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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

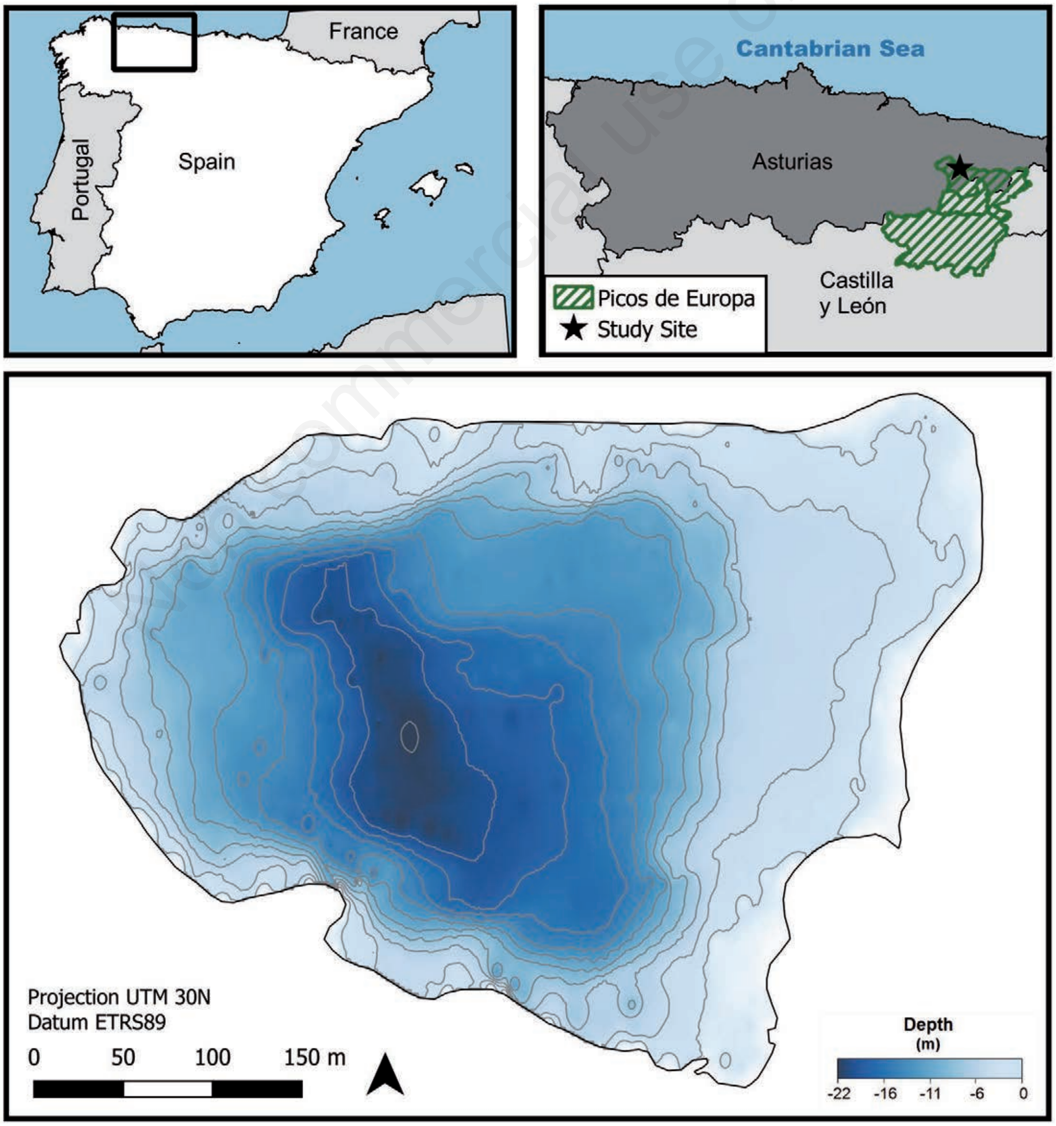

Fig. 1. Enol Lake location map. Bathymetry adapted from Rodríguez-García *et al.* (2016); with permission.

