PALEOLIMNOLOGICAL EVIDENCE OF GLOBAL CHANGE IN HIGH MOUNTAIN ECOSYSTEMS OF SIERRA NEVADA

Laura Jiménez Liébanas
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Memoria presentada por Laura Jiménez Liébanas para optar al Grado de Doctor con Mención Internacional en Ciencias Biológicas por la Universidad de Granada. Esta memoria ha sido realizada bajo la dirección de Dra. Carmen Pérez Martínez y Dr. Jose María Conde Porcuna, Doctores en Ciencias Biológicas por la Universidad de Granada.

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"El más bello sentimiento que uno puede experimentar es sentir misterio. Esta es la fuente de todo arte verdadero, de toda verdadera ciencia. Aquel que nunca ha conocido esta emoción, que no posee el don de maravillarse ni encantarse… sus ojos están cerrados…"

Albert Einstein (1879 - 1955)

A la ciencia

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Materials and Methods

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Abbreviation

ACL - Average Chain Length

AD - Anno Domini

AICc - Akaike Information Criterion adjusted for sample size

AP San Fernando - total annual precipitation from the San Fernando climate station

$\boldsymbol{\delta^{13}C_{org}}$ - carbon isotope

Ca - Calcium

C/P - cyperaceae/poaceae pollen ratio

C/N - carbon/nitrogen ratio

CD/TC - chlorophyll derivative: total carotenoid

chl *a* - chlorophyll-*a*

CONISS- constrained incremental sum of squares

CPI - Carbon Preference Index

CRS - constant rate of supply

DCA - detrended correspondence analysis

DCCA - detrended canonical correspondence analysis

DOC - dissolved organic carbon

HPLC - high-performance liquid chromatography

LIA - Little Ice Age

$\mathbf{LOI_{550}}$ - loss on ignition at 550 ºC

MAAT Madrid - mean annual air temperature from the Madrid climate station

MJAT - mean July air temperature

pRDA - partial redundancy analysis

TN - Total nitrogen

TP -Total phosphorus

$\mathbf{P_{aq}}$ - Portion aquatic

PCA - principal component analysis

RDA - redundancy analysis

SPI - Sahel Precipitation Index

TLE - total lipid extract

VIF - variance inflation factors

WA-PLS - Weighted averaging - partial least squares regression

wNAO - winter North Atlantic Oscillation

Zr/Al ratio - Zirconium/Aluminum ratio

Summary

In this thesis, we seek a deeper and broader understanding of how Mediterranean alpine ecosystems responds to global change impacts over the past ~200 years. High mountain lakes constitute an excellent witness of global change and are amongst the most sensitive ecosystems to anthropogenic climatic change and environmental changes. Nowadays, changes in air temperature and precipitation have direct effects on the physical, chemical, and biological characteristics of high mountain lakes, and indirectly by modifying in-lake and catchment processes. In this thesis, we show that Mediterranean high mountain ecosystems experience major changes in response to recent warming. Besides, the interaction between factors of global change promotes a series of direct and indirect effects able to affect changes in biotic community composition and in the functioning and structure of alpine ecosystems.

Spread over four data chapters, we explore distinct potential drivers of local to regional-scale variability in the climate response of biological and geochemical variables in the Sierra Nevada. More specifically, we study the effects of climate-driven changes (such us ice cover duration, water residence time or water temperature, among others) in the ecology and environment in an alpine lake and its catchment, as well as the interaction between climate and nutrient inputs in biological communities, and the role of lake-specific characteristic in the distribution of subfossil cladocerans across Sierra Nevada lakes.

In the first data chapter (**Chapter 1**), we estimated changes in the algal community by analyzing sedimentary photosynthesis pigments from an alpine Mediterranean lake of the Sierra Nevada (Río Seco Lake) over the past 200 years. The results shows that the main algal groups identified were cyanobacteria (zeaxanthin, echinenone and myxoxanthophyll), diatoms and chrysophytes (fucoxanthin and diadinoxanthin) and green algae (lutein), and

that the most important change occurred from the 1950s onward. The main change in algal community was the marked decrease in zeaxanthin, mainly attributed to picoplanktonic cyanobacteria. This change appears to be driven by factors linked to global warming, such as increased zooplankton grazing pressure and reduced water residence time. This chapter also provides information of direct catchment perturbations by analyzing geochemical variables. This period of human pressure occurred during 30-year, between the construction of a dirt road and a mountain hut close to the lake shoreline in the 1960s and their destruction in the late 1990s, resulting in a catchment erosion and consequent dilution of chlorophylls and labile carotenoids over recent decades.

Chapter 2 provides a high-resolution multi-proxy reconstruction of recent environmental and ecological changes from Río Seco Lake by the combination of stratigraphic records of sub-fossil chironomid assemblages, leaf waxes biomarkers (*n*-alkanes), spectrally-inferred chlorophyll-*a* concentrations, pollen, cladocera and diatoms data, organic matter content, atomic C/N ratio and organic carbon isotopic. According to main shifts in climatic data, consistent with noticeable changes in major biogeochemical proxies, two distinct climatic stages are indicated: Stage One (1820- ~1920s) characterized by colder and wetter conditions than the more recent Stage Two (~1920s to the present), characterized by warmer and drier conditions. When comparing both Stages, independent proxies in Stage One indicated a longer ice-cover period, colder water temperature and more pronounced accumulation of snow in the catchment, resulting in a reduced growing season for aquatic and/or terrestrial primary production; and the dominance of cold-water chironomid taxa. However, biogeochemical changes in Stage Two are mainly attributable to longer ice-free seasons, warmer summer water temperatures, and more frequent thawing events and thus available water in the catchment. These conditions led to a longer growing season for aquatic primary production and development of wetland plants in the catchment.

These changes seem to be related to the temperature increase and precipitation decrease that started in the 1920s and intensified over the past 50-years. This chapter also provide useful information of the chironomid-inferred mean July air temperature, which evidenced a 2ºC increase from ~1950 onwards. This implies a pronounced increase in summer air and water temperature at the lake as evidenced by the decline of a cold-water taxon, and arrival of new taxa characteristic of warmer water. Chironomids changes are also coincident with shifts in cladoceran and diatom assemblages indicating a similar response of the lacustrine biota to the effects of climatic warming. Overall, this chapter indicates that the in-lake and catchment related processes are mainly attributed to direct and indirect climate-driven changes.

In **Chapter 3**, we track changes in sedimentary cladoceran assemblages and sedimentary chlorophyll-*a* concentrations for well-dated sediment cores retrieved from six remote alpine lakes of the Sierra Nevada over the past ~150 years. This study indicated that the common trend in the six lakes were the abrupt increase in the relative abundance of the benthic cladoceran *Alona quadrangularis* at the expense of *Chydorus sphaericus*, and a significant increase in *Daphnia pulex* gr. The results suggest that the chlorophyll-*a* production increased since the 1970s, consistent with a response to rising air temperatures and the intensification of atmospheric deposition of Saharan P. On the other hand, similar shifts in cladoceran taxa across lakes began over a century ago, but intensified over the past ~50 years, concurrent with trends in regional air temperature, precipitation, and increased Saharan dust deposition. This study also determined that the differences observed in the magnitude and timing of these biological changes are likely due to catchment and lake-specific differences. Overall, this chapter demonstrates that the long-term changes in both cladoceran assemblages and primary production in recent decades indicated a regional-scale response to climate. Also, in contrast with other alpine lakes that are often affected by acid deposition, atmospheric Ca and P deposition appears to be also a significant explanatory factor for the changes in the lake biota of Sierra Nevada.

Finally, **Chapter 4** provide a detailed descriptions of cladoceran assemblages (including pelagic and littoral taxa) and their relationships to physical, chemical and limnological parameters from surface sediment of a set of 17 alpine lakes across Sierra Nevada by using multivariate techniques. The results indicates the distribution of cladoceran assemblages is mainly associated to morphometric variables (e.g. extent of the catchment vegetation in the basin, lake depth and size and the presence of inlets and/or outlets), while the influence of chemical parameters play a secondary role as explanatory variables, being total nitrogen (TN) the most important variable. Among cladoceran taxa, *Alona quadrangularis* was strongly associated to lakes with inlets and/or outlets and surrounded by meadows. *A. quadrangularis* and *C. sphaericus* relative abundance showed a significant negative relationship Higher abundance of *Chydorus sphaericus* was found in closed or open/closed basins with less or no meadows surrounding the lake, mainly associated to their advantage to inhabiting littoral and water pelagic column, while *Alona elegans* was linked to open and shallow lakes with a high nutrient and ion concentration, likely attributed to the development of vegetation. There was a strong influence of two morphometric variables (maximum depth and presence of inlets and outlets) on *Daphnia* presence. The absence of *Daphnia* in open systems was related to the negative effect of dilution on *Daphnia* population development associated to a high water renewal in these alpine lakes.

In conclusion, we found direct and indirect effects on biota communities and catchment-related processes across Sierra Nevada over the past 200 years with special focus on the last decades. Changes in biota communities provide evidence that temperature-driven ecological thresholds have been crossed. Besides, this thesis also demonstrate the importance of understanding the environmental and ecological context when interpreting species changes and distribution as well as shifts in geochemical variables in paleolimnological records. This thesis is the first high-resolution paleoclimatic study for Sierra Nevada lakes, providing important long-term context for the more recent climate changes (past 60 years) experienced in the region, and provide a more comprehensive picture of the extant cladoceran assemblages over recent years than would otherwise be obtained by routine monitoring programs.

Resumen

En esta tesis, exploramos los efectos del cambio global en los ecosistemas mediterráneos alpinos durante los últimos 200 años. Los lagos de alta montaña constituyen un excelente testigo del cambio global, y se encuentran entre los ecosistemas más sensibles al cambio climático antropogénico y cambios ambientales. Hoy en día, los cambios en la temperatura del aire y la precipitación tienen efectos directos sobre las características físicas, químicas y biológicas de los lagos de alta montaña, e indirectamente mediante la modificación de los procesos internos del lago y su cuenca. En esta tesis, mostramos que los ecosistemas mediterráneos de alta montaña experimentan cambios importantes en respuesta al cambio climático actual. Además, la interacción entre factores de cambio global promueve una serie de efectos directos e indirectos que producen cambios en la composición de la comunidad biológica, y en el funcionamiento y estructura de los ecosistemas alpinos.

A lo largo de cuatro capítulos, exploramos distintos factores potenciales de variabilidad desde una escala local a regional asociados a la respuesta climática de variables biológicas y geoquímicas en Sierra Nevada. Concretamente, estudiamos los efectos de los cambios climáticos (como la duración de la capa de hielo, el tiempo de residencia del agua o la temperatura del agua) en un lago de alta montaña y su cuenca, así como la interacción entre el clima y la entrada de nutrientes en las comunidades biológicas, y el papel de las características específicas de los lagos en la distribución de cladóceros fósiles en Sierra Nevada.

En el **capítulo 1,** se estima los cambios en la comunidad algal usando como técnica paleolimnológica los pigmentos fotosintéticos sedimentarios de la laguna de Río Seco desde los últimos 200 años. Los resultados indican que los principales grupos algales identificados fueron las cianobacterias (zeaxantina, equinenona y mixoxantofila), diatomeas y crisofíceas (fucoxantina y diadinoxantina) y algas verdes (luteína), y que los cambios más importantes ocurrieron a partir de 1950. El cambio principal de la comunidad algal está asociados al abrupto descenso de la zeaxantina, pigmentos atribuido a las cianobacterias picoplantónicas en dicha laguna. Este descenso puede producirse por factores dependientes del clima que afectan a la presión de herbivoría y al tiempo de residencia del agua. Este capítulo también proporciona valiosa información relacionada con las perturbaciones que ha sufrido la cuenca a través del análisis de variables geoquímicas. Este periodo de mayor presión antrópica ocurrió en un periodo de 30 años, comprendido entre la construcción de una carretera y un refugio, situados a la orilla de la laguna a principios de 1960, y su posterior destrucción a finales de 1990. Esta creciente actividad humana alrededor de la laguna probablemente generó un alto aporte de materia orgánica pobremente pigmentada, provocando una dilución de clorofilas y carotenoides lábiles en décadas recientes.

En el **capítulo 2**, se realiza un estudio de alta resolución usando variables paleolimnológica independientes para reconstruir los cambios ambientales más recientes en la laguna de Río Seco. La combinación de variables paleolimnológicas incluyen quironómidos fósiles, biomarcadores foliares (*n*-alcanos), concentración de clorofila mediante técnicas de espectroscopia de reflectancia, datos de polen, cladóceros y diatomeas fósiles, contenido de materia orgánica e isótopos de carbono orgánico. En este estudio se indican dos escenarios climáticos distintos en función de los principales cambios en los datos de clima, y que a su vez, coinciden con los principales cambios de las variables biogeoquímicas: el escenario 1 (desde 1820 hasta 1920) que se caracteriza por unas condiciones más frías y húmedas que el periodo más reciente, el escenario 2 (desde 1920 hasta la actualidad), caracterizado por unas condiciones más cálidas y secas. Comparando ambos escenarios, las variables sedimentarias independientes en el escenario 1 indicaron un período de cobertura de hielo más largo, temperatura del agua más fría y una acumulación de nieve en la cuenca más acusada. Estas condiciones podría resultar en una reducción del período de crecimiento para la producción primaria tanto acuáticas como terrestre; así como la dominancia de especies de quironómidos adaptados a aguas más frías. Sin embargo, los cambios en las variables biogeoquímicas en el escenario 2 están principalmente relacionadas con un período libre de hielo más prolongado, temperaturas del agua más cálidas durante la estación libre de hielo, eventos de deshielo más frecuentes y como consecuencia, mayor disponibilidad de agua en la cuenca. Las condiciones que reinan en este período hacen pensar en la existencia de un período de crecimiento más prolongado para la producción primaria del lago, y el desarrollo de plantas terrestres en la cuenca. Estos cambios parecen estás relacionados con el incremento de la temperatura y reducción de la precipitación que empieza a partir de 1920, pero que se intensifica en los últimos 50 años. Este capítulo también suministra nuevos datos relacionados con la temperatura media del aire en julio obtenido mediante datos de quironómidos fósiles, en el que se evidencia un incremento de la temperatura de 2ºC desde 1940-50 hasta la actualidad. Esto implica un pronunciado incremento de la temperatura del agua y del aire durante el verano, que queda registrado por el descenso de un especie adaptada a aguas más frías, y el aumento de nuevas especies más asociadas a aguas cálidas. Los cambios observados en los quironómidos fósiles coinciden también con los cambios observados en el conjunto de cladóceros y diatomeas fósiles, indicando una respuesta similar en la biota de la laguna relacionada con el reciente cambio climático. En general, este capítulo indica que los procesos internos de la laguna y los relacionados con la cuenca están principalmente asociados a cambios directos e indirectos del clima.

En el **capítulo 3**, exploramos los cambios en el conjunto de cladóceros fósiles y clorofila-*a* sedimentaria, procedente de testigos de sedimento extraídos de seis lagunas de Sierra Nevada, durante los últimos 150 años. Este estudio indicó que el cambio más común en las seis lagunas fue el importante incremento de la especies bentónica *Alona quadrangularis* a expensas de la especies *Chydorus sphaericus*, y un incremento significativo de especies del grupo *Daphnia pulex*. Los resultados sugieren que la producción de clorofila-*a* aumentó desde 1970, estando este cambio relacionado con un incremento en la temperatura del aire y la intensificación de la deposición atmosférica de fósforo sahariano. Por otro lado, los cambios similares en la especies de cladóceros entre las lagunas comienzan a principio de siglo, y se intensifican en los últimos 50 años, simultáneos con las tendencias observadas para la temperatura regional del aire, la precipitación, y el aumento de la deposición de polvo sahariano. Este estudio también determinó que las diferencias observadas en la magnitud y cronología de estos cambios biológicos están probablemente relacionado con la cuenca y las características específicas de las lagunas. En general, este capítulo demuestra que los cambios observados en el tiempo tanto en el conjunto de cladóceros fósiles como la producción primaria durante las décadas más recientes indicaron una respuesta a escala regional del clima. Además, al contrario que en otros lagos alpinos que si están afectados por la deposición ácida, la deposición atmosférica de calcio y fósforo parece ser también un factor explicativo en los cambios observados en la biota de los lagos de Sierra Nevada.

Por último, en el **capítulo 4** se describen detalladamente la relación entre el conjunto de cladóceros (incluidos taxones pelágicos y litorales) con los parámetros físicos, químicos y limnológicos de los sedimentos superficiales de un conjunto de 17 lagos alpinos de Sierra Nevada, mediante el uso de técnicas multivariantes. Los resultados indican que la distribución del conjunto de cladóceros está relacionada principalmente con variables morfométricas como la extensión de la vegetación en la cuenca de captación, profundidad y tamaño del lago o presencia de afluentes y/o efluentes, mientras que la influencia de parámetros químicos desempeñan un papel secundario como variables explicativas, siendo el nitrógeno total la principal variable. *Alona quadrangularis* está asociada a lagunas con afluentes y/o efluentes, así como aquellas rodeadas de prado alpino, y presenta una relación significativa negativa con la especies *Chydorus sphaericus*. La mayor abundancia de *C. sphaericus* se presenta en lagunas con cuencas cerradas o abiertas/cerradas con baja abundancia o ausencia de prado alpino en su cuenca, relacionado principalmente por su disposición a habitar no sólo la zona litoral sino también la zona pelágica de las lagunas, mientras que *Alona elegans* se ha relacionado con lagos abiertos y poco profundos con alto contenido de nutrientes y concentración de iones, probablemente asociado al desarrollo de la vegetación. Existe una relación importante de dos variables morfométricas (profundidad máxima y presencia de afluentes y/o efluentes) en la presencia de *Daphnia*. La ausencia de *Daphnia* en sistemas abiertos está relacionada con la alta tasa de renovación del agua, provocando un descenso de la población y impidiendo su desarrollo en estas lagunas de alta montaña.

En conclusión, se han observado efectos directos e indirectos sobre las comunidades biológicas y los procesos relacionado con la cuenca en Sierra Nevada durante los últimos 200 años con especial interés en las últimas décadas. Los cambios en las comunidades biológicas asociados al aumento de temperatura proporcionan evidencia de que se han superado umbrales ecológicos. Además, esta tesis también demuestra la importancia de entender el contexto ambiental y ecológico al interpretar los cambios de especies y su distribución, así como los cambios en variables geoquímicas en registros paleolimnológicos. Esta tesis es el primer estudio paleoclimático de alta resolución en la lagunas de Sierra Nevada, que aporta información muy importante sobre el cambio climático en la región, especialmente en los últimos 60 años, y proporciona una visión más completa de la distribución de las comunidades de cladóceros en los últimos años que sólo podría obtenerse mediante un intenso programa de monitoreo.

Introducción general

Introducción general

El cambio global en la región Mediterránea

El cambio global se refiere a todas aquellas transformaciones ambientales de gran escala que afectan al funcionamiento del sistema Tierra, y que se derivan de las actividades humanas sobre el planeta (Petschel-Held y Reusswig, 1999). Actualmente, los problemas medioambientales y socioeconómicos derivados del cambio global constituye el principal reto que enfrenta la sociedad (Gibson *et al.*, 2000), y la magnitud de los cambios asociados a las actividades antrópicas están en continuo debate. Las evidencias de un acelerado calentamiento global que afecta a la atmósfera y el océano son inequívocas, y las emisiones de gases de efecto invernadero, principalmente dióxido de carbono CO_2, han aumentado hasta niveles sin precedentes (IPCC 2013). La temperatura media global de la superficie de la Tierra ha aumentado alrededor de 0.8 ºC durante los últimos 150 años (IPCC, 2013), y los modelos de circulación general predicen que la temperatura global de la superficie aumentará entre 1.4 y 5.8 ºC en el año 2100 (Houghton *et al.*, 2001). Podemos considerar que estamos en una era de cambio, una nueva etapa en la historia de la Tierra, dominada por los seres humanos: el Antropoceno (Steffen *et al.*, 2011)

El cambio global está teniendo un gran impacto en los sistemas físicos (criosfera, hidrología y procesos costeros) y biológicos (sistemas acuáticos marinos y continentales, sistemas terrestres) a nivel mundial. Las respuestas de los sistemas físicos al calentamiento global se ven reflejados en cambios en los regímenes de hidrología y temperatura del agua, cambios en el patrón de precipitación, disminución en la extensión de permafrost, retroceso de glaciares o aumento del nivel del mar (Adrian *et al.*, 2009, EEA, 2012, Dokulil, 2014). Estos cambios se han visto especialmente reflejados en el Hemisferio Norte, donde el registro de la temperatura de la superficie del aire ha aumentado 1.3 ºC durante la última década, considerándose el período más cálido jamás registrado (Friedman *et al*., 2013).

