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UNIVERSITAT DE BARCELONA

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TESI DOCTORAL

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Chironomids as lake paleoenvironmental indicators: the role of present fauna to understand the past

Els quironòmids com a indicadors paleoambientals lacustres: el paper de la fauna actual per entendre el passat

Memòria presentada per Pol Tarrats Sada per optar al títol de Doctor per la Universitat de Barcelona

Pol Tarrats Sada
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Vist-i-plau dels directors de la tesi:

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Als fars que em guien:
els meus pares i la meva germana

A la Maria,
per inculcar-me la passió per l'ecologia

After climbing a great hill,
one only finds that there are many more hills to climb.

Nelson Mandela

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ADVISORS' REPORT

Dr. Narcís Prat Fornells and Dr. Miguel Cañedo-Argüelles, advisors of the PhD thesis entitled "**Chironomids as lake paleoenvironmental indicators: the role of present fauna to understand the past**",

CERTIFY that it has been carried out by Pol Tarrats Sada in its totality. The PhD candidate is the main author of the 4 chapters, and has actively participated in all tasks regarding them: setting the research objectives and the experimental design, conducting fieldwork and laboratory analyses, analysing the data, interpreting the results, writing the manuscripts, and reviewing and editing during the publication process.

INFORM that none of the information included in this thesis will be used, implicitly or explicitly, to elaborate another PhD thesis.

Below, we detail the publication status of the chapters and indicate the impact factor of the journals where the chapters have been published or submitted.

Chapter 1 (published)

Tarrats, P., M. Cañedo-Argüelles, M. Rieradevall and N. Prat. 2017. Chironomids as indicators of local and global changes in an oligotrophic high mountain lake (Enol Lake, northwestern Spain). *Journal of Limnology*, 76(2): 355-365. 5-year impact factor (2016): 1.569.

Chapter 2 (under revision)

Tarrats, P., M. Cañedo-Argüelles, M. Rieradevall and N. Prat. The influence of depth and macrophyte habitat on paleoecological studies using chironomids: Enol Lake (Spain) as a case study. *Journal of Paleolimnology*. 5-year impact factor (2016): 2.309.

Chapter 3 (to be submitted)

Tarrats, P., P.G. Langdon, M. Cañedo-Argüelles, M.J. Leng, R.T. Jones, M. Rieradevall and N. Prat. Chironomid-depth relationships provide environmental reconstructions from northern Spain. *The Holocene.* 5-year impact factor (2016): 2.773.

Chapter 4 (under revision)

Tarrats, P., O. Heiri, B. Valero-Garcés, M. Cañedo-Argüelles, M. Rieradevall, N. Prat and P. González-Sampériz. Chironomid-inferred Holocene temperature reconstruction in Basa de la Mora Lake (Central Pyrenees). *The Holocene.* 5-year impact factor (2016): 2.773.

For all of the above, we consider that the work of the PhD candidate grants him the right to defend his PhD thesis in front of a scientific committee.

Barcelona, November 2017

Dr. Narcís Prat Fornells Dr. Miguel Cañedo-Argüelles

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ABSTRACT

The study of past ecological changes is highly useful for understanding how ecosystems have responded to climate and human-induced disturbances. It is thus relevant in the context of climate change, as it allows several ecological processes and properties, such as ecosystem baseline conditions or resilience, to be evaluated. This thesis focuses on the study of past ecological changes in mountain lakes, which are widely considered to be sentinel ecosystems due to their ability to effectively record signals of climate change and anthropogenic impacts in their sediments.

To do this we use Chironomidae (Diptera) as model organisms. The use of chironomids as lake paleoenvironmental indicators has a long tradition, mainly in terms of quantitative temperature reconstructions. However, there are still many uncertainties involving their reliable use as paleoindicators that need to be further tested. Moreover, their use in paleoecological studies in the Iberian Peninsula is still scarce. In this context, this thesis focuses on two main issues. On the one hand, it reveals the main drivers affecting the chironomid paleorecord by comparing living and recent subfossil assemblages. On the other hand, past changes (mainly temperature and hydrological changes) are reconstructed in two morphologically different lakes from the Iberian Peninsula.

This thesis demonstrates the key influence of macrophyte habitat in shaping the chironomid community, both in the living larvae community and acting as a major confounding factor in the subfossil chironomid record. The presence of *Chara* in Enol Lake is identified as one of the main drivers affecting the living community. These effects are amplified in the recent subfossil record due to the high chironomid productivity related to this habitat. These results warn against the use of recent subfossil data without further questioning, as they can be greatly affected by a single factor, biasing qualitative or quantitative environmental reconstructions. Moreover, they indicate the importance of studying the present-day fauna before conducting paleoenvironmental studies.

Our results also show that, in many cases (e.g. deep lakes), it might be necessary to analyse more than one core (which is usually retrieved at maximum lake depth) to perform complete and reliable paleoenvironmental reconstructions. The comparison of two sediment cores retrieved from different lake zones (sublittoral and profundal) shows several differences in terms of how chironomids recorded past changes. The deeper core records some hydrological fluctuations, but is also characterized by low head capsule densities, which could lead to less reliable interpretations of downcore changes. The shallower core records the main anthropogenic impacts, in addition to some hydrological oscillations. In this case, results are more robust due to the high chironomid densities found in this core.

Finally, this thesis provides the first chironomid-based temperature reconstruction covering the entire Holocene in the Iberian Peninsula. The comparison of these results with other local and regional studies highlights similarities and differences between this new reconstruction and previous records. These differences can be attributed to different patterns of temperature, hydrology and seasonality changes throughout the Holocene in our study region.

Taken as a whole, this thesis constitutes one of the first complete studies regarding chironomid remains in the Iberian Peninsula and opens up many new research opportunities, in this region and elsewhere. According to the main findings of this study, the combination of ecological and paleoecological approaches is advised for carrying out complete paleoenvironmental studies.

RESUM

L'estudi dels canvis ecològics esdevinguts en el passat és de gran utilitat per entendre com han respost els ecosistemes davant de pertorbacions de tipus climàtic i humà. Per aquest motiu és rellevant en el context del canvi climàtic, ja que permet avaluar diverses propietats i processos ecològics, com ara les condicions basals o la resiliència dels ecosistemes. Aquesta tesi se centra en l'estudi de l'evolució dels canvis ecològics en llacs de muntanya que, per l'eficàcia en la seva capacitat d'enregistrar senyals de canvis climàtics i impactes antròpics en els seus sediments, són àmpliament acceptats com a ecosistemes sentinella.

Per fer-ho, s'utilitzen els quironòmids (Diptera) com a organismes model. L'ús dels quironòmids com a indicadors paleoambientals lacustres té una llarga tradició, principalment pel que fa a reconstruccions quantitatives de temperatura. Tanmateix, encara hi ha diverses incerteses respecte a la seva fiabilitat com a paleoindicadors que aconsellen un estudi més profund. A més, el seu ús en estudis paleoecològics a la Península Ibèrica encara és escàs. En aquest context, aquesta tesi se centra en dos aspectes principals. D'una banda, revela els principals factors que modelen el registre subfòssil mitjançant la comparació entre les comunitats actuals i les subfòssils recents. D'una altra banda, es reconstrueixen els canvis esdevinguts en el passat (principalment hidrològics i de temperatura) en dos llacs de diferents característiques de la Península Ibèrica.

Aquesta tesi demostra la influència clau de l'hàbitat de macròfits en el modelatge de la comunitat de quironòmids, tant en la comunitat actual de larves com actuant com a principal factor de confusió en el registre subfòssil. La presència de *Chara* al Llac Enol s'identifica com un dels principals elements que afecta la comunitat actual. Aquests efectes s'amplifiquen en el registre subfòssil recent a causa de la gran productivitat de quironòmids relacionada amb aquest hàbitat. Aquests resultats indiquen que no es poden utilitzar dades subfòssils recents sense qüestionar-les prèviament, ja que es poden veure afectades en gran mesura per un únic factor, fet que podria generar un biaix en les reconstruccions ambientals qualitatives i/o quantitatives. A més, indiquen que és molt important estudiar la fauna actual abans de dur a terme estudis paleoambientals.

Els resultats obtinguts també mostren que, en molts casos (p. e. llacs profunds), caldria analitzar més d'un testimoni de sediment, que normalment s'obté a la zona més profunda del llac, per a dur a terme reconstruccions paleoambientals completes i fiables. La comparació de dos testimonis de sediment recuperats en diferents zones d'un llac (sublitoral i profunda) mostra nombroses diferències en la manera en què els quironòmids van enregistrar els canvis esdevinguts en el passat. El testimoni profund registra algunes fluctuacions hidrològiques, però també es caracteritza per unes densitats de càpsules cefàliques més baixes, fet que podria comportar una interpretació menys fiable dels canvis. El testimoni més somer registra els principals impactes antròpics, a més de diverses oscil·lacions hidrològiques. En aquest cas, els resultats són més sòlids gràcies a les elevades densitats de quironòmids trobades en aquest testimoni.

Finalment, aquesta tesi aporta la primera reconstrucció de temperatura a la Península Ibèrica a partir de quironòmids que abarca tot l'Holocè. La comparació d'aquests resultats amb d'altres estudis locals i regionals permet destacar similituds i diferències entre aquesta nova reconstrucció i altres registres previs. Aquestes diferències es poden atribuir a diferents patrons de canvis de temperatura, hidrologia i estacionalitat al llarg de l'Holocè en la regió objecte del nostre estudi.

En general, aquesta tesi constitueix un dels primers estudis complets sobre restes de quironòmids a la Península Ibèrica i obre diverses noves oportunitats de recerca, tant en aquesta regió com en d'altres. D'acord amb els principals resultats obtinguts en aquest estudi seria, doncs, recomanable combinar aproximacions ecològiques i paleoecològiques per dur a terme estudis paleoambientals complets.

GENERAL INTRODUCTION

CLIMATE CHANGE FROM A PALEOCLIMATIC PERSPECTIVE

Although the study of climate change has a long tradition (Plass 1959), increasing scientific knowledge and social awareness have transformed it into possibly the main environmental research topic of the last two decades (e.g. Oreskes 2004; Cook et al. 2016). This has resulted in major efforts (e.g. policy development, research funding schemes) designed to face up to the issues related to climate change (e.g. warmer temperatures, sea-level rise) and climate system dynamics (Bord et al. 2000; Grieneisen and Zhang 2011). However, this is not an easy task. The study of climate change is based on multiple disciplines, which deal with different spatiotemporal frameworks. Smol (2008) proposed four main approaches: i) to trace indicatory parameters of change, ii) to substitute time for space, iii) to use models to predict future climate changes, and iv) to use paleoenvironmental data.

Paleoclimatology can be defined as the discipline aimed at understanding the fundamental patterns and processes of the Earth's climate across all temporal and spatial scales (Cronin 2010). In this respect, the quantitative information provided by paleoclimatic data and modelling is essential for understanding the main forces that shaped the Earth's system prior to instrumental records (Masson-Delmotte et al. 2013). Past climate studies document transitions between different climatic states, including abrupt events occurring in narrow time scales (from decades to a few centuries). Moreover, they inform of multi-centennial to millennial baseline variability, which can be compared with recent changes to assess whether or not it is unusual (Masson-Delmotte et al. 2013). Thus, this discipline constitutes a key framework in the study of climate change (Alverson et al. 2003).

Paleoclimatology is particularly relevant for studying the current interglacial period, the Holocene, which covers the last 11500 years. Although considered relatively stable in climatic terms, this period comprises several climatic oscillations and phases (e.g. Mayewski et al. 2004; Alley and Ágústsdóttir 2005; Wanner et al. 2008; Ahmed et al. 2013; Marcott et al. 2013). More importantly, this period has been characterized by an increasing anthropogenic impact, especially since the beginning of the Industrial Era. Thus, paleoclimatic studies of this period can be very useful for distinguishing between climatic and human disturbances that have shaped modern environmental conditions (Seddon et al. 2014).

Paleoclimatic studies cover a wide range of approaches. One of them is paleoecology, which can be etymologically defined as the ecology of the past (Birks and Birks 1980). More precisely, Rull (2010) stated that it is the branch of ecology that studies past ecological systems and their trends in time using fossils and other proxies. In practice, paleoecology is largely concerned with the reconstruction of past ecosystems (Birks and Birks 1980). Many authors have emphasized the importance of the paleoecological record, as it can help us to understand certain key ecological processes and properties, such as baseline conditions, resilience, thresholds and biotic responses to environmental change (Willis et al. 2007, 2010; Birks 2011).

The most common empirical approach used to perform quantitative reconstructions of past changes is based on the development of ecological response functions or transfer functions (Battarbee et al. 2001). These functions are based on the relationship between species and the environment in a modern training set (Juggins and Birks 2012). Thus, the main aim of a training set is to cover the full range of taxa and environmental parameters in a given study region. This data is analysed to build the transfer function, which correlates a set of modern biological responses (i.e. taxon abundances) with the environmental parameter of interest. Finally, this transfer function is applied to the fossil biostratigraphical record in order to obtain the environmental reconstruction. To carry out the paleoreconstructions, these functions are built upon different types of archives, including lake sediments.

LAKES AS SENTINELS OF GLOBAL CHANGE

Lakes have been widely considered as sentinel ecosystems, as their sediments collect and comprise both regional and local environmental signals (Carpenter and Cottingham 2002; Adrian et al. 2009; Williamson et al. 2009). They also provide durable containers of environmental processes that may persist long after the lake or its geomorphology has disappeared (Cohen 2003). Thus, analysing lake sediments represents one of the best ways of undertaking paleoenvironmental continental reconstructions (Last and Smol 2001), as they provide valuable information about geomorphological, hydrological and vegetation changes related to climate variability on different time scales (Cohen 2003). Moreover, lacustrine sediments preserve a wide range of organism remains (e.g. Chironomidae, Cladocera, Ostracoda, diatoms) that are sensitive to hydrological and other environmental changes, either of a local or regional nature (see Fig. I.1).

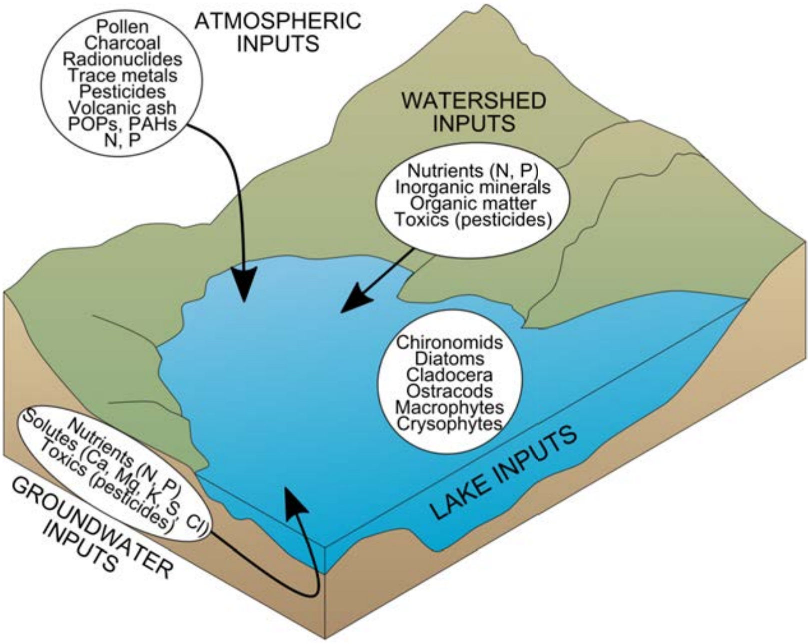


Figure I.1 Allochthonous and autochthonous sources of chemical and biological elements represented in lake sediments. Adapted from Last and Smol (2001).

Among lakes, those located in mountain areas have been widely considered as offering early warning signs of climate and environmental changes (Lotter and Psenner 2004; Parker et al. 2008). Their intrinsic characteristics (higher altitude, higher UV radiation, lower nutrients, etc.) make them very sensitive to external forces (Catalan et al. 2006). Although these lakes are normally located in remote areas, they are still subject to human influence, mainly through atmospheric fertilization (Bergström and Jansson 2006; Camarero and Catalan 2012), atmospheric pollutants (Grimalt et al. 2004; Gallego et al. 2007; Catalan

2015), pasturing (Ruggiero et al. 2004) and tourism (Catalan et al. 2017). In fact, some studies have shown that few, if any, mountain lakes can be considered to be pristine (Battarbee 2005). For this reason, this type of lakes have received special scientific attention in recent decades (e.g. Battarbee 2000; Battarbee and Binney 2009; Catalan et al. 2013).

When studying lake ecosystems, it is important to differentiate between shallow and deep lakes. Shallow lakes can be defined as those that have complete mixing during the whole year (Scheffer 2004), with very few exceptions (e.g. those protected by ice or aquatic vegetation (Löffler 2003)). In contrast, deep lakes are stratified during different periods depending on the lake characteristics, although it is usually during summer. This circumstance implies several differences in terms of functioning. In the case of shallow lakes, the physicochemical parameters remain almost constant throughout the water column and over time, while in the case of deep lakes these parameters change considerably during the stratification period, when the lake is divided into two layers: epilimnion and hypolimnion. The epilimnion is the upper and warmer layer, while the hypolimnion is characterized by dense, cooler water. Both layers are separated by a steep thermal gradient called the thermocline or metalimnion (Wetzel 2001). This stratification period is crucial for all the physicochemical parameters (e.g. a decrease in oxygen content can cause anoxia in the hypolimnion), but especially for nutrients. In shallow lakes nutrients are always available for aquatic vegetation and algae, while in the case of deep lakes, some nutrients are trapped in the hypolimnetic zone during the stratification period and cannot be used by the organisms growing in the upper layer. Thus, when studying oligotrophic mountain lakes, shallow lakes can be expected to have more constant water chemistry and environmental conditions throughout the water column and over time than deep lakes.

CHIRONOMIDS AS (PALEO)ECOLOGICAL INDICATORS

Chironomidae constitute a family of true flies (Diptera), whose larvae dominate the benthic invertebrate communities of virtually all freshwater biotopes (Pinder 1986; Armitage et al. 1995; Ferrington 2008). It is a highly diverse group with between 4,000 and 5,000 described species worldwide (Ferrington 2008) and more than 1,000 in Europe (Lindegaard 1997), although it has been estimated that there may be more than 15,000 species worldwide (Armitage et al. 1995). The Chironomidae family is composed of eleven subfamilies (SF.), although the majority of taxa encountered in lake sediments belong to three of them: SF. Tanypodinae, SF. Orthocladiinae, and SF. Chironominae.

As holometabolous insects, the chironomid life cycle is divided into four stages: egg, larva, pupa and imago (Fig. I.2). Of these, the last two have a very short duration, whereas the egg and larval stages are longer and vary both within and between species (Tokeshi 1995). Thus, the duration of the whole life cycle varies greatly among taxa and habitats. For example, arctic taxa are characterized by long life cycles, occasionally lasting up to seven years (Butler 1982), whereas several generations are common during a single season in warmer climates (e.g. Prat and Rieradevall 1995).

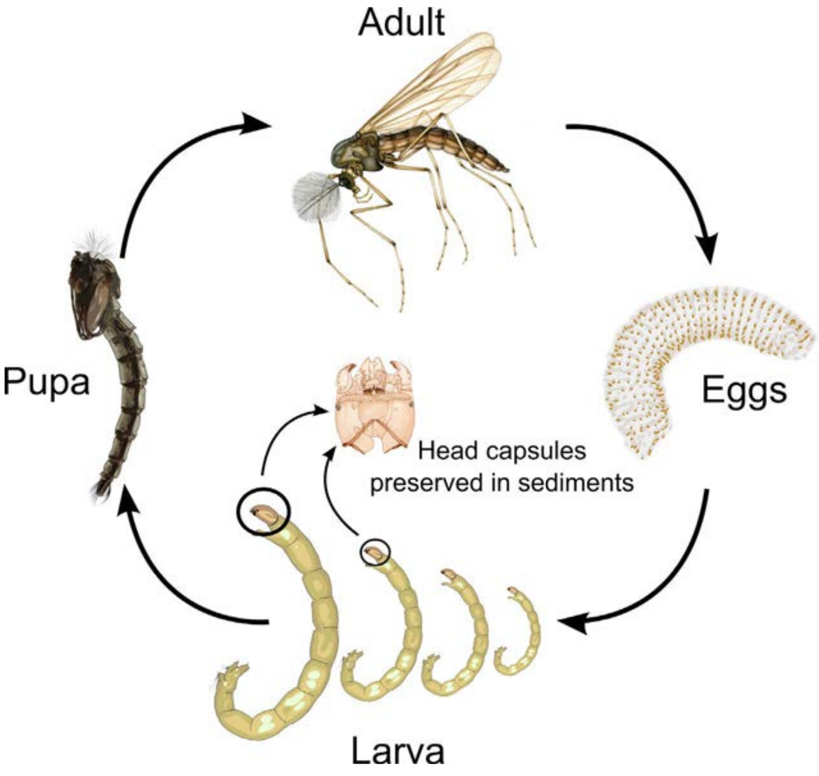


Figure I.2 Diagram representing the four stages of a chironomid life cycle: i) egg, ii) larva, iii) pupa, and iv) adult. Head capsules preserved in sediments mainly come from 3rd and 4th instars.

The survival of chironomid larvae depends on a number of different factors, including water temperature, habitat and food availability, as well as the chemical characteristics of the lake water, such as oxygen content or pH (Pinder 1986; Armitage et al. 1995). Predation on microcrustaceans and chironomid early instars occurs among free-living Tanypodinae and in some genera of the Harnischia complex, whereas most other chironomids combine algae and detritus to make up their diet (Walker 1987). Chironomid larvae produce four moults during their life, and the chitinous head capsules of the last three are well preserved in the sediment (Hofmann 1988; Walker 1995). Thus, they are suitable tools for paleoecological research through the study of subfossil head capsules (Fig. I.3).

The main reasons for using chironomids as a paleoenvironmental indicator were summarized by Hofmann (1988) and Brooks (2003):

- *Diversity and ubiquity*: chironomids are highly diverse (both species and individuals) and occur in almost all aquatic biotopes.
- *Stenotopicity*: the species are adapted to particular ecological conditions. Thus, due to the ecological requirements of the taxa, the subfossil record is indicative of the ecological conditions at the time of sedimentation.
- *Abundance and preservation*: the head capsules of the moulted skins are preserved in the sediment and are usually abundant, even in relatively small sediment samples.
- *Identification*: the head capsules are usually well preserved and thus the identification of the remains is possible.
- *Complementarity*: inferences and interpretations obtained by analysing chironomids can complement the information inferred from other indicators. This constitutes the best and most complete approach in paleoecology (i.e. multiproxy studies).

Thus, chironomids have been widely used to reconstruct past environmental conditions, such as:

- *Temperature*: temperature reconstruction is the most significant application of chironomids in paleoecology, as the relationship between chironomid species and temperature has been studied in depth and is well established (Eggermont and Heiri 2012). Through calibration sets and transfer functions (Barley et al. 2006; Heiri et al. 2011; Massaferro and Larocque-Tobler 2013), many chironomid-based temperature paleoreconstructions have been carried out (e.g. Brooks and Birks 2000; Heiri et al. 2003a; Porinchu et al. 2008; Tóth et al. 2015).

- *Lake level*: almost all the environmental parameters affecting chironomids change with depth. That is why many studies have focused on the reconstruction of lake level changes using chironomids (e.g. Korhola et al. 2000; Kurek and Cwynar 2008; Engels et al. 2012; Velle et al. 2012).

- *Eutrophication*: chironomid communities respond strongly to nutrient enrichment and trophic alterations. Several studies have focused on the changes of lake trophic status over time (e.g. Brodersen and Lindegaard 1999; Little and Smol 2001; Langdon et al. 2006).

- *Salinity*: changing salinity represents a dramatic disturbance in the lake environment, which can be correctly read in the subfossil assemblage (Heinrichs et al. 2001). It is especially relevant in arid zones, where several studies have been conducted (e.g. Verschuren et al. 2000; Eggermont et al. 2006).

- *Acidification*: several studies have documented chironomid communities inhabiting waters of different acidities, as pH is an important variable affecting chironomid species. The first studies related to pH changes in a paleolimnological approach were conducted by Henrikson et al. (1982) and Brodin (1990).

In addition to their paleoenvironmental applications, chironomids have been used to evaluate water quality in lotic (Wilson and Bright 1973) and lentic (Ruse 2010; Cañedo-Argüelles et al. 2012) ecosystems. Thus, they also constitute a powerful tool for assessing the impact of human activities on lake ecosystems.

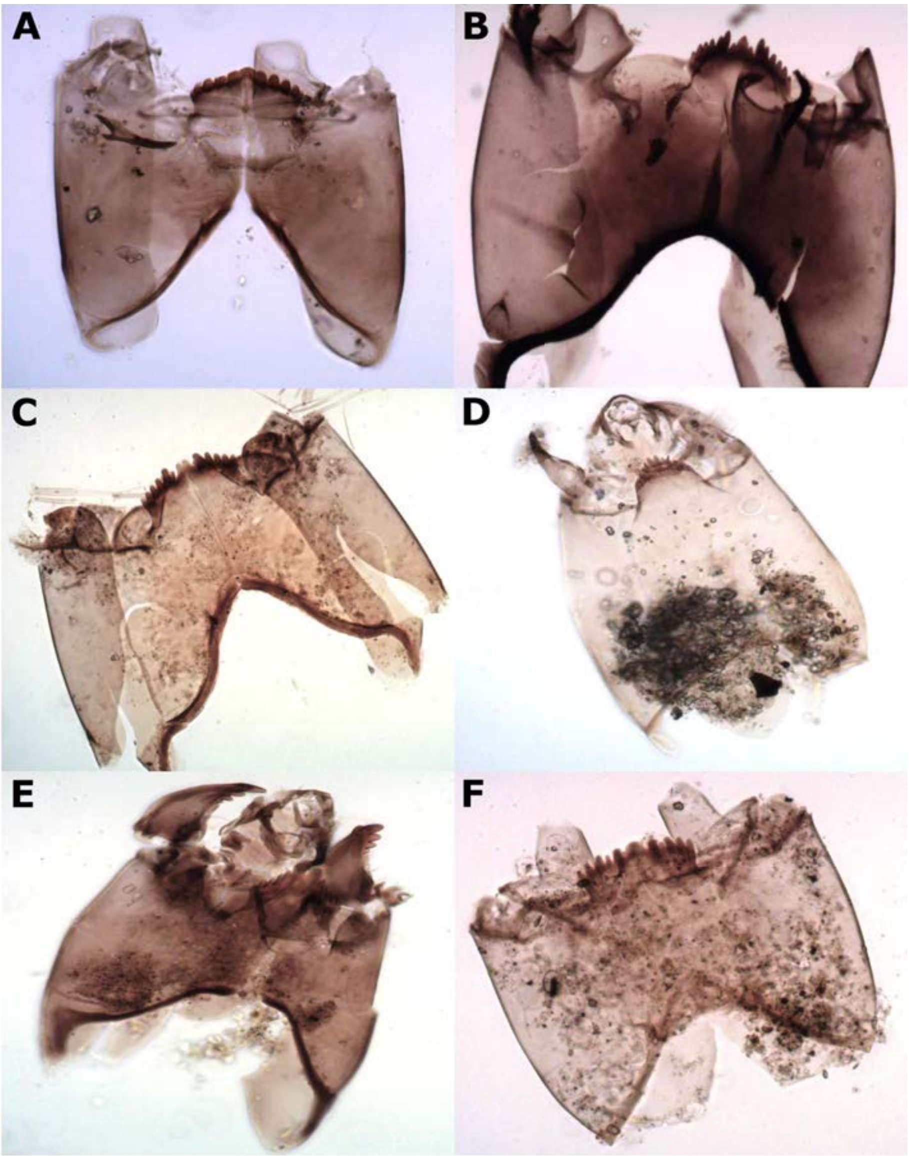


Figure I.3 Photographs of chironomid head capsules remains from Enol Lake sediments. a) *Paratanytarsus austriacus*-type; b) *Chironomus plumosus*-type; c) *Microtendipes pedellus*-type; d) *Corynoneura lobata*-type, e) *Polypedilum* type A; f) *Cladotanytarsus mancus*-type. Photographs credits: P. Tarrats.

UNCERTAINTIES AND OPEN QUESTIONS

There are still many assumptions and open questions that need to be tested regarding the use of chironomids as paleoecological indicators.

One central question is the correct representation of the Chironomidae community of a given lake by a single sediment core, especially in deep lakes. It is widely known that chironomid diversity decreases with depth. Thus, many taxa inhabiting littoral zones do not occur in the deep-water benthos. When larvae die or moult, their remains can stay on site or be moved offshore as a consequence of several factors: wind, currents, sediment focusing, lake morphology, substrate composition, and species taphonomy (Walker et al. 1984; Frey 1988; Schmäh 1993; Heiri 2004; Walker 2007; Holmes et al. 2009; Luoto 2010) (see Fig. I.4). Paleoecological studies usually rely on a single core taken from the deepest part of the lake, as it can capture all the environmental processes that have affected the lake (Glew et al. 2002; Smol 2008). However, head capsule transport and deposition processes are complex and differ greatly among species and lakes. Thus, it is not yet clear if a single core from the deepest part of the lake can properly represent the whole Chironomidae community. Several studies have tried to answer this question, but have obtained contradictory results. Some authors have claimed that there are no significant differences between the Chironomidae subfossil assemblages along the depth transect, meaning that the chironomids are transported and deposited offshore (Frey 1988; Schmäh 1993; Eggermont et al. 2007; Langdon et al. 2008; Holmes et al. 2009; van Hardenbroek et al. 2011; Heggen et al. 2012). Others found depth-related thresholds in the subfossil chironomid community (Walker et al. 1984; Heiri 2004; Kurek and Cwynar 2009; Luoto 2010; Cao et al. 2012).

Another key question that needs further exploration is the misrepresentation of some important variables when building transfer functions or performing quantitative reconstructions (Brooks 2003). For example, Sayer et al. (2010) or Velle et al. (2010) claimed that training sets usually focus on physicochemical variables and that other key parameters affecting chironomid distribution (e.g. macrophyte abundance) are neglected. In fact, other authors have proved that macrophytes can play a key role in transfer functions when taken into account

(Langdon et al. 2010). Since the factors affecting the Chironomidae community can differ greatly from lake to lake, many authors have claimed that a good characterization of the environmental drivers and ecological processes operating in each lake needs to be explored before building transfer functions or interpreting subfossil data (Frey 1988; van Hardenbroek et al. 2011).

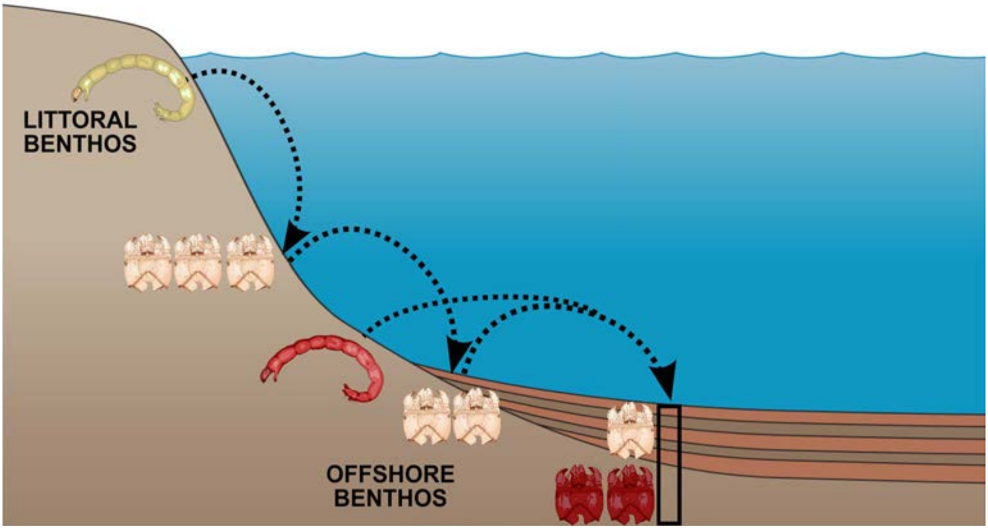


Figure I.4 Schematic and theoretical diagram representing the transport and accumulation of different chironomid larvae and their remains in lake sediments. Adapted from (Frey 1988).

BACKGROUND OF CHIRONOMID STUDIES IN THE IBERIAN PENINSULA

The study of midges in the Iberian Peninsula is relatively new. Since the first initial studies of adults conducted by Margalef (1944), no studies focusing on larvae are to be found until Prat (1978), who studied the chironomid fauna of reservoirs, including their subfossil remains (Prat and Daroca 1983). In mountain lakes, the first studies were also conducted by Ramón Margalef (1949). Since then, a few ecological studies have focused on chironomids (Rieradevall and Prat 1999; Rieradevall et al. 1999; Real et al. 2000), while others have included chironomid analysis among other macroinvertebrate groups (Prat et al. 1992; Rieradevall et al. 1998; Rieradevall and Prat 2000;

Catalan et al. 2006, 2009a, 2009b; Toro et al. 2006; de Mendoza and Catalan 2010; Martínez-Sanz et al. 2012; de Mendoza 2013).

Despite the work of Maria Rieradevall (Rieradevall and Brooks 2001), the use of chironomids as paleolimnological indicators in the Iberian Peninsula is still scarce. A few studies have been carried out to reconstruct past environmental changes occurring in the Lateglacial period (Muñoz Sobrino et al. 2013) or the last 200 (Granados and Toro 2000; Battarbee et al. 2002a, 2002b) and 100 (Catalan et al. 2002) years. Additionally, other multiproxy studies have used chironomids as a secondary source of information to support and detail the main inferences obtained by other proxies (Morellón et al. 2009a, 2012; Pérez-Sanz et al. 2013).

OBJECTIVES

This PhD thesis has two overriding goals. On the one hand, we aimed to unravel how chironomids are represented in the subfossil record by understanding the factors affecting the living community and the transport and deposition patterns of chironomid head capsules. On the other hand, we aimed at using chironomids to reconstruct past environmental changes. In this respect, we focused on two main aspects: i) to reconstruct recent past changes to disentangle climate and human impacts and ii) to perform the first quantitative chironomid-based temperature reconstruction in the Iberian Peninsula covering the entire Holocene period. To achieve these objectives, we selected two morphologically different mountain lakes from the Iberian Peninsula. A deep lake (Enol Lake, Picos de Europa) was used to study how chironomid head capsules are deposited and preserved, as we expected the chironomid community to change with depth, and to reconstruct recent environmental changes. Conversely, an oligotrophic shallow lake (Basa de la Mora Lake, Central Pyrenees) was used to perform the chironomid-based temperature reconstruction, since its more spatially and temporally stable environmental conditions were more suitable for the application of a transfer function without interfering factors.

The thesis is divided into four independent chapters, each addressing specific objectives.

In Chapter 1, we explore the spatial and temporal variation in the living Chironomidae larvae communities of the mountain lake Enol and assess which environmental factors control them.

In Chapter 2, we assess the relationship between the living and recent subfossil chironomid assemblage of Enol Lake in order to understand transport and deposition patterns of chironomid head capsules. In particular, we explore the presence and relative abundance of midge remains along the depth transect, aiming at elucidating at which depth the subfossil assemblage best represents the whole community.

In Chapter 3, we reconstruct past hydrological and climate changes and human impacts recorded in Enol Lake by means of chironomids, comparing them with different geochemical proxies. We also analyse differences between cores retrieved at different depths to ascertain whether a single core retrieved from the deepest part of the lake can adequately represent past environmental changes.

In Chapter 4, we present the first chironomid-based Holocene temperature reconstruction for the Iberian Peninsula, using a Pyrenean multiproxy lacustrine sequence from the shallow lake of Basa de la Mora.

GENERAL MATERIALS AND METHODS

In this section, we aim to summarize all the materials and methods that are analysed and used in this thesis, which are further detailed in each Chapter (see Table M.1, at the end of this section, for a summary).

STUDY SITES

Enol Lake

Enol Lake (43º 16' N, 4º 59' W, 1070 m a.s.l.) (Fig. M.1 and M.2) is a karstic lake of glacial origin located in the northwestern part of Spain (Asturias), in the western massif of Picos de Europa National Park. It has a water surface of 12.2 ha, a volume of 10^6 m^3, a maximum depth of 22 m and a small watershed (1.5 km^2). The lake is fed by groundwater and surface runoff and it has no permanent inlets. Water losses occur through evaporation, groundwater discharges and an outlet located at the northeast border of the lake, which is regulated by a small dam. Geologically, the lake basin is dominated by calcareous outcrops of several types of limestone (Moreno et al. 2011).

