Ecosystem responses to climate-related changes in a Mediterranean alpine environment over the last ~180 years

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ABSTRACT

The effect of recent climatic warming is significant in the Mediterranean region, especially in high mountain areas. This study uses multiple sedimentary proxies from Río Seco Lake, a remote alpine lake in the Sierra Nevada, southeastern Spain, to reconstruct recent environmental and ecological changes in the lake and catchment. Two main climatic periods can be distinguished during the past 180 years: Period One (1820- ~1920s) characterized by colder and wetter conditions than the more recent Period Two (~1920s to the present), characterized by warmer and drier conditions. Independent proxies such as subfossil chironomid assemblages, *n*-alkane indices, pollen data and/or spectrally-inferred chlorophyll-*a* concentrations indicate a longer ice-cover period, colder water temperature and more pronounced accumulation of snow in the catchment during Period One than in Period Two, likely producing water stress for catchment plant growth because of the low rate of ice melting in Period One. As temperatures increase and precipitation decreases from the 1920s onwards, a wider development of wetland plants observed, which is associated with the longer warm season that contributed to snow and ice melting in the catchment. This continuing temperature rise and precipitation decrease over the past 60-years by ~0.24 °C per decade and –0.92 mm/yr, respectively, lead to an important increase in chlorophyll-*a* and changes in lake biotic assemblages. Major chironomid community structure changes to warmer water taxa were recorded, resulting in a 2 ºC increase in mean July air temperature inferred by chironomids from ~1950 onwards. An inferred increase in primary production for the past few decades is consistent with higher temperatures, whilst wider development of wetland plants is associated to longer warm season. The coherence between independent environmental proxies, each associated with distinct mechanistic linkages to climatic shifts, strengthens our interpretations of a recent warming trend and an intensification of summer drought in this high mountain area leading to distinct changes in the lake and its catchment. The impact of this climate change on the summits of Sierra Nevada and its influence transcends its

geographical limits because these systems provide ecosystem services to a vast area.

Keywords: alpine lakes, Sierra Nevada, warming, chironomids, *n*-alkanes, chlorophyll-*a*, primary production

INTRODUCTION

Over the last 150 years, the global average air temperature at the Earth's surface has increased by ~0.8 ºC, while in the Northern Hemisphere the last 30-year period was the warmest period on record (IPCC 2013). High mountain areas are among the most sensitive to anthropogenic climate change and are experiencing some of the highest rates of warming due to the elevation-dependent warming (EDW), i.e. the rate of warming is amplified with altitude (Pepin and others 2015). The Mediterranean region is considered to be the largest "climate change hot-spot" in the world (Giorgi 2006). In particular, Mediterranean high mountain ecosystems have been identified as especially susceptible to global warming (Lionello, 2012; Pauli and others, 2012). This susceptibility is partially associated to the increased risk of summer drought in this region, caused by the rise in average summer air temperature and the reduction in annual rainfall (Nogués-Bravo and others, 2012).

Remote areas, referred as unpopulated high altitude regions above the tree line as well as high latitude areas, are excellent sites to study climate change effects because the climate signal is not as strongly obscured by other human impacts as in more populated areas (Battarbee and others 2002) and because they are sensitive to both natural and anthropogenic factors (Pauli and others 1996; Adrian and others, 2009; Smol, 2008).High mountain ecosystems are strongly influenced by physical conditions and strongly limited by nutrients, exhibiting steep ecological gradients and narrow ecotonal boundaries. Therefore, changes in environmental conditions (e.g. temperature patterns, ice-free period duration, water thermal regime, habitat and water availability) are expected to have great effects in them (Beniston 2003; Rühland and others 2015).

One way to assess the pressures influencing these remote sites is through the study of lake sediments, which are excellent archives of long-term environmental changes and allow environmental and ecosystem conditions to be reconstructed from limnological, ecological and geochemical lake-sediment proxies (Smol 2008). Ecosystem responses to warming are complex and show many direct and indirect interactions, with numerous climate-related processes affecting to different biotic and abiotic parameters. The analysis of independent proxies allows for the tracking of processes within lakes and their catchment. Since individual proxies can have different mechanistic links that determine their response to external stressors, studies based on multiple proxies offer a holistic approach to interpreting past lake and catchment-related changes.

The Sierra Nevada of southernmost Spain is a protected high-mountain area situated where alpine conditions and the influence of Mediterranean climate coexists. It is one of the most important biodiversity hotspots in Europe and plant species loss in its summits attributed to climate change in the last decades has been reported (Pauli and others 2012). This Mediterranean mountain range has shown a rapid response to the recent warming with the disappearance of permanent ice from the highest north-facing cirques (Oliva and others 2016). A trend in declining mean annual rainfall (Ruiz-Sinoga and others 2011) and a reduction of snow and ice cover since the 1960s (Pérez-Palazón and others 2015) has become more pronounced since the onset of the 21st century (Bonet and others 2016). In addition, climate models project an ongoing warming trend in this region for the end of the 21st century (Pérez-Luque and others 2016).

With this background, alpine ecosystems in the Sierra Nevada have become the focus of several paleoecological research projects over the Holocene in order to understand their post-glacial environmental responses. Most of the wetlands and peatlands in the Sierra Nevada developed in depressions carved during glacial times. Actually, the studied site, Río Seco Lake, bears the longest sedimentary record in Sierra Nevada, registering the Pleistocene-Holocene transition (between ~11000-12000 cal yr BP) (Anderson and others 2011). All the paleoenvironmental studies in Sierra Nevada alpine wetlands agree with the Holocene climatic evolution of the western Mediterranean region, with an humid early-middle Holocene that changes towards more arid conditions in the middle-late Holocene. Saharan dust inputs in the Sierra Nevada after 6000 cal yr BP affected alpine aquatic primary production (Jiménez-Espejo and others 2014; García-Alix and others 2017), as well as local vegetation and landscape (Anderson and others 2011; Jiménez-Moreno and Anderson 2012; García-Alix and others 2017). Most of the available long-term studies are not available at high resolution for the last hundred years, more specifically for the period after the end of the Little Ice Age, when the current environmental conditions of Sierra Nevada were established (García-Alix and others 2017; Oliva and others 2018). Some short-term surveys (~150 years) based on biological proxies, such as cladocerans, diatoms and sedimentary algal pigments (Jiménez and others 2015; Pérez-Martínez 2016; Jiménez and others 2018), have shown significant response of biotic assemblages to direct and indirect effects of temperature increases at the turn of 20th century, and especially over the past ~50 years. As yet, detailed comparisons of the communal response of chironomids to climate variability have not been conducted in the Sierra Nevada. Paleolimnological studies combining chironomids with organic geochemical indices may further our understanding of climate and landscape processes in this alpine ecosystem.

This study aims to provide a high-resolution multi-proxy reconstruction of recent palaeoenvironmental conditions from an alpine Mediterranean environment of the Sierra Nevada for the first time, helping to understand past changes and constrain future environmental scenarios. The aim is to put effects of recent climate changes in the context of a record extending back over the last 180 years to assess the extent that lake and catchment-related processes are linked to direct and indirect climate-driven changes. To accomplish this, we use a combination of stratigraphic records of sub-fossil chironomid assemblages, leaf wax biomarkers (*n*-alkanes), spectrally-inferred chlorophyll-*a* concentrations, pollen, cladocera, diatoms, organic matter content, organic matter C/N ratio and organic carbon isotopic composition (Online Appendix 3).

MATERIALS AND METHODS

Study site

Sierra Nevada (SE Spain, maximum altitude 3482 masl) is the highest mountain range of the Southern Europe. Sierra Nevada summits experience a high mountain Mediterranean semi-arid climate characterized by a warm and dry season (from ~June to October). The meteorological station at the summit (2507 masl) reports a mean annual temperature of 3.9 ºC and total precipitation of 693 mm, with 80% occurring as snow between October and April (Worldwide Bioclimatic Classification System, 1996-2018). There are ~50 small lakes of glacial origin and many alpine meadows around lakes, streams and depressions. These high-mountain meadow ecosystems represents a small area of the mountain range but have a high rate of plant endemicity and host many threatened taxa (Pérez-Luque and others 2015).

Río Seco (RS) Lake (37º 03’N, 3º 20’W) is a small, low primary production and shallow lake of glacial origin located above tree line at 3020 masl in the Sierra Nevada (southern Spain) (Fig. 1). The catchment bedrock is siliceous and largely comprised of mica-schist with graphite and/or feldspar. The soil is poorly developed and does not support agriculture or forestry. The catchment area is partially covered (~15%) by alpine meadows, consisting primarily of sedges (Cyperaceae) and grasses (Poaceae). The lake has no clearly differentiated littoral zone, but its shoreline is covered by bryophytes. With the exception of the meadows, most of the catchment vegetation consists of scarce xerophytic shrubland (see Anderson and others 2011).

The lake has diffuse inflows that provide water to the basin, and a small outlet. The inflow and outflow of water can disappear as the ice-free season progresses. The lake is ice covered from around October-November until June-July with a large interannual variability. Further details of physicochemical and biological features are shown in Online Appendix 1. During the ice-free period, Secchi disk visibility exceeds the water depth, the lake is not thermally stratified, and the maximum temperature is 16–18 ºC. It is a fishless lake, with low plankton diversity. Among chironomids, the species *Psectrocladius limbatellus* and *Micropsectra radialis* (as *M. coracina*) have been recorded in RS Lake (Laville and Vílchez-Quero 1986).

