*) *angulata* (55-92%) while the other species are by far subordinated.

Cluster 5 is made of 8 samples (samples 130-123 from 177,5 to 161,5 cm) in which *D. stevensoni* occurs at very high frequencies (63-93%) accompanied by subordinated *C.* (*N.*) *angulata* and *C. torosa*.

Using constrained-based cluster analyses, the Holocene part of the Co1320 Trasimeno sediment core was subdivided in 6 biozones (Fig. 5). They were described and interpreted hereinafter following the autoecological and synecological characteristics of the species and assemblages (i.e. results of the cluster analyses Q-mode), as well as other metadata such as the lithological and TOC analyses (Fig. 5).

Interval A (338 to 314 cm, 11,700-9,200 cal yr BP) represents the oldest part of the Holocene sediment core. Ostracods are very abundant and can even be detected by naked-eye between 338 and 330 cm sediment depth (Fig. 2). The assemblages are dominated at the bottom of the interval by *S. aculeata* accompanied by *H. salina*

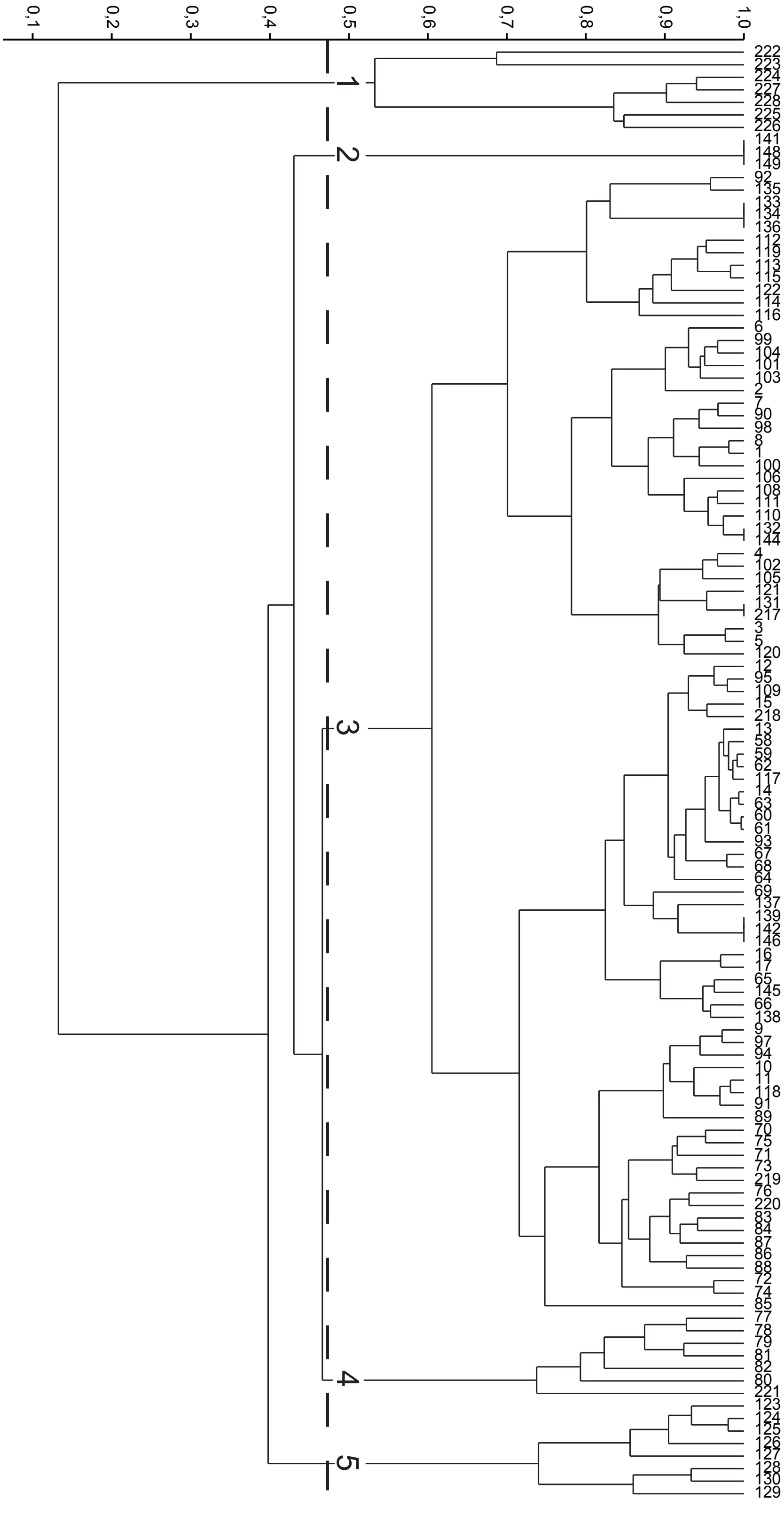


Figure 4: Dendrogram resulting from the cluster analysis in Q-mode (samples) using the UPGMA method and the Bray-Curtis dissimilarity applied to the Holocene samples from the sediment core Co1320. The dotted line indicates the distance level of the separation of the four clusters.

and *L. inopinata* (cluster 1). *C.* (*N.*) *angulata, C. torosa, E. mareotica* and *Amnicythere* sp. are also present at very low frequencies. Further upwards, *S. aculeata*, *H. salina* and *L. inopinata* progressively decrease, and *E. mareotica* and *Amnicythere* sp. disappear, whereas *C.* (*N.*) *angulata* and *C. torosa* become dominant, accompanied by *D. stevensoni*, *C. vidua* and *H. helenae* (cluster 3, 4). The changes in the assemblages observed in Interval A indicate a progressive increase of the lake level, from very shallow temporary conditions to a permanent lacustrine waterbody. The increase of ostracods abundance could be linked to increasing eutrophication as suggested by *D. stevensoni*, *C. vidua* and *H. helenae* that in present day Lake Trasimeno are strictly associated to macrophytes (Marchegiano et al., 2017b). Moreover, the TOC content increases from the bottom to the top of this interval, reaching values of around 11%, which represent the highest content in the entire sediment core (Marchegiano et al., 2017a).

Interval B spans from 314 to 216 cm (9,200-4,200 cal yr BP) and is characterised by the complete absence of ostracods whilst TOC is still high.

Interval C spans from 216 to 189.5 cm (4,200-3,000 cal yr BP; 2260-1050 BC). This interval shows the re-appearance of ostracods even if at low abundances. The assemblages are dominated by *C. torosa* accompanied by *C.* (*N.*) *angulata* (clusters 2, 3), while *D. stevensoni* is almost absent except for a short interval at around 205 cm in which it attains 20%. According to Marchegiano et al. (2017b) the *torosa-angulata* association occurs in modern conditions only in the deepest areas of the lake, deprived of vegetation (e.g. 4-5 m water depth). Therefore, relatively deep-water conditions at the coring location are inferred for this interval. This low ostracod abundance could be explained by a reduction in nutrient availability due to decreasing runoff into the lake. This is also confirmed by the TOC values, which display a decreasing trend around 4-6% on average. This change could be due to drier conditions and thus to low precipitation/evaporation ratio that also caused a lowering of the lake-level.

Interval D (from 189.5 to 163.5 cm; 3,000-1,700 cal yr BP; 1050 BC-220 AD), ostracods are present at low abundance and with assemblages that still testify a decreasing trend of the lake level. The abundance of *D. stevensoni* that is living in modern Lake Trasimeno at around 1.5-2.5 m (Marchegiano et al., 2017b) progressively increases while *C. torosa* and *C.* (*N.*) *angulata* become subordinated (clusters 3, 5). TOC continues to decrease showing average values of around 4%, suggesting lower nutrient supply and runoff from the catchment, and thus, lower

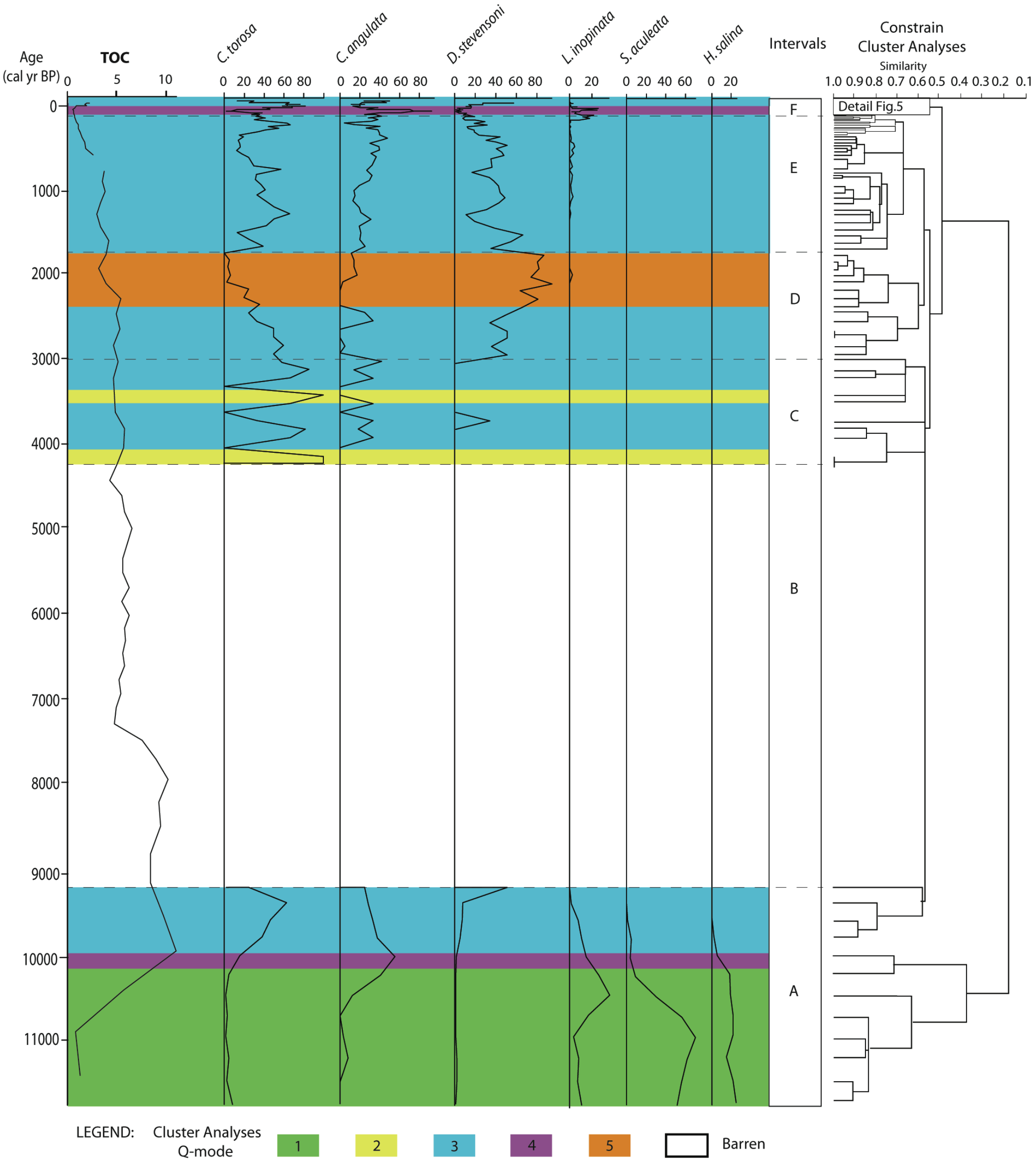


Figure 5: Palaeoenvironmental reconstruction of the sediment core Co1320 using ostracods (percentage curves) plotted against the TOC curve. Rare ostracods species are excluded here because negligible. Refer to the text for the discussion.

precipitation. However, in correspondence of the short peak of C. (*N.*) *angulata,* occurring between 181.5 and 177.5 cm (2,500-2,400 cal yr BP; 580-480 BC), TOC (ca. 5%) increases, indicating an augmentation of the runoff and thus a possible lake level highstand. Further upwards in this interval *D. stevensoni* increases (cluster 5) up to 93% of abundance, suggesting a decrease of the lake level.

Interval E spans from 163.5 to 83.5 cm (ca. 1,700-78 cal yr BP; ca. 220–1872 AD) displaying almost constant ostracod frequencies with only small variability in the relative abundance of species. The occurring species are *C. torosa*, *C.* (*N.*) *angulata*, *D. stevensoni* and, secondarily, *L. inopinata*. In the first part of the interval, until 1080 AD, *C. torosa* and *D. stevensoni* are almost equally represented, suggesting a relatively low lake level. At ca. 750 cal yr BP (1190 AD) they reach 57% and 16%, respectively, probably indicating a small increase in water level. In this lower part of the interval, *C.* (*N.*) *angulata* is subordinated with percentages comprised between 13 and 29%. This species starts increasing in the middle portion of the interval from ca. 700 cal yr BP (1250 AD) (with values around 40-48%) indicating a progressive deepening of the lake accompanied by *D. stevensoni*, while *C. torosa* becomes very scarce. The latter becomes again the most abundant species from ca. 310 cal yr BP (1640 AD) whilst *D. stevensoni* decreases suggesting highstand conditions. Interval E encompasses lower TOC values (i.e. around 3-4% to 1%) with respect to the previous interval D, indicating dry conditions.

Interval F is recognized from 83.5 cm to the top of the core (from ca. 80 to -60 cal yr BP; ca. 1870-2013 AD). In this interval (Fig. 6), all four common species *C. torosa*, *C.* (*N.*) *angulata*, *D. stevensoni* and *L. inopinata* are represented, although the latter is present only in the lower and middle part. The first assemblage (cluster 4), from 1870 to 1899 AD is totally dominated by *C.* (*N.*) *angulata* with a maximum frequency of 92%, pointing to a high lake level. Subsequently, the ostracod associations are dominated by abundant *C. torosa* (on average 70% and up to 82%) while *C.* (*N.*) *neglecta* decreases and *D. stevensoni* is rare until ca. 1940 AD and then starts to increase; the opposite is shown by *L. inopinata* that is more abundant in the lower part and almost disappears at ca. 1940 AD. This middle portion of Interval F records a low lake level that is interrupted around 1980 AD by a short highstand period recorded by the increase of *C.* (*N.*) *angulata* up to around 28-47%. The drastic increase of *D. stevensoni* in the last portion of the sediment core, up to 56%, testifies a prolonged lake level lowstand.