La región Mediterránea se encuentra en una zona de transición entre el clima árido del norte de África y el clima templado y lluvioso de Europa central y se ve afectado por las interacciones entre la latitud media y los procesos tropicales (Giorgi y Lionello, 2008). Por lo tanto, pequeñas modificaciones en los procesos meteorológicos de las latitudes medias y subtropicales como sequías, tormentas u olas de calor, pueden generar importantes cambios climáticos en esta región (Giorgi y Lionello, 2008). Debido a estas características, la región Mediterránea se ha identificado como uno de los "puntos calientes" (del inglés *Hot-spots*) más prominentes en las proyecciones futuras del cambio climático (Giorgi, 2006; Diffenbaugh y Giorgi, 2012). De esta manera, los recientes estudios de modelización sugieren que el clima de la cuenca mediterránea (inviernos suaves y húmedos y veranos calurosos y secos) pueden llegar a ser más extremos durante el siglo XXI (Alessandri *et al*., 2014; García-Herrera *et al*., 2014). Estas simulaciones climáticas predicen una sequía generalizada y un calentamiento global, especialmente en la estación cálida con una descenso de las precipitaciones superior al 25-30% y un aumento de la temperatura de 4-5 ºC hasta el año 2100 (Giorgi y Lionello, 2008).

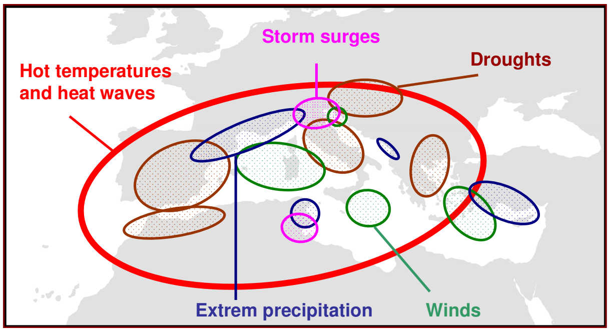


Figura 1. Ilustración simplificada que muestra las áreas de la región Mediterránea con mayor intensidad de eventos climáticos extremos obtenidos de las tendencias previamente observadas y de los modelos simulados (García-Herrera *et al*., 2014, Fig. 2 modificada).

Aunque ésta es la predicción generalizada para toda la región Mediterránea, el cambio climático en la Península Ibérica, y más concretamente en el sur de la Península es notable. Las temperaturas han aumentado de forma general durante el siglo XX, principalmente en invierno (Brunet y López-Bonillo, 2001), y las precipitaciones anuales en Andalucía muestran una tendencia decreciente, aunque con considerable variabilidad (Rodrigo *et al*. 2000). Los datos instrumentales actualmente disponibles, así como los modelos regionales para 2071-2100, indican que la Península Ibérica es especialmente sensible a cambios climáticos rápidos a escala de décadas. Para la España meridional continental se predice un progresivo aumento de las temperaturas medias con aumentos térmicos considerables en verano así como primaveras más secas y una disminución de la precipitación acumulada anual (Moreno *et al*., 2005).

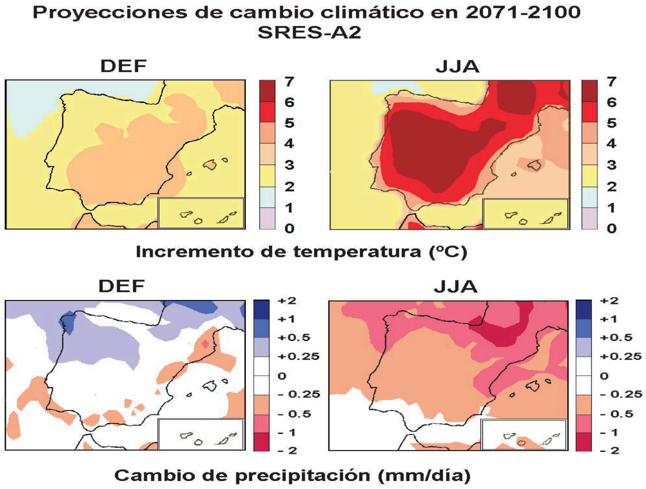


Figura 2. Proyecciones de cambio de temperatura media diaria del aire superficial (ºC) y de cambio de precipitación (en mm/día), promediadas para dos estaciones del año (DEF invierno y JJA verano) en la Península Ibérica, Baleares y Canarias (esquina inferior derecha en cada mapa), correspondientes al escenario SRES de emisiones A2. Los valores corresponden a diferencias entre la simulación del periodo 2071-2100 y la de control (1961-1990) (Moreno *et al*., 2005).

Las lagunas de alta montaña en la región Mediterránea. Factores de cambio global

El cambio global actual es de naturaleza multidimensional (Rockström *et al.* 2009), cuyos factores estresantes se caracterizan por tener mecanismos de retroalimentación, y expresar comportamientos a menudo sinérgicos, acumulativos y no lineales con el cambio climático, que dificultan su predicción (Wagner y Adrian 2009). Además, no son fácilmente evaluables, ya que su efecto interactivo puede ser mayor (sinérgico) o menor (antagonista) que el propio efecto aditivo que cabría esperar (Folt *et al.*, 1999). De igual manera, el potencial de dichos factores para actuar en algunas regiones de manera independiente y en otras sinérgicamente, hace que exista una creciente preocupación por intentar resolver todas las cuestiones relacionadas con estos factores climáticos indirectos (Adrian *et al.*, 2009). Hoy en día, la combinación de los múltiples factores antropogénicos ha creado complejas situaciones de estrés que pueden influir sustancialmente en la función y biodiversidad de los lagos (Christensen *et al.*, 2006; Ormerod *et al.*, 2010).

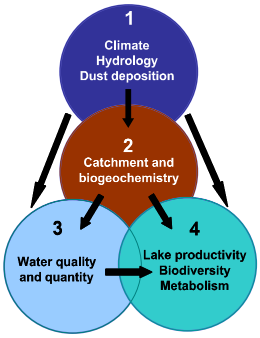


Figure 3. Los lagos responden al clima, la deposición atmosférica y las propiedades de sus cuencas. 1) Influencia de factores atmosféricos (temperatura, precipitación y deposición atmosférica de nutrientes); 2) La cuenca responde a los factores climáticos y la entrada de nutrientes atmosféricos por medio de la vegetación y los procesos del suelo; 3) Esto determina las entradas de materia orgánica, nutrientes y elementos clave que determinan parámetros como tiempo de retención, transparencia, pH y temperatura del agua; 4) La biota responde en términos de fenología, productividad, metabolismo, composición de la comunidad, diversidad e interacciones de la red trófica, a la influencia de los factores atmosféricos directamente (1), las propiedades de la cuenca (2) y del agua (3) (Adrian *et al.*, 2016, Fig. 10.1 modificada).

Muchos de los múltiples factores de estrés a escala local y global que pueden afectar de manera individual o combinada en los ecosistemas acuáticos son: 1) la acidificación (Curtis *et al.*, 2009); 2) eutrofización (Schindler, 2012); 3) pérdida de hábitat (Kurek *et al.*, 2012); 4) invasión de especies no nativas (McIsaac, 2001; Miró y Ventura, 2015); 5) cambios en el uso del suelo (Zhao *et al.*, 2012) o 6) aumento de la radiación UV (Vinebrooke y Leavitt, 2005; Villar-Argaiz *et al.*, 2009).

Muchos de los factores estresantes se potencian en los ecosistemas acuáticos de alta montaña (Pepin *et al.*, 2015). Aunque estos ecosistemas se encuentran en zonas poco alteradas por el hombre, están amenazados y bajo el impacto de numerosos factores como la deposición ácida, los contaminantes tóxicos atmosféricos, entrada de nutrientes por distintas vías, cambios en la radiación solar y cambio climático (Battarbee *et al.*, 2009). El hecho de que estos ecosistemas de alta montaña estén tan restringidos físicamente, tan limitados por nutrientes y presenten bajas tasas de producción y diversidad determina que cualquier cambio en las condiciones ambientales se manifieste de manera mucho más visible. Además, estos sistemas son muy dependientes de su propia cuenca hidrográfica, y por lo tanto los efectos indirectos del clima pueden ejercer una influencia significativa sobre ellos. Los efectos climáticos indirectos se ven especialmente reflejados en cambios del nivel de agua, alteración de la capa de nieve, entrada alóctona de material orgánico e inorgánico, duración del período libre de hielo, aumento de la temperatura del agua, cambios en las comunidades biológicas o en ciclos biogeoquímicos (Thies *et al.*, 2007; Parker *et al.*, 2008; Gobiet *et al.*, 2014; Sospedra-Alfonso *et al.*, 2015).

Los ecosistemas de alta montaña en la región Mediterránea se hacen particularmente vulnerables a los efectos del cambio global (Nogués-Bravo *et al.*, 2008), debido al particular aumento de la temperatura de aire en esta región, y en especialmente en áreas remotas de alta altitud (Dokulil, 2014). A medio plazo, una de las amenazas que penden sobre estas lagunas es su cada vez más frecuente agotamiento estival, debido tanto al descenso de nevadas, como al adelanto del deshielo y aumento de pérdidas por evaporación (Pimentel *et al.*, 2015). No obstante, el proceso más irreversible puede ser el de su progresiva colmatación, por un incremento de las tasas de sedimentación por arroyamientos pluviales, que serán más intensos y numerosos sin la capa protectora de la nieve (Castillo, 2009; Gobiet *et al.*, 2014).

La formación de esta cubierta de hielo invernal es probablemente una de las características más relevantes de los lagos de alta montaña, destacando su potencial como factor en el funcionamiento de dichos lagos al inducir respuestas hidrológicas, químicas y biológicas a los factores climáticos (Thompson *et al.*, 2005). La disminución de la cubierta de nieve debido al aumento de la temperatura, se ha asociado a aumentos en los tiempos de residencia del agua, estratificación térmica, concentraciones iónicas, nitrógeno disuelto, pH o clorofila *a* en los lagos de alta montaña (Koinig *et al.*, 1997; Preston *et al.*, 2016). La disminución de precipitación en forma sólida en primavera y el aumento de las temperaturas del aire también promueven la fusión del hielo glacial y del permafrost (Gómez-Ortíz *et al.*, 2014; Oliva *et al.*, 2016). El adelanto en la retirada del hielo y/o nieve se ha potenciado en las últimas décadas en este tipo de ecosistemas (Beniston, 1997; Pimentel *et al.*, 2015), y modelos predictivos sugieren un deshielo cada vez más temprano (Rammig *et al.*, 2010). Esta fusión de los hielos también induce a un aumento en el transporte de solutos hacia la cuenca aumentando las concentraciones iónicas (Koinig *et al.*, 1997) y carbono orgánico disuelto.

Bajo un escenario de cambio global, se fomentará también un incremento del contenido global de aerosoles derivado principalmente de suelos áridos (Prospero y Lamb, 2003). Las persistentes sequías en el Sahara, Sahel y la cuenca del lago Chad están incrementando sustancialmente la frecuencia e intensidad de los eventos de polvo con consecuencias potenciales para la productividad primaria y el estado trófico (Duarte *et al*., 2006; Morales-Baquero *et al*., 2006a).

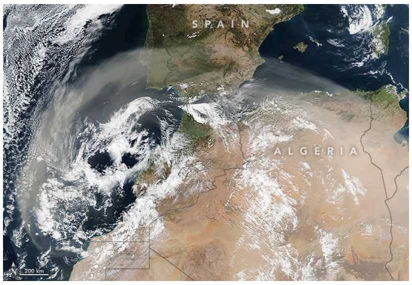


Figure 4. Imagen satélite de plumas masivas de polvo del Desierto del Sahara aerotransportadas. Fuente: National Aeronautics and Space Administration (NASA).

Estas entradas de polvo sahariano suponen una fuente significativa de nutrientes minerales como N, P y Ca, que suelen llegar a ser limitantes para el crecimiento de los organismos acuáticos. De esta manera, se ha evidenciado un efecto fertilizador directamente asociada a episodios atmosféricos de polvo sahariano enriquecido en P sobre el fitoplancton (Morales-Baquero *et al*., 2006a; Pulido-Villena et al., 2008) y bacterias (Reche *et al*., 2008), y su efecto sobre la biogeoquímica de las lagunas de alta montaña de Sierra Nevada (Pulido-Villena *et al*., 2006). Por otro lado, estas entradas de material alóctono son una importante fuente de alcalinidad en los lagos de alta montaña (Psenner y Nickus, 1986), pudiendo ejercer cambios en las comunidades de los organismos, hacia aquellos más adaptados a ambientes más alcalino.

En este tipo de ecosistemas, la biota está sometida a otras condiciones físicas extremas, tales como bajas temperaturas y elevada radiación ultravioleta incidente (Caldwell *et al*., 1980). Además, la vulnerabilidad de los ecosistemas de alta montaña se acentúa debido al bajo contenido de nutrientes y carbono orgánico disuelto que hace que la UVR penetre hasta las capas más profundas (Parker *et al*. 2008; Sommaruga y Psenner, 1997).

El cambio en la temperatura del agua de los lagos de alta montaña es otro factor, que aunque depende del régimen térmico actual y de la región geográfica, puede sufrir cambios significativos. Modelos predictivos en lagos de los Alpes han evidenciado un aumento de la temperatura del agua en 2ºC hasta 2050 (Dokulil, 2013a; 2013b). Este cambio, a su vez, puede afectar directamente a las características de estratificación y los procesos de mezcla. Se ha observado período de estratificación térmica prolongados debido al aumento de la temperatura en años muy cálidos, y un posterior agotamiento de oxígeno hipolimnético en lagos alpinos de la región Mediterránea (Jankowski *et al*., 2006). Además, se requerirá una mayor cantidad de energía (p. ej. fuertes tormentas de viento) para inicializar la mezcla completa, ya que el calentamiento probablemente inhibirá la mezcla en aguas profundas y templada (Ambrosetti *et al*., 2010, Rempfer *et al*., 2010).

La magnitud y dirección de los cambios observados en la biota dependen en gran medida de los diferentes umbrales de intensidad de cada factor estresor, de la tolerancia específica de la especie y de los mecanismos de compensación en los diferentes niveles de organización de las comunidades biológicas (Srivastava y Vellend, 2005). Los lagos de alta montaña sitúa probablemente a los recursos nutritivos como los principales mecanismos de control de la red trófica, dado su naturaleza oligotrófica y bajos aportes de nutrientes tanto minerales como orgánicos (Reche *et al*., 2001). En lagos de alta montaña de Sierra Nevada se ha observado un predominio del control del zooplancton en años más secos (en inglés "*top-down control*"), y una regulación del nivel trófico inferior (en inglés "*bottom-up control*") en años más húmedos, a consecuencia de una mayor limitación de nutrientes (Morales-Baquero *et al*., 2006b; García-Jurado *et al*., 2011). De esta manera, bajo un escenario de calentamiento global, los productores secundarios pueden llegar a ser uno de los principales mecanismos de regulación de los productores primarios. Sin embargo, un aumento de la producción primaria (p. ej. debido a la entrada de nutrientes por deposición atmosférica o un período de crecimiento mayor) también puede generar mayores tasas de reproducción de productores secundarios.

Paleolimnología: herramienta para la reconstrucción de ambientes pasados

La evaluación de los principales factores del cambio global y sus efectos en los ecosistemas requiere el uso de bases de datos extensas en el tiempo, proporcionando información valiosa sobre el modo y el ritmo con que se están produciendo dichos cambios. Sin embargo, los cambios en los ecosistemas y la respuesta de la biosfera a múltiples factores de estrés, usando exclusivamente estas bases de datos impide determinar la variabilidad de los cambios ambientales pasados y el seguimiento de su trayectoria, ya que estas series ofrecen perspectivas temporales relativamente cortas a veces de pocas décadas. Además, estas bases de datos no están disponibles en la mayoría de los sistemas, y en aquellos sistemas donde si lo están, sólo se presentan un reducido número de variables relacionadas con los procesos del ecosistema (Smol, 2008; Battarbee *et al.*, 2012). De este manera, la Paleolimnología es la única herramienta útil que puede proporcionar una visión complementaria y global, con una perspectiva temporal mucho más larga (Axford *et al.*, 2009).

La huella de los cambios climáticos puede quedar registrada en el sedimento de los lagos, y el análisis de sus diversas variables físico-químicas y biológicas a lo largo del tiempo nos permite realizar una reconstrucción de las condiciones del sistema y las características de la comunidad biológica.

La composición y abundancia de restos de organismos indican diferentes condiciones de composición iónica y pH, salinidad y estado trófico del sistema (Smol, 2010). En estas inferencias resultan de gran utilidad las diatomeas, cladóceros o quironómidos como indicadores biológicos para el cambio ambiental (Korhola 1999; Battarbee, 2000; Heiri & Lotter, 2003), de los cuales los dos últimos desempeñan un papel fundamental en esta tesis y se discuten más detalladamente en los capítulos específicos.

Además, una reconstrucción fiable de las condiciones ambientales del pasado depende del conocimiento de la biología de las especies de estos microfósiles. Así, se han generado ecuaciones de transferencia para inferir condiciones ambientales pasadas en base a presencia/ausencia y abundancia de las especies consideradas bajo diferentes condiciones ambientales actuales (Mackay *et al*., 2005; Heiri *et al*., 2011). Por otro lado, la determinación de especies en el sedimento superficial y su relación con las condiciones ambientales recientes suponen una alternativa a los programas intensos de monitoreo, y proporcionan una visión más completa de las distribución de la biota, teniendo en cuenta no sólo los factores de escala global o regional sino también las características específicas de los sistemas (factores locales) donde se encuentran. Estos trabajos ayudan a comprender mejor la interpretación paleolimnológica de dichas variables biológicas. En el caso específico de la comunidad algal, existe otro atributo complementario y de gran importancia, los pigmentos fotosintéticos. La diversidad y concentración de pigmentos fotosintéticos en sedimentos provee información sobre la composición histórica de grupos biológicos que no se depositan como microfósiles (Leavitt *et al*., 1993; Reuss, 2005).

La aplicabilidad de la Paleolimnología depende en gran medida de dos factores: 1) del uso de diversas aproximaciones y de la sensibilidad y precisión de las distintos indicadores que se usen para la reconstrucción del clima y condiciones ambientales pasadas, y 2) del grado al cual las señales climáticas en el registro sedimentario estén oscurecidas por ruido procedente de otras perturbaciones o influencias humanas (Battarbee *et al*., 2002, Catalan *et al*., 2013). Por lo tanto, existe la necesidad de centinelas biosféricos del cambio global (Williamson *et al*., 2009; Adrian *et al*. 2009). Los ecosistemas en sitios aislados de la presión humana inmediata pueden proporcionar una visión mucho más completa de estos cambios.

Los sistemas más apropiados para estudiar cambios climáticos (pasados y presentes) y sus efectos son ecosistemas remotos, prístinos, como son los lagos árticos y antárticos y los de alta montaña por encima de la línea de árboles, lagos que no han sufrido graves perturbaciones en la cuenca y no estén afectados en gran medida por la polución del aire (p. ej. lluvia ácida), siendo especialmente sensibles al calentamiento climático (Hauer *et al*. 1997, Sorvari y Korhola, 1998, Smol *et al*. 2005.) Estos ecosistemas pueden servirnos de torres de vigía proporcionando oportunidades únicas para abordar estudios de respuesta climática (Battarbee *et al*., 2002). De esta manera, los efectos del cambio global en la biota, así como en las características hidrológicas y biogeoquímicas, se han documentado en el Ártico (Douglas *et al*., 1994, Smol *et al*., 2005, Rühland *et al*., 2013) y en sistemas de alta montaña (Sommaruga-Wögrath *et al*. 1997; Hundey *et al*., 2014).

Más concretamente, las técnicas paleolimnológicas se han utilizado eficazmente en las regiones de alta montaña para evaluar los efectos el cambio climático (Catalan et al., 2013), el transporte de nutrientes y contaminantes (Camarero *et al*. 2009; Camarero y Catalan 2012), la acidificación (Stuchlík *et al*., 2002), cambios en la vegetación e historia de los incendios (Anderson *et al*., 2012;), cambios en las condiciones de humedad (Jiménez-Espejo *et al*., 2014), etc.

En este sentido, las lagunas de Sierra Nevada cumplen especialmente estos requisitos y se revelan como sistemas de referencia para este tipo de investigación, puesto que son sistemas de alta montaña situados por encima de la línea de árboles donde no han existido asentamientos humanos y la influencia de la polución ambiental es débil, generándose ambientes de sedimentación uniformes e inalterados. Además, su posición meridional y su enclave geográfico particular, entre dos regiones biogeográficas muy diferentes (Europa y África), los sitúa como lugares de referencia ideales en el seguimiento del cambio climático global.