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.*, ρ≥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*

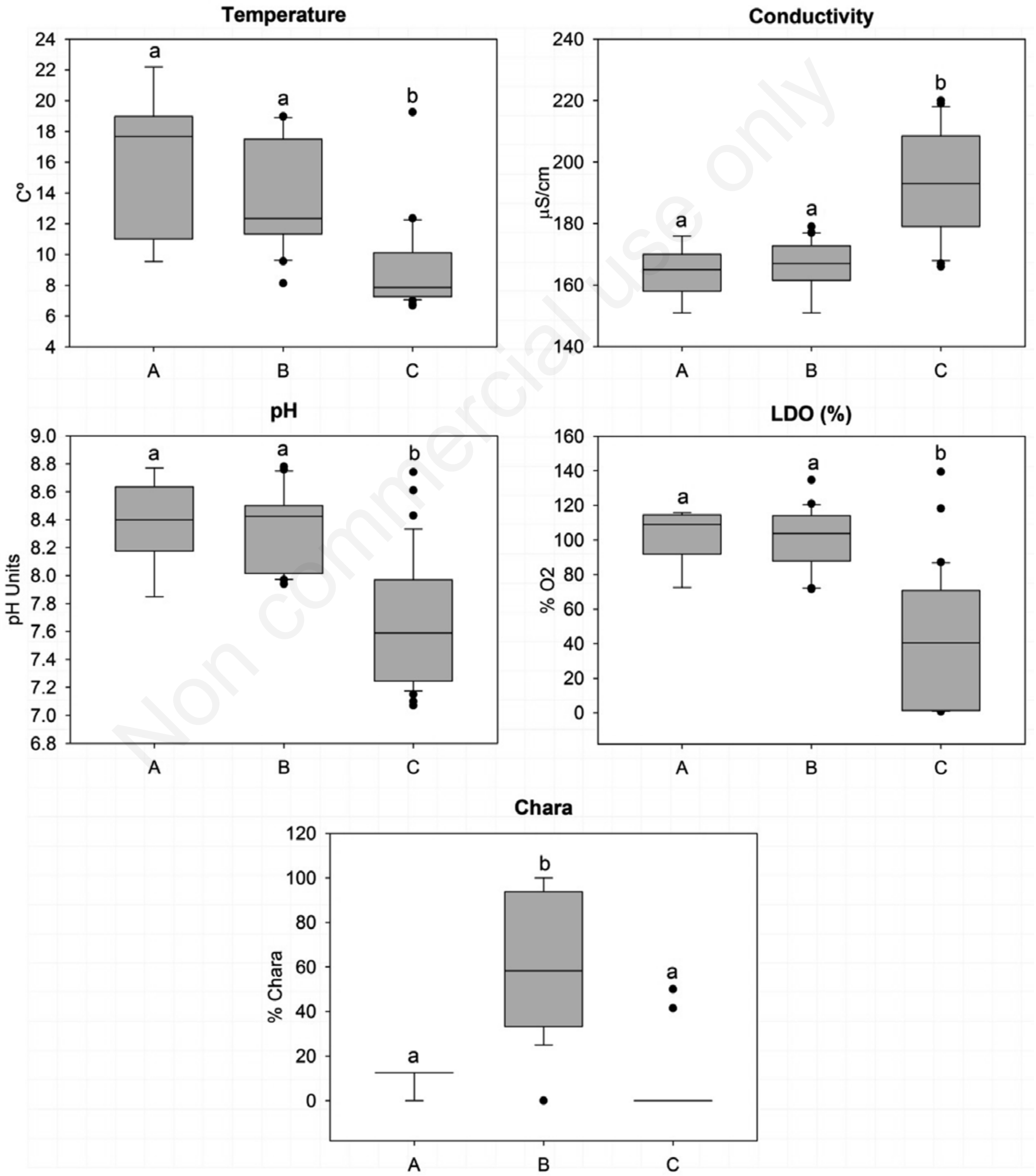

Fig. 2. Box plots representing temperature, conductivity, pH, Dissolved Oxygen and *Chara* sp. abundance according to the identified groups: a) Littoral, b) *Chara* and c) Profundal. Tukey Test results are specified above each box, with the same letter (a) representing non-significant differences between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