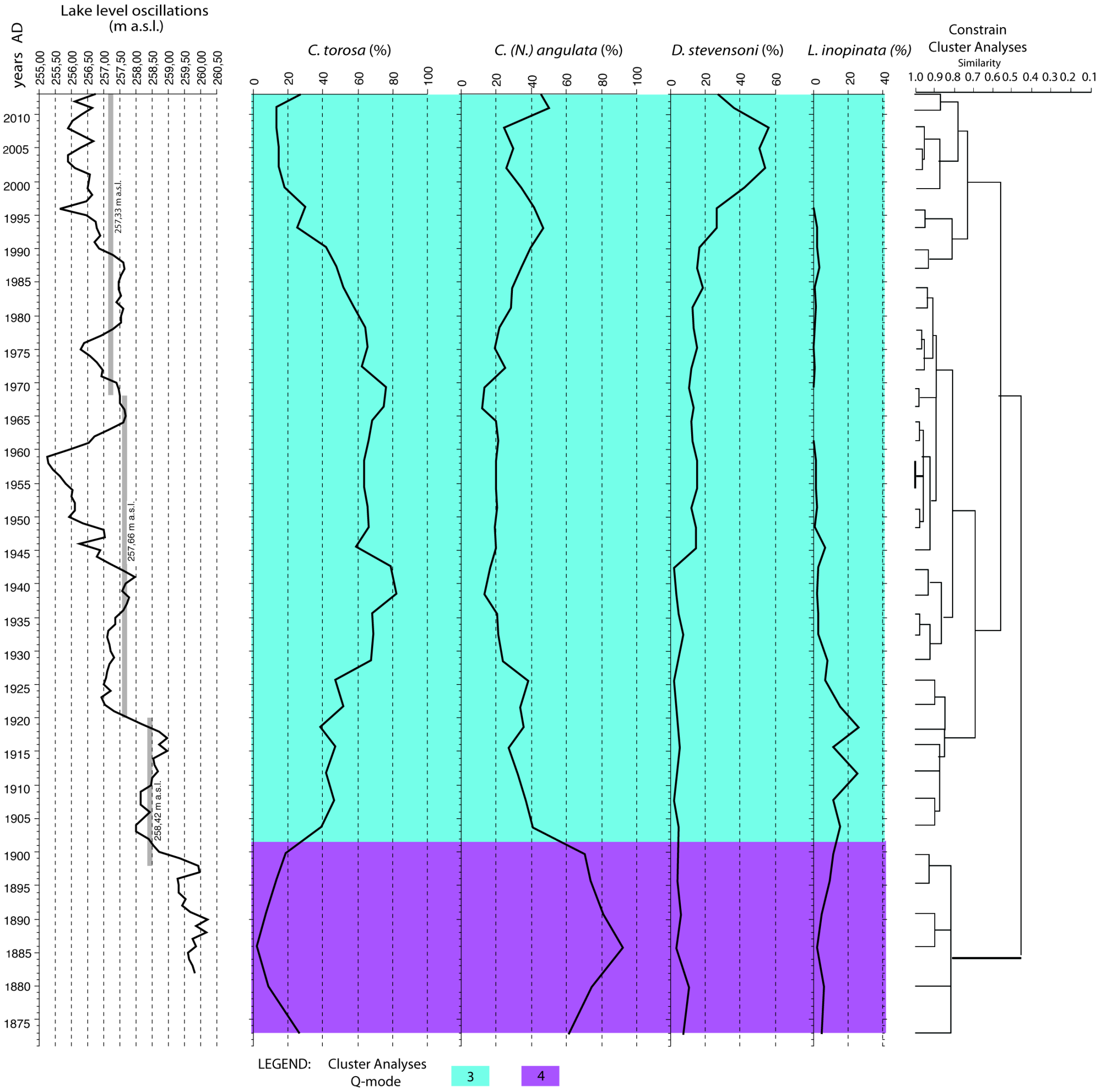


Figure 6: Detailed of the period from ca. 1700 to 2013 AD (Interval F). Ostracod percentage curves were compared with the measured lake level oscillations of Lake Trasimeno (from Gambini, 2005). Refer to the text for the discussion.

5.4.1 Palaeoenvironmental/palaeoclimate interpretation

The analysis of the ostracod assemblages occurring in the Holocene part of the Co1320 sedimentary core along with variations in TOC indicate a sequence of repeated lake level changes. These variations in water level can be explained by the natural reaction of the lacustrine system to regional climate changes as well as to the human interventions on the lake hydrology as recorded by historical documents.

At the Pleistocene/Holocene transition and at the beginning of the early Holocene between 11,700 and 9,200 cal yr BP the *S. aculeata*-dominated ostracod assemblages indicate a very shallow/temporary waterbody, as a consequence of the long dry period that occurred during the MIS 2 (Marchegiano et al., in 2017a). The replacement of the *S. aculeata* assemblage by *C. torosa*, *C.* (*N.*) *angulata*, and *D. stevensoni*, the high ostracod frequencies, and high TOC values are probably linked to the high abundance of macrophytes, documented the gradual establishment of full lacustrine conditions at the end of Interval A. A similar pattern was also recorded at Lago di Mezzano (Lazio, central Italy) at the Pleistocene/Holocene transition, where TOC values increased from 7 to 13% (Ramrath et al., 2000). The increasing TOC values are contemporaneous to a climatic amelioration that enhanced both weathering and runoff in the catchment. Thus, the ampunt of allochtonous OM inscreased as well as the nutrient supply into the lake promoting aquatic productivity.

As already shown in Marchegiano et al. (in 2017a) the ostracod communities indicate that due to the climate amelioration, Lake Trasimeno reached its highest lake level at around 9,000 cal yr BP recording a delay of about 2,700 years with respect to global climate changes as provided by the North Greenland Ice Core temperature record (NGRIP). However, the change from a temporary lake during MIS 2, to a permanent waterbody was already attained at around 10,000 cal yr BP as shown by the shift of the ostracod assemblages from the *aculeata*-dominated assemblages to *C.* (*N.*) *neglecta*, *C. torosa* and *D. stevensoni*. In fact, the latter two species do not tolerate temporary conditions (Griffiths, and Butlin, 1994; Meisch, 2000). A similar change in the ostracod assemblages at the Late glacial/Post glacial transition has also been recognized in other European and Mediterranean lakes. For example, Griffiths and Evans (1995 and references therein) interpreted the change from the *C. candida* to the *M. cordata*-fauna in several lakes of central Europe (Great Britain, Germany, Poland) as the result of increasing ambient temperature, productivity and trophic state. In the Mediterranean area, Belis et al. (2008) observed the switch between the *C. candida* to the *C. ophtalmica* dominated assemblages at Lago Piccolo di Avigliana (northern Italy), suggesting an increase of the lake trophic state. Similar changes were also observed in central Italy at Lake Ripa Sottile (Ricci Lucchi et al., 2000) and at the Valle di Castiglione maar (Gliozzi and Mazzini, 1998; Anadon et al., 2012). In all cases, the Late Glacial/Holocene transition was characterised by the appearance/abundance of more termophilic species and increasing primary productivity, similar as it occurs in Lake Trasimeno. However, only ostracod assemblages of Lake Trasimeno indicate a clear change in water level and a progressive deepening of the lake that is mostly related to changes in the precipitation regime and not in temperature, whereas the other archives record an increase in temperature.

The high lake level conditions continued up to 4,200 cal yr BP as suggested by the high amount of TOC (interval B). Evidence of ostracods absence during the Early-Mid Holocene are also reported in other localities such as the maar lakes of the Eifel region (Scharf, 1993), Lake Gorgo Basso (Curry et al., 2016) and Lake Skhodra (Mazzini et al., 2016). Two main explanations are possible: (a) the shells were dissolved after deposition; b) the ion concentration in the lake water was strongly diluted due to enhanced runoff from the Lake Trasimeno catchment where limestones are absent.

Examples of shell decalcification (hypothesis a) due to high lake productivity are reported by McNabb (1979) at Lake Lansing (MI, U.S.A.), Behbehan et al., 1986 at Lake Attersee (Austria), Scharf (1993) at the Eifel maar lakes (Germany) and Curry et al. (2016) at Preola and Gorgo Basso lakes (Sicily). The last authors suggest that shell dissolution could be due to both low Ca^{2+} water concentration and to elevated levels of dissolved CO_2 related to high primary productivity. In contrast, Belmecheri et al. (2009) suggest a strong calcite undersaturation at Lake Ohrid (Albania) coupled with low pH of the interstitial water into the sediments as the cause of the shells dissolution during the Last Glacial Period.

The high amount of TOC recorded in interval B could support the hypothesis (a). However, the shallow nature of Lake Trasimeno excludes the stratification of the water column and thus the development of anoxia and comparatively low pH even during highstand conditions. Furthermore, the hypothesis of shell dissolution is quite unlikely given the absolute absence of ostracods. Hypothesis (b) is in fact the most plausible since present day data show that Lake Trasimeno water chemistry composition is very sensitive to the precipitation regime. Recent data for instance show that prolonged humid phases could cause changes in the total dissolved solutes (TDS) of the order of 100 mg L^{-1} (Ludovisi and Gaino, 2010). In particular, the chemical analyses of Lake Trasimeno during the 1969 highstand indicated a Ca^{2+} concentration of 1,04 meq L^{-1}, while it doubled this value during the 2005 lowstand (2.03 meq L^{-1}) (Ludovisi and Gaino, 2010). Furthermore, although during the humid climatic phases the runoff from the catchment area was enhanced, and therefore mobilized a major amount of solutes, the surroundings and the substrate of the lake are composed of Oligocene-Miocene silicoclastic turbidites deprived of carbonates (Barchi and Marroni, 2014; Gasperini et al., 2010). The latter is confirmed by the absence of $CaCO_3$ in this lithological unit (Fig. 2) suggesting reduced carbonate precipitation. A similar pattern was also described by Scharf (1993) that ascribed the absence of *C. lacustris* from the post-glacial sediments of the Weinfelder Maar, Gemundener Maar and Pulver Maar (Germany) to the low amount of dissolved Ca^{2+} making these lakes unsuitable for the formation of ostracod shells.

Both previously described hypotheses would imply that humid conditions prevailed at Lake Trasimeno between 9,200 and 4,200 cal yr BP. The high TOC values appear to confirm this hypothesis suggesting enhanced runoff and nutrient supply from the catchment. However, at around 7,200 cal yr BP the TOC values decreased, suggesting the beginning of slightly drier conditions. This interpretation is supported by the general wet conditions recorded for the first part of the Holocene (until 5,000 cal yr BP) (Abrantes et al., 2012) as shown by the high lake levels attained in south European lacustrine basins during the first part of the Holocene (Harrison and

Digerfeldt, 1993). In Italy, lake level highstands and enhanced rainfall during the first part of the Holocene were also recorded at lakes Ledro, Trifoglietta and Pergusa (Peyron et al., 2013) as well as at Lago Velino (De Angelis, 2014). Conversely, other lakes such as Lake Fucino reacted differently and low lake levels are reported for the first part of this period (9,000 to 6,000 cal yr BP; Giraudi, 1998; Giraudi et al., 2011).

Around 4,200 cal yr BP ostracods appeared again, with the deep *torosa-angulata* association (Interval C), which still recorded high lake-level conditions but along a shallowing trend linked to progressively increasing dry conditions. This period (ca. 1350-1000 BC; ca. 3,300-2,900 cal yr BP) witnessed the settlement of late Bronze communities along the Trasimeno lakeshores. In central Italy, De Angelis et al., (2014) reported the change from a permanent to a temporary basin at Lake Velino at around 2000 BC (ca. 4,000 cal yr BP), which promoted Prehistoric human settlements in the area. According to Ramrath et al. (2000 and references therein) and Kelts (1997) a mid-Holocene (between 5,000 and 4,000 cal yr BP) climate shift towards drier conditions has affected all the Mediterranean area. This change in climate has possibly driven some of the major changes in human settlements of archaic cultures in Greece, Mesopotamia and Egypt. A similar trend towards slightly dryer conditions was also recognized at Lake Mezzano by Ramrath et al. (2000) between 5,000 and 4,200 cal yr BP, as well as in lakes Castiglione and Lagaccione, (around 3,500 cal yr BP; Alessio et al., 1996; Bonadonna and Leone, 1995; Magri, 1989), Vico (around 4,000 cal yr BP; Magri and Sadori, 1999), Trifoglietti and Pergusa (after 4,000 cal yr BP; Peyron et al., 2013) as recorded by pollen and lake level reconstructions.

This shallowing trend continued up to the top of the sediment core (Intervals D, E and F) alternating with high lake level oscillations. Some of these oscillations have been linked to alternate dry/humid climatic conditions, such as the short highstand around 2,500 to 2,400 cal yr BP (ca. 580-480 years BC) (Interval D) also recorded at Lake Mezzano (central Italy) by Ramrath et al. (2000); the lowstand conditions attained in Lake Trasimeno around 930-700 cal yr BP (1000 to 1250 AD) that could correspond to the warm and dry MCA (1000 to 1250 AD); and the increase of lake levels from ca. 700-100 cal yr BP (1250 to 1850 AD) during the cool and wet LIA

(Interval E). Conversely, other observed changes in lake level such as the prolonged lowstand phase recorded by interval D shown by the abundance of *D. stevensoni*, are most probably due to the introduction and proper functioning of the Etruscan-Roman artificial outlet. However, dry conditions recorded during the end of Interval C were also observed at the Rieti Plain (central Italy), where the Lago di Ripasottile and Lago Lungo showed a reduced areal extension that started to enlarge again around the beginning of the second millennium (Ricci Lucchi et al., 2000). A shift to wetter conditions starting from ca. 560 cal yr BP (1400 AD), thus slightly later with respect to Lake Trasimeno (i.e. ca. 700 cal yr BP; 1250 AD), is also recorded in pollen records of southern Italy (Sadori et al., 2015) and tree-ring reconstructions by Esper et al. (2007). In its middle portion, interval E mirrors a lake level highstand, shown by increasing valves of *C.* (*N.*) *angulata* that, with some oscillations, has started around 700 cal yr BP (1200 AD) and continued until the first part of the 17th century. The latter is recording lake levels above the artificial threshold, thus confirming the dysfunction of both the Fortebraccio da Montone new artificial outlet and the deviation westwards of the ditch Tresa and Rigo Maggiore waters. Humid conditions in central Italy are shown by the high lake levels attained by Lago di Ripasottile and Lago Lungo which, during the 1600s, were merged into only one lake (Mensing et al., 2016). Thus, notwithstanding human attempts to regulate the lake hydrology, Lake Trasimeno continued to undergo lake level variations mostly ruled by climatic changes at least until 1989 when the new “Pompili” artificial outlet became operational. Even if the chronology of the most recent sediments lacks the required resolution, the changes observed in the ostracod assemblages can be tentatively correlated with existing historical and documentary data. Fig. 6 shows the lake level oscillations measured during the last 150 years as reported by Gambini (1995; 2000) and Burzigotti et al. (2003). They seem to match our proposed trend of lake level variations reconstructed using the observed changes in ostracod assemblages. They show the lack of marked highstand (because regulated by the artificial outlet), as suggested by the decrease of *C.* (*N.*) *angulata* and the increase of *C. torosa* and *D. stevensoni*, and strong lowstands matching prolonged dry periods.

5.5.2 The Lake Trasimeno record within the framework of central Mediterranean climate

Lake Trasimeno (43°09’N) is located in a crucial position to understand the past climate variations of the central Mediterranean area. During the entire Holocene, the lake experienced several water level variations as recorded by changes in both TOC content and ostracod assemblages. Very low water levels at the beginning of the Holocene, from 11,700 cal yr BP (Interval A), are still recording prolonged dry and cold climate conditions that dominated the deglaciation, as shown by the *S. aculeata* dominated association which indicate very shallow/temporary conditions (lower part of Interval A). Further on, around ca. 10,000 cal yr BP, the lake slowly reacted to the climate amelioration of the Early Holocene achieving the status of a permanent lake, recorded by the change in ostracod assemblages in particular by the appearance of *C. angulata*, *C. torosa* and *D. stevensoni* (upper part of Interval A). However, it is only around 9,000 cal yr BP that Lake Trasimeno reached its maximum highstand that persisted until 4,200 cal yr BP (Interval B). This very long humid phase encompass high TOC values and was characterized by the total absence of ostracods, possibly due to very diluted waters with low $CaCO_3$ content. No particular climate evidences were recognized in this lake corresponding to the 8.2 kyr cooling event (Alley et al., 1997) confirming the hypothesis of Magny et al. (2013) that in the central Mediterranean area the main changes in lake sedimentation occurred at 10,500 cal yr. BP and 9,000 cal yr. BP at south and north of latitude 40°N respectively, rather than at 8.2 kyr. At ca. 4,200 cal yr BP (Interval C), Lake Trasimeno underwent a remarkable change as shown by the reappearance of ostracoda fauna, after almost 5,000 years of total absence. The lake level was still high as recorded by the ostracod assemblage recovered in this interval (*C. torosa* and *C. angulata*), corresponding to today assemblages recognised in the lake at its maximum depths (4-5 m, Marchegiano et al., 2017b), but with enough calcium dissolved in the water to allow ostracods to form their shells. This apparently confirms the hypothesis of Peyron et al. (2017) that around 4,200 cal yr BP in central Italy annual precipitations were similar to present. After 4,200 cal yr BP until present, a general drying trend was recorded by the ostracod assemblages of Lake Trasimeno represented by the presence of *C. (N) angulata*, *C. torosa* and *D. stevensoni,* whose alternate abundances indicated short-term climatic changes. In particular, the Lake Trasimeno record shows decreasing water levels during the dry MCA (1000 to 1250 AD) (abundance of *D. stevensoni)* and high water levels to the humid LIA (1250 to 1850 AD) (dominance of *C. (N) angulata).* Only by the end of the 19th century efficient human interventions on the lake water regime allowed solving the problem of the catastrophic inundations interrupting the tight link between rainfall and lake level variations. At present, Lake Trasimeno water level reacts naturally only to dry periods.