There are a few signs of significant human activity in the area. RS Lake is relatively remote, with local human activity currently limited to infrequent sheep herding around surrounding meadows during summer months. A dirt road, constructed between 1964 and 1965 and lying upgradient from RS Lake, experiences only foot traffic since the establishment of the Sierra Nevada National Park in 1999. A mountain hut was situated close to the shoreline and operated for three decades (1967-1997). The demolition of the hut at 1997 produced a large amount of inorganic material, which clouded the lake and had a major effect on biota and geochemical variables (Jiménez and others 2015).

Sediment sampling, analyses and dating

A sediment core was collected from the deepest part of the lake in September 2008 using a slide-hammer gravity corer (Aquatic Research Instruments, Hope, Idaho, USA) with an inner core-tube diameter of 6.8 cm. The core (16 cm long), was extracted in a methacrylate cylinder and immediately wrapped in a dark bag to keep it protected from the light, sectioned into 0.5 cm slices using an extruder, and sealed in sterile Whirlpak® bags, which were stored and transported in a cold box to the laboratory. Subsamples were collected at each interval in the laboratory and kept in a cold (4 ºC) and dark room until analysis. The sediment was dated by gamma (^{210}Pb, ^{137}Cs, and ^{226}Ra) and alpha spectroscopy (^{210}Pb in deepest part of core) (Eakins and Morrison 1978) establishing a chronology for the past ~180 years. The dating and sedimentation rate were calculated by using the constant flux: constant sedimentation (cf:cs) model (Appleby and Oldfield 1983). The core was analyzed at the Center for Research, Innovation and Technology (CITIUS) of the University of Sevilla, Spain.

Instrumental climate data

We use MAAT Madrid (mean annual air temperature series from Madrid station) and AP San Fernando (annual precipitation series from San Fernando station) as representative of air temperature and precipitation tendencies of the larger region around the Sierra Nevada during the last 170 years throughout the analyses. Geographical distance between Madrid and San Fernando climate stations from RS Lake are 376 and 262 km, respectively, while altitude differences are 2356 and 2992 m, respectively (Online Appendix 2).

Sedimentary proxy record

Sedimentary chlorophyll-*a* was inferred by visible reflectance spectroscopy using a FOSS NIRSystems Model 6500 series Rapid Content Analyzer (Tidestone Technologies, Inc.) to measure spectral reflectance of sediments that had been freeze-dried and sieved through a 125 µm-mesh, following the methods described by Michelutti and others (2005). The chlorophyll-*a* concentration includes native chlorophyll-*a*, as well as all chlorophyll isomers and its major derivatives (pheophytin *a* and pheophorbide *a*), and therefore accounts for the major diagenetic products (Michelutti and Smol 2016).

Loss on ignition (LOI) was measured to calculate the organic matter and carbonate content in the sediments (Heiri and others 2001). LOI was assessed sequentially on all core intervals (every 0.5 cm) using a muffle furnace. Samples were dried in an oven at 105 ºC for 24 h and weighed. The content of the organic and carbonate matter was analysed by incinerating the samples at 550 ºC for 4 h and at 900 ºC for 2 h, respectively (see detailed methods in Dean 1974 and Heiri and others 2001).

Total and inorganic carbon, and nitrogen content were analyzed with a CARLO ERBA EA 1108 CHNSO Elemental Analyzer system. The organic fraction was determined as the difference between the total and the inorganic carbon fraction (Meyers and Teranes 2001). The carbon/nitrogen (C/N) ratio was calculated from the mass data and expressed as atomic ratio.

Prior to the carbon isotope analysis from the bulk sediment organic matter ($\delta^{13}C_{org}$), 1 g of freeze-dried sediment was extracted by drying the samples (50 ºC) for 24h. The carbonate fraction was then removed by addition of 10% HCl to the solution. The C isotopic composition ($^{13}C/^{12}C$) of acid-treated samples was analyzed using a mass multicollector spectrometer (Isoprime; GV Instruments) equipped with a EuroVector elemental analyzer (mod. Euro EA 3000) and continuous flow inlet. The results are expressed as $\delta^{13}C_{org}$ in the conventional delta (δ) notation versus Vienna PeeDee Belemnite (V-PDB). Reproducibility measured for working standards during each run was better than ± 0.15 ‰.

The total lipid extract (TLE) from 32 freeze-dried sediment samples was extracted with a Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor system using 9:1 DCM:methanol. The obtained TLE was separated in neutral and acid fractions by aminopropyl-silica gel chromatography using 1:1 DCM:isopropanol and ether with 4% acetic acid, respectively. Afterwards, the *n*-alkanes were obtained by eluting the neutral fraction with hexane through a 230-400 mesh/35-70 micron silica-gel chromatographic column and analyzed using a GC-FID (Shimadzu 2010) and a GC-MS (Shimadzu QP2010-Plus Mass Spectrometer interfaced with a Shimadzu 2010 GC). To check the reproducibility of the measurements and to quantify the *n*-alkane content, a mixture of *n*-alkanes (C_{16}, C_{18}, C_{19}, C_{20}, C_{23}, C_{25}, C_{26}, C_{28}, C_{30}, C_{32}, and C_{37}) was measured every five samples. The measurement error was lower than 1.5%.

For fossil pollen, a modified Faegri and Iversen (1989) procedure was followed using 1 cm^3 of sediment. Pre-treatment included $(NaPO_3)_6$ to deflocculate clays and the addition of *Lycopodium* spores for calculation of pollen concentration. Sediments were suspended in $Na_4P_2O_7$ and sieved, then treated with HCl, HF and acetolysis solution. Samples were stained and suspended in silicone oil and identified at 400-1,000x to their lowest taxonomic level – mostly genus, sometimes family or other grouping – using a light microscope. For more details on the methodology see Anderson and others (2011).

For chironomid analysis, samples (~0.3 g dry weight) for each analysed interval of sediment were immersed in 10% KOH for 2-3 hours and subsequently sieved through a 100 µm fraction. The head capsules were sorted from other sieve residue under a dissection microscope. Chironomids were prepared in Eurapal mounting medium and identified at 100-400x magnification with a compound microscope. The minimum count threshold was 40 (range 43-83.5), except for the uppermost sample, which only consisted of 32 head capsules. Taxonomy mainly followed Oliver and Roussel (1983) and Brooks and others (2007). Comparatively, fewer intervals were counted for chironomids (10 samples) than for the other proxies (32 samples). Chironomid samples were analyzed every 2 cm from 0 to 16 cm.

Cladoceran and diatom assemblages from the sediment core of RS Lake were previously analyzed and published in Pérez-Martínez (2016) and Jiménez and others (2018). In this study we use the PCA axis 1 sample scores of both assemblages to compare with the rest of the proxies.

Interpretation of selected sedimentary proxies in RS Lake can be found in Online Appendix 3.

Data analyses

Principal component analysis (PCA) was used to summarize the dominant pattern of assemblage variability in chironomid, cladocera and diatom assemblages, as detrended correspondence analysis (DCA) indicated relatively short lengths of the first two compositional gradients (1.43 and 0.73 standard deviation units on the DCA axis 1 and 2, respectively). Chironomid relative abundances were square root transformed prior to analyses to equalize variance among taxa. Ordinations were conducted using the vegan package (Oksanen and others 2015) for the R software environment (R Development Core Team, 2015). The annually resolved climate series (MAAT Madrid and AP San Fernando) were averaged over the period of accumulation for each dated interval, thereby integrating the instrumental data with the paleolimnological data (Sorvari and others 2002). The relationships between sedimentary proxies (downcore PCA axis 1 sample scores, organic geochemical proxies and sedimentary chlorophyll-*a* record) and changes in climatic series (MAAT Madrid and AP San Fernando) were then examined. STATISTICA v.7 (Statsoft) software was used to test the data normality and calculate Pearson correlations. The Kolmogorov-Smirnov test with Lilliefor's correction was performed to determine the normality of the data distribution. Pearson correlation coefficients were used to provide an indication of the strength of the relationships between parameters. For the sedimentary chlorophyll-*a* record, the uppermost sedimentary interval (0-0.5 cm) was excluded because it could not be reliably identified as exclusively representing sedimentary chlorophyll-*a* due to the presence of algal mat material. Lake sediment records can be affected by bioturbation and other factors which can to some extent smooth out short-term variability (e.g. between year variability). We therefore do not report *p* values for correlations between lake sediment records as well as between lake sediment records and instrumental data series, since statistical testing of correlation coefficients assumes statistical independence of the data points. However, all of the discussed relationships would have been statistically significant if tested using standard tests for statistical significance of *p* values.

A stratigraphically constrained cluster analysis was carried out in the R software environment (R Development Core Team 2015), using the Rioja package (Juggins, 2012) to identify the periods with homogeneous response of the different proxies. Stratigraphic zonation was done by a cluster analysis with a constrained incremental sum of squares (coniss method), using the chclust() function (method='coniss') of the Rioja package. The cluster analysis was applied using the combination of all biogeochemical proxies. To characterize the timing of largest change in MAAT Madrid and AP San Fernando series data, breakpoint analyses using a two-segment piecewise linear regression were applied to each series to identify where the slope changes (Toms and Lesperance 2003).