Research objectives

Research objectives

The main objective of this thesis is to determine the effects of global change in the Mediterranean alpine lakes and environments (Sierra Nevada) over the last ~200 year by analyzing the sediment record. To address this, we use multiple biogeochemical variables to provide an integrated and comprehensive view of the effects of these multiple factors on in-lake and catchment related processes. More specifically, the following objectives are addressed in this thesis:

In **Chapter 1**, we seek to understand the multiple factors that influence changes in algal communities, combining the effects associated to recent global warming and those related to an probably catchment alteration by analyzing geochemical variables. To address this, we focus on the changes in sedimentary photosynthesis pigments in Río Seco Lake.

In **Chapter 2**, we evaluated the extent that the in-lake and catchment-related processes in Río Seco Lake are linked to direct and indirect climate-driven changes. To do this, we provide a high-resolution paleolimnological reconstruction by the combination of multiple biological and geochemical variables, including sub-fossil chironomid assemblages and leaf waxes biomarkers (*n*-alkanes), among others.

In **Chapter 3**, we analyze the changes within the sedimentary cladoceran assemblages and sedimentary chlorophyll-*a* concentrations in six high-mountain lakes of Sierra Nevada. The principal objective is to know whether the nature, magnitude, direction and timing of cladoceran assemblages changes are consistent with recent warming. Moreover, we analyze whether the changes of *Daphnia* relative abundance and sedimentary chlorophyll-*a* are partially related to increased delivery of nutrient from Saharan dust.

In **Chapter 4**, we explore the influence of physical, chemical and limnological parameters in the distribution of sedimentary cladoceran assemblages under a context of global change. Here we address this issue by analyzing the composition of sedimentary cladocerans (including pelagic and littoral taxa) from surface sediment of a set of 17 alpine lakes.

Objetivos

El principal objetivo del presente proyecto de Tesis Doctoral es determinar los efectos de cambio global en los ecosistemas acuáticos mediterráneos de alta montaña de Sierra Nevada en una escala temporal de 200 años mediante el análisis del registro fósil de sedimentos. Para abordar esto, usamos múltiples variables biogeoquímicas ofreciendo así una visión integrada y completa de los efectos de los múltiples factores sobre las procesos relacionado con las lagunas y sus cuencas. Más concretamente, en esta tesis se abordan los siguientes objetivos específicos:

En el **capítulo 1**, determinamos los diferentes factores que pueden influir en los cambios de las comunidades algales, combinando los posibles efectos producidos por el calentamiento global y aquellos relacionado con una posible alteración de la cuenca analizado mediante variables geoquímicas. Para llevar a cabo este objetivo, nos centramos en los cambios de los pigmentos fotosintéticos fósiles en la laguna de Río Seco.

En el **capítulo 2**, evaluamos la medida en que los procesos relacionados con la laguna de Río Seco y su cuenca están vinculados a los cambios directos e indirectos provocados por el clima. Para ello realizamos una reconstrucción paleolimnológica de alta resolución mediante el uso de múltiples variables biológicas y geoquímicas, en el que se incluyen quironómidos fósiles, y biomarcadores de ceras foliares (*n*-alcanos), entre otras variables paleolimnológicas.

En el **capítulo 3**, analizamos los cambios en los conjuntos de cladóceros fósiles y las concentraciones de clorofila-*a* sedimentaria en seis lagunas de alta montaña de Sierra Nevada. El principal objetivo es conocer si la naturaleza, magnitud, dirección y momento de los cambios del conjuntos de cladóceros se relacionan con el calentamiento global. Además, estudiamos si los cambios en la abundancia relativa de *Daphnia* y clorofila-*a* sedimentaria están parcialmente asociados a la entrada de nutrientes asociados a la deposición atmosférica procedente del Sáhara.

En el **capítulo 4**, exploramos la influencia de los parámetros físicos, químicos y limnológicos en la distribución del conjunto de cladóceros fósiles bajo un escenario de cambio global. Se lleva a cabo este objetivo mediante el análisis de la composición de cladóceros fósiles (incluyendo los taxones pelágicos y litorales) procedentes de sedimento superficial de un conjunto de 17 lagunas alpinas.

Sistema de estudio

Sistema de estudio

El macizo montañoso de Sierra Nevada

El macizo montañoso de Sierra Nevada se encuentra en el sureste de la Península Ibérica entre 36° 55' y 37° 15° de latitud N y 2° 56' y 3° 38' de longitud E. Sierra Nevada pertenece a la cordillera Penibética situada en el extremo sur del sistema Bético, y se considera una de las cordilleras más altas del sur de Europa con más de un 20% situada por encima de los 3000 m sobre el nivel del mar. El macizo se extiende aproximadamente unos 80 km en sentido oeste-este, siendo el Mulhacén (3479 m), Veleta (3396 m) y Alcazaba (3366 m) sus picos más altos, así como los tres picos más altos de la Península Ibérica.

Geología y geomorfología

Sierra Nevada constituye una de las regiones glaciares europeas más meridionales durante el Pleistoceno tardío (Superior) (Gómez Ortiz *et al.*, 1996), siendo su actual paisaje fuertemente condicionado por la acción erosiva resultante de la morfodinámica glacial de dicho período (Schulte, 2002). La posterior retirada de los glaciares de circo llevó a la creación de pequeños lagos y humedales, que actualmente se encuentran ubicados en un área de unos 100 km^2 por encima de los 2600 metros sobre el nivel del mar, y se asientan sobre la roca madre metamórfica (principalmente esquistos) (Castillo, 2009). La elevación media de los lagos más importantes es de unos 2900 m; sin embargo la superficie y profundidad de dichos lagos son de dimensiones moderadas debido a la relativa dureza de los materiales geológicos frente a la acción erosiva de los glaciares.

Desde la última deglaciación, los procesos periglaciares controlan los procesos geomorfológicos en las zonas altas de Sierra Nevada, a pesar de que registros sedimentológicos (Oliva y Gómez-Ortiz, 2012) juntos con datos históricos (Gómez-Ortiz *et al.*, 2009) reportaron evidencias de la presencia de pequeños glaciares en los picos más altos de Sierra Nevada durante la pequeña Edad del Hielo. Siendo a su vez, la cordillera más meridional con glaciales durante el Holoceno en Europa (Schulte, 2002).

En la actualidad, no hay trazas visibles de glaciares en la zonas norte del macizo, sin embargo estudios recientes detectaron la existencia de permafrost en el corral del Veleta (3150 metros sobre el nivel del mar) bajo estratos de clastos sometidos a un continuo proceso de degradación (Gómez-Ortiz *et al.*, 2012; 2014), hasta su completa desaparición hace escasamente unos años debido al continuo aumento de las temperaturas durante las últimas décadas (Oliva *et al.* 2016).

El núcleo central de la sierra, donde se encuentran las cumbres más elevadas y las lagunas de mayor altitud, se desarrolla sobre los materiales del *Complejo Nevado-Filábride,* formado por rocas metamórficas y se incluye dentro de la unidad tectónica denominada *Unidad Veleta*. Dicha unidad está constituida por micaesquistos oscuros y cuarcitas, formadas por un metamorfismo de grado bajo (bajas temperaturas y presiones) (Martín-Martín *et al.*, 2010).

Aspectos climáticos y meteorológicos

El clima regional del sur de España se engloba dentro del clima mediterráneo, caracterizado por un fuerte contraste estacional con veranos calurosos y secos, e inviernos suaves y húmedos. Sin embargo, dentro de esta misma zona, un estudio más detallado conduce a cierta regionalización climática, como la que presenta Sierra Nevada, propia de un clima de montaña. En general, el clima de Sierra Nevada se caracteriza por una marcada insolación y aridez. Las condiciones climáticas en el macizo montañoso están determinadas por una marcada estacionalidad, que se presenta entre una estación cálida y seca (de mayo a septiembre) y una estación fría y húmeda (de octubre a abril). Su posición meridional, hacen de Sierra Nevada un sistema montañoso bastante seco, en invierno las precipitaciones son en forma de nieve, mientras en verano la pluviosidad es escasa. De esta manera, Diciembre suele ser el mes más húmedo, y Julio, seguido de Agosto se consideran los meses más secos (Castillo *et al.*, 1996). Sin embargo, episodios intensos de granizadas suelen producirse a final de primavera y en verano (con un máximo entre los meses de Julio y Octubre).

En el caso de altas cumbres, las estaciones meteorológicas del Albergue Universitario (2507 m) y Cetursa Sierra Nevada S. A. (2170 m) subrayan una temperatura media anual de 4.4°C, y una precipitación de 700 mm/año, con más del 75% del total concentrada principalmente en los meses más fríos en forma de nieve por encima de los 2000 m (Castillo *et al.*, 1996). Febrero se considera el mes con el máximo nival, tanto en espesor de nieve número de días como número de días (Castillo *et al.*, 1996). Los patrones de precipitación regional están fuertemente influenciados por las fuentes de humedad atlántica occidental y la precipitación orográfica (Anderson *et al.*, 2011). Los régimen de los vientos establece vientos predominantes en dirección noroeste durante el invierno, y vientos del sur y del suroeste durante el verano (Anderson *et al.*, 2011).

Los recursos hídricos medios drenados (principalmente superficial y subterráneamente) por el sistema se estima en unos 750 hm^3/año. Dichas aportaciones pluviométricas se caracterizan por una marcada irregularidad espacial ligada a la fuerte influencia orográfica del macizo y una irregularidad temporal, asociada a las precipitaciones propias del clima mediterráneo (Castillo *et al.*, 1996). Existe un tipo de flujo subsuperficial, especialmente importante en cotas superiores a los 2.000 m de altitud. Estas zonas están intensamente afectadas por la acción ejercida por procesos glaciares y periglaciares, y la lenta liberación de agua por el deshielo suele favorecer los procesos de infiltración (Castillo *et al.*, 1996). Esta red de drenaje da lugar a surgencias con distintos grados de abundancia y duración sobre todo en el período de deshielo, de las cuales las más permanentes son denominadas en el argot local como "borreguiles" generando a veces extensas praderas hidrófilas próximas a las lagunas (Castillo y Fedeli, 2002).

Vegetación de alta montaña

La composición de la vegetación en Sierra Nevada está fuertemente influenciada por los gradientes térmicos y por la precipitación (Fletcher *et al.*, 2010). A día de hoy, la línea de árboles se alcanza a los 2.550 m (Anderson *et al.*, 2011). Los suelos están muy pobremente desarrollados y no han soportado ni agricultura ni uso forestal (Morales-Baquero *et al.*, 2006a). En la zona de cumbres, por encima de los 2900 m, las extremas condiciones climáticas caracterizadas por una intensa radiación solar y nevadas, fuertes vientos, inviernos muy fríos junto con temperaturas muy bajas y una estación de crecimiento corta condicionan un limitado desarrollo de la vegetación (Valle, 2003). A efectos de una meteorización del sustrato, la escasa cobertura vegetal no impide la intensa acción de los procesos glaciares y periglaciares por encima de los 2.500 m, mencionados previamente. Esta cobertura vegetal (flora crioromediterránea) está determinada por un gran número de especies endémicas (Molero-Mesa y Pérez-Raya, 1987) cuya cobertura es escasa y suele está localizada en los suelos de los valles y a las orillas de las lagunas (borreguiles) (Oliva *et al.* 2009). Estudios recientes establecen un descenso en la riqueza de especies endémicas en las zonas de altas cumbres y cambios en su abundancia (Fernández-Calzado y Molero-Mesa, 2013), además de un patrón creciente y gradual de plantas vasculares endémicas desde la cima más baja a la más alta debido a los efectos del cambio climático (Fernández-Calzado *et al.*, 2013).

Importancia ecológica y su conservación

El macizo montañoso de Sierra Nevada es una espacio de alta montaña de enorme valor ambiental. Sierra Nevada fue declarada Reserva de la Biosfera en 1986, Parque Natural en 1989 y Parque Nacional en 1999. Esta última categoría de protección supuso una acción clave para la conservación de sus valores naturales y culturales. El especial atractivo de este espacio se debe principalmente a su altitud, paisaje y morfología glacial, siendo las lagunas de especial importancia entre los elementos paisajísticos y morfológicos (Castillo *et al.*, 2005).

Este complejo de lagunas de alta montaña en Sierra Nevada representa todavía uno de los pocos reductos de ecosistemas prístinos que actualmente conservan gran parte de sus características originales, únicamente afectados por los propios mecanismos de regulación natural (Reche y Casamayor, 2011). Debido a las duras condiciones climáticas, que a su vez impiden un asentamiento permanente y a la escasez de recursos naturales susceptibles de explotación entre otros factores, estos ecosistemas no han sufrido una influencia antrópica intensa durante el pasado. Entre estos signos de actividad humana destacaron la práctica de la trashumancia con ganado ovino y vacuno bordeando normalmente los borreguiles, la intensa influencia de un refugio de montaña cerca de la laguna de Río Seco que estuvo operativo durante tres décadas (1967-1997), o la construcción de la carretera más altas de la Península Ibérica entre 1964-1965 que conectaba la cara norte del macizo con la Alpujarras, situada relativamente cerca de lagunas como Aguas Verdes, Río Seco y Río Seco Superior. Su especial atractivo turístico hizo que, tras la declaración como Parque Nacional, se tomaran medidas restrictivas de acceso, cerrando el tránsito de vehículos por la carretera y demoliendo dicho refugio, dada la aceleración que suponía los procesos erosivos por la creciente afluencia de visitantes. Estas lagunas y humedales de alta montaña en Sierra Nevada han sido sistema de estudio de múltiples proyectos de investigación en un esfuerzo por aumenta el conocimiento de su dinámica y funcionamiento, así como contribuir a su gestión y conservación.

Lagunas de estudio

Existen unas 50 lagunas de origen glacial en Sierra Nevada comprendidos ente los 2800 y 3100 m sobre el nivel del mar, de las cuales 42 son de aguas relativamente permanentes, situadas la mayor parte en la vertiente Sur. En la vertiente Norte, tan solo 10 lagunas son consideradas de aguas estables (Castillo, 1993).

En esta tesis se seleccionaron estratégicamente 17 lagunas teniendo en cuenta la ubicación de las mismas en diferentes valles para caracterizar la mayor heterogeneidad regional posible en Sierra Nevada. Para el Capítulo 3, se tuvo en cuenta también que los cuerpos de agua fueran permanentes con profundidades superiores a 2 metros y que no presentaran un fondo rocoso, con el fin de obtener un registro sedimentario de alta calidad y resolución. Las lagunas seleccionadas (junto con sus correspondientes nomenclaturas) fueron: 1) la laguna de Río Seco (RS) ampliamente estudiada en esta tesis e incluida en todos los capítulos, 2) Cuadrada (CD), Aguas Verdes (AV), Río Seco Superior (RSS), Mosca (MC) y Borreguil (BG) analizadas en el Capítulo 3 mediante un testigo de sedimento largo y de alta resolución y 3) Caballo (CB), Nájera (NJ), Lanjarón (LJ), Ermita Baja (EB), Ermita Media (EM), Virgen Superior (VS), Virgen Media (VM), Majano (MJ), Culo Perro (CP), Hondera (HN) y Peñón Negro (PN) analizadas junto con las anteriormente mencionadas mediante un testigo de sedimento superficial.

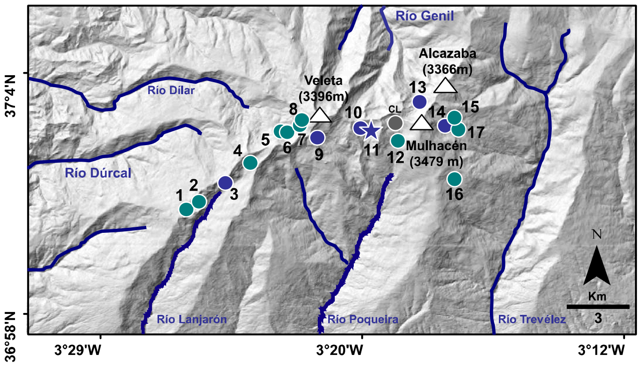


Figura 5. Localización de las lagunas estudiadas en esta tesis. Los círculos azules representan las lagunas incluidas en el ***Capítulo 3*** (testigos de sedimento de mayor profundidad). Los círculos verdes representan las lagunas estudiadas únicamente en el ***Capítulo 4*** (testigos de sedimento superficiales). También se ha analizado datos de la laguna de la Caldera (CL) en éste capítulo (círculo gris). La laguna de Río Seco se representa con una estrella, ampliamente estudiada en esta tesis e incluida en todos los ***Capítulos 1-4***. Las enumeración de las lagunas es la siguiente: 1. Laguna del Caballo; 2. Laguna de Nájera; 3. Laguna Cuadrada; 4. Laguna de Lanjarón; 5-6. Lagunas de la Ermita Baja y Media; 7-8. Lagunas de la Virgen Superior y Media; 9. Laguna de Aguas Verdes; 10. Laguna de Río Seco Superior; 11. Laguna de Río Seco; 12. Laguna del Majano; 13. Laguna de la Mosca; 14. Laguna de Borreguil; 15. Laguna Culo Perro; 16. Laguna de Peñón Negro; 17. Laguna Hondera.

Las lagunas de estudio se encuentran en un radio de 13 km desde la laguna del Caballo a Laguna Hondera; y la mayoría se ubican en la cara sur del macizo a excepción de las lagunas Ermita Baja y Media, Virgen Media y Superior, y Mosca (Figura 5). Las lagunas de estudio son cubetas someras (profundidad máxima <10 m) y pequeñas (áreas de la superficie <1 ha), aunque el tamaño individual de las lagunas y la profundidad máxima varían significativamente con la precipitación y la temperatura anual (por ejemplo, Villar-Argaiz *et al*, 2001, Sánchez-Castillo *et al.*, 2008). Las lagunas permanecen cubiertas de hielo durante 8-9 meses al año, normalmente desde noviembre hasta julio, siendo los aportes hídricos procedentes del deshielo y la escorrentía superficial. Sin embargo, las duración de la cobertura de hielo se han relacionado con las variaciones anuales de las condiciones climáticas (Barea-Arco *et al.*, 2001; Morales-Baquero *et al.*, 2006a; Pérez-Martínez *et al.* 2007) pudiendo existir grandes diferencias interanuales.

La mayoría de los lagunas se sitúan en un circo glacial y presentan una cuenca abierta. Aunque la presencia de afluentes y efluentes naturales superficiales dependen de las características específicas de cada laguna como la localización o el tamaño relativo del área de captación, la mayoría de las lagunas estudiadas presentan entrada y salida de aguas. De entre las lagunas que suelen reciben aguas superficiales durante todo el período libre de hielo se encuentran las lagunas de Virgen Superior o Aguas Verdes, a pesar de ser ésta última, la que se sitúan a mayor cota (3030 m de altitud) con respecto a las demás lagunas presentes en el estudio. En relación a los efluentes, pueden presentarse en forma de emisarios como el caso de la laguna de Río Seco y otros en forma de chorreras, como la laguna Hondera, aunque en años secos este tipo de aliviaderos suelen ir desapareciendo con el avance del período libre del hielo. Debido a la naturaleza dura del sustrato geológico sobre el que se asientan, en general micaesquistos, no existen acuíferos y por lo tanto, los aportes de agua subterráneas son mínimos. Sin embargo, se puede hablar de la existencia de un reducido flujo subsuperficial generado de entre las fracturas de micaesquistos que puede general cierto aporte a las lagunas. De igual manera, puede existir pérdida de agua en algunas cubetas debido a la existencia de estas fracturas, sumado a la pérdida generada por evaporación.

Estas lagunas presentan normalmente praderas de pastizales de alta montaña (borreguiles) a excepción de la Cuadrada (Figura 6 y 7).

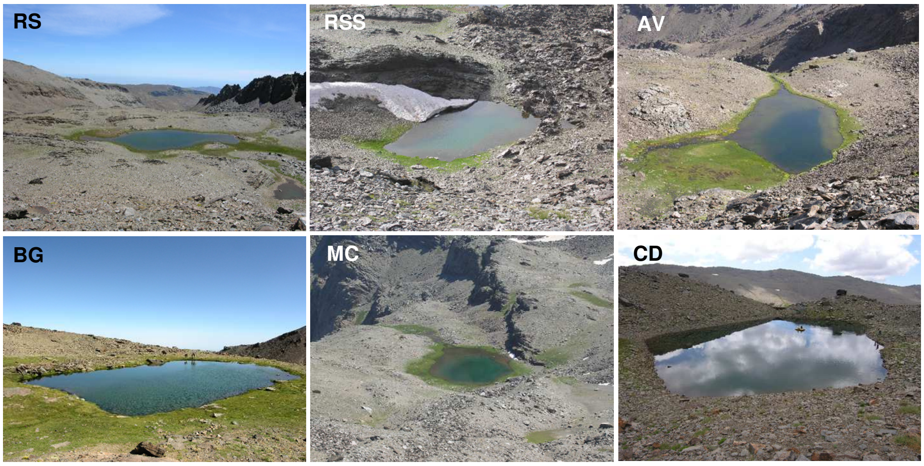


Figura 6. Las lagunas de estudio analizadas en el Capítulo 3 mediante un testigo de sedimento largo y de alta resolución. Lagunas de Río Seco (RS), Río Seco Superior (RSS), Aguas Verdes (AV), Borreguil (BG), Mosca (MC) y Cuadrada (CD). Las imágenes corresponden al mismo día de muestreo en agosto de 2011, excepto la laguna de Río Seco, cuyo muestreo fue realizado en septiembre de 2008.