The reconstructed lake level trend issued from this study shows that during the Holocene Lake Trasimeno generally behaved as other central, eastern and western lacustrine and marine basins in the Mediterranean area located south of 40°N (refer to Magny et al. 2013). However, the major change towards higher water levels at 9,000 cal yr BP was contemporaneous to those observed in other lakes situated north of 40°N (Magny et al. 2013). Furthermore, several (palaeo)-lakes located in central Italy, such as Lago di Vico (42°19'N; Magri and Sadori, 1999), Lago di Mezzano (42°36'N; Ramrath et al., 2000), Lago Velino (42°26'N; De Angelis, 2014), Valle di Castiglione (41°53'N; Follieri et al., 1988), Lagaccione (42°12'N; Magri 1999) and Lago di Albano (41°45'N; Lowe et al., 1996) display the same behaviour of Lake Trasimeno (43°09'N). This suggest that the moisture boundary for the central Mediterranean proposed by Magny et al. (2013) at latitude 40°N should be slightly shifted northwards at least up to 43°N. The latter would be more in line with the atmospheric reconstruction proposed by Mauri et al. (2015) and Peyron et al. (2017) that locate this boundary at 45°N. However, data from Lake Accesa (41°59'N; Marchal et al., 2002; Kim et al., 2004; Magny et al., 2006) display an opposite behaviour than Lake Trasimeno, being more comparable to northern Mediterranean inland waterbodies. Hence, more investigations are needed to fully understand the complex and often opposite reaction of lacustrine basins to precipitation regimes.

5.6 Conclusions

As highlighted in the previous section, the reconstructed lake level changes have had a significant impact on the human settlements in Lake Trasimeno shores since the Neolithic. Starting at the Roman Imperial Age several attempts have been done to control continuous lake level changes although without major results before 1889 AD. Thus, Lake Trasimeno is a very important site for palaeoclimatic and palaeoenvironmental reconstructions, as human impact during most of the Holocene has been minimal and most of all identified using historical data. Thus, the observed variations in ostracod associations can be mostly linked to lake level changes driven by climate. They further allow us to obtain a more pristine climatic signal in comparison to other proxies commonly used in palaeoclimatic and palaeoenvironmental reconstructions that are more sensitive to changes in the catchment (e.g. pollen). At ca. 10,000 cal yr BP the ostracod association changed from *S. aculeata* to *C. angulata* highlighting the shift from a temporary to a permanent waterbody. The Late glacial/Holocene transition in several lacustrine records was characterised by the appearance and increasing abundance of more termophilic ostracods. Conversely, closed Lake Trasimeno presents species more sensible to lake level variations indicating changes in the precipitations regime. Moreover, at ca. 9,000 cal yr BP the total absence of ostracods along with the highest TOC values indicate that the lake reached the highest water level. At ca. 4,200 cal yr BP the constant presence of *angulata-torosa* and *stevensoni* associations indicate a continue decreasing water level trend until today. Changes in the relative abundance of the main species (i.e. *C. angulata*, *C. torosa* and *D. stevensoni*) underline centennial variations such as the MCA (1000 to 1250 AD) and the LIA (1250 to 1850 AD).

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CHAPTER 6

Coeval Mediterranean rainfall and global marine circulation patterns during the Last Glacial*

Rapid climate oscillations in the central Mediterranean region during the Last Glacial have frequently been attributed to Dansgaard-Oeschger (D/O) cycles mirrored in the Greenland ice core records. In the central Mediterranean region, these oscillations have mostly been reported to be expressed as temperature fluctuations. Here, we show for the first time that distinct lake level fluctuations at Lake Trasimeno (central Italy) can be linked to modeled and reconstructed intensities of the Atlantic Meridional Overturning Circulation (AMOC), which are a result of changing fresh water flux to the North Atlantic associated with D/O cycles. During the Last Glacial, a strong AMOC associated with Greenland interstadial periods (D/O warm periods) promotes more precipitation in central Italy, as inferred from high lake levels at Lake Trasimeno. Periods of weak AMOC intensities, such as during Greenland stadials (D/O cold periods) or during Heinrich events are correlated with lake level low stands, which imply relatively dry conditions in central Italy. The coupling of AMOC intensity and lake level at Lake Trasimeno reveals a close ocean-atmosphere interaction associated with Northern Hemisphere climate variability during the Last Glacial period.

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6.1 Main Text

Rapid climate variability on millennial time scales during the last glacial period is widely recognized to comprise so-called Dansgaard-Oeschger (D/O) cycles (Dansgaard et al., 1984) and Heinrich (H) 6 events (Heinrich H., 1988). D/O events recurring every 2,000 to 3,000 years are finely resolved in the Greenland ice core record and are associated with a rapid warming in the order of 8-15°C (Steffensen et al., 2008) (Greenland interstadials, GI) and a subsequent gradual cooling trend (Greenland stadials, GS) (NGRIP-members, 2004; Rohling et al., 2003). H-events mark the deposition of ice rafted debris layers in the North Atlantic recurring every ~7,000 years and are explained by enhanced iceberg discharge mainly from the Laurentide Ice Sheet (Bond et al., 1995).

The impact of D/O (or GI/GS)–like millennial-scale climate variability during the Last Glacial maximum (LGM) has been described in many terrestrial records across the world (Harrison and Sanchez Goñi 2010). Given its proximity to the North Atlantic, the European continent is able to provide the most robust evidence of these millennial-scale oscillations, and several palynological studies from the central Mediterranean region (Fletcher et al., 2010) have revealed that vegetation adapted quickly to centennial- and millennial-scale environmental modifications. The pollen records suggest that warm and humid conditions prevailed in the Mediterranean during GI periods, with a cold and dry regional setting during GS periods (Allen et al., 1999; Follieri et al., 1998). However, there is a paucity of detailed information on hydrological variability, detached from temperature-driven influences, and the response of the environmental archive to rapid millennial-scale climate change in the central Mediterranean region. In order to better understand hydrological variability in the central Mediterranean region in response to rapid Northern Hemisphere climate change, we investigated the (bio-) geochemical, mineralogical and stable isotope composition of a 859-cm long sediment core (Co1320) from Lake Trasimeno (Fig. 1) during the last ~47,100 years (see supplement for more details about the age model). In concert with modeled data (Manviel et al., 2014) and marine proxy records from offshore the Iberian Margin (Skinner et al., 2013), our

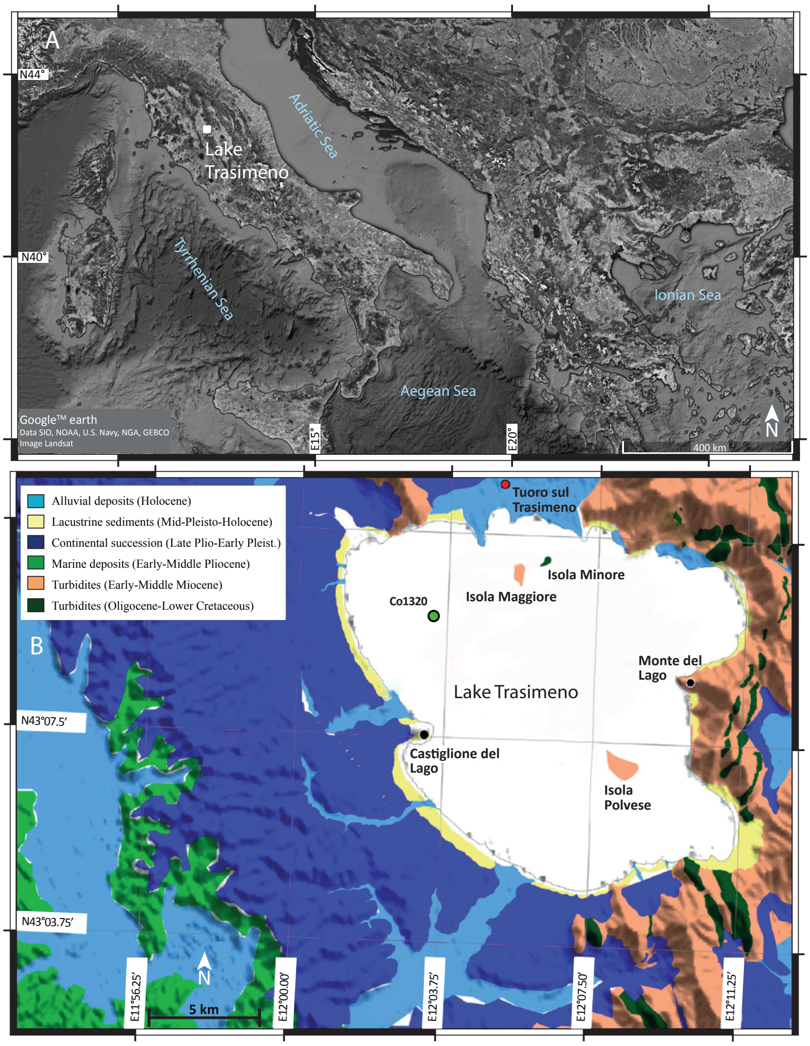

Figure 1: A: Geographical map showing the location of Lake Trasimeno in central Italy. White squares indicate the location of cores MD99-2334/MD01-2444, and of Lago Grande di Monticchio and Valle di Castiglione. **B**: Geological map of the Lake Trasimeno area14. The green circle marks the location of core Co1320.

data imply that Trasimeno’s lake level fluctuations during the last glacial period are closely connected to Northern Hemisphere ice sheet variability and its associated modification of the Atlantic meridional overturning circulation (AMOC) strength (Kageyama et al., 2010). Due to the connection between hydrologic variability in central Italy and the AMOC strength, we find for the first time evidence for a close oceanic-atmospheric coupling with intensified/more frequent (reduced/less frequent) mid latitude cyclogenesis during GI (GS) boundary conditions in terrestrial, non-pollen terrestrial records during the Last Glacial.

Lake Trasimeno is an excellent target for the reconstruction of past hydrologic variability in central Italy (Gasperini et al., 2010) where historical archives document the occurrence of severe flood and drought events (Ludovisi and Gaino, 2010). Modern limnological data of this shallow (<5 m water depth), meso- to eutrophic lake have shown that Lake Trasimeno is very sensitive to variations in local hydrologic conditions (Taticchi, 1992). Ion concentrations ($Ca^{2+}HCO_3$, Mg^{2+}) vary on seasonal and decadal time scales and indicate increasing trends associated with a higher evaporation to precipitation ratio (E/P) and a lower lake level (Ludovisi and Gaino, 2010). High Ca^{2+} and HCO^{-}_3 concentrations first promote calcite precipitation in the water column, whereas ongoing mineral formation (low lake level) and high Mg concentrations subsequently foster Mg-calcite and finally aragonite formation in the lake water (De Choudens-Sanchez and Gonzalez, 2009). Hence, we use the amount of endogenic carbonates (TIC, Ca) and the relative contribution of Mg-calcite and aragonite as a proxy for lake level fluctuations at Lake Trasimeno (cf. Figs. 2, 3). Mg-calcite and aragonite preferentially incorporate Sr in their crystalline lattices (Sanchez and Gonzalez, 2009; Cohen, 2003) (Fig. 3A, 3B). In order to obtain a high-resolution estimate for the amount of Mg-calcite and aragonite in the sediments we therefore normalize Sr intensities to the immobile element Rb (Chen et al., 1999), with low (high) Rb/Sr ratios pointing to low (high) lake levels at Lake Trasimeno.

In addition, we use stable isotopes ($\delta^{18}O_{carb}$, $\delta^{13}C_{carb}$) as proxies for variations of the E/P ratio. Given the high lake surface area to water volume ratio of Lake Trasimeno, that $\delta^{18}O$ of modern precipitation in Italy averages ~ −7 ‰ (Giustini et al., 2016), and the covariance of $\delta^{18}O_{carb}$ and $\delta^{13}C_{carb}$, stable isotope variability in core Co1320 is most likely driven predominantly by changes in the E/P ratio (Lacey et al., 2016; Leng et al., 2013). We account for the different fractionation between calcite and aragonite by introducing error bars of +0.6 ‰ ($\delta^{18}O_{carb}$) and +1.8 ‰ ($\delta^{13}C_{carb}$) of aragonite-bearing sediments (Rb/Sr<0.4, cf. 2, 3, see methods for more details).