Zonation of the stratigraphic profiles of chironomid data was performed by a cluster analysis with a constrained incremental sum of squares (CONISS), square root transformation of data and chord distance as the dissimilarity coefficient, using Tiliagraph View (TGView) version 2.02 (Grimm 2004) and determining the number of significant zones by means of the broken stick model (Bennett 1996). Chironomid-inferred mean July air temperature (MJAT) reconstruction was performed using the program C2 (Juggins 2007) based on a 274-lake chironomid-temperature calibration dataset from Switzerland and Norway (Heiri and others 2011) and a temperature inference model (transfer function) developed from these data. The calibration dataset covers a mean July air temperature gradient from 4 to 18.4°C and a wide range of arctic, alpine, subalpine and temperate lowland lakes. The applied transfer function was based on weighted averaging-partial least squares regression (WA-PLS; ter Braak and Juggins 1993; ter Braak and others 1993). The model featured a cross-validated r^2 of 0.84 and a root mean square error of prediction (RMSEP) of 1.55°C. RMSEP, r^2, and sample-specific errors of prediction (eSEPs) were calculated based on 9999 bootstrapping cycles in C2. Chironomid assemblage percentage data were square root transformed before calculation of WA-PLS and distance metrics. To assess the trajectory of RS chironomid assemblages relative to summer temperature, we compare the RS data to mountain lakes in the Swiss Alps, see details in Online Appendix 4 (Fig S2).

RESULTS

Chronology

The ^{210}Pb dating of the sediment core from RS Lake shows sedimentation rates of 0.9-1.1 mm year^{-1} from 0 to 6 cm depth (from ca. 2008 to 1948) and 0.7-0.8 mm year^{-1} from 6 cm to 15.5 cm depth (from ca. 1948 to 1821) (Jiménez and others 2018). For further details see Online Appendix 5.

Instrumental climate data

Over the 143-year record, mean annual air temperature (MAAT) from the Madrid climate station indicates a warming trend beginning at the turn of the 20th century (Fig. 2 and Fig. S1 in Online Appendix 2). Total annual precipitation (AP) from the San Fernando climate station indicates that over the 172-year record, the second half of the 19th century was wet, reaching a maximum around 1860-70 and then decreasing from the late 19th century to the present, interrupted only by positive anomalies in the 1960s (Fig. 2 and Fig. S1 in Online Appendix 2). The last 40 years of the AP San Fernando record exhibit persistent low precipitation values that were particularly low from 1985-1995.

According to main shifts in climatic data, consistent with noticeable changes in direction, magnitude and timing in the major paleoenvironmental proxies in the present study, two distinct climatic periods are indicated for the climate data: Period One from 1820 to ~1920s, a period of relatively high precipitation and low and decreasing temperature; and Period Two from ~1920s to the present, a warmer and drier period, particularly since the mid-70s (Fig. 2).

Sedimentary proxy record

A similar trend is observed in % organic matter content (estimated by Loss on ignition; LOI_{550}) and sedimentary chlorophyll-*a* through the entire profile (Pearson r=0.52, n=32). The two variables show a progressive decrease from 1820 to ~1920s followed by an increase to the present, except for the abrupt decrease of LOI_{550} in the 1990s (Fig. 2). The trend of sedimentary chlorophyll-*a* is parallel to MAAT Madrid for the entire record (Pearson r=0.76, n=24), and also similar between LOI_{550} and MAAT Madrid until the late 1980s (Pearson r=0.46, n=20). The abrupt decrease of LOI_{550} values from ~1990s is responsible for the decoupling of trends between LOI_{550} and sedimentary chlorophyll-*a* record and MAAT Madrid, and is associated with the demolition of the mountain hut in 1998, altering sediment composition as is also apparent in the ^{210}Pb activity profile (Jiménez and others 2015) (Fig. S3; Online Appendix 5).

From 1860 to the ~1920s, the C/N ratio shows the lowest values of the entire period (11.7±2.1, mean±SD), coincident with high values of AP San Fernando and low values of MAAT Madrid, while higher values are recorded from ~1920s to the present (15.6±2.6) coincident with a warmer and drier period. C/N ratio and $\delta^{13}C_{org}$ show opposite tendencies for almost all the record. C/N peaks in the mid-19th century (1860-1870s) and ~1920s, associated with $\delta^{13}C_{org}$ decreases, except for the last four decades (from mid-1970s to the present), a period in which the C/N ratio shows the highest persistent values and $\delta^{13}C_{org}$ exhibits a decreasing trend after maximum values (Fig. 2).

The RS Lake record shows a predominance of *n*-alkanes with odd carbon chains. The CPI ranges from 3.6 to 4.4 and P_{aq} values are higher than 0.28 for the whole period (Fig. 2). From 1820 to ~1920, P_{aq} shows the lowest and very homogeneous (~0.3) values, while ACL shows the highest values of the record. ACL values show an opposite trend than P_{aq} values (Pearson r=-0.97, n=32) for the entire record. The main difference between ACL and P_{aq} values is observed during the first period, between ~1850 and ~1880, with a small increase (0.32) of P_{aq} in the 1860s, agreeing with decreasing ACL values during this period (~1850-1880) and coincident with the highest persistent precipitation. CPI and P_{aq} do not show any correspondence before ~1915, but from ~1920s to the present CPI and P_{aq} have an opposite trend. The most important change in the *n*-alkane record is indicated by the maximum P_{aq} value (~0.48) and minimum ACL values (~28.30) recorded at ~1963, agreeing with increase in the AP San Fernando record, followed by a P_{aq} decrease and ACL increase after ~1970s (Fig. 2). This P_{aq} decrease is coeval with the drop of $\delta^{13}C_{org}$ and increase of the C/N ratio of bulk sediment around 1978. Just after ~1988, the increase in CPI and ACL values, as well as the decrease in P_{aq} agrees with the MAAT Madrid increase and AP

San Fernando decrease which reflects a severe and long drought period from the late 1980s to mid-1990 (Fig. 2).

In the pollen assemblages, the Cyperaceae/Poaceae (C/P) ratio shows low values (0.17±0.04) from 1820 to 1920s coincident with low values of P_{aq} and MAAT Madrid, together with high values of AP San Fernando. Higher values (0.27±0.1) are recorded from ~1920s to the present, agreeing with MAAT Madrid increase and AP San Fernando decrease. A similar trend is observed between C/P ratio and P_{aq} (Pearson r=0.41, n=31). In addition, the appearance of the green alga *Pediastrum* from ca. 1950 onwards is noticeable (Fig 2).

Major changes in aquatic organism groups are observed for the last 60 years preceded by a period of minor changes. A total number of 7 morphotypes of chironomids were identified. The taxa *Micropsectra radialis*-type and *Psectrocladius sordidellus*-type are abundant throughout the sedimentary intervals (Fig. 3). Based on cluster analysis, the most significant change is observed around 1950s and consists in the new arrival of *Chironomus plumosus*-type, *Heterotrissocladius marcidus*-type and *Micropsectra insignilobus*-type (Fig. 3). One major significant zone boundary in the chironomid record ~1940-1950 was identified based on comparison with the broken-stick model, coincident with a major change of PCA axis 1 sample scores for chironomids (which explain 51% of the variance). The chironomid-based MJAT reconstruction suggests a trend of increasing MJAT from ~1940-1950s onwards, presumably driven by increasing summer water temperature in RS from this period onwards (Fig. 3 and 4, and Fig. S2 in Online Appendix 4).

Cladoceran PCA axis 1 sample scores identified the greatest change at ~1990, while a significantly shift in diatom PCA axis 1 sample scores is observed from ~1960 onwards (Fig. 4). These changes consisted in changes in species relative abundance but also in appearance and disappearance of certain species. The taxon-specific cladoceran and diatom changes (not shown) started at the turn of the

20^{th} century (~1920s), but became especially striking in the last five decades (Pérez-Martínez and others 2012; Jiménez and others 2018). The first PCA axis explained 38% (cladocera) and 26% (diatoms) of the variance of the biological assemblage data. Cladoceran and diatom PCA axis 1 sample scores are consistent with major changes observed for MAAT Madrid (increasing trend) and AP San Fernando (decreasing trend).

Overall, major changes for geochemical proxies started after the ~1920s consistent with the rise in temperature and preceded by a period of minor changes; however the main changes in biological proxies seem to be delayed, and their response intensified after the ~1940-1950s onwards, consistent with recent intensification of warming.

DISCUSSION

The combination of analysis of C/N ratio, $\delta^{13}C_{org}$ values and *n*-alkanes indices from the sediments from the RS Lake core provides an opportunity to identify the main sources of organic matter in the study area. The values of C/N ratio (9.5 to 19.7), $\delta^{13}C_{org}$ (-23.63 to -20.57 ‰) and CPI (~3.6 to 4.5) indicate the deposition of a mixed source of algal-derived and terrestrial organic matter for the entire record. This is expected for this system due to the small lake size (0.42 ha) and catchment area (9.9 ha), together with its partial coverage (~15%) by alpine meadows. It is worth noting that the mixed sources that led to the observed $\delta^{13}C_{org}$ values in RS Lake are also in accordance with a water-column study of this system (Pulido-Villena and others 2005), and with long-term and modern surveys of plants and lacustrine algae in other alpine lakes of the Sierra Nevada (García-Alix and others 2012; Jiménez-Moreno and others 2013).

Three factors suggest a predominance of wet environments in the catchment basin, in agreement with the previously discussed C/N ratio values. These include the low CPI values (<4.5), which show a dominance of *n*-alkanes with odd carbon chains indicating vascular plant input (Bush and McInerney

2013) and algal contributions (Han and Calvin 1969) and the P_{aq} values, which exceed 0.28 pointing to an emergent aquatic plant predominance (Ficken and others 2000) for the entire record. In RS Lake there are no emergent aquatic plants, but this P_{aq} value likely indicates bryophyte and others semi-aquatic vegetation, that formed the alpine meadows and surrounded the catchment (García-Alix and others 2017). However, variations in the different proxies indicate differences in the degree of wet conditions in the RS catchment basin over the study period (discussed below).