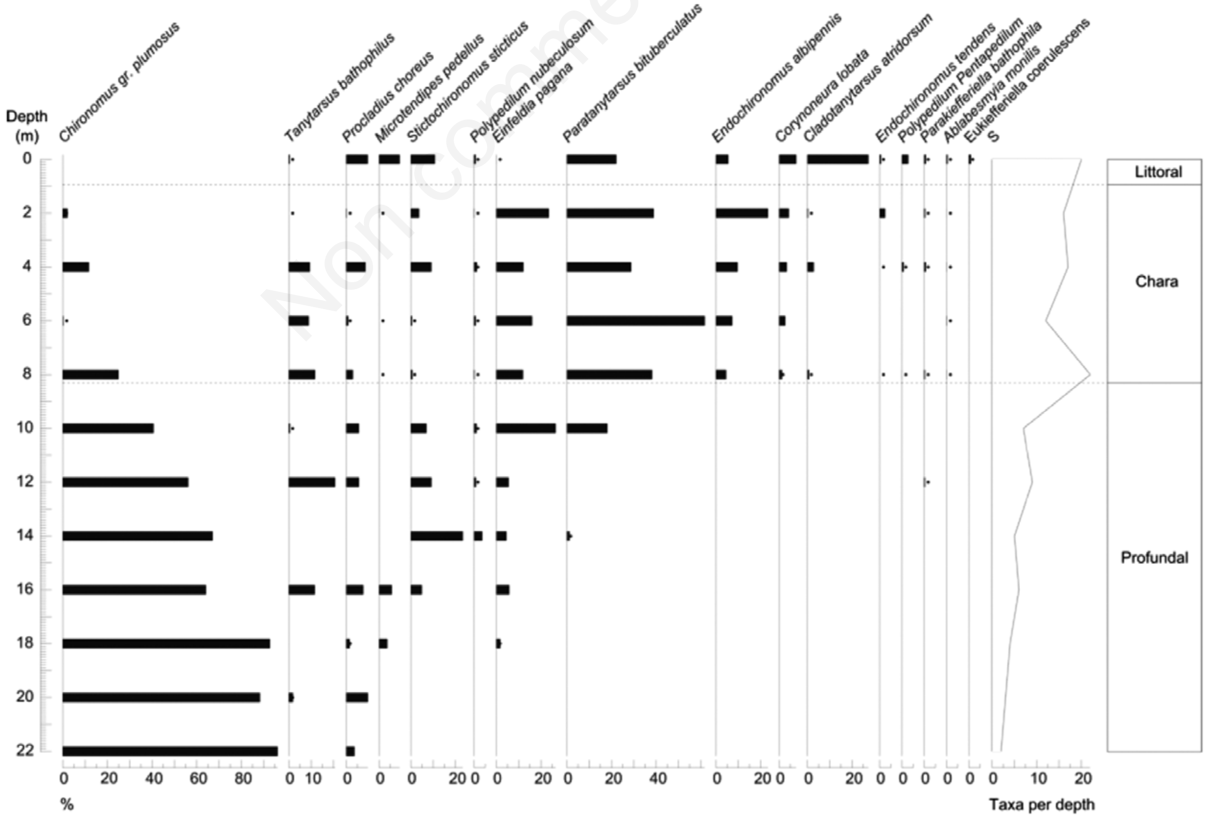

Fig. 3. Depth distribution of Chironomidae in Enol Lake. The percentage of each taxon for each depth is shown.