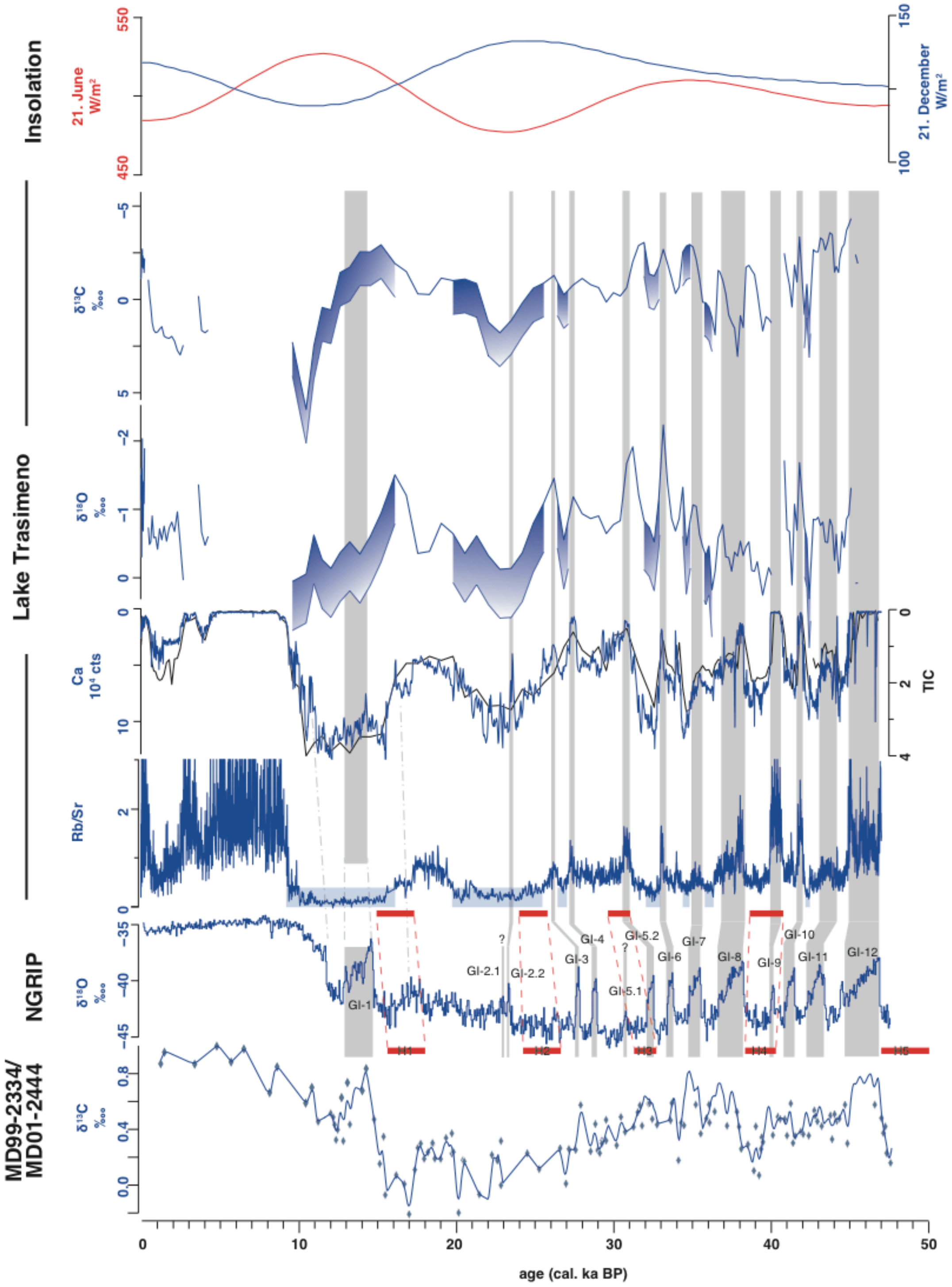

Figure 2: TIC content, Ca intensities, Rb/Sr ratio, $\delta^{18}O_{carb}$, and $\delta^{13}C_{carb}$ plotted against age (cal ka BP). Also shown are the summer and winter insolation at the latitude of Lake Trasimeno (43°N), the NGRIP $\delta^{18}O$ ice core record (NGRIP members, 2004 based on the chronology of the INTIMATE (Integration of Ice-core, Marine and Terrestrial records) working group (Rasmussen et al., 2014), and the MD99-2334/MD01-244 $\delta^{13}C_{benthic}$ record from the Iberian Margin (Skinner et al., 2013). Grey bars mark the suggested correlation between GI warm periods GI-12 to GI-1 of the NGRIP ice core record (Rasmussen et al., 2014) and the Lake Trasimeno proxy data. The ages for Heinrich events H5 to H1 are from Sanchez Goñi and Harrison (2010). Blue shaded areas define where Rb/Sr is <0.4 and mitigates for mineral-water fractionation differences between the carbonate phases.

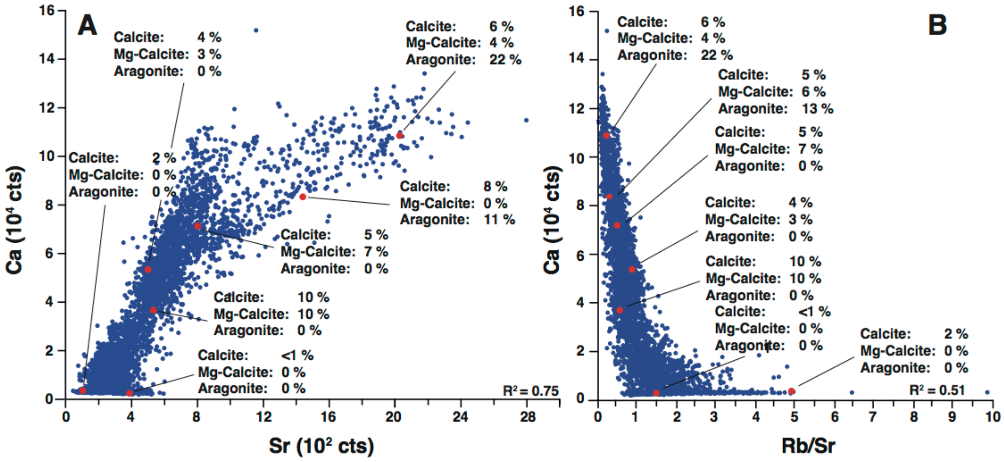

Figure 3: **A**: Cross plot of the Ca and Sr intensities as derived from XRF core scanning. Marked in red are the selected samples for XRD analyses. Shown are the calcite, Mg-calcite, and aragonite concentrations as obtained from XRD analyses. This cross plot shows that Mg-calcite and aragonite-bearing sediments show elevated Sr intensities, as Mg-rich calcium carbonates preferentially incorporate Sr in its crystal lattice (cf. main text and supplement information) **B**: Cross plot of the Ca intensities and the Rb/Sr ratio. Calcite, Mg-calcite, and aragonite concentrations as obtained from XRD analyses are also displayed by red dots. The data imply that aragonite is dominant when Rb/Sr is <0.4 (cf. also Table S3).

Pre-LGM (47,100 cal a BP to 30,000 cal a BP)

A comparison of the geochemical data of core Co1320 to the NGRIP ice core record reveals that the sedimentary proxies from Lake Trasimeno respond to Northern Hemisphere climate variability on glacial-interglacial and short-term time scales (cf. Fig. 2). Indeed, Lake Trasimeno has experienced rapid lake level fluctuations between 47,100 and 30,000 cal a BP, which can be correlated to GI/GS variability in NGRIP. Low Ca, $\delta^{18}O_{carb}$, and $\delta^{13}C_{carb}$ corresponding to high Rb/Sr imply high lake levels and a low E/P ratio at Lake Trasimeno during GI periods, with highest levels reaching almost interglacial-like levels during GI-12, GI-9, and GI-8. Low lake levels and a high E/P ratio as indicated by high Ca, $\delta^{18}O_{carb}$, and $\delta^{13}C_{carb}$, and low Rb/Sr, are associated with GS time periods and Heinrich events H4 and H3.

These periods of rapid aridification in central Italy correspond well with abrupt slow-downs of the Atlantic Meridional Overturning Circulation (AMOC). This can be concluded from a comparison of the Lake Trasimeno proxy data to the AMOC intensity as inferred from the MD99-2334/MD01-244 record (cf. Fig. 2), where low

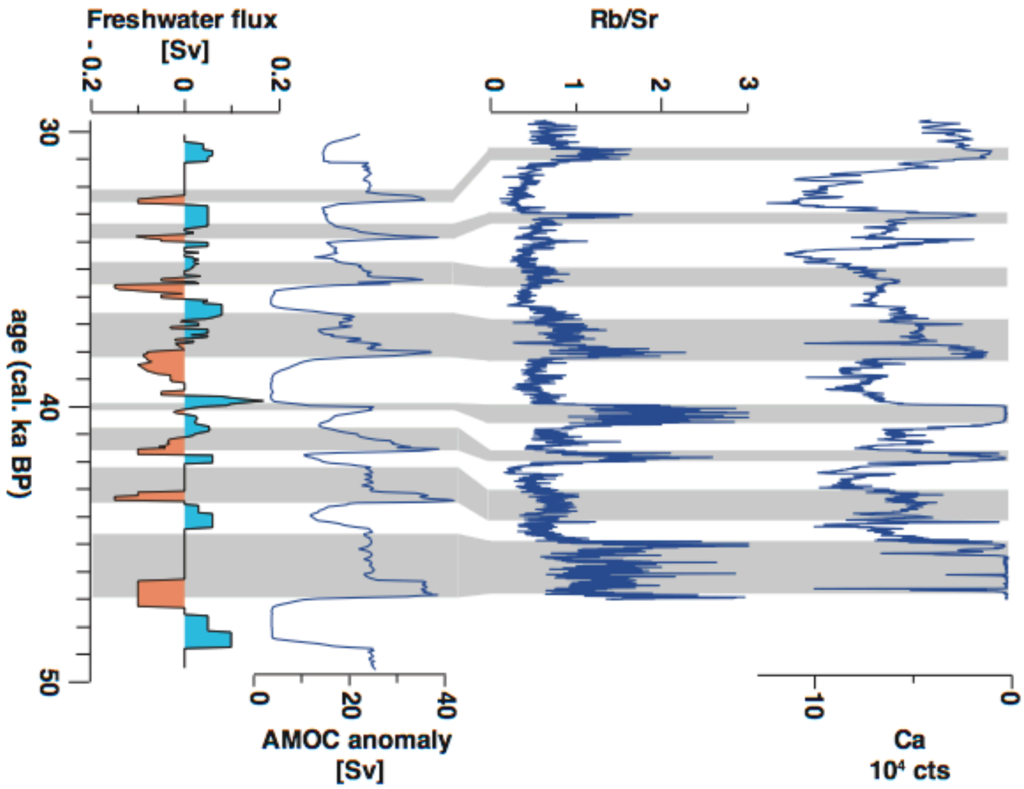

Figure 4: Freshwater flux to the North Atlantic and AMOC intensity modeled using the LOVECLIM climate model11 compared to the Ca intensities and the Rb/Sr ratio of core Co1320. Note that based on the correlation of core Co1320 to the NGRIP ice core record (cf. Fig. 3), low Ca intensities and high Rb/Sr ratios after 34 ka BP should switch towards younger ages, which further supports the match between Ca, Rb and the AMOC variability.

benthic δ^{13}C values reflect the incursion of Antarctic bottom water towards the Iberian Margin correlated to a break-down of the AMOC intensity (Skinner et al., 2013). The dependency of the hydrologic conditions at Lake Trasimeno on the AMOC is further supported by a comparison to the AMOC LOVECLIM model (cf. Fig. 4), forced by greenhouse gas concentration, orbital forcing, ice sheet orography, albedo, and North Atlantic freshwater flux from Northern Hemisphere ice caps (Manviel et al., 2014). The model results imply that ice-sheet instability across Eurasia, Iceland, and Greenland may have triggered perturbations of the AMOC. A close correspondence between a strengthening (weakening) of the AMOC during GI (GS) periods has recently been underpinned by Gottschalk et al. (2015) reporting that the majority of GI periods are accompanied by a rapid incursion of North Atlantic Deep Water into the South Atlantic. The good correspondence between modeled AMOC variability and Lake Trasimeno water level fluctuations supports the close link of ocean circulation patterns and hydrological conditions in the central Mediterranean and, thus, between ocean and atmospheric circulation during the last glacial period. During GS periods and Heinrich events (weak AMOC), cooling is centered over Scandinavia but also spreads from Greenland and northern Europe to southern Europe, North Africa, and Asia, triggering a southward displacement of the atmospheric circulation pattern (Manviel et al., 2014). A southward displacement of atmospheric circulation could have promoted outbreaks of cold and dry polar air masses from northern and northeastern directions, explaining the low lake levels and the shift towards higher $\delta^{18}O_{carb}$ during GS periods and during Heinrich events H4 and H3. In contrast, enhanced rainfall across the Mediterranean region during warm GI intervals is mostly associated with enhanced storm track intensities and/or frequency from the Westerlies and the delivery of low $\delta^{18}O$ precipitation from the Atlantic (Zanchetta et al., 2007; Roberts et al., 2008; Chondrogianni et al., 2004), which would promote reduced E/P conditions and lower $\delta^{18}O_{carb}$ at Lake Trasimeno. The close connection between central Mediterranean precipitation amount and AMOC intensity imply a close ocean-atmosphere coupling with intensified / more frequent (reduced / less frequent) mid-latitude cyclogenesis during GI (GS) boundary conditions. However, it is still unclear whether the position of the northern ice sheet (high latitude forcing) or the orbital configuration (low latitude precession forcing) has stronger influence on the amplitude of hydrologic variability in the central Mediterranean region. The excellent match between the Lake Trasimeno proxy record and the AMOC variability now underpins the importance of high latitude forcing (Fletcher et al., 2010). Highest lake levels with almost interglacial-like conditions can be inferred for GI-12, GI-10, and GI-9, which occur prior to the precession minima (maximum in summer insolation, Fig. 2) normally coinciding with enhanced precipitation in the Mediterranean region (Bosmans et al., 2015; Kutzbach et al., 2013). Moreover, lower lake levels after 35,000 cal a BP (between GI-7 and GI-6, i.e. during the summer insolation maximum, cf. Fig. 2) occur during a time of lower variability of the AMOC intensity, as suggested by benthic $\delta^{13}C$ values at the Iberian Margin (cf. Fig. 2). The proposed dominance of high-latitude forcing via AMOC variability at Lake Trasimeno at a latitude of 43°N is in line with pollen records from Italy (Fletcher et al., 2010) showing a stronger response of temperate forest taxa compared to Mediterranean-type taxa (and associated Mediterranean-type climate conditions) north of 40°N.

The pollen records also imply increasing aridity over the course of longer interstadial periods (GI-12, GI-8) (Fletcher et al., 2010), whereas it remains unclear if such trends can be observed during shorter GI intervals due to a delayed adaption of the vegetation belts to rapid climate change. Lake level fluctuations of Lake Trasimeno likely responded much quicker to changes in moisture availability in central Italy compared to pollen records, since decreasing lake levels (increasing aridity) towards GS periods can also be inferred for GI-11, GI-7, and GI-5.2. More rapid aridification patterns can be identified for GI-10, GI-9, and GI-6. An opposite pattern with highest sea surface temperatures occurring at the end of GI periods haven been reported from the Alboran Sea (MD95-2043, Fig. 1) (Cacho et al., 1999). This supports that the intensification and/or higher frequency in Westerlies of Atlantic origin drive the hydrologic variability in central Italy rather than (regional) Mediterranean cyclones.

LGM and termination 1 (30,000 cal a BP to 11,600 cal a BP).

The core Co1320 proxy data points to more stable hydrological conditions at Lake Trasimeno between 30,000 and 11,600 cal a BP (cf. Fig. 2). Lowermost lake levels and driest conditions can be inferred for the periods between ~25,000 and ~20,000 cal a BP contemporaneous with Heinrich event H2, and between ~17,000 and ~11,600 cal a BP. A somewhat higher lake level is indicated between ~20,000 and ~17,000 cal a BP. These long-term fluctuations of the hydrological conditions at Lake Trasimeno are overprinted by rapid climate oscillations associated with higher lake levels during GI-4, GI-3, and GI-1 (cf. Fig. 2). The relatively weak GI-2.2 is only mirrored in the Ca intensities implying just slightly higher lake levels whereas GI-2.1 is not expressed in our data. H1 is contemporaneous with decreasing moisture availability in central Italy inferred by decreasing Rb/Sr and increasing Ca, $\delta^{18}O_{carb}$, and $\delta^{13}C_{carb}$ prior to GI-1.