The lake area is characterized by a mountainous oceanic climate, which is defined by high annual precipitation and small annual temperature range, with mild winters and cool summers (Moreno et al. 2011). According to the Iberian Peninsula Climatic Atlas (Ninyerola et al. 2005), annual average rainfall is 1,500 mm, with a clear minimum in summer and a maximum in winter associated with mid-latitude Atlantic Ocean storms (Moreno et al. 2011). Mean air temperature ranges from 2.5 (January) to 16ºC (July-August).

Lake Enol region is located within the Eurosiberian biogeographical region. According to the vegetation description in Moreno et al. (2011), the climatic characteristics of the zone (i.e. humid conditions) enhance the development of dense deciduous forests, mainly composed by *Quercus* species, *Betula alba*, *Corylus avellana*, *Fraxinus excelsior*, *Alnus glutinosa* or *Acer* sp., while *Fagus sylvatica* occurs in even more humid conditions and evergreen sclerophyllous formations dominate sunny and exposed calcareous ridges. The intense human activity for animal grazing that have affected the lake catchment and the entire region during historical times has lead to deforestation and the resulting present landscapes of alpine grassland and meadows (Montserrat and Fillat 1990). In this respect, the lake surroundings are dominated by a vegetation belt of Poaceae, Fabaceae, Asteraceae and Cyperaceae species (Moreno et al. 2011).

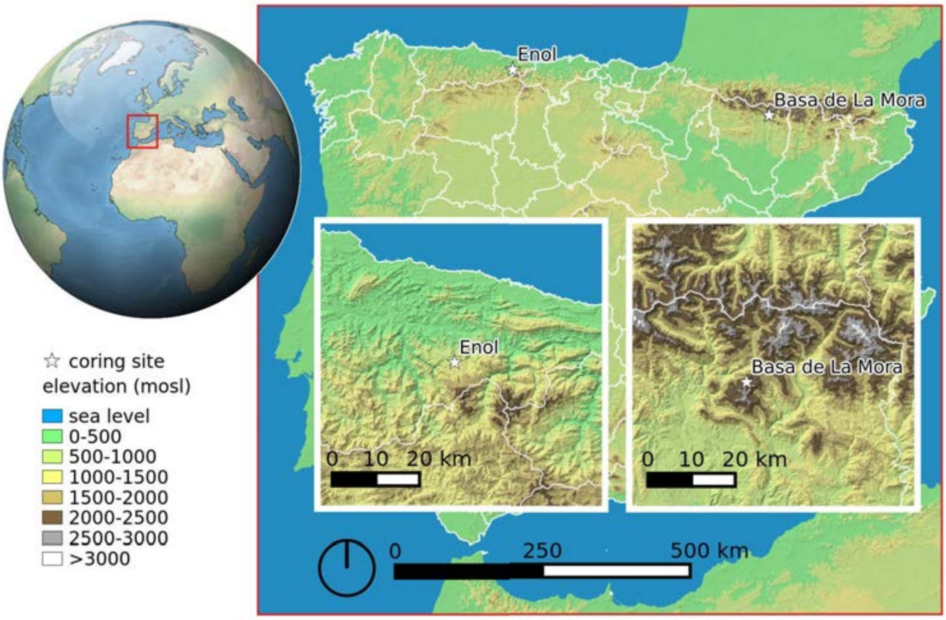


Figure M.1 Enol Lake and Basa de la Mora Lake general location map. Map plotted by M. Sevilla-Callejo (IPE-CSIC) for CLAM project.

Previous surveys (Velasco et al. 1999; Moreno et al. 2011) and the data collected during the fieldwork performed in this thesis (Sánchez-España et al. 2017) characterize the lake as warm monomictic (with a thermocline located between 8 and 12 m from early July until early November). The lake is oligotrophic (total phosphorous 8 µg l^{-1}, Chl-a 0.5-1 µg l^{-1}), with moderately hard water (alkalinity 2.4 meq l^{-1} and 24-37 mg Ca l^{-1}) and it has a conductivity ranging between 150 µS cm^{-1} at the surface and 227 µS cm^{-1} at the bottom. It has a surrounding karst bench system, that likely progrades out into the lake. It is almost fully covered with a dense carpet of *Chara* sp. between 2 and 8 m of depth, while *Potamogeton* sp. occurs between 1 and 3 m of depth. Despite this oligotrophy the bottom of the lake is anoxic during 4-6 months every year during the summer stratification period (García-Criado and Martínez-Sanz 2010; Sánchez-España et al. 2017).

Apart from its environmental importance, Enol Lake is one of the most important touristic attractions of Picos de Europa National Park, which was the first National Park settled in Spain. Its creation dates from 1918 under the name of Covadonga National Park, which was renamed in 1995 to the current Picos de Europa National Park. Enol Lake constitutes, together with Ercina Lake, the Covadonga Lakes area, the most visited part of Picos de Europa National Park, which receives up to 2 million visitors per year according to the National Park data (Picos de Europa National Park 2015).

Basa de la Mora Lake

Basa de la Mora Lake (42°32'N, 0°19'E, 1914 m a.s.l.) (Fig. M.1 and M.2) is a small (flooded surface: 3 ha) and shallow lake (depth: 2.5-4.5 m) of glacial origin located in the Central Pyrenees (Spain). It is placed in the Cotiella Massif, which is one of the largest calcareous massifs in the Pyrenees. The lake occupies a glacial over-deepened basin enclosed by a frontal moraine (Belmonte 2003) and surrounded by steep limestone walls. The catchment consists of Mesozoic limestones and sandy limestones affected by several thrust sheets (reverse faults) (Pérez-Sanz 2014). Moreover, Basa de la Mora Lake is part of the Sobrarbe Geopark, which is one of the 12 Geoparks located in Spain. This circumstance illustrates the great interest of this zone, mainly in terms of geological heritage.

The lake region is characterized by a sub-Mediterranean climate with continental features (Pérez-Sanz et al. 2013), although some areas of the Cotiella massif are influenced by local oceanic climate depending on altitudinal and orientation conditions (Izard et al. 1985). According to the Iberian Peninsula Climatic Atlas (Ninyerola et al. 2005), mean air temperatures range from -3.4 (January) to 15°C (July-August). Annual average rainfall is approximately 1,300 mm, with peaks following the Mediterranean pattern (i.e. during spring an autumn) (García-Ruiz et al. 1985). However, summers are not as dry as is typical in Mediterranean climates due to frontal and convective precipitation affecting the mountainous areas in July and August (Pérez-Sanz 2014).

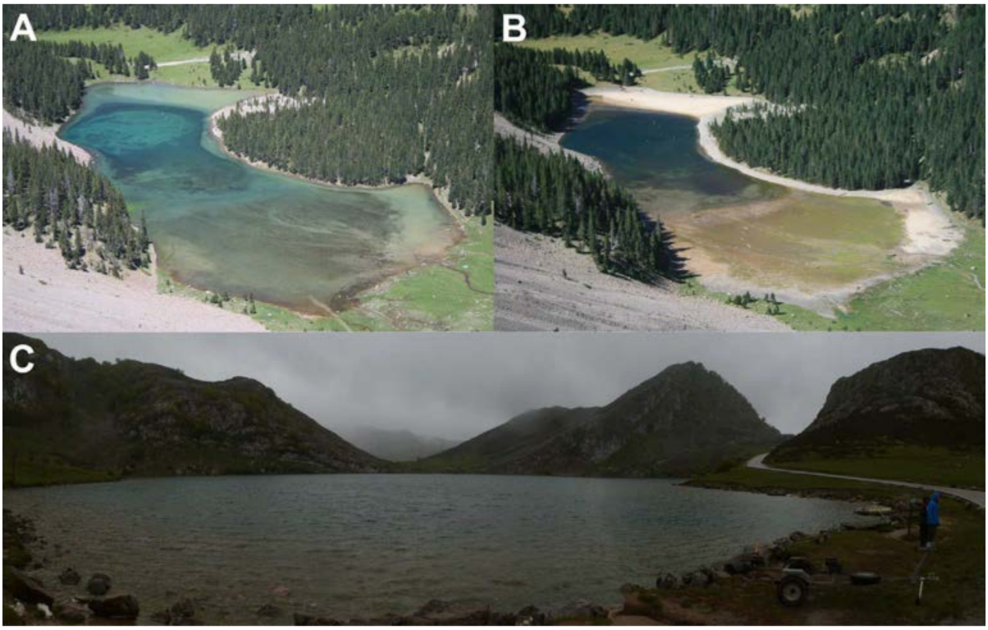


Figure M.2 Photographs of Basa de la Mora Lake during humid (a) and dry (b) seasons, and Enol Lake (c). Photographs credits: a) and b) A. Belmonte, c) P. Tarrats.

Vegetation of the area widely varies from Mediterranean vegetation at southern slopes (e.g. evergreen *Quercus* formations and sclerophyllous shrubland), to mixed forests at northern slopes consisting of *Pinus sylvestris* and deciduous taxa such as *Betula alba, Corylus avellana, Fagus sylvatica, Quercus faginea* and *Quercus petraea*, among others (Pérez-Sanz et al. 2013). The lake is located near the current treeline of the area and the local vegetation in the lake catchment is mainly composed of alpine grassland (43%), *Pinus uncinata* forest (9%) and *Juniperus communis-Rhododendron sp.* shrubland. Basa de la Mora includes aquatic vegetation in its basin with several *Potamogeton* spp. and *Ranunculus* sp. (Goñi and Benito 2009). It is characterized by seasonal water level fluctuation, which can be of the order of 2 m. Little information is available on the limnological parameters of the lake beyond the physicochemical data collected by the EMERGE project (EMERGE 2002) and during the fieldwork conducted to retrieve the sequence analysed in this thesis. According to these data the lake is oligotrophic (TP= 9.2 μg l^{-1}, TN= 918 μg l^{-1}), with a pH of 8.96, a conductivity of almost 200 μS cm^{-1} and DOC concentrations of 3.5 mgC l^{-1}.

SAMPLING STRATEGIES

This thesis focuses on 3 different types of benthic sediment records: i) living chironomid community, ii) recent subfossil chironomid community, and iii) downcore subfossil chironomid community.

In order to evaluate the living chironomid community of Enol Lake used in Chapters 1 and 2, we performed 8 fieldwork campaigns in two consecutive years (2013 and 2014), in May, July, September and November of each year. In every sampling campaign, both littoral and bottom benthic samples were collected. In the upper littoral zone, we took 3 samples per campaign belonging to the three different dominant habitats found in the lake: i) sediment, ii) stones and iii) blocks. Samples were collected using the kick-sampling method (sampling surface: 1 m^2) with a 250 μm mesh net and the filtered sample was preserved in formaldehyde at 4%. In the case of the sublittoral and profundal zones (2-22 m), we used an Ekman grab (sampling surface: 225 cm^2), with three replicates per sample following a depth transect, collecting samples every 2 m. These samples were also sieved in the field using a 250 μm mesh net and preserved in formaldehyde at 4%.

The recent subfossil record from Enol Lake used in Chapter 2 was obtained in two sampling campaigns performed in July of 2013 and 2014. Samples were obtained in July basing on the convention adopted for chironomid-based temperature reconstructions, which are supposed to estimate mean July temperatures. Thus, all studies aiming at studying chironomid recent subfossil records should to perform the fieldwork in July. In this campaigns, we collected 3 samples per depth following a depth transect every 4 meters (at 4, 8, 12, 16 and 20 m) in July 2013, and every 2 m of depth following two transects in July 2014. By that, we aimed to capture spatial variability, either longitudinal or transversal. Surface sediment samples for subfossil midge analysis were taken using a UWITEC gravity corer. The first centimetre of each core was subsampled in the field using a UWITEC core cutter and the material was transferred to zip bags and preserved in the refrigerator until its analysis in the laboratory.

Finally, the subfossil material analysed in Chapters 3 and 4 comes from different lake sediment cores. In Chapter 3, 2 sediment cores from Enol Lake were retrieved in September of 2014 using a UWITEC gravity corer at different depths: 8 and 20 m, respectively. On the other hand, the Basa de la Mora Lake paleoenvironmental sequence analysed in Chapter 4 comes from two parallel cores retrieved in July 2008 by the Quaternary Environments Group of the Pyrenean Institute of Ecology (IPE-CSIC) using a UWITEC coring system with platform (BSM08-1A-1U, core length=11.75 m). Additionally, a gravity core for completing the upper part of the sequence (BSM08-1B-1G, core length=60 cm) was collected using a UWITEC short gravity corer.

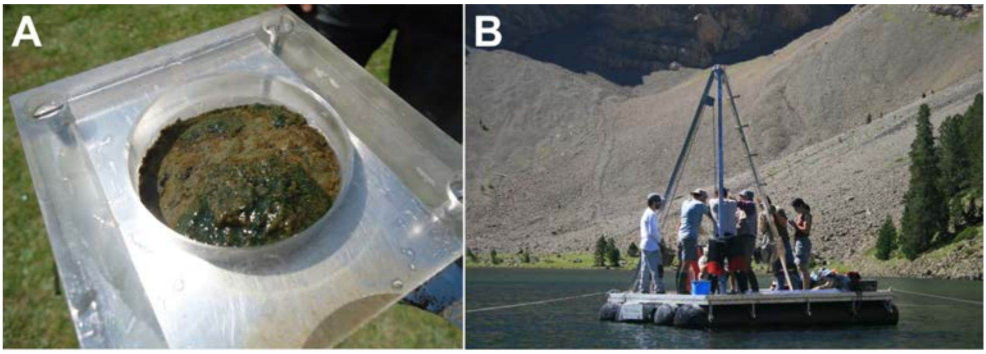


Figure M.3 Cutting the first centimetre of a sediment core from Enol Lake for recent subfossil chironomid community analysis (a), and UWITEC platform used to retrieve the sequence from Basa de la Mora Lake (b). Photographs credits: a) P. Tarrats, b) A. Belmonte.

LABORATORY WORK

This thesis is exclusively focused on the analysis of chironomids from lake sediments. However, we have analysed different type of samples: kick littoral samples, grab samples, and sediment cores. Thus, these analyses relied on slightly different laboratory protocols depending on the analysed record (i.e. living larvae or subfossil).

For the living chironomid community, all the Chironomidae larvae present in each sample were firstly sorted up to a maximum of 300 individuals. Initially, we classified the chironomid specimens by morphotypes. A certain number of larvae of each morphotype were treated with 10% potassium hydroxide (KOH) at 70 ºC and, after dehydration, were mounted on microscope slides in Euparal® for their identification. Later, once morphotypes were identified, several taxa were counted directly from samples without further processing. Those morphotypes that were not previously identified, were treated and mounted as explained above.

The laboratory protocol for the subfossil samples, both recent and downcore, followed standard procedures (Walker 2001): i) wet sediment was weighted, deflocculated in warm KOH (70 ºC) and stirred at 300 rpm for 20 minutes; ii) the sediment was sieved through a 90 μm mesh size sieve; iii) Chironomidae head capsules (HC) were picked out under a stereo microscope at 40x magnification, dehydrated in 96% ethanol and mounted in Euparal®; and iv) Chironomidae head capsules (HC) were identified.

Both living larvae and subfossil Chironomidae specimens were identified using an optical microscope (Olympus CX41) at 400x magnification and several taxonomic keys (Wiederholm 1983; Rieradevall and Brooks 2001; Brooks et al. 2007). Moreover, the identification of living larvae to species level was validated through the examination of a large collection of pupal exuviae from Enol Lake using the key of Langton and Visser (2003).

DATA ANALYSIS

Statistical analyses were performed using different specialized softwares: R (R Core Team 2016), Paleontological Statistics (PAST) (Hammer et al. 2001), CANOCO (ter Braak and Smilauer 2002) and C2 (Juggins 2007). Stratigraphic diagrams were plotted using Psimpoll 4.27 software (Bennett 2009).

Multivariate analyses constituted the core of the data analysis performed in this thesis and were mainly carried out to understand compositional changes in the chironomid assemblages. In Chapter 1 and 3, a Nonmetric Multidimensional Scaling (NMDS) was employed to evaluate the similarity in the chironomid community between different assemblages (Chapter 1) and sediment cores

(Chapter 3). Forward selection Redundancy Analysis (RDA) was used in Chapter 1 in order to understand the temporal variations in living chironomid assemblages in relation to environmental variable at different depths. Principal Component Analysis (PCA) was performed in Chapter 2 and 3 to explore the distribution patterns of different samples. In Chapter 2, it was used to understand the relation between living and subfossil samples, while in Chapter 3 it was performed to assess changes in downcore distribution. Finally, cluster analysis was performed to determine groups of samples or downcore zones. In Chapter 1, a k-means cluster analysis was used to classify the samples in different groups. In this same chapter, the differences in the environmental characteristics of each group were tested by means of non-parametric Kruskal-Wallis tests. The zonation of sediment core sequences for Chapter 3 and 4 was performed through CONISS (Constrained Incremental Sums of Squares) cluster analysis (Grimm 1987), and the number of significant zones was tested by means of a broken-stick model.

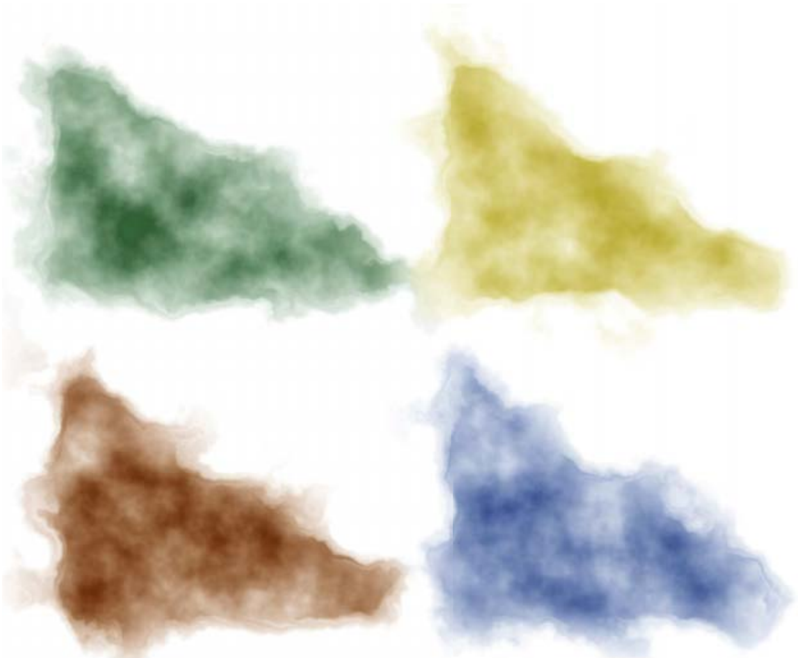
Chironomid-based temperature estimates in Chapter 4 were obtained using Weighted Averaging-Partial Least Squares (WAPLS) regression (ter Braak and Juggins 1993; ter Braak et al. 1993). Finally, Pearson correlations were calculated in Chapter 2 to estimate the correlation between samples and records.

Table M.1 Summary of materials and methods used in this thesis.

	Location	Community	Sampling	Laboratory	Data analysis
Chapter 1	Enol Lake	Living larvae	8 campaigns (2013, 2014) - 3 littoral samples/campaign, - 11 sublittoral and profundal samples/campaign, 3 replicates	Larvae: - Sorting - Treatment - Mounting - Identification	- Diagram representation - Multivariate analysis (NMDS, RDA) - Kruskal-Wallis tests - Clustering, IndVal
Chapter 2	Enol Lake	Living larvae Recent subfossil	39 gravity cores (2013, 2014) - 1 cm sediment Samples from Chapter 1	Head capsules: - Sorting - Treatment - Mounting - Identification	- Diagram representation - Multivariate analysis (PCA) - Correlation analyses
Chapter 3	Enol Lake	Subfossil	2 gravity cores (2014): - 8 m depth: 38 samples - 20 m depth: 41 samples	Head capsules: - Sorting - Treatment - Mounting - Identification	- Diagram representation - CONISS clustering - Multivariate analysis (NMDS, PCA) - Mantel test
Chapter 4	Basa de la Mora Lake	Subfossil	1 UWITEC long core (2008): - 12.35 m long - 69 samples	Head capsules: - Sorting - Treatment - Mounting - Identification	- Diagram representation - CONISS clustering - Temperature estimates (WAPLS)

CHAPTER 1

Chironomid communities as indicators of local and global changes in an oligotrophic mountain lake (Enol Lake, northwestern Spain)



The benthos of the mountain Enol Lake (Picos de Europa National Park, Spain) was analysed in order to understand the spatiotemporal factors and patterns controlling its current Chironomidae community. In total, more than 14,000 chironomid larvae were identified, belonging to 27 taxa. The results have pointed out the presence of 3 main chironomid assemblages in the lake: i) littoral community, which is mainly controlled by temperature and oxygen seasonal changes, ii) *Chara*-dominant community, which is mainly controlled by the presence and abundance of Characeae in the lake, and iii) profundal community, which is affected by low oxygen levels caused by sediment and organic matter discharge to the lake due to human pressures in the lake basin. We provide valuable insights for the managers to understand the current ecological status of Enol Lake and to evaluate which measures should be implemented to preserve or improve it. Moreover, our results constitute an essential step forward to improve the interpretation of the past changes of the lake by means of the subfossil chironomid community.

INTRODUCTION

Chironomidae (Insecta: Diptera) are the most widespread of all freshwater macroinvertebrate families (Ferrington 2008) occurring in almost all kinds of freshwater bodies of all zoogeographic regions over the world (Sæther 2000). They are also often the most abundant and diverse group in freshwaters, representing up to 50% of the macroinvertebrate community (Armitage et al. 1995). Chironomids are widely regarded as effective indicators of water quality and changes in habitat conditions, since they exhibit a great variety of ecological traits and can be present over a wide range of environmental conditions (Battarbee 2000; Walker 2001; Cañedo-Argüelles et al. 2012).

Temperature has been traditionally considered one of the most important drivers controlling chironomid distribution (Eggermont and Heiri 2012; Marziali and Rossaro 2013), but other factors such as water depth (Korhola et al. 2000; Engels et al. 2012), dissolved oxygen (Little and Smol 2001) or trophic status (Brodersen and Quinlan 2006) can also be important. Moreover Chironomidae communities experience marked temporal and spatial variations in their composition. The temporal variability of the Chironomidae community is mainly related to the life cycle characteristics (Heinis and Davids 1993), which lead to different emergence patterns that are known to be principally controlled by water temperature and light intensity (Kureck 1980). Chironomidae spatial changes result from different factors (e.g. aquatic vegetation, dissolved oxygen) affecting the community at different depths and habitats (Prat and Rieradevall 1995; Eggermont et al. 2008; Frossard et al. 2013).

The key factors controlling Chironomidae communities may change among lakes according to local and global conditions. Understanding the response of the current communities can be helpful to interpret the past and predict future changes, since Chironomidae capsules are well preserved through time in the sediment, allowing us to conduct paleolimnological studies (e.g. Walker 2001). In this regard, local and regional studies of the present fauna are necessary to calibrate how the community has changed and will change in the future if Chironomidae want to be used for predictive studies of global change (e.g. using transfer functions) (Luoto 2010). At the same time, by exploring the spatial and temporal variations in community composition, the influence of local factors (e.g. land use, organic enrichment) can be disentangled. Thus, Chironomidae can provide useful information for water managers, helping them to adopt appropriate measures to achieve and maintain the good ecological status of lakes, e.g. as required in Europe by the Water Framework Directive.

Lakes have been widely considered as sentinels of climate and environmental changes (Adrian et al. 2009; Schindler 2009). Among them, mountain lakes are specially sensitive to external forcing due to their characteristics (higher altitude, higher UV radiation, lower nutrients, etc.) (Catalan et al. 2006). Thus, special scientific attention has been paid to this type of lakes (Battarbee 2005; Catalan et al. 2009b). In the Iberian Peninsula, there are some studies focused on chironomid communities from mountain lakes (Real and Prat 1992; Rieradevall and Prat 1999; Real et al. 2000), although the majority took place in the Pyrenees (Catalan et al. 2009a; de Mendoza and Catalan 2010) or in the Central Range (Toro et al. 2006). Furthermore, these studies usually focus on the littoral part of the lake, with only a few covering the whole depth transect (Rieradevall et al. 1999; Rieradevall and Prat 1999) and usually only sampling once or twice, which implies the loss of information related to seasonal variability. Here we address seasonal and spatial variability of the Chironomidae communities, providing with certainty one of the most intensive work conducted on a single mountain lake of the Iberian Peninsula to date.

Our main hypothesis was that depth would play an important role in explaining chironomid community changes in Enol Lake through changes in environmental factors. Together with depth, we hypothesized that the presence of macrophytes and algae in the lake should be important, since the macrophyte and algae-related chironomid species tend to be very different than the sediment-related ones (Langdon et al. 2010; Vermaire et al. 2013). The knowledge of the factors that control the community in the lake today are a preliminary step in understanding the past changes in the community (using the subfossil midges present in the sediment) and the possible future changes related to local, regional and global drivers, which will be the objective of the forthcoming studies in the lake.

MATERIALS AND METHODS

Study site

Enol Lake (43º 16’ N, 4º 59’ W, 1070 m a.s.l.) (Fig. 1.1) is a karstic lake of glacial origin located in the northern part of Spain (Asturias), in the western massif of Picos de Europa National Park. It has a water surface of 12.2 ha, a maximum depth of 22 m and a small watershed (1.5 km^2). The lake is fed by groundwater and surface runoff and it has no permanent inlets. Water looses occur through evaporation, groundwater discharges and an outlet located at the northeast border of the lake, which is regulated by a small dam. Previous surveys (Velasco et al. 1999; Moreno et al. 2011) and the data collected in our study (Sánchez-España et al. 2017) characterize the lake as warm monomictic (with a thermocline located between 8 and 12 m from early July until early November). The lake is oligotrophic (total phosphorous 8 μg l^{-1}, Chl-a 0.5-1 μg l^{-1}), moderately hard (alkalinity 2.4 meq l^{-1} and 24-37 mg Ca l^{-1}) and with a conductivity between 150 μS cm^{-1} at the surface and 227 μS cm^{-1} at the bottom. It is almost fully covered with a great carpet of *Chara* sp. between 2 and 8 m of depth, while *Potamogeton natans* occurs between 1 and 3 m of depth. Despite this oligotrophy the bottom of the lake is devoid of oxygen for 4-6 months every year during the stratification period (García-Criado and Martínez-Sanz 2010).

Sampling

Samplings were performed in two consecutive years (2013 and 2014), in May, July, September and November of each year. In every sampling campaign, both littoral and bottom samples were collected. In the upper littoral zone, we took 3 samples per campaign belonging to the three different dominant habitats found in the lake: i) sediment, ii) stones and iii) blocks. Samples were collected using the kick-sampling method (sampling surface: 1 m^2) with a 250 μm mesh net and the filtered sample was preserved in formaldehyde at 4%. In the case of the sublittoral and profundal zones (2-22 m), we used an Ekman grab (sampling surface: 225 cm^2), with three replicates per sample following a depth transect, collecting samples every 2 m. These samples were also sieved in the field using a 250 μm mesh net and preserved in formaldehyde at 4%.

In the laboratory we sorted all the Chironomidae larvae present in the sample up to a maximum of 300 individuals. Initially, we sorted the chironomid specimens by morphotypes. A certain number of larvae of each morphotype were treated with 10% potassium hydroxide (KOH) at 70 ºC and, after dehydration, were mounted on microscope slides in Euparal®. The Chironomidae specimens were identified using an optical microscope (Olympus CX41) at 400x magnification and several taxonomic keys (Wiederholm 1983; Rieradevall and Brooks 2001; Brooks et al. 2007). Later, when morphotypes were identified, several taxa were counted directly from samples without further processing. The identification of larvae to species level was validated through the examination of a large collection of pupal exuviae from the lake using the key of Langton and Visser (2003).

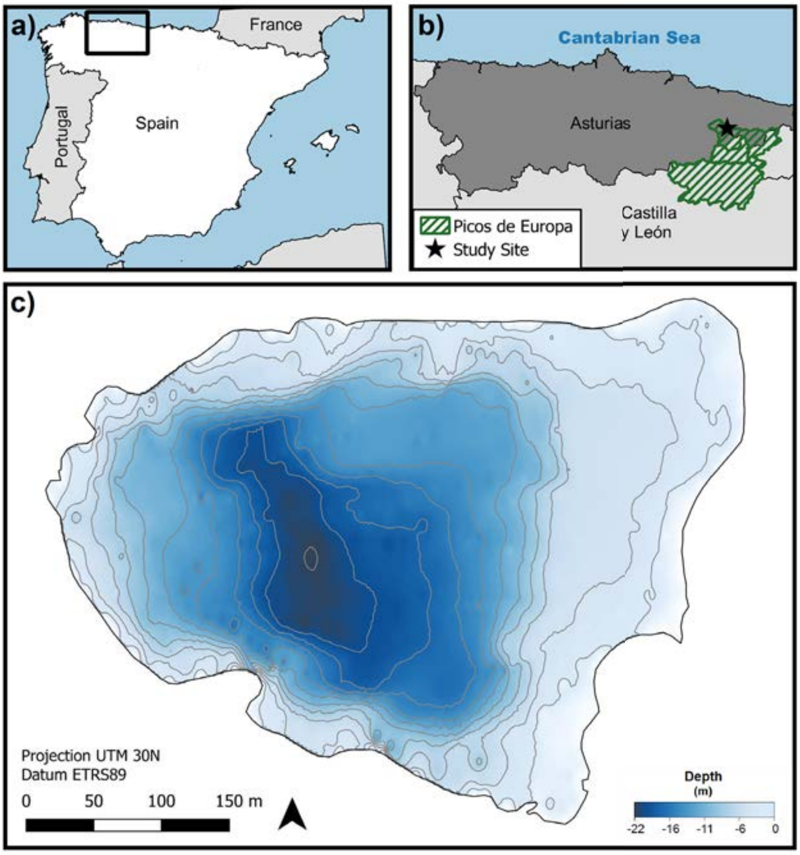


Figure 1.1 Enol Lake location map within the northern region of the Iberian Peninsula (a, b). Bathymetry of Enol Lake (c) adapted from Rodríguez-García et al. (2016).

Environmental variables were collected by researchers of the Department of Geological Resources Research of the Geological and Mining Institute of Spain (IGME) in the same 8 campaigns. Temperature, pH, dissolved oxygen and conductivity were measured every 0.5 m of depth using a multiparametric sensor (Hydrolab MS5 and DS5). Water samples were collected every 5 m of depth for total organic carbon (TOC), total inorganic carbon (TIC), nutrients, major ions and trace metals analyses. These samples were analysed in the laboratory following standard methods (Rice et al. 2012).

Data analysis

The general distribution of the chironomid taxa along depth was represented by the program Psimpoll (Bennett 2009), using the mean of the relative abundances of each taxon per depth. Statistical analyses were performed using R software (R Core Team 2016). Rare taxa, defined by those that did not reach a relative abundance of ≥2% in at least two samples, were removed from the analyses. For the statistical analyses we used total abundances, which were previously transformed to log (x+1).

Assemblage ordination was explored through Nonmetric Multidimensional Scaling (NMDS) of the species matrix using the function 'metaMDS' of the R package "vegan" (Oksanen et al. 2016) and the Bray-Curtis dissimilarity index to calculate the resemblance matrix. We classified the samples in 3 groups through a k-means clustering, then the indicator taxa of each group were identified through an IndVal analysis (Dufrêne and Legendre 1997) using the 'multipatt' function in the R package "indicspecies" (De Cáceres and Legendre 2009). This analysis assigned each taxon to a most probable group based on its relative abundance and relative frequency and provided an indicator value (IV, varying between 0 and 1) and an alpha value obtained by Monte Carlo permutations (9999 runs). The differences in the environmental characteristics of each group were tested by means of non-parametric Kruskal-Wallis tests, as variables were not normally distributed. In this case, we used temperature, pH, dissolved oxygen, conductivity and *Chara* sp. abundance as environmental descriptors. When Kruskal-Wallis tests yielded significant results, pairwise Mann-Whitney tests were performed to detect significant differences between groups. Finally, temporal variations in the chironomid assemblages were explored through a Redundancy Analysis (RDA) at 5 depths (0.5, 5, 10, 15 and 20m) with forward selection of explanatory variables using the 'rda' and 'ordistep' functions of the "vegan" package (Oksanen et al. 2016) respectively. For these analyses, all environmental variables measured were included. Prior to this, all variables were normalized and Spearman correlation matrices were calculated in order to discard those variables that were significantly correlated (i.e. $\varrho \geq 0.85$).

Table 1.1 Chironomid taxa list of Enol Lake and equivalent code proposed by Schnell et al. (1999).

Sub-family	Name	Code
Chironominae	*Chironomus (Chironomus) plumosus*	Chir plu
	Einfeldia pagana	Einf pag
	Endochironomus albipennis	Endo alb
	Endochironomus tendens	Endo ten
	Glyptotendipes (Glyptotendipes) pallens	Glyp pal
	Microchironomus tener	Micc ten
	Microtendipes pedellus	Mict ped
	Parachironomus arcuatus	Parc arc
	Paratendipes albimanus	Patd alb
	Polypedilum (Polypedilum) nubeculosum	Poly nuc
	Polypedilum (Pentapedilum) nubens	Poly nub
	Stictochironomus sticticus	Stic sti
	Cladotanytarsus atridorsum	Clat atr
	Micropsectra sp.	Micrind
	Paratanytarsus bituberculatus	Part bit
	Tanytarsus bathophilus	Tany bat
Orthocladiinae	*Corynoneura lobata*	Cory lob
	Eukiefferiella coerulescens	Euki coe
	Eukiefferiella sp.	Eukiind
	Orthocladius sp.	Orthort
	Parakiefferiella bathophila	Park bat
	Parametriocnemus stylatus	Pare sty
	Paratrichocladius rufiventris	Patr ruf
Tanypodinae	*Ablabesmyia monilis*	Abla mon
	Procladius choreus	Proc cho
	Zavrelimyia sp.	Zavyind
Prodiamesinae	*Prodiamesa olivacea*	Prod oli

RESULTS

Assemblage composition

We identified a total of 14,248 chironomid larvae belonging to 27 species from 4 subfamilies. The most diverse and abundant was the Chironominae subfamily, with 12 species of the Tribe Chironomini and 4 species of the Tribe Tanytarsini, followed by Orthocladiinae (7 species), Tanypodinae (3 species) and Prodiamesinae (1 species). Among them, we identified *Einfeldia pagana* (Meigen, 1838), which is considered a rare species in the Iberian Peninsula with only one previous citation (de Mendoza 2013). All the identified taxa are shown in Table 1.1, with their names following the Fauna Europaea database nomenclature (Sæther and Spies 2013) and the equivalent code proposed by Schnell et al. (1999).

Spatial variation

According to the k-means clustering, samples were classified as follows: i) profundal samples (10-22 m), ii) *Chara*-dominant samples (2-8 m) and iii) littoral samples (0.5 m). All environmental variables included in the Kruskal-Wallis tests significantly differed among these groups (Fig. 1.2). Temperature, conductivity, pH and dissolved oxygen showed non-significant differences between the littoral and the *Chara*-zone group and significant differences between these two groups and the profundal samples. *Chara* sp. abundance showed non-significant differences between the littoral and the profundal groups and significant differences between these two groups and the *Chara*-dominant one.

Only a few taxa were present in the profundal zone, mainly represented by *Chironomus (Chironomus) plumosus* (Linnaeus, 1758) (Fig. 1.3). However, the IndVal analysis did not get any taxon exclusively associated with this group, as *C. plumosus* is also present in other zones (although with lower abundances) (Table S.1). *E. pagana* was considered to be exclusively indicator of the *Chara*-dominant zone while *Corynoneura lobata* (Edwards, 1924), *Polypedilum (Polypedilum) nubeculosum* (Meigen, 1804), *Stictochironomus sticticus* (Fabricius, 1781), *Procladius (Holotanypus) choreus* (Meigen, 1804), *Ablabesmyia (Ablabesmyia) monilis* (Linnaeus, 1758), *Parakiefferiella bathophila* (Kieffer, 1912), *Paratanytarus bituberculatus* (Edwards, 1929) and *Endochironomus albipennis* (Meigen, 1830) were significantly associated with both *Chara* and littoral zones, although the last two species were significantly more abundant where *Chara* sp. was present (45 and 22 % respectively for *P. bituberculatus* and 12 and 5% for *E. albipennis*). Finally, *Cladotanytarsus (Cladotanytarsus) atridorsum* (Kieffer, 1924), *Microtendipes pedellus* (De Geer, 1776), *Polypedilum (Pentapedilum) nubens* (Edwards, 1929), *Endochironomus tendens* (Fabricius, 1775) and *Eukiefferiella coerulescens* (Kieffer, 1926), were significantly associated with the littoral zone, with *C. atridorsum* being the most abundant (27%).