Related to biological proxies, the observed low taxonomic richness in chironomids is consistent with an oligotrophic alpine lake, and is similar to conditions found in other alpine lakes (Heiri and others 2011). The chironomid assemblages were heavily dominated by the two taxa: *Psectrocladius sordidellus*-type (~77%), which often dominates in alpine lakes (Heiri and Lotter 2010) and *Micropsectra radialis* (~15%), which in small lakes is restricted to cold arctic and alpine habitats (Heiri and others 2011). However, the arrival of chironomid groups and the most marked changes in chironomid community composition are observed for the last 60 years, coincident with the main observed changes in cladoceran and diatom assemblages. The similar timing in changes indicates a parallel response of the lacustrine biota to the effects related to climate change. Most of the chironomid taxa are typical for cold, nutrient poor and oxygen rich environments (Lotter and others 1998; Heiri and others 2011). However, *Chironomus plumosus*-type is typically found in more nutrient rich, warmer and oxygen poor environments (Lotter and others 1998; Heiri and others 2011). The appearance of this taxon agrees with higher nutrient availability and higher oxygen depletion (e.g. in the sediments) in RS Lake from ~1960 onwards.

The general environmental trends deduced from RS Lake organic proxies follow the long-term late Holocene changes described in the same site and in neighbor alpine wetlands (Anderson and others 2011; Jiménez-Moreno and Anderson 2012; Jiménez-Moreno and others 2013; García-Alix and others

2017). The Period One, from 1820 to the 1920s, with colder and wetter conditions, registers the environmental response to the warming after the LIA conditions, and Period Two, from 1920s to the present, with drier and warmer conditions, records the response of RS Lake to the recent and ongoing climate change. The identification of these two different environmental periods agrees with an abrupt environmental change registered in other alpine peatlands in the region (Borreguil de la Virgen and Borreguil de la Caldera) that occurred at ~1920s (García-Alix and others, 2017). Furthermore, high-resolution cladoceran records from 6 lakes of the Sierra Nevada region, including RS Lake (Jiménez and others 2018), indicate that the onset cladoceran changes also occurred at the turn of the 20th century and intensified in the past 50 years. All these results indicate a regional-scale response to climate change.

Period One: *period between 1820 and ~1920s*

The paleolimnological changes observed in Period One may have been promoted by the seasonal character of the Mediterranean precipitation, mainly concentrated during winter, as well as a longer cold season in southern Iberia coeval with lower temperature than experienced during Period Two. Hence, because precipitation occurs mainly during the cold winter season, the combined effects of relatively high precipitation and decreasing air temperatures (Fig. S1; Online Appendix 2) during the Period One probably led to later seasonal lake ice-off period, colder water temperatures and larger accumulation of snow in the catchment basin, and as a consequence, the reduction of aquatic and/or terrestrial primary production, as can be observed by the gradual decrease of LOI_{550} and sedimentary chlorophyll-*a* in parallel with MAAT Madrid. However, despite the overall decrease of aquatic primary production during Period One, the C/N ratio indicates a higher contribution of algae to the bulk organic matter than in the most recent climatic period, which agrees with the homogeneous P_{aq} values (~0.3), which indicate there is a predominance of emergent aquatic plants (Ficken and others 2000). This is probably the consequence of a reduced catchment surface and growing season for wetland plants (lower C/N values) and a reduced water availability (lower P_{aq} values in Period One than in Period Two). This may have been caused by delayed ice and snow melting in the catchment basin and higher snow accumulation by the precipitation increase and the presumably low temperatures. Besides, any shortening of the ice-free period in the catchment may also hinder input of terrestrial organic matter into the lake.

The highest ACL and lowest C/P ratio values of the entire record also indicate less water availability (Fig. 2). The main difference in Period One between P_{aq} and ACL values is characterized by a small increase (0.32) of P_{aq} in the decade of 1860, concomitant with the ACL decrease (average of 28.93) from 1850 to 1880, indicating a preponderance of *n*-alkanes with lower chain length. This response of P_{aq} and ACL can be read as indicating relatively wetter environment compared to the rest of Period One, likely induced by the combination of persistent high precipitation coeval with milder temperature.

This scenario (cold and wet conditions) presumably fostered the maintenance of glacial and other perennial ice banks in the highest north-facing cirque of Sierra Nevada during the final periods of the Little Ice Age (LIA) (Oliva and Gómez-Ortiz 2012), which began to disappear around the ~1920s (Grunewald and Scheithauer 2010). Colder water conditions would explain the chironomid community composition in Period One (Fig. 3), with a high abundance of cold-tolerant taxa such as *M. radialis*-type and *P. sordidellus*-type, a very low diversity and the absence of warm-water chironomids. This is also supported by the dominance of the cladoceran species *Chydorus sphaericus*, which has been mainly associated with long ice cover period in the Sierra Nevada (Jiménez and others 2018). PCA axis 1 sample scores of cladocerans, diatoms and chironomids show minor changes during this period (Fig. 4).

Period Two: period from ~1920s to the present

Warmer and drier climate conditions during Period Two produced substantial changes in biological and organic geochemical proxies. The increasing values of the sedimentary chlorophyll-*a* and LOI_{550} may indicate a progressive increase of aquatic and terrestrial primary production probably associated with longer growing seasons and higher water temperatures with the onset of the 20th century rise in air temperature (Fig. 2). An increase in aquatic primary production in remote lakes by warming has been reported (Adrian and others 2009). Warming may cause a longer ice-free season which increases light availability and mean water temperature, while also increasing water lake residence time through reduced inflows and increasing evaporation but enhanced melting of snow and weathering (increasing lake solute inputs; Preston and others 2016). These processes may enhance biological production in lakes, and a longer growing season could also increase annual biomass accumulation (Fee and others 1992) in lakes and catchment. This effect may have been enhanced by atmospheric deposition of Saharan dust at these low-productive lakes. For example, the delivery of atmospheric P-rich Saharan dust during the last 50 years may partially explain the trends in the sedimentary chlorophyll-*a* record in RS Lake, a phenomenon that has been demonstrated in Sierra Nevada lakes (Morales-Baquero and others 2006; Jiménez and others 2018). Hence, it is likely that the combination of warmer temperatures, longer growing seasons and increased delivery of P-laden dust has resulted in notable increases in chlorophyll-*a* in RS Lake. These observations are consistent with the appearance of the green alga *Pediastrum* from ~1950 onwards and the chironomid *Chironomus plumosus*-type from ~1960 onwards, also agreeing with an increase of primary production (Lotter and others 1998; Weckström and others 2010).

The previous findings are consistent with the higher C/N values from the entire period indicating a higher contribution of vascular land plants to bulk organic matter. A longer warm season with increased temperatures probably enhanced snow and ice melting in the catchment basin and, as a consequence, the catchment surface and growing season for wetland plants. This is supported by the increasing values of P_{aq} after ~1921, reaching 0.38 at ~1928 simultaneous to the decreasing values of ACL. The maximum P_{aq} value (~0.48) and minimum ACL values (~28.30) are recorded by ~1963, agreeing with recorded periods of elevated precipitation (Fig. 2). Unlike conditions with higher precipitation and colder temperatures around ~1850 and the 1880s, climate during the 1960s shows high precipitation with higher temperatures, thereby enhancing the melting season and providing more net water availability. This combination of a longer growing season and greater water availability triggered the development of larger wetland areas, as shown by higher C/P ratio values. This is in concordance with Pérez-Palazón and others (2015) indicating a decreasing extent and persistence of the ice and snow covered area over the Sierra Nevada from the 1960s onwards.

Warmer conditions are likely responsible for the transition in cladocera, diatoms and chironomid assemblages from the 1940-1950s onwards, following the shifts in P_{aq} and ACL values after the 1920s. Assemblage shifts as a consequence of the rise of temperature in the first part of the 20th century have been observed in many others remote areas (Sorvari and others 2002; Rühland and others 2015). Over the last ~60 years the most notable changes in lacustrine biota are shown by the trend of PCA axis 1 scores coincident with major shift in MAAT Madrid and AP San Fernando (Fig. 4). Lacustrine biota apparently exhibited a delayed response to changes in air temperatures and precipitation, and significant responses to climate change are observed when the climatic shift intensified for the last ~60 years.

Temperature is particularly important in determining shifts in chironomid assemblage composition (Heiri and others 2003; Bigler and others 2006). Cluster and PCA analyses indicate that the most relevant changes were characterized by the reduction of cold-tolerant taxa *P. sordidellus*-type and *M. radialis*-type and the increase of taxa better adapted to warmer condition such as *C. plumosus*-type, *H. marcidus*-type, *M. insignilobus*-type in the uppermost section of the sediment core. The taxon *C. plumosus*-type includes a number of species, and is generally considered to be thermophilic and indicative of relatively warm lakes (Brooks and Heiri 2013), although it can also occur in lakes in the subalpine vegetation belt at low abundances (Heiri and Lotter 2010). Hence, the new arrival and increase of *C. plumosus*-type in RS Lake is probably, at least partially, related to water temperature rise and possibly promoted by the increase in in-lake nutrient availability discussed above. This represents the first occurrence of the genus *Chironomus* in alpine lakes of Sierra Nevada (Laville and Vílchez-Quero 1986; Real and others 2000), yet it does occur in subalpine lakes of Central Europe (Heiri and Lotter 2010). Similarly, *M. insignilobus*-type has its maximum abundances at lower altitudes in the Alps (Bigler and others 2006). Overall, the timing of appearance and major contribution to change of these two taxa in RS Lake suggest warmer summer water temperature, which is also reflected in a warming of chironomid-inferred mean July air temperatures by about 2 ºC from ~1950s onwards. This warming in climatic conditions is consistent with changes in cladoceran and diatom community composition at RS Lake (Pérez-Martínez and others 2012; Jiménez and others 2018). The similar timing and direction of changes in chironomid community composition as observed for cladoceran and diatom assemblages, coincident with changes in other Sierra Nevada lakes (Jiménez and others 2018), corroborate the hypothesis of climate-driven shifts in the ecological status of distinct trophic levels in these alpine lakes. These changes are also in good agreement with changes in aquatic community structure in others remote ecosystems (Rühland and others 2014), coinciding with recent warming.