The onset of more stable and dry conditions at Lake Trasimeno after ~30,000 cal a BP correspond to the start of full glacial conditions in marine cores (marine isotope stage 2, MIS2), concomitant with the expansion of the global ice sheets (Lisiecki and Raymo, 2005), and a weakening of the AMOC (cf. Fig. 2). However, a precise correlation of the marine chronology to GI/GS climate variability in the Greenland ice cores is still under debate. Recently, the onset of full glacial conditions was placed between GI-4 and GI-3, at ~27,800 cal a BP (Sanchez Goñi and Harrison, 2010). Full glacial climate in the Mediterranean with very arid conditions based on pollen reconstructions from Lago Grande di Monticchio emerged after 25,900 cal a BP, i.e. subsequent to GI-3 (Allen et al., 2000). This is in agreement with the lake level lowering at Lake Trasimeno between ~25,000 and 20,000 cal a BP and contemporaneous with the H2 event. Despite some age uncertainties in the sedimentary sequences of lakes Monticchio and Trasimeno, the latter implies a delayed aridification of central and southern Italy compared to the establishment of full glacial conditions in Greenland. The aridification at Lake Trasimeno is likely due to a weakening of the AMOC during H2, such as recorded in core MD99-2334/MD01-2444 from the Iberian Margin, and recently confirmed from the South Atlantic (Zanchetta et al., 2007). Similarly, the subsequent breakdown of the AMOC associated with H1 (corresponding to the Oldest Dryas (Naughton et al., 2007) is well reflected in the northeast Atlantic by low $\delta^{13}C$ fitting with a significant drop in water levels at Lake Trasimeno, as inferred from increasing Ca, $^{18}O_{carb}$, and $\delta^{13}C_{carb}$ and decreasing Rb/Sr between 17,000 and 13,000 cal a BP (cf. Fig. 2). The higher lake level at Lake Trasimeno between 20,000 and 17,000 cal a BP matches increasing temperatures in Greenland subsequent to the LGM and a re-enforcement of the AMOC at the Iberian margin during the same time interval (cf. Fig. 2). However, this AMOC reinforcement is not evident at southernmost sites (South Atlantic) (Gottschalk et al., 2015), which can be explained by the progressively decreasing influence of southern deep waters on both the North and South Atlantic during the last deglaciation (Lippold et al., 2016). This supports a high sensitively of moisture variability in central Italy to North Atlantic AMOC intensity. Interestingly, the climate oscillation towards more humid conditions is not evident in the temperate pollen taxa from Lago Grande di Monticchio (Fletcher et al., 2010; Brauer et al., 2007) and Valle di Castiglione (Fletcher et al., 2010; Follieri et al., 1998), supporting that it was mostly driven by precipitation with no or negligible impact of temperature.

The reinforcement of the AMOC circulation associated with GI-1 (cf. Fig. 2), a climate oscillation correlated to the Bølling/Allerød interstadial (Naughton et al., 2007), and subsequent cold and dry conditions of Younger Dryas time period until 11,700 cal a BP is only mirrored in the Ca intensities of core Co1320, whereas Rb/Sr ratios remained low due to considerable amounts of aragonite in the sediments. This implies an overall low lake level at Lake Trasimeno despite a strengthening of the AMOC (cf. Fig. 2) and, thus, an overall low impact of deglaciation subsequent to the LGM on moisture availability in central Italy. Dry conditions in this area during the Late Glacial period are also supported by Lago Grande di Monticchio, where pollen data indicate a strong seasonality (Allen et al., 2000). This strong seasonality with dry winter conditions during the Late Glacial may be explained by a low winter insolation (cf. Fig. 2) and a southward displacement of polar air during winter. These conditions provide cold and dry air masses towards central Italy in particular during winter, a season critical for moisture availability in the Mediterranean region.

Holocene (11,600 to Present Days)

During the Holocene, a relatively high lake level is inferred from low to negligible Ca and high Rb/Sr. Higher moisture availability during this period compared to Glacial times is consistent with various palaeoclimate records in the Mediterranean region (Roberts et al., 2008). The data show a delayed lake level increase during the first part of the Holocene, with a highest lake level occurring only after ~9,000 cal a BP. A delayed maximum in humidity compared to the onset of interglacial conditions has been reported for the Mediterranean region south of 40°N (Magny et al., 2013) and is explained by strong Hadley cell activity in response to enhanced summer insolation forcing (cf. Fig. 2) (Tinner et al., 2009). A strong Hadley cell and an intensive African monsoon system acts to blocks the westerly transport of wet air to the Mediterranean region (Magny et al., 2013), probably enabling the supply of cold and dry air masses from a northeastern direction to Lake Trasimeno. In addition, a low lake level at Lake Trasimeno during the first two millennia of the Holocene could have been amplified by enhanced evaporation due to high summer temperatures, which is consistent with decreasing $\delta^{18}O_{carb}$ and $\delta^{13}C_{carb}$ until ~9,000 cal a BP.

6.2. Methods

A hydro-acoustic survey and drilling at Lake Trasimeno were conducted in September and November 2014. Coring was carried out from a floating platform using a UWITEC (Austria) gravity and piston corer. Core Co1320 was retrieved at a water depth of ~4.9 m in the northwestern part of the lake (N43° 09.624', E12° 03.491' cf. Fig. 1B) where hydro-acoustic data indicated undisturbed, horizontal bedded sediments down to at least ~12 m sediment depth. However, the hydro-acoustic signal is in most places biased by finely dispersed gas. During drilling activities, a stiff layer at ~8.6 m sediment depth hampered deeper penetration of the corer. Overlapping core segments (up to 3 m) where cut into 1 m long sections in the field and subsequently shipped to the University of Cologne for storage (4°C) and further processing.

In the laboratory, the core sections were split lengthwise into two halves. One halve was sealed for archiving, the other half was used for high resolution X-Ray fluorescence (XRF,) core scanning (ITRAX core scanner, Cox Analytics, Sweden) at 2 mm resolution using a Voltage and Amperage of 30 kV and 55 mA, respectively. XRF core scanning provides semi-quantitative estimates of the elemental composition in the investigated sediments. Inaccuracies compared to conventional XRF analyses on powdered sample material arise due to variations in water content, grain-size, mineralogy, porosity, and the surface structure of the core (Croudace et al., 2006). Suspicious layers, which yielded significant variations in their chemical composition, color and/or grain size, were directly sampled for smear slide analyses and investigated under the microscope for the occurrence of volcanic glass shards.

Based on the XRF core scanning data and on lithological information, core correlation and splicing was carried out on the individual core segments. The established composite profile suggests that core Co1320 penetrates down to 859 cm composite depth. Sub-sampling was then carried out on the composite profile at 2 cm resolution. The 2 cm thick sub-samples were freeze-dried, homogenized, and an aliquot of ~100 mg was ground to <63 µm. For total inorganic carbon (TIC) analyses on every other sample, 10 mg of these aliquots were dispersed in 10 ml DI water. The TIC content was determined by a DIMATOC 100 carbon analyzer (Dimatec Corp., Germany) as released CO_2 after treatment with phosphoric acid (H_3PO_4) and combustion at 160°C.

In order to investigate the mineralogical composition of the lacustrine sediments as well as the origin of the TIC in the deposits of core Co1320, 1.5 g of seven selected samples were ground to <63 µm and prepared for X-ray diffraction (XRD) analyses. XRD pattern were recorded using a PANalytical X'Pert PRO MPD Θ-Θ diffractometer (Co-Kα radiation generated at 40 kV and 40 mA), equipped with a variable divergence slit (20 mm irradiated length), primary and secondary soller, Scientific X'Celerator detector (active length 0.59°), and a sample changer (sample diameter 28 mm). The samples were investigated from 5° to 85° 2Θ with a step size of 0.0167° 2Θ and a total measuring time of 1 h. For specimen preparation the back loading technique was used. Rietveld refinement of the experimental XRD data was conducted using the software BGMN (Bergmann et al., 1998). The coexisting calcites could be distinguished from each other by slightly different lattice constants and hence different peak positions.

For stable isotope analysis on carbonate ($\delta^{18}O$ and $\delta^{13}C$), between 30 and 300 mg of sediment (dependent on TIC %) was disaggregated in 5 % sodium hypochlorite solution for 24 h to oxidize reactive organic material, following sieving to 63 µm to remove any biogenic carbonate. The <63 µm fraction was then rinsed in deionized water to neutral pH, dried at 40°C, and ground in agate. The processed samples were reacted overnight inside a vacuum with anhydrous phosphoric acid at a constant 25°C, and then cryogenically separated from water vapor under vacuum before being analyzed using a VG Optima dual inlet mass spectrometer. The mineral-gas fractionation factor of 1.01025 (Friedman and O'Neil, 1977) was used to account for isotopic fractionation during the reaction of carbonate with phosphoric acid. Unlike for other carbonate minerals, the mineral-gas fractionation factors for calcite and aragonite are not significantly different (Tarutani et al., 1969) and, therefore, the same correction was applied to all samples. Experimental data suggests there is a positive equilibrium oxygen isotope fractionation between inorganically precipitated aragonite and calcite of +0.6 ‰(Tarutani et al., 1969; Kim et al., 2007) to +0.8 ‰ (Kim et al., 2007) at 25°C, which is consistent with semi-empirical calculated values of between +0.5 ‰ Kim et al., 2007; Golyshev et al., 1981) and +0.8 ‰ (Tarutani et al., 1969; Kim et al., 2007). Empirical studies on the aragonite-calcite fractionation are more extensive for $\delta^{18}O$ compared to investigations for $\delta^{13}C$. Theoretical calculation of the carbon isotope fractionation between aragonite and calcite indicates an offset of +0.9 ‰ (Kim et al., 2007) between the two experimental determination suggests the fractionation is between +1.4 ‰ (Kim et al., 2007; Romanek et al., 1992; Turner, 1982) and +1.8 ‰ (Kim et al., 2007; Rubinson and Clayton, 1969). We therefore apply an error bar of +0.8 ‰ (Kim et al., 2007) for $\delta^{18}O$ and +1.8 ‰ (Kim et al., 2007) for $\delta^{13}C$ to those data where low Rb/Sr (< 0.4) indicates that aragonite is most likely a major sediment constituent.

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CHAPTER 6.1

Supplementary material

6.1.1 Site Information Lake Trasimeno

Lake Trasimeno (N43°08’, E12°06’, cf. Fig. 1, Chapter 6) is a tectonic lake located in the region of Umbria (Italy) at an altitude of ~250 m above sea level (a.s.l.). The lake basin developed from a marine gulf on the continental shelf of the Tyrrhenian Sea during the Early Pliocene via a wide fluvial plain to a freshwater lake during the Middle Pleistocene (Gasperini et al., 2010). The extensional tectonic regime is still active today and ongoing subsidence prevents the lake from completely silting up (Gasperini et al., 2010). Lake Trasimeno is one of the largest lakes of Italy and covers a surface area of 124.3 km^2 with a maximum diameter of ~10 km^2. The flat-bottom bathymetry is simple with a maximum water depth of 6 m and an average of 4 m (Gasperini et al., 2010; Ambrosetti et al., 2003). The total water volume calculates to 0.59 km^3. Seasonal, decadal, and long-term lake level fluctuations are common, although the lake level has been controlled by an artificial, underground outlet since Etruscan and Roman times (Ludovisi and Gaino, 2010; Burzigotti et al., 2003). Prior to anthropogenic influence, Lake Trasimeno was a closed lake (Burzigotti et al., 2003). Overprinted by decadal variations, long-term water balance fluctuations display an almost continuous decline of the water level since 1880 (Ludovisi and Gaino, 2010; Ludovisi et al., 2013) caused by variations in the evaporation/precipitation (E/P) ratio (as recorded by local meteoric stations), and water extraction for irrigation. An artificial enlargement of the catchment area of Lake Trasimeno in the 1960s led to a cessation and recovery of the lake level decline. However, water level will most likely continue to decline in the future given the predicted extent of climate change impacts across the Mediterranean region (Ludovisi and Gaino, 2010; Ludovisi et al., 2013). Lake level fluctuations considerably influence the water quality of Lake Trasimeno (Taticchi, 1992). Salinity and total alkalinity (measured as mgL^{-1} CaCO3) vary on seasonal and decadal time scales, indicating a general increase associated with a higher E/P ratio and a lower lake level (Ludovisi and Gaino, 2010). The trophic state of the lake has not changed and Lake Trasimeno remained a meso- to eutrophic freshwater lake (Ludovisi and Gaino, 2010; Taticchi, 1992) with a mean annual pH between 8.2 and 8.9 (Ludovisi and Gaino, 2010). This implies that variations in water transparency following the recorded lake level variations are a result of increased sediment re-suspension from the surface sediments during lake level low stands rather than being a product of higher algae concentrations (Taticchi, 1992). Due to the shallow water depth and large surface area, Lake Trasimeno is not thermally stratified even during prolonged hot summer months (Ludovisi and Gaino, 2010). The catchment area of Lake Trasimeno covers about 376 km^2. Whereas the topography to the West of the lake is characterized by a wide, flat plain area, high mountains (up to ~790 m a.s.l.) can be found in the North, East and South of the lake. These mountains predominantly consist of Oligocene to Miocene turbidites with marine sandstones, claystones and some marly claystones (cf. also Fig. 1 Chapter 6) (Burzigotti et al., 2003). The turbidite successions also form the three islands Maggiore, Polvese, and Minore in northern and southeastern part of Lake Trasimeno (Gasperini et al., 2010). To the West of the lake, Pliocene marine sandstones and claystones crop out. The wide flat areas that surround the lake are covered by Pliocene to Holocene lacustrine and fluvial deposits (Gasperini et al., 2010; Burzigotti et al., 2003). The catchment area of Lake Trasimeno is drained by small rivers, which occasionally form prograding wedges in the lake basin (cf. Fig. 1 Chapter 6). For example, a delta topset enters the lake close to the city of Tuoro sul Trasimeno and extends for more than ~1 km in comparison to a hypothetical direct shoreline. A submerged prograding wedge covered by younger lacustrine deposits close to the city Castiglione del Lago records a significantly lower lake level in the geological history of the lake (Gasperini et al., 2010). Mediterranean-type conditions with hot-warm summers and mild-humid winters control the climate regime at Lake Trasimeno. The mean annual air temperature averages at 13.3°C, with the hottest and coldest month being July (mean temperature 22.4°C) and January (4.7°C), respectively. The mean annual precipitation is 783 mm.

6.1.2. Methods for age depth modeling

Terrestrial plant materials recovered from the surface of core halves following opening and/or during sub-sampling, along with five bulk organic carbon samples, were radiocarbon dated at the CologneAMS facility (Germany). Sample pre-treatment and graphitization followed the guidelines described in more detail by Rethemeyer et al. (2012). The software package clam2.2 (Blaauw, 2010) and the IntCAL13 calibration curve (Reimer et al., 2013) were used for age-depth modeling and for the calibration of the radiocarbon ages into calendar years before present (cal a BP). For biomarker analyses, lipids were extracted from 1.0 to 5.2 g freeze-dried and homogenized sediments using accelerated solvent extraction (Dionex ASE 300) with 9:1 dichloromethane: methanol (v:v) maintained at 120°C and 120 bar. The total lipid extract was dried using rotary evaporation and a C_{46} GDGT Standard was added prior to filtering over PTFE filters (0,45µm x 4mm) using 95:5 hexane: isopropanol (v:v). GDGTs were analysed using an Agilent 1290 UHPLC connected to an Agilent 6460 QQQ equipped with an APCI ion source following the method of (Schouten et al., 2007). GDGTs were analysed in SIM mode and quantified according to (Huguet et al., 2006) including normalization to TOC. The br/(cren + cren') ratio was defined as the sum of brGDGTs IIIa, IIIa', IIIb, IIIb', IIIc, IIIc', IIa, IIa', IIb, IIb', IIc, IIc', Ia, Ib and Ic over the sum of crenarchaeol and its regio-isomer (Table S2).