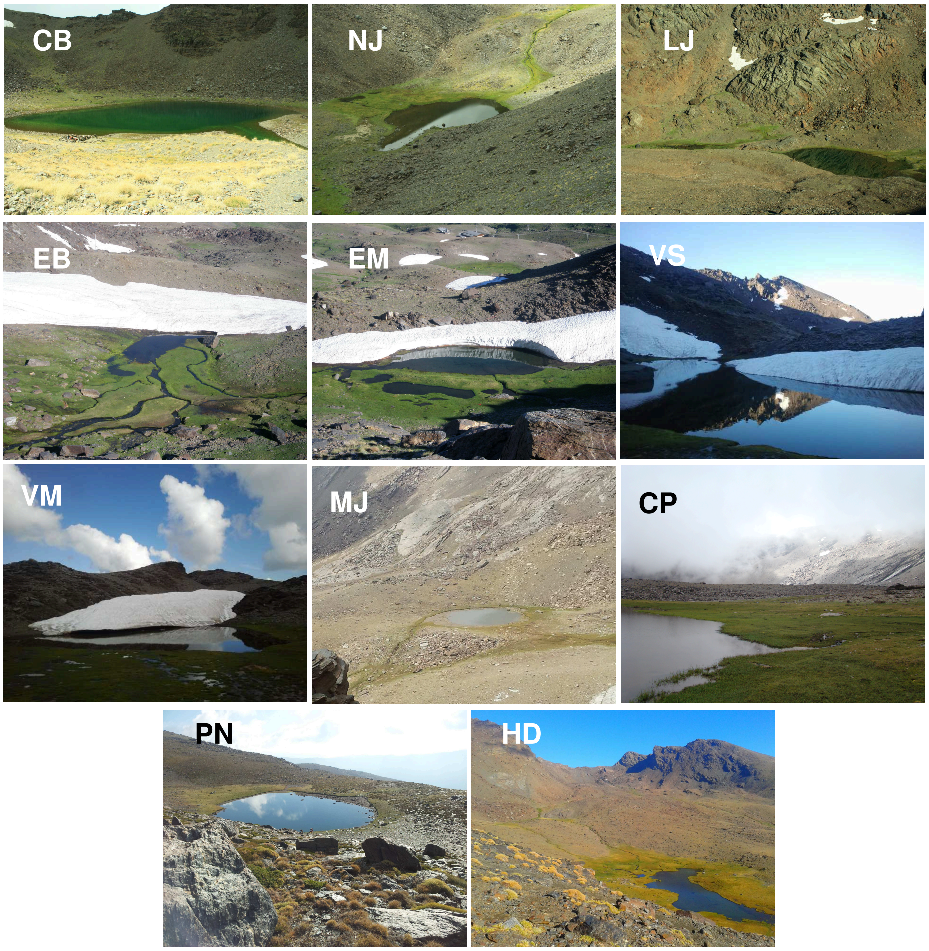


Figura 7. La lagunas de estudio analizadas en el Capítulo 4 mediante un testigo de sedimento superficial. Lagunas de Caballo (CD), Nájera (NJ), Lanjarón (LJ), Ermita baja (EB), Ermita Media (EM), Virgen Superior (VS), Virgen Media (VM), Majano (MJ), Culo Perro (CP), Peñón Negro (PN) y Hondera (HN). Las imágenes corresponden al mismo día de muestreo en el período libre de hielo en 2013.

Aspectos hidroquímicos

El conjunto de lagunas de estudio representan una particular tipología hidroquímica, normalmente condicionada por aspectos climatológicos, procesos biogeoquímicos, deposiciones atmosféricas, procesos biológicos y finalmente, variables hidrológicas como la evaporación o la tasa de renovación de sus aguas.

Las lagunas de Sierra Nevada son aguas débilmente mineralizadas, con baja concentración en sales y no presentan estratificación térmica. Las conductividad de las lagunas de estudio son igualmente muy bajas con valores comprendidos entre 7.4 y 60.4 μS cm^{-1} (datos de este estudio). La alta resistencia a la erosión hídrica del sustrato rocoso hace que el aporte de iones por disolución del sustrato sea reducido. Así como la baja variabilidad en la composición química debido a la naturaleza homogénea del sustrato. En cuanto a la composición aniónica, se trata de aguas bicarbonatas en las que los iones cloruro y sulfato se encuentra en bajas concentraciones (Pulido-Villena, 2004). Con respecto a la composición catiónica, el calcio es el mayoritario. El potasio, aunque también suele aumentar su concentración en verano, es más conservativo que el resto, siendo claramente minoritario. Así pues, las lagunas de Sierra Nevada presentan secuencias del tipo $[K^+] < [Mg^{2+}] < [Na^+] < [Ca^{2+}]$.

Las lagunas presenta baja alcalinidad y capacidad reguladora del pH. Sin embargo, son sistemas que presentan unas concentraciones de calcio y capacidad de tamponamiento relativamente más elevada que lo esperable dado la naturaleza rocosa sobre las que se asientan las cubetas, con valores de alcalinidad entre 0.02 y 0.35 meq L^{-1}, y de pH entre 6.2 y 7.8 (datos de este estudio).

Los lagos de alta montaña de Sierra Nevada son oligotróficos ya que reciben pocos aportes de nutrientes tanto minerales como orgánicos (Morales-Baquero *et al.*, 1999; Reche *et al.*, 2001). A su vez, la cantidad de sólidos en suspensión es baja, favoreciendo una visibilidad total del disco de Secchi, y presentan tasas de sedimentación bajas, propias de este tipo de ecosistemas de alta montaña.

Este conjunto de lagunas son ecosistemas potencialmente sensibles a la deposición atmosférica debido a su carácter oligotrófico. De esta manera, estas lagunas situados en un enclave geográfico particular, en el límite entre

Europa y África, dos regiones biogeográficas muy diferentes, junto con su posición meridional los sitúa como lugares de referencia ideales en el seguimiento del cambio climático global. Su localización, a menos de 2000 km del desierto del Sahara, hacen que reciban altas tasas de deposición de polvo atmosférico procedente del Sahara, suponiendo una fuente significativa de nutrientes minerales como nitrógeno, fósforo y calcio (Morales-Baquero *et al.*, 2006a; Pulido-Villena *et al.*, 2006; Reche *et al.*, 2009)

Aspectos biológicos

En las lagunas de estudio, las comunidades biológicas son extremadamente simple, siendo el zooplancton el máximo nivel trófico. El cladócero planctónico *Daphnia pulicaria* domina en términos de biomasa en aquellas lagunas en las que está presente con respecto a las demás especies como el copépodo *Mixodiaptomus laciniatus* que es muy abundante en la mayoría de las lagunas de Sierra Nevada. Las especies de fitoplancton suelen ser de pequeño tamaño y presentan altas tasa de renovación (Cruz-Pizarro *et al*. 1994).

Materials and Methods

Materials and Methods

The sediment coring, field measurements and sediment chronology are described in more details in this section for Chapter 1, 2 and 3 of this thesis.

Sediment coring and field measurements

Six sediment cores were collected from Río Seco Superior (RSS), Aguas Verdes (AV), Borreguil (BG), Mosca (MC) and Cuadrada (CD) during the summer of 2011 (except for Río Seco (RS) Lake, which was sampled in 2008) from the deepest area of each lake using a slide-hammer gravity corer (Aquatic Research Instruments, Hope, Idaho, USA) with an inner core-tube diameter of 6.8 cm. All cores, with the exception of RS, were extruded on-site at 0.25 cm intervals for the upper 5-10 cm, and then at 0.5 cm intervals from 5-10 cm to the base of the core (RSS: 8,5 cm; AV: 11,5 cm; BG: 11,25 cm; MC: 7,75 cm; CD: 11 cm). The sediment core retrieved from RS was sectioned into 0.5 cm intervals for the entire core (16 cm long).

On the other hand, surface sediment cores (1 cm) were collected during the summer of 2013, from Caballo (CB), Nájera (NJ), Lanjarón (LJ), Ermita Baja (EB), Ermita Media (EM), Virgen Superior (VS), Virgen Media (VM), Majano (MJ), Culo Perro (CP), Hondera (HN) y Peñón Negro (PN) from the deepest basin of each lake. The top 1-cm of sediment (representing approximately 2–5 years of accumulated material) was collected from each lake, except for RS, RSS, AV, BG, MC and CD, which average values (from 0 to 1 cm) were calculated (see Figure 3.2 of Chapter 3 and Appendix E).

Following extrusion, sediment samples were immediately sealed in sterile Whirlpak® bags, wrapped in a dark bag and placed in a cooler until they were transported to the University of Granada (Spain) where they were stored in a cold room at ~4 ºC until analysis. Subsampling was performed at each intervals at the laboratory, except for the sedimentary photosynthetic pigments analysis for RS, which was frozen (-80ºC).

Tube samplers (6.7 cm diameter) of different lengths were used to collect the whole water column in an integrated sample from the deepest point of each lake and analyzed for a suite of limnological variables following the techniques detailed in Barea-Arco *et al*. (2001) and Morales-Baquero *et al*. (2006b). For nutrient concentration analyses and, alkalinity and calcium determination, water samples were collected in acid-washed polyethylene bottles and immediately cooled. For dissolved organic carbon (DOC) analyses, samples were stored in precombusted amber glass bottles at approximately 4ºC in the dark until laboratory analysis. Specific conductivity and pH were measured on site with a Waterproof PC 300 meter. For pH and conductivity, equipment was calibrated at each lake prior to measurements. Water for nutrient analyses was frozen until analysis.

Figure 8. Left: a photo of core sampling in RS during September 2008; right: a photo of the core extruding in Río Seco Lake.

Analytical methods

Water chemistry analyses

Dissolved calcium (Ca) concentration was analyzed by atomic absorption. Dissolved organic carbon (DOC) concentrations were carried out by thermal oxidation with a ShimadzuTOC-5000 equipped with a Shimadzu platinised-quartz catalyst (Mladenov *et al*., 2008). Previous to the analyses Ca and DOC samples of all the study lakes where filtered through precombusted (2 h at 500 ºC) Whatman GF/F glass fiber filters (20µm).

Concentrations of silicon oxide (SiO_2), total nitrogen (TN) and total phosphorus (TP) were measured according to standard methods [American Public Health Association (APHA), 1995]. TN and TP measurement were measured in unfiltered water samples after acidic digestion in an autoclave for 2h at 120ºC. Total alkalinity was measured according to titration method (APHA, 1995).

Lake-morphometric data

Catchment, meadow and lake surface area for each lake were obtained from Morales-Baquero *et al.* (1999) and also by analyzing images captured from Google Maps with a microscope imaging software (Leica Application Suite).

Sediment chronology

Sediment cores from RSS, RS, AV, BG, MC and CD were dated using gamma spectroscopy to measure the activities of radioisotopes and establish a chronology for the past ~150 years. For each core, a selection of 15-20 sedimentary intervals was analyzed for ^{210}Pb activity following the technique outlined in Schelske *et al.* (1994). Chronologies for each core were calculated from excess ^{210}Pb activities using the constant-rate-of-supply (CRS) model (Appleby & Oldfield, 1978). Additionally, ^{137}Cs was used as an independent chronological marker of 1963 (global nuclear weapons testing ban) and used to corroborate the ^{210}Pb dates (Appleby, 2001). The RS core was analyzed at the Center for Research, Innovation and Technology (CITIUS), University of Seville, Spain and the other cores were analyzed at the Paleoecological Environmental Assessment and Research Laboratory (PEARL), Queen's University, Kingston ON, Canada using the same methodology (see Appendix E for details on dated intervals).

Sedimentary proxy record

The sedimentary proxy record are described in more details in the specific data chapters of this thesis. See table 1 of this section to view correspondence from each sedimentary proxy and specific data chapters.

Biological proxies

Sedimentary photosynthetic pigments

The photosynthetic pigments were analysed according to Airs *et al.*, (2001). In brief, thawed samples (between 0.92 and 2.05 wet/wt) were extracted in 10 mL of acetone (100 %) by sonication (10 min, 50/60 Hz, Ultrason-H, Selecta) under darkness and then centrifuged (5 min, 3500 rpm) to remove cellular particle debris. The supernatant was filtered through a cotton wool plug. This was repeated up to a maximum of six times until the supernatant was colourless. The pigment extracts were dried under an N2 stream and then stored at 4 ºC until ready for analysis with high-performance liquid chromatography (HPLC). Before the HPLC analysis, the acetone extracts were dissolved in 500 µL of MeOH (10% AcNH4 5 M) and 40 µL were injected. The HPLC system was equipped with a 1525 Binary Pump separation module and 2996 photodiode-array detector (Waters, Milford, USA). Chromatography analyses were performed with two Spherisorb S3ODS2 columns (4.6 × 150 mm, 3 µm, Waters) using a Spherisorb ODS2 guard cartridge (4.6× 10 mm, 5 µm, Waters) to maintain the performance of the analytical column. The pigments were eluted using a mobile phase gradient starting with mixture A (MeOH: AcN: AcNNH4 0.1 M, 80:15:5) for 5 min, and then from t = 5 min to t = 100 min, a linear gradient from 100% mixture A to 100% mixture B (MeOH: AcN: ethyl acetate, 20:15:65) at a constant flow of 0.7 mL/min and following the method of Airs *et al.* (2001).

Subfossil Cladocera

Subfossil cladoceran samples were analyzed and identified using the methods described in Szeroczyńska & Sarmaja-Korjonen (2007). For each sediment core interval, ~1 cm^3 of fresh sediment was heated for 20 minutes in 10% KOH to remove humic matter, and then washed and sieved through a 38 µm-mesh under tap water. The samples were centrifuged (5 min, 3000 rpm) to concentrate the fossil cladoceran remains, and mounted onto microscope slides using glycerol gelatin dyed with safranin. A minimum of 200 remains (carapaces, headshields, postabdomens, postabdominal claws, valves, shells and ephippia) were identified and enumerated from each sediment interval under a light microscope at 200x magnification (Kurek *et al.*, 2010). The most abundant remain from each taxon was used to calculate the number of individuals present in the sample (Frey, 1986). Counts of individual cladocerans are presented as relative abundances and all the cladoceran taxa from each site are included in the stratigraphic plots.

Subfossil chironomids

For chironomid analysis, samples (~0.3 g dry weight) for each interval of sediment were immersed in 10 % KOH for 2-3 hours and subsequently sieved through a 100 µm fraction. The head capsules were separated under a dissection microscope. Chironomids were prepared in Eurapal mounting medium after dehydration and identified at 100-400x magnification with a compound microscope.

Subfossil diatoms

In this thesis we use the PCA axis 1 sample scores of diatoms (Chapter 2) to compare with the rest of the proxies. In brief, the sediment samples were acid digested and the siliceous remains were mounted in slides with Naphrax for counting (Battarbee *et al.*, 2001). A minimum of 300 diatom frustules were identified and enumerated in each interval, using a light microscope at 1000x and differential interference contrast (DIC).

Sedimentary chlorophyll-*a*

For each core (RSS, RS, AV, BG, MC and CD), sedimentary chlorophyll-*a* concentrations were 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 *et al.* (2005, 2010).

Fossil pollen

For fossil pollen, a modified Faegri & Iversen (1989) procedure was followed using 1 cm^3 of sediment. Processing included pre-treatment with $(NaPO_3)_6$ 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 using a light microscope.

Organic geochemical proxies

Loss on ignition

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

$$1)\ LOI_{550}=((DW_{105}-DW_{550})/DW_{105})*100$$

In a second step, the carbonate matter is calculated as:

$$2)\ LOI_{950}=((DW_{550}-DW_{950})/DW_{105})*100$$

where LOI_{550} and LOI_{950} are the LOI at 550ºC and 950ºC respectively (both as a percentage), DW_{105} is the dry weight of the sample before the organic carbon combustion, DW_{550} and DW_{950} are the represents the dry weight of the sample after heating to 550ºC and 950ºC, respectively (all in grams).

Carbon/nitrogen (C/N)

Analysis of total and inorganic carbon, and nitrogen content were carried out by combustion on a CARLO ERBA EA 1108 CHNSO Elemental Analyzer system. Correcting by the organic fraction was determined as the difference between the total and the inorganic carbon fraction (Meyers & Teranes, 2001). The carbon/nitrogen (C/N) ratio was calculated from the mass data and expressed as atomic ratio.

Carbon isotope

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 24 h. 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 analysed using a mass multicollector spectrometer (Isoprime; GV Instruments) equipped with a EuroVector elemental analyser (mod. Euro EA 3000) and continuous flow inlet. The results are expressed as $\delta^{13}C_{org}$ described as:

$$\delta^{13}C_{org} = (R_{sample}/R_{standard}-1)*1000$$

where R equals $^{13}C/^{12}C$.

Data are reported 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 ‰.

n-Alkanes

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 means of 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. The *n*-alkanes were analysed 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 (C16, C18, C19, C20, C23, C25, C26, C28, C30, C32, and C37) was measured every five samples. The measurement error was lower than 1.5%.

Granulometry

Prior to the grain size analyses, 0.5 g wet sediment samples were extracted and sieved through 150 µm mesh; the size was determined as the cumulative mass percent by X-ray diffraction (XRD) using GALAY model CIS-1, which measures particles ranging from 0 to 150 µm.

Major and trace elements

Al was measured by atomic absorption spectrometry (AAS) using a Perkin-Elmer 5100 spectrometer. Zr was performed using Inductively Coupled Plasma–Mass Spectrometry (ICP–MS) after digestion in HNO_3 (65%) + HF (48%) of 0.1 g of sample powder in a Teflon-lined vessel for 150 min at high pressure and temperature, evaporated to dryness, and subsequent dissolved in 100 ml of 4 vol.% HNO3. Instrumental measurements were carried out in triplicate with a NexION 300d (Perkin Elmer) spectrometer.

	Method	**Chapter**
Dating of sediment	Gamma spectroscopy	1, 2, 3
Pigments	High performance liquid chromatography	1
Cladocera	Microscope	1, 2, 3, 4
Chironomids	Microscope	2
Diatoms	Microscope	2
Chlorophyll-*a*	Visible reflectance spectroscope	2, 3
Pollen	Microscope	2
Organic matter content	Loss on ignition (LOI) at 550ºC	1, 2, 4
C and N content	Elemental Analyzer	1, 2
Isotopes carbon	Mass spectrometer	2
n-Alkanes	Gas-chromatograph	2
Granulometry	X-ray difraction	1
Major elements (Al)	Atomic absorption spectrometry	2
Trace elements (Zr)	Inductively Coupled Plasma–Mass Spectrometry	2

Table 1. Analysis for sediment cores subsamples in the sediment of study lakes.

Instrumental climate data

The instrumental climate data is described in more details in this section for Chapter 1, 2 and 3 of this thesis.

Temperature and precipitation series

Long-term temperature and precipitation records do not exist for Sierra Nevada summits. The closest instrumental data for the high mountain lakes comes from meteorological stations located under 2000 m a.s.l. and they cover no more than the past 50 years. Moreover, these data series are not reliable because of the numerous gaps and poor quality of the information. One of the longest temperature series in the Sierra Nevada is provided by Cerecillo station (Láujar, Almería), which is located at 1800 m a.s.l. and has offered data since 1960, although there are numerous gaps. A strong correlation was found between the Cerecillo temperature series and short homogenized series of mean annual temperatures from meteorological stations (http://www.aemet.es) in low areas, but less than 20 km from the Sierra Nevada summit (Armilla $r = 0.67$, $p < 0.001$, $n = 35$; Lanjarón $r = 0.78$, $p < 0.001$, $n = 35$).

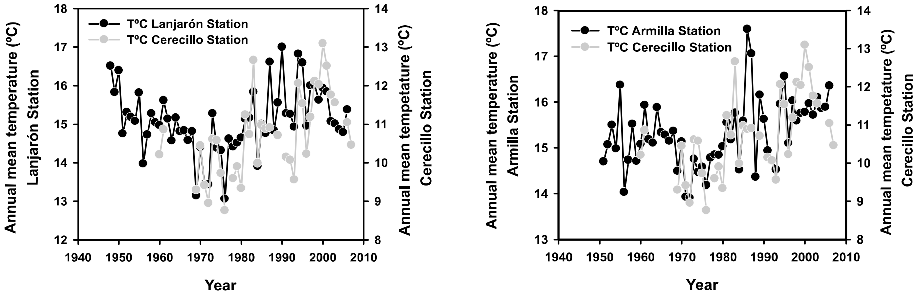


Figure 9. Trend of mean annual temperature of Cerecillo, Lanjarón and Armilla stations.