Maximum values of C/N ratio and decreasing $\delta^{13}C_{org}$ values from mid-1970s to the end of the 80s are interpreted as a major vascular land plant contribution to bulk organic matter. There was a decrease in this time in the P_{aq} values (simultaneous with ACL values increase), and therefore apparently a decrease in the water availability in the catchment, agreeing with unprecedented high temperatures and a precipitation decrease. This suggests lesser water availability induced by greater evaporation rates, enhanced by higher frequency of intense summer droughts as a consequence of intensified warming in the Mediterranean area (Giorgi 2006). Even though this warming promoted a longer growing season and increased lake primary production (more *Pediastrum*, higher sedimentary chlorophyll-*a* and LOI_{550}), the C/N ratio suggests a higher vascular land plant contribution to the bulk organic matter.

After ~1988, CPI and ACL values sharply increase and P_{aq} decreases, which agrees with the extreme droughts of the early 1990s in the Mediterranean region, and is in concordance with the temperature rise and precipitation decrease. It suggest less water availability in the catchment likely affecting those meadow plant species with highest water requirements. These observations, together with the decrease of the C/N ratio values, point towards a relative decrease in terrestrial vegetation production in the catchment, probably related to the regional drought. By contrast, these conditions of increasing temperatures and less water availability in the catchment likely enhanced biological production in the lake by different processes such as longer ice-free season, higher mean water temperature and higher solute concentrations. Cladocera results strongly support this findings considering that the main shift within the sedimentary cladoceran assemblages occurred at the 90s in RS Lake (Jiménez and others 2018) and is coincident with the severe periods of drought during the late-1980s and 1990s in Southern Spain (Udelhoven and others 2009).

This short-core multiproxy study provides a valuable high-resolution record of paleoenvironmental and paleolimnological change for the last ~180 years. The climate-driven changes from Period One to Period Two – shorter duration of ice-cover period, higher summer water temperature and greater water availability for catchment plant growth – are responsible for the primary changes in lake and catchment history between both distinguished periods. From ~1970 forward the steep rising in temperature and decrease in precipitation likely lead to a drier ambient in the catchment, with less water availability for plant growth and increasing drought as the summer advances. Pauli and others (2012) evidence that flora species richness has declined on the southern European summits (including Sierra Nevada) within the 2000s but increased in European boreal-temperate mountain regions. Differences are attributed to the decrease of the availability of water in the European south. In this study, we show a tendency to increased aridity in the Sierra Nevada, starting at the turn of the 20th century and intensified from the 70s onward. Consequences for the vegetation are serious in terms of species loss considering that the flora of Sierra Nevada summits comprises a sizable percentage of endemic species in Europe, implying therefore an important loss of endemic European species.

Consequences of the changes observed in climate for lakes are also pronounced. We show an increase in chlorophyll-*a* and changes in biota assemblages from the mid-20th century onward, mainly governed by different processes as longer growing season, increasing water temperature and reduced water level because of higher evaporation rates and reduced water inflow. All these processes, and the additional P enrichment due to Saharan dust affecting this region, may lead to further trophic state changes of the Sierra Nevada lakes. An increase in algal biomass and the appearance and disappearance of lake species signify deep changes in the ecosystem functioning as both primary producer biomass and lake trophic web are major components of the ecosystem structure. The similar timing and direction of changes in chironomid community composition as observed for cladoceran and diatom assemblages, coincident with changes in other Sierra Nevada lakes (Jiménez and others 2018), corroborate the hypothesis of climate-driven shifts in the ecological status of distinct trophic levels in these alpine lakes. Hence, it is likely the ecological thresholds for biotic communities were crossed after the intensification of temperature and precipitation changes since the last decades.

If, as predicted by climate models, the rising of temperature and decrease in rainfall continue in the Sierra Nevada region and drought processes observed in this study intensify, physical and biological transformations can be expected in the catchment ecosystem of RS Lake and in other glacial valleys of Sierra Nevada, even modifying the Sierra Nevada summits' image of glacier valleys with clear water lakes and green alpine meadows. The impact of this climate change on the summits of Sierra Nevada and its influence transcends its geographical limits because these systems provide ecosystem services as important as being the largest source of water for the population living in the lowlands, for agricultural uses, for the generation of hydroelectric power, habitat for the species (many of them endemic), ecotourism, and the aesthetic value and source of scientific knowledge (Palomo and others 2013). The beneficiaries of water resources are primarily the inhabitants of the large cities near Sierra Nevada (Granada and Almería) and many other smaller towns since this is a very populated area. Moreover, South East of Spain is a preeminently agricultural and tourist area and the numerous rivers whose sources are in Sierra Nevada supply water for these activities. For millennia, humans have inhabited the Sierra Nevada environment and have benefited from these services, however the magnitude of human pressures, including climate change, could exceed the resilience of these ecosystems.

This is the first study at short-time scale (180 years) to use multiples proxies to provide an integrated view of how this and similar alpine ecosystems are responding to climate change. Because so little is known concerning the effects of recent warming on these alpine ecosystems, further investigations on similar lakes in the region are needed to provide a more comprehensive understanding of the effect of climate change on these vulnerable ecosystems and their surroundings.

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REFERENCES

Adrian R, O'Reilly CM, Zagarese H, Baines SB, Hessen DO, Keller W, Livingstone DM, Sommaruga R, Straile D, Van Donk E, Weyhenmeyer GA, Winder M. 2009. Lakes as sentinels of climate change. Limnology and Oceanography 54:2283–2297.

Anderson RS, Jiménez-Moreno G, Carrión JS, Pérez-Martínez C. 2011. Postglacial history of alpine vegetation, fire, and climate from Laguna de Río Seco, Sierra Nevada, southern Spain. Quaternary Science Reviews 30:1615–1629.

Appleby PG, Oldfield F. 1983. The assessment of ^{210}Pb data from sites with varying sediment accumulation rates. Hydrobiologia 103:29–35.

Battarbee RW, Grytnes JA, Thompson R, Appleby PG, Catalan J, Korhola A, Birks HJB, Heegaard E, Lami A. 2002. Comparing palaeolimnological and instrumental evidence of climate change for remote mountain lakes over the last 200 years. Journal of Paleolimnology 28:161–179.

Beniston M. 2003. Climatic change in mountain regions: A review of possible impacts. Climate Change 59:5–31.

Bennett KD. 1996. Determination of the number of zones in a biostratigraphical sequence. New Phytologist 132:155–70.

Bigler C, Heiri O, Krskova R, Lotter AF, Sturm M. 2006. Distribution of diatoms, chironomids and cladocera in surface sediments of thirty mountain lakes in south-eastern Switzerland. Aquatic Sciences 68:154–171. Birks HJB, Simpson GL. 2013. Diatoms and pH reconstructions (1990) revisited. Journal of Paleolimnology 49:363–371.

Bonet FJ, Pérez-Luque AJ, Pérez-Pérez R. 2016. Trend analysis (2000–2014) of the snow cover by satellite (MODIS sensor). Zamora R, Pérez-Luque AJ, Bonet FJ, Barea-Azcón JM, Aspizua R, editors.

Global Change Impacts in Sierra Nevada: Challenges for Conservation. Sevilla: Consejería de Medio Ambiente y Ordenación del Territorio, Junta de Andalucía. p43–46.

Brooks S, Heiri O. 2013. Response of chironomid assemblages to environmental change during the early Late-glacial at Gerzensee, Switzerland. Palaeogeography, Palaeoclimatology, Palaeoecology 391:90–98.

Brooks SJ, Langdon PG, Heiri O. 2007. The identification and use of Palaearctic Chironomidae larvae in palaeoecology (Issue 10 of Technical Guide). Cambridge: Quaternary Research Association. 276p.

Bush RT, McInerney FA. 2013. Leaf wax *n*-alkane distributions in and across modern plants: Implications for paleoecology and chemotaxonomy. Geochimica et Cosmochimica Acta 117:161–179.

Dean WE. 1974. Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition: comparison with other methods. Journal of Sediment Petrology 44: 242–248.

Eakins JD, Morrison RT. 1978. A new procedure for the determination of lead-210 in lake and marine sediments. International Journal of Applied Radioactive Isotopes 29:531–536.

Faegri K, Iversen J. 1989. Textbook of Pollen Analysis. New York: Wiley. 328p.

Fee EJ, Shearer JA, DeBruyn ER, Schindler EU. 1992. Effects of lake size on phytoplankton photosynthesis. Canadian Journal of Fisheries and Aquatic Sciences 49:2445–2459.