6.1.3 Lithology and Chronology of core Co1320

Between 859 and 320 cm composite depth, core Co1320 predominantly consists of silty clay material with finely dispersed fine sand. Fine sand and sand layers of less than 1 cm are common, whereas two thick sand horizons between 852.0 and 826.0 cm and between 705.5 and 686.5 cm were excluded from the composite profile for further age depth modeling, as they likely represent event layers and are considered to be temporally instantaneous. The sediments appear mottled and the color

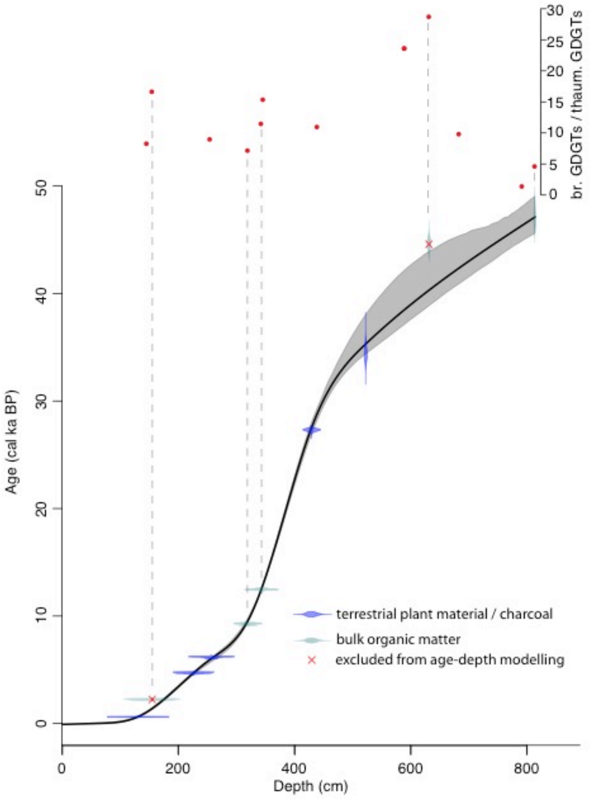

Figure S1: Age model and br/(cren + cren') GDGT ratios of core Co1320. The age model of core Co1320 is based on 4 terrestrial plant material samples, 1 charcoal sample, and 5 bulk organic carbon samples. The interpolation was carried out using the software package clam2.28. Bulk organic carbon samples COL3665.1.1 (631 cm) and COL3662.1.1 (154.5 cm) were excluded as they have high br/(cren + cren') GDGT ratios (upper panel), implying that theses samples contain significant amounts of soil organic matter that is likely pre-aged.

changes between greenish grey, olive grey and grey, which is due to varying carbonate (reaction with 10% HCl) and organic matter (OM) content. Sand-sized material is present between 332 and 320 cm sediment depth. Above 320 cm composite depth, core Co1320 predominantly consists of silty clay material. The sediments appear massive, which imply intensive bioturbation, and dark greenish grey to olive grey colors indicate high OM concentrations. Weak to absent reaction with 10% HCl indicate overall low to negligible carbonate concentrations above 320 cm composite depth. A detailed description of the lithology of core Co1320 can be found in Marchegiano et al. (2017a).

The age model of core Co1320 is based on 10 radiocarbon ages, 4 of which are derived from terrestrial plant remains, 1 from charcoal, and 5 from bulk organic carbon samples (cf. Fig. S1 and Table S1). The 4 radiocarbon ages derived from terrestrial plant remains and the age from the charcoal sample provide a robust basis for the chronological framework of core Co1320, since they are not affected by reservoir and/or hard water effects. Several studies have shown that reservoir and/or hard water effects on bulk lacustrine OM can vary significantly over time (Vogel et al., 2010a; Aufgebauer et al., 2010; Francke et al., 2013). However, reservoir and/or hard water effects for lacustrine OM are expected to be low for Lake Trasimeno, as carbonate bedrock is not widespread in the catchment and as the high lake surface to water volume ratio promotes exchange of atmospheric ^{14}C. Radiocarbon ages can also be biased if the analyzed bulk OM sample includes a considerable amount of pre-aged or fossil soil organic matter (SOM) (Martin et al., 1995). In order to estimate the contribution of (potentially pre-aged) SOM to Lake Trasimeno sediments, we analyzed the relative contribution of branched glycerol dialkyl glycerol tetraethers (brGDGTs) and the isoprenoid GDGT crenarchaeol cren (and its regio-isomer cren’) in 12 samples of core Co1320 (Table S2), out of which give were used for bulk organic matter radiocarbon dating (cf. Fig. S1, Table S2). BrGDGTs are bacterial membrane lipids predominantly found in soils, whereas crenarchaeol is primarily produced by (chemo-)autotrophic planktonic Thaumarchaeota (Schouten et al., 2013). Since the branched and isoprenoid tetraether (BIT) index, a proxy for SOM input (Hopmans et al., 2004), may be biased by variable crenarchaeol concentrations, we report the ratio of absolute branched GDGTs to the isoprenoid GDGT crenarchaeol cren and cren’ (br/(cren + cren’) GDGT ratio) in order to identify those samples containing considerable amounts of SOM. The basal age of core Co1320 is constrained by sample COL3666.1.1 at 827 cm composite depth, which has an age of 47,200 ± 1,800 cal a BP. The low br/(cren + cren’) GDGT ratio implies that sample COL3666.1.1 does not incorporate a considerable amount of SOM and the radiocarbon age is therefore considered to be reliable. The bulk organic sample at 631 cm depth (COL3665.1.1) has an age of 44,900 ± 880 cal a BP, which would suggest extraordinarily high sedimentation rates or even an age reversal between the two lowermost radiocarbon ages. However, the high br/(cren + cren’) GDGT ratio of sample COL3665.1.1 at 631 cm depth (cf. Fig. S1) indicates a substantial SOM contribution, which likely distorts the ^{14}C age due to the incorporation of pre-aged SOM. Thus, sample COL3655.1.1 was excluded from the age depth model. As inferred from low br/(cren + cren') GDGT ratios, samples COL3664.1.1 (343 cm, 12,500 ± 120 cal a BP) and COL3663.1.1 (319 cm, 9,280 ± 155 cal a BP) do not contain considerable amounts of potentially pre-aged SOM. Thus, both samples were included in the age-depth model and indicate that the deposition of sand-sized material between 332 and 320 cm sediment depth and the related distinct shifts in total inorganic carbon (TIC) content and Ca intensities (see Fig. 2 Chapter 6) is not due to an erosional discordance. The high br/(cren + cren') GDGT ratio of sample COL3662.1.1 at 154.5 cm (2,250 ± 100 cal a BP) and the age from the terrestrial plant material at 130.5 cm (COL3171.1.1, 625 ± 65 cal a BP) imply that sample COL3662.1.1 is probably affected by the contribution of old/pre-aged SOM. Thus, sample COL3662.1.1 was excluded from the age depth model. The mid to late Holocene is well constrained by the radiocarbon ages of COL3173.1.1, COL3172.1.1 and COL3171.1.1. Excluding sample COL3665.1.1 and COL 3662.1.1, interpolation between the radiocarbon ages and the sediment surface, which has an age of -64.75 cal a BP, was carried out using a smooth spline function. Several test runs revealed that a smoothing of 0.2 provided the best fit to the radiocarbon ages of core Co1320. The established age model yields a basal age of ~47,100 cal a BP for core Co1320. The sedimentation rate does not exceed 0.03 cm/yr between 47,100 and ~1000 cal a BP, with lowest rates between 34,000 and 10,000 cal a BP. During the last ~1000 years cal a BP, the sedimentation rate increases to a maximum value of 0.21 cm/yr.

6.1.4. Additional information on the carbonate species in core Co1320

The good correspondence of the total inorganic carbon (TIC) content and the Ca intensities as well as the XRD results in core Co1320 (cf. Fig. 2 Chapter 6) imply that calcium carbonate ($CaCO_3$) is the dominant carbonate phase in the sediments of Lake Trasimeno. In order to verify the correlation between TIC content and Ca intensities, the high-resolution XRF core scanner data (Ca intensities at 2 mm resolution) were converted to the same depth scale as the biogeochemical data (TIC content at 2 cm resolution). For this purpose, XRF core scanning data were averaged over the thickness of each biogeochemical sample (i.e. every 2 cm). The results are presented in Fig. S2A and Table S3. The strong correlation between total inorganic carbon (TIC) content and Ca intensities (R^2 = 0.85) supports that $CaCO_3$ is the dominate calcium carbonate species in the sediments of Lake Trasimeno and that only minor contributions come from other carbonates, such as siderite ($FeCO_3$) and/or ankerite ($CaFe[CO_3]_2$). Additionally, Ca can be incorporated in detrital feldspars (Ca-rich

plagioclases). XRD analyses suggest that ankerite is only present in one sample, whereas plagioclase occurs in all analyzed samples (Table S3). Although this might imply that Ca can also be bound to plagioclase, the strong correlation between Ca and TIC still support the interpretation that Ca is predominately incorporated in calcium carbonates. The cross-plot of Ca and TIC also show two slightly different correlation lines, which characterize two different intervals in core Co1320. Dots in linear correlation, which characterize two different intervals in core Co1320. Dots blue and red (Fig. S2A) mark samples older (blue dots) and younger (red dots) than ~4,200 cal a BP, respectively. This could imply that human activity in the catchment had a considerable impact on the lake water chemistry and/or on the supply of detrital matter to the lake, which may have particular significance following the construction of the Etruscan artificial outlet (Burzigotti et al., 2003). Furthermore, it has been reported from other lakes in the Mediterranean region that human-induced wood clearance significantly enhanced clastic matter supply (Aufgebauer et al., 2012; Vogel et al., 2010b; Wagner et al., 2008). Excluding the Late Holocene samples younger than ~4,200 cal a BP, the correlation coefficient (R^2) between TIC and Ca increases to 0.88 (cf. Fig. S2). As the bedrock in the catchment of the lake predominately consists of siliciclastic sediments, the supply of detrital calcium carbonate to the lake is most likely negligible, which suggests that changes in the amount of endogenic and/or biogenic carbonates drive TIC and Ca variability in core

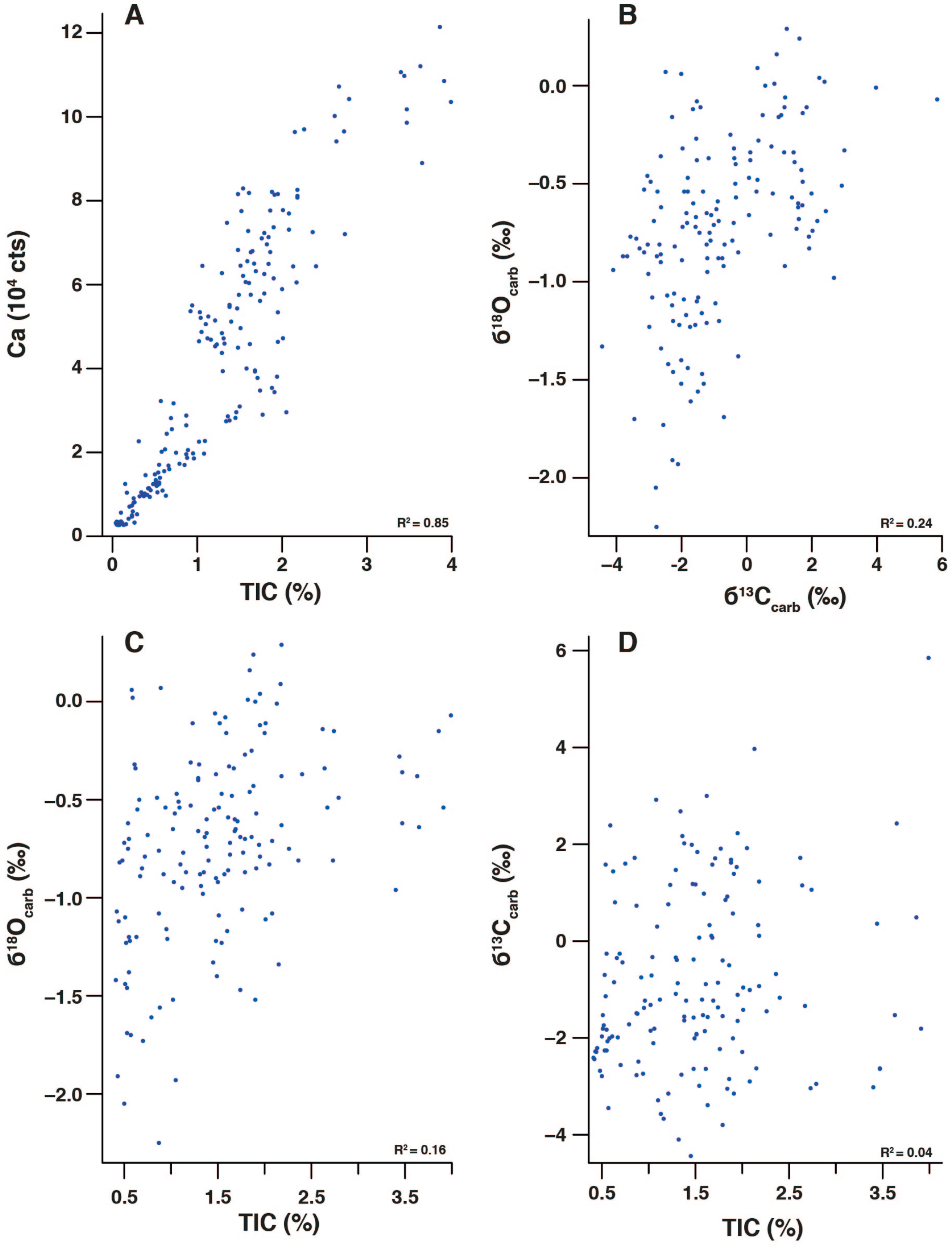

Figure S2: Cross plots TIC content against Ca intensities **(A)**, $\delta^{13}C_{carb}$ against $\delta^{18}O_{carb}$ **(B)**, TIC against $\delta^{18}O_{carb}$ **(C)**, and TIC against $\delta^{13}C_{carb}$ **(D)**.

Co1320. In most lakes, endogenic CaCO3 is preferentially precipitated as calcite during algae blooms in spring and summer (Matzinger et al., 2007), however, in high-Mg low-Ca lake water regimes, Mg-calcite and aragonite can become the dominant carbonate species (Dean et al., 2006; Roeser et al., 2016; De Choudens-Sanchez and Gonzalez, 2009). XRD analyses on 7 representative samples (Fig. 3, Table S3) confirm the presence of calcite, Mg-calcite and aragonite in the sediments of Lake Trasimeno. Aragonite-bearing samples from Lake Trasimeno show also elevated Sr intensities, which indicates that Mg-calcite and aragonite preferentially incorporate Sr in their crystalline lattices (De Choudens-Sanchez and Gonzalez, 2009; Cohen, 2003) (Fig. 3A). In order to account for the concentrations of the different carbonate types in the sediments of Lake Trasimeno, we normalize the Sr intensities to the immobile element Rb (Chen et al., 1999) and we use the Ca intensity and the Rb/Sr ratio as a proxy for the amount of calcite, Mg-calcite, and aragonite in the sediments of core Co1320 (Fig. 3B and Table S3). Biogenic carbonates (bivalves, mollusks) can also contribute to the CaCO3 content in the sediments of Lake Trasimeno, and moreover, even can influence the relative contribution of calcite, Mg-calcite, and aragonite. Thus, macroscopic shell fragments were carefully removed prior to TIC and stable isotope analyses. At Lake Dojran (Macedonia, Greece), a medium- sized, shallow (<7 m water depth), meso- to eutrophic lake, high abundances of macroscopic shell fragments have been reported during lower lake levels (Francke et al., 2013). The respective intervals yield an elevated TIC concentration, however, this is restricted to the few centimeters corresponding to the occurrence of the macroscopic fragments. Such a pattern could not be observed in the sediments of core Co1320, although lower lake levels at Lake Trasimeno could have also promoted high abundances of bivalves and/or mollusks at the coring location (and thus higher TIC and/or low Rb/Sr). A major impact of ostracode carbonate, which is abundant in the sediments of Lake Trasimeno (Marchegiano et al., 2017a,b) on TIC can also be excluded, as ostracodes incorporate low Mg-calcite into their shells (Cohen, 2003) and XRD results shows that the Mg-calcite found in the sediments of Lake Trasimeno predominantly consists of high-Mg calcite. Endogenic $CaCO_3$ precipitation only takes place as long Ca^{2+} and HCO_3^- are not short in supply (Regattieri et al., 2015; Wagner et al., 2010). The lack of limestones in the catchment and enhanced runoff resulting in higher lake levels would lead to a diluted ion concentration in the lake water and, thus, in a reduction or cessation of endogenic CaCO3 precipitation. Modern observations (Ludovisi and Gaino, 2010) have shown that on decadal timescales variations in total alkalinity of Lake Trasimeno depends on fluctuations of the E/P ratio and lake level, with dry conditions (low E/P) leading to lake level low stands and increased ion (Ca^{2+}, HCO_3^-, Mg^{2+}) concentrations in the lake water. In particular during dry periods, a further reduction in lake level and ongoing calcite precipitation can cause Mg- calcite and aragonite formation due to the progressive depletion of Ca^{2+} in the lake water (De Choudens-Sanchez and Gonzalez, 2009). Therefore, the TIC content, the Ca intensities and Rb/Sr can be used as an indicator for lake level fluctuations at Lake Trasimeno.