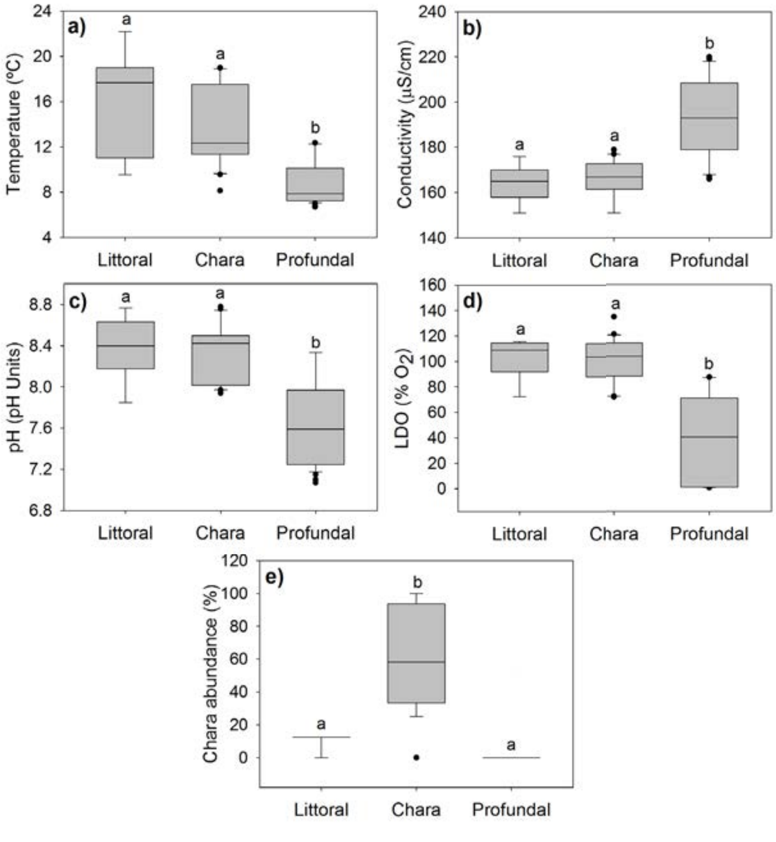


Figure 1.2 Box plots representing temperature (a), conductivity (b), pH (c), dissolved oxygen (d) and *Chara* sp. Abundance (d) according to the identified groups: littoral, *Chara* and profundal. Mann-Whitney results are specified above each box, with the same letter (a) representing non-significant differences between groups and different letter (b) representing significant differences.

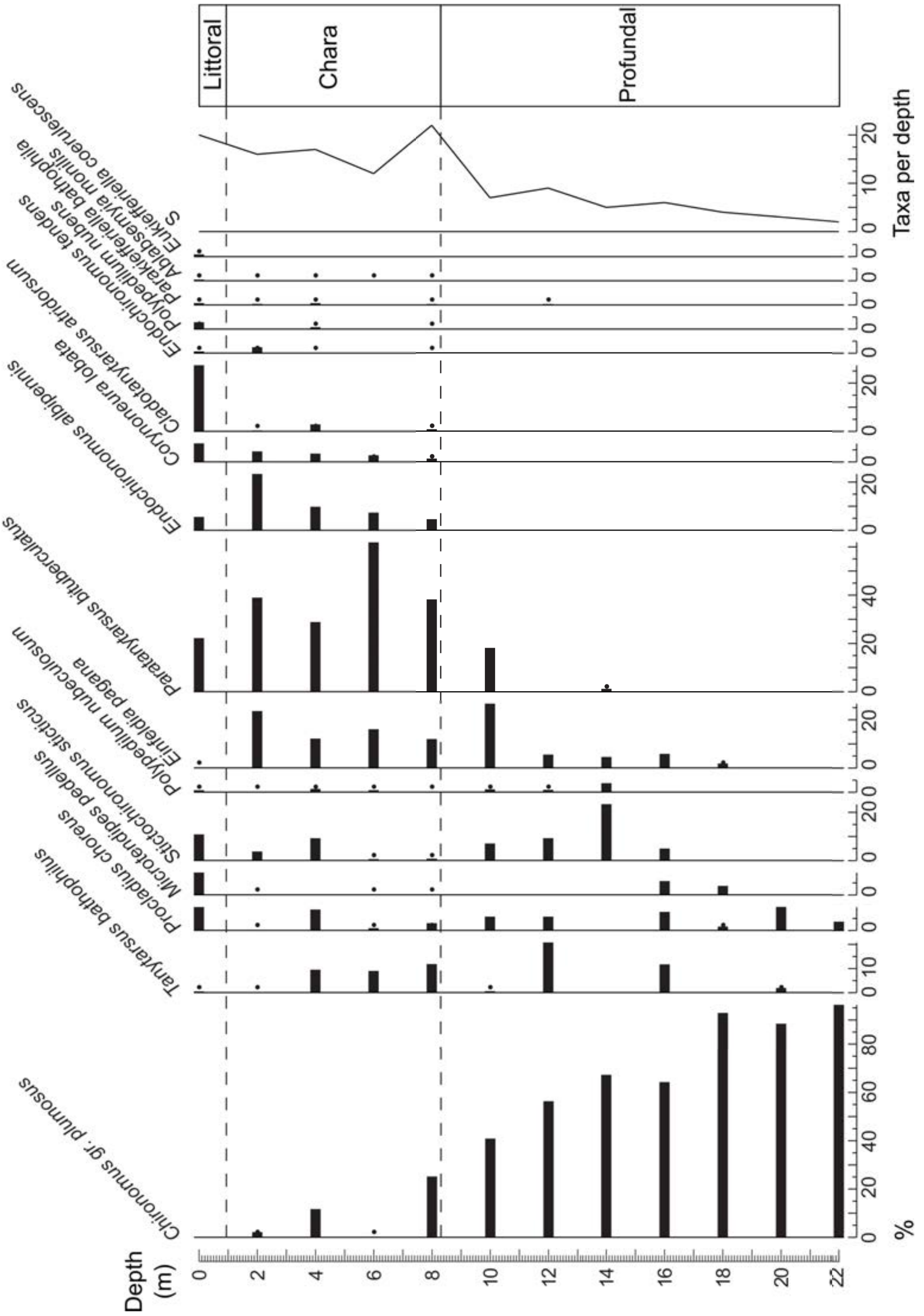


Figure 1.3 Depth distribution of Chironomidae in Enol Lake. The percentage of each taxon for each depth is shown.

According to the NMDS 3 groups of assemblages could be identified (Fig. 1.4), similar to those obtained with the k-means clustering (littoral, *Chara*-dominant and profundal assemblages). The 8 samples belonging to the littoral group appear well separated in the NMDS plot. The *Chara*-dominant group included samples located between 2 and 8 m of depth, but also few samples at 10 m depth, on the edge of *Chara* sp. zone. Finally, the profundal group mainly included samples located below 10 m of depth, although three samples included in this group were collected at lower depth. These lower-depth samples were collected in soft sediment substrate, devoid of vegetation.

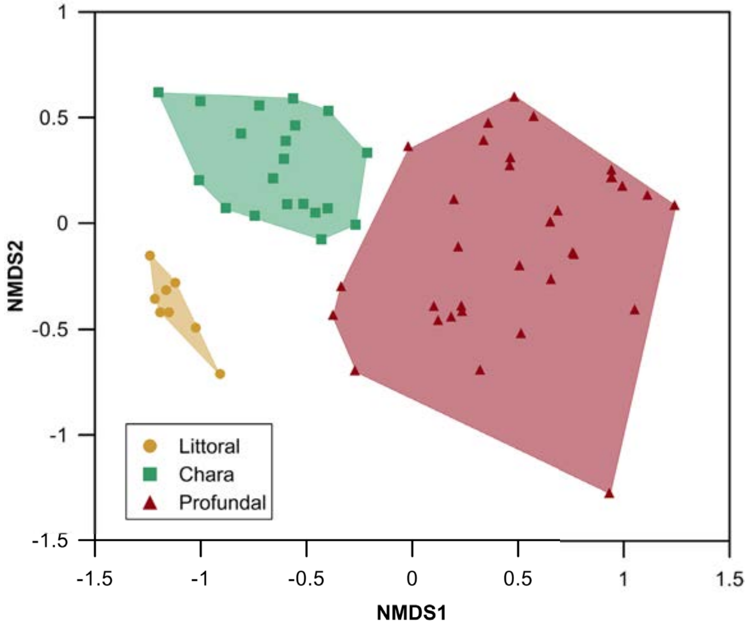


Figure 1.4 NMDS analysis of the Chironomidae community of Enol Lake. The samples are separated into three groups: i) Littoral, ii) *Chara* and iii) Profundal. Stress = 0.12.

Temporal variation

According to forward selection, temperature and dissolved oxygen significantly explained the temporal variation in the Chironomidae assemblages in the littoral zone (Fig. 1.5). As a consequence, the sites were ordered by sampling date, with higher temperatures and dissolved oxygen summer samples plotted in the upper right part of Fig. 1.5. Although magnesium (Mg) was selected at 5 m of depth and sulphates (SO_4^{2-}) and bicarbonate ($HC0_3^-$) were significantly selected at 10 m, they showed very narrow concentration ranges (i.e. 0-3 mg L^{-1} of Mg at 5 m, 2-15 mg L^{-1} of SO_4^{2-} and 84-101 mg L^{-1} of $HC0_3^-$ at 10 m). Finally, no environmental variable was significantly related with the temporal changes in Chironomidae assemblages in the profundal zone (i.e. 15 and 20 m).

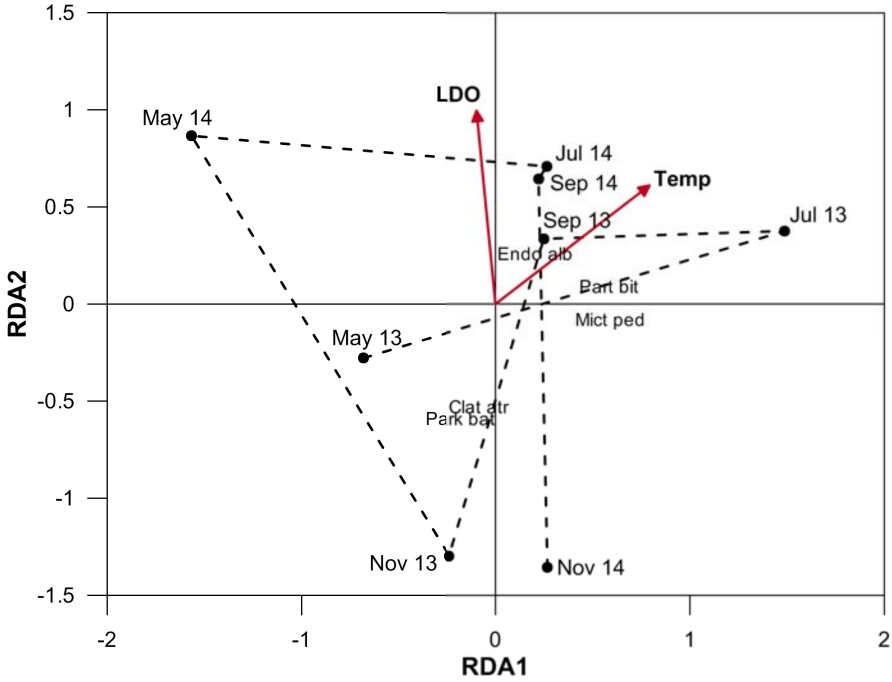


Figure 1.5 Redundancy Analysis plot showing the relationships between all sampling campaigns and the statistically significant variables in the littoral zone.

DISCUSSION

Our results show that, according to Chironomidae assemblages, Enol Lake is clearly differentiated in 3 zones: a littoral zone, a sublittoral zone dominated by *Chara* sp., and a profundal zone. In the littoral zone, both abundance and composition of Chironomidae changed in time. In the *Chara*-zone, Chironomidae assemblages were constantly dominated by a single species: *P. bituberculatus*. The profundal zone was dominated by *C. plumosus*, indicator of low oxygen concentration. Overall, the diversity of midges decreased with depth, as previously reported in many other lakes (e.g. Lindegaard 1992; Korhola et al. 2000; Chen et al. 2014). Furthermore, the spatial variation in the assemblages' composition was consistent with the known autoecology of the identified taxa (Pinder and Reiss 1983; Pinder 1986; Brodin 1986; Prat and Rieradevall 1995; Brodersen and Lindegaard 1997; Brodersen et al. 2001; Brooks et al. 2007). *C. plumosus* is known to live in water with low oxygen content (e.g. Brooks et al. 2007), whereas *P. bituberculatus* has been often associated with the presence of macrophytes (Brodersen et al. 2001).

C. plumosus has been commonly regarded as an indicator of eutrophication (Brodersen and Quinlan 2006) due to its tolerance to low oxygen concentrations and even anoxia (Real et al. 2000). The dominance of this species in the profundal zone of Enol Lake would suggest that the lake is eutrophic, although according to primary production rates the lake is oligotrophic. Since the nutrient content of the water is low (Velasco et al. 1999; García-Criado and Martínez-Sanz 2010; Moreno et al. 2011; Sánchez-España et al. 2017), the deoxygenation of the bottom should be related to a source of organic matter different from phytoplankton production. The lake basin has been traditionally used for intensive pasture of cow (Rodríguez Castañón 1996). This cattle grazing leads to important soil erosion, which increases the input of allochthonous sediment rich in organic matter, and a direct input of cow manure into the lake, which constitutes an additional source of organic carbon and nitrogen (Sánchez-España et al. 2017). Thus, according to Sánchez-España et al. (2017), this factor is largely responsible for the high oxygen consumption of hypolimnetic waters in Enol Lake. Organic matter measures in sediment cores from Enol Lake emphasized an increase of TOC levels in the sediment during the recent decades (Ortiz et al. 2016), supporting the idea of an increase in organic matter input to the lake due to human land uses in the basin area. The oxygen consumption is enhanced by the relative low volume of water in the hypolimnion and the extended period of the hypolimnetic anoxia. In this regard, Sánchez-España et al. (2017) showed that the spatial and temporal extent of the anoxic period is increasing over the last years, suggesting a deterioration of the lake trophic conditions. The absence of oxygen in the deep zone has been also noted in several mountain lakes but usually without such temporal extension and dominance of *C. plumosus,* as it is not commonly found in these type of lakes (Walker 1993). In this respect, the presence of *Chironomus* in oligotrophic environments has been usually associated with oxygen depletion caused by the ice-cover period (e.g. in the Pyrenees (Capblancq and Laville 1983) or in Laguna Cimera (Central Range) (Granados and Toro 2000)). However, this circumstance is not responsible of the anoxic conditions found in Enol Lake, since the stratification period caused by ice-cover is very short if present. Moreover, the temporal stability of the profundal chironomid community (i.e. always dominated by *C. plumosus*) suggests that the extension of the anoxic period prevents the presence of taxa non-adapted to anoxic conditions even during the mixing period. Thus, the Chironomidae seem to be clearly reacting to the lake ecological conditions and changes, making them useful as ecological indicators. Overall, Enol Lake might constitute a good example of what could occur in mountain lakes if human activities occurring in the lake basin are not properly managed. This is a hypothesis that should be further tested since other factors (e.g. water temperature increase) could play an important role in oxygen depletion. In fact, the study conducted by Sánchez-España et al. (2017) showed a significant correlation between the extension of the anoxic period and the climatic conditions (i.e. temperature increase and precipitation decrease) in the last years. Thus, it seems clear that, if no measures are taken in Enol Lake, the lake trophic status will deteriorate in the future within a context of global change.

The presence of *Chara* sp. beds was mainly responsible for explaining changes in the chironomid community of the littoral and sublittoral zones. *P. bituberculatus*, *E. albipennis, E. pagana* and *C. lobata* were more abundant in *Chara* sp. beds, whereas *C. atridorsum* was present in littoral areas devoid of Characeae, similar to the results found by van den Berg et al. (1997). Since the littoral and *Chara*-dominant zones were not different in terms of key environmental variables (e.g. oxygen contents), our results suggest that the presence of *Chara* sp. was the key driver of Chironomidae assemblages in the littoral and sublittoral zones (i.e. the species commonly associated with macrophytes increased their dominance when *Chara* sp. was present). The importance of aquatic vegetation as a prime driver of Chironomidae biodiversity in lakes has been pointed out by several studies (Brodersen et al. 2001; Langdon et al. 2010; Cañedo-Argüelles and Rieradevall 2011). Characeae density has also been reported to structure lake macroinvertebrate communities (van den Berg et al. 1997, 1998).

On the other hand, temperature has been widely acknowledged as one of the main drivers affecting chironomid community composition in mountain lakes (e.g. Heiri and Lotter 2003). In Enol Lake, the temporal variability of the littoral zone assemblage was mainly driven by seasonality. This is not surprising, since seasonal changes in temperature, light incidence and food availability, are known to have a great influence on Chironomidae assemblages through changes in adult emergence (e.g. Armitage 1995; Tokeshi 1995) and habitat conditions, e.g. higher temperature levels can enhance macrophyte production (highest oxygen values) during the summer (Hering et al. 2010). In our case, spring and autumn samples were mainly represented by *C. atridorsum* and *P. bathophila*, whereas *P.bituberculatus*, *M. pedellus* and *E. albipennis* dominated the summer samples, suggesting different emergence patterns of the littoral community.

CONCLUSIONS

The results obtained in this study are crucial to understand the temporal and spatial dynamics of Chironomidae assemblages in Enol Lake, constituting an essential step forward to improve the interpretation of the past changes of the lake by means of the subfossil chironomid community. Future studies should focus on the relationship between the living and the recent subfossil communities in order to understand head capsules' transport and deposition processes. Further knowledge of these processes would constitute the key point to understand how the living community is recorded in the sediments, which would allow a correct interpretation of downcore changes. Disentangling the importance of local, regional and global factors affecting the community is necessary to calibrate past changes and making future predictions. In this regard, we have shown that the littoral community is mainly driven by regional and global processes (e.g. temperature), whereas the profundal assemblages are mainly driven by local factors, i.e. sediment and organic matter discharge into the lake caused by pasture, leading to oxygen depletion in the hypolimnion. Our results also provide valuable insights for the managers to understand the current ecological status of Enol Lake and to evaluate which measures should be implemented to preserve or improve it. For example, the dominance of pollution tolerant species in the bottom of the lake suggests that its trophic status may be changing from oligotrophy to mesothrophy in the near future if no measures are taken to counteract the direct entrance of organic matter to the lake bottom caused by pasture in the lake basin.

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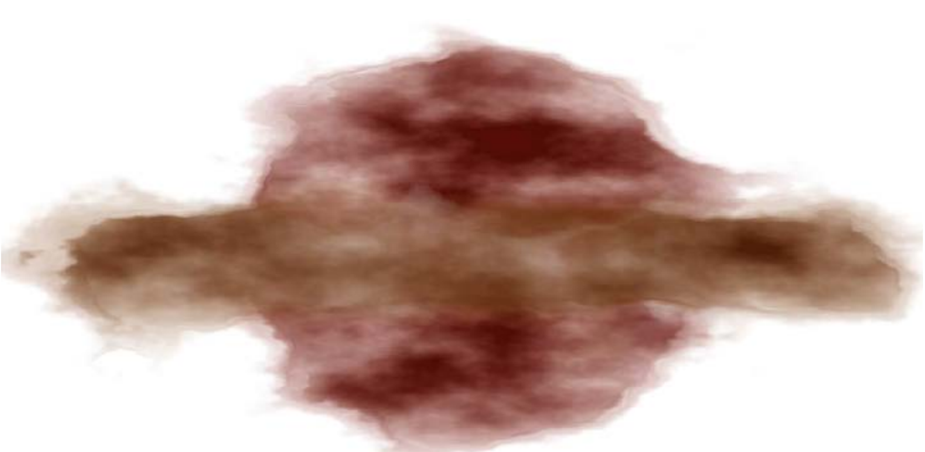
CHAPTER 2

The influence of depth and macrophyte habitat on paleoecological studies using chironomids: Enol Lake (Spain) as a case study



Paleolimnological studies often rely on a single sediment core for reconstructing past environmental changes of an entire lake system. This involves a number of assumptions about the correct representativeness of the living assemblage by the subfossil record. This study is aimed at understanding the main drivers affecting the dispersion and transportation of Chironomidae head capsules, which may affect the correct interpretation of downcore changes through over- or underrepresentation of certain taxa. We analysed the chironomid living assemblage of Enol Lake (Picos de Europa National Park, Spain) and compared to the recent subfossil assemblage at different depths. We found a highly homogeneous composition and density of recent subfossil assemblage along the depth transect (i.e. dominance of the Tanytarsini *Paratanytarsus austriacus*-type), which would indicate that a single core retrieved at any depth would be representative of the lake community. However, the composition of the benthic living assemblage changed significantly with depth, suggesting the existence of a driving force behind the dominance of *P. austriacus*-type in the subfossil assemblage. We argue that the dense mats of Characeae present in the sublittoral area (from 2 to 8m) are most likely responsible for this homogenization, since this is the preferred habitat of *Paratanytarsus*, which was found at very high densities at this depth. Thus, we conclude that the interpretation of past changes in the lake conditions should be made with caution due to the overrepresentation of *P. austriacus*-type head capsules along the depth transect. Our findings show that it is important to explore the relationship between the living and the recent subfossil fauna of each lake in paleolimnological studies, since understanding deposition and transport patterns can help to avoid misinferring past environmental and limnological conditions.

INTRODUCTION

Chironomids (Insecta: Diptera) have been traditionally used in paleolimnology due to their abundance, ubiquity, and the good preservation of their chitinous larval remains (Walker 2001). Paleolimnological studies often rely on a single sediment core for reconstructing past environmental changes of an entire lake system. This involves a number of assumptions about the similarity in composition and abundance between the living assemblage and the subfossil assemblage (van Hardenbroek et al. 2011). Moreover, this single core is usually retrieved in the lake centre, as it is believed to record all the processes and remains originated from the entire lake basin (Smol 2008).

However, it is widely known that chironomid living assemblages may experience great spatial and temporal changes, which are mainly related to different habitat preferences (Frey 1988; Eggermont et al. 2008), changes in the emergence patterns (Heinis and Davids 1993), and other ecological traits (dispersion, predation, competence, etc.). Depth is usually considered a key variable that helps to explain chironomid distribution (Lindegaard 1992; Korhola et al. 2000; Brooks et al. 2007) because it is strongly linked to several factors that affect chironomid composition and diversity (e.g. temperature, oxygen, pH, substrate, aquatic vegetation, etc.) (Prat and Rieradevall 1995; Brodersen and Quinlan 2006; Kurek and Cwynar 2009).

Subfossil chironomid remains are considered to represent the living assemblage at the moment of the sediment deposition. However, once chironomids die or moult, different diagenetic and transport processes can alter the composition of subfossil remains. In general, these processes are mainly driven by three factors: i) lake morphology (Frey 1988) and substrate composition (Heiri 2004), ii) species morphology and taphonomy (Walker et al. 1984), and iii) environmental factors, mainly wind (Frey 1988; Holmes et al. 2009), currents (Bigler et al. 2006; Luoto 2010) and sediment focusing (Schmäh 1993). All these factors indicate that each lake may respond individually and heterogeneously to these processes, as their chironomid assemblage composition differ from one to another, as well as their morphological and environmental characteristics. Moreover, such processes may affect certain taxa more strongly than others, leading to an over- or underrepresentation of certain taxa (Frey 1988; Brodersen and Lindegaard 1999; Eggermont et al. 2007). In fact, redeposition processes by sediment erosion and transportation are considered one of the most important sources of disturbance of chironomid remains (Kansanen 1986), which may lead to a misinterpretation of the environmental factors shaping subfossil assemblages.

Since each lake has distinctive geomorphological and environmental characteristics, many authors have claimed that each lake should be studied separately to disentangle the local processes that affect the subfossil assemblage used for interpreting past environmental changes (Frey 1988). Ideally, the study of the subfossil assemblage should be complemented with information on the living assemblage to obtain complete and reliable information to infer past changes (van Hardenbroek et al. 2011). However, only a few studies have explored the relationship between living and subfossil chironomid assemblages, leading to contradictory results. Whereas some studies found that most subfossil chironomids tend to remain where the larvae lived (Iovino 1975; Walker et al. 1984), van Hardenbroek et al. (2011) found that the remains are transported and redeposited offshore. The same contradictory patterns have been shown by studies focusing exclusively on the subfossil assemblage. Whereas several studies found significant changes of the subfossil assemblage with depth (Frey 1988; Heiri et al. 2003b; Heiri 2004; Kurek and Cwynar 2009; Luoto 2010; Cao et al. 2012; Karmakar et al. 2014), others (Frey 1988; Schmäh 1993; Brodersen and Lindegaard 1999; Eggermont et al. 2007; Langdon et al. 2008; Holmes et al. 2009) did not found any significant depth pattern. These contradictory results support the idea that each lake system should be intensively studied before making inferences about past climatic conditions based on subfossil chironomids.

The main objective of this study was to understand the relationship between the living and recent subfossil Chironomidae assemblage of Enol Lake (northwestern Spain). In particular, we aimed to explore the presence and relative abundance of midge remains along the depth transect, as well as to elucidate at which depth the subfossil assemblage best represents the whole community. Previous studies in Enol Lake (Tarrats et al. 2017) have shown that the living chironomid assemblage changes significantly along depth, with three main groups: i) littoral (0-2 m), ii) *Chara*-related (2-8 m), and iii) profundal (8-22 m). Our two alternative hypotheses were that: i) most of the species would remain in the same lake depth zone, and ii) some or many species would be transported to other lake zones. According to the first hypothesis, the subfossil assemblage would be reliable to infer past environmental and limnological conditions, but different cores would have to be retrieved in different zones to ensure a good representation of the overall living assemblage. According to the second hypothesis, a single core would accurately represent the whole living assemblage, but the over- or under-representation of certain taxa could lead to misleading inferences of past environmental and limnological conditions.

MATERIALS AND METHODS

Study site

Enol Lake (43º 16' N, 4º 59' W, 1070 m a.s.l.) (Fig. 2.1) is a karstic lake of glacial origin located in the northwestern part of Spain (Asturias), in the western massif of Picos de Europa National Park. It has a water surface of 12.2 ha, a maximum depth of 22 m and a small watershed (1.5 km^2). The lake is fed by groundwater and surface runoff and it has no permanent inlets. Water loses occur through evaporation, groundwater discharges and an outlet located at the northeast border of the lake, which is regulated by a small dam. Previous surveys (Velasco et al. 1999; Moreno et al. 2011) and the data collected in our study characterize the lake as warm monomictic (with a thermocline located between 8 and 12 m from early July until early November). The lake is oligotrophic (total phosphorous 8 μg l^{-1}, Chl-a 0.5-1 μg l^{-1}), moderately hard (alkalinity 2.4 meq l^{-1} and 24-37 mg Ca l^{-1}) and with a conductivity ranging between 150 μS cm^{-1} at the surface and 227 μS cm^{-1} at the bottom. It is almost fully covered with a dense carpet of *Chara sp.* between 2 and 8 m of depth, while *Potamogeton sp.* occurs between 1 and 3 m depth. Despite this oligotrophy the bottom of the lake is anoxic during 4-6 months every year during the stratification period (García-Criado and Martínez-Sanz 2010).

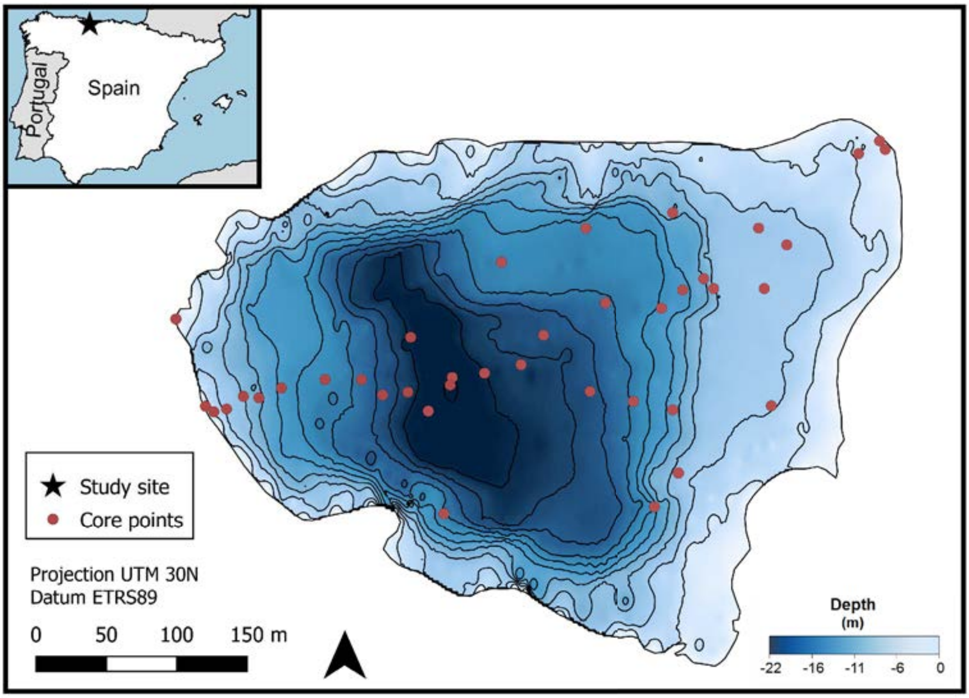


Figure 2.1 Enol Lake location map showing sampling points for recent subfossil chironomid assemblage analysis. Bathymetry adapted from Rodríguez-García et al. (2016).

Sampling and laboratory procedures

Surface sediment sampling of Enol Lake was performed in two different campaigns: i) July 2013 and ii) July 2014. The surface samples for studying the subfossil assemblage correspond to the topmost (0-1 cm) of the sediment, and their subfossil assemblage composition is considered to be analogue of the modern assemblage (Frey 1988). We collected 3 samples per depth following a depth transect every 4 meters (at 4, 8, 12, 16 and 20 m) in July 2013, and every 2 m of depth following two transects in July 2014 (Fig. 2.1). By that, we aimed to cover the whole spatial variability, either longitudinal or transversal. Surface sediment samples for subfossil midge analysis were taken using a UWITEC gravity corer. The first centimetre of each core was subsampled in the field using a UWITEC core cutter and the material was transferred to zip bags and preserved in the refrigerator until its analysis. The laboratory protocol for the subfossil samples followed a standard procedure (Walker 2001): i) wet sediment was weighted, deflocculated in warm KOH (70 ºC) and stirred at 300 rpm for 20 minutes; ii) the sediment was sieved through a 90 µm mesh size sieve; iii) Chironomidae head capsules were picked out under a stereo microscope at 40x magnification, dehydrated in 96% ethanol and mounted on microscope slides using Euparal®; iv) Chironomidae head capsules were identified under an optical microscope (Olympus CX41) at 400x magnification using several specialized guides (Wiederholm 1983; Rieradevall and Brooks 2001; Brooks et al. 2007).

Living chironomid larvae samples came from 8 sampling campaigns performed between 2013 and 2014 in May, July, September and November of each year. These samples included 3 littoral samples per campaign and 3 replicates per depth every 2 m per campaign. Littoral samples were collected using the kick-sampling method (sampling surface: 1 m^2) with a 250 µm mesh net. In the case of the sublittoral and profundal zones (2-22 m), an Ekman grab was used (sampling surface: 225 cm^2), and the samples were sieved in the field using a 250 µm mesh net and preserved in formaldehyde at 4%. For each sample, we picked up all the Chironomidae larvae present to a maximum of 300 individuals, which were firstly sorted by morphotypes. A minimum of 50 larvae of each morphotype were treated with 10% potassium hydroxide (KOH) at 70 ºC and, after dehydration, were mounted on microscope slides in Euparal®. The Chironomidae specimens were identified using an optical microscope (Olympus CX41) at 400x magnification and several taxonomic keys (Wiederholm 1983; Rieradevall and Brooks, 2001; Brooks et al. 2007). Once morphotypes were identified, the most abundant taxa were counted directly from samples without further processing. The identification of larvae was validated through the examination of a large collection of pupal exuviae from the lake using the key of Langton and Visser (2003). The results of this study can be found in Tarrats et al. (2017).

Data analysis

We merged living and subfossil data matrices by defining equivalent taxon in living and subfossil samples (Table S.2). This required some arrangements. For example, as we could not identify all subfossil Pentaneurini tribe to genus level due to head capsules remains bad preservation, we decided to combine the living identified larvae *Ablabesmyia (Ablabesmyia) monilis* (Linnaeus, 1758) and *Zavrelimyia sp.* into a single category (Pentaneurini). This circumstance is not surprising, as many authors have already emphasized the difficulty to identify some chironomid subfossil taxa, specially Tanypodinae (Walker et al. 1984), due to bad preservation of head capsules (Brooks et al. 2007; van Hardenbroek et al. 2011). Rare taxa, i.e. relative abundance ≥2% in at least two samples, were removed from all analyses.

We performed a Detrended Correspondence Analysis (DCA) to explore the distribution patterns of both living and subfossil samples. We chose between linear or unimodal-based methods by estimating the lengths of the compositional gradients (i.e. axes 1 and 2). If the length of the gradients is <2 SD, linear methods (Principal Component Analysis, PCA) are recommended, whereas if the length is >4.0 SD, unimodal methods (Correspondence Analysis, CA) should be used (Legendre and Legendre 1998; ter Braak and Šmilauer 2002). Given that the first two axes were 2.4 and 1.3 SD respectively, a PCA was performed on Chironomidae relative abundances using the software CANOCO version 4.52 (ter Braak and Šmilauer 2002).

We performed Pearson correlation tests between the average relative taxon abundances of the subfossil assemblage at every depth and the whole average relative taxon abundances of the living assemblage to explore if the subfossil samples were appropriately explaining the total current assemblage. The same correlation analyses were performed to understand the representativeness of a single sample as a reference to the selection of the coring point in paleolimnological downcore studies. In this case, we performed Pearson correlation tests between the average relative taxon abundances of the subfossil assemblage at every depth with the whole average relative taxon abundances of the subfossil assemblage.

The comparison between the concentrations of living taxa and subfossil remains was plotted using the software Psimpoll (Bennett 2009). In order to understand the relationship between the larvae and the subfossil remains, as well as to understand the transport and deposition of the different taxa, we developed a ‘transport index’. This index compares the difference between the abundance of larvae and head capsules for each taxon at every depth category. These categories were established following the different significant groups observed in the living assemblage (Tarrats et al. 2017): i) Littoral, ii) *Chara* zone and iii) profundal. We calculated percentages of each taxon per depth in relation with its total concentrations along the depth transect. Positive values of this index are related to an overrepresentation of larvae, whereas negative values are related to an overrepresentation of head capsules.

RESULTS

A total of 5,500 chironomid head capsules and 14,000 chironomid larvae were identified, belonging to 4 subfamilies (Chironominae, Orthocladiinae, Tanypodinae and Prodiamesinae). We identified 24 taxa in the case of subfossil chironomids, belonging to 21 genera, and 27 taxa in the case of living larvae, belonging to 24 genera. Overall, both subfossil and living communities were similar in terms of taxonomic composition (Table S.2). Most taxa were identified both in the living and the subfossil assemblages; exclusive taxa for any of these assemblages were rare (did not reach 2% in at least 2 samples). Thus the assemblage composition of both living and recent subfossil chironomids can be considered as close analogues.

Community ordination and correlation

The PCA using all samples (Fig. 2.2) explained 49.1% and 12.1% of variance in axis 1 and 2, respectively. Living assemblage samples were arranged according to depth in the first axis, with *Chironomus (Chironomus) plumosus* (Linnaeus, 1758) showing a clear preference for deeper zones when compared with the rest of the taxa. The second axis was mainly related with the abundances of

Cladotanytarsus (Cladotanytarsus) atridorsum (Kieffer, 1924), *Paratanytarsus bituberculatus* (Edwards, 1929), *Corynoneura lobata* (Edwards, 1924) and *Endochironomus albipennis* (Meigen, 1830). The subfossil assemblage showed very little dispersion in the PCA, especially for the first axis, and no depth pattern could be observed. In fact, the sample scores indicated that all subfossil samples were strongly related to the littoral and sublittoral living samples located between 0.5 and 6 m of depth.