Because Armilla and Lanjarón are short series, we also analysed the annual mean air temperatures of ten longer homogenized series from Central and South climate stations across Spain (Staudt *et al.*, 2007). We also found strong correlations (all *r* > 0.60) between the available data from the Cerecillo, Armilla, and Lanjarón stations and the long-term temperature series of Staudt *et al.* (2007). This indicated that the annual trends in these long series can be considered representative of the Sierra Nevada region, especially those located in the central and southwest areas of Spain (Cerecillo all r > 0.66, *n* = 35; Armilla all r > 0.82, *n* = 51 and Lanjarón all r > 0.68, n = 54). One of the strongest correlations found was with the Madrid temperature series (AEMET 3195), which dates from 1869, and this series was used to represent annual mean temperature trends in the Sierra Nevada.

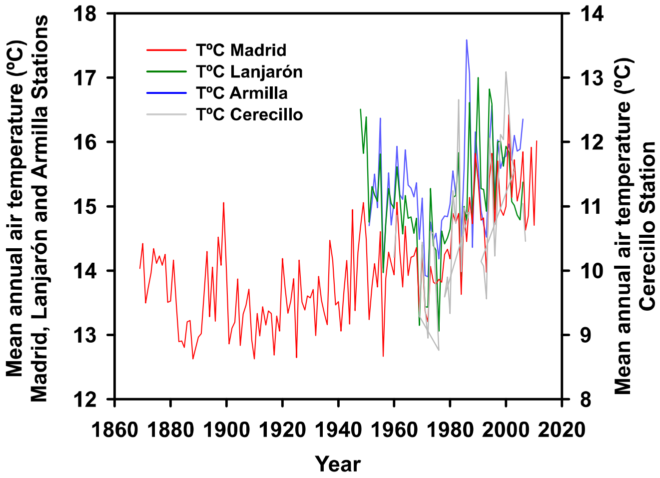


Figure 10. The mean annual air temperature (ºC) from Madrid (red line), Lanjarón (green line), Armilla (blue line) and Cerecillo (grey line) climate stations.

There are no useful series of precipitation data at the Sierra Nevada summit. Therefore, we analysed the correlation between a short homogenized series of total annual precipitation data from Armilla (since 1940) and five long series from southern Spain. The best correlation was obtained with the series of San Fernando (Naval Base of the Spanish Army, Cádiz, since 1839 $r = 0.62$, $p < 0.001$, $n = 65$); hence, this series was used to represent total annual rainfall trends in Sierra Nevada. Esteban-Parra *et al.* (1997) found that the time course of precipitation is similar among the different areas in the south of Spain.

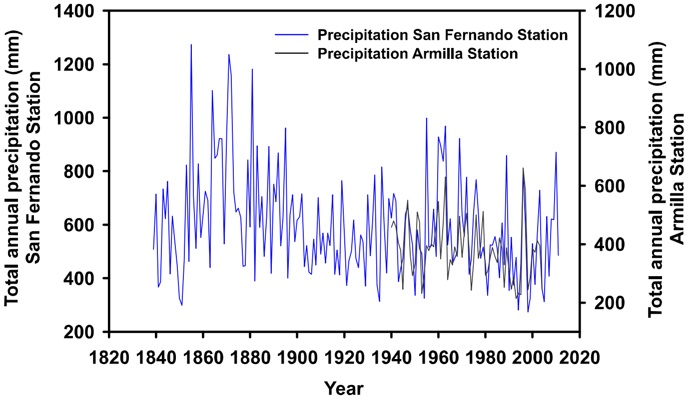


Figure 11. The annual precipitation (mm) from San Fernando (blue line) and Armilla (black line) climate stations

Thus, we use MAAT Madrid (mean annual air temperature series from Madrid station) and AP San Fernando (annual precipitation series from San Fernando station) as representatives of air temperature and precipitation tendencies of the Sierra Nevada region throughout the analyses.

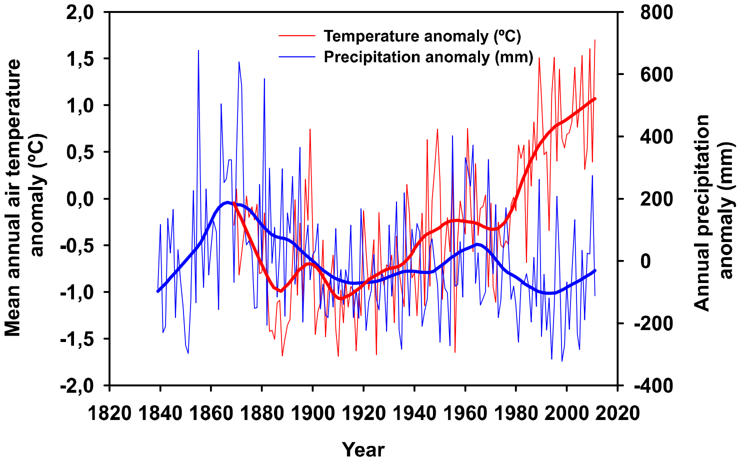


Figure 12. The mean annual air temperature anomalies from the Madrid climate station from 1869 to 2011 (red line); anomalies are relative to the 1961-1990 period. The annual precipitation anomalies from the San Fernando climate station from 1839 to 2011 (blue line); anomalies are relative to the whole period. A LOWESS smoother (span = 0.25) was applied to the climate data to improve the clarity of the figure and highlight trends.

Saharan calcium deposition data and drivers

To determine Saharan atmospheric Ca deposition, we used ice core data obtained from the French Alps glacier by Preunkert & Legrand (2013), who chemically identified Saharan dust events and quantified the Ca concentrations. Their data set spans almost 100 years from 1906 to 2004, with an 18 year gap between 1953 and 1970. Hence, we used the wNAO (winter North Atlantic Oscillation) index and the Sahel precipitation index records (both series longer than ice core Ca record) as representatives of Saharan and Ca deposition tendencies in the Sierra Nevada. The wNAO index (DJFM) defined by Hurrell (1995) is based on the difference of normalized sea level pressure between the Azores High (Lisbon, Portugal) and the Iceland Low (Stykkisholmur, Iceland) stations. Hurrell's wNAO index data set extends back to 1864 on a monthly mean basis. The Sahel precipitation index (SPI), extending back to 1900, provided by the University of Washington and the Joint Institute for the Study of the Atmosphere and Ocean, provided a standardized rainfall index data for the Sahelian zone of northern Africa. The stations are situated within 20-10N, 20W-10E, and the data set was obtained from the NOAA Global Historical Climatology Network.

wNAO data retrieved from: http://climatedataguide.ucar.edu/guidance/hurrell-north-atlantic-oscillation-nao-index-station-based

SPI data retrieved from: http://jisao.washington.edu/data/sahel/

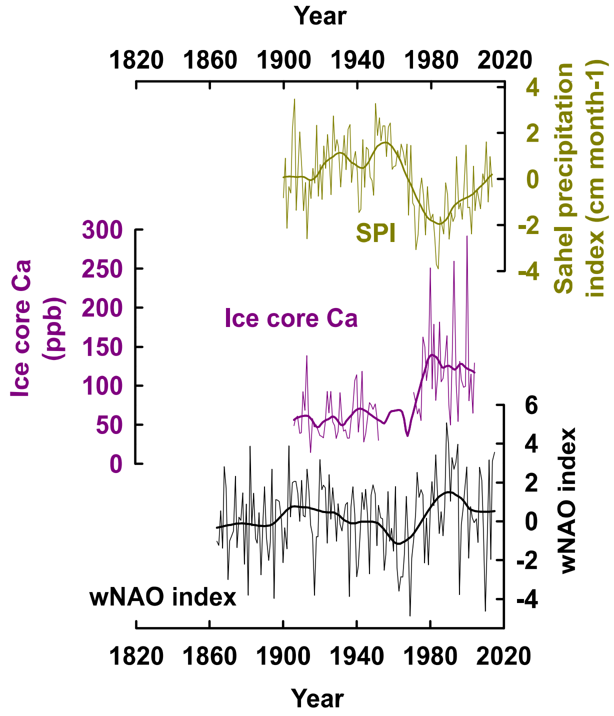
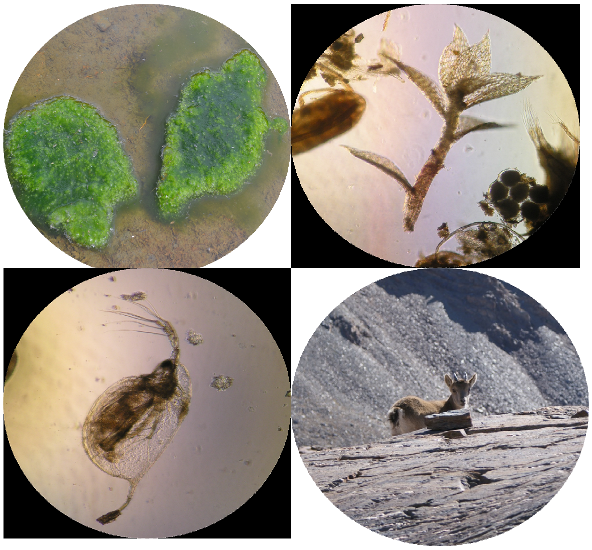


Figure 13. The wNAO index and the Sahel precipitation index (SPI) and Saharan calcium values (ppb) from an ice core from the French Alps (Preunkert & Legrand, 2013). The anomalies of the Sahel precipitation index (SPI) are with respect to 1900 and 2013, and based on June through October averages for each year. A LOESS smoother (span = 0.2) was applied to all the variables to improve the clarity of the figure and highlight trends.

Chapter 1

Sedimentary photosynthetic pigments as indicators of climate and watershed perturbations in an alpine lake in southern Spain



ABSTRACT

A short core was collected in Río Seco Lake, an alpine and oligotrophic lake located in the Sierra Nevada Mountains (in the southeast region of Spain) to determine the algal group changes over the past 200 years. In particular, it was analysed for fossil pigments and their derivatives and the geochemical (C/N ratio, grain-size analyses and isotopic dating) and climatic (temperature and precipitation values obtained from a long instrumental series) variables. The main pigments were carotenoids that indicate cyanobacteria (zeaxanthin, echinenone and myxoxanthophyll), diatoms and chrysophytes (fucoxanthin and diadinoxanthin) and green algae (lutein). The changes in pigment abundance over time were mainly explained by the temperature. Zeaxanthin showed a marked decrease from the 19th century to the present and is attributed to picoplanktonic cyanobacteria in Río Seco Lake. This decrease may result from climate-driven factors affecting herbivorous grazing pressure and water residence time. The increasing human activity around the lake likely generated a high input of carotenoid-poor pigmented organic matter and led to the dilution of chlorophylls and labile carotenoids observed over recent decades.

Keywords: Paleolimnology, sedimentary pigments, HPLC, alpine lake, Sierra Nevada

INTRODUCTION

Remote lakes are considered excellent ecosystems to study the effects of climate and environmental changes (Sorvari & Korhola, 1998; Smol, 2008) because they are above the treeline with no severe perturbations in their catchment area and are extremely sensitive to climatic warming (Hauer *et al.,* 1997; Smol *et al.*, 2005). The effects of recent warming are documented in the Arctic (Douglas *et al.*, 1994; Smol *et al.*, 2005; Rühland *et al.*, 2013) and in high-mountain systems in the Alps and Pyrenees (Sommaruga-Wögrath *et al.*, 1997; Lotter *et al.*, 1997; Catalan *et al.*, 2002), Rocky Mountains (Hobbs *et al.*, 2010; Hundey *et al.*, 2014), Himalayas (Rühland *et al.*, 2006) and Andes (Michelutti *et al.*, 2015). Among the numerous proxies employed in paleolimnological studies, sedimentary photosynthesis pigments can provide reliable records of changes in primary production and algal community composition in lakes (e.g., Romero-Viana *et al.*, 2009, 2010) and modifications in their biotic and physical environment (e.g., Verleyen *et al.*, 2005). Sedimentary pigment studies in remote lakes have revealed an increase in primary production as a result of climate warming (Battarbee *et al.*, 2002; Michelutti *et al.*, 2005; Lami *et al.*, 2010), and an increase in cyanobacterial abundance was shown in many of the lakes in the MOLAR European project (Lami *et al.*,1998; Lami *et al.*, 2000). These changes have been attributed to a lengthening of the ice-free period and growing season, a greater water column stability, and an increase in catchment nutrient fluxes and algal habitat availability. However, some authors have reported that pigment composition changes were small and independent of temperature (Koinig *et al.*, 2002). It appears that the amount and direction of change may depend upon the site including the lake's limnologic characteristics and the geographical setting (Sommaruga-Wögrath *et al.*, 1997; Corbett & Munroe, 2010; Luoto & Nevalainen, 2013a). Furthermore, the climate signal may sometimes be obscured in the sediment record due to the impact of other factors, notably those related to direct human disturbance (Battarbee *et al.*, 2002; Catalan *et* *al*., 2013). These include: watershed and shoreline erosion caused by an increased number of visitors (Toro *et al*., 2006), long-distance air transport of pollutants and/or nutrients that produces acid deposition and eutrophication problems (Lotter & Birks, 1997; Wolfe *et al*., 2001; Battarbee *et al*., 2009; Smol, 2010; Catalan *et al*., 2013), and fisheries management (Alric *et al.,* 2013), among other human activities. Differentiating between limnological responses of lake ecosystems to human and climate-change stressors is a challenging task for paleolimnologists and limnologists (Smol, 2010) and may be facilitated by the utilization of a combination of paleolimnological proxies. The Sierra Nevada mountain range is a unique environment for analyzing phenomena directly related to global change. It is the southernmost mountain range in Europe and the highest on the Iberian Peninsula with elevations greater than 3000 m a.s.l. It is also situated between two biogeographic regions (Europe and Africa) and its longitudinal west-east shape creates watersheds with different influences. The Sierra Nevada is approximately 60 km from the coast and is governed by a semi-arid Mediterranean climate. These characteristics have led high-mountain lakes of the Sierra Nevada to be considered ideal reference sites for climate monitoring. Limnological studies have previously described the influence of interannual climatic variations (temperature/precipitation and Saharan deposition) on the biogeochemical variables and biota in the lakes of the Sierra Nevada (Morales-Baquero *et al*., 2006a; Pérez-Martínez *et al*., 2007), but no data are available on the direct and indirect effects of climate change on century time scales. The present study was designed to determine the changes in sedimentary photosynthesis pigments in Río Seco Lake over the past 200 years. Our research group has accumulated an abundance of information on the chemical and physical conditions of Río Seco Lake and is engaged in an intensive program to monitor planktonic communities and physicochemical variables. It is also possible to identify a specific period of human pressure on the catchment area that occurred between the construction of a dirt road and a mountain hut close to the lake shoreline in the 1960s and their destruction in the late 1990s.

Vehicular access produced a major increase in the number of same-day and overnight visitors during this 30-year period. With this background knowledge, we hypothesized that the algal community in Río Seco Lake could be affected by recent warming and by direct catchment perturbations and that these changes are recorded in the lake sediment. The specific objectives of this study, using a sediment core from the Rio Seco Lake, were to estimate changes in the algal community over the past 200 years by analyzing the pigment record and identifying signs of catchment perturbation by analyzing geochemical variables. The aim was to determine the effects of global warming and those of human-induced catchment perturbation.

MATERIALS AND METHODS

Site description

Río Seco (RS) (37◦03_N, 3◦20_W) is a small (0.4 ha surface and 9.9 ha catchment area), oligotrophic, and shallow (Zmax = 2.90 m) lake of glacial origin located at 3020 m a.s.l. in the Sierra Nevada Mountains (southern Spain) above the tree-line (Fig. 1. 1). The maximum depth was determined by a surface outflow. The lake is ice-covered from around October-November until June-July with a large interannual variability. Data have been published on the physicochemical characteristics of the lake in different years (Barea-Arco *et al.*, 2001; Morales-Baquero *et al.*, 2006a; Pérez-Martínez *et al.*, 2013). During the ice-free period, Secchi disk visibility exceeds the water depth, the lake is not thermally stratified (see thermistor data in García-Jurado *et al.*, 2011), and the maximum temperature is 16-18 ◦C. Dissolved organic carbon values range from 62.1 to 283.5 µmol L^{-1}, conductivity values from 9.84 to 16.29 µS/cm, pH values from 6 to 7.4 and acid neutralizing capacity values from 0.05 to 0.20 meq L^{-1}. It is a fishless lake, the chlorophyll concentration is approximately 0.5-2 µg L^{-1} (Morales-Baquero *et al.*, 2006a), and the phytoplankton biomass is less than 20 µgC L^{-1} (Peréz-Martínez *et al.*, 2012). The lake bedrock basin is siliceous and largely comprised of micaschists. The catchment area is partially covered (~15 %) by alpine meadows, and the lake border is covered by bryophytes.

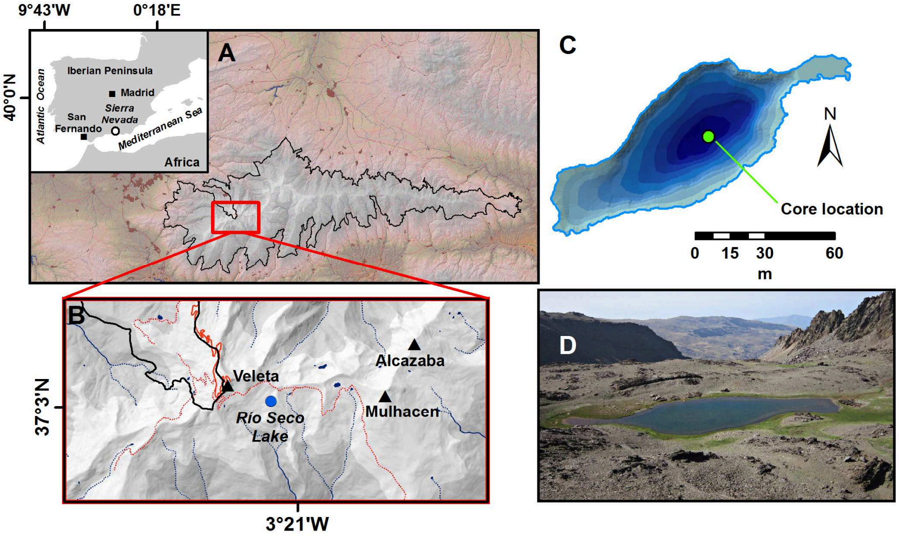


Figure 1.1. A) Inset map: Contour map of Iberian Peninsula showing the location of the study area; Contour of Sierra Nevada National Park indicating the study area B) map of the Sierra Nevada mountain range showing geographical locations of Río Seco Lake (circle) and highest mountain peaks (white triangles); C) Río Seco Lake bathymetry (digitalized map of bathymetry report from Egmasa S.A.); D) photo of Río Seco Lake taken in August 2012.

Sediment coring and sampling

A sediment core was collected from the deepest part of the lake in September 2008. For further details see section "Sediment coring and field measurments" in Materials & Methods (see p. 73).

Analytical methods

The dating analysis is described in details in section "Sediment chronology" in Analytical methods, see p. 75. The photosynthetic pigments were analysed according to Airs *et al.*, (2001). In brief, thawed samples (between 0.92 and 2.05 wet/wt) were extracted in 10 mL of acetone (100 %)

by sonication (10 min, 50/60 Hz, Ultrason-H, Selecta) under darkness and then centrifuged (5 min, 3500 rpm) to remove cellular particle debris. The supernatant was filtered through a cotton wool plug. This was repeated up to a maximum of six times until the supernatant was colourless. The pigment extracts were dried under an N_2 stream and then stored at 4 ºC until ready for analysis with high-performance liquid chromatography (HPLC). Before the HPLC analysis, the acetone extracts were dissolved in 500 µL of MeOH (10% AcNH4 5 M) and 40 µL were injected. The HPLC system was equipped with a 1525 Binary Pump separation module and 2996 photodiode-array detector (Waters, Milford, USA). Chromatography analyses were performed with two Spherisorb S3ODS2 columns (4.6 × 150 mm, 3 µm, Waters) using a Spherisorb ODS2 guard cartridge (4.6 × 10 mm, 5 µm, Waters) to maintain the performance of the analytical column. The pigments were eluted using a mobile phase gradient starting with mixture A (MeOH: AcN: AcNNH4 0.1 M, 80:15:5) for 5 min, and then from t = 5 min to t = 100 min, a linear gradient from 100% mixture A to 100% mixture B (MeOH: AcN: ethyl acetate, 20:15:65) at a constant flow of 0.7 mL/min and following the method of Airs *et al.*, (2001). The compounds were identified from the absorption spectra (Romero-Viana *et al.*, 2009) and their concentration was expressed in µg per g of organic matter, based on specific absorption coefficients published in Jeffrey (1997). The CD/TC (chlorophyll derivative: total carotenoid) ratio was analysed as the sum of all of the breakdown products of native chlorophyll a divided by the sum of all of the carotenoids present throughout the core. The sediment intervals were subsampled for LOI analyses and placed into pre-weighed crucibles.