6.1.5. Additional information on stable isotope in core Co1320

Stable isotope analysis was carried out on core sections where TIC is > 0.4% (cf. Fig. 2 Chapter 6). The moderate variability of ~2.5 ‰ (−2.2 to +0.3 ‰; x̄ = −0.7 ‰) in $\delta^{18}O_{carb}$ suggests that the lake did not experience complete hydrological closure during the last ~47,100 cal a BP. $\delta^{18}O_{carb}$ variability in core Co1320 can be explained by changes in the lake water residence time, in the proportion of evaporation versus precipitation (E/P ratio) or by changes in the isotopic composition of the precipitation (Roberts et al., 2008; Leng et al., 2004). Given the high lake surface area to water volume ratio of Lake Trasimeno, and that $\delta^{18}O$ of modern precipitation in Italy averages ~ −7 ‰ (Giustini et al., 2006), $\delta^{18}O_{carb}$ variability in core Co1320 is most likely driven predominantly by changes in the E/P ratio. An overriding influence of E/P is supported by the good agreement of $\delta^{18}O_{carb}$ and $\delta^{13}C_{carb}$ (Fig. 2, S2B), as evaporation promotes isotopic exchange between the lake water and the atmosphere (Lacey et al., 2016; Leng et al., 2013). In addition, high $\delta^{13}C_{carb}$ can be triggered by the supply of dissolved carbon from the catchment, which is, however, likely insignificant for Lake Trasimeno due to the bedrock lithology, and by an increased primary productivity and the preferential uptake and burial of ^{13}C by algae material (Lacey et al., 2016). Low $\delta^{13}C_{carb}$ are supported by a greater contribution of ^{12}C rich soil derived, respired CO_2 from the catchment and/or by the decomposition and recycling of organic material in lacustrine surface sediments (Leng et al., 2010).

Isotope analyses have been carried out under the assumption that calcite is the dominant $CaCO_3$ species in the sediments of Lake Trasimeno. However, XRD analyses and the Rb/Sr ratio evidence the presence of aragonite in some intervals of core

Co1320 (cf. Fig. 3 Chapter 6). Aragonite-bearing sediment successions were defined by Rb/Sr ratios <0.4, as XRD results suggest that samples with Rb/Sr >0.31 only contain calcite (Table S3). As the isotopic fractionation between calcite and aragonite is +0.6 ‰ (Tarutani et al., 1969) for $\delta^{18}O_{carb}$ and +1.8 ‰ (Rubinson and Clayton, 1969) for $\delta^{13}C_{carb}$ (see Methods section), both stable isotope ratios may potentially be underestimated in aragonite-bearing sediments of core Co1320. However, as our XRD analyses show a mixture of calcite, Mg-calcite, and aragonite, with aragonite contributing ~70 % of the total carbonate present in samples 223 and 424 (Table S3), $\delta^{18}O_{carb}$ and $\delta^{13}C_{carb}$ records are likely biased by less than +0.6 ‰ and +1.8 ‰, respectively. Although the true error is likely lower, we refer to these errors in Fig. 2. The mixture of $CaCO_3$ species in Lakes Trasimeno sediments can also explain the moderate to poor correlations despite similar trends (cf. Fig. 2) between $\delta^{18}O_{carb}$ and $\delta^{13}C_{carb}$ (due to the different fractionation factors, R2 = 0.24), $\delta^{13}C_{carb}$ and TIC content (R2 = 0.04), and $\delta^{18}O_{carb}$ and TIC content (R2 = 0.16, Fig. S2). Given that aragonite-bearing sediment successions in core Co1320 correspond to low Rb/Sr and high TIC, these intervals likely correspond to phases of low lake level and dry conditions at Lake Trasimeno. A low lake level and dry conditions are consistent with high E/P ratios, as indicated by the high $\delta^{18}O_{carb}$ and $\delta^{13}C_{carb}$ during these time periods, even when the potential bias of different fractionation factors is not taken into account (cf. Fig. 2). If the potential bias is considered, the amplitude of excursions in $\delta^{18}O_{carb}$ and $\delta^{13}C_{carb}$ that indicate high E/P ratios during lake level low stands is amplified, which further supports the interpretation of the stable isotope record.

AMS Lab ID	Core depth (cm)	Material	C weight (µg)	^{14}C age (a BP)	Calibrated age (cal a BP)
COL3171.1.1	130.5	terrestrial plant	988	686 ± 37	623 ± 64
COL3662.1.1	154.5	bulk organic matter	995	2261 ± 39	2252 ± 97
COL3172.1.1	225.5	charcoal	1000	4195 ± 43	4728 ± 118
COL3173.1.1	256.5	terrestrial plant	575	5409 ± 51	6156.5 ± 142.5
COL3663.1.1	319	bulk organic carbon	999	8288 ± 50	9281 ± 153
COL3664.1.1	343	bulk organic carbon	993	10535 ± 57	12503 ± 125
COL3174.1.1	429	terrestrial plant	791	23008 ± 100	27688.5 ± 720.5
COL3175.1.1	522	terrestrial plant	320	30305 ± 810	34948 ± 1619
COL3665.1.1	631	bulk organic carbon	999	41113 ± 622	44878 ± 881
COL3666.1.1	827	bulk organic carbon	992	43671 ± 827	47223 ± 1773

Table S1: Radiocarbon and calendar ages from core Co1320 (Lake Trasimeno). Calibration into calendar ages was carried out using the software package clam2.1 (Blaauw, 2010) and the INTCAL13 (Reimer et al., 2013) calibration curve. Core depth referrers to the composite depth excluding the two sandy layer between 852 and 826 cm, and between 785 and 686.5 cm, respectively.

		isoGDGTs					brGDGTs									
Depth	**GDGT-0**	**GDGT-1**	**GDGT-2**	**GDGT-3**	**cren**	**cren'**	**IIIa+IIIa'**	**IIIb+IIIb'**	**IIIc+IIIc'**	**IIa+IIa'**	**IIb+IIb'**	**IIc+IIc'**	**Ia**	**Ib**	**Ic**	**br/(cren + cren')**
146.5	18503.0	1852.7	1347.9	280.6	4726.3	64.7	7347.7	1001.2	146.1	11582.7	6557.9	779.5	7860.3	3713.9	972.9	8.3
154.5 *	58654.1	6632.5	3823.7	1562.4	6501.4	136.8	18170.1	3324.5	591.9	31171.5	20477.5	2635.0	21110.5	10558.0	3098.0	16.7
255	38996.4	4302.0	1757.3	681.1	6755.3	80.8	11272.6	1590.4	218.9	16859.9	9995.9	1236.4	11300.0	6608.5	1794.6	8.9
319*	191529.5	8974.3	7526.5	1042.1	14654.1	231.3	18316.6	2190.7	343.8	29623.9	14549.6	1265.4	29059.2	9192.8	1977.9	7.2
343*	26421.8	911.6	1167.8	147.0	1098.0	62.6	2376.7	185.9	0.0	4727.6	875.3	88.8	4173.4	714.8	188.9	11.5
347	13219.8	470.0	501.9	73.5	492.0	30.6	1231.8	111.1	0.0	3235.7	527.5	64.7	2295.5	467.6	132.4	15.4
440	7445.9	466.0	362.8	72.8	824.9	24.5	1424.7	244.1	0.0	2371.5	1544.4	176.2	2037.7	1120.0	401.9	11.0
590	663.2	80.2	73.8	30.1	184.2	6.3	420.2	62.2	0.0	807.9	858.9	117.7	857.7	1004.2	370.0	23.6
631*	19107.8	1830.1	572.2	215.1	1757.1	36.7	15350.7	1523.0	250.9	14894.6	7309.1	854.0	7525.5	2818.8	882.1	28.7
683	23487.6	1189.0	677.2	113.1	1052.4	58.5	1823.4	283.4	0.0	3708.3	1021.7	101.9	2992.2	719.3	253.5	9.8
971	9457.2	731.0	597.5	401.9	5963.7	121.3	1084.2	142.6	0.0	1536.8	432.8	99.4	3695.1	520.5	291.7	1.3
827*	360.1	195.8	229.9	120.6	607.4	88.4	708.5	71.9	0.0	1044.3	482.0	41.7	491.2	311.8	77.6	4.6

Table S2: GDGT concentrations (in ng/g TOC) of 12 selected samples from core Co1320, out of which 5 correspond to samples used for bulk organic matter radiocarbon dating (marked with an asterisk). The ratio of branched GDGTs to the isoprenoid GDGT crenarchaeol (cren and its regio-isomer cren') was used to estimate the relative contribution of (potentially pre-aged) soil organic matter (SOM) on the bulk organic radiocarbon ages of core Co1320. BrGDGTs are bacterial membrane lipids predominantly found in soils, whereas crenarchaeol (cren) and its regio-isomer (cren') are primarily produced by (chemo-)autotrophic planktonic Thaumarchaeota (thaum GDGTs). Core depth refers to the composite depth excluding the two sandy layers between 852 and 826 cm, and between 705.5 and 686.5 cm, respectively.

Vial	Sediment Depth	Age	TIC	Ca	Rb/Sr	Calcite	Mg-calcite	Aragonite	Ankerite	Pyrite	Gipsum	Anatase	Rutile	Quarz	K-feld	Plagio	Kaoli	Chlo	Musc (2M1)
	(cm)	(cal yr BP)	(%)	(cts)		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
115	146.5	1,120	1.9	38039	0.65	10	10			<1		1	<1	16	6	6	6	11	33
233	347	13,200	3.9	108530	0.12	6	4	22	6	<1	<1	<1	<1	16		9		6	27
280	440	29,000	1	53417	0.72	4	3			1		<1	<1	36	6	16		7	25
378	590	38,500	1.5	64502	0.58	5	7					1	<1	27	6	14		9	30
398	631	40,300	0.1	3270	2.43	2				1		1	2	28	4	12		11	39
424	683	42,400	2.2	82578	0.31	5	6	13		1		2	1	16	5	8		12	31
619	810	46,300	0.1	2749	1.32	<1						1	1	41	7	17		8	25

Table S3: Results of X-ray Diffraction (XRD) analyses and TIC content, Ca intensities and Rb/Sr ratios from 7 selected samples for core Co1320. Percentages for calcite, Mg-calcite, and aragonite are also presented in Fig. 3.

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CHAPTER 7

Conclusions and Outlook

An 859 cm long sedimentary core retrieved from Lake Trasimeno, central Italy, has been used for a palaeoenvironmental reconstruction since the Late Pleistocene. Because of its endorheic nature and its central position within the Mediterranean area, Lake Trasimeno represents an outstanding place for this kind of study.

The results presented in this thesis allowed reconstructing past climatic and hydrological changes in Lake Trasimeno using ostracod assemblages as main tool while comparing them with other proxies analyzed in the same sedimentary core. The almost continuous presence of ostracod species that are good palaeoenvironmental indicators led to the high-resolution reconstruction of lake level and associated salinity changes. Moreover, the occurrence of a temperature indicator taxa allowed the successful application of the Mutual Ostracod Temperature Range (MOTR) method to the Late Pleistocene record. Furthermore, the calibration of the living fauna of Lake Trasimeno with their ecological preferences was indispensable for the interpretation of the fossil fauna and has given additional robustness to this ostracod study. Archeological and historical data show evidence of human activity in the catchment of the lake since ca. 7500-7000 cal yr BP. The copious historical documentation was fundamental to attain the challenging goal of separating climatically- and anthropologically-driven environmental changes.

The sedimentary core covers the last 47,000 cal yr BP and encompasses several of the major global climatic changes occurring within this time interval.

During Marine Isotopic Stage (MIS) 3 Interglacial phase (ca. 47,000 – 33,000 cal yr B.P.) Lake Trasimeno was characterized by an alternation of humid and dry periods (i.e., GI and GS/H4, respectively) that are shown by changes in ostracod associations (Chapter 3). Consequently, Greenland Interstadial (GI) phases show low salinity waters and prolonged high lake level conditions (permanent lake, *C. torosa* association). Conversely, Greenland Stadial (GS) periods and Heinrich event H4 are especially characterized by lowstands with more saline waters (very shallow and/or temporary lake, *S. aculeata* and *S. aculeata-E. mareotica* association). Moreover, during MIS 3 the presence of *C. fuscata* and *L. blankenbergensis* revealed colder winter and summer temperatures than present (Chapter 4). Besides, the Jan T_{min} and Jul T_{min} MOTR curves mirror the humid/arid as well as the GI/GS-H events alternation confirming a correlation between precipitation and temperature patterns (i.e., humid/warm and arid/cold). These results also show that the ostracod record matches the Rb/Sr variability, which in turn corresponds to variations in Ca^{2+}, $\delta^{18}O$ and $\delta^{13}C$ (Chapter 6), measured in the same core. More specifically, low Rb/Sr values correspond to the *S. aculeata* and *E. mareotica-S. aculeata* associations (low lake levels); while high Rb/Sr values match with the *C. torosa* association (high lake level).

The interval between MIS 2 and the early Holocene (ca. 33,000 – 10,400 cal yr B.P.) is characterized by more homogeneous and prevalently cold and arid periods. They correspond to a shallow lake or temporary water conditions as shown by the dominance of the *S. aculeata* association (Chapter 3). Furthermore, short cold and dry episodes correlate well with the H3 and H2 events and, probably, with the Younger Dryas and are shown by an increase of *E. mareotica.* The Jan T_{min} and Jul T_{min} curves continue to follow the lake level (i.e., precipitation) and NGRIP trend.
In comparison with the previous interval (MIS3), more stable hydrological conditions are also indicated by the low and more uniform values of the Rb/Sr ratio confirming dry conditions and low lake levels depicted by the ostracod associations (Chapter 6). Nevertheless, some high Rb/Sr values were recorded during short and comparatively higher lake levels mirroring the presence of the *C. torosa* association.

The Pleistocene/Holocene transition (ca. 11,700 cal yr BP) marks out the onset of a climatic amelioration as shown by an increase in ostracod abundance, total organic carbon, temperatures (MOTR) (chapters 3 and 4) and the Rb/Sr ratio (Chapter 6). However, the lake level started increasing at ca. 10,000 cal yr BP as suggested by a clear change in ostracod assemblages from *S. aculeata*, (i.e., very shallow/temporary conditions) to *C. angulata* (i.e., permanent lake conditions) association. The maximum lake level is reached at ca. 9,000 cal yr BP (highest Rb/Sr ratio and disappearance of ostracods) showing a delay of ca. 2,700 yr with respect to the NGRIP (chapters 3, 5 and 6). This high lake level (i.e., humid conditions) continued until 4,200 cal yr BP as suggested by high TOC values and Rb/Sr ratios. After almost 4,800 yr of absence, ostracod reappeared with the *angulata-torosa* association, which still indicates relatively high lake levels but also a shallowing trend that is linked to progressively increasing dry conditions. The latter is also suggested by a decreasing trend in TOC and Rb/Sr ratios (chapters 5 and 6).