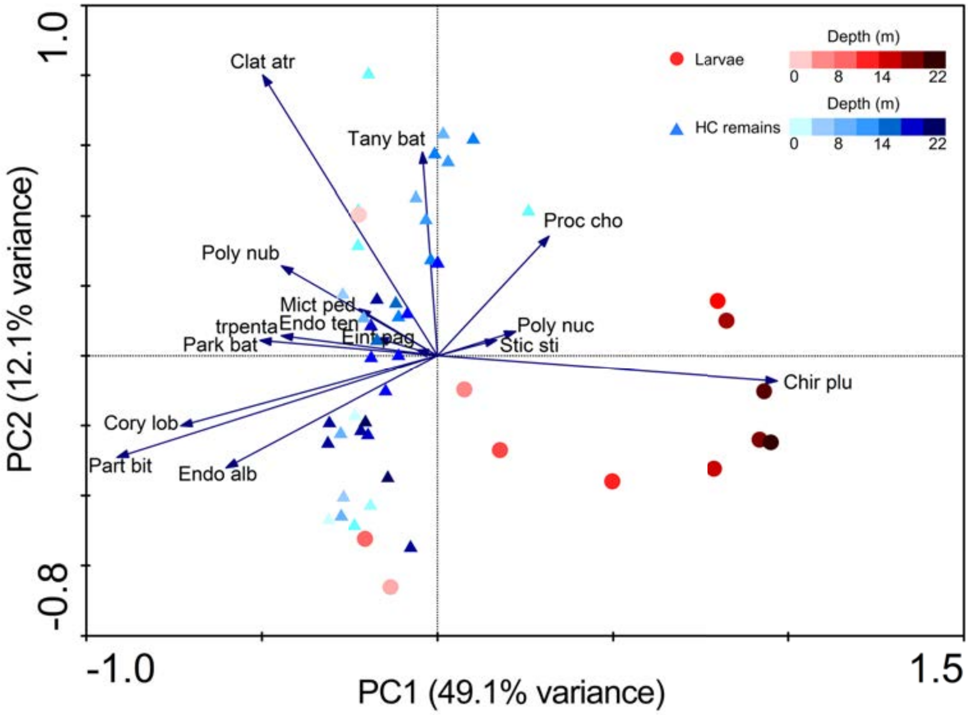


Figure 2.2 PCA analysis representing living and subfossil chironomid assemblages of Enol Lake. Red circles represent living samples, following a colour gradation from light red (littoral samples) to dark red (lake bottom samples). Blue triangles represent recent subfossil samples, following a colour gradation along the depth transect, from light blue (littoral samples) to dark blue (lake bottom samples). Taxa codes can be found in Table S.2 following Schnell et al. (1999).

We found significant differences in the relative abundances of living and surface subfossil midges (Table 2.1), when samples are grouped according to its location in the littoral, *Chara* or profundal zone. The living assemblage showed a clear depth arrangement, with *Cladotanytarsus*, *Paratanytarsus* and *C. plumosus* dominating the littoral, *Chara* and profundal zones, respectively; whereas the subfossil assemblage was dominated by *Paratanytarsus* all along the depth transect. However, we found a relatively strong correlation between the recent subfossil assemblage at each depth and the overall living assemblage (Fig. 2.3a). The Pearson correlation r values were relatively high (i.e. ranging between 0.6 and 0.8) and significant (*p*-values ranging between 0.0001-0.008) at all depths except at 10 m ($r = 0.4$; $p = 0.11$). The correlation between the recent subfossil assemblage at every depth and the overall subfossil assemblage (Fig. 2.3b) was strong (0.8-0.98) and significant ($p = 1\text{x}10^{-5}$-0.001), although it decreased at 2 and 10 m ($r = 0.7$; $p = 0.001$).

Table 2.1 Relative abundance of most abundant living and subfossil taxa for each significant zone identified in the living assemblage (Tarrats et al. 2017): i) littoral, ii) *Chara*, and iii) profundal.

Zone	**Taxa**	**Living (%)**	**Subfossil (%)**
Littoral	*Cladotanytarsus*	**31.6**	4.4
	Paratanytarsus	24.1	**46.3**
	Microtendipes	10.7	12.8
	Stictochironomus	9.3	3.5
	Corynoneura	7	2.7
Chara	*Paratanytarsus*	**50.5**	**49.3**
	E. pagana	22.5	7
	E. albipennis	14	10.1
	Tanytarsus	3.3	2.9
	Corynoneura	2.5	9.6
Profundal	*C. plumosus*	**66.8**	4.5
	Paratanytarsus	6.6	**38.7**
	Procladius	6.6	3.4
	Tanytarsus	6.2	7.7
	E. pagana	5.8	8

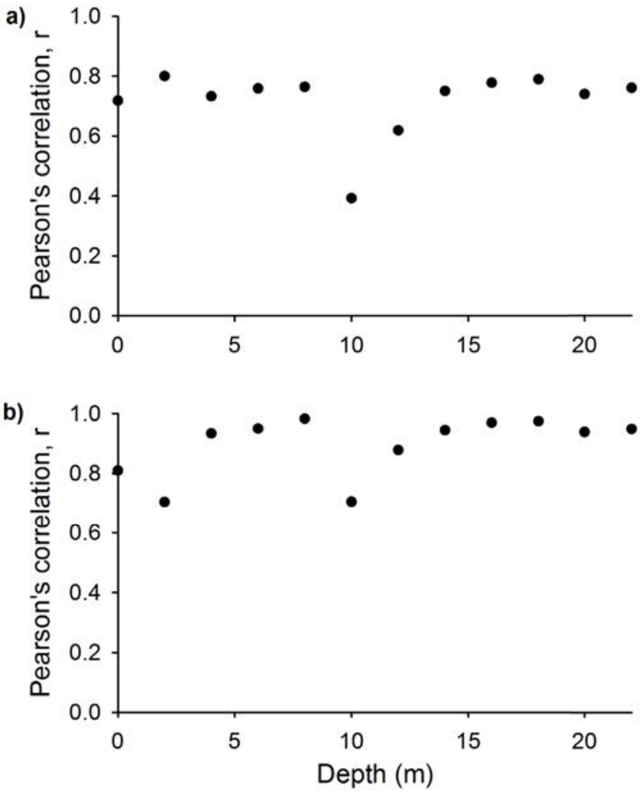


Figure 2.3 Pearson correlation r values of a) subfossil assemblage at every depth vs. living overall assemblage and b) subfossil assemblage at every depth vs. subfossil overall assemblage.

Taxa deposition

Although the overall recent subfossil and the living assemblages were relatively similar in terms of taxa composition, large differences emerged when looking at the depth distribution of taxa densities (Fig. 2.4). The transport index showed 3 main patterns related with differences in the deposition of subfossil taxa along the depth transect (Fig. 2.5). The first was characterized by taxa with a balanced ratio between head capsules and larvae along all zones, suggesting a low transport range (i.e. transport index values around 0). Within this group, we found taxa with different depth preferences: some taxa (e.g. *C. plumosus* and *Stictochironomus sticticus* (Fabricius, 1781)) were deposited along the whole depth transect, whereas others were almost constrained to the *Chara* zone (*E. albipennis* and *Polypedilum (Polypedilum) nubeculosum* (Meigen, 1804)).

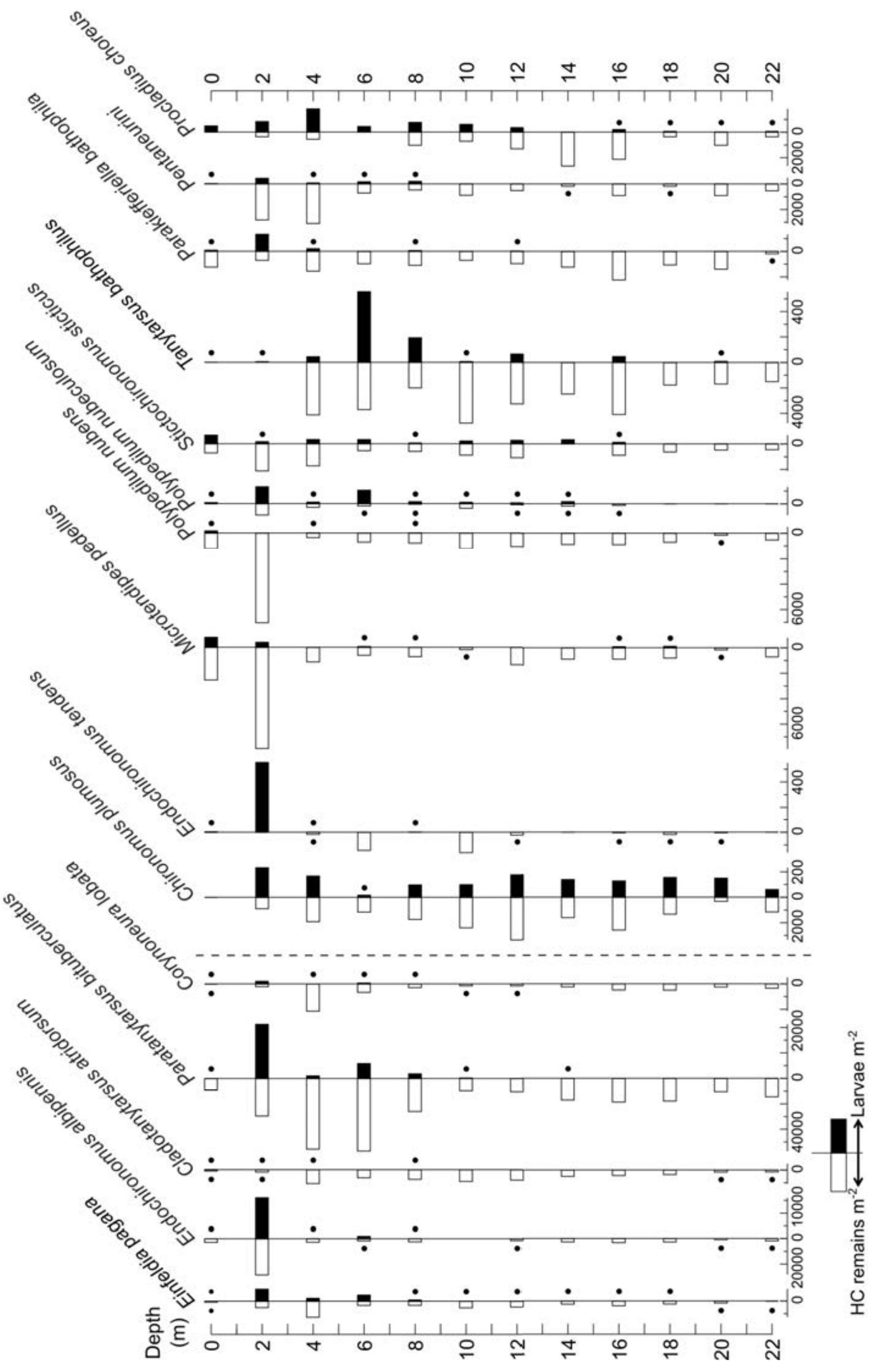


Figure 2.4 Comparison between concentration of head capsule remains (white) and larvae (black) from Enol Lake at different depths. Taxa labels follow the living assemblage nomenclature.

The second pattern consisted of taxa occurring almost exclusively as living larvae in the littoral zone, which were transported and deposited to deeper levels. In this group, two patterns could be observed: i) taxa that were deposited along the depth transect, showing a deeper transport range (e.g. *Cladotanytarsus*), and ii) taxa that were almost completely deposited in the *Chara* zone, showing a shorter transport range (e.g. *P. nubens* and *Microtendipes pedellus* (De Geer, 1776)). The third pattern consisted of taxa with the highest living larvae abundances in the *Chara* zone, which were mainly deposited in the profundal zone (e.g. *Parakiefferiella bathophila* (Kieffer, 1912), *Tanytarsus bathophilus* (Kieffer, 1911), *Procladius (Holotanypus) choreus* (Meigen, 1804) or along the depth transect (e.g. *P. bituberculatus*, *C. lobata*, Pentaneurini tribe).

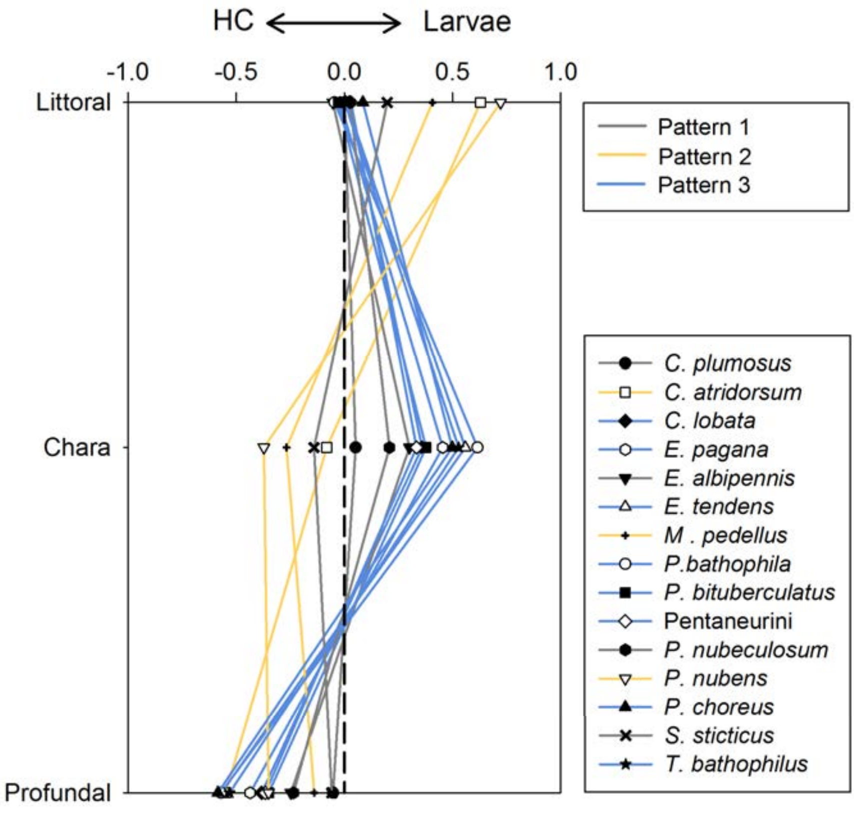


Figure 2.5 'Transport Index' representing the overrepresentation of larvae or chironomid head capsules remains at every significant zone. The index compares the difference between the abundance of larvae and head capsules for each taxa at every depth category. Positive values of this index are related to an overrepresentation of larvae, whereas negative values are related to an overrepresentation of head capsules. Taxa labels follow the living assemblage nomenclature.

DISCUSSION

The similarity between the overall living and subfossil assemblages in terms of taxonomic composition makes Enol Lake a good case study for exploring possible transport and deposition processes of chironomid head capsules. According to our results, none of the initial hypothesis could be completely rejected. On one side, the subfossil assemblage of Enol Lake had a relatively homogeneous composition along the depth transect. This suggests that a single core retrieved at any part of the lake (except at 0.5, 2 and 10 m of depth) could properly explain the whole assemblage. Moreover, we found that the recent subfossil assemblage was properly representing the overall living assemblage (except at 10 m of depth, mainly due to the low head capsules (HC) abundances of *E. albipennis* found at this level). This is an important finding, since assessing the ability of a single core to properly represent the whole assemblage should be a prerequisite to any paleolimnological study. Concordantly, other studies did not find any depth-related pattern or threshold of the recent subfossil assemblage (Schmäh 1993; Brodersen and Lindegaard 1999; van Hardenbroek et al. 2011). Also, Heiri (2004) found that sediment cores taken at any part of the basin in shallow Norwegian lakes included the dominant taxa, although with varying abundances. Thus, our results suggest that a core retrieved at any depth except the littoral zone (0-2 m) and at 10-12 m could properly represent the whole chironomid community.

However, it is very important to notice that the lack of changes reported in the subfossil assemblage along the depth transect was largely driven by the dominance of a single taxon: *Paratanytarsus*. It was by far the most abundant species in the subfossil assemblages, both in terms of relative abundance (mean relative abundance = 45%, Table 2.1) and densities (more than 2 times higher than the second most abundant taxa). This was also the case for the living community of Enol Lake, as *Paratanytarsus* also reported the highest densities among chironomid larvae (Fig 2.4), and may explain the good correlation between the living overall community and the recent subfossil community. This taxon was strongly associated with the *Chara* zone in the living community (Table 2.1). This matches the habitat preferences of *Paratanytarsus* larvae, which are known to live preferentially in aquatic vegetation (Brodersen et al. 2001; Boggero et al. 2006). Thus, the dominance of *Paratanytarsus* head capsules remains along the depth transect was most likely related to a single factor: the presence of Characeae in the lake. The high productivity in terms of chironomid individuals related to this habitat, and the great dominance and densities of *Paratanytarsus* in the *Chara* zone resulted in the homogenization of the recent subfossil assemblage along the depth transect. Other factors could be enhancing the transport of *Paratanytarsus* remains. For example, we observed tubes built by *Paratanytarsus* on *Chara* branches when processing living larvae samples. This tube-building behaviour could facilitate the dispersion and transport of their remains, since a large proportion of head capsules were not initially buried in the sediment but hanging from the branches of the aquatic macrophytes.

As it was reported in a previous study (Tarrats et al. 2017), *Chara* presence and abundance is one of the main factors currently shaping chironomid assemblages in Enol Lake, both in terms of abundance and composition. The importance of macrophytes as a driver of the subfossil assemblage has been already reported in several studies (Langdon et al. 2010; Luoto 2010; Rumes 2010; van Hardenbroek et al. 2011). Among macrophytes, *Chara* has been shown to be especially relevant for living (Hargeby et al. 1994; van den Berg et al. 1997, 1998; Cañedo-Argüelles and Rieradevall 2011) and subfossil chironomid communities (Brodersen et al. 2001; Ruiz et al. 2006). Thus, although in our study the recent subfossil assemblages offered a good representation of one of the key drivers of Chironomidae assemblages operating at the lake scale (i.e. the presence of *Chara*), other environmental factors that are currently affecting chironomids (e.g. anoxic conditions in deeper layers, temperature fluctuations in the littoral zone (Tarrats et al. 2017)) were masked. This circumstance points out the major effect that a single factor can have on the subfossil chironomid community, and may hinder the use of these results to reconstruct past changes in other parameters rather than the presence of *Chara* (e.g. temperature, lake level). Our results suggest that using the recent subfossil chironomid community to build a transfer function could be misleading, since the environmental preferences of *Paratanytarsus* would have a great weight and the preferences of other taxa would be underrepresented. Moreover, since the presence and abundance of *Characeae* have been widely reported to fluctuate along time (e.g. Jeppesen et al. 1998; Rip et al. 2007; Scheffer and Jeppesen 2007; van Nes et al. 2007), recent subfossil assemblages may be excessively affected by a temporal and unstable situation.

Although the overall recent subfossil assemblage was homogeneous, each taxon had different deposition patterns, as Fig. 2.4 and 2.5 show. Some taxa showed a lower transport range (i.e. living and subfossil abundances were very similar at a given depth) but others had a higher transport to other lake zones. As it was expected, the littoral zone was overrepresented by living larvae compared to the subfossil HC. This fact can be explained by a higher transport affecting littoral chironomids to deeper parts of the lake and can also explain the lower correlation levels reported at 2 m (Fig. 2.3a). The heterogeneous patterns reported in the *Chara* zone (Fig. 2.5) are related to the deposition of littoral taxa at these depths and the high productivity of certain *Chara*-related taxa, which are transported and deposited to deeper levels. At the same time, the low diversity in terms of chironomid living larvae caused an overrepresentation of the subfossil chironomids that are transported to the deepest areas. These heterogeneous deposition patterns suggest certain complexity regarding chironomid HC deposition and transport, which was masked by the great dominance of *Paratanytarsus* in the overall community.

CONCLUSIONS

We found a great spatial homogeneity of the recent subfossil assemblage and a relatively strong correlation between the overall living assemblage (i.e. mean abundances of taxa) and the subfossil assemblage both in terms of taxa composition and abundance. Thus, according to our results, in Enol Lake a single core taken at any depth would properly represent the whole living community (except at 0-2 and 10-12 m of depth). However, the homogeneity of the subfossil assemblage along the depth transect did not match with the wide variety of habitat preferences of the living assemblage and was mainly explained by the great presence of *Chara*, which seems to promote the dominance of *Paratanytarsus* along the depth transect. Thus, caution should be applied when inferring past environmental conditions using the recent subfossil assemblage, at least in *Chara*-dominated lakes like Enol Lake. Overall, the results found in this study point out the potential confounding effect of a single factor in paleolimnological studies using chironomids. In this regard, we strongly recommend including studies aiming at understanding head capsules transport and deposition patterns before using subfossil remains to infer past environmental conditions, especially in deep lakes.

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CHAPTER 3

Chironomid-depth relationships provide environmental reconstructions from northern Spain

The study of recent environmental change is essential to disentangle climate and human-induced influences on ecosystems. There are still many uncertainties involving the comprehensive representation of past changes from single cores retrieved from the deepest part of lacustrine environments, especially when analysing biological benthic paleoenvironmental indicators such as chironomids. Here, we provide an environmental reconstruction for the last 600 years from Enol Lake (northwestern Spain) using chironomids by comparing signals from two cores retrieved from different depths (8 m and 22 m). Moreover, we compare the chironomid data both to carbon and nitrogen content from bulk sediment and to carbon and oxygen isotopes from carbonate, which allows us to improve the chironomid-based interpretations. Our results identify major differences between the two cores. Several hydrological changes can be observed in the deeper core, although with greater uncertainty due to low head capsule concentrations. The shallower core is likely more reliable, as the chironomid data shows a better community representativeness and relative stability. This core records both human-induced influences and hydrological changes in the lake. Overall, we suggest that cores from different depths should be analysed to perform complete paleoenvironmental reconstructions using chironomids.

INTRODUCTION

Understanding the drivers of past environmental change is of crucial importance for disentangling climate and human influences on modern environmental conditions (Seddon et al. 2014). The time period investigated in this study (last 600 years) comprises both relatively colder and warmer periods within the Late Holocene climate. On the one hand, it includes the Little Ice Age (LIA), that was characterized by a global decrease in temperatures lasting from ca. 1300 to 1850 yr AD (Jones et al. 2001, 2009; Mann and Jones 2003), although the precise timing and magnitude of change is known to vary spatially (Mann et al. 2009; Ahmed et al. 2013). Several studies from the Iberian Peninsula have already identified this cold phase, together with more humid conditions (Martín-Puertas et al. 2008; Moreno et al. 2008; Morellón et al. 2009a; Smith et al. 2016). Following the LIA, the Industrial Era (1850 AD-present) is a period of steady warming trend, which has been reported both in the Iberian Peninsula (Brunet et al. 2007; Büntgen et al. 2008) and elsewhere (Agustí-Panareda and Thompson 2002; Mann and Jones 2003).

Together with the known climatic variability, the last 600 years have also been characterized by an increasing anthropogenic impact, especially over the last ca. 250 years since the Industrial Revolution. In our study area (Enol Lake, NW Spain), the main anthropogenic disturbance has been livestock grazing and shepherding (López-Merino et al. 2011), which is (and has been) one of the main economic activities in the Cantabrian region (Domínguez Martín and de la Puente Fernández 1995). A recent study established the effects of livestock on the lake area since the seventeenth century, or even earlier (Ortiz et al. 2016), and showed that the effects have intensified during recent decades (López-Merino et al. 2011). In addition, since the middle of the nineteenth century, there has been mining downstream close to the lake (Buferrera mines) (Rodríguez Terente et al. 2006), for iron and manganese. The main impacts caused by the mining included the construction of a small dam in the lake in 1891 (Suárez Antuña et al. 2005), which caused a significant lake level rise (ca. 4 m), and a channel built for water and energy supply. The mine was operational until 1970, although there were temporal fluctuations in its production rates (Rodríguez Terente et al. 2006). In parallel to these activities, the Covadonga National Park (the first National Park in Spain) was established in 1918, which evolved into the current Picos de Europa National Park (established in 1995). Different management measures and conservation policies were imposed during this period, including periods of relatively higher and lower protection (García Dory 1977; Suárez Antuña et al. 2005). Finally, tourism constitutes the most recent impact in the lake area, as the Park receives ca. 2 million visitors per year mainly focused around lakes Enol and Ercina (Picos de Europa National Park 2015).

In order to assess paleoenvironmental changes in Enol Lake, we undertook an examination of subfossil Chironomidae from the lake sediments. Chironomidae are aquatic insects widely used in paleolimnology due to their stenotopic nature, ubiquity and abundance (Brooks 2003). Midges respond fast to environmental changes, making them useful to reconstruct past changes, mainly temperature (e.g. Eggermont and Heiri 2012), water depth (e.g. Korhola et al. 2000; Velle et al. 2012) and trophic status (e.g. Brodersen and Lindegaard 1999; Brooks et al. 2001; Langdon et al. 2006). One of the main controversies regarding the use of chironomids in paleolimnology focuses on the correct and reliable representation of past changes by a single core retrieved in the deepest part of the lake, on which paleolimnological studies often rely. In this respect, several studies aiming at answering that question have obtained contradicting results. Some studies conclude that the deposition of chironomid head capsules (HC) is equal at all depths, meaning that a single core retrieved at any part would properly explain past changes (Frey 1988; Schmäh 1993; Eggermont et al. 2007; Holmes et al. 2009; van Hardenbroek et al. 2011; Heggen et al. 2012), while others show several differences related to depth (Walker et al. 1984; Heiri 2004; Kurek and Cwynar 2009; Luoto 2010; Cao et al. 2012; Luoto 2012; Frossard et al. 2013). This ambiguity is the focus of this study.

There have been few attempts to reconstruct past environmental change using Chironomidae in the Iberian Peninsula, which cover the Lateglacial period (Muñoz Sobrino et al. 2013), the last 200 (Granados and Toro 2000) and the last 100 (Catalan et al. 2002) years. The first chironomid-based temperature reconstruction covering the entire Holocene for this region has only recently been completed (Tarrats et al. submitted), although other multiproxy studies have used chironomids as a secondary source of information to support the main inferences obtained by other proxies (Morellón et al. 2009a; Pérez-Sanz et al. 2013). In Enol Lake, only a few palaeolimnological studies have been conducted, which looked at the environmental changes since the Pleistocene/Holocene transition until 2200 yr BP (Moreno et al. 2011), the last 400 years variability using geochemical proxies (Ballesteros-Lázaro 2014) and the last 2 centuries from a palynological perspective (López-Merino et al. 2011).

Here, subfossil Chironomidae were used to reconstruct past hydrological and climate changes in Enol Lake, and to assess the impact of recent human-induced changes at the catchment scale. Differences between cores retrieved from the lake bed at different depths were analysed to test if a single core retrieved in the deepest part of the lake (which is the usual method in paleolimnology) would adequately represent past environmental changes. We test two hypotheses: i) that all depths in Enol Lake equally record past changes (i.e. there are no differences between cores), or ii) that different depths in Enol Lake distinctively record past changes (i.e. there are differences between cores). We focused on two zones considered ecologically different: one located in the deepest part of the lake (20 m of depth), which we argue should be more stable throughout time, and another located in the present thermocline depth (8 m of depth), which is more likely to be affected by possible lake level changes and disturbances occurring in and around the lake basin. We also compare the Chironomidae data with the geochemical proxy data, where we assume that total organic carbon (TOC) is related to productivity, total inorganic carbon (TIC) to the amount of bicarbonate brought into the lake (freshwater), the ratio carbon/nitrogen (C/N ratio) to the source of the organic matter (Meyers 2003), and stable isotopes to productivity ($\delta^{13}C$) and precipitation:evaporation balance ($\delta^{18}O$) (Leng and Marshall 2004).

MATERIALS AND METHODS

Study site

Enol Lake (43º 16’ N, 4º 59’ W, 1070 m a.s.l.) (Fig. 3.1) is a karstic lake of glacial origin located in the northwestern part of Spain (Asturias), in the western massif of Picos de Europa National Park. It has a water surface of 12.2 ha, a maximum depth of 22 m and a small watershed (1.5 km^2). The lake is fed by groundwater and surface runoff and it has no permanent inlets. Water losses occur through evaporation, groundwater discharges and an outlet located at the northeast border of the lake, which is regulated by a small dam. Previous surveys (Velasco et al. 1999; Moreno et al. 2011) and the data collected in a recent study (Sánchez-España et al. 2017) characterize the lake as warm monomictic (with a thermocline located between 8 and 12 m from early July until early November). The lake is oligotrophic (total phosphorous 8 µg l^{-1}, Chl-a 0.5-1 µg l^{-1}), with moderately hard water (alkalinity 2.4 meq l^{-1} and 24-37 mg Ca l^{-1}) and its conductivity ranges between 150 µS cm^{-1} at the surface and 227 µS cm^{-1} at the bottom. It has a surrounding karst bench system, that progrades out into the lake. It is almost fully covered with a dense carpet of *Chara sp.* between 2 and 8 m of depth, while *Potamogeton sp.* occurs between 1 and 3 m of depth. Despite this oligotrophy the bottom of the lake is anoxic for 4-6 months every year during the summer stratification period (García-Criado and Martínez-Sanz 2010; Sánchez-España et al. 2017).

Core drilling and laboratory analyses

This study analyses two short cores retrieved from Enol Lake in July 2014, using a UWITEC gravity corer (Fig. 3.1). Core ENO14-2-1B-1G (80 cm long) was drilled from the deepest part of the lake (20 m), whereas core ENO14-2-2B-1G (75 cm long) was obtained at 8 m of depth, on the carbonate bench. Both cores were longitudinally cut, described and subsampled for dating (^{210}Pb and ^{14}C), geochemical (TIC, TOC, TN) and chironomid analyses. In addition, we used another core taken at 8 m of depth previously in 2006 (ENO-06-FG1) with a UWITEC gravity corer with hammer action. This core was used for the stable isotope (δ^{13}C and δ^{18}O) analyses.

Chironomid samples were analysed every 2 cm in each core, which made a total of 41 subfossil samples from core ENO14-2-1B-1G and 38 samples from core ENO14-2-2B-1G. The laboratory protocol for the subfossil samples followed standard procedures (Walker 2001): i) 4-13 g of wet sediment were weighted, deflocculated in warm KOH (70 ºC) and stirred at 300 rpm for 20 minutes; iii) the sediment was sieved through a 90 µm mesh size sieve; iii) Chironomidae head capsules were picked out under a stereo microscope at 40x magnification, dehydrated in 96% ethanol and mounted in Euparal®; iv) Chironomidae head capsules were identified under a microscope at 400x magnification using several specialized guides (Wiederholm 1983; Rieradevall and Brooks 2001; Brooks et al. 2007).

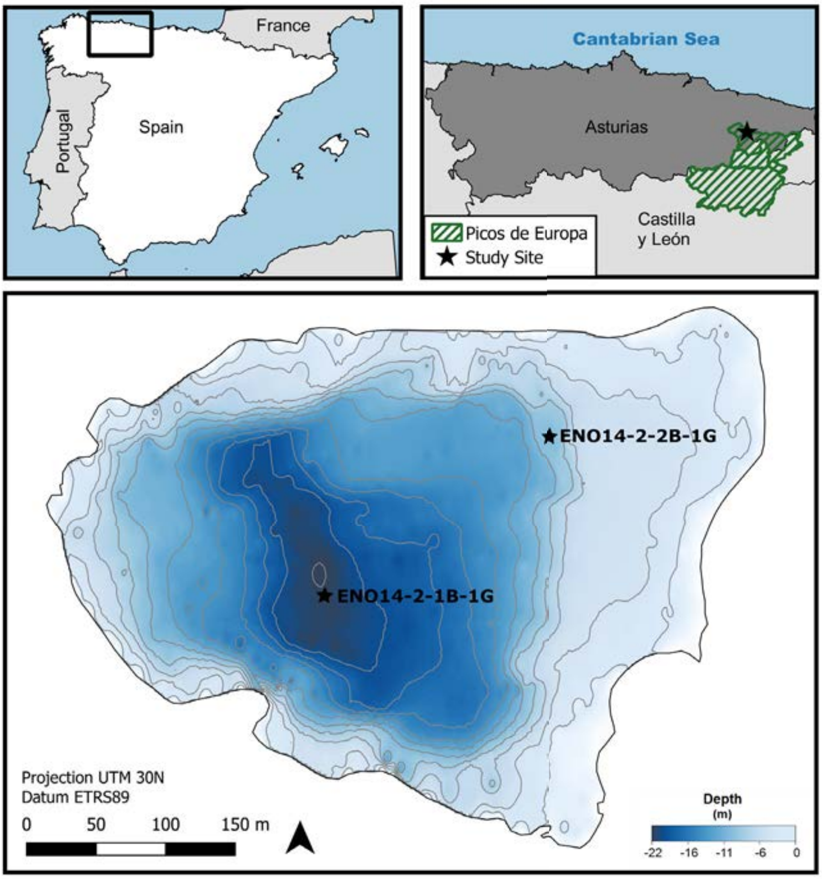

Figure 3.1 Enol Lake location map and core sampling points. Bathymetry adapted from Rodríguez-García et al. (2016).

TOC, TIC and TN values from cores ENO14-2-1B-1G and ENO14-2-2B-1G were provided by the Pyrenean Institute of Ecology (IPE-CSIC). TOC and TIC were measured using a LECO SC144 DR furnace, while TN (used to calculate C/N ratio) was analysed by a VARIO MAX CN elemental analyzer. Loss-on-ignition was undertaken on the ENO-06-FG1core in order to cross-correlate this sequence with the 2014 cores.

The stable isotope analyses were carried out at the NERC Isotope Geosciences Laboratory (NIGL), Keyworth, UK on the ENO-06-FG1 core. Bulk carbonate samples from both the open water and bench core were analysed for $\delta^{18}O$ and $\delta^{13}C$ by gently disaggregating the sediments in 5% sodium hypochlorite solution (10% chlorox) for 24 h to oxidise reactive organic material. Samples were then washed three times in distilled water and sieved at 63 µm to remove any shelly material (Leng et al. 2006). The <63 µm fraction was filtered, washed with deionised water and dried at 40°C and ground in agate. The isolated material was reacted with anhydrous phosphoric acid in vacuo overnight at a constant 25°C. The CO_2 liberated was separated from water vapour under vacuum and collected for analysis. Measurements were made on a VG Optima mass spectrometer. Isotope measurements used internal standards calibrated against the international standards. Errors are typically +/– 0.05‰ for $\delta^{18}O$ and $\delta\ ^{13}C$.

Data analysis

All statistical analyses were performed using R software packages (R Core Team 2016) and the Paleontological Statistics (PAST) software (Hammer et al. 2001). Chironomid relative abundances were plotted using Psimpoll 4.27 software (Bennett 2009), and the core zonation was performed by a CONISS (Constrained Incremental Sums of Squares) cluster analysis (Grimm 1987) using the function ‘chclust’ of the R package “rioja” version 0.9-9 (Juggins 2015). The number of significant zones was tested by means of a broken-stick model using the function ‘bstick’ of the “rioja” R package.

A Mantel test was performed to evaluate differences between cores, both in terms of community composition and downcore changes. In addition, Nonmetric Multidimensional Scaling (NMDS) was employed to represent the assemblage ordination of both cores on the Bray-Curtis distance matrix.

For each core, a Detrended Correspondence Analysis (DCA) was performed using the function ‘decorana’ of the “vegan” 2.4-0 package (Oksanen et al. 2016) in order to discriminate between linear or unimodal-based methods by estimating the lengths of the compositional gradients (i.e. axes 1 and 2). Since the lengths of the gradients were lower than 2 in both cases, a Principal Components Analysis (PCA) was performed using the function ‘rda’ of the “vegan” 2.4-0 package. For all these analyses, rare taxa (those that did not reach a relative abundance of ≥2% in at least two samples) were removed, and the square root transformation was previously applied to the matrix.

Age model

For core ENO14-2-1B-1G, three samples (29.5, 46.5 and 71.5 cm of depth) were radiocarbon dated, but were discarded as the analyses resulted in modern ages. Radiocarbon dating cores rich in carbonate sediments is typically challenging. As such, we focused on dating the upper parts of the different cores through short lived radionuclides, and then attempted to cross-correlate these sequences with previously published sequences from the lake. For core ENO14-2-2B-1G, the first 18 cm were dated using ^{210}Pb analysis, while for ENO-06-FG1 the upper sediments were dated by ^{137}Cs, which showed an agreement in terms of sedimentation rates with core ENO14-2-2B-1G. In order to build the age models of both the shallow and deep cores, we correlated the TOC content of our cores with the TOC reported by previous studies on ^{210}Pb dated and correlated cores (López-Merino et al. 2011; Ballesteros-Lázaro 2014) (Fig. 3.2a). However, this correlation could only be performed for the upper 40 cm of the sequence due to the lack of data thereafter. Thus, in order to build the rest of the age model we applied a linear interpolation between the previous ages. This linear interpolation resulted in an r^2 of 0.98 for core ENO14-2-1B-1G and 0.99 for core ENO14-2-2B-1G, allowing its use for completing the age model. The final age model can be shown in Fig. 3.2b and 3.2c, and spans until ca. 1380 A.D. in the case of the deeper core (ENO14-2-1B-1G, Fig. 3.2b) and ca. 1390 A.D. in the case of the shallower core (ENO14-2-2B-1G, Fig. 3.2c). Note for ENO-06-FG1 we have correlated the sequence with ENO14-2-2B-1G sequence, and as such the stable isotope data is shown against the other proxies from the ENO14-2-2B-1G sequence. The resulting age models should be taken with caution, especially from the top 20 cm on, as the uncertainties associated with the model (i.e. correlation with other cores, linear interpolation) could cause significant error.