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

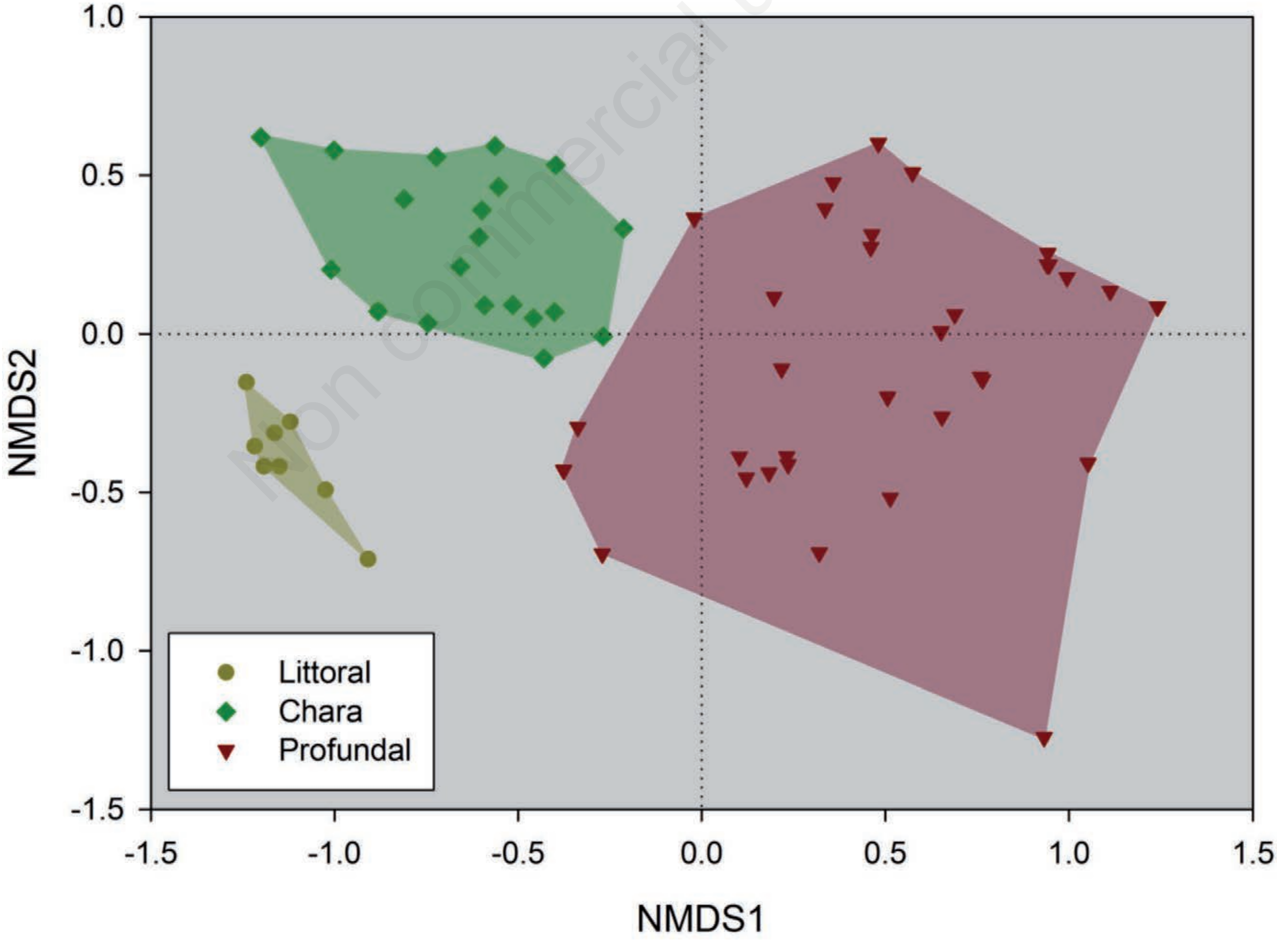

Fig. 4. NMDS analysis of the Chironomidae community of Enol Lake. The samples are separated into three groups: i) Littoral, ii) *Chara* and iii) Profundal. Stress: 0.12.