From 4,200 cal yr BP until present the composition of the ostracod assemblages did not change (i.e., *C. angulata*, *C. torosa*, *D. stevensoni* and *L. inopinata*) suggesting no major climatic changes. The latter also prevented the application of the MOTR method, which takes into account only the presence and not the abundance of different ostracod species. However, variations in the relative percentages of the recovered species indicate the alternation of dry and humid periods at centennial scale, such as the warm and dry Medieval Climate Anomaly (1000 to 1250 AD) and the cool and wet Little Ice Age (1250 to 1850 AD) (Chapter 5).

Lake Trasimeno changes in water level have always triggered inundations and droughts in its catchment affecting the evolution and distribution of ostracods species as well as human activities such as fishing and agriculture. Consequently, since the Roman Imperial Age several engineering constructions of different magnitude were built focused on controlling the already known lake level changes. However, all the efforts done before 1889 AD failed to reach the expected results. As a consequence of successive failures in artificially controlling lake level changes it is possible to confidently affirm that the reconstructed lake level curve represents climatically-induced changes in moisture availability for the region.

The study of the living fauna of Lake Trasimeno was fundamental for the interpretation of the fossil record. As some of the species recovered can tolerate very wide range of the ecological parameters (e.g. salinity, temperature, depth), a detailed comprehension of the Lake Trasimeno palaeoenvironment required the knowledge of the modern analogue. The analyses of the physical and chemical parameters associated to the living ostracod fauna allowed to characterize the ecological niches preferred by those species. In particular, the presence of *C. torosa* in Lake Trasimeno living fauna is a very interesting discovery as it is the first time that is recovered in an inland lake with low salinity waters (0.8 g L^{-1}) (Chapter 2). This species was always widely used as an indicator of brackish conditions. In Lake Trasimeno, its abundance could be explained by the peculiar chemistry of the lake waters, which are rich in chlorine and sodium. The recovery of *C. torosa* in the fossil record could thus suggest water chemistry similar to the present one with low salinity conditions.

The absence of *E. mareotica* and *S. aculeata* in the living fauna confirms the peculiar situation recovered during MIS 2 linked to dryer and colder conditions than present ones. Moreover, the lack of *C. fuscata* and *L. blanckenbergensis* in the present-day permanent lake validates them as cold temperature indicators.

Starting from the Holocene, the ostracod fossil assemblages became very similar to the ones recovered in 2014 in the deepest area of the lake with the presence of only *C. torosa* and *C. angulata.* Intervals containing more diversified species such as *D. stevensoni* and *L. inopinata* as observed along the present day lakeshore (Chapter 2) indicate relatively shallower conditions.

In summary, the identified variations in Lake Trasimeno ostracod assemblages throughout time have allowed reconstructing lake level changes that appear mostly in phase with those recorded in Greenland ice cores for the same time window. Furthermore, these results are challenging existing views about moisture distribution in the Italian peninsula and, thus, in this area of the Mediterranean realm.

Outlook

The study of a sedimentary core from Lake Trasimeno has provided several hints about the evolution of local environmental conditions for the last ca. 47 cal. ka BP. Its correlation with other central Mediterranean and the NGRIP records allowed evaluating the connection between local and global events. However, some additional analyses could still be performed to enhance the results of this study. They are summarized in the following paragraphs:

1) Seasonal sampling of living ostracods together with the measurement of the prevailing physico-chemical parameters. This will not only improve the existing database but also will allow excluding potential seasonal controls in the assemblage variability.
2) Analyses of trace elements (especially Mg and Sr) and stable isotopes ($\delta^{18}O$ and $\delta^{13}C$) on the ostracod shells could be use to reconstruct lake hydrochemistry and the temperatures of the water in which they were formed. Moreover, it will represent an additional method to validate the results of the already performed MOTR method.
3) Application of the carbonate clumped isotope method on ostracods shells to reconstruct paleotemperatures excluding the limitations of more conventional techniques such as $\delta^{18}O$, $\delta^{13}C$ stable isotopes and trace metal distributions. The temperature values estimated with this innovative technique do not depend on the isotopic composition of the water and it also seems to be independent of any ‘vital effect’ of the organisms used for the analyses (e.g., ostracoda and foraminifera) (Eiler, 2011).

   In our knowledge this technique has not been used on ostracod shells yet. The almost continuous presence and abundance of ostracod shells in the Lake Trasimeno sedimentary core is providing an ideal record to apply this method. Moreover, the high-resolution paleoenvironmental reconstruction already achieved is providing a unique dataset that can be used to interpret the isotopic results. In particular, it can be also used to validate the results of the already performed MOTR method.
4) Improvement of the still in progress age model using tephras that will enhance the present paleoenvironmental reconstruction.

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OSTRACOD TABLE

OSTRACOD SPECIES	LIVING	HOLOCENE	PLEISTOCENE
Amnicythere sp		*	*
Candona (Neglecandona) angulata	*	*	*
Candona (Candona) candida	*	*	*
Candona (Neglecandona) neglecta			*
Cytheromorpha fuscata			*
Cypria ophtalmica	*		
Cyprideis torosa	*	*	*
Cypridopsis vidua	*	*	*
Darwinula stevensoni	*	*	*
Eucypris mareotica		*	*
Eucypris virens	*		
Fabaeformiscandona fabaeformis	*		
Herpetocypris helenae	*	*	*
Heterocypris incongruens	*		*
Heterocypris salina	*		*
Ilyocypris bradyi			*
Ilyocypris getica	*		
Ilyocypris gibba	*		
Ilyocypris salebrosa	*		
Isocypris beauchampi	*		
Limnocythere blankenbergensis			*
Limnocythere inopinata	*	*	*
Limnocythere stationis	*		
Plesiocypridopsis newtoni			*
Pseudocandona marchica	*		
Potamocypris paludum			*
Sarscypridopsis aculeata		*	*
Trajancypris clavata	*		
Trajancypris serrata			*

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FIELD AND LABORATORY COURSES

- 2017 (4-12 September)-ESPP fieldtrip to Israel- The impact of tectonics and climate in the dead sea area: A land bridge for humans out of Africa
- 2016 (2-11 September) –ESPP fieldtrip to Utah- Sequence stratigrapy and reservoir architectures of continental and marginal marine clastic depositional systems: the Mesozoic and Tertiary sequences of central Utah- U.S.
- 2016 (27-30 May) Freshwater ostracods workshop meeting at Malham Tarn Field Studies centre- Ostracod Group of The Micropaleontological Society
- 2016 (7-11 March) European School on Ostracoda, Jena, Germany
- 2015 (5 – 11 July) ESSP Fieldtrip to Wilhelmshaven, Germany – Sedimentology and ecology of the intertidal area -
- 2015 (16 – 30 April) – ESSP Fieldtrip to Barbados – Geology of Barbados –
- 2014 (10-14 October) – ESSP Fieldtrip to Sicily – Mixed carbonate ramps: tectonic and paleogeographic settings (Sicily, Southern Italy)
- 2014 (22 - 30 September) – IAS Summer School of Sedimentology, Beijing “Lacustrine sediments through time: An archive for paleoenvironment analysis of Luanping Basin in China”
- 2014 (1-6 September)- ESPP workshop- Cyclostratigraphy the origin of cyclicity and orbital tuning in sediments
- 2014 (31 March – 12 April) – ESPP Fieldtrip to California – The active continental margin of California and its sedimentary record –
- 2008 (8-15 May): - Field Course on Coral Reef Biogeology, Marsa Alam, Egypte.

TEACHING

- 2014 – until present – Assistant at Palaeobiology and palaeontology course (1st Bachelor year)
- 2015 – 2016 - Assistant in the Micropaleontology course (2nd- 3rd Bachelor year)
- 2013 – 2015 Assistant in the General Geology (1st Bachelor year)
- 2013 – 2015 Assistant in the Limnogeology (2nd Bachelor year)
- 2013 – 2014 Assistant in the Geomorphology course (2nd Bachelor year)

AWARDS

- 2017 - Special Poster Award for a creative presentation- 18 th Swiss Global Change day
- 2016 - Scholarship Grant -Fondation Ernst et Lucie Schmidheiny
- 2014 – Scolarship Grant – Fondation A. Lombard
- 2014 – Scolarship Grant – Fondation M. Birkigt
- 2012 – Swiss Government Excellence Scholarship for Foreign Scholars

NOTABLE EXPERIENCES

- 2014 – 2016 CUSO delegate for Phd students of the University of Geneva
- 2016 – Staff member during the Swiss Geoscience Meeting Geneva
- 2014 – Staff member during the International Sedimentology Congress, Geneva, Switzerland

LANGUAGES COMPETENCES

Italian : native speaker
French: C1
English: B2
Spanish: A2

- 2015 (24 August - 4 September) English intensive summer course - level B2, Geneva, Switzerland.

LABORATORY EQUIPMENT USAGE

- In charge of the PerkinElmer CHNS/O elemental analyses
- Usage of the Particle size analyzers CILAS
- Preparation of micropaleontological samples
- Preparation of palynological and palynomorph samples

OUTPUT LIST

JOURNAL ARTICLES

PUBLISHED

- 2017 Marchegiano M., Gliozzi E., Ceschin S., Mazzini I., Adatte T., Mazza R., Gliozzi S., Ariztegui D. "Ecology and distribution of living ostracod assemblages in a shallow endorheic lake: the example of Lake Trasimeno (Umbria, central Italy)" Journal of Limnology 76(3), 457-475 **DOI:** http://dx.doi.org/10.4081/jlimnol.2017.1478

- 2017 Marchegiano M., Francke A., Gliozzi E. Ariztegui D., "Arid and humid phases in central Italy during the Late Pleistocene revealed by the Lake Trasimeno ostracod record" Palaeogeography, Palaeoclimatology, Palaeoecology DOI: doi.org/10.1016/j.palaeo.2017.09.033

IN REVIEW

- 2017 Francke A., Lacey J. H., Marchegiano M., Wagner B., Ariztegui D., Zanchetta G., Kusch S., Ufer K., Baneschi I., and Knödgen K., "Coeval Mediterranean rainfall and global marine circulation patterns during the Last Glacial" in review to Nature Scientific Report.
- 2017 Marchegiano M., Francke A., Gliozzi E. Wagner B., Ariztegui D., "Lake Trasimeno (central Italy): A record of society, environment and climate interactions during the Holocene" in review to The Holocene
- 2017 Marchegiano M., Horne D., Gliozzi E., Ariztegui D., "Rapid Late Pleistocene climatic changes reconstructed from a lacustrine ostracod record (Lake Trasimeno, Umbria, central Italy) submittet to Quaternary Science Reviews

CONFERENCE PAPERS AND ABSTRACT

- 2017 (9-12 October): International Meeting of Sedimentology – Oral presentation: Lake level fluctuation and rainfall variability at Lake Trasimeno (Italy) inferred from ostracod assemblages. Marchegiano M., Francke A., Gliozzi E., Ariztegui D.
- 2017 (27- 31 August): International Symposium of Ostracosa ISO18 – Oral presentation: The climate history of Lake Trasimeno (Italy) during Late Glacial-Holocene transition revealed from ostracod assemblages. Marchegiano M., Francke A., Gliozzi E., Ariztegui D.
- 2017 (9-13 May) 5th PAGES OSM-Saragoza- Abstract: "The interplay of climate change and human activity in the central Mediterranean región during the last millennia: the varved, multiproxy record of Lake Butrint (Albania). Morellon M., Vegas J., Anselmetti F.S., Sinopoli G., Marchegiano M., Garcia-Arnay A., Sadori L., Sanchez-Moya Y., Wagner B., Brushulli B., Pambuku A., Ariztegui D.
- 2017 (11 April) Swiss Global Change Day – Poster: The climate history of Lake Trasimeno (Italy) during Late Glacial-Holocene transition revealed from ostracod assemblages. Marchegiano M., Francke A., Gliozzi E., Ariztegui D.

- 2016 (18-19 November): Swiss Geoscience Meeting Geneva - Poster: Application of the Mutual Ostracod Temperature Range method (MOTR) to Lake Trasimeno (Umbria, central Italy) sediment core. Marchegiano M., Horne D., Gliozzi E., Francke A., Ariztegui D.
- 2016 (27 February): SwissSed, Fribourg, Switzerland - Poster: Ecology and distribution of living ostracod assemblages in a shallow endorheic lake: the example of Lake Trasimeno (Umbria, central Italy) Marchegiano M., Gliozzi E., Ceschin S., Mazzini I., Adatte T., Mazza R., Ariztegui D.

- 2015 (22-30 July): European Ostracod Meeting, Tartu, Estonia – Oral presentation: "Living ostracod assemblages of Lake Trasimeno (Umbria, central Italy) Marchegiano M., Gliozzi E., Ceschin S., Mazzini I., Mazza R., Ariztegui D.
- 2015 (28 February): SwissSed, Fribourg, Switzerland – Poster: "Disentangling the relationship between environmental conditions and ostracod assemblages in modern Lake Trasimeno, Italy" Marchegiano M., Gliozzi E., Ceschin S., Mazzini I., Mazza R., Ariztegui D.

- 2014 (21-22 November): Swiss Geoscience Meeting, Fribourg, Switzerland Poster: “Detailed analysis of Middle Pleistocene ostracod assemblages from Lake Trasimeno, Perugia, (Italy)” Marchegiano M., Gliozzi E., Buratti N., Ariztegui D., & Cirilli S.
- 2014 (18 -22 August): International Sedimentology Congress 2014, Geneva, Switzerland Oral presentation: “Paleoenvironmental changes in Lake Trasimeno (central Italy) since the middle Pleistocene” Marchegiano M., Gliozzi E., Buratti N., Ariztegui D. & Cirilli S.
- 2014 (27 April – 2 May): European Geosciences Union General Assembly 2014, Vienna, Austria Poster: “Middle Pleistocene ostracod assemblages from Lake Trasimeno, Perugia (Italy)” M.Marchegiano, E.Gliozzi, N.Buratti, D.Ariztegui, S.Cirilli.
- 2014 (22 February): - SwissSed, Fribourg, Switzerland Poster: “Detailed analysis of Middle Pleistocene ostracod assemblages from Lake Trasimeno, Perugia, (Italy)” M.Marchegiano, E.Gliozzi, N.Buratti, D.Ariztegui, S.Cirilli.

- 2013 (15-16 November): – Swiss Geoscience Meeting, Lausanne, Switzerland Poster:” Middle Pleistocene ostracod assemblages from Lake Trasimeno Perugia, (Italy)” M.Marchegiano, E.Gliozzi, N.Buratti, D.Ariztegui, S.Cirilli
- 2013 (23-26 July) – International Symposium on Ostracoda, Rome, Italy Poster: “Ostracod assemblages from Middle Pleistocene sequence of Lake Trasimeno (Perugia, Italy)- Preliminary results” M.Marchegiano, N.Buratti, D.Ariztegui, S.Cirilli.
- 2013 (23-25 May) – Giornate di Paleonologia XIII Edition – Perugia, Italy Poster: “Ostracod assemblages from Middle Pleistocene sequence of Lake Trasimeno (Perugia, Italy)- Preliminary results” M.Marchegiano, N.Buratti, D.Ariztegui, S.Cirilli.
- 2013 (23 February) - SwissSed, Fribourg, Switzerland - Poster: “Lacustrine organic facies and pollen record from Lake Trasimeno (central Italy)” M.Marchegiano, D.Ariztegui, N.Buratti, M.Barchi, A.Bertini, S.Cirilli, L.Gasperini, F.Pazzaglia.