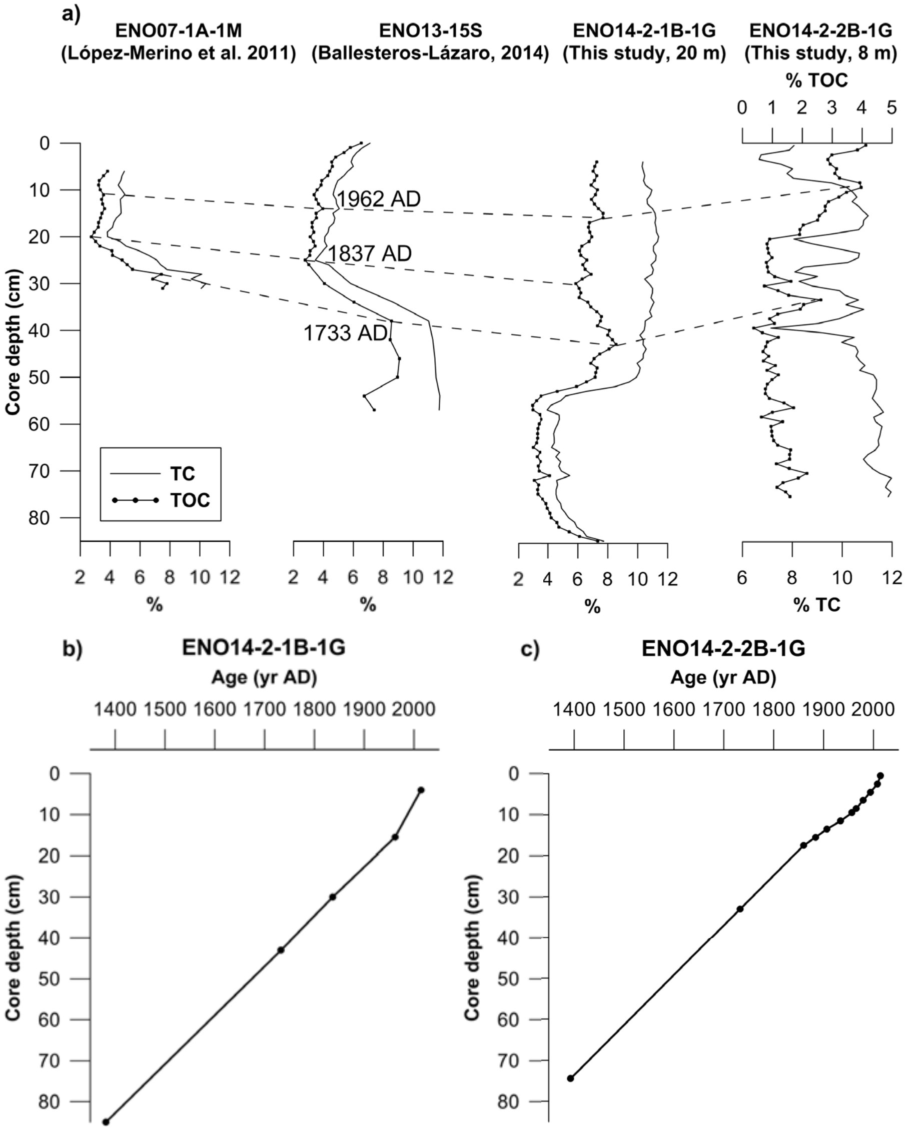

Figure 3.2 a) Total carbon (TC) and total organic carbon (TOC) cross-dating for age model, b) ENO14-2-1B-1G age model, and c) ENO14-2-2B-1G age model.

RESULTS

A total of 8,185 chironomid head capsules were identified from the 2014 cores, belonging to 26 taxa from 3 subfamilies (Tanypodinae, Chironominae and Orthocladiinae). Overall, 3 taxa dominated both sequences: *Cladotanytarsus mancus-type*, *Procladius* and *Tanytarsus lugens*-type. However, the Mantel test showed significant differences between cores (ENO14-2-1B-1G, 20m-depth or profundal core from now on, and ENO14-2-2B-1G, 8m-depth or shallow core from now on), both in terms of community composition and downcore changes. The NMDS (Fig. 3.3) also showed clear differences in the assemblage ordination of both cores, with a higher dispersion in the deeper core.

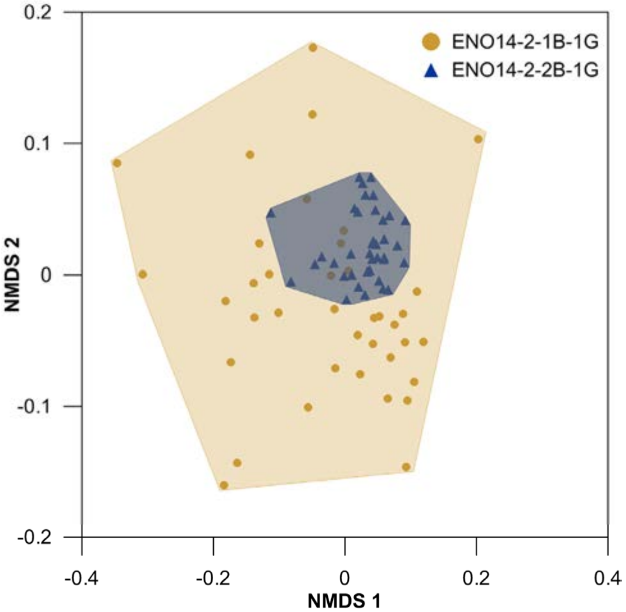

Figure 3.3 Nonmetric multidimensional scaling (NMDS) ordination of chironomid assemblages of core ENO14-2-1B-1G (yellow circles) and core ENO14-2-2B-1G (blue triangles).

20m-depth core (ENO14-2-1B-1G)

Chironomids

1,182 head capsules were identified from the profundal core, belonging to 26 taxa (Fig. 3.4). *Cladotanytarsus mancus*-type, *Procladius* and *Tanytarsus lugens*-type were the most abundant taxa throughout the sequence. Other taxa, such as *Microtendipes pedellus*-type or *Glyptotendipes pallens*-type, were also important at certain depths. Chironomid densities were low (5-16 HC/ g wet sediment) along the sequence.

The broken-stick model divided the sequence into 3 significant zones:

CZ1 (71.5-84.5 cm; ca. 1380-1490 AD)

The lowermost zone was characterized by the highest abundances of *Cladotanytarsus mancus*-type throughout the sequence (mean relative abundance = 30%), as well as by the lowest abundances of *Procladius* (<10%) and relatively high levels of *Chironomus plumosus*-type (10-20%). Moreover, the two lowermost samples also reported the highest abundances of *Paratanytarsus austriacus*-type (40-60%), which suddenly decreased to very low levels in the other zones. The diversity of chironomid taxa in this zone was the highest throughout the sequence, with 19 identified taxa.

CZ2 (53.5-71.5 cm; ca. 1490-1640 AD)

The second zone was characterized by a significant increase of *Procladius* (mean relative abundance = 25%) as well as an important decline of *Cladotanytarsus mancus*-type (10%).

CZ3 (4.5-53.5 cm; ca. 1640-2014 AD)

The uppermost zone was mainly characterized by the presence of *Tanytarsus lugens*-type (10-30%), *Glyptotendipes pallens*-type (10-20%) and *Microtendipes pedellus*-type (10-25%), which were either not present or with very low abundances in the previous zones, and a decline in *Procladius* (10-20%) and *Chironomus plumosus*-type (0-5%). Overall, this zone was characterized by the co-dominance of several taxa with similar abundances. The lower part of this zone reported a significant increase on chironomid densities, reaching the highest levels throughout the sequence (16 HC/g wet sediment).

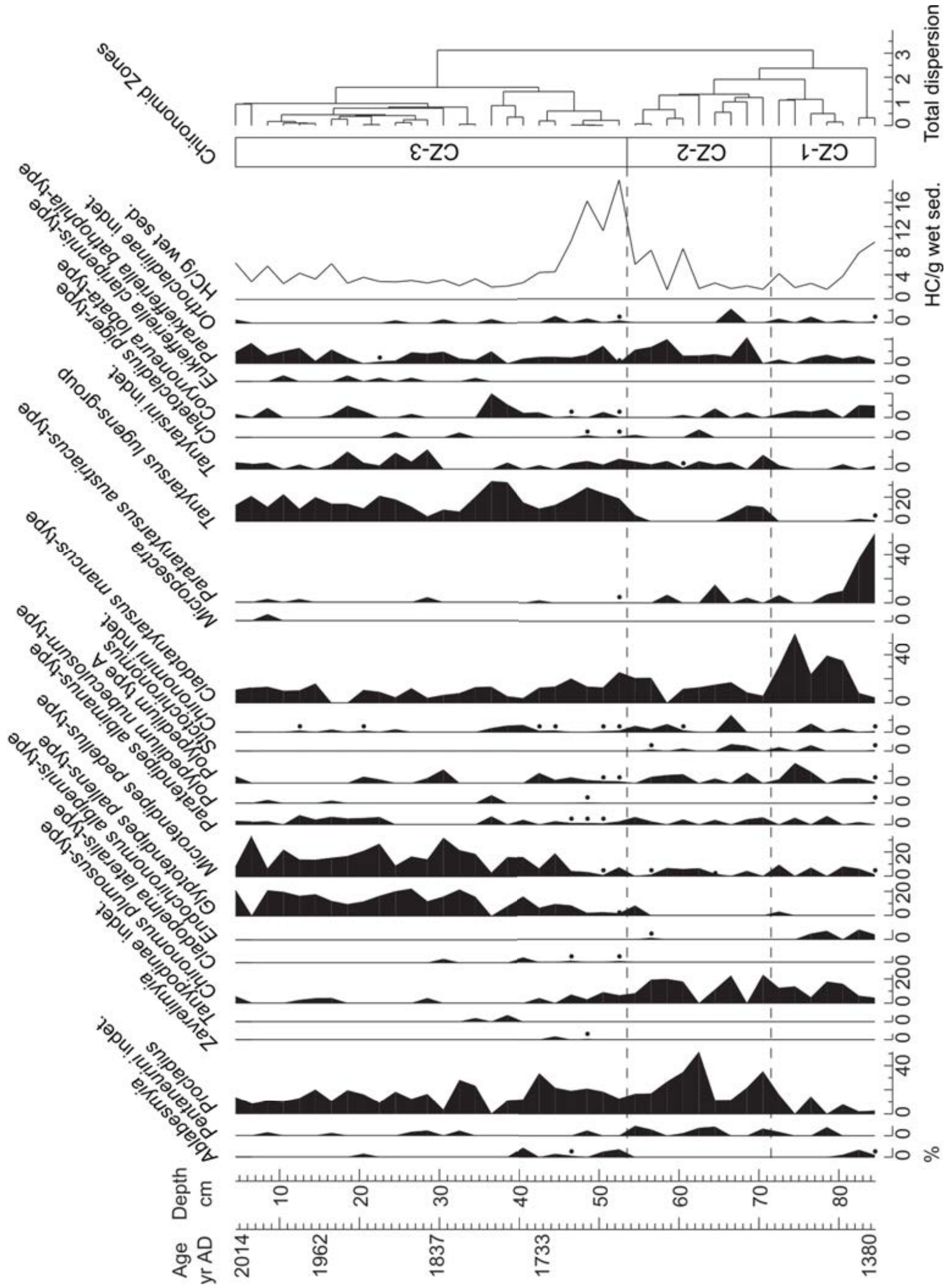

Figure 3.4 ENO14-2-1B-1G chironomid diagram.

PC1 and PC2 explained 32.1% and 12.6% of the total variance, respectively (Fig. 3.5). Negative scores of the PC1 were related to *Chironomus plumosus*-type, *Cladotanytarsus mancus*-type and *Paratanytarsus austriacus*-type, while positive values were mainly related to *Tanytarsus lugens*-type, *Microtendipes pedellus*-type and *Glyptotendipes pallens*-type. Regarding PC2, *Procladius* explained most of the variation, being related with positive values. Samples were organized following a clear gradient in PC1, with negative values for lower samples (CZ1) and positive values for upper samples (CZ3), whereas PC2 did not show any clear trend.

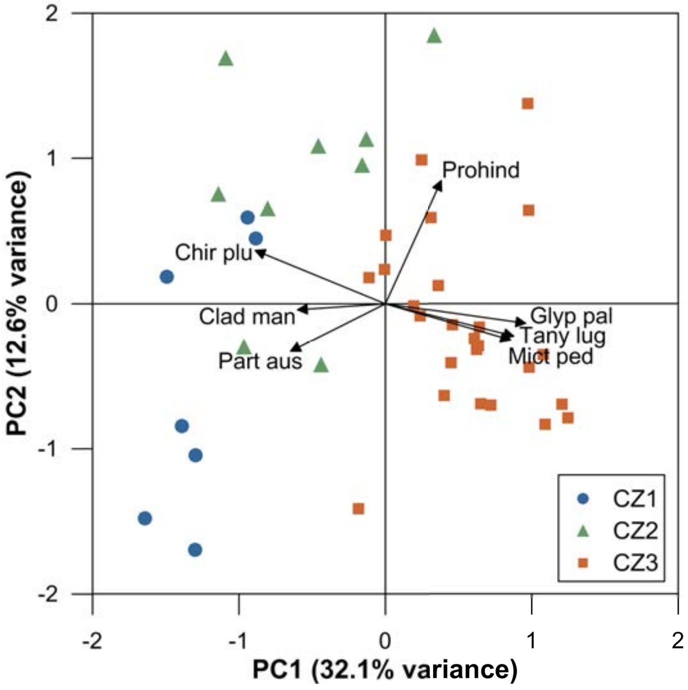

Figure 3.5 PCA analysis of core ENO14-2-1B-1G.

Geochemical indicators

The lowermost zone (CZ1) was characterized by low TIC levels (2%) (Fig. 3.6), a progressively decrease in TOC values (from 7.5% to 3%) and constant C/N ratios <10. The second zone (CZ2) maintained constant and low TIC and TOC levels (2% and 3% respectively), and the C/N remained low. Finally, the uppermost zone (CZ3) showed an increase in C/N ratios (10-11), whereas TIC and TOC reported heterogeneous patterns. TIC initially increased (from 2 to 5%) then progressively decreased to ca. 3%. TOC initially increased (from 3 to 8%) and then decreased to 6% until the top of the core.

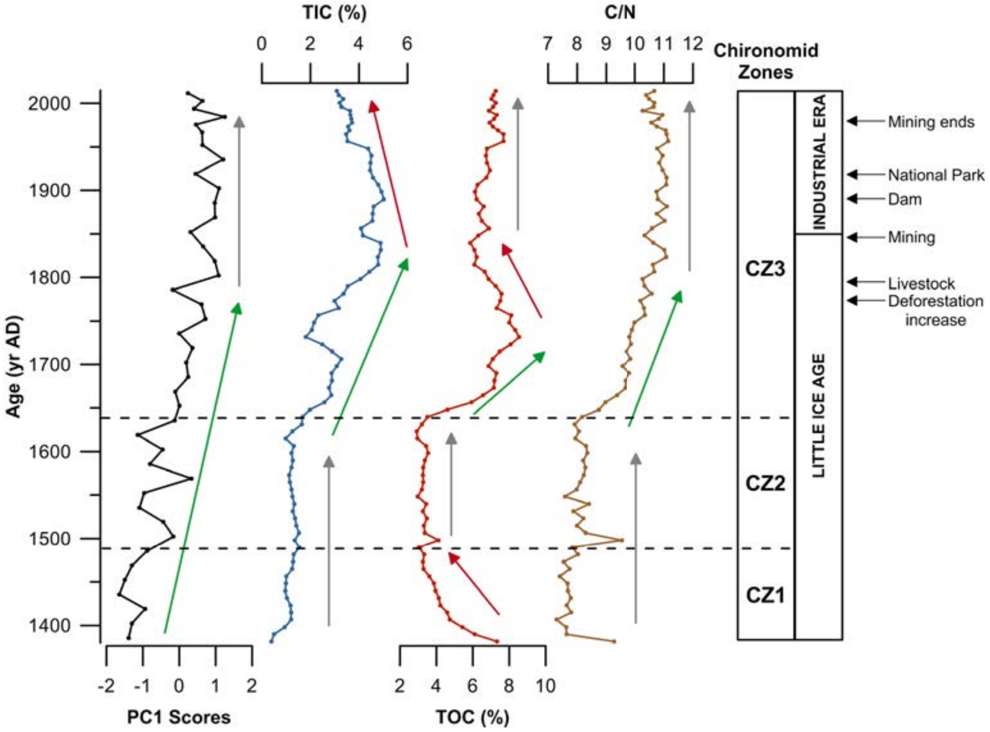

Figure 3.6 PC1 scores and geochemical parameters (TIC, TOC and C/N) of core ENO14-2-1B-1G.

8m-depth core (ENO14-2-2B-1G)

Chironomids

A total of 6,363 head capsules were identified from the 8m-depth core, belonging to 26 different taxa (Fig. 3.7). As in the case of the profundal core, *Cladotanytarsus mancus-type*, *Procladius* and *Tanytarsus lugens*-type were the most abundant taxa, followed by *Chironomus plumosus*-type, *Microtendipes pedellus*-type and *Glyptotendipes pallens*-type. Chironomid densities were higher than in the deeper core, reaching values from 20 to 70 HC/ g wet sediment (i.e. approximately 5 times more than in the profundal core).

The broken-stick model divided the sequence into the following 4 significant zones:

CZ1 (59.5-74.5 cm; ca. 1390-1510 AD)

The lowermost zone was characterized by a gradual increase in *Cladotanytarsus mancus*-type relative abundances (from 15 at the bottom to 30% at the top) together with a decrease in *Procladius* relative abundances (from 50 to 15%). Moreover, *Tanytarsus lugens*-type showed constantly high abundances (mean relative abundance = 25%).

CZ2 (19.5-59.5 cm; ca. 1510-1850 AD)

The second zone was characterized by a decrease in *Cladotanytarsus mancus*-type abundances (from 20 to <10%) and relatively constant levels both of *Procladius* and *Tanytarsus lugens*-type in relation to the CZ1 zone. Moreover, this zone was characterized by the first appearance of *Microtendipes pedellus*-type and *Glyptotendipes pallens*-type (maximum relative abundances = 20%).

CZ3 (9.5-19.5 cm; ca. 1850-1950 AD)

The third zone was mainly characterized by an early and pronounced increase of *Chironomus plumosus*-type (25%) and an important decrease in *Cladotanytarsus mancus*-type (5-10%).

CZ4 (0-9.5 cm; ca. 1950-2014 AD)

The uppermost zone from the shallower core was characterized by an increase of *Chironomus plumosus*-type (up to 30%) and *Cladotanytarsus mancus*-type (25%) and a significant decrease of *Tanytarsus lugens*-type (from 35 to 5%). Moreover, chironomid densities were the lowest throughout the sequence.

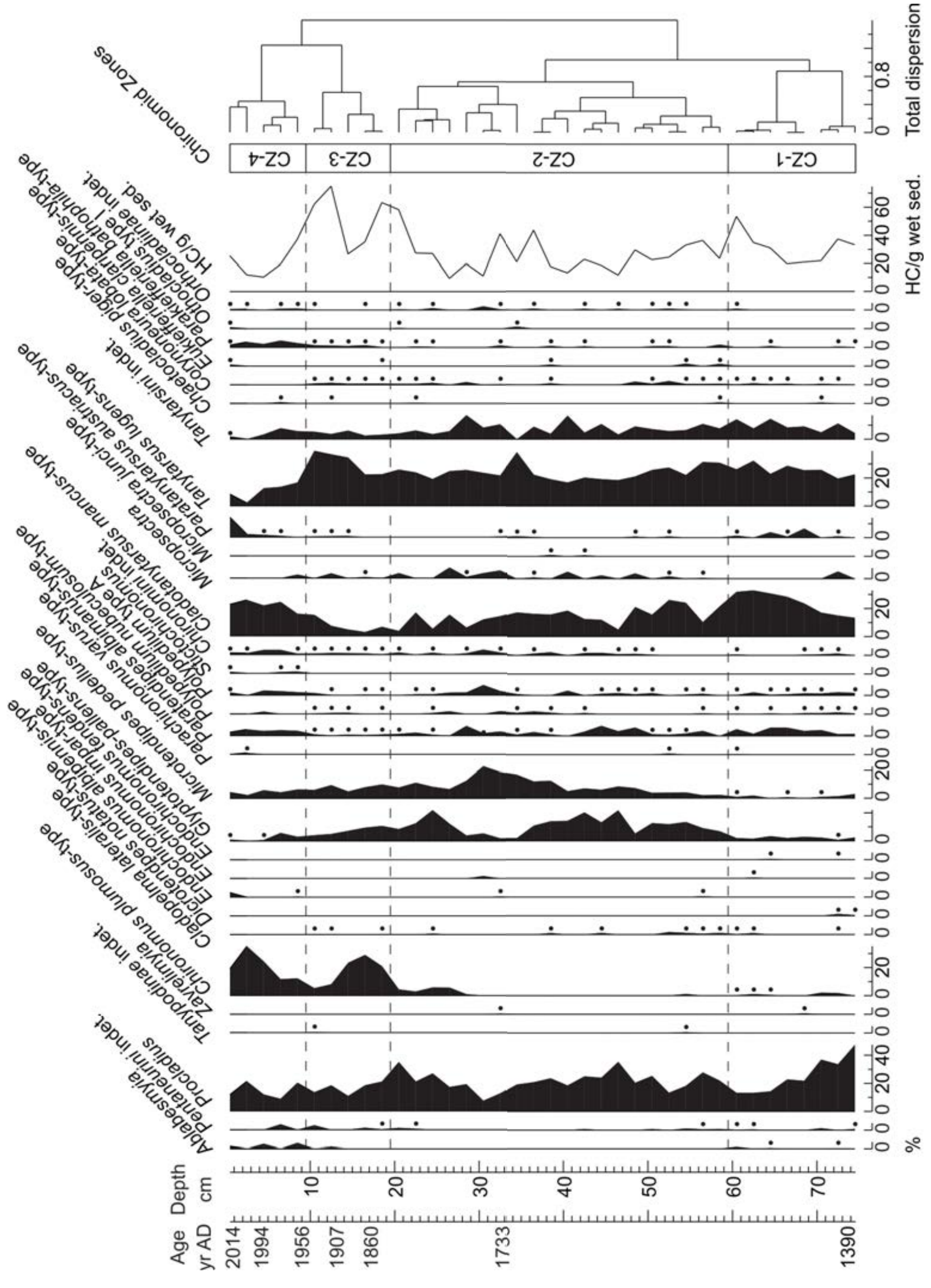

Figure 3.7 ENO14-2-2B-1G chironomid diagram.

PC1 and PC2 explained 32.6 and 20.4% of total variance, respectively (Fig. 3.8). PC1 negative values were related to *Chironomus plumosus*-type and *Cladotanytarsus mancus*-type, while positive values were mainly correlated with *Tanytarsus lugens*-type and *Glyptotendipes pallens*-type. In PC2, negative values were related to *Cladotanytarsus mancus*-type and positive values to *Microtendipes pedellus*-type and *Chironomus plumosus*-type. Samples were placed around 0 in PC1 for CZ1 and CZ2 but showed a clear trend to negative values at the top of the sequence (CZ3 and CZ4). Regarding PC2, samples showed a clear trend from negative to positive values, especially for zones CZ1, CZ2 and CZ3.

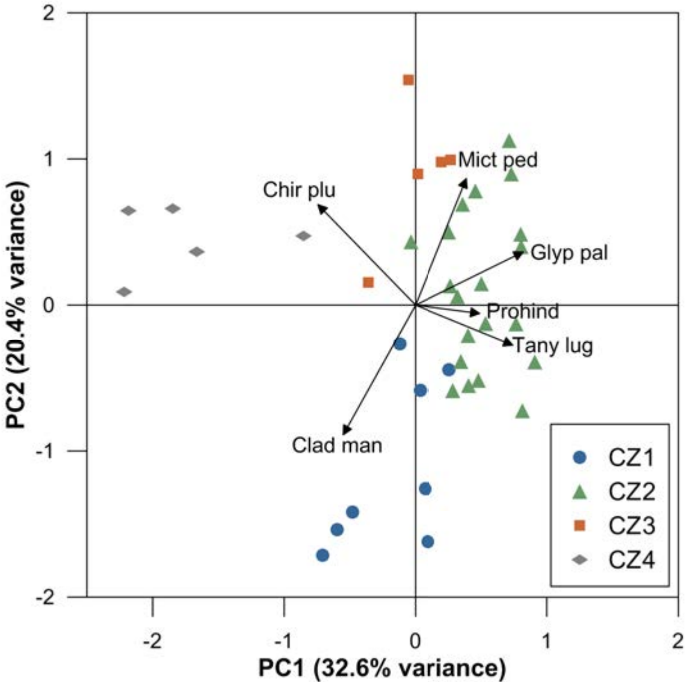

Figure 3.8 PCA analysis of core ENO14-2-2B-1G.

Geochemical indicators

The C/N ratio remained quite constant (mean value = 12.9±2.7) along the sequence (Fig. 3.9). TIC and TOC values also remained constant along the first and second zones (CZ1 and CZ2), with high TIC values (8-10%) and low TOC values (1-2%). The most important change occurred in the last two zones (CZ3 and CZ4), with a progressive decline of TIC values (from 8 to 4%) and a progressive increase of TOC values (from 1 to 4%).

The lowermost zone (CZ1) was characterized by the highest carbonate δ^{13}C values throughout the sequence (–2‰), and the lowest δ^{18}O values (–8‰). δ^{13}C and δ^{18}O recorded opposite trends in the second zone (CZ2), with δ^{13}C progressively decreasing (from –2 to –3‰) and δ^{18}O increasing at the base (from –8 to –6.5‰) with constant and higher values above. δ^{18}O remained constant throughout the third zone (CZ3), while δ^{13}C continued to decrease (up to –4‰). The uppermost zone (CZ4) was characterized by an increase in δ^{13}C (from –4‰ to –2.5‰) and a high variability in both isotopes.

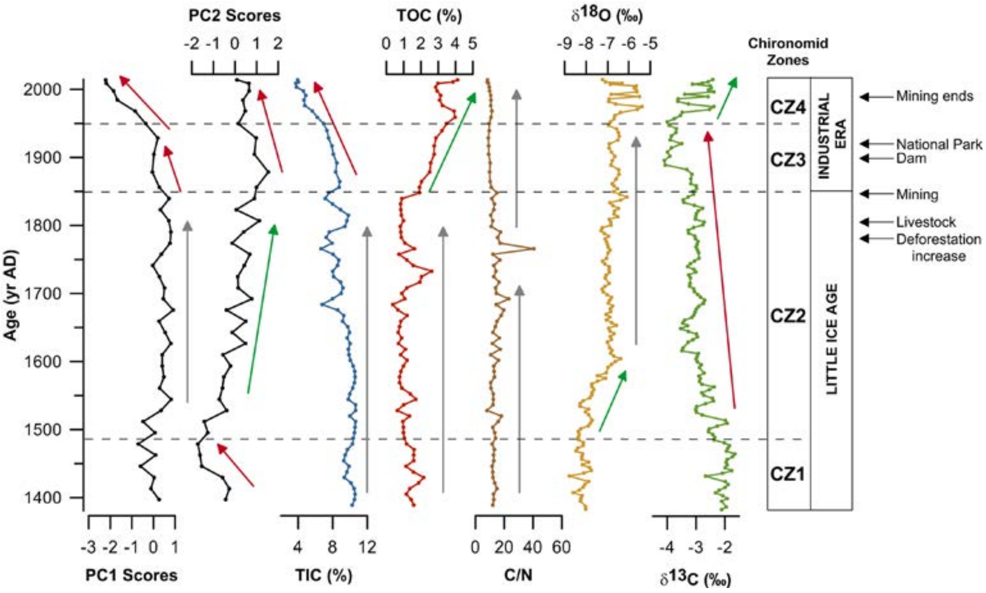

Figure 3.9 PC1 and PC2 scores, geochemical parameters (TIC, TOC and C/N), and δ^{18}O and δ^{13}C of core ENO14-2-2B-1G.

DISCUSSION

All the identified chironomid taxa were also found in the previous studies of Enol Lake, both regarding the living chironomid community (Tarrats et al. 2017) and recent subfossil head capsules (Tarrats et al. submitted). However, one of the most abundant taxon in both cores (i.e. *Glyptotendipes pallens*-type) was considered to be a rare taxon (i.e. low abundances) in these previous studies (living and recent), which indicates that environmental conditions have likely significantly changed during the last 600 years.

One of the main aims of this study was to test whether two cores retrieved at different depths and lake environments (deep and sublittoral zones) would record the same past environmental changes. Our results show that although both cores shared the same dominant taxa, their abundances, composition and changes throughout the sequences were significantly different. Thus, it seems like cores retrieved from different depths may not provide the same signal of chironomid-inferred past environmental change, at least in Enol Lake. The two cores provided complementary (and sometimes contradicting) information to explain past environmental changes, which is detailed below. This is an important finding, and overall we question the use of profundal cores to reconstruct catchment scale changes when much of the impact on the lake ecosystem may occur in the littoral zone.

Paleoenvironmental insights from the profundal zone

When interpreting the deeper core it is important to notice the low HCs densities recorded within the sequence. Only 7 out of 41 samples reached the minimum number (50) of HC required to apply quantitative reconstructions (Larocque 2001). Therefore, changes in the chironomid community composition in the 20m-depth core should be taken with caution, since they could be affected by the patchy distribution of Chironomidae, thus biasing or adding uncertainty to the interpretation of these past changes. However, the correlation between the PC1 scores and the C/N ratio along the sequence suggests that the chironomid community did respond to lake hydrological changes.

The presence of *Cladotanytarsus mancus*-type and *Paratanytarsus austriacus*-type in the bottommost zone of the 20m-depth core (15th century) suggests a period of littoral region expansion (Korhola et al. 2000) with an increased presence of macrophytes (Brodersen et al. 2001; Langdon et al. 2010; Tarrats et al. 2017).

This littoral expansion could be caused by a wetter climate. In this regard, geochemical indicators suggested contradicting trends. Low TIC values could indicate a period characterized by lower precipitation (less inwash of dissolved IC) or dilution by a wetter climate. Low C/N ratios suggest more autochthonous organic matter produced by freshwater algae (Meyers 2003), which would indicate greater algal productivity and a greater distance to the terrestrial zone (i.e. higher lake levels). Most of the sequences analysed in the Iberian Peninsula characterized this period with high water availability and higher lake levels (e.g. Morellón et al. 2012; González-Sampériz et al. 2017 and references there in). Moreover, the decrease in TOC levels may be related to lower productivity, which could be an effect of colder temperatures, although this interpretation does not match with the temperature preferences of the Chironomidae assemblages but is in agreement with colder temperatures reported since the onset of the LIA (Pla and Catalan 2005; Martín-Chivelet et al. 2011; Corella et al. 2012).

The increase of *Procladius* in the second zone (16th century and first half of the 17th century) indicates an increase in lake levels, since *Procladius* has been associated with deeper preferences in Enol Lake (Tarrats et al. 2017). This fact is in agreement with the previously mentioned period of higher water availability that has been reported in the Iberian Peninsula during the LIA but especially between 1500 and 1850, both in lakes (Julià et al. 1998; Moreno et al. 2008; Morellón et al. 2009a) and rivers (Benito et al. 2003; Lebreiro et al. 2006; Sancho et al. 2008). Low C/N values, which suggest algal productivity, also support this interpretation.

Finally, the third zone comprises the latter part of the LIA and the Industrial Era. The first part of this zone is characterized by the significant increase of *Tanytarsus lugens*-type and the relatively constant values of *Procladius*, suggesting the maintenance of high lake levels. In this case, geochemical proxies are in agreement with the Chironomidae assemblages, as both TOC and TIC significantly increased, suggesting greater productivity and perhaps precipitation. At the beginning of the Industrial Era, there is another major change in the sequence represented by the rise in *Glyptotendipes pallens*-type and *Microtendipes pedellus*-type, a situation that is maintained until the present. This change would suggest an increase in temperatures and a decrease in lake levels, as these taxa have been reported as having intermediate to warm preferences (Brooks and Birks 2001; Barley et al. 2006; Heiri et al. 2011) and being inhabitants of the littoral zone of lakes (Płóciennik et al. 2011). This circumstance matches with the reported temperature increase during the Industrial Era but not with the lake level increase caused by the construction of the dam at the end of the nineteenth century, suggesting that it did not modify the hydrological and sedimentary conditions of the distal zone (Ballesteros-Lázaro 2014).

Paleoenvironmental insights from the shallower core

The HC densities found in the shallower core were significantly higher than in the deeper core, with all samples above the minimum number of HC required for quantitative reconstructions and reaching up to 400 HC/sample. This arguably allows a more reliable interpretation of past changes. As noted previously, although the dominant taxa were the same as in the deeper core, their distribution and changes remain much more constant. The C/N ratio of >10 along the sequence indicates its shallower characteristics, with a high influence of allochthonous organic matter produced by terrestrial plants (Meyers 2003). PC1 scores appear closely related to the TOC and TIC content of the lake along the whole sequence. Thus, the chironomid assemblages from this core seem to have responded both to organic matter inputs to the lake and to hydrological changes.

The lowermost zone of this core is broadly similar with the deeper sequence, both in terms of duration (15th century) and environmental conditions. The presence of relatively high abundances of *Cladotanytarsus mancus*-type suggests an expansion of the littoral region, supporting the interpretation of the deeper core. In this case, the relatively low $\delta^{18}O$ values could indicate a wetter climate (assuming $\delta^{18}O$ records a balance between precipitation and evaporation (Leng and Marshall 2004)), which would be consistent with high TIC values bringing more bicarbonate into the system from the aquifer.

The second zone spans until the end of the LIA (ca. 1850) and only differs from the previous by the replacement of *Cladotanytarsus mancus*-type by *Glyptotendipes pallens*-type and *Microtendipes pedellus*-type*,* which are also considered littoral taxa. TIC and TOC values remained constant. Thus, the data suggest no relative change in environmental conditions, contradicting the lake level rise suggested by the deeper core.

The major change in the sequence is placed at the beginning of the Industrial Era, and is represented by two main changes in the chironomid community. The increase in the abundance of *Tanytarsus lugens*-type and the low abundances of littoral and sublittoral taxa, such as *Cladotanytarsus mancus*-type, *Glyptotendipes pallens*-type and *Microtendipes pedellus*-type, would suggest lake level rise. This could be related to the establishment of mining activities and the later construction of the dam. At the same time, the appearance of *Chironomus plumosus-type* for the first time in the sequence is indicative of productivity increase and trophic level change, suggesting a transition from oligotrophy to mesotrophy. All the changes reported by the chironomid community were also supported by geochemical parameters. The combined rise of TOC (greater productivity) and decrease of TIC (fresher lake conditions) would indicate deeper lake levels. Moreover, the increase of TOC is consistent with the increase in lake productivity suggested by *Chironomus plumosus*-type. In this regard, López-Merino et al. (2011) reported changes in diatoms of Enol Lake in the second half of the nineteenth century and the beginnings of the twentieth century as a consequence of both climate improvement and mining activities. Our results thus provide new evidences of these anthropogenic impacts on the lake.

High lake productivity seems to have been maintained during the last 60 years (CZ-4, ca. 1950-2014 AD), with the presence of *Chironomus plumosus*-type, high levels of TOC (high productivity) and $\delta^{13}C$ increase (perhaps due to high aquatic productivity). This is in agreement with present day observed conditions, with high lake productivity probably resulting from an eutrophication process driven by the increase of cattle activity and tourism in the lake basin (Rodríguez Castañón 1996; Picos de Europa National Park 2015; Sánchez-España et al. 2017). Another major change in the topmost zone is related to the abundance recovery of a littoral taxon, such as *Cladotanytarsus mancus*-type, which may indicate lower lake levels than the previous zone. TIC values decrease would suggest less bicarbonate-rich water entering the lake caused by lower rainfall. δ^{18}O increases are likely due to increased evaporation (Leng and Marshall 2004). This interpretation is supported by instrumental records reporting lower precipitation and higher temperatures during this period (Brunet et al. 2007; López-Merino et al. 2011). Thus, the top of the sequence would be characterized by lower lake levels than before and a continuous rise in lake productivity and eutrophication processes. Both δ^{13}C and δ^{18}O show the greatest variability towards the top of the sequence, probably as a result of the greater variability in human impacts, but potentially also related to climate change.