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

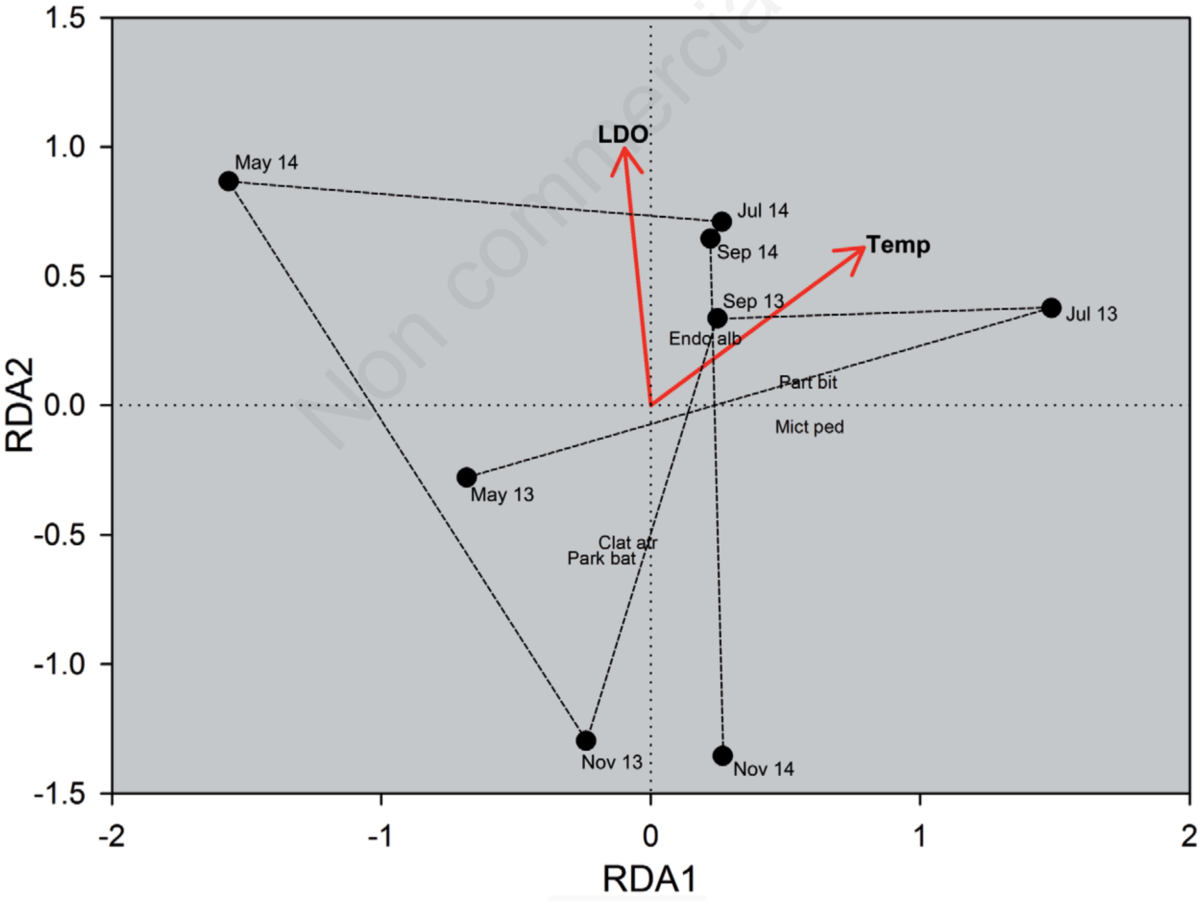

Fig. 5. Redundancy Analysis plots showing the relationships between all sampling campaigns and the statistically significant variables in the littoral zone.

(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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REFERENCES

Adrian R, Reilly C, Zagarese H, Baines S, Dag O, 2009. Lakes as sentinels of climate change. Limnol. Oceanogr. 54:2283-2297.

Armitage PD, 1995. Behaviour and ecology of adults, p. 194-224. In: P.D. Armitage, L.C.V. Pinder and P. S. Cranston (eds.), The Chironomidae: biology and ecology of non-biting midges. Chapman & Hall, London: 572 pp.

Armitage PD, Pinder LCV, Cranston PS, 1995. The Chironomidae: biology and ecology of non-biting midges. Chapman & Hall, London: 572 pp.

Battarbee RW, 2000. Palaeolimnological approaches to climate change, with special regard to the biological record. Quaternary Sci. Rev. 19:107-124.

Battarbee RW, 2005. Mountain lakes, pristine or polluted? Limnetica 24:1-8.

Bennett KD, 2009. Documentation for psimpoll 4.27 and pscomb 1.03: C programs for plotting and analyzing pollen data. http://chrono.qub.ac.uk. Unknown Publisher.

Brodersen KP, Lindegaard C, 1997. Significance of subfossile chironomid remains in classification of shallow lakes. Hydrobiologia 342:125-132.

Brodersen KP, Odgaard BV, Vestergaard O, Anderson NJ, 2001. Chironomid stratigraphy in the shallow and eutrophic Lake Søbygaard, Denmark: chironomid – macrophyte co-occurrence. Freshwater Biol. 46:253-267.

Brodersen KP, Quinlan R, 2006. Midges as palaeoindicators of lake productivity, eutrophication and hypolimnetic oxygen. Quaternary Sci. Rev. 25:1995-2012.

Brodin YW, 1986. The postglacial history of Lake Flarken, Southern Sweden, interpreted from subfossil insect remains. Int. Rev. Ges. Hydrobio. 71:371-432.

Brooks SJ, Langdon PG, Heiri O. 2007. The identification and use of Palaearctic Chironomidae larvae in Palaeoecology (QRA Technical Guide No. 10). Quaternary Research Association, London: 276 pp.