Ficken KJ, Li B, Swain DL, Eglinton G. 2000. An *n*-alkane proxy for the sedimentary input of submerged/floating freshwater aquatic macrophytes. Organic Geochemistry 31:745–749.

García-Alix A, Jiménez Espejo FJ, Toney JL, Jiménez-Moreno G, Ramos-Román MJ, Anderson RS, Ruano P, Queralt I, Delgado Huertas A, Kuroda J. 2017. Alpine bogs of southern Spain show human-induced environmental change superimposed on long-term natural variations. Scientific Reports, 7:7439.

García-Alix A, Jiménez-Moreno G, Anderson RS, Jiménez-Espejo FJ, Delgado Huertas A. 2012.

Holocene environmental change in southern Spain deduced from the isotopic record of a high-elevation wetland in Sierra Nevada. Journal of Paleolimnology 48:471–484.

Giorgi F. 2006. Climate change hot-spots. Geophysical Research Letters 33:1–4.

Grimm, EC, editor. 2004. Tilia and TG View Version 2.0.2. Springfield, IL, USA: Illinois State Museum, Research and Collection Center.

Grunewald K, Scheithauer J. 2010. Europe's southernmost glaciers: response and adaptation to climate change. Journal of Glaciology 56:129–142.

Han J, Calvin M. 1969. Hydrocarbon distribution of algae and bacteria, and microbiological activity in sediments. Proceedings of the National Academy of Sciences 64:436–443.

Heiri O, Brooks SJ, Birks JB, Lotter AF. 2011. A 274-lake calibration data-set and inference model for chironomid-based summer air temperature reconstruction in Europe. Quaternary Science Reviews 30:3445–3456.

Heiri O, Lotter AF. 2010. How does taxonomic resolution affect chironomid-based temperature reconstruction?. Journal of Paleolimnology 44:589-601.

Heiri O, Lotter AF, Hausmann S, Kienast F. 2003. A chironomid-based Holocene summer air temperature reconstruction from the Swiss Alps. The Holocene 4:477–484.

Heiri O, Lotter AF, Lemcke G. 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. Journal of Paleolimnology 25:101–110.

IPCC. 2013. Summary for policymakers. Stocker TF, Qin D, Plattner G-K, Tignor MMB, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM. Climate Change 2013: The Physical Science Basis.

Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge: Cambridge University Press. p3–17.

Jiménez L, Romero-Viana L, Conde-Porcuna JM, Perez-Martinez C. 2015. Sedimentary photosynthetic pigments as indicators of climate and watershed perturbations in an alpine lake in southern Spain. Limnetica 34:439–454.

Jiménez L, Rühland KM, Jeziorski A, Smol JP, Pérez-Martínez C. 2018. Climate change and Saharan dust drive recent cladoceran and primary production changes in remote alpine lakes of Sierra Nevada, Spain. Global Change Biology, 24:e139–e158.

Jiménez-Espejo FJ, García-Alix A, Jiménez-Moreno G, Rodrigo-Gámiz M, Anderson RS, Rodríguez-Tovar FJ, Martínez-Ruiz F, Giralt S, Delgado Huertas A, Pardo-Igúzquiza EP. 2014. Saharan aeolian input and effective humidity variations over western Europe during the Holocene from a high altitude record. Chemical Geology 374-375:1–12.

Jiménez-Moreno G, Anderson RS. 2012. Holocene vegetation and climate change recorded in alpine bog sediments from the Borreguiles de la Virgen, Sierra Nevada, southern Spain. Quaternary Research 77:44–53.

Jiménez-Moreno G, García-Alix A, Hernández-Corbalán MD, Anderson RS, Delgado-Huertas A. 2013. Vegetation, fire, climate and human disturbance history in the southwestern Mediterranean area during the late Holocene. Quaternary Research 79:110–122.

Juggins S. 2007. C2 Version 1.5 User Guide. Software for Ecological and Palaeoecological Data Analysis and Visualisation. Newcastle upon Tyne: Newcastle University. 73p.

Juggins S. 2012. Rioja: Analysis of Quaternary Science Data. R package version 0.9-9. Retrieved from http://cran.r-project.org/package= rioja.

Laville H, Vílchez-Quero A. 1986. Les Chironomidés (Diptera) de quelques «lagunas» de haute altitude de la Sierra Nevada (Granada, Espagne). Annales de Limnologie- International Journal of Limnology 22:53–63.

Lionello P. 2012. The climate of the Mediterranean region: From the past to the future. Amsterdam, Netherlands: Elsevier. 502p.

Lotter AF, Birks HJB, Hofmann W, Marchetto A. 1998. Modern diatom, cladocera, chironomid, and chrysophyte cyst assemblages as quantitative indicators for the reconstruction of past environmental conditions in the Alps. II. Nutrients. Journal of Paleolimnology 19:443–463.

Meyers, PA, Teranes JL. 2001. Sediment organic matter. Last WM, Smol JP, editors. Tracking Environmental Change Using Lake Sediments. New York: Springer. p239–269.

Michelutti N, Smol JP. 2016. Visible spectroscopy reliably tracks trends in paleo-production. Journal of Paleolimnology 56:253–265.

Michelutti N, Wolfe AP, Vinebrooke RD, Rivard B, Briner JP. 2005. Recent primary production increases in arctic lakes. Geophysical Research Letters, 32, L19715.

Morales-Baquero R, Pulido-Villena E, Reche I. 2006. Atmospheric inputs of phosphorus and nitrogen to the southwest Mediterranean region: Biogeochemical responses of high mountain lakes. Limnology and Oceanography 51:830–837.

Nogués-Bravo D, López-Moreno JI, Vicente-Serrano SM. 2012. Climate change and its impact. Vogiatzakis IN, editor. Mediterranean Mountain Environments. Chichester, UK: Wiley-Blackwell. p185–201.

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. 2015. Vegan: Community Ecology

Package. R package version 2.4-0. Retrieved from http://CRAN.R-project.org/package=vegan.

Oliva M, Gómez-Ortiz A. 2012. Late Holocene environmental dynamics and climate variability in a Mediterranean high mountain environment (Sierra Nevada, Spain) inferred from lake sediments and historical sources. The Holocene 22:915–927.

Oliva M, Gómez-Ortiz A, Salvador-Franch F, Salvà-Catarineu M, Palacios D, Tanarro L, Ramos M, Pereira P, Ruiz-Fernández J. 2016. Inexistence of permafrost at the top of the Veleta peak (Sierra Nevada, Spain). Science of the Total Environment 550:484–494.

Oliva M, Ruiz-Fernández J, Barriendos M, Benito G, Cuadrat JM, Domínguez-Castro F, García-Ruiz JM, Giralt S, Gómez-Ortíz A, Hernández A, López-Costas O, López-Moreno JI, López-Sáez JA, Martínez-Cortizas A, Moreno A, Prohom M, Saz MA, Serrano E, Tejedor E, Trigo R, Valero-Garcés B, Vicente-Serrano SM. 2018. The Little Ice Age in Iberian mountains. Earth-Science Reviews 177:175–208.

Oliver DR, Roussel ME. 1983. Redescription of *Brillia Kieffer* (Diptera, Chironomidae) with Descriptions of Nearctic Species. Canadian Entomologist 115:257–279.

Palomo I, Martín-López B, Potschin M, Haines-Young R, Montes C. 2013. National Parks, buffer zones and surrounding lands: mapping ecosystem service flows. Ecosystem Services 4:104–116.

Pauli H, Gottfried M, Grabherr G. 1996. Effects of climate change on mountain ecosystems – Upward shifting of alpine plants. World Resource Review 8:382–390.

Pauli H, Gottfried M, Dullinger S, Abdaladze O, Akhalkatsi M, Alonso JLB, Coldea G, Dick J, Erschbamer B, Calzado RF, Ghosn D, Holten JI, Kanka R, Kazakis G, Kollár J, Larsson P, Moiseev P, Moiseev D, Molau U, Mesa JM, Nagy L, Pelino G, Puscas M, Rossi G, Stanisci A, Syverhuset AO, Theurillat JP, Tomaselli M, Unterluggauer P, Villar L, Vittoz P, Grabherr G. 2012. Recent plant diversity changes on Europe's mountain summits. Science 336:353–355.

Pepin N, Bradley RS, Diaz HF, Baraer M, Caceres EB, Forsythe N, Fowler H, Greenwood G, Hashmi MZ, Liu XD, Miller JR, Ning L, Ohmura A, Palazzi E, Rangwala I, Schöner W, Severskiy I, Shahgedanova M, Wang MB, Williamson SN, Yang DQ. 2015. Elevation-dependent warming in mountain regions of the world. Nature Climate Change 5:424–430.

Pérez-Luque AJ, Pérez-Pérez R, Bonet FJ. 2016. Climate change over the last 50 years in Sierra Nevada. Zamora R, Pérez-Luque AJ, Bonet FJ, Barea-Azcón JM, Aspizua R, editors. Global Change Impacts in Sierra Nevada: Challenges for Conservation. Sevilla: Consejería de Medio Ambiente y Ordenación del Territorio, Junta de Andalucía. p24–26.

Pérez-Luque AJ, Sánchez-Rojas CP, Zamora R, Pérez-Pérez R, Bonet FJ. 2015. Dataset of Phenology of Mediterranean high-mountain meadows flora (Sierra Nevada, Spain). PhytoKeys 46:89–107.

Pérez-Martínez C. 2016. Analysis of the palaeolimnological indicators in the lakes of Sierra Nevada. Zamora R, Pérez-Luque AJ, Bonet FJ, Barea-Azcón JM, Aspizua R, editors. Global Change Impacts in Sierra Nevada: Challenges for Conservation. Sevilla: Consejería de Medio Ambiente y Ordenación del Territorio, Junta de Andalucía. p53–55.