Paleoenvironmental lessons from different environments and final remarks

The analysis of two cores retrieved from different depositional environments has revealed significant differences in terms of how paleoenvironmental changes are recorded in lake sediments. Although both cores have reported signals of environmental changes, their nature, drivers and implications greatly differed.

The first consideration is related to the reliability of the interpretation obtained by both cores. While HC densities obtained in the shallower core were enough to suggest that the resulting community was representative and reliable, the relative low densities found in the deeper core did not allow interpreting the results with such confidence. Moreover, the high dispersion of the NMDS suggests that changes in the deeper core are highly stochastic and abrupt, probably due to these low densities, which likely led to important community changes caused by a few individuals of a given taxon.

Another important finding is that the deeper core did not record the main human-induced disturbances reported in the lake (i.e. organic matter inputs, lake level rise caused by the dam) but did record some climatic and hydrological fluctuations. Conversely, the shallower core mainly recorded these anthropogenic impacts, in addition of some hydrological oscillations. Thus, we suggest that these differences are closely influenced by the sampling point. The distal zone may be less sensitive to human-induced hydrological impacts, as its characteristics remain constant despite minor local changes, but can record major climatic and hydrological impacts. The shallower core is directly affected by these local impacts due to its proximity to the littoral zone. Thus, at least in Enol Lake, it is essential to understand and include these differences in order to build up a precise interpretation of past environmental changes.

ACKNOWLEDGEMENTS

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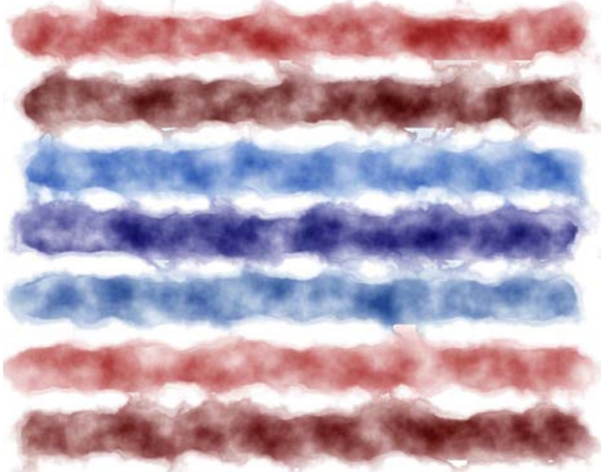
CHAPTER 4

Chironomid-inferred Holocene temperature reconstruction in Basa de la Mora Lake (Central Pyrenees)



We present the first Holocene chironomid-based summer temperature reconstruction for the Iberian Peninsula. A sequence from the shallow mountain lake Basa de la Mora (1914 m a.s.l., Central Pyrenees) was analysed and the reconstruction was performed by means of a merged Norwegian-Swiss chironomid calibration data set. The presence of a multiproxy study conducted in the same lake, as well as other available regional temperature estimations, allowed the comparison of our results with distinct local and regional records in order to disentangle different temperature, hydrological and seasonal patterns throughout the Holocene. The sampling resolution does not allow analysing rapid climatic oscillations but provides insights into general Holocene trends. Overall, we found increasing temperatures at the onset of the Holocene, reaching the highest values during the Holocene Climatic Optimum (in our record ca. 7800 cal yr BP). High temperatures were observed until ca. 6000 yr BP, when a decline of ca. 1.5 ºC was inferred and the lowest temperature values throughout the sequence were reconstructed during the period 4200-2000 cal yr BP coinciding with the first part of the Late Holocene. Finally, an increasing trend in temperature values characterized the last two millennia, although we interpret this reconstructed temperature rise with caution as distinguishing between climatic and anthropogenic influences on the chironomid record in this youngest section of the Holocene is challenging.

INTRODUCTION

The Holocene, the current interglacial period, is widely regarded as warm and relatively stable (Tóth et al. 2015). However, it was also characterized by several rapid climatic oscillations (Bond et al. 1997; Mayewski et al. 2004; Alley and Ágústsdóttir 2005). Reconstructions of temperature evolution in the Mediterranean region during the Holocene are scarce and, particularly for the Iberian Peninsula, available data sets are mainly based on sea surface temperatures (SST) inferred from adjacent marine records (Cacho et al. 2001). In fact, in continental series, temperature is not easily reconstructed and most proxies reflect precipitation (or more generally "moisture availability"), based on paleohydrological fluctuations inferred in lake level reconstructions (Morellón et al. 2009b, submitted; González-Sampériz et al. 2017). Similarly, Holocene vegetation dynamics reported by palynological sequences are usually interpreted as indicating qualitative fluctuations in humid/drier and warmer/colder conditions, as well as anthropogenic impact (e.g. Carrión et al. 2010 and references therein).

In general, warmer temperatures have been recorded during the onset of the Holocene in marine records (Cacho et al. 2001). More humid conditions occurred in Atlantic-influenced environments (e.g. González-Sampériz et al. 2006; Moreno et al. 2009; Carrión et al. 2010; Gil-Romera et al. 2014) but aridity continued in continental Mediterranean ones during the first two millennia of the Holocene (e.g. Morellón et al. 2009b; Pérez-Sanz et al. 2013; Aranbarri et al. 2014; González-Sampériz et al. 2017). Thus, a complex scenario regarding moisture availability has been reported for Iberia during the Early Holocene (Morellón et al. submitted). In respect to Mid-Holocene climatic conditions (8.2-4.2 cal kyr BP following Walker et al. (2012) Holocene subdivision), vegetation based reconstructions also suggest intense differences between both Atlantic (north and northwestern) and Mediterranean Iberian areas (eastern, southern and inner continental sites). Slightly cooler summers and more humid conditions have been inferred for northwestern Spain (e.g. Martínez-Cortizas et al. 2009; Mauri et al. 2015) while northeastern and southern sequences show a first maximum development of mesophytes until ca. 6 ka cal BP but more xerophytic expansion afterwards, probably due to both drier climate and human impact increase (Carrión et al. 2010; Aranbarri et al. 2014, 2015; González-Sampériz et al. 2017). Finally, a greater climatic variability characterizes the Late Holocene period (since 4.2 cal ka BP), which could be related to the difficulty of disentangling climate and anthropogenic forcings and the seasonality signal of the different proxies (Cacho et al. 2010; Carrión et al. 2010).

The use of Chironomidae (Insecta: Diptera) in paleolimnological studies is widely known and developed (Battarbee 2000; Walker 2001). Many factors influence chironomid presence and distribution (Velle et al. 2005) but among them, temperature has been regarded as one of the key drivers due to the strong relationship between summer air and water temperatures and chironomid assemblages (Eggermont and Heiri 2012). Thus, in temperate, boreal and arctic regions this proxy has been traditionally used for temperature reconstructions, particularly on longer, multicentennial to millennial-time scales and for time intervals preceding major human impacts (e.g. eutrophication events) on lakes. Several studies have already established temperature transfer functions for chironomid records in different regions of the world (Barley et al. 2006; Heiri et al. 2011; Massaferro and Larocque-Tobler 2013). In Europe, chironomid temperature calibration datasets (so-called training sets) have been developed in a range of regions such as the Swiss Alps (Heiri and Lotter 2003; 2010), Norway (Brooks and Birks 2001), Sweden (Larocque et al. 2001), Finland (Luoto 2009) or Iceland (Langdon et al. 2008). More recently, the Norwegian and Swiss training sets were merged (Heiri et al. 2011) in order to integrate a wider latitudinal and altitudinal range. However, southern European sets for, i.e., Mediterranean mountain areas, are still not available. Since the existing Norwegian-Swiss training set covers a wide range of environments and lake types, the transfer function developed from these data has become widely used in regions where local chironomid temperature training sets and transfer functions are not available (e.g. Muñoz Sobrino et al. 2013; Tóth et al. 2015; Hájková et al. 2016)

The distribution of quantitative paleoreconstructions in Europe is still irregular and unequal (Millet et al. 2012). In the case of chironomid-based reconstructions, the vast majority are located in the Alps and Central Europe, Scandinavia and the British Isles. Thus the south-western part of Europe remains mostly unexplored (Millet et al. 2012). Chironomid records in the Iberian Peninsula are scarce. The only temperature reconstructions available for the moment are focused on the Lateglacial period (Millet et al. 2012 (French Pyrenees); Muñoz Sobrino et al. 2013 (NW Iberia)) or the last centuries (Granados and Toro 2000), whereas other chironomid records covering the last centuries (Battarbee et al. 2002a; Catalan et al. 2002) or millennia (Morellón et al. 2009a; Pérez-Sanz et al. 2013) are not linked to quantitative temperature reconstructions. Thus, chironomid records or chironomid-based temperature reconstructions covering large sections of the Holocene are not yet available for Iberia.

Here we present the first Holocene chironomid-based Holocene temperature reconstruction for the Iberian Peninsula, using a Pyrenean multiproxy lacustrine sequence: the Basa de la Mora record (Pérez-Sanz et al. 2013). The advantage presented by this sequence is that main paleoenvironmental changes were already characterized (Pérez-Sanz et al. 2013) thus allowing to better isolate the temperature signal from the new chironomid study in comparison with previous interpretations on the hydrological variability. Since no local transfer function for reconstructing chironomid paleotemperatures in this region is available, we use the merged Norwegian-Swiss transfer function (Heiri et al. 2011) which includes temperature preferences for the vast majority of chironomid taxa presently observed in Pyrenean mountain lakes. Our results contribute to the knowledge on Holocene climate variability in Europe and will help to improve the spatial distribution of palaeotemperature reconstructions in southwest Europe. The specific objectives of this study are to: i) describe the subfossil chironomid sequence of a high mountain lake in the Pyrenees (Basa de la Mora) and chironomid assemblage changes throughout the Holocene, ii) reconstruct Holocene mean July air temperature trends using the merged Norwegian-Swiss transfer function, iii) compare our results with other local proxies in order to discriminate among different climate variables and to test the performance of the transfer function, and iv) compare our results with other regional records and explore the potential seasonality signals of different proxies.

MATERIALS AND METHODS

Study Area

Basa de la Mora Lake (42º32'N, 0º19'E, 1914 m a.s.l.) is a small and shallow lake (flooded surface: 3 ha, depth: 2.5-4.5 m) of glacial origin located in the Central Pyrenees (Spain) (Fig. 4.1). It is placed in the Cotiella Massif, which is one of the largest calcareous massifs in the Pyrenees. The region lies under a sub-Mediterranean climate with continental features (Pérez-Sanz et al. 2013), although some areas of the Cotiella massif are influenced by local oceanic climate depending on altitudinal and orientation conditions (Izard et al. 1985).

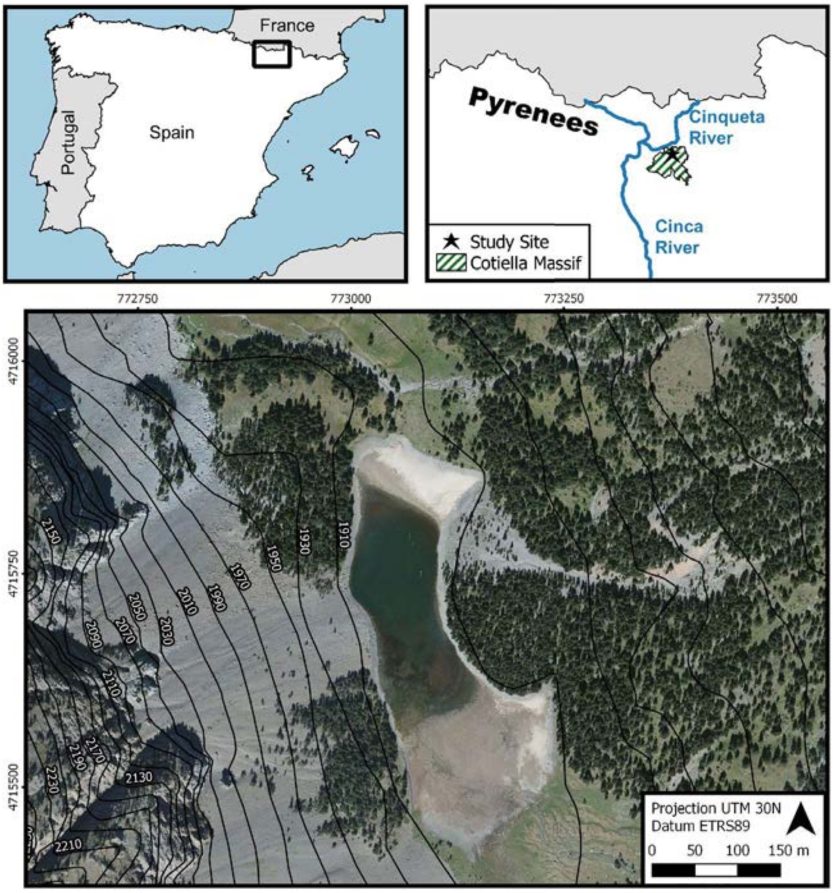


Figure 4.1 Basa de la Mora Lake location map and orthophotograph.

Vegetation of the area widely varies from Mediterranean vegetation at southern slopes (e.g. evergreen *Quercus* formations and sclerophyllous shrubland), to mixed forests at northern slopes consisting of *Pinus sylvestris* and deciduous taxa such as *Betula alba, Corylus avellana, Fagus sylvatica, Quercus faginea* and *Quercus petraea*, among others (Pérez-Sanz et al. 2013). Basa de la Mora is located near the current treeline of the area and the local vegetation in the lake catchment is mainly composed of alpine grassland (43%), *Pinus uncinata* forest (9%) and *Juniperus communis-Rhododendron sp.* shrubland. The lake includes the presence of aquatic vegetation in its basin with several *Potamogeton* spp. and *Ranunculus* sp. (Goñi and Benito 2009), which are considered rare species in the Pyrenean region.

Basa de la Mora Lake is characterized by seasonal water level fluctuation, which can be of the order of 2 m. Little information is available on the limnological parameters of the lake beyond the physicochemical data collected by the EMERGE project (EMERGE 2002) and during the fieldwork conducted to retrieve the sequence presented in this study. According to these data the lake is oligotrophic (TP= 9.2 μg l^{-1}, TN= 918 μg l^{-1}), with a pH of 8.96, a conductivity of almost 200 μS cm^{-1} and DOC concentrations of 3.5 mgC l^{-1}.

Sampling and laboratory analyses

The Basa de la Mora Lake paleoenvironmental sequence comes from two parallel cores retrieved in July 2008 by the Quaternary Environments Group of the Pyrenean Institute of Ecology (IPE-CSIC) using a UWITEC coring system with platform (BSM08-1A-1U, core length=11.75 m). A gravity core for completing the upper part of the sequence (BSM08-1B-1G, core length=60 cm) was collected using a UWITEC short gravity corer. Another gravity core (BSM08-1A-1G) was retrieved and sampled in the field for ^{210}Pb and ^{137}Cs analyses. Further details on core sampling, sedimentology and geochemical composition are described in Pérez-Sanz et al. (2013). Chironomid samples of the lacustrine sequence were analysed every 20 cm in core BSM08-1A-1U and every 5 cm in core BSM08-1B-1G, which made a total of 69 subfossil samples. The laboratory protocol for the subfossil samples followed standard procedures (Walker 2001): i) 2.5-5 g of wet sediment were weighted, deflocculated in warm KOH (70 °C) and stirred at 300 rpm for 20 minutes; ii) the sediment was sieved through a 90 μm mesh size sieve; iii) Chironomidae head capsules (HC) were picked out under a stereo microscope at 40x magnification, dehydrated in 96% ethanol and mounted in Euparal®; iv) Chironomidae head capsules (HC) were identified under a microscope at 400x magnification using several specialized guides (Wiederholm 1983; Rieradevall and Brooks 2001; Brooks et al. 2007).

Some identification problems appeared regarding the Pentaneurini tribe, as their head capsules were generally poorly preserved. We decided to identify to genus levels those HC that were well preserved, and to merge the others into the category Pentaneurini. Selected taxa of the whole Chironomidae content have been already published in Pérez-Sanz et al. (2013).

Data analysis

Data analyses were performed using R software (R Core Team 2016) and a series of specialized programs developed for analysing Quaternary palaeoecological data. Chironomid relative abundances were plotted using Psimpoll 4.27 software (Bennett 2009), and the core zonation was performed by a CONISS (Constrained Incremental Sums of Squares) cluster analysis (Grimm 1987) using the function 'chclust' of the R package "rioja" version 0.9-9 (Juggins 2015). The number of significant zones was tested by means of a broken-stick model using the function 'bstick' of the "rioja" R package.

Chironomid-inferred estimates of past mean July air temperatures were reconstructed from the record based on a merged chironomid temperature calibration dataset (Heiri et al. 2011) describing the relationship between July air temperature and chironomid taxa in 274 lakes in the Swiss Alps and their foreland (Heiri and Lotter 2010) and Norway (Brooks and Birks 2001). Chironomid-based temperature estimates were obtained using Weighted Averaging-Partial Least Squares regression (ter Braak and Juggins 1993; ter Braak et al. 1993) as implemented in the program C2 (Juggins 2007). The chironomid data were available at coarser taxonomic resolution in the downcore record than in the calibration dataset. Therefore, several taxa had to be merged into higher taxonomic categories before application of the transfer function (e.g. several *Cricotopus* morphotypes as well as several *Orthocladius* morphotypes). Furthermore, due to the high number of unidentified Pentaneurini in the down-core record, *Zavrelimyia* and *Ablabesmyia* were merged for temperature reconstruction.

After outlier deletion (following Heiri et al. 2011) and taxonomic harmonization the applied two-component WAPLS transfer function predicted mean July air temperatures within the calibration data with a bootstrapped r^2 of 0.87 and a root mean square error of prediction of 1.42°C. Several samples in the record were characterized by a low number of chironomid head capsules. To reach more reliable counts adjacent samples were therefore pooled before temperature reconstruction resulting in 51 samples with count sums above the 40-50 head capsules usually recommended for chironomid-based temperature reconstruction (Heiri and Lotter 2001; Larocque 2001; Quinlan and Smol 2001) as well as 5 samples with counts in the range of 31-40 HCs. It has recently been shown that counts in this range may provide acceptable results for temperature reconstructions in situations with very low chironomid concentrations (Larocque-Tobler et al. 2015). Two samples were deleted from the record since they were characterized by very low chironomid counts and there were no close samples to pool the chironomids with. This resulted in a gap in the chironomid-based temperature reconstruction between 456.5 and 476.5 cm (4850 to 5300 cal BP).

Fossil samples were screened for samples with an unusual composition relative to the calibration data based on the recommendations by Birks et al. (1990). No samples were identified as having no good or close analogues (based on threshold values representing the 2nd and 5th percentile of all distances in the modern calibration data; (Birks et al. 1990). The record was also examined for samples with a poor fit with temperature by including the fossil samples passively in a CCA of the modern calibration data with July air temperature as only constraining variable. No samples exceeded the squared residual distance to axis one exceeding the 90th percentile of the distances of the modern calibration set samples and no samples were therefore identified as having a poor fit with temperature. No chironomid taxa in the fossil record were identified as rare in the modern calibration data (N2<5). One single taxon (*Polypedilum* type A), which only occurs in a single sample with an abundance of 3.8%, was not represented in the calibration dataset.

Chironomid abundances were square root transformed before numerical analysis and both chi-square and squared chord distance were used for calculating analogue statistics. Residual distances were calculated using the program CANOCO (ter Braak and Smilauer 2002) and reconstructions using C2 (Juggins 2007).

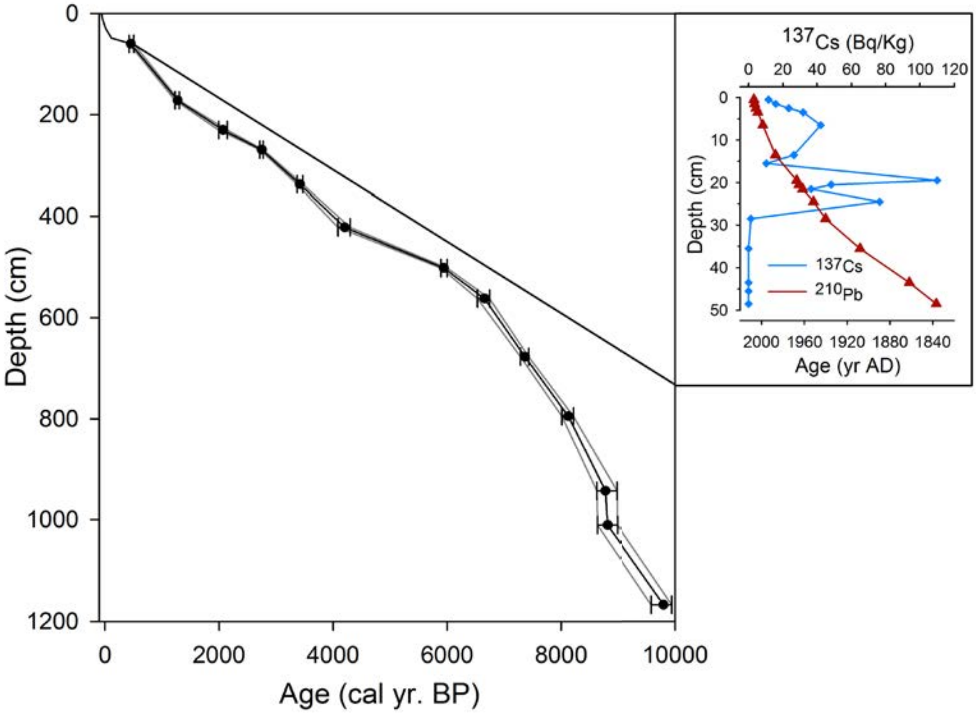


Figure 4.2 Basa de la Mora Lake sequence age-depth model. Adapted from Pérez-Sanz et al. (2013).

Age model

The age model for the lacustrine sequence of Basa de la Mora Lake (Fig. 4.2) is based on 13 AMS ^{14}C dates (11 dates based on macrofossils and 2 on charcoal) and ^{210}Pb and ^{137}Cs chronologies for the upper section. The age model indicates that the record presented here spans the past 9900 cal yr BP. Further details on dating and age model are included in Pérez-Sanz et al. (2013).

RESULTS

Chironomid assemblages and zonation

A total of 6,303 chironomid head capsules were identified belonging to 18 different taxa representing three subfamilies (Tanypodinae, Orthocladiinae and Chironominae) (Fig. 4.3).

Tanytarsus lugens-type was the most abundant taxon throughout the core, followed by *Procladius*, *Chironomus anthracinus*-type and *Paratanytarsus austriacus*-type. Due to the low taxonomic resolution, fossil samples had relatively low taxon diversity (3 to 9 morphotypes per sample). The sequence was divided into 3 significant zones using the broken-stick model; zone 2 was further subdivided into two subzones based on abundance changes between the dominant chironomid groups.

CZ1: Chironomid Zone 1 (1168.5-521 cm depth; 9900-6200 cal yr BP)

This zone was characterized by low head capsule densities and dominated by *Procladius*, reaching its maximum relative abundances throughout the sequence (30-60%). *T. lugens*-type abundance fluctuated strongly whereas the Pentaneurini tribe was present in most samples. The Orthocladiinae subfamily was quite diverse, with an early representation of *Psectrocladius sordidellus*-type and *Corynoneura arctica*-type and a moderate representation of other Orthocladiinae (5-7%), including taxa related with water runoff and seepages (i.e. *Smittia foliacea*-type).

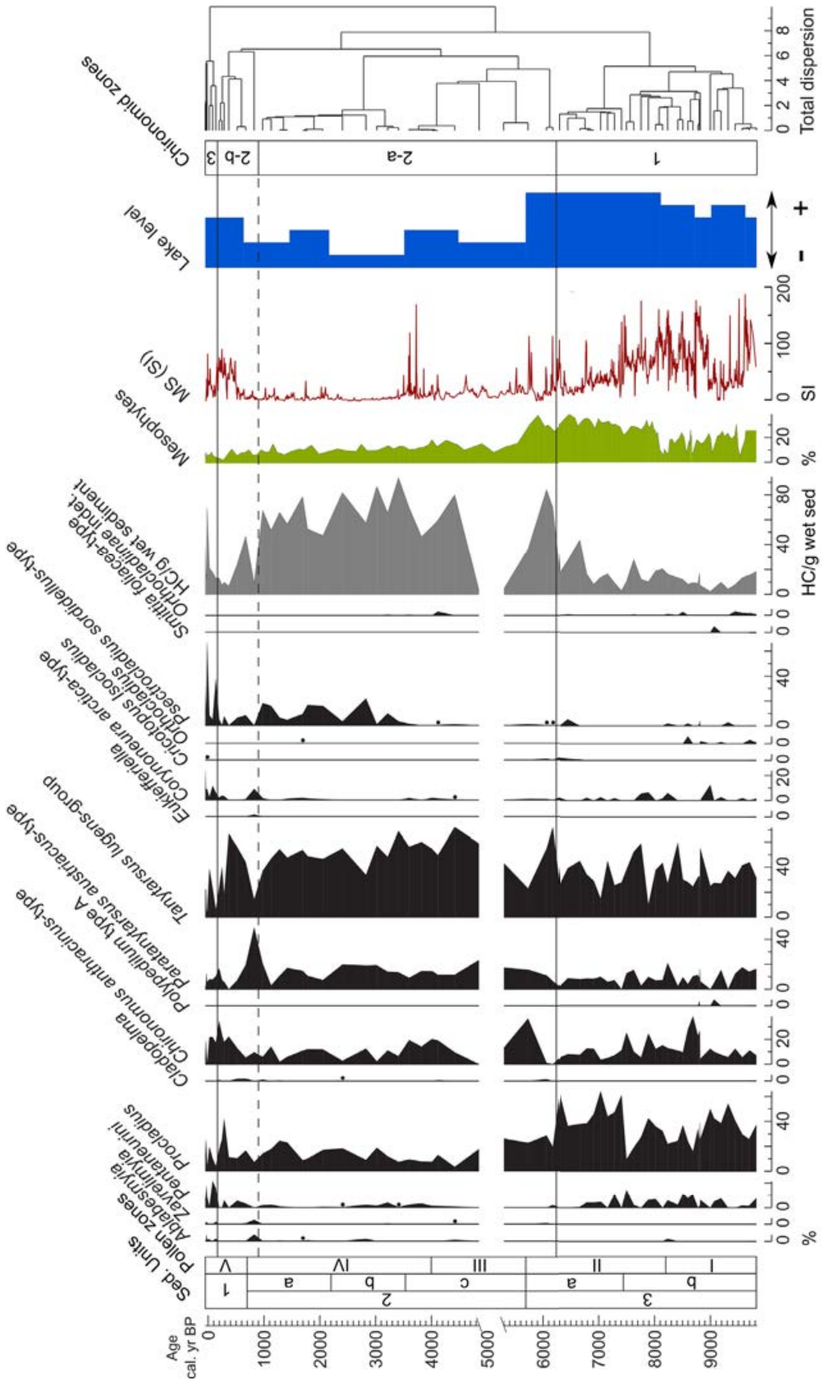


Figure 4.3 Diagram including all chironomid taxa relative abundances, chironomid zones, sedimentary units, pollen zones, mesophytes abundances, MS, and lake level reconstruction from Basa de la Mora Lake sequence (Central Pyrenees, Spain).

CZ2-a: Chironomid Zone 2-a (521-132 cm depth; 6200-1000 cal yr BP)

This zone was characterized by high head capsule densities, although an interval with too low density of head capsules was present in the period 5300-4900 cal yr BP. Abundances of head capsules of the Tanypodinae subfamily (*Procladius* and Pentaneurini) clearly decreased, whereas the abundance of *T. lugens*-type increased and remained constant throughout the zone (50-60%). The main change occurred at 3500 cal yr BP with an increase of *P. sordidellus*-type, reaching abundances up to 20%.

CZ2-b: Chironomid Zone 2-b (132-37.5 cm depth; 1000-200 cal yr BP)

This zone was characterized by a decline in overall density values as well as *P. austriacus*-type and *P. sordidellus*-type abundances, as well as a progressive increase of *C. anthracinus*-type (from 5 to 35%).

CZ3: Chironomid Zone 3 (37.5-0 cm depth; 200 cal yr BP-present; 1750-2008 AD)

The uppermost zone was mainly characterized by a strong increase of *P. sordidellus*-type, reaching up to 35%, together with Pentaneurini and *C. arctica*-type (up to 20% both). A reduction in *T. lugens*-type and *C. anthracinus*-type is also apparent.

Temperature reconstruction

The reconstructed mean July air temperatures ranged between 7.6 and 10.0 ºC (Fig. 4.4a). The Early Holocene and the first part of the Mid-Holocene (chironomid zone CZ1, ca. 10000-6000 cal yr BP), had temperature values in the range of 8.2 to 10.0 °C. During the Late Holocene (ca. 4500-2000 cal yr BP) temperatures were lower in the range of 7.6 to 8.6 °C. Finally, during the last two millennia the reconstructed temperature increased from ca. 8.3 to 9.7 °C.

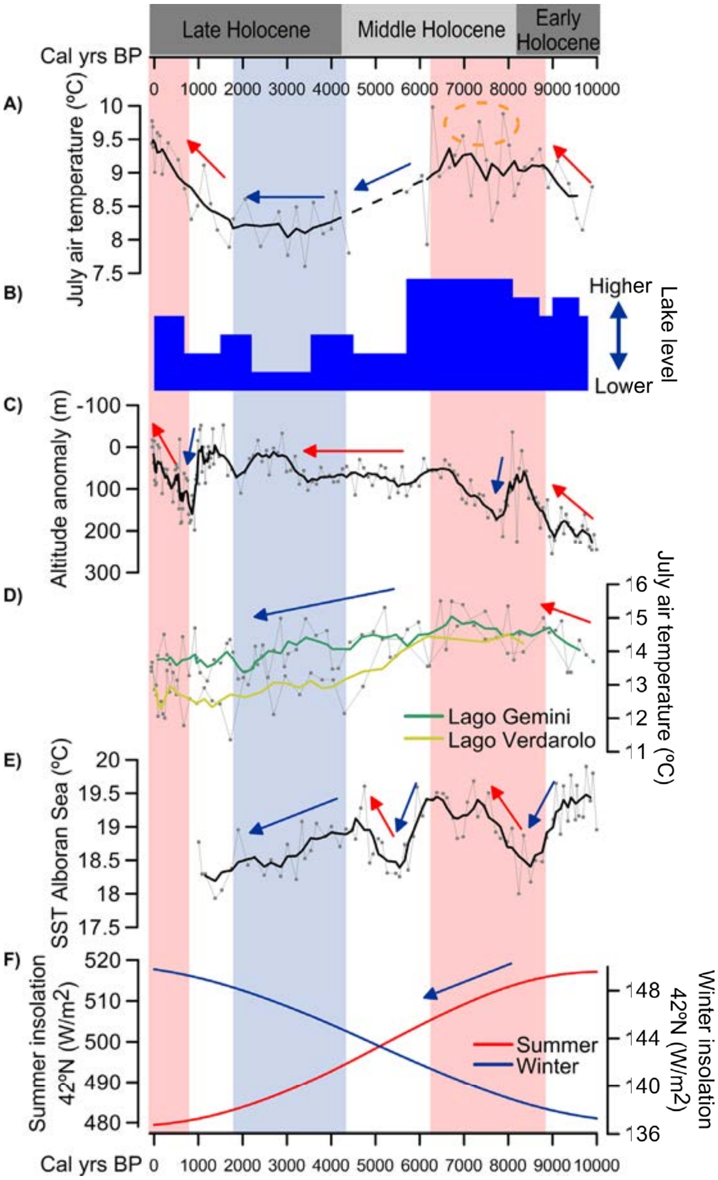


Figure 4.4 a) Basa de la Mora Lake chironomid-inferred July temperature estimates reconstructed based on a WA-PLS based transfer function developed from the merged Norwegian–Swiss calibration data set (Heiri et al. 2011); b) Basa de la Mora lake level reconstruction (González-Sampériz et al. 2017); c) reconstructed altitude anomaly at Lake Redón (2240 m a.s.l., Central Pyrenees) throughout the Holocene by means of chrysophyte cysts (Pla and Catalan 2005); d) chironomid-based Holocene temperature reconstruction from Lago Gemini and Lago Verdarolo (Italy) (Samartin et al. 2017); e) SST reconstruction during the Holocene in the Alboran Sea (Cacho et al. 2001); and f) summer and winter insolation at 42°N (Laskar et al. 2004). Curves smoothed by means of a LOESS smoothing (second degree, span=0.2).

The analogue statistics, goodness of fit statistics and the absence of rare taxa in the chironomid record all indicate that the fossil assemblages were well represented in the modern calibration data. However, these favourable analogue statistics may to some extent be related to the amalgamation of taxa for temperature reconstruction, since this results in a higher likelihood of finding good modern analogues for the fossil samples.

DISCUSSION

The Basa de la Mora sequence provides a unique opportunity to investigate past long-term temperature dynamics and seasonality changes in the Central Pyrenees, as the chironomid-based temperature reconstruction obtained in this study can be compared with qualitative moisture reconstruction based on previous multiproxy studies in the region.

Holocene hydrological changes in Basa de la Mora Lake

The composition of the Holocene chironomid assemblages in Basa de la Mora Lake is typical of a high mountain lake in southern Europe. Taxa as *Procladius*, *T. lugens*-type, *P. austriacus*-type, *C. arctica*-type or *P. sordidellus*-type have been previously found at high abundances in lakes located above 1500 m in the Swiss Alps (Lotter et al. 1997; Heiri and Lotter 2003) or in the Pyrenees (Catalan et al. 2002; de Mendoza and Catalan 2010). At the same time, the dominance of *T. lugens*-type throughout the record is most likely related with the shallowness of Basa de la Mora. Heiri (2001) showed that high *T. lugens*-type abundances (>30%) characterized many shallow lakes (maximum depth <7 m) in the Swiss Alps, and Laville (1971) found *Tanytarsus bathophilus* (*T. lugens*-type subfossil morphotype) in cold and shallow Pyrenean lakes located between 2100 and 2400 m a.s.l. The high abundances of *T. lugens*-type could also be related with the oligotrophic conditions of the lake (Brooks et al. 2007).

Procladius dominated the chironomid assemblage during most of the Early and first part of the Mid-Holocene (10000-6000 cal yr BP), which could possibly be related to relatively deeper lake levels (Wiederholm 1983; Brooks et al. 2007). Moreover, the presence of non-lacustrine Orthocladiinae taxa during this period supports increased runoff to the lake, in agreement with higher detrital input and the highest values of magnetic susceptibility (MS: Fig. 4.3) (Pérez-Sanz et al. 2013). These sedimentological features suggest the highest lake levels of the sequence during this period (González-Sampériz et al. 2017), and the significant increase and high abundance of Mesophytes reconstructed by pollen analysis also support the idea of humid conditions and increasing water availability in the area (Fig. 4.3). Higher lake levels (probably related to increase rainfall) have been also identified in Atlantic-influenced regions of the Iberian Peninsula since ca. 11 ka cal BP, but not till much later —since ca. 9 ka cal BP— in Mediterranean-influenced areas (Morellón et al. 2009b, submitted; Moreno et al. 2009). Similar periods of relatively high lake levels have been recorded between 8.5-6.5 cal ka BP in the central Mediterranean region (Magny et al. 2013) and between 9-6 cal ka BP in south and central Europe (Verschuren and Charman 2008).

The decline of *Procladius* observed during 6000-1000 cal yr BP together with the high and constant abundances of *T. lugens*-type, may be related to generally lower lake levels. Sedimentological features (Fig. 4.3) indicated the lowest lake levels of the sequence during the Middle and Late Holocene, mainly between ca. 4-3 ka cal BP (González-Sampériz et al. 2017). Pollen analyses showed a reduction of deciduous forest and an expansion of conifers during this period (Pérez-Sanz et al. 2013), coherent with increased aridity. These changes in Basa de la Mora are in agreement with an arid phase that has been reported in other areas of the Iberian Peninsula (Reed et al. 2001; Morellón et al. 2008; Corella et al. 2011; González-Sampériz et al. 2017) and southern Europe (Magny et al. 2002; Magny 2004; Jalut et al. 2009) during this period.

The increase registered at 3000 cal yr BP in the abundance of *P. sordidellus*-type could be related to the development of macrophytes in the lake basin (Brodersen et al. 2001). This increase in macrophytes could be in relation with these reported lower lake levels, which would lead to a larger area suitable for macrophyte colonization. However, pollen spectra do not necessarily support this hypothesis as Cyperaceae and hydrophytes (*Potamogeton, Ranunculus,* *Myriophyllum*) show similar percentages as in previous periods (Pérez-Sanz et al. 2013).