Cañedo-Argüelles M, Rieradevall M, 2011. Early succession of the macroinvertebrate community in a shallow lake: response to changes in the habitat condition. Limnologica 41:363-370.

Cañedo-Argüelles M, Boix D, Sánchez-Millaruelo N, Sala J, Caiola N, Nebra A, Rieradevall M, 2012. A rapid bioassessment tool for the evaluation of the water quality of transitional waters. Estuar. Coast. Shelf S. 111:129-138.

Capblancq J, Laville H, 1983. [Le lac de Port-Bielh (Pyrénées):

exemple de fonctionnement d'un écosysteme lacustre de haute montagne, p. 51-80]. [Article in French]. In: M. Lamotte and F. Bourliére (eds.), Problèmes d'écologie: structure et fonctionnement des écosystèmes limniques. Masson.

Catalan J, Camarero L, Felip M, Pla S, Ventura M, Buchaca T, Bartumeus F, De Mendoza G, Miró A, Casamayor EO, Medina-Sánchez JM, Bacardit M, Altuna M, Bartrons M, De Quijano DD, 2006. High mountain lakes: extreme habitats and witnesses of environmental changes. Limnetica 25:551-584.

Catalan J, Barbieri MG, Bartumeus F, Bitušík P, Botev I, Brancelj A, Cogalniceanu D, Manca M, Marchetto A, Ognjanova-Rumenova N, Pla S, Rieradevall M, Sorvari S, Štefková E, Stuchlík E, Ventura M, 2009a. Ecological thresholds in European alpine lakes. Freshwater Biol. 54:2494-2517.

Catalan J, Curtis CJ, Kernan M, 2009b. Remote European mountain lake ecosystems: regionalisation and ecological status. Freshwater Biol. 54:2419-2432.

Chen J, Zhang E, Brooks SJ, Huang X, Wang H, Liu J, Chen F, 2014. Relationships between chironomids and water depth in Bosten Lake, Xinjiang, northwest China. J. Paleolimnol. 51:313-323.

De Cáceres M, Legendre P, 2009. Associations between species and groups of sites: indices and statistical inference. Ecology 90:3566-3574.

De Mendoza G, Catalan J, 2010. Lake macroinvertebrates and the altitudinal environmental gradient in the Pyrenees. Hydrobiologia 648:51-72.

De Mendoza G, 2013. Lake macroinvertebrates and the altitudinal gradient in the Pyrenees. PhD thesis, Universitat de Barcelona: 559 pp.

Dufrêne M, Legendre P, 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol. Monogr. 67:345-366.

Eggermont H, Kennedy D, Hasiotis ST, Verschuren D, Cohen A, 2008. Distribution of living larval Chironomidae (Insecta: Diptera) along a depth transect at Kigoma Bay, Lake Tanganyika: implications for palaeoenvironmental reconstruction. Afr. Entomol. 16:162-184.

Eggermont H, Heiri O, 2012. The chironomid-temperature relationship: expression in nature and palaeoenvironmental implications. Biol. Rev. Biol. P. Cam. 87:430-456.

Engels S, Cwynar LC, Rees ABH, Shuman BN, 2012. Chironomid-based water depth reconstructions: an independent evaluation of site-specific and local inference models. J. Paleolimnol. 48:693-709.

Ferrington LC, 2008. Global diversity of non-biting midges (Chironomidae; Insecta-Diptera) in freshwater. Hydrobiologia 595:447-455.

Frossard V, Millet L, Verneaux V, Jenny JP, Arnaud F, Magny M, Poulenard J, Perga ME, 2013. Chironomid assemblages in cores from multiple water depths reflect oxygen-driven changes in a deep French lake over the last 150 years. J. Paleolimnol. 50:257-273.

García-Criado F, Martínez-Sanz C, 2010. [Estudio limnológico de los lagos Enol y La Ercina]. [Article in Spanish]. Departamento de Biodiversidad y Gestión Ambiental, Universidad de León, León: 73 pp.

Granados I, Toro M, 2000. Recent warming in a high mountain lake (Laguna Cimera, Central Spain) inferred by means of fossil chironomids. J. Limnol. 59:109-119.

Heinis F, Davids C, 1993. Factors governing the spatial and temporal distribution of chironomid larvae in the Maarsseveen lakes with special emphasis on the role of oxygen conditions. Neth. J. Aquat. Ecol. 27:21-34.