The LOI was determined by the method of Heiri *et al.* (2001). The sedimentary C and N content were assessed by combustion in a CARLO ERBA EA 1108 CHNSO Elemental Analyzer system. Prior to the grain size analyses, 0.5 g wet sediment samples were extracted and sieved through 150 µm mesh; the size was determined as the cumulative mass percent by X-ray diffraction (XRD) using GALAY model CIS-1, which measures particles ranging from 0 to 150 µm.

Subfossil cladoceran samples were analysed and identified using the methods described by Szeroczýnska and Sarmaja-Korjonen (2007). In brief, 1 cm3 of fresh sediment of each interval was heated for 20 min in 10% KOH to remove the humic matter and were then washed and sieved through 38 µm-mesh under tap water, followed by centrifugation (5 min, 3000 rpm) to concentrate the fossil cladoceran remains. The *Daphnia* remains were counted under a light microscope with 200x magnification; a minimum of 200 subfossil cladoceran remains were counted and identified per interval, and the cladoceran counts were expressed as a percent relative abundance of the total number of individuals counted for each interval.

Long-term climatic instrumental records are not maintained in the Sierra Nevada Mountain range. For further details of intrumental climate data (temperature and precipitation series) see pp. 81-84. The average of temperature (MAAT-Madrid) and precipitation (AP San Fernando) data was calculated for each dating interval of the Río Seco Lake core (see Appendix A and B).

Statistical analyses

STATISTICA v.7 (Statsoft) software was used to test the data normality and Pearson correlations. The Kolmogorov-Smirnov test with Lilliefor's correction was performed to determine the normality of the data distribution. Non-normally distributed variables were log or square-root transformed and Pearson correlation coefficients were used to test the correlations between transformed variables by applying the Bonferroni correction. We used a redundancy analysis (RDA) to relate the pigment matrix (Hellinger-transformed variables) to the environmental variables, temperature, precipitation and *Daphnia* relative abundance (log transformed). This analysis was followed by a permutation test produced by the *anova.cca* function of the Vegan package (Oksanen *et al.*, 2015) in R software (R Development Core Team, 2015). The permutations method was used to test for significance in the canonical analysis (e.g., redundancy analysis) (Legendre& Legendre, 1998; Borcard *et al.*, 2011). Permutation is the method of choice because it avoids making assumptions regarding the distribution of the data (Legendre & Legendre, 1998). The uppermost sediment sample (0-0.5 cm) was eliminated from the statistical analyses because it could not be reliably identified as exclusively sedimentary pigment. Zonation of the stratigraphic profiles of the pigment concentrations 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).

RESULTS

Climate data

The mean annual air temperature data show a marked warming trend since the 1920s that was especially pronounced after the early 1970s. The second half of the 19th century was wet and reached a maximum in approximately 1860-70. Since the late 19th century, precipitation has progressively decreased, except during the 1960s, and the last 40 years have been especially dry (Fig. 12 in section "Temperature and precipitation series" of Instrumental climate data, see p.84).

Chronological model

The ^{210}Pb total activity profile shows a decreasing trend. The ^{210}Pb dated sediment core represents 187 years of accumulation, and the deepest part of the core was dated at 1821 A. D. The sedimentation rates were 0.9-1.1 mm years^{-1} from 0 to 6 cm depth (from ca. A. D. 2008 to A. D. 1948) and 0.7-0.8 mm years^{-1} from 6 cm to 15.5 cm depth (from ca. A. D. 1948 to A. D. 1821). The ^{137}Cs activity versus the depth profile showed a single significant peak between 4 and 4.5 cm, which corresponded to 1962-63 by the estimated ^{210}Pb age (Fig. 1.2).

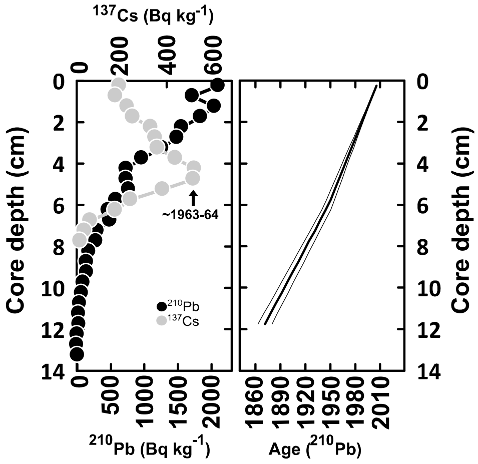


Figure 1. 2. Radiometric chronology showing ^{210}Pb (black circle) and ^{137}Cs (grey circle) activity (Bq Kg^{-1} dried sediment). On the right, A. D. year for Río Seco Lake sediment core; continuous lines represent the dating errors (1 SD in sediment age) associated with each dated interval.

Sedimentary proxy record

The atomic C/N ratio, which yields information on the source of the organic matter, ranged between 10.39 and 19.73 and showed a significant increase from the 1970s onward. The sand/(clay+silt) ratio showed strong fluctuations over time with a significant increase from the 1940s to the present. The mean ^{226}Ra activity throughout the stratigraphic profile was 50 Bq/kg with highest values in the uppermost intervals (Fig. 1.3).

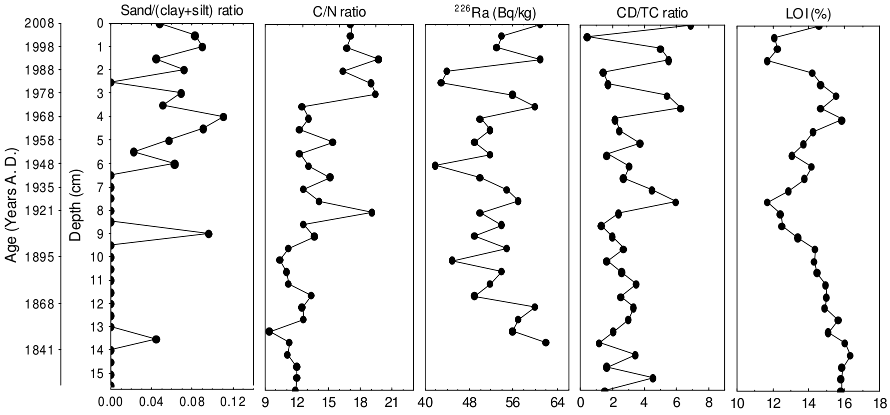


Figure 1. 3. The Sand/(clay + silt) ratio, C/N ratio, ^{226}Ra activity, CD/TC ratio and LOI (%) in the sediment of Río Seco Lake.

The Río Seco Lake sediment sample exhibited a narrow variety of sedimentary photosynthetic pigments (Fig. 1.4). Twenty different pigments were identified, including native chlorophyll a and b and their degradation products, which were pheophytins, pyropheophytins and pheophorbides. The most abundant carotenoids were zeaxanthin, echinenone and myxoxanthophyll (specific pigments from cyanobacteria), fucoxanthin and diadinoxanthin (from diatoms and chrysophytes), and lutein (from chlorophytes). Less abundant carotenoids included cryptoxanthin (from chlorophytes and cyanobacteria) and b-carotene (from all of the algal groups). Studying the main patterns in the pigment composition in relation to the environmental variables, we found that the pigment matrix was only significantly related with temperature, although the relative abundance of *Daphnia* was marginally related (Table 1. 1). The stratigraphic pigment signal in Río Seco Lake shows a vertical profile marked by two distinct zones (Fig. 1.4).

	Df	Variance	F	*p*-values
Temperature	1	1.007	5.010	0.003
Precipitation	1	0.141	0.705	0.542
Daphnia	1	0.484	2.408	0.059
Residual	19	3.819		

Adjusted $R^2 = 0.148$

Table 1. 1. The results of the redundancy analyses (RDA) with temperature, precipitation and the relative abundance of *Daphnia pulex* gr. as predictor variables and pigment data as response variables.

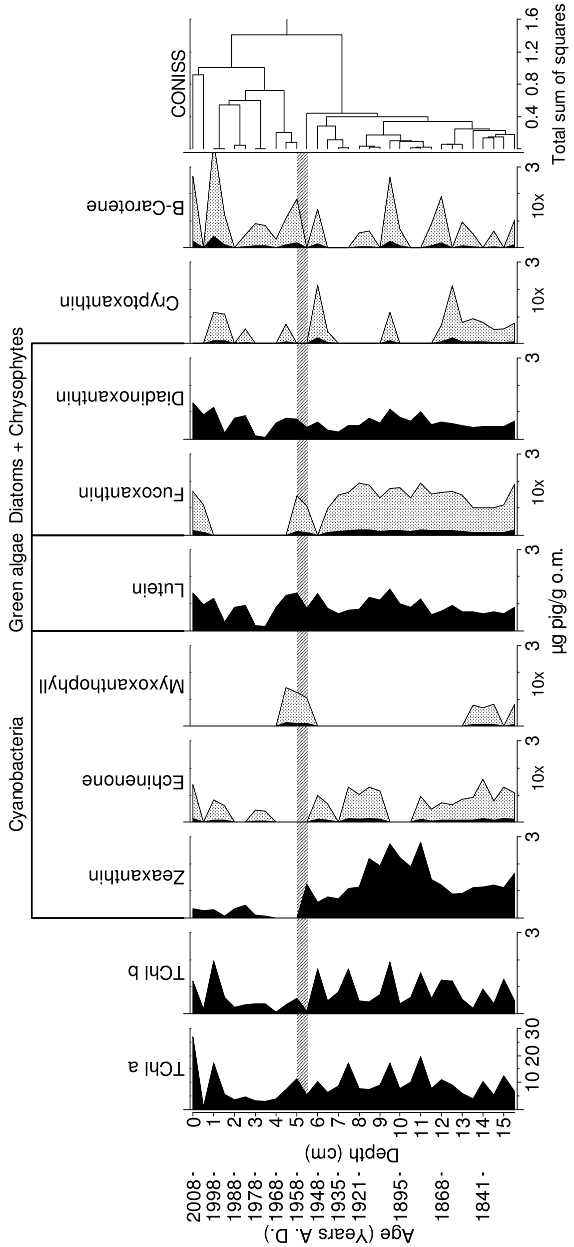


Figure 1. 4. A stratigraphic profile of specific pigments (TChl a, TChl b, zeaxanthin, echinenone, myxoxanthophyll, lutein, fucoxanthin, diadinoxanthin, cryptoxanthin and β-carotene). All of the compounds are expressed as µg pigment/g of organic matter. The exaggerated areas are dotted (each exaggeration scale is indicated below). A horizontal line delineates different zones determined by cluster analysis using the Constrained Incremental Sum of Squares (CONISS). The uppermost sediment sample (0-0.5 cm) is also shown, although it is not exclusively composed of sedimentary pigments.

The lower zone from 1820 to the 1950s, is characterized by an abundant signal of carotenoids that are specific indicators of the cyanobacteria community (mainly zeaxanthin and echinenone), which represent approximately 45% of the total carotenoid concentration in the sediment profile. In the upper zone, there is a drastic reduction in the cyanobacteria community signal. Hence, there has been a sharp decrease in the contribution percentage of cyanobacteria indicator pigments since the 1940s in Río Seco Lake and a marked increase in the relative abundance of the *Daphnia pulex* group since the late 1970s (Fig. 1.5).

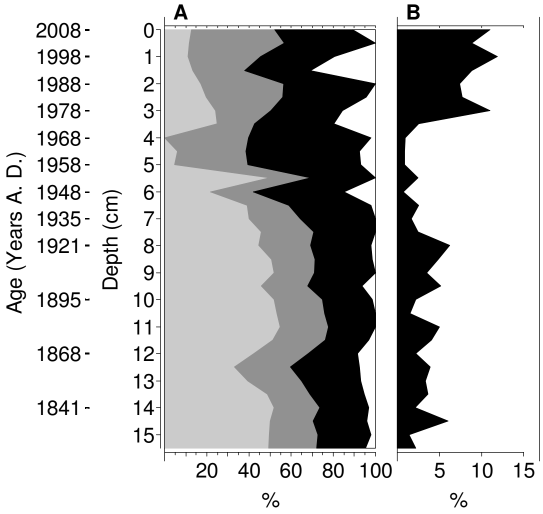


Figure 1. 5. (A) The percentage contribution of the sum of the main algal carotenoids; from left to right: zeaxanthin, echinone and myxoxanthophyll (light grey), diadinoxanthin and fucoxanthin (medium grey), lutein (black) and other carotenoids (white). (B) The relative abundance, expressed as a percentage, of Daphnia pulex gr. in the sediment of RS Lake.

The concentration of zeaxanthin was higher during wet and cold periods than during dry and warm periods over the past few decades (Fig. 1.6).

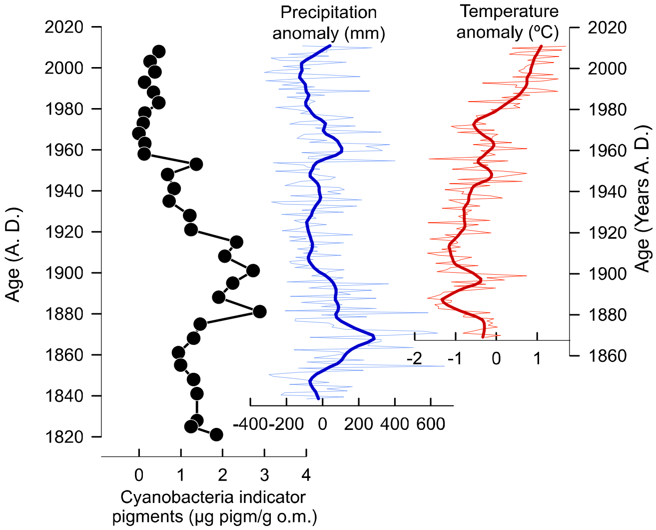


Figure 1. 6. A sedimentary profile of cyanobacteria indicator pigments and climate data representing the mean temperature and precipitation anomalies for the dating intervals of Río Seco Lake. A LOESS smoother (span = 0.1) was applied to the climate data.

The cyanobacteria pigment profile was negatively correlated with temperature values ($r = -0.54$, $p < 0.05$). There was a major reduction in the total sum of chlorophyll a and b and the concentration of some carotenoids (mainly fucoxanthin) between the late 1950s and the 1990s. The CD/TC ratio ranged from 0.4 to 6.9 with the highest values in the uppermost layers (Fig. 1.3). The TC values showed a significant negative correlation with the C/N ratio ($r = -0.46$, $p < 0.05$).

DISCUSSION

The low variety and concentration of sedimentary pigments in the Río Seco Lake sediment reflects the typical primary production of oligotrophic and high-mountain lakes (Lami *et al*., 2000; Kamenik *et al*., 2000; Buchaca *et al*., 2005). The presence of specific photosynthetic pigments throughout the stratigraphic sedimentary profile indicates that cyanobacteria, diatoms, chrysophytes and chlorophytes have been the most abundant algal groups in this lake over the past 200 years. It is noteworthy to mention that several years of plankton sampling in Río Seco Lake have demonstrated that *Synechococcus nidulans* is one of the dominant planktonic species (Conde-Porcuna *et al.,* 2014) and is the most important planktonic cyanobacteria species. Although we have not quantified the abundance of benthic cyanobacteria species in Río Seco Lake, occasional samplings show their low contribution to the benthic algal community. Benthic cyanobacteria species are filamentous species belonging to the genera *Oscillatoria*, *Anabaena*, *Nostoc,* and *Calothrix* and non-filamentous species of the genus *Aphanothece*. The endosymbiont cyanobacteria *Paulinella chromatophora* is also found in the lake (Sánchez-Castillo, 1988). The green algae assemblage shows a major contribution rather than other algae groups; and they largely comprise planktonic Chlorophyceae species, notably the epibiont *Korshikoviella gracilipes* and benthic filaments of a species of Zygnemataceae (Pérez-Martínez *et al*., 2013). The present data show a significant abundance of diatom-derived pigments, such as diadinoxanthin and fucoxanthin, and marker pigments of diatoms, chrysophytes and dinoflagellates (Leavitt & Hodgson, 2001; Buchaca & Catalan, 2007). Studies of siliceous subfossil samples in Río Seco Lake showed a predominance of benthic diatom species and a relatively low abundance of stomatocyst types (Pérez-Martínez *et al.,* 2012).

The presence of dinoflagellates in the plankton of Río Seco Lake is almost exclusively reduced due to a low concentration of *Gymnodinium* sp., whereas species of the genus *Chromulina* only show a peak of abundance after the thaw (Barea-Arco *et al*., 2001). Therefore, the diadinoxanthin and fucoxanthin in Río Seco Lake is mainly attributed to benthic diatoms. Other marker pigments of chrysophytes (violaxanthin) and dinoflagellates (peridinin and dinoxanthin) were not detected in our sediment record.

The most significant changes observed in the pigment stratigraphy of Río Seco Lake are the decrease in zeaxanthin pigment from the 19th century to the present time and the decrease in chlorophylls and labile carotenoids from the 1960s to the early 1990s. Zeaxanthin are observed in large amounts in picocyanobacteria than in other cyanobacterial groups (Bonilla *et al*., 2005; Romero-Viana *et al.,* 2009), and *S. nidulans* is the main planktonic species of cyanobacteria in Río Seco Lake. Hence, the observed decrease of zeaxanthin in Río Seco Lake can be mainly attributed to a decrease in picoplanktonic cyanobacteria species, such as *S. nidulans*, which would be controlled by abiotic factors (e.g., temperature and/or nutrients) or by top-down resource control. According to the classification of Reynolds *et al.* (2002), picoplanktonic cyanobacteria species are tolerant to low nutrient water and sensitive to grazing. With regard to nutrients, the decrease in livestock activity in the high mountain of Sierra Nevada since the late 1950s, which is shown by the decrease in herbivore dung fungus *Sporormiella* (Anderson *et al*., 2011), may have reduced the nutrient inputs into the lake. However, the increasing number of visitors and recreation activities since the 1960s is likely to have increased nutrient input to the lake offsetting the above reduction. The atmospheric deposition of N and P may have increased in the last decades because of pollution and Saharan dust events. The deposition N:P ratio is lower than 16 (Redfield ratio) during the spring-summer period (Morales-Baquero *et al*., 2006b), which could have favoured cyanobacteria rather than undermine them. Hence, we cannot propose nutrient availability as a direct cause of zeaxanthin changes based on the available data. Alternatively, the zeaxanthin concentration in Río Seco Lake sediment may have been related to climate conditions. This is supported by the significant correlation between cyanobacteria indicator pigments with temperature and by the significant relationship of global pigment data with temperature (RDA analyses). Reduced water residence time and nutrient limitation during cold and wet periods may have favoured species with elevated growth rates and high competitive ability for nutrients, such as picoplanktonic species (Schlesinger *et al.*, 1981; Reynolds *et al.*, 2002; Rigosi *et al.*, 2014), whereas no advantage is obtained under warmer and drier conditions. Additionally, under the latter conditions, picoplanktonic species may have suffered from high grazing pressure in Río Seco Lake. Morales-Baquero *et al.* (2006a) showed that the constrained zooplankton growth in Río Seco Lake during the cold years produced a weaker top-down control of phytoplankton biomass and therefore a higher algal biomass in comparison to warmer years. Likewise, the low concentration of zeaxanthin since the 1980s could also be attributed to an increase in grazing pressure due to a greater abundance of zooplankton; this hypothesis is supported by the marked increase since the late 1970s in the relative abundance of the efficient grazer *D. pulicaria,* which is the only *Daphnia* species of the *pulex* group present in Río Seco Lake and the main planktonic Cladocera found in the sedimentary profile. Similarly, grazing on other important planktonic algal groups could be expected to reduce pigments in Río Seco Lake such as green algae. However, the main chlorophyte planktonic species is *K. gracilipes*, an epibiont species on *D. pulicaria* with which it shows a mutualistic relationship and its density is therefore favoured by a higher *D. pulicaria* density (Barea-Arco *et al*., 2001; Pérez-Martínez *et al.*, 2001). The other main algal group in Río Seco Lake is represented by diatoms, which do not seem to be affected by zooplankton herbivorous grazing because they are mainly found in the benthos and littoral zone. Therefore, the changes observed in the zeaxanthin concentration may result from the effect of climate-driven factors on herbivorous grazing pressure and water residence time. An increase in the cyanobacteria sedimentary signal over recent decades has been reported in many European arctic and alpine lakes in the MOLAR project (Lami *et al.*, 1998, 2000). A warming-linked increase in cyanobacteria has been repeatedly recorded and predicted (Reynolds, 2006; Jöhnk *et al.*, 2008; Gallina *et al.*, 2011) because of the benefits they obtain in warmer water and a stabilized water column (Carey *et al.,* 2012; Rigosi *et al.,* 2014). These studies mainly refer to filamentous cyanobacteria species in relatively deep lakes that stratify in the summer time. However, Río Seco Lake is a shallow lake that does not stratify, and the cyanobacteria are mainly picoplanktonic species, which are affected by other factors including grazing pressure.