The last millennium is characterized by two zones. The decrease of *P. sordidellus*-type reported in zone 2b (1000-200 cal yrs BP, 950-1750 AD), next to the moderate increase of *C. anthracinus*-type, suggest a slight change in the trophic status, from an oligotrophic lake dominated by macrophytes to a mesotrophic lake. Finally, the last 200 years are characterized by a major change (Zone 3). The increase in both *P. sordidellus*-type and *C. arctica*-type suggest a significant expansion of macrophytes, similar to the current lake status with *Potamogeton* spp. and *Ranunculus* sp meadows at least seasonally when lake levels were low. However, both sedimentological data and the aquatic pollen content did not record a trend towards lower lake levels (Pérez-Sanz et al. 2013), possibly due to the different seasonal response of chironomids, aquatic plants and sedimentological proxies.

Understanding seasonality in temperature reconstruction records using the case of Basa de la Mora

The Basa de la Mora chironomid-based reconstruction represents one of the first quantified temperatures records in the terrestrial domains of the Iberian Peninsula based on vegetation records. It generally agrees, with the timing of the northern Hemisphere Holocene Thermal Maximum period previously put forward based on climate modelling results (Renssen et al. 2009; Renssen et al. 2012) and the July temperature reconstructions for central Italy made by Samartin et al. (2017), at least until the last 2000 years (Fig. 4.4).

Previous vegetation-based reconstructions of past Holocene temperature changes for southern Europe have suggested a very different pattern of summer temperature change in comparison to northern regions (Davis et al. 2003; Mauri et al. 2015). However, pollen-based summer temperature reconstructions in Iberia and most parts of the Mediterranean —including mountain areas— may be problematic for the Holocene, as vegetation in the Mediterranean region has to a large extent been mainly driven by moisture availability (Carrión et al. 2010; González-Sampériz et al. 2017; Morellón et al.

submitted), and not principally by temperature. For this reason, the Holocene Climatic Optimum in the Iberian Peninsula, for example, has been frequently interpreted in respect to humidity conditions rather than to temperatures (Cacho et al. 2010). On the contrary, vegetation-independent reconstructions, such as the recent comparison of different proxy indicators (e.g. chironomids, glacier records) and climate modelling results made for Italy and adjacent regions by Samartin et al. (2017), have provided consistent and mutually supporting results for the northern Central Mediterranean region, indicating a Mid-Holocene optimum in summer temperatures in agreement with our results from the Central Pyrenees.

The increasing trend in the reconstructed summer temperatures in Basa de la Mora Lake (Fig. 4.4a) during the Early Holocene (ca. 10000-9000 cal yr BP) was also reported in Italian lakes such as Lago Gemini and Lago Verdarolo (Samartin et al. 2017; Fig. 4.4d). In the Pyrenees, semi-quantitative reconstruction of winter-spring temperature at Lake Redón (Pla and Catalan 2005; Fig. 4.4c) also show an increasing Early Holocene trend. Interestingly, the data suggest some delay in terrestrial environments (summer temperatures) with respect to annual sea surface temperatures reconstructed in the Alboran Sea (Cacho et al. 2001) (Fig. 4.4e).

High and relatively constant July air temperature (despite some fluctuations) occurred in BSM until approximately 6200 cal yr BP, similarly again to Lago Gemini and Lago Verdarolo (Fig. 4d). In broad agreement with the previously mentioned Holocene Thermal Maximum reconstructed by Renssen et al. (2009), which suggest a Holocene thermal maximum in Iberia around 7-6 ka, the BSM maximum temperatures have been reconstructed between 8-6.5 cal kyr BP (Fig. 4.4a). This timing agrees with Italian lakes (Fig. 4.4d) but it is later again than in southern Iberia (Alboran SST, Cacho et al. 2001). Although a latitudinal time lag from southern (Alboran) to northern Mediterranean areas (BSM in the Pyrenees, Lago Gemini and Lago Verdarolo in northern Apennines) could be the explanation, other factors have to be evaluated (e.g. seasonality, marine versus terrestrial environments).

Considering the insolation orbital forcing (Fig. 4.4f), the Mid-Holocene (between 8-6.5 cal kyr) corresponds with still high summer and low winter values in northern mid latitudes which can, therefore, be expected to have been characterized by a high seasonality. High run off reconstructed for Basa de la Mora, likely caused by snow melt, suggests winters with higher snow precipitation and higher water availability, coherent with pollen data showing high abundances of mesophytes. Summer temperature effects could have been more intense in mountain areas accounting for some of the discrepancies with marine average annual temperature (Sachs et al. 2000).

Considering seasonality, Lake Redón temperatures reconstructed from chrysophyte cysts, mainly reflect winter-spring temperatures (Kamenik and Schmidt 2005; Pla and Catalan 2005) while chironomids reconstruct temperatures during the summer months (Eggermont and Heiri 2012). Therefore, the different annual and/or seasonal signal of different proxies may explain the distinct patterns of alkenones, chyrsophyte and chironomid-inferred temperature reconstructions for the Holocene in different parts of Iberia.

The BSM temperature record also agrees with the widespread cooling trend in summer temperatures inferred by climate models for different parts of Europe starting at ca. 7000-5500 cal yr BP (Renssen et al. 2009; Samartin et al. 2017) and other chironomid-based temperature reconstructions throughout Europe (e.g. Larocque-Tobler et al. 2010; Ilyashuk et al. 2011; Tóth et al. 2015). The Redón sequence points to more stable winter/spring temperatures inferred by chrysophyte cysts while the Alborán SST record also reflects, although with fluctuations, a general cooling trend during the Middle and Late Holocene (Fig. 4.4).

A low-density chironomid period in BSM (ca. 6000-5000 cal yr BP) coincides with this marked regional decrease in temperatures and a transition from humid to arid conditions for the western Mediterranean (Jalut et al. 2009). This humidity decrease also occurred in Basa de la Mora Lake as documented by mesopythes, MS and reduced reconstructed lake levels (Figs. 4.3 and 4.4b). Unfortunately, low chironomid content prevents a temperature reconstruction for this period. However, during the Late Holocene inferred temperatures were ca. 1 ºC lower (Fig. 4.4a) with the lowest BSM values throughout the Holocene reconstructed during two millennia (ca. 4200-2000 cal yr BP). This implies that a major, centennial to millennial-scale cooling trend or shift occurred at Basa de la Mora between ca. 6000 and 4200 cal yr BP. At a regional scale, similar trends occurred in Lago Gemini and Verdarolo and the Alboran Sea, while in Lake Redón values were more stable (Fig. 4.4). Again, seasonal differences in respect to the proxies' sensitivities must be taken into account when interpreting these apparent disagreements. This reconstructed colder phase, which would imply lower summer temperatures and consequently reduced evaporation, contrasts with low lake levels reconstructed in BSM by sedimentological and geochemical proxies (Fig. 4.4b). A plausible scenario would therefore be a decrease in annual precipitation during this phase — mostly during winter— while summer temperatures remained relatively cool.

Finally, the last two millennia are characterized in BSM by an increase in temperature of about 1.5°C (Fig. 4.4a). Similar increases in chironomid-inferred temperatures in Central (Alps) and Eastern European (Carpaths) mountain ranges (Heiri et al. 2003a; Tóth et al. 2015, respectively) have been interpreted as a potential consequence of increasing human impact on mountain lakes (e.g. via pasturing in their catchment), which may have led to higher nutrient loading to the lakes and changes in chironomid communities not related to climatic features. However, this trend has not been documented in Lago Gemini or Verdarolo (Fig. 4.4d) but it appears in Redón (Fig. 4.4c). Bearing in mind the mentioned potential anthropogenic effect, we must also take into account that the chronological resolution of both BSM and Redón reconstructions does not permit to identify temperature differences between the well-known Medieval Climate Anomaly (MCA) and Little Ice Age (LIA) during the last millennium in the region (Morellón et al. 2009a). Besides, in Basa de la Mora Lake, pollen evidence does not indicate an increase in plant types typically associated with grazing and pasturing until the last 700 years (Pérez-Sanz et al. 2013). Nevertheless, without additional quantitative palaeoclimatic evidences supporting a continuous warming of summer temperatures during the last millennium, the higher recent chironomid-inferred temperatures in BSM compared to the Mid-Holocene values should therefore be interpreted with caution.

CONCLUSIONS

We developed the first chironomid-based Holocene summer temperature for the Iberian Peninsula, based on a sequence retrieved from Basa de la Mora Lake (Central Pyrenees). The comparison of our results with other local and regional records have highlighted similarities and differences between our new reconstruction and previous records which we attribute to different patterns regarding temperature, hydrology and seasonality changes throughout the Holocene in our study region.

The onset of the Holocene was characterized by an increasing trend in summer temperature, which has also been reported elsewhere, although in the central Pyrenees there is apparently some delay in this warming compared with marine environments and other chironomid sequences from Eastern Europe. The high and relatively constant temperature values reported until ca. 6000 cal yr BP are in agreement with the established northern Hemisphere Holocene summer temperature Maximum. Considering together the reported high summer temperatures, sedimentological and pollen proxies from BSM, and insolation forcing (high summer and low winter insolation values), we suggest that this period was characterized by two main and contrasted seasons, with warm summers and cold winters, and high rainfall.

The temperature decline reported after ca. 6000 cal yr BP is consistent with other regional and chironomid-based reconstructions throughout Europe. However, the overall warmer Mid-Holocene reported in Basa de la Mora Lake contrasts with colder estimations made by vegetation-based reconstructions for southern Europe but is consistent with recent chironomid-based reconstructions conducted in the Mediterranean region (Italy).

Finally, the Late Holocene was characterized by a first period (ca. 4200-2000) with colder temperatures, which in the Iberian Peninsula has been mainly suggested as a transition from humid to arid conditions. Thus, a combination of lower temperatures and a significant decrease of annual precipitation could characterize this period. During the last two millennia, we reported an increase in reconstructed temperatures of ca. 1.5 ºC, although it is difficult to disentangle whether this really represents reaction of chironomid assemblages to higher temperatures or is a consequence of human activities.

This study adds important information about the Holocene climatic evolution in the south-western part of Europe and the chironomid-based temperature reconstruction establishes a valuable precedent in the Iberian Peninsula for future studies.

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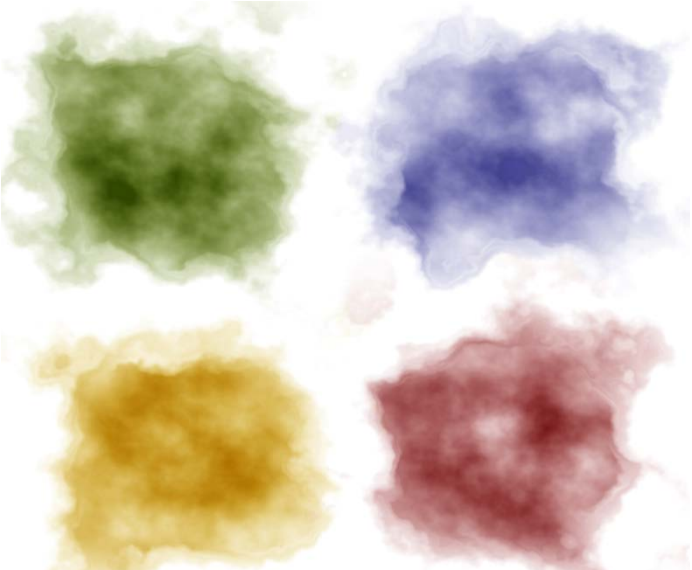
GENERAL DISCUSSION AND CONCLUSIONS



This PhD thesis constitutes one of the most detailed studies carried out to date on subfossil chironomids in the Iberian Peninsula. It aimed to provide an understanding of all the factors involved in paleoecological studies of subfossil chironomids (e.g. sedimentation, habitat characteristics, and environmental variables). On the one hand, it explored how chironomid remains are recorded in lake sediments, their relationship with their living communities, and the implications in paleoenvironmental reconstructions (Chapters 1 and 2). On the other hand, paleoenvironmental reconstructions using chironomids were performed in two different ways: i) by comparing two cores retrieved from different lake environments (Chapter 3) and ii) by obtaining a temperature reconstruction through a quantitative approach (i.e. applying a transfer function) (Chapter 4). As a whole, this thesis offers a valuable contribution to the use of Chironomidae in paleoecology and the knowledge of the particularities of the Holocene in the Iberian Peninsula.

In this general discussion we unravel the main contributions and implications of this thesis in a wider perspective. Specifically, we address the following take-home messages: i) the present conditions need to be understood in depth to be able to properly infer past conditions; ii) a single factor (e.g. habitat condition) can have a major effect on paleoenvironmental reconstructions; iii) complete paleoenvironmental reconstructions can be performed using Chironomidae subfossil remains. Finally, future research directions are provided based on the main findings of this thesis.

UNDERSTANDING THE PRESENT TO EXPLAIN THE PAST

The most common expression defining Hutton's principle of uniformitarianism states that "the present is the key to the past" (Tomkeieff 1962), which means that the natural processes operating in the present are the same as those that operated in the past. Although being a geological principle, and even considering that geological and ecological processes are different (biological evolution makes the difference), it is reasonable to believe that ecological processes have been operating in a similar way in a geological time frame (i.e. ecological uniformitarianism) (Rull 2010). In fact, this assumption is one of the main cornerstones of paleoecology, and points out the great importance of properly understanding the present ecological drivers and processes before conducting paleoecological studies.

Unfortunately, paleoecology and contemporary ecology have rarely been combined, despite being complementary disciplines (Jackson 2001; Sayer et al. 2010). Although during the 1990s some authors suggested that they were beginning to merge (Davis 1994), it has only been in the last decade that considerable progress has been made in this direction (Flessa and Jackson 2005; Willis et al. 2007). Rull (2010) tried to unravel the reasons why these disciplines have rarely worked together. From the psychological point of view, the conceptual dissociation between the past and the present has led to an underestimation of both disciplines in relation to each other. At the same time, the different methodological approaches used in each discipline have led to a barrier between them.

Pollen studies constitute the most important effort made to combine paleoecology and contemporary ecology by calibrating paleoreconstructions with the evaluation of modern pollen rain (Jackson and Williams 2004). In the case of Chironomidae, studies addressing the relationship between living and subfossil assemblages are scarce. Moreover, most of these studies focus on how head capsules are transported, deposited and recorded in lake sediments, neglecting the main drivers that shape living assemblages and how this knowledge can be useful in the study of past changes.

The results of this thesis have proved that it is essential to properly understand the current ecological factors shaping living assemblages before interpreting paleoecological information. Knowing the autoecology of each taxon at the specific study site is essential for calibrating and improving the interpretation of downcore changes (Chapter 1). Moreover, ecological information on living assemblages is useful for understanding the factors affecting chironomid deposition and its possible effects on quantitative reconstructions using transfer functions (Chapter 2).

The scarcity of this information in chironomid studies has usually been justified by the large amount of time needed to obtain results, mainly in sampling and laboratory work. Although it is true that working with living and subfossil Chironomidae can be time-consuming, the results of this thesis suggest that the amount and the quality of the information they can provide makes it worthwhile. Thus, we encourage paleolimnological studies to incorporate Chironomidae as study organisms. In this regard, given our results, paleoecology and contemporary ecology should be combined to obtain more complete and accurate paleolimnological inferences.

Finally, this thesis provides an example on the major importance of paleoenvironmental studies in explaining present and even future conditions. As stated above, the traditional dissociation between past and present applies in both ways. Thus, the study of past changes is essential for differentiating between the climate and human drivers that have affected Earth's ecosystems. Moreover, the study of past environments allows us to understand the response, resistance and resilience of the ecosystem and its components when faced with disturbances of different kinds. This information is therefore crucial for understanding present-day conditions and extrapolating future responses to similar disturbances (Alverson et al. 2000, 2003).

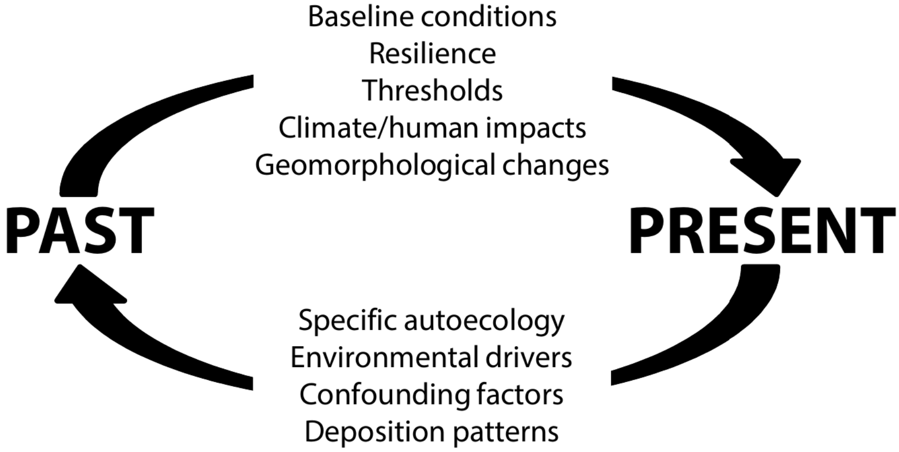


Figure D.1 Diagram representing sources of relevant information obtained by ecological studies for the study of paleoenvironmental changes and viceversa.

WHEN A SINGLE FACTOR MODIFIES PALEOENVIRONMENTAL RECONSTRUCTIONS

There are still many uncertainties and open questions that deserve further research regarding the use of chironomids in paleoecological studies. One of them is related to their consistence and reliability as quantitative indicators. Many authors have discussed this question and their opinions vary. For example, Gaute Velle and Stephen Brooks recently discussed the question in several papers (Velle et al. 2010, 2012a; Brooks et al. 2012). In this discussion, they both agreed on the fact that different confounding factors may influence the reliability of chironomids as past temperature indicators. However, they disagreed on the magnitude of this influence. While Velle et al. (2010, 2012a) argued that these factors could have a major influence, Brooks et al. (2012) considered that, despite these factors, chironomids can provide reliable estimates of past temperatures. This circumstance is not only restricted to temperature reconstructions, but also to other parameters (e.g. lake level, trophic status, pH, dissolved oxygen).

Velle et al. (2010) carried out a full and precise analysis of these confounding factors, which were summarized in: i) the spatial heterogeneity of organisms, ii) the complexity of ecological interactions between biological communities and concurrent environments, iii) the use of an over-coarse taxonomic resolution, iv) an inaccurate chronology, and v) the choice of numerical models. This thesis has dealt with the first two factors, obtaining results that add information in this respect, but that also open up new questions and uncertainties.

Our results prove the major influence of macrophytes on current chironomid community composition and the huge implications for paleoenvironmental reconstructions. As stated in Chapter 1, the influence of aquatic vegetation in current communities is well known (Dvořak and Best 1982; van den Berg et al. 1997). In this regard, we have identified the presence of *Chara* as one of the main drivers affecting the chironomid community in Enol Lake, since it can cause the homogenization of the whole subfossil chironomid community. The high densities of *Chara*-associated chironomids (especially *Paratanytarsus*) greatly affected the results obtained when analysing recent subfossil head capsules. This circumstance has already been pointed out by other authors, suggesting that macrophytes could have a major impact in quantitative reconstructions (Langdon et al. 2010).

According to our results, using the recent subfossil community to perform quantitative transfer functions in Enol Lake (e.g. temperature, lake level) could be misleading, since macrophyte-related taxa would act as a confounding factor, biasing the results and their interpretation. The homogenization of the recent subfossil community composition would hinder the identification of the other factors affecting the current community (i.e. temperature, oxygen, organic matter), preventing the use of these results in quantitative reconstructions. Moreover, due to the great abundance of macrophyte-related taxa, subfossil head capsules were evenly distributed along the depth transect, suggesting that a single core would properly and equally explain past environmental changes. However, as we will discuss below, this is a misleading conclusion.

As a whole, this thesis shows that paleoecological studies using chironomids are complex, and that just one factor may be enough to greatly influence the final results and interpretations. Thus, we encourage researchers to understand and deal with this complexity by evaluating each step involved in paleoecological studies and combining both ecological and paleoecological approaches.

TOWARDS A COMPLETE ENVIRONMENTAL RECONSTRUCTION

Another key question regarding the use of chironomids as paleoenvironmental indicators is related to the correct and complete representation of past changes by a single core taken from the lake centre, on which paleolimnological studies often rely. This circumstance is greatly affected by the geological view that dominates paleolimnological studies. However, as has already been pointed out, the spatial heterogeneity of organisms is one of the key confounding factors in paleoreconstructions involving biological indicators.

As we have shown in Chapter 3, the record of subfossil Chironomidae in lake sediments can vary widely along the depth transect. This is probably related to differences in environmental and hydrological factors. The littoral and sublittoral zones are directly exposed to external forces (e.g. surface water runoff, habitat modification, wind) and the dynamism of these zones, whereas profundal zones should experience more constant environmental and hydrological conditions throughout time. Thus, it is rather surprising that the most accepted premise still considers that obtaining a sample from the centre of the lake basin is representative of the whole subfossil assemblage (e.g. van Hardenbroek et al. 2011). This is probably due to the time-consuming nature of chironomid analysis (Heiri 2004), and it applies both to time-stratigraphic studies and the development of training sets (Holmes 2014). The contradictory conclusions obtained by studies on chironomid transport and depositional patterns (i.e. some found different patterns in relation to depth, while others did not) reveal the uncertainty of this premise.

The need of retrieving and analysing more than a single core when performing complete environmental reconstructions can be especially important in deep lakes. As shown in Chapter 1, the differences between chironomid assemblages in relation to depth can be very important in this type of lakes. Moreover, transport and deposition patterns can greatly affect the chironomid subfossil record. Although we found a homogeneous recent subfossil community (Chapter 2), assemblage turnover was largely explained by a single factor (the massive presence of *Chara*) that was masking other complex heterogeneous patterns. When analysing a longer period in which the recent influence of *Chara* went unnoticed (Chapter 3), the spatial differences of subfossil assemblages were clearly revealed. These spatial differences can cause a major bias in the interpretation of downcore changes, by overestimating some environmental factors and underestimating others. Thus, the results obtained in this thesis clearly suggest that more than one core should be retrieved within the same lake to perform complete environmental reconstructions using subfossil chironomids.

As Velle et al. (2010) argued, this circumstance may have important implications, not only when qualitatively interpreting downcore changes, but also when performing quantitative reconstructions. This is why the temperature transfer function was applied in a small, shallow lake such as Basa de la Mora (Chapter 4). In this type of lakes, the spatial heterogeneity affecting the distribution of chironomids can be considered to be less important, although there are other factors that can have a major effect on quantitative reconstructions, such as the presence of macrophytes. However, the subfossil chironomid community of Basa de la Mora Lake was dominated by non-macrophyte related taxa throughout the sequence, and those related to aquatic vegetation were only significant in the upper part of the sequence. Moreover, the pollen analysis did not show an important presence of macrophytes along the record. This circumstance adds reliability to the application of the temperature transfer function in Basa de la Mora Lake.

CONTRIBUTIONS TO THE STUDY OF THE HOLOCENE IN THE IBERIAN PENINSULA

Cacho et al. (2010) pointed out several gaps in paleoclimate reconstructions in the Iberian Peninsula since the last glacial period. According to them, the vast majority of the Holocene reconstructions in Iberian Peninsula continental sequences have so far focused on hydrological changes and water availability. In this respect, there are only two studies on quantitative temperature reconstructions covering the entire Holocene for this region. The first (Davis et al. 2003) was a European pollen-based reconstruction, which has been considered as non-representative due to the sparse number of sites included from the Iberian Peninsula. The second was a crysophyte-based reconstruction conducted in the Pyrenees, which reflected changes in winter-spring climate conditions (Pla and Catalan 2005). Thus, this thesis has been an excellent opportunity to deal with this major gap, by exploring temperature changes in the Iberian Peninsula during the Holocene, taking advantage of the widely established relationship between chironomids and temperature (Eggermont and Heiri 2012). The reliability of quantitative paleoreconstructions, including chironomid-based reconstructions, for representing past changes is still questioned (Velle et al. 2005, 2010; Birks 2011). However, it can be considered as one of the most interesting and useful approaches for reconstructing past environmental conditions using biological indicators. Thus, although being aware of these weaknesses and potential multiple drivers affecting chironomid reconstructions, it is still an interesting tool to develop and explore.

In this respect, this thesis represents an important contribution to the study of the Holocene in the Iberian Peninsula, as it provides the first chironomid-inferred temperature reconstruction covering the entire Holocene (as well as the first reconstruction focusing on summer conditions). It provides new and complementary information and opens up new horizons in the discussion of climate evolution during the current interglacial period in southwestern Europe. As we saw in Chapter 4, the combination of this temperature reconstruction with other local, regional and global records allows the exploration of other interesting climate features, such as seasonality, which can be essential for properly understanding climate evolution in our region.

Besides this major contribution, Chapter 3 also provides complementary information about the environmental dynamics of the last 600 years, an especially interesting and complex period due to the mixture of climate and anthropogenic signals recorded in lake sediments. Reconstructions dealing with this period are challenging, since these signals need to be differentiated. This thesis has shown evidences of the response of the chironomid community to several local anthropogenic impacts on the lake during this period, as well as the close relationship between chironomids and organic matter and trophic status changes. Moreover, some hydrological and climatic patterns have been also observed. Thus, subfossil Chironomidae seem to be a promising tool for understanding human influence on lake ecosystems within the context of global change.

FUTURE RESEARCH OPPORTUNITIES

As indicated at the beginning of this section, this PhD thesis is one of the first studies conducted in the Iberian Peninsula attempting to evaluate the use of Chironomidae as lake paleoenvironmental indicators. It is a comprehensive and ambitious study of the different factors affecting the midge paleoenvironmental record. Thus it provides useful information on the application of subfossil Chironomidae as both qualitative and quantitative indicators. Based on the results presented here, several future research opportunities, both in the Iberian Peninsula and elsewhere, are suggested below.

Similar studies (i.e. combining ecological and paleoecological information) could be replicated in other lakes and regions in order to properly recognize the factors shaping the midge paleoenvironmental record. In this respect, it would be interesting to test differences among different types of lakes (e.g. shallow and deep, macrophyte-related and non-macrophyte-related) in order to explore how lake morphology and other key drivers can affect chironomid distribution and deposition. These studies would add very valuable information, as they could establish target lakes at which to perform chironomid-based environmental reconstructions (i.e. those that are more homogeneous in terms of the spatial distribution of subfossil assemblages).

Another key issue that needs to be addressed is related to quantitative reconstructions using chironomids. In this respect, it would be very interesting to build a temperature transfer function for the Iberian Peninsula. As Chapter 4 shows, there are several chironomid-based transfer functions in Europe, although the most used come from the Alps and Norway. This thesis has proved that it is possible to apply these transfer functions to data from the Pyrenees with good results. However, the Iberian Peninsula provides excellent conditions for building a good temperature transfer function (i.e. several mountain ranges with many lakes). In this regard, 82 Pyrenean lakes have already been sampled as part of the Emerge Project (EMERGE 2002), and the resulting data is suitable for building a reliable transfer function. Thus, we encourage its development in the Pyrenean region, as well as expanding the scope to other regions, such as Picos de Europa, the Central Range or Sierra Nevada.

Another interesting application that should be explored, as it can be extremely relevant in the context of the Iberian Peninsula, is the use of chironomids as past lake level indicators. As previously indicated, the vast majority of lake environmental reconstructions in the Iberian Peninsula are related to hydrological changes. Thus, developing quantitative chironomid-based lake level reconstructions would allow the comparison of chironomid-based reconstructions with all those sequences, adding new and relevant information.

This application would somehow merge what has been suggested in the previous paragraphs. In order to establish the appropriate lakes at which to perform this kind of studies, it would be necessary to study both the living and the recent subfossil communities, as lakes showing homogeneous depth distribution patterns of chironomid assemblages would not be suitable for carrying out lake level reconstructions. Additionally, a transfer function should be built to obtain the quantitative reconstructions. In this respect, the studies conducted by Kurek and Cwynar (2008) or Engels et al. (2012), in which they tested the differences between site-specific and regional lake-level inference models, could serve as a good starting point. As chironomid depth patterns are greatly affected by local conditions, it would be interesting to further test whether it is possible to correctly apply a lake level transfer function relying on a single lake, since this would reduce the large amount of time needed to carry out this kind of studies.

Overall, we encourage the use of chironomids as lake paleoenvironmental indicators in the Iberian Peninsula, as they constitute a largely neglected but promising tool for understanding the past. In this regard, there are several innovative research lines that could expand the use of Chironomidae. One good example is the analysis of the isotopes contained in their chitinous head capsules. On the one hand, chironomid carbon isotopes are believed to provide insights into lake carbon cycles (Jones et al. 2008), and so $\delta^{13}C$ analysis is increasingly being applied to the subfossil record (Wooller et al. 2012; van Hardenbroek et al. 2012; Frossard et al. 2014). Given the strong relationship that this thesis and several other studies have shown between chironomids and organic matter, it would be very interesting to perform this carbon isotope analysis not only in Enol Lake, but in other mountain lakes around the world. On the other hand, $\delta^{18}O$ analysis on chironomid head capsules has been considered a good proxy for reconstructing temperature changes (Verbruggen et al. 2011). Although its use is still scarce (Wooller et al. 2004; Verbruggen et al. 2010), it would be interesting to compare the temperature reconstructions obtained by transfer functions with those obtained by $\delta^{18}O$ analysis. Thus, the use of chironomids as lake paleoenvironmental tools provides many opportunities in general, but in particular in those regions where it remains largely unexplored, as is the case of the Iberian Peninsula.

CONCLUSIONS

General Conclusions

- The use and application of chironomids as paleoenvironmental indicators is complex, as many factors (e.g. macrophytes, lake morphology, substrate composition, and currents) can have a major influence on how chironomids are recorded in lake sediments and this could greatly bias and mislead the interpretation of downcore analyses.

- It is essential to undertake complete studies when using chironomids to reconstruct past environmental changes. These studies should firstly focus on understanding the living community and its drivers and secondly on how their chitinous remains are transported, deposited and recorded in lake sediments. This information is essential both for understanding the specific autoecology of the species at the study site and for establishing the most representative depth(s) at which cores should be retrieved for undertaking chironomid analysis.

Chapter 1

- The living chironomid community in Enol Lake is divided into 3 main assemblages: littoral, *Chara*-related, and profundal. These assemblages differ greatly in terms of taxa composition and temporal changes.

- The littoral zone is mainly driven by temperature and dissolved oxygen seasonal changes. As expected, it is the most diverse zone of the lake in terms of taxa richness.

- The results highlight the considerable importance of Characeae for chironomid communities. In this respect, the reported abundances characterize this zone as highly productive in terms of chironomid individuals.

- The profundal zone is characterized by the presence and dominance of *Chironomus plumosus*. This circumstance can be explained by the anoxia reported in the bottom layers of Enol Lake during several months, which was mainly caused by the organic matter inputs due to cattle grazing in the lake surroundings.

- The results show that chironomids are good indicators of the different ecological drivers affecting the lake at different depths and, more importantly, that they are reacting to human impacts (e.g. eutrophication).

Chapter 2

- We found a considerable spatial homogeneity in the subfossil assemblage and a relatively strong correlation between the overall living assemblage (i.e. mean abundances of taxa) and the subfossil assemblage, both in terms of taxa composition and abundance.

- However, this homogeneity was driven by a single factor (i.e. the major presence of *Chara*), which seems to promote the dominance of *Paratanytarsus* along the depth transect. This circumstance masks other more complex patterns of different taxa.

- Although these results would suggest that a single core retrieved from any part of the lake would equally and properly record past environmental changes, caution should be applied, as the considerable influence of a single and fluctuating factor (i.e. *Chara* presence) could lead to a major bias, both when using the recent subfossil record in quantitative inferences and when interpreting past changes through a single core.

Chapter 3

- The analysis of two cores retrieved from different parts of the lake (sublittoral and profundal) showed several differences in terms of how different sampling points can record chironomid-inferred past changes. In this respect, the results contradict what was reported in Chapter 2, meaning that it is necessary to analyse more than one core to perform complete paleoenvironmental reconstructions using chironomids, at least in Enol Lake.

- The profundal core did not record the main human-induced disturbances reported in the lake, but did record some climatic and hydrological fluctuations. However, the low densities found in this core hindered confident interpretation of the results, as they may be highly influenced by changes produced by a few individuals.

- The shallower core reported the main anthropogenic impacts, in addition to some hydrological oscillations. In this respect, we have observed the response of the chironomid community to an increase in organic matter. Moreover, the high densities found in this core make the results more reliable.

Chapter 4

- The first chironomid-based temperature reconstruction in the Iberian Peninsula covering the entire Holocene was performed successfully.
- The comparison of these results with other local and regional records has highlighted similarities and differences between this new reconstruction and the previous records. These can be attributed to different patterns regarding temperature, hydrology and seasonality changes throughout the Holocene in our study region.
- We found an increasing trend in summer temperature at the onset of the Holocene, maintaining high values until ca. 6000 cal yr BP. This is in agreement with the established northern Hemisphere Holocene summer Temperature Maximum. We suggest that this period was characterized by two main and contrasting seasons, with warm summers and cold winters, and high rainfall.
- The temperature decline reported after ca. 6000 cal yr BP is consistent with other regional and chironomid-based reconstructions throughout Europe.
- Finally, the Late Holocene was characterized by a first period (ca. 4200-2000) with colder temperatures, which in the Iberian Peninsula has been mainly suggested as a transition from humid to arid conditions. During the last two millennia, we reported an increase in reconstructed temperatures of ca. 1.5 ºC, although it is difficult to disentangle whether this really represents a response of chironomid assemblages to higher temperatures or is a consequence of human activities.

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SUPPORTING INFORMATION

This section comprises supporting information for Chapter 1 and Chapter 2.

Appendix A provides the IndVal results for Chapter 1 and the equivalence between living and recent subfossil taxa identified in Chapter 2.

Appendix B provides the original publication of Chapter 1.

Chironomid databases of each chapter are available upon request through the following links:

- Chapter 1: https://goo.gl/6vjSMa
- Chapter 2: https://goo.gl/mKDrdW
- Chapter 3: https://goo.gl/erhrTZ
- Chapter 4: https://goo.gl/M5Hb6y

Appendix A

This appendix provides supplementary information regarding Chapter 1 and Chapter 2. It comprises 2 tables.

Table S.1 IndVal results of living Chironomidae community from Enol Lake.

Group	Taxa	IndVal	*p*
Littoral	*C. atridorsum*	91.1	0.005
	M. pedellus	85.9	0.005
	P. nubens	82.6	0.005
	E. tendens	73.6	0.005
	E. coerulescens	57.7	0.005
Chara	*E. pagana*	91.4	0.005
Littoral+Chara	*P. bituberculatus*	98.9	0.005
	E. albipennis	97.7	0.005
	C. lobata	88.9	0.005
	P. nubeculosum	78.4	0.005
	S. sticticus	75.1	0.010
	P. choreus	72.6	0.005
	A. monilis	64.0	0.015
	P. bathophila	60.2	0.015
Profundal + Chara	*C. plumosus*	95.3	0.005

Table S.2 Equivalence between the identified chironomid taxa of living and subfossil assembalges of Enol Lake. Rare taxa are marked with an asterisk. Taxa equivalent codes following Schnell et al. (1999).

Sub-family	Living assemblage	Subfossil assemblage	Rare taxa	Code
Chironominae	*Chironomus plumosus*	*Chironomus plumosus*-type		Chir plu
		Dicrotendipes nervosus-type	*	
	Einfeldia pagana	*Einfeldia pagana*-type		Einf pag
	Endochironomus albipennis	*Endochironomus albipennis*-type		Endo alb
	Endochironomus tendens	*Endochironomus tendens*-type		Endo ten
	Glyptotendipes pallens	*Glyptotendipes pallens*-type	*	
	Microchironomus tener		*	
	Microtendipes pedellus	*Microtendipes pedellus*-type		Mict ped
	Parachironomus arcuatus	*Parachironomus varus*-type	*	
	Paratendipes albimanus	*Paratendipes albimanus*-type	*	
	Polypedilum nubeculosum	*Polypedilum nubeculosum*-type		Poly nuc
	Polypedilum nubens	*Polypedilum* type A		Poly nub
	Stictochironomus sticticus	*Stictochironomus*		Stic sti
	Cladotanytarsus atridorsum	*Cladotanytarsus mancus*-type		Clat atr
	Micropsectra sp.	*Micropsectra*	*	
	Paratanytarsus bituberculatus	*Paratanytarsus austriacus*-type		Part bit
	Tanytarsus bathophilus	*Tanytarsus lugens*-type		Tany bat
Orthocladiinae		*Chaetocladius piger*-type	*	
	Corynoneura lobata	*Corynoneura lobata*-type		Cory lob
	Eukiefferiella coerulescens	*Eukiefferiella claripennis*-type	*	
	Eukiefferiella sp.			
	Orthocladius sp.	*Orthocladius*	*	
	Parakiefferiella bathophila	*Parakiefferiella bathophila*-type		Park bat
	Parametriocnemus stylatus		*	
	Paratrichocladius rufiventris		*	
		Psectrocladius sordidellus-type	*	
Tanypodinae	*Ablabesmyia monilis*	Pentaneurini		trpenta
	Zavrelimyia sp.			
	Procladius choreus	*Procladius*		Proc cho
Prodiamesinae	*Prodiamesa olivacea*		*	

Appendix B

This appendix provides the original publication of Chapter 1.