Heiri O, Lotter AF, 2003. 9000 years of chironomid assemblage dynamics in an Alpine lake : long-term trends , sensitivity to disturbance , and resilience of the fauna. J. Paleolimnol. 30:273-289.

Hering D, Haidekker A, Schmidt-Kloiber A, Barker T, Buisson L, Graf W, Grenouillet G, Lorenz A, Sandin L, Stendera S. 2010. Monitoring the responses of freshwater ecosystems to Climate Change, p. 84-118. In: M. Kernan, R.W. Battarbee and B. Moss (eds.), Climate change impacts on freshwater ecosystems. Wiley-Blackwell Publishing.

Korhola A, Olander H, Blom T, 2000. Cladoceran and chironomid assemblages as quantitative indicators of water depth in subarctic Fennoscandian lakes. J. Paleolimnol. 24:43-54.

Kureck A. 1980. Circadian eclosion rhythm in *Chironomus thummi*; ecological adjustment to different temperature levels and the role of temperature cycles, p. 73-80. In D.A. Murray (ed.), Chironomidae. Ecology, Systematics, Cytology and Physiology. Pergamon Press.

Langdon PG, Ruiz Z, Wynne S, Sayer CD, Davidson TA, 2010. Ecological influences on larval chironomid communities in shallow lakes: Implications for palaeolimnological interpretations. Freshwater Biol. 55:531-545.

Langton PH, Visser H. 2003. Chironomidae exuviae: a key to pupal exuviae of the West Palaearctic Region. World Biodiversity Database, CD-ROM Series. Expert Center for Taxonomic Identification, University of Amsterdam, Amsterdam.

Lindegaard C, 1992. Zoobenthos ecology of Thingvallavatn: vertical distribution, abundance, population dynamics and production. Oikos:257-304.

Little JL, Smol JP, 2001. A chironomid-based model for inferring late-summer hypolimnetic oxygen in southeastern Ontario lakes. J. Paleolimnol. 26:259-270.

Luoto TP, 2010. Hydrological change in lakes inferred from midge assemblages through use of an intralake calibration set. Ecol. Monogr. 80:303-329.

Marziali L, Rossaro B, 2013. Response of chironomid species (Diptera, Chironomidae) to water temperature: effects on species distribution in specific habitats. J. Entomol. Acarol. Res. 45:73-89.

Moreno A, López-Merino L, Leira M, Marco-Barba J, González-Sampériz P, Valero-Garcés BL, López-Sáez JA, Santos L, Mata P, Ito E, 2011. Revealing the last 13,500 years of environmental history from the multiproxy record of a mountain lake (Lago Enol, northern Iberian Peninsula). J. Paleolimnol. 46:327-349.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, Mcglinn D, Minchin PR, O'hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H. 2016. vegan: community ecology package. R package version 2.4-0.

Ortiz JE, Sánchez-Palencia Y, Torres T, Domingo L, Mata MP, Vegas J, Sánchez España J, Morellón M, Blanco L, 2016. Lipid biomarkers in Lake Enol (Asturias, Northern Spain): coupled natural and human induced environmental history. Org. Geochem. 92:70-83.

Pinder LCV, Reiss F, 1983. The larvae of Chironominae (Diptera, Chironomidae) of the Holarctic region, p. 293-435.

In: T. Wiederholm (ed.), Chironomidae of the Holarctic region: keys and diagnoses, Part 1: Larvae. Entomol. Scand. Suppl. 19.

Pinder LCV, 1986. Biology of freshwater Chironomidae, p. 1-23. In: T.E. Miller, F.J. Radovsky, and V.H. Rash (eds.), Annual review of entomology, Vol. 31. Annual Reviews Inc.

Prat N, Rieradevall M, 1995. Life-cycle and production of Chironomidae (Diptera) from Lake Banyoles (Ne Spain). Freshwater Biol. 33:511-524.

R Core Team, 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Real M, Prat N, 1992. Factors influencing the distribution of chironomids and oligochaetes in profundal areas of Spanish reservoirs. Neth. J. Aquat. Ecol. 26:405-410.

Real M, Rieradevall M, Prat N, 2000. Chironomus species (Diptera: Chironomidae) in the profundal benthos of Spanish reservoirs and lakes: factors affecting distribution patterns. Freshwater Biol. 43:1-18.

Rice, EW, Baird, AD, Eaton AD, Clesceri LS, 2012. Standard Methods for the Examination of Water and Wastewater. American Public Health Association: 1496 pp.