Another major change observed in Río Seco sedimentary pigment is the decrease in total chlorophylls and fucoxanthin from the early 1960s to the 1990s. This is a striking result given the numerous reports of an increase in primary production due to longer summer growing seasons and higher nutrient input in remote lakes under recent warming conditions (Battarbee *et al.*, 2002; Michelutti *et al.*, 2005; Reuss *et al.*, 2010). These discrepancies may be explained by the substantial human disturbances in the watershed of Río Seco Lake since the 1960s. The intense activity of mountaineers and vehicle traffic around the lake likely resulted in a considerable alteration of the shoreline and the surrounding area. Thus, increases in the proportion of sand and the sedimentation rate since the 1940s, and the higher ^{226}Ra activity detected since the 1960s, might indicate the occurrence of shoreline erosion that facilitated the mobilization and transport of inorganic particulate matter from the land to the water (Lami *et al.*, 1998; Toro & Granados, 2002; Brenner *et al.*, 2004). The marked increase in the C/N ratio over the past few decades indicates the inputs of organic matter into the lake. Their negative correlation with total carotenoids and the peaks of the CD/TC ratio in the uppermost layers of Río Seco Lake sediment indicate inputs of carotenoid-poor pigmented organic matter (Gorham & Sanger, 1972; Sanger, 1988), which probably consists of littoral plant fragments (macroscopic bryophytes remains were observed throughout the core) and/or littoral sediment from the dried shoreline. This would have resulted in a dilution of the concentration of chlorophylls and labile carotenoids. Finally, the two high peaks of the sand/(clay + silt) ratio around the 1960s and 1990s may be due to the building and destruction, respectively, of the mountain hut and road. The drop in sedimentary pigment concentration during the second interval might have been produced by the huge amounts of external material generated by the demolition, which clouded the lake and had a major effect on all of the organisms including algae. A reduction in the ^{210}Pb profile was also observed, which reflected an abrupt change in the sediment composition. The most important change in Río Seco Lake over the past two centuries occurred from the 1950s onward. Unlike other alpine and high mountain lakes, where an increase in cyanobacteria abundance has been reported over recent decades, the sedimentary pigments in Río Seco Lake indicate the opposite trend. Change in the algae community in Rio Seco Lake appears to be driven by factors linked to global warming, such as increased zooplankton grazing pressure and reduced water residence time. Human disturbance have been recognized in the core profile, resulting in an intense erosion of the catchment and consequent dilution of chlorophylls and labile carotenoids over recent decades. This is the first study on sedimentary pigments in Sierra Nevada National Park, and its results have been served to elucidate how a high-mountain lake responds to natural and human processes over a 200-year time-scale.

Chapter 2

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

ABSTRACT

The effect of recent climatic warming is significant in the Mediterranean region, especially in high mountain regions. 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 stages can be distinguished during the past 180 years: Stage One (1820- ~1920s) characterized by colder and wetter conditions than the more recent Stage 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 Stage One than Stage Two. Major biogeochemical changes occurred as a result of the temperature increase and precipitation decrease that started in the 1920s, and intensified over the past 50-years to ~0.28 °C per decade and –0.07 mm/yr. 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 ~1940-50 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 that contributed to snow and ice melting in the catchment. The coherent level between independent environmental proxies, each associated with distinct mechanistic linkages to climatic shifts, confirm a common response of the ecosystem towards in the direction of assemblages and an ecosystem state representative of warmer climate.

Keywords: High mountain lakes, Sierra Nevada,
Warming, Chironomids, *n*-alkanes, Chlorophyll-*a*

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). The effect on remote areas has been amplified, with increasingly faster warming rates (Beniston, 2003, Serreze & Francis, 2006; Parker *et al.*, 2008). Hence, mountain ecosystems are expected to experience disproportionately changes in response to climate change (Battarbee *et al.*, 2009; Pepin *et al.*, 2015). Modeling studies suggest that future climates of the Mediterranean basin (mild, wet winters and hot, dry summers) may become more extreme during the 21st century (Giorgi & Lionello, 2008; Alessandri *et al.*, 2014). The Mediterranean region is considered to be the largest "Hot-Spot" in the world (Giorgi, 2006), and its high mountain ecosystems are expected to become particularly vulnerable to global warming (Nogués-Bravo *et al.*, 2008).

Increases in global temperatures have an increasingly significant impact on aquatic and terrestrial ecosystems worldwide. One of the most visible physical changes associated with climate forcing is the duration of ice cover (Magnuson *et al.* 2000), influencing the ecology and environment of lakes and wetlands (Catalan *et al.*, 2006, Thompson *et al.*, 2009, Ramming *et al.*, 2010). This is in accordance with studies in remote areas such as the Arctic (Rühland *et al.*, 2003; 2008; Guilizzoni *et al.*, 2006), Lapland (Sorvari *et al.*, 2002), Rocky Mountains (Karst-Riddoch *et al.*, 2005), Tibetan Plateau (Wang *et al.*, 2015), Sierra Nevada, California (Franklin, 2013) and the Alps (Sommaruga e*t al.*, 1999; Ramming *et al.*, 2010).

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 recorded as limnological, ecological and geochemical proxies (Smol, 2008; Williamson *et al.*, 2009). The analysis of independent proxies allows for the tracking of processes within lakes and their catchment. Since individual proxies can have a 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. Among the proxies used in this study, subfossil assemblages of chironomids have been successfully used as reliable temperature indicators (Battarbee, 2000), which make chironomid-based inference models for reconstructing temperature of interest in many regions (Heiri *et al.*, 2003; Self *et al.*, 2011; Samartin *et al.* 2017). Other biological groups such as subfossil cladocera and diatoms are also widely used as paleoecological indicators of various environmental parameters, such as water level or lake productivity changes, due to their abilities to respond rapidly to environmental change (Battarbee *et al.*, 2001a; Jeppesen *et al.*, 2001; Rühland *et al.*, 2013). Among sedimentary pigments, spectrally-inferred chlorophyll-*a* is increasingly applied to track past trends in aquatic primary production in many recent studies (see Michelutti & Smol [2016] for a review). In addition to this suite of proxies, organic geochemical proxies (i.e., organic matter content, carbon isotope ratios or *n*-alkanes) incorporate a signal of regional and broader environmental changes as apparent in the distribution, amount and isotopic signatures of organic molecules (Meyers, 1997; 2003), and can therefore be used as indicators of vegetation or algal changes associated with climatic changes (Meyers, 1994; Meyers & Teranes, 2001). Recently, organic biomarkers such as *n*-alkanes have also been used as novel indicators of environmental and paleoenvironmental changes. Hence, *n*-alkanes are ideal for differentiating changes between dry and wet environmental conditions (Ficken *et al.*, 2000; Schefub *et al.*, 2003; Hou *et al.*, 2008). Simultaneously, the reconstruction of catchment vegetation based on pollen analyses provides a further approach that can provide insights into past climate conditions (Bennett & Willis, 2001).

The Sierra Nevada of southernmost Spain is a protected high-mountain area situated where alpine conditions and the influence of Mediterranean climate coexists. This Mediterranean mountain range has shown a rapid response to the recent global warming with the disappearance of permanent ice from the highest north-facing cirques (Gómez-Ortiz *et al.*, 2014; Oliva *et al.*, 2016). Moreover, a trend in declining mean annual rainfall (Ruiz-Sinoga *et al.*, 2011) and a reduction of snow and ice cover since the 1960s (Pérez-Palazón *et al.*, 2015) has become more pronounced since the onset of the 21st century (Bonet *et al.*, 2016). In addition, climate models project an ongoing warming trend in the Sierra Nevada for the end of the 21st century (Pérez-Luque *et al.*, 2016) that could enhance the already rapid warming and increase in aridity.

With this background, alpine ecosystems in the Sierra Nevada have become the focus of many paleoecological research projects over Holocene timescale. These studies have reported an increasing aridity (Anderson *et al.*, 2011; Oliva *et al.*, 2011; García-Alix *et al.*, 2012), Saharan dust input effects (Jiménez-Espejo *et al.*, 2014) and local vegetation and landscape changes (Jiménez-Moreno & Anderson, 2012) as well as a human disturbance history directly associated with pasturing herbivores or repopulation at lowest elevations (Anderson *et al.*, 2011; Jiménez-Moreno *et al.*, 2013). Parallel to long-term studies, short-term and high-resolution paleolimnological surveys provide a strong and reliable history of the lake and environmental region over a decadal to centennial timescale.

Until now, short-term surveys based on biological proxies, such as cladocera, diatoms and sedimentary algal pigments (Pérez-Martínez *et al.*, 2012; Pérez-Martínez, 2016; see Chapter 1 and 3), have shown significant responses of biotic assemblages due to direct and indirect effects of temperature increases at the turn of 20th century, and especially over the past ~50 years. However, short-term studies based on the communal response of chironomids to climate variability are lacking, even though this group of organisms has an excellent potential as indicator of past temperature change. Hence, chironomids may provide a valuable complement to other biological proxies in an attempt to better understand past and predict future consequences of climate-driven changes on lacustrine biota. Moreover, paleolimnological studies using chironomids together with organic geochemical indices are inexistent in the Sierra Nevada. Many different mechanistic linkages to climate variations of these proxies and their applicability as indicators of both in-lake and catchment related processes, may provide an integrated view of how alpine ecosystems function, and, ultimately, enhance the reliability of the paleolimnological record in Río Seco Lake. Little is know concerning the effects of recent warming on Sierra Nevada lakes at short, multidecadal to centennial timescales. This study may therefore significantly improve knowledge about the effects of climate change on these ecosystems.

This study aims to provide a high-resolution multi-proxy reconstruction of recent palaeoenvironmental conditions from an alpine Mediterranean environment of the Sierra Nevada. 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, by presenting a combination of stratigraphic records of sub-fossil chironomid assemblages, leaf wax biomarkers (*n*-alkanes), spectrally-inferred chlorophyll-*a* concentrations, pollen, cladocera and diatoms data, organic matter content, atomic C/N ratio and organic carbon isotopic data.

MATERIALS AND METHODS

Study site

Río Seco Lake (37° 03’N, 3° 20’W) is a small (0.4 ha lake surface area and 9.9 ha catchment area), low productivity and shallow ($Z_{max} = 2.90$ m) lake of glacial origin located at 3020 masl in the Sierra Nevada (southern Spain) above the tree-line (see Fig. 1.1 - Chapter 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.

There are a few signs of minor human activity in the area. Río Seco Lake is relatively remote, with local human activity currently limited to some transhumant sheep herding within the surrounding meadows during summer months. A mountain hut was situated close to the shoreline and operated for three decades (1967-1997) and a dirt road constructed between 1964 and 1965 is relatively close to Río Seco Lake, and has experienced limited summer traffic activity. The road is only frequented by pedestrians since the establishment of the Sierra Nevada National Park in 1999. The demolition of the mountain hut in 1997 resulted in the transport of large amounts of external material into the lake, which decreased lake water transparency and had a major effect on biota and geochemical variables (see Chapter 1). The catchment area is partially covered (~15%) by alpine meadows, and the lake has no clearly differentiated littoral zone, but its shoreline is covered by bryophytes. Catchment vegetation consists of xerophytic shrublands mainly dominated by the wetland plants Cyperaceae and grasses, Poaceae. 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. The physicochemical and biological features of this lake have been published in several papers (Barea-Arco *et al.*, 2001; Morales-Baquero *et al.*, 2006b; Pérez-Martínez *et al.*, 2001; 2007; 2013). During the ice-free period, Secchi disk visibility exceeds the water depth, the lake is not thermally stratified (García-Jurado *et al.*, 2011), and the maximum temperature is 16–18 ºC. Dissolved organic carbon values range from 62.1 to 283.5 µmol/L, conductivity values range from 9.84 to 16.29 µS cm^{-1}, pH values range from 6 to 7.4, and acid neutralizing capacity values range from 0.05 to 0.20 meq L^{-1}, the chlorophyll-*a* concentration ranges from 0.5 to 2 µg L^{-1}, and the phytoplankton biomass is less than 20 µg C L^{-1}. It is a fishless lake, and the phytoplankton and zooplankton communities are extremely simple. Among chironomids, the species *Psectrocladius limbatellus* and *Micropsectra radialis* (as *M. coracina*) have been recorded in Río Seco Lake

(Laville & Vílchez-Quero, 1986).

Sediment sampling, analyses and dating

A sediment core was collected from the deepest part of the lake in September 2008. For further details see section "Sediment coring and field measurments" in Materials & Methods (p. 73).

The sediment was dated by gamma spectroscopy (^{210}Pb, ^{137}Cs, and ^{226}Ra) and alpha spectroscopy (^{210}Pb in deepest part of core) techniques according to the method of Eakins & Morrison (1978). For further details see section "Sediment cronology" in Analytical methods (p. 75).

Instrumental climate data

For further details of intrumental climate data (temperature and precipitation series) see pp. 81-84. The average of temperature (MAAT-Madrid) and precipitation (AP San Fernando) data was calculated for each dating interval of the Río Seco Lake core (see Appendix A and B).

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 *et al*. (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 & Smol, 2016).

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

Analysis of total and inorganic carbon, and nitrogen content were carried out by combustion on a CARLO ERBA EA 1108 CHNSO Elemental Analyzer system. Correcting by the organic fraction was determined as the difference between the total and the inorganic carbon fraction (Meyers & 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 24 h. 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 analysed using a mass multicollector spectrometer (Isoprime; GV Instruments) equipped with a EuroVector elemental analyser (mod. Euro EA 3000) and continuous flow inlet. The results are expressed as $\delta^{13}C_{org}$ described as $(R_{sample}/R_{standard}-1) \times 1000$, where R equals $^{13}C/^{12}C$. Data are reported 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 means of 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. The *n*-alkanes were analysed 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 (C16, C18, C19, C20, C23, C25, C26, C28, C30, C32, and C37) was measured every five samples. The measurement error was lower than 1.5%.

For fossil pollen, a modified Faegri & Iversen (1989) procedure was followed using 1 cm^3 of sediment. Processing included pre-treatment with $(NaPO_3)_6$ 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 *et al.* (2011).

For chironomid analysis, samples (~0.3 g dry weight) for each interval of sediment were immersed in 10 % KOH for 2-3 hours and subsequently sieved through a 100 µm fraction. The head capsules were separated under a dissection microscope. Chironomids were prepared in Eurapal mounting medium after dehydration and identified at 100-400x magnification with a compound microscope. Taxonomy mainly followed Wiederholm (1983), Brooks *et al.* (2007), and Oliver & Roussel (1983). 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.

Cladocera and diatom assemblages from the sediment core of Río Seco Lake were previously analyzed and published in Pérez-Martínez *et al.* (2012), Pérez-Martínez (2016) and Chapter 3. 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 Río Seco Lake

Sedimentary chlorophyll-*a* concentration is used to track past trends in aquatic and terrestrial primary production (Leavitt & Hodgson, 2001; Hundey *et al.*, 2014; Michelutti & Smol, 2016). LOI_{550} values were used as an estimate of organic matter content for each sediment interval (Heiri *et al.* 2001). LOI_{550} has been widely used as indicative of primary production (Dean, 1974; Battarbee *et al.*, 2001b; Smol, 2008), but can also be influenced by changes in catchment erosion (Battarbee *et al.*, 2002). Hence, sedimentary chlorophyll-*a* and LOI_{550} values have been used to infer changes in both aquatic and terrestrial primary production in the present study.

The atomic C/N ratio is used as an indicator of organic matter source in lacustrine sediments (Meyers & Ishiwatari, 1993; Kaushal & Binford, 1999). Organic matter from terrestrial vascular land plants (cellulose-rich and protein-poor) is usually characterized by C/N values higher than 20, while algal-derived OM (cellulose-poor and protein-rich) typically features values between 4-10. Intermediate values (C/N rates between 10 and 20) are typical for sediments influenced by both sources (Meyers & Teranes, 2001).

The $\delta^{13}C_{org}$ values of sediment cores indicate past changes in productivity levels in lacustrine environments (Schelske & Hodell 1991, 1995). Higher $\delta^{13}C$ of organic matter may result from increased aquatic productivity levels (Hodell & Schelske, 1998). Lacustrine algae preferentially take up the light carbon isotope (^{12}C) from the water's dissolved inorganic carbon (DIC) pool during photosynthesis. However, with enhanced productivity, this discrimination leads to an increase in the heavier isotope in the water DIC pools. Consequently, the algae that use ^{13}C are enriched in the heavier isotope (O'Leary 1988; Wolfe *et al.*, 2001) and show, therefore, higher values of $\delta^{13}C$. Nevertheless, the carbon isotopic composition of algal-derived organic matter is similar from that of organic matter from C_3 vascular plants (Meyers & Teranes, 2001), where heavier isotopic compositions are usually related to a decrease in the water-use efficiency under dry conditions

(Farquhar *et al.* 1982). Hence, the additional use of the atomic C/N ratio to support the interpretation of $\delta^{13}C$ values allows better discrimination of organic matter sources in systems where both algal and vascular plants organic matter are present (Meyers, 1994).

The main source of the *n*-alkanes in the sediment are the epicuticular leaf waxes of plants that protect the water balance of leaves, reduce the mechanical damage to leaf cells, and prevent fungal and insect attack (Eglinton & Hamilton, 1967; Post-Beittenmiller, 1996). *n*-Alkane carbon chains from leaf waxes usually range from 21 to 37 C atoms, with a predominance of odd carbon numbers. Long chain *n*-alkanes (higher than C_{27}) are usually found in vascular terrestrial plants (Eglinton & Hamilton, 1967). Aquatic and semiaquatic plants, such as macrophytes, usually maximise the C_{21}, C_{23}, and C_{25} *n*-alkanes (Cranwell, 1984; Ficken *et al.*, 2000). Shorter *n*-alkanes, such as C_{15}, C_{17}, and C_{19} as well as low odd/even carbon ratios are usually related to autochthonous aquatic organic matter, originating from organisms such as algae and bacteria (Cranwell, 1982; 1984). Several *n*-alkane indices have been calculated to summarize their distributions. The Average Chain Length (ACL) is a measurement of the weighted average of the carbon chain lengths. The portion aquatic (P_{aq}) is the ratio between typical aquatic plant *n*-alkanes (C_{23} and C_{25}) and terrestrial plant waxes (C_{29} and C_{31}) (Ficken *et al.*, 2000). For modern plants, terrestrial plants < P_{aq} 0.23 > emergent aquatic plants < P_{aq} 0.48 > floating/submerged aquatic plants (Ficken *et al.*, 2000). Although other *n*-alkane indices can also provide information on the aquatic or terrestrial source of organic matter (e.g. the ratio of short odd *n*-alkanes vs. long odd *n*-alkanes; C_{17-25}/C_{29-35}), the present study focusses on P_{aq} to simplify the discussion. However, P_{aq} shows a very similar trend as the C_{17-25}/C_{29-35} ratio in the Río Seco *n*-alkane record (Pearson r= -0.96; n=32).

The Carbon Preference Index (CPI) represents the relative abundance of odd vs. even carbon chain lengths (see Bush & McInerney, 2013 for a review). Values lower than 2 suggest an even *n*-alkane preference (indicating diagenetic alteration or algal/bacterial influence), and values higher than 2 point towards an odd preference (indicating plant sources, and thermal immaturity). *n*-alkane distributions usually vary depending on the environmental conditions, such as water availability, precipitation or temperature (Ficken *et al.*, 2000; Schefuß *et al.*, 2003). However, since *n*-alkanes can be also affected by regional conditions, we will follow a regional interpretation for *n*-alkanes based on an unpublished *n*-alkane plant survey in high elevation wetlands in the Sierra Nevada. This pointed out that there is a predominance of the short carbon chains distribution in and near water pools, and a predominance of longer *n*-alkane chains furthest away from such pools. Therefore, lower ACL values are usually recorded in areas closer to the water pools, where the P_{aq} values are usually high, pointing towards higher water availability. The same data showed that plants in environments with high water availability usually recorded low CPI values (6.5±2.5), while plants from fully terrestrial environments in Sierra Nevada, with lower water availability, usually recorded higher CPI values (higher than 13.6±8.6).

Cyperaceae/Poaceae pollen ratio (C/P ratio) is used in this study as a sedimentary proxy of changes in climatic conditions (Turney *et al.*, 2004). High C/P ratio usually indicates wetter conditions, whilst low C/P ratios represent drier environments (Jiménez-Moreno *et al.*, 2008; Ramos-Román *et al.*, 2016).