Tarrats, P., Cañedo-Argüelles, M., Rieradevall, M. And Prat, N. (2017). Chironomids as indicators of local and global changes in an oligotrophic high mountain lake (Enol Lake, northwestern Spain). *Journal of Limnology*, 76(2): 355-365. doi: 10.4081/jlimnol.2016.1590

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ORIGINAL ARTICLE

Chironomid communities as indicators of local and global changes in an oligotrophic high mountain lake (Enol Lake, Northwestern Spain)

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ABSTRACT

The benthos of the high mountain Enol Lake (Picos de Europa National Park, Spain) was analyzed in order to understand the spatiotemporal factors and patterns controlling its current Chironomidae community. In total, more than 14,000 chironomid larvae were identified, belonging to 27 taxa. The results have pointed out the presence of 3 main chironomid assemblages in the lake: i) littoral community, which is mainly controlled by temperature and oxygen seasonal changes, ii) *Chara*-dominant community, which is mainly controlled by the presence and abundance of Characeae in the lake, and iii) profundal community, which is affected by low oxygen levels caused by sediment and organic matter discharge to the lake due to human pressures in the lake basin. We provide valuable insights for the managers to understand the current ecological status of Enol Lake and to evaluate which measures should be implemented to preserve or improve it. Moreover, our results constitute an essential step forward to improve the interpretation of the past changes of the lake by means of the subfossil chironomid community.

Key words: Chironomidae; high mountain lakes; biological indicators; Characeae; eutrophication; oxygen.

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INTRODUCTION

Chironomidae (Insecta: Diptera) are the most widespread of all freshwater macroinvertebrate families (Ferrington, 2008) occurring in almost all kinds of freshwater bodies of all zoogeographic regions over the world (Sæther, 2000). They are also often the most abundant and diverse group in freshwaters, representing up to 50% of the macroinvertebrate community (Armitage *et al.,* 1995). Chironomids are widely regarded as effective indicators of water quality and changes in habitat conditions since they exhibit a great variety of ecological traits and can be present over a wide range of environmental conditions (Battarbee, 2000; Walker, 2001; Cañedo-Argüelles *et al.,* 2012).

Temperature has been traditionally considered one of the most important factors controlling chironomid distribution (Eggermont and Heiri, 2012; Marziali and Rossaro, 2013), but other factors such as water depth (Korhola *et al.,* 2000; Engels *et al.,* 2012), dissolved oxygen (Little and Smol, 2001) or trophic status (Brodersen and Quinlan, 2006) can also be important. Moreover, Chironomidae communities experience marked temporal and spatial variations in their composition. The temporal variability of the Chironomidae community is mainly related to the life cycle characteristics (Heinis and Davids, 1993), which lead to different emergence patterns that are known to be principally controlled by water temperature and light intensity (Kureck, 1980). Chironomidae spatial changes result from different factors (*e.g.*, aquatic vegetation, dissolved oxygen) affecting the community at different depths and habitats (Prat and Rieradevall, 1995; Eggermont *et al.,* 2008; Frossard *et al.,* 2013).

In each lake the key factors controlling Chironomidae communities may change according to local and global conditions. Understanding the response of the current communities can be helpful to interpret the past and predict future changes, since Chironomidae capsules are well preserved through time in the sediment, allowing us to conduct paleolimnological studies (*e.g.*, Walker, 2001). In this regard, local and regional studies of the actual fauna are necessary to calibrate how the community has changed and will change in the future if Chironomidae want to be used for predictive studies of global change (*e.g.*, using transfer functions) (Luoto, 2010). At the same time, by exploring the spatial and temporal variations in community composition, the influence of local factors (*e.g.*, land use, organic enrichment) can be disentangled. Thus, Chironomidae can provide useful information for water managers, helping them to adopt appropriate measures to achieve and maintain the good ecological status of lakes, *e.g.*, as required in Europe by the Water Framework Directive.

Lakes have been widely considered as sentinels of climate and environmental changes (Adrian *et al.,* 2009; Schindler, 2009). Among them, high mountain lakes are especially sensitive to external forcing due to their characteristics (high altitude, high UV radiation, low nutrients, *etc*.) (Catalan *et al.,* 2006). Thus, special scientific attention has been paid to this type of lakes (Battarbee, 2005; Catalan *et al.,* 2009a). In the Iberian Peninsula, there are several studies focused on chironomid communities of high mountain lakes (Real and Prat, 1992; Rieradevall and Prat, 1999; Real *et al.,* 2000), although the majority took place in the Pyrenees (Catalan *et al.,* 2009b; De Mendoza and Catalan, 2010) or in the Central Range (Toro *et al.,* 2006). Furthermore, these studies usually focus on the littoral part of the lake, with only a few covering the whole depth transect (Rieradevall *et al.,* 1999; Rieradevall and Prat, 1999) and usually only sampling once or twice, which implies the loss of information related to seasonal variability. Here we address seasonal and spatial variability of the Chironomidae communities, providing with certainty the most intensive work conducted on a single high mountain lake of the Iberian Peninsula to date.

Our main hypothesis was that depth would play an important role in explaining chironomid community changes in Enol Lake through changes in environmental factors. Together with depth, we hypothesized that the presence of macrophytes and algae in the lake should be important, since the macrophyte and algae-related chironomid species tend to be very different than the sediment-related ones (Langdon *et al.,* 2010; Vermaire *et al.,* 2013). The knowledge of the factors that control the community in the lake today are a preliminary step in understanding the past changes in the community (using the subfossil midges present in the sediment) and the possible future changes related to local, regional and global drivers, which will be the objective of the forthcoming studies in the lake.

METHODS

Study site

Enol Lake (43° 16' N, 4° 59' W, 1070 m asl) (Fig. 1) is a karstic lake of glacial origin located in the northern part of Spain (Asturias), in the western massif of Picos de Europa National Park. It has a water surface of 12.2 ha, a maximum depth of 22 m and a small watershed (1.5 km^2). The lake is fed by groundwater and surface runoff and it has no permanent inlets. Water looses occur through evaporation, groundwater discharges and an outlet located at the northeast border of the lake, which is regulated by a small dam. Previous surveys (Velasco *et al.,* 1999; Moreno *et al.,* 2011) and the data collected in our study (see sampling subsection) characterize the lake as warm monomictic (with a thermocline located between 8 and 12 m from early July until early November). The lake is oligotrophic (total phosphorous 8 µg L^{-1}, Chl-a 0.5-1 µg L^{-1}), moderately hard (alkalinity 2.4 meq L^{-1} and 24-37 mg Ca L^{-1}) and with a conductivity between 150 µS cm^{-1} at the surface and 227 µS cm^{-1} at the bottom. It is almost fully covered with a great carpet of *Chara* sp. between 2 and 8 m of depth, while *Potamogeton natans* occurs between 1 and 3 m of depth. Despite this oligotrophy the bottom of the lake is devoid of oxygen for 4 months every year during the stratification period (García-Criado and Martínez-Sanz, 2010).

Sampling

Samplings were performed in two consecutive years (2013 and 2014), in May, July, September and November of each year. In every sampling campaign, both littoral and bottom samples were collected. In the upper littoral zone, we took 3 samples per campaign belonging to the three different dominant habitats found in the lake: i) sediment, ii) stones and iii) blocks. Samples were collected using the kick-sampling method (sampling surface: 1 m^2) with a 250 µm mesh net and the filtered sample was preserved in formaldehyde at 4%. In the case of the sublittoral and profundal zones (2-22 m), we used an Ekman grab (sampling surface: 225 cm^2), with three replicates per sample following a depth transect, collecting samples every 2 m. These samples were also sieved in the field using a 250 µm mesh net and preserved in formaldehyde at 4%.

In the laboratory, we sorted all the Chironomidae larvae present in the sample up to a maximum of 300 individuals. Initially, we sorted the chironomid specimens by morphotypes. A certain number of larvae of each morphotype were treated with 10% potassium hydroxide (KOH) at 70°C and, after dehydration, were mounted on microscope slides in Euparal®. The Chironomidae specimens were identified using an optical microscope (Olympus CX41) at 400x magnification and several taxonomic keys (Wiederholm, 1983; Rieradevall and Brooks, 2001; Brooks *et al.,* 2007). Later, when morphotypes were identified, several taxa were counted directly from samples without further processing. The identification of larvae to species level was validated through the examination of a large collection of pupal exuviae from the lake using the key of Langton and Visser (2003).

Environmental variables were collected by researchers of the Department of Geological Resources Research of the Geological and Mining Institute of Spain (IGME) in the same 8 campaigns. Temperature, pH, dissolved oxygen and conductivity were measured every 0.5 m of depth using a multiparametric sensor (Hydrolab MS5 and DS5). Water samples were collected every 5 m of depth for total organic carbon (TOC), total inorganic carbon (TIC), nutrients, major ions and trace metals analyses. These samples were analyzed in the laboratory following standard methods (Rice *et al.,* 2012).

Data analysis

The general distribution of the chironomid taxa along depth was represented by the program Psimpoll (Bennett, 2009), using the mean of the relative abundances of each taxa per depth. Statistical analyses were performed using R software (R Core Team, 2016). Rare taxa, defined by those that did not reach a relative abundance of ≥2% in at least two samples, were removed from the analyses. For the statistical analyses, we used total abundances, which were previously transformed to log (x+1).

Assemblage ordination was explored through Non-metric Multidimensional Scaling (NMDS) of the species matrix using the function ‘metaMDS’ of the R package ‘vegan’ version 2.4-0 (Oksanen *et al.,* 2016) and the Bray Curtis dissimilarity index to calculate the resemblance matrix. We classified the samples in 3 groups through a k-means clustering, then the indicator taxa of each group were identified through an IndVal analysis (Dufrêne and Legendre, 1997) using the ‘multipatt’ function in the R package ‘indicspecies’ (De Cáceres and Legendre, 2009). This analysis assigned each taxon to a most probable group based on its relative abundance and relative frequency and provided an indicator value (IV, varying between 0 and 1) and an alpha value obtained by Monte Carlo permutations (9999 runs). The differences in the environmental characteristics of each group were tested by

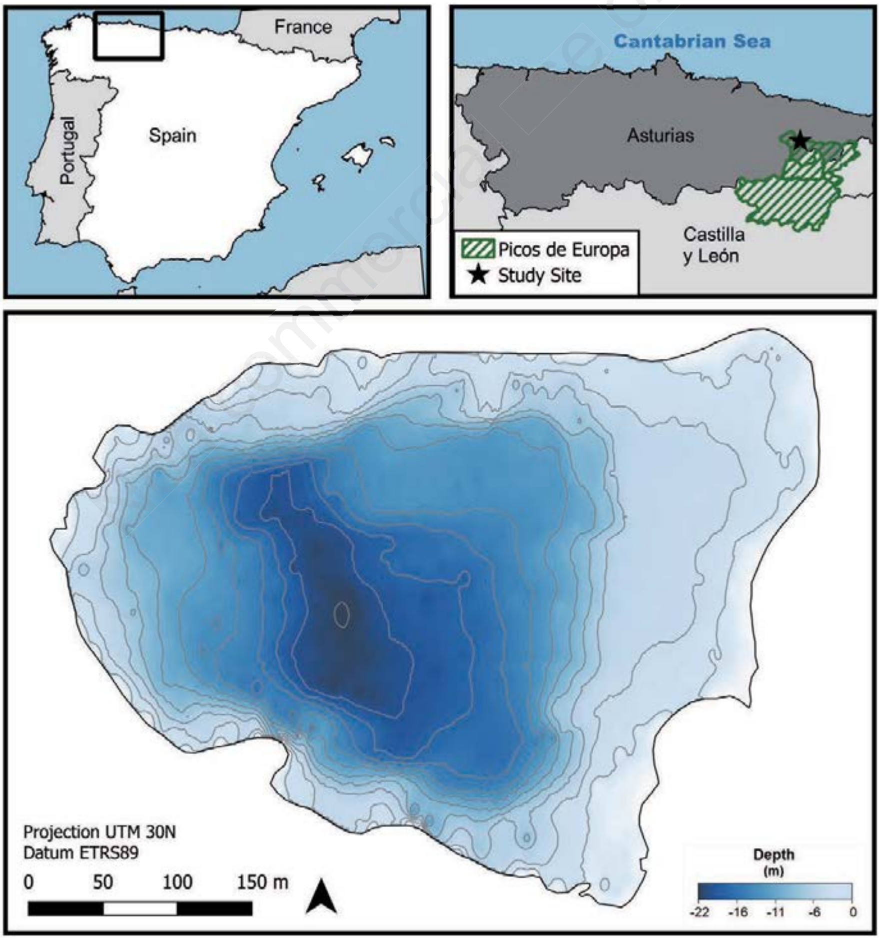

Fig. 1. Enol Lake location map. Bathymetry adapted from Rodríguez-García *et al.* (2016); with permission.

means of one-way ANOVA of normalized variables. In this case, we used temperature, pH, dissolved oxygen, conductivity and *Chara* sp. abundance as environmental descriptors. When ANOVA yielded significant results, Tukey tests were performed to evaluate pairwise comparisons. Finally, temporal variations in the chironomid assemblages were explored through a Redundancy Analysis (RDA) at 5 depths (0.5 m, 5m, 10m, 15m and 20m) with forward selection of explanatory variables using the 'rda' and 'ordistep' functions of the 'vegan' 2.4-0 package (Oksanen *et al.,* 2016) respectively. For these analyses, all environmental variables measured were included. Prior to this, Spearman correlation matrices were calculated in order to discard those variables that were significantly correlated (*i.e*., $\rho \geq 0.85$).

RESULTS

Assemblage composition

We identified a total of 14,248 chironomid larvae belonging to 27 species from 4 sub-families. The most diverse and abundant was the Chironominae sub-family, with 12 species of the Tribe Chironomini and 4 species of the Tribe Tanytarsini, followed by Orthocladiinae (7 species), Tanypodinae (3 species) and Prodiamesinae (1 species). Among them, we identified *Einfeldia pagana* (Meigen, 1838), which is considered a rare species in the Iberian Peninsula with only one previous citation (de Mendoza, 2013). All the identified taxa are shown in Tab. 1, with their names following the Fauna Europaea database nomenclature (Sæther and Spies, 2013) and the equivalent code proposed by Schnell *et al*. (1999) (Tab. 1).

Spatial variation

According to the k-means clustering, samples were classified as follows: i) profundal samples (10-22 m), ii) *Chara*-dominant samples (2-8 m) and iii) littoral samples (0.5 m). All environmental variables included in the ANOVA significantly differed between these groups (Fig. 2). Temperature, conductivity, pH and dissolved Oxygen showed non-significant differences between the littoral and the *Chara*-zone group and significant differences between these two groups and the profundal samples. *Chara* sp. abundance showed non-significant differences between the littoral and the profundal groups and significant differences between these two groups and the *Chara*-dominant one.

Tab. 1. Chironomidae taxa list of Enol Lake and equivalent code proposed by Schnell *et al*., (1999).

Sub-family	Name	Code
Chironominae	*Chironomus (Chironomus) plumosus*	Chir plu
	Einfeldia pagana	Einf pag
	Endochironomus albipennis	Endo alb
	Endochironomus tendens	Endo ten
	Glyptotendipes (Glyptotendipes) pallens	Glyp pal
	Microchironomus tener	Micc ten
	Microtendipes pedellus	Mict ped
	Parachironomus arcuatus	Parc arc
	Paratendipes albimanus	Patd alb
	Polypedilum (Polypedilum) nubeculosum	Poly nuc
	Polypedilum (Pentapedilum) nubens	Poly nub
	Stictochironomus sticticus	Stic sti
	Cladotanytarsus atridorsum	Clat atr
	Micropsectra sp.	Micrind
	Paratanytarsus bituberculatus	Part bit
	Tanytarsus bathophilus	Tany bat
Orthocladiinae	*Corynoneura lobata*	Cory lob
	Eukiefferiella coerulescens	Euki coe
	Eukiefferiella sp.	Eukiind
	Orthocladius sp.	Orthort
	Parakiefferiella bathophila	Park bat
	Parametriocnemus stylatus	Pare sty
	Paratrichocladius rufiventris	Patr ruf
Tanypodinae	*Ablabesmyia monilis*	Abla mon
	Procladius choreus	Proc cho
	Zavrelimyia sp.	Zavyind
Prodiamesinae	*Prodiamesa olivacea*	Prod oli

Only a few taxa were present in the profundal zone, mainly represented by *Chironomus (Chironomus) plumosus* (Linnaeus, 1758) (Fig. 3). However, the IndVal analysis did not get any taxa exclusively associated with this group, as *C. plumosus* is also present in other zones (although with lower abundances). *E. pagana* was considered to be exclusively indicator of the *Chara*-dominant zone while *Corynoneura lobata* (Edwards, 1924), *Polypedilum (Polypedilum) nubeculosum* (Meigen, 1804), *Stictochironomus sticticus* (Fabricius, 1781), *Procladius (Holotanypus) choreus* (Meigen, 1804), *Ablabesmyia (Ablabesmyia) monilis* (Linnaeus, 1758), *Parakiefferiella bathophila* (Kieffer, 1912), *Paratanytarus bituberculatus* (Edwards, 1929) and *Endochironomus albipennis* (Meigen, 1830) were significantly associated with both *Chara* and littoral zones, although the last two species were significantly more abundant where *Chara* sp. was present (45 and 22 % respectively for *P. bituberculatus*

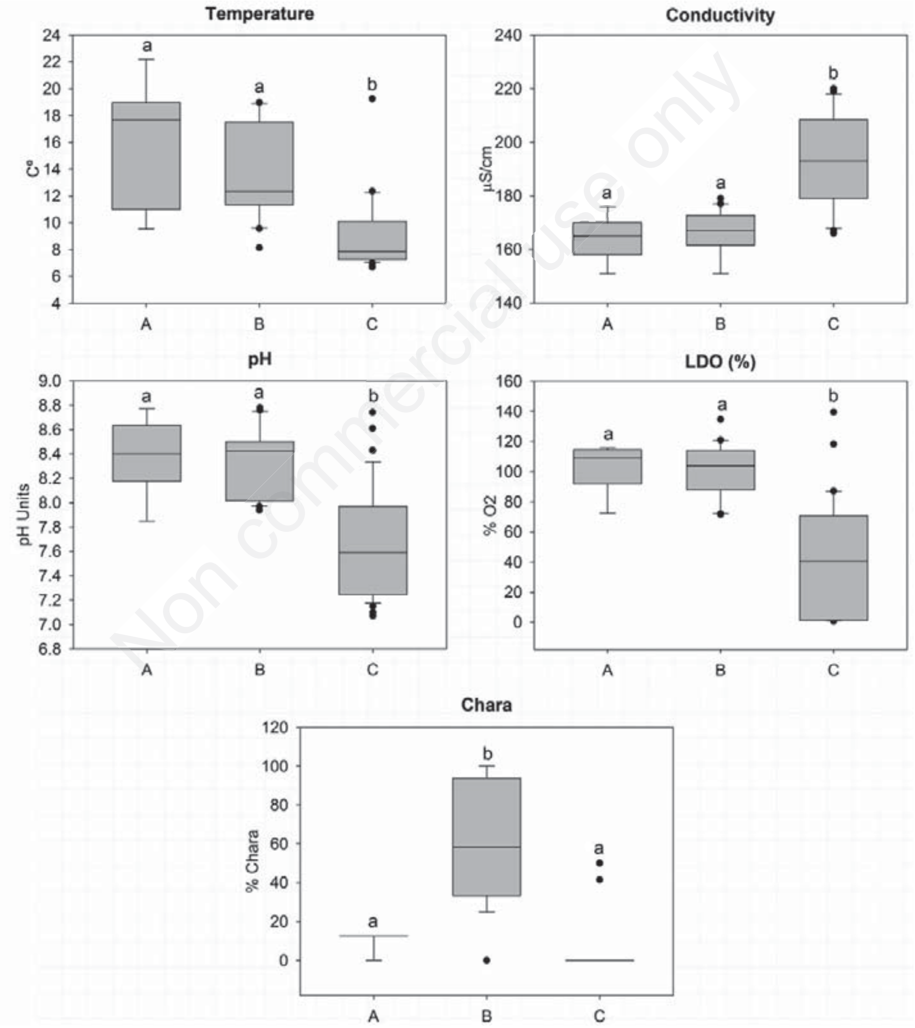

Fig. 2. Box plots representing temperature, conductivity, pH, Dissolved Oxygen and *Chara* sp. abundance according to the identified groups: a) Littoral, b) *Chara* and c) Profundal. Tukey Test results are specified above each box, with the same letter (a) representing non-significant differences between groups and different letter (b) representing significant differences.

and 12 and 5% for *E. albipennis*). Finally, *Cladotanytarsus (Cladotanytarsus) atridorsum* (Kieffer, 1924), *Microtendipes pedellus* (De Geer, 1776), *Polypedilum (Pentapedilum) nubens* (Edwards, 1929), *Endochironomus tendens* (Fabricius, 1775) and *Eukiefferiella coerulescens* (Kieffer, 1926), were significantly and exclusively associated with the littoral zone, with *C. atridorsum* being the most abundant (27%).

According to the NMDS 3 groups of assemblages could be identified (Fig. 4), similar to those obtained with the k-means clustering (littoral, *Chara*-dominant and profundal assemblages). The 8 samples belonging to the littoral group appear well separated in the NMDS plot. The *Chara*-dominant group included samples located between 4 and 8 m of depth, but also few samples at 10 m depth, on the edge of *Chara* sp. zone. Finally, the profundal group mainly included samples located below 10 m of depth, although three samples included in this group were collected at lower depth. These lower-depth samples were collected in soft sediment substrate, devoid of vegetation.

Temporal variation

According to forward selection, temperature and dissolved oxygen significantly explained the temporal variation in the Chironomidae assemblages in the littoral zone (Fig. 5). As a consequence, the sites were ordered by sampling date, with higher temperatures and dissolved oxygen summer samples plotted in the upper right part of Fig. 5. Although magnesium (Mg) was selected at 5 m of depth and sulphates (SO_4^{2-}) and bicarbonate ($HC0_3^-$) were significantly selected at 10 m, they showed very narrow concentration ranges (*i.e.*, 0-3 mg L^{-1} of Mg at 5 m, 2-15 mg L^{-1} of SO_4^{2-} and 84-101 mg L^{-1} of $HC0_3^-$ at 10 m). Finally, no environmental variable was significantly related with the temporal changes in Chironomidae assemblages in the profundal zone (*i.e.*, 15 and 20 m).

DISCUSSION

Our results show that, according to Chironomidae assemblages, Enol Lake is clearly differentiated in 3 zones: a littoral zone, a sublittoral zone dominated by *Chara* sp., and a profundal zone. In the littoral zone, both abundance and composition of Chironomidae changed in time. In the *Chara*-zone, Chironomidae assemblages were constantly dominated by a single species: *P. bituberculatus*. The pro-

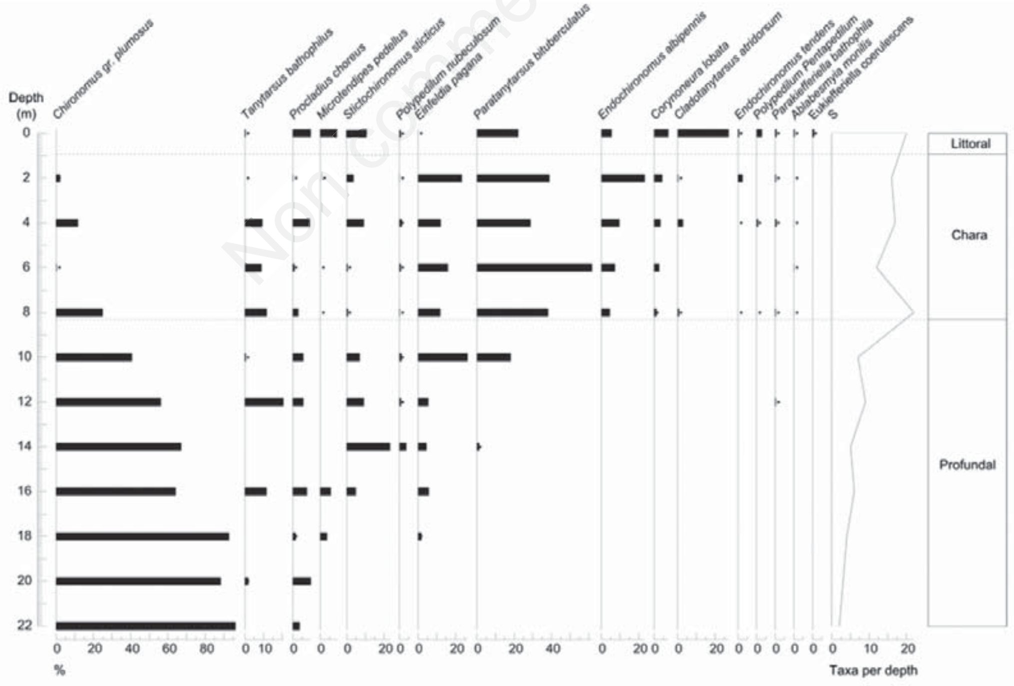

Fig. 3. Depth distribution of Chironomidae in Enol Lake. The percentage of each taxon for each depth is shown.

fundal zone was dominated by *C. plumosus*, indicator of low oxygen concentration. Overall the diversity of midges decreased with depth, as previously reported in many other lakes (Lindegaard, 1992; Korhola *et al.,* 2000; Chen *et al.,* 2014). Furthermore, the spatial variation in the assemblages' composition was consistent with the known autoecology of the identified taxa (Pinder and Reiss, 1983; Brodin, 1986; Pinder, 1986; Prat and Rieradevall, 1995; Brodersen and Lindegaard, 1997; Brodersen *et al.,* 2001; Brooks *et al.,* 2007). *C. plumosus* is known to live in water with low oxygen content (Brooks *et al.,* 2007), whereas *P. bituberculatus* has been often associated with the presence of macrophytes (Brodersen *et al.,* 2001).

C. plumosus is also commonly regarded as an indicator of eutrophication (Brodersen and Quinlan, 2006) due to its tolerance to low oxygen concentrations and even anoxia (Real *et al.,* 2000). The dominance of this species in the deepest part of Enol Lake should suggest that the lake is eutrophic, but according to primary production rates the lake is oligotrophic. Since the nutrient content of the water is low (Velasco *et al.,* 1999), the deoxygenation of the bottom should be related to a source of organic matter different from phytoplankton production. The lake basin is used for intensive pasture of cow (Rodríguez Castañón, 1996), which leads to important soil erosion and a direct input of cow manure into the lake. These factors increase the sediment and organic matter direct inputs into the lake bottom, as they are directly transported to the sediment. This process is very likely responsible for the high oxygen consumption of hypolimnetic waters. Organic matter measures in sediment cores from Enol Lake emphasized an increase of TOC levels in the sediment during the recent decades (Ortiz *et al.,* 2016), supporting the idea of an increase in organic matter input to the lake due to human land uses in the basin area. The oxygen consumption is enhanced by the relative low volume of water in the hypolimnion and the

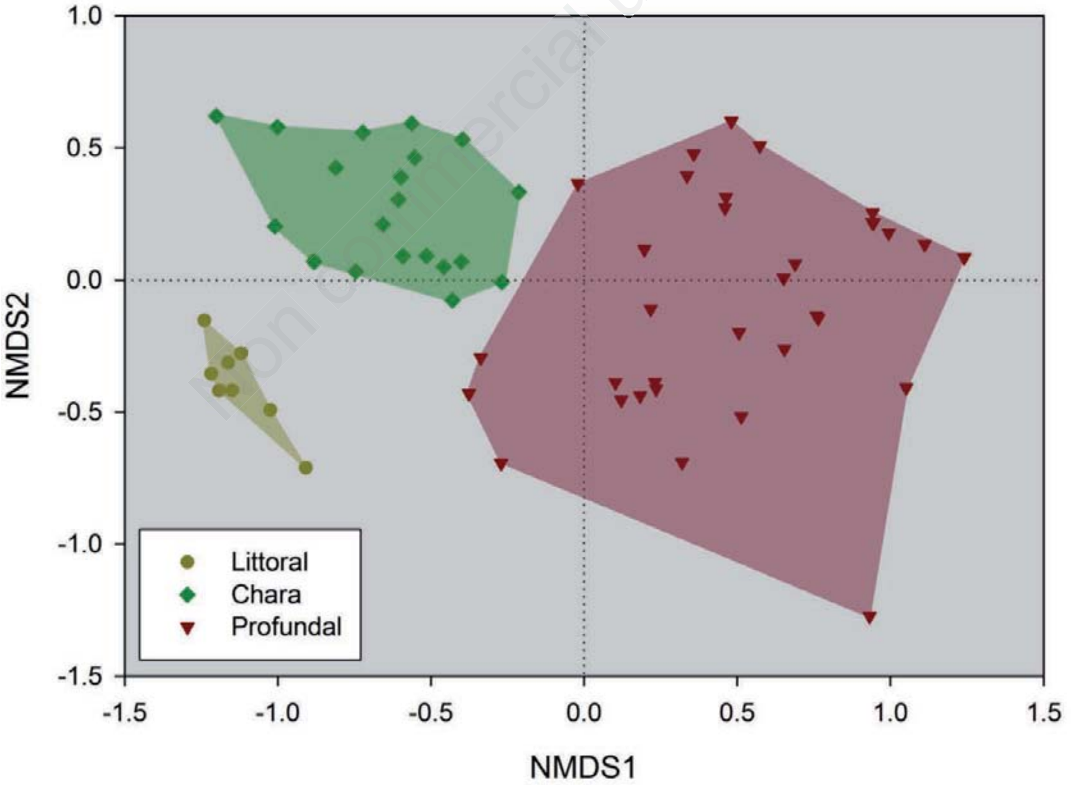

Fig. 4. NMDS analysis of the Chironomidae community of Enol Lake. The samples are separated into three groups: i) Littoral, ii) *Chara* and iii) Profundal. Stress: 0.12.

extended period of the thermocline presence, lasting from early July to early November (García-Criado and Martínez-Sanz, 2010). The absence of oxygen in the deep zone has been also noted in many mountain lakes but usually without such dominance of *C. plumosus,* as it is not commonly found in these type of lakes (Walker, 1993). In the Pyrenees (Capblancq and Laville, 1983) and in Laguna Cimera (Central Range) (Granados and Toro, 2000), its presence was associated with oxygen depletion during the ice-cover period. However, this can't explain the anoxic conditions found in Enol Lake, since the stratification period caused by ice-cover is very short if present. Thus, Enol Lake might constitute a good example of what could occur in mountain lakes if human activities occurring in the lake basin are not properly managed. This is a hypothesis that should be further tested since other factors (*e.g.*, water temperature increase) could play an important role in oxygen depletion.

The presence of *Chara* sp. beds was mainly responsible for explaining changes in the chironomid community of the littoral and sublittoral zones. *P. bituberculatus*, *E. albipennis, E. pagana* and *C. lobata* were more abundant in *Chara* sp. beds, whereas *C. atridorsum* was present in littoral areas devoid of Characeae, similar to the results found by Van Den Berg *et al.* (1997). Since the littoral and *Chara*-dominant zones were not different in terms of key environmental variables (*e.g.*, oxygen contents), our results suggest that the presence of *Chara* sp. was the key driver of Chironomidae assemblages in the littoral and sublittoral zones (*i.e.*, the species commonly associated with macrophytes increased their dominance when *Chara* sp. was present). The importance of aquatic vegetation as a prime driver of Chironomidae biodiversity in lakes has been pointed out by several studies (Brodersen *et al.,* 2001; Langdon *et al.,* 2010; Cañedo-Argüelles and Rieradevall, 2011). Characeae density has also been reported to structure lake macroinvertebrate communities (Van Den Berg *et al.,* 1997; Van Den Berg *et al.,* 1998).

On the other hand, temperature has been widely acknowledged as one of the main drivers affecting chironomid community composition in high mountain lakes

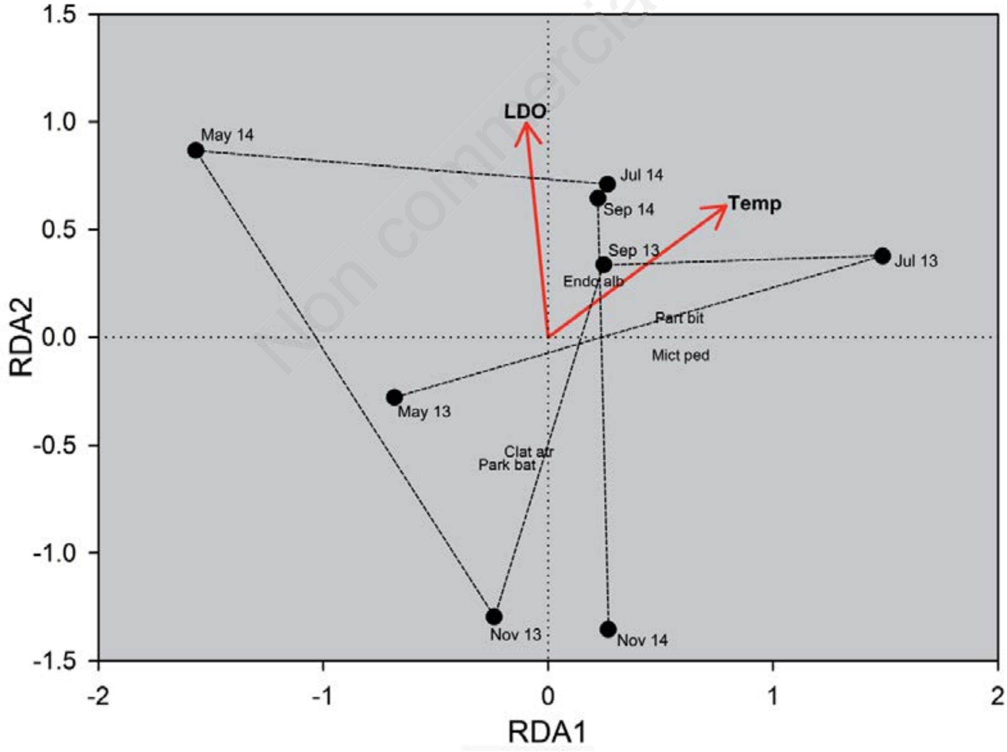

Fig. 5. Redundancy Analysis plots showing the relationships between all sampling campaigns and the statistically significant variables in the littoral zone.

(Heiri and Lotter, 2003). In Enol Lake, the temporal variability of the littoral zone assemblage was mainly driven by seasonality. This is not surprising, since seasonal changes in temperature, light incidence and food availability, are known to have a great influence on Chironomidae assemblages through changes in adult emergence (Armitage, 1995; Tokeshi, 1995) and habitat conditions, *e.g.*, higher temperature levels can enhance macrophyte production (highest Oxygen values) during the summer (Hering *et al.,* 2010). In our case, spring and autumn samples were mainly represented by *C. atridorsum* and *P. bathophila*, whereas *P.bituberculatus*, *M. pedellus* and *E. albipennis* dominated the summer samples, suggesting different emergence patterns of the littoral community.

CONCLUSIONS

The results obtained in this study are crucial to understand the temporal and spatial dynamics of Chironomidae assemblages in Enol Lake, constituting an essential step forward to improve the interpretation of the past changes of the lake by means of the subfossil chironomid community. Future studies should focus on the relationship between the living and the recent subfossil communities in order to understand head capsules' transportation and deposition processes. It would constitute the key point to understand how the living community is recorded in the sediments, which would allow a correct interpretation of downcore changes. Disentangling the importance of local, regional and global factors affecting the community is necessary to calibrate past changes and making future predictions. In this regard, we have shown that the littoral community is mainly driven by regional and global processes (*e.g.*, temperature), whereas the profundal assemblages are mainly driven by local factors, *i.e.*, sediment and organic matter discharge into the lake caused by pasture, leading to oxygen depletion in the hypolimnion. Our results also provide valuable insights for the managers to understand the current ecological status of Enol Lake and to evaluate which measures should be implemented to preserve or improve it. For example, the dominance of pollution tolerant species in the bottom of the lake suggests that its trophic status may be changing from oligotrophy to mesothrophy in the near future if no measures are taken to counteract the direct entrance of organic matter to the lake bottom caused by pasture in the lake basin.

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