Pérez-Martínez C, Jiménez L, Conde-Porcuna JM, Moreno E, Ramos-Rodríguez E, Heiri O, Jiménez-Moreno G, R Scott Anderson. 2012. Efectos del cambio climático en los ecosistemas acuáticos y terrestres de alta montaña de Sierra Nevada: Análisis del registro fósil en los sedimentos. Organismo Autónomo de Parques Naturales, editor. Proyectos de Investigación en parque nacionales: 2008-2011. Madrid: Ministerio de Agricultura, Alimentación y Medio ambiente. p71–93.

Pérez-Palazón MJ, Pimentel R, Herrero J, Aguilar C, Perales JM, Polo MJ. 2015. Extreme values of snow-related variables in Mediterranean regions: trends and long-term forecasting in Sierra Nevada (Spain). Proceedings of the International Association of Hydrological Sciences 369:157–162.

Preston DL, Caine N, McKnight DM, Williams MW, Hell K, Miller MP, Hart SJ, Johnson PTJ. 2016. Climate regulates alpine lake ice cover phenology and aquatic ecosystem structure. Geophysical Research Letters 43:5353–5360.

Pulido-Villena E, Reche I, Morales-Baquero R. 2005. Food web reliance on allochthonous carbon in two high mountain lakes with contrasting catchments: a stable isotope approach. Canadian Journal of Fisheries and Aquatic Sciences 62:2640–2648.

R Development Core Team. 2015. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.

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 Biology 43:1–18.

Rühland KM, Hargan KE, Jeziorski A, Paterson AM, Keller W, Smol JP. 2014. A multi-trophic exploratory survey of recent environmental changes using lake sediments in the Hudson Bay Lowlands, Ontario, Canada. Arctic, Antarctic, and Alpine Research 46:139–158.

Rühland KM, Paterson AM, Smol JP. 2015. Lake diatom responses to warming: reviewing the evidence. Journal of Paleolimnology 54:1–135.

Ruiz-Sinoga JD, Garcia Marin R, Martinez Murillo F, Gabarron Galeote MA. 2011. Precipitation dynamics in southern Spain: trends and cycles. International journal of climatology 31:2281–2289.

Smol, JP. 2008. Pollution of Lakes and Rivers: a Paleoenvironmental Perspective. 2nd edition. Oxford, UK: Willey-Blackwell publishing. 396p.

Sorvari S, Korhola A, Thompson R. 2002. Lake diatom response to recent Arctic warming in Finnish Lapland. Global Change Biology 8:171–181.

ter Braak CJF, Juggins S. 1993. Weighted averaging partial least squares regression (WA-PLS): an improved method for reconstructing environmental variables from species assemblages. Hydrobiologia 269:485–502.

ter Braak CJF, Juggins S, Birks HJB, van der Voet H. 1993. Weighted averaging partial least squares regression (WA-PLS): definition and comparison with other methods for species-environmental calibration. Patil GP, Rao CR, editors. Multivariate Environmental Statistics. Amsterdam: Elsevier Science Publishers. p525–560.

Toms JD, Lesperance ML. 2003. Piecewise regression: a tool for identifying ecological thresholds. Ecology 84:2034–2041.

Udelhoven T, Stellmes M, del Barrio G, Hill J. 2009. Assessment of rainfall and NDVI anomalies in Spain (1989–1999) using distributed lag models. International Journal of Remote Sensing 30:1961–1976.

Weckström K, Weckström J, Yliniemi L-M, Korhola A. 2010. The ecology of *Pediastrum* (Chlorophyceae) in subarctic lakes and their potential as paleobioindicators. Journal of Paleolimnology 43:61–73.

FIGURES

Figure 1. Geographical location of the study site. A) Inset map: Contour map of Iberian Peninsula showing the location of the study area; Contour of Sierra Nevada National Park indicating the study area; B) map of the Sierra Nevada mountain range showing locations of Río Seco (RS) Lake (circle) and highest mountain peaks (white triangles); C) RS Lake bathymetry (digitized map of bathymetry report from Egmasa S.A.); D) photo of RS Lake (August 2012).

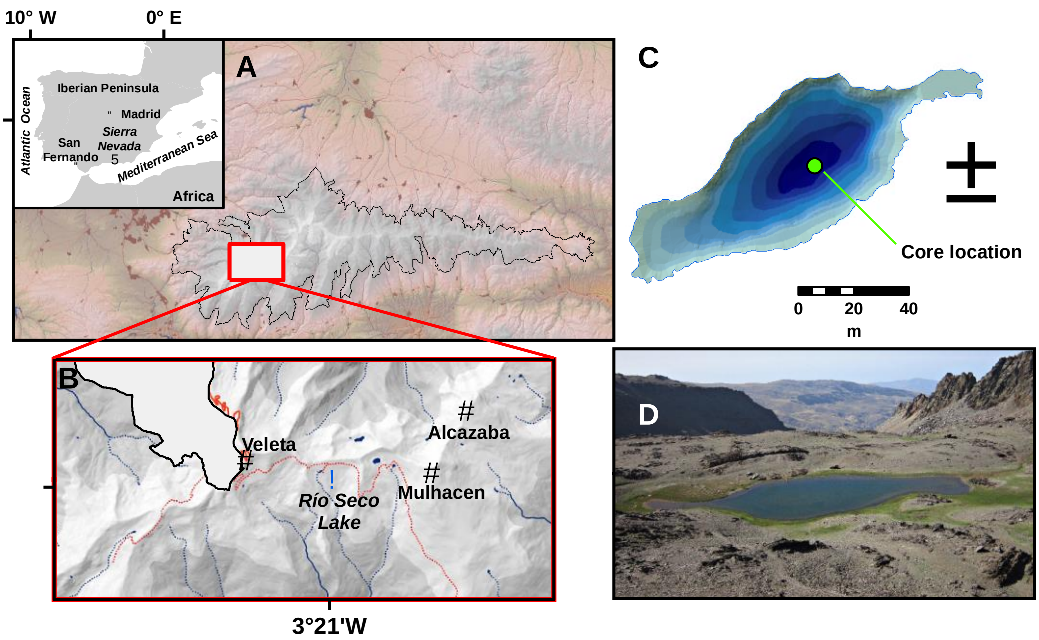
Figure 2. Comparison of the downcore sedimentary proxies. Profiles of organic matter content (LOI_{550}), sedimentary chlorophyll *a* (Chl a) (mg g^{-1} DW); $\delta^{13}C_{org}$ (V-PDB), atomic C/N ratio, biomarkers (CPI, P_{aq}

and ACL) and pollen data (C/P ratio and *Pediastrum* %) from 1820 to 2008 A.D. Two distinct climatic period are defined based on the climate data: Period One (from 1820 to ~1920s) and Period Two (from ~1920s to the present). Stratigraphically constrained cluster analyses using biological and geochemical proxies is also shown. The mean annual air temperature anomaly from Madrid climate station (MAAT Madrid) and annual precipitation anomaly from San Fernando climate station (AP San Fernando) is shown since 1860 and 1840, respectively. Temperature anomalies are related to the period 1961-1990 and precipitation anomalies are related to the whole period. A LOESS smoother (span = 0.2) was applied to all the variables (bold line). Applying a two-segment, piecewise linear regression to the MAAT Madrid series identified a threshold change to higher mean temperatures in the early 1970s (breakpoint= 1972 ± 4.7, $p < 0.0001$), while a potential additional breakpoint, not considered statistically significant, is also identified in the time interval of 1912-1915. For precipitation data, no significant breakpoint was identified.

Figure 3. Chironomid remains in the sediment core from RS Lake, together with a cluster analysis of assemblage data using Constrained Incremental Sum of Squares (CONISS). Light grey silhouettes show ×10 exaggeration. The horizontal grey-shaded area represent the period post-1820 A.D.

Figure 4. Comparison of Cladocera, diatom and chironomid PCA axis 1 sample scores for RS Lake sediment core, together with chironomid-inferred mean July air temperatures based on the chironomid records (see text for details). The error bar lines indicate the sample-specific estimated standard error of prediction**.** Note the inverted scales in the axis scores for Cladocera and chironomids.