Rieradevall M, Prat N. 1999. Chironomidae from high mountain lakes in Spain and Portugal, p. 605-613. In: O. Hoffrichter (ed.) Late 20th century research on Chironomidae: an anthology from the 13th International Symposium on Chironomidae. Shaker Verlag.

Rieradevall M, Bonada N, Prat N, 1999. Substrate and depth preferences of macroinvertebrates along a transect in a Pyrenean high mountain lake (Lake Redó, NE Spain). Limnetica 17:127-134.

Rieradevall M, Brooks SJ, 2001. An identification guide to subfossil Tanypodinae larvae (Insecta: Diptera: Chironomidae) based on cephalic setation. J. Paleolimnol. 25:81-99.

Rodríguez-García J, Vegas J, López-Vicente M, Mata MP, Morellón M, Navas A, Salazar Á, Sánchez-España J, 2016. [Lake Enol (Asturias, NW Spain): origin, evolution and geomorphological dynamics, p. 151-158]. [Article in Spanish]. In: J.J. Durán, M. Montes, A. Robador, A. Salazar (eds.), Comprendiendo el relieve: del pasado al futuro. Instituto Geológico y Minero de España.

Rodríguez Castañón AA, 1996. [La producción de vacuno con rebaños de Asturiana de la Montaña: ganadería extensiva en la Cordillera Cantábrica].[Article in Spanish]. Agricultura 764:214-217.

Sæther OA, 2000. Zoogeographical patterns in Chironomidae (Diptera). Int. Ver. The. 27:290-302.

Sæther OA, Spies M, 2013. Fauna Europaea: Chironomidae. In: Beuk P, Pape T (Eds.), Fauna Europaea: Diptera. Fauna Europaea version 2.6, http://www.fauna-eu.org

Schindler DW, 2009. Lakes as sentinels and integrators for the effects of climate change on watersheds, airsheds, and landscapes. Limnol. Oceanogr. 54:2349-2358.

Schnell ØA, Rieradevall M, Granados I, Hanssen O, 1999. A chironomid taxa coding system for use in ecological and palaeoecological databases. Project Manual, Annex B. NIVA Report SNO:3710-3797.

Tokeshi M. 1995. Life cycles and population dynamics, p. 225-268. In P.D. Armitage, P.S. Cranston and L.C.V. Pinder [eds.], The Chironomidae: biology and ecology of non-biting midges. Chapman & Hall, London: 572 pp.

Toro M, Granados I, Robles S, Montes C, 2006. High mountain lakes of the Central Range (Iberian Peninsula): Regional limnology & environmental changes. Limnetica 25:217-252.

Van Den Berg MS Coops H, Noordhuis R, Vanschie J, Simons J, 1997. Macroinvertebrate communities in relation to submerged vegetation in two Chara-dominated lakes. Hydrobiologia 342:143-150.

Van Den Berg MS, Scheffer M, Coops H, Simons J, 1998. The role of Characean algae in the management of eutrophic shallow lakes. J. Phycol. 34:750-756.

Velasco JL, Araujo R, Álvarez M, Colomer M, Baltanás Á, 1999. [Aportación al conocimiento limnológico de ocho lagos y lagunas de Asturias (España)]. [Article in Spanish]. Boletín de la Real Sociedad Española de Historia Natural. Sección Biológica. 95 (1-2):181-191.

Vermaire JC, Greffard MH, Saulnier-Talbot É, Gregory-Eaves I, 2013. Changes in submerged macrophyte abundance altered diatom and chironomid assemblages in a shallow lake. J. Paleolimnol. 50:447-456.

Walker IR, 1993. Paleolimnological biomonitoring using freshwater benthic macroinvertebrates, p. 306-343. In D.M. Rosenberg and V.H. Resh (eds.), Freshwater biomonitoring and benthic macroinvertebrates. Chapman & Hall.

Walker IR, 2001. Midges: Chironomidae and related diptera, p. 43-66. In J.P. Smol, H.J.B. Birks and W.M. Last (eds.), Tracking environmental change using lake sediments, Volume 4: Zoological Indicators. Kluwer Academic Publishers.

Wiederholm T, 1983. Chironomidae of the Holarctic region: keys and diagnoses. Part 1: Larvae. Entomol. Scand. Suppl. 19: 457 pp.