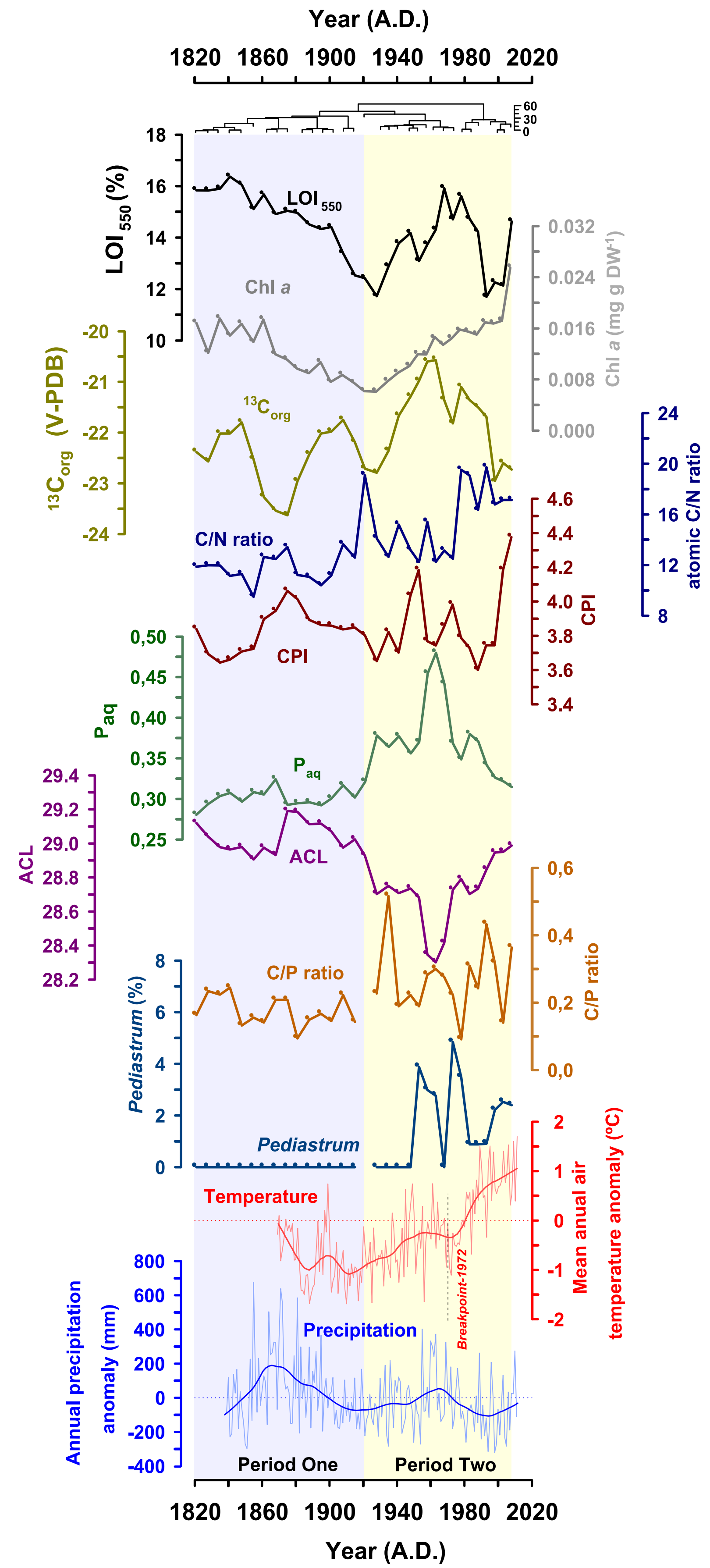
Fig 1.



10° W
0° E
A
Iberian Peninsula
Madrid
Atlantic Ocean
Sierra
Nevada
San
Fernando
5
Mediterranean Sea
Africa
C
Core location
0
20
40
m
B
Veleta
Alcazaba
Mulhacen
Río Seco
Lake
D
3°21'W

791

792 **Fig 2.**



Year (A.D.)
1820 1860 1900 1940 1980 2020
LOI 550 (%)
LOI 550
Chl a
Chl a (mg g DW-1)
13Corg (V-PDB)
13Corg
C/N ratio
atomic C/N ratio
CPI
Paq
ACL
C/P ratio
Pediastrum (%)
Pediastrum
Temperature
Mean anual air temperature anomaly (ºC)
Breakpoint-1972
Precipitation
Annual precipitation anomaly (mm)
Period One
Period Two

Fig 3.

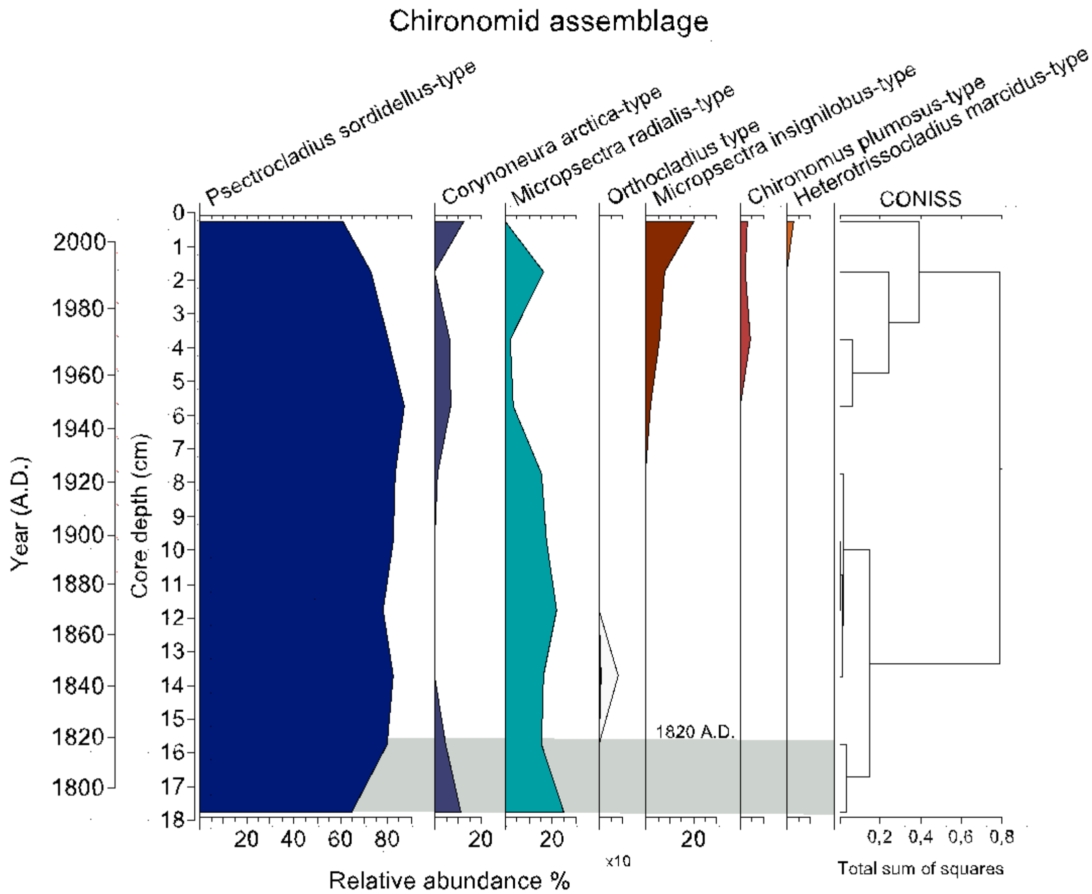
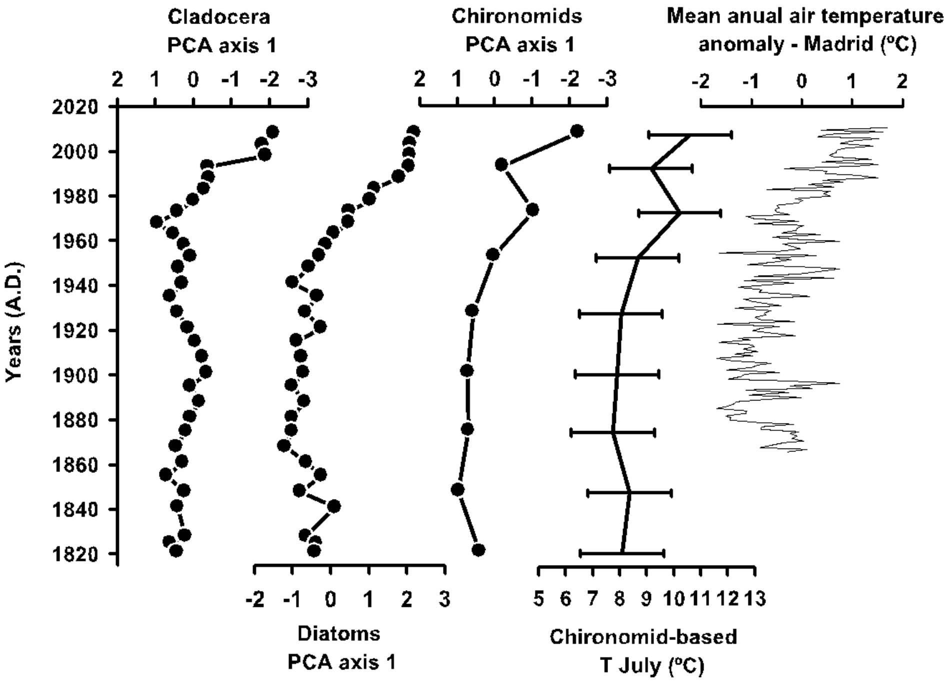

Chironomid assemblage
Psectrocladius sordidellus-type
Corynoneura arctica-type
Micropsectra radialis-type
Orthocladius type
Micropsectra insignilobus-type
Chironomus plumosus-type
Heterotrissocladius marcidus-type
CONISS
Year (A.D.)
Core depth (cm)
2000
1980
1960
1940
1920
1900
1880
1860
1840
1820
1800
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
1820 A.D.
20 40 60 80
20
20
x10
20
0,2 0,4 0,6 0,8
Relative abundance %
Total sum of squares

Fig 4.


Cladocera
PCA axis 1
Chironomids
PCA axis 1
Mean anual air temperature
anomaly - Madrid (ºC)
Years (A.D.)
2020
2000
1980
1960
1940
1920
1900
1880
1860
1840
1820
2 1 0 -1 -2 -3
2 1 0 -1 -2 -3
-2 -1 0 1 2
-2 -1 0 1 2 3
Diatoms
PCA axis 1
5 6 7 8 9 10 11 12 13
Chironomid-based
T July (ºC)