Data analyses

The linear and indirect ordination technique, principal component analysis (PCA), was chosen to summarize the dominant pattern of assemblage variability in chironomid communities, 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). The chironomid relative abundances were square root transformed prior to analyses to equalize variance among taxa. Ordinations were conducted using the vegan package (Oksanen *et al.*, 2015) for the R software environment (R Development Core Team, 2015). The annually resolved climate metrics (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 *et al.*, 2002). The relationships between sedimentary proxies (downcore PCA axis 1 sample scores, organic geochemical proxies and sedimentary chlorophyll-*a* record) and changes in climatic metrics (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 show the coherence between parameters. For the sedimentary chlorophyll-*a* record, the uppermost sedimentary interval (0-0.5 cm) was excluded from the statistical analyses because it could not be reliably identified as exclusively representing sedimentary chlorophyll-*a* due to the presence of algal mat material in these uppermost intervals. 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.

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 biogeochemical response. Stratigraphic zonation was done 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.

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 *et al.*, 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 & Juggins, 1993; ter Braak *et al.*, 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. The modern distribution of chironomid assemblages in lakes in the Swiss Alps (Heiri & Lotter 2010) was used to assess the trajectory Rio Seco chironomid assemblages relative to summer temperature. For this purpose fossil samples were plotted passively in a Detrended Canonical Correpondence Analysis (DCCA) with mean July air temperature as the sole constraining variable. Variations of site scores of Rio Seco assemblages towards high or low DCCA axis values 1 represent changes in assemblage composition towards assemblages typical for cold or warm mountain lakes in the Swiss Alps, respectively. Chironomid assemblage percentage data were square root transformed before calculation of DCCA.

RESULTS

Chronology

The ^{210}Pb dating of the sediment core from Río Seco Lake shows sedimentation rates of 0.9-1.1 mm years^{-1} from 0 to 6 cm depth (from ca. 2008 to 1948) and 0.7-0.8 mm years^{-1} from 6 cm to 15.5 cm depth (from ca. 1948 to 1821). The ^{210}Pb activity profile shows a typical exponential decline towards the deepest part of the core. Based on the sedimentation rate, the ^{210}Pb dated sediment core indicates 187 years of accumulation and each interval of the sediment core represents approximately 5 and 7 years of accumulation from 0 to 6 cm and from 6 to 15.5 cm depth, respectively. A significant peak in ^{137}Cs activity profile is observed between 4 and 4.5 cm of sediment samples, recording the 1963 fallout maximum from atmospheric nuclear weapons testing (see Fig. 1.2 - Chapter 1).

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. 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. 12 in section "Temperature and precipitation series" of Instrumental climate data, see p. 84). The last 40 years of the AP San Fernando record exhibit persistent low precipitation values that were particularly low from 1985-1995. Further, 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.

According to main shifts in climatic data, consistent with noticeable changes in direction, magnitude and timing in the major biogeochemical proxies in the present study, two distinct climatic stages are indicated for the climate data: Stage One from 1820 to ~1920s, a stage of relatively high precipitation and low and decreasing temperature; and Stage Two from ~1920s to the present, a warmer and drier stage, particularly since mid-70s (Fig. 12).

Sedimentary proxy record

A similar trend is observed in % organic matter content (estimated by Loss on ignition-LOI_{550}) and sedimentary chlorophyll-*a* record 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 organic matter content (LOI_{550}) values in the 1990s (Fig. 2.1). 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. 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 according to the trend break of ^{210}Pb activity profile (see Fig 1.2 - Chapter 1)

From 1860 to the ~1920s, the atomic C/N ratio shows the lowest values for all the period (11.7±2.1, mean ± SD), coincident with high values of AP San Fernando and low values of MAAT Madrid, while the higher values are recorded from ~1920s to the present (15.6±2.6) coincident with a warmer and drier period. Atomic C/N ratio and $\delta^{13}C_{org}$ variables show opposite tendencies for almost all the records. The C/N peaks in the mid-19th century (1860-1870s) and ~1920s correspond with $\delta^{13}C_{org}$ drops at the same periods, except for the last four decades (from mid-1970s to the present), a period in which atomic C/N ratio shows the highest persistent values and

$\delta^{13}C_{org}$ exhibits a decreasing trend after a period of highest values (Fig. 2.1).

The Río Seco 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. 4). 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 that P_{aq} values (Pearson r = -0.97, n=32) for the entire record. However, the main difference between ACL and P_{aq} values is observed between ~1850 and ~1880, where there is a small increase (0.32) of P_{aq} in the 1860s, agreeing with decrease of ACL values during this period (~1850-1880) and coincident with the highest persistent precipitation. Moreover, CPI and P_{aq} do not follow any correlation before ~1915, but from ~1920s to the present CPI and P_{aq} have an opposite trend. However, 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. 3). This P_{aq} decrease is coeval with the drop of $\delta^{13}C_{org}$ and increase of the atomic 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 due to a severe and long drought period from the late 1980s to mid-1990 (Fig. 12 and 2.1).

In relation to the pollen assemblage, the 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). Besides, the appearance of the green alga *Pediastrum* from ca. 1950 onwards is noticeable (Fig 2.1).

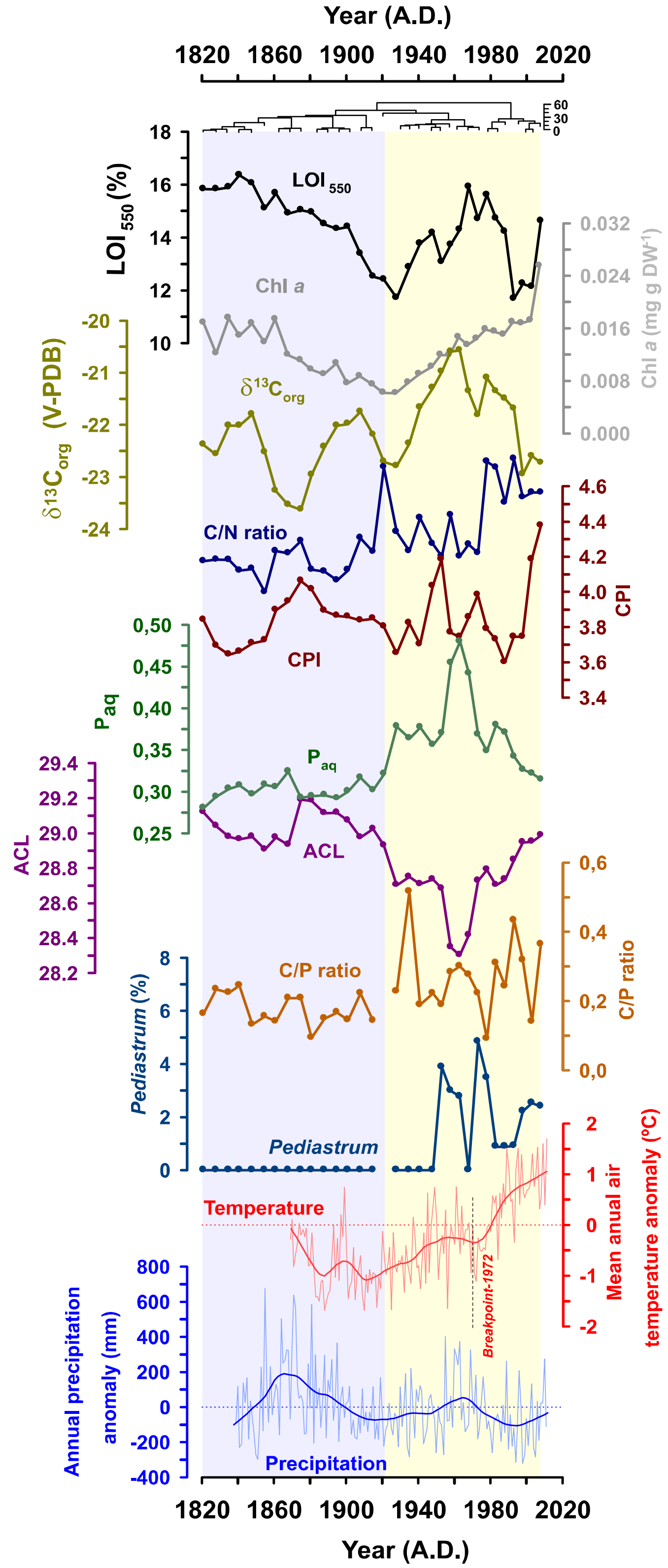

Year (A.D.)
1820 1860 1900 1940 1980 2020
60
30
0
LOI₅₅₀ (%)
18 16 14 12 10
LOI 550
Chl a
0.032 0.024 0.016 0.008 0.000
Chl a (mg g DW⁻¹)
δ13Corg (V-PDB)
-20 -21 -22 -23 -24
δ13Corg
24 20 16 12 8
atomic C/N ratio
C/N ratio
4.6 4.4 4.2 4.0 3.8 3.6 3.4
CPI
Paq
0,50 0,45 0,40 0,35 0,30 0,25
ACL
29.4 29.2 29.0 28.8 28.6 28.4 28.2
0,6 0,4 0,2 0,0
C/P ratio
Pediastrum (%)
8 6 4 2 0
Pediastrum
Temperature
2 1 0 -1 -2
Mean anual air temperature anomaly (ºC)
Breakpoint-1972
Annual precipitation anomaly (mm)
800 600 400 200 0 -200 -400
Precipitation
1820 1860 1900 1940 1980 2020
Year (A.D.)

Figure 2.1. Profile of organic matter content (LOI_{550}), sedimentary 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 %) are shown from 1820 to 2008 A.D. Two distinct climatic stages are defined based on the climate data: Stage One (from 1820 to ~1920s) and Stage Two (from ~1920s to the present). Stratigraphically constrained cluster analyses using the biogeochemical proxies is also shown. The mean annual air temperature anomaly and annual precipitation anomaly are also presented. A threshold change to higher mean temperature is identified (breakpoint= 1972 ± 4.7, $p < 0.0001$).

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. 5). Major changes in biological proxies are observed for the last 60 years preceded by a period of minor changes. Based on cluster analysis, the most significant change is observed around 1940-50s and consists in the new arrival of *Chironomus plumosus*-type, *Heterotrissocladius marcidus*-type and *Micropsectra insignilobus*-type (Fig 2.2). The broken-stick model application identified one major zone boundary in the chironomid record ~1940-50, coincident with major change of PCA axis 1 sample scores for chironomids (which explain 51 % of the variance).

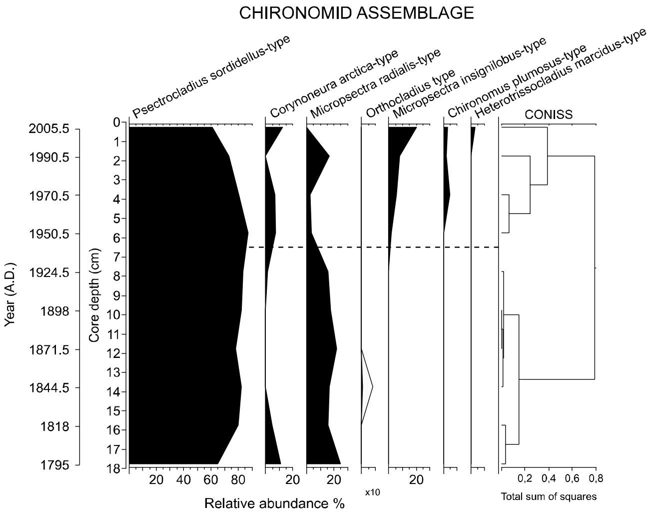


Figure 2.2. Chironomid remains in the sediment core from Río Seco Lake, together with a cluster analysis of assemblage data using Constrained Incremental Sum of Squares (CONISS). The broken line represents the main zonation identified by the broken-stick model. Light grey silhouettes show ×10 exaggeration.

The chironomid-based MJAT reconstruction suggests a trend of increasing MJAT from ~1940-50s onwards, presumably driven by increasing summer water temperature in Rio Seco form this period onwards (Fig 2.3 and 2.4).

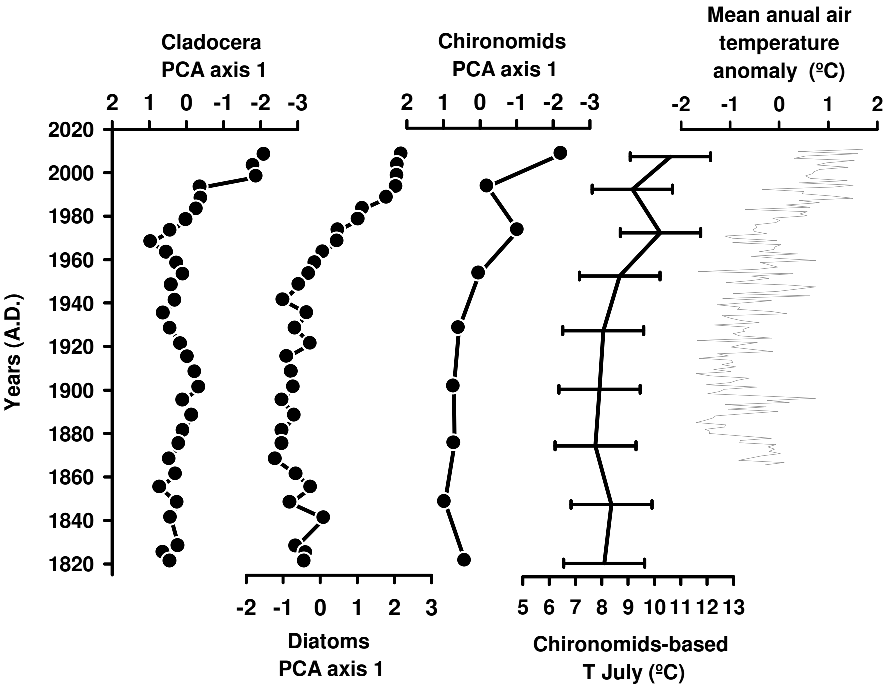


Figure 2.3.- Comparison of Cladocera, Diatoms and Chironomids PCA axis 1 sample scores for Río Seco Lake sediment core, together with chironomid-inferred mean July air temperatures based on the chironomid records (see text for details). The error bars lines indicate the sample-specific estimated standard error of prediction**.** Note the inverted scales in the axis scores for Cladocera and chironomids.

This interpretation is supported by the changes in DCCA axis 1 of the Rio Seco chironomid assemblages if added passively into a DCCA of modern chironomid assemblages from small lakes in Switzerland (Figure 2.5). Before ~1940-50 DCCA axis 1 values represent assemblages presently found in small Swiss lakes in the alpine zone with temperatures mostly below 10°C. Between ~1940-1970 DCCA axis 1 scores change to values more typical for Swiss lakes in the upper subalpine zone with July air temperatures in the 10-12°C range.

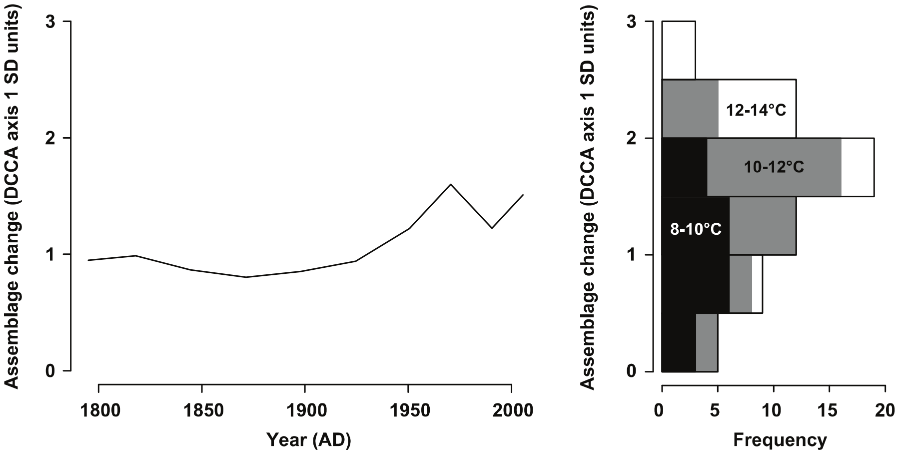


Figure 2.4. Axis 1 scores of the Rio Seco chironomid samples added passively to a Detrended Canonical Correspondence Analysis (DCCA) of chironomid surface sediment samples from 117 sites in the Alps with July air temperature as only constraining variable. For comparison the distribution of DCCA axis 1 scores of the surface sediment samples for three groups of lakes with mean July air temperatures in the range of 8-10, 10-12 and 12-14°C are shown, which represents the transition of chironomid assemblages in lakes across the treeline elevation.

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. 2.4). However, the taxon-specific cladoceran and diatom changes (not shown) started at the turn of the 20th century (~1920s), but became especially striking in the last five decades (Pérez-Martínez *et al*., 2012; see Chapter 3). 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 preceded by a period of minor changes; however the main changes in biological proxies seem to be delayed, and its response are intensified after the ~1940-50s onwards, consistent with recent warming.

DISCUSSION

Organic geochemical and biological proxies in Río Seco Lake have shown important environmental changes in and around the lake over the last ~180 years, which took place in accordance with the major shifts in climate data. Our records confirm the role of climate as a primary driver of physical and biological properties of the lake and its catchment.

The combination of analysis of atomic C/N ratio, $\delta^{13}C_{org}$ values and *n*-alkanes indices from the sediments from the Río Seco Lake core provides an opportunity to identify the main sources of organic matter in the study area. The values of atomic 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 size of lake (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 of $\delta^{13}C_{org}$ values in Río Seco Lake is also in accordance with a water-column study of this system (Pulido-Villena *et al.*, 2005), and with long-term and modern surveys of plants and lacustrine algae in other alpine lakes of Sierra Nevada (García-Alix *et al*, 2012; Jiménez-Moreno *et al.*, 2013).

Three factors suggest a predominance of wet environments in the catchment basin, in agreement with the previous atomic 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, 2013) and algal contributions (Han & Kelvin, 1969) and the P_{aq} values, which exceed 0.28 pointing to an emergent aquatic plant predominance (Ficken *et al.* 2000) for the entire record. In Río Seco Lake there are no emergent aquatic plants, but this P_{aq} value likely indicates bryophyte and others semi-aquatic vegetation, that composed the alpine meadows and surround the catchment. However, variations in the multiple proxies indicate differences in the degree of wet conditions in the Río Seco 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 (Lotter *et al*., 2002; Heiri *et al*., 2011). The chironomid assemblages were heavily dominated by the two taxa: *Psectrocladius sordidellus*-type (~77%), which often dominates in alpine lakes (Heiri & Lotter, 2010) and *Micropsectra radialis* (~15%), which in small lakes is restricted to cold arctic and alpine habitats (Heiri & Lotter 2010; Heiri *et al*., 2011). However, the arrival of chironomid groups and the most marked changes in chironomids 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 (discussed below).

Two different environmental stages can be distinguished according to the main shift in direction, magnitude and timing in biogeochemical proxies along with the precipitation and temperature data (Fig. 2.1): Stage One, from 1820 to the 1920s, with colder and wetter conditions, and Stage Two, from 1920s to the present, with drier and warmer conditions.

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

The paleolimnological changes observed in Stage 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 Stage Two. Hence, because precipitation occurs mainly during the cold winter season, the combined effects of relatively high precipitation and decreasing air temperatures (Fig. 12) during the Stage 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. The gradual decrease of LOI_{550} and sedimentary chlorophyll-*a* in parallel with MAAT Madrid supports the hypothesis of a reduction of primary production in response to a temperature decrease. It is noteworthy that relatively high values of both the sedimentary chlorophyll-*a* record and LOI_{550} occurred from ~1820 to 1860, likely related to a milder temperature in those years. However, despite the overall decrease of aquatic primary production during Stage One, atomic C/N ratio indicate a higher contribution of algae to the bulk organic matter than in the most recent climatic stage, which agrees with the P_{aq} values. This is probably the consequence of a reduced catchment surface and growing season for wetland plants, due to delayed ice and snow melting in the catchment basin and higher snow accumulation during this wetter and cooler period. Any shortening of the ice-free period may also hinder input of terrestrial organic matter into the lake.

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 (Oliva & Gómez-Ortiz, 2012), which began to disappear around the ~1920s (Grunewald & Scheithauer, 2010). Although there is no record of glaciers or persistent permafrost conditions in the south-facing cirque of Río Seco, the last active solifluction processes related to cold phases may have been during the Little Ice Age (LIA) (from 1550 to 1800 AD) (Oliva *et al.*, 2014).

Colder water conditions would explain the chironomid community composition in Stage One (Fig 2.3), 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 (see Chapter 3). PCA axis 1 sample scores of cladocerans, diatoms and chironomids show minor changes during this period (Fig 2.4).

The *n*-alkanes analyses also support our environmental reconstruction. The homogeneous P_{aq} values (~0.3) indicate there is a predominance of emergent aquatic plants (Ficken *et al.*, 2000). However, lower P_{aq} values in Stage One than in Stage Two point towards reduced water availability that might have resulted from the lower melting caused by the precipitation increase and the presumably low temperatures that continued after the LIA together with the shorter growing period (Fig. 12). This condition is also consistent with the highest ACL and lowest C/P ratio values of all the record, which indicate less water availability due to a shorter ice-free season and cold environment. However, the highest precipitation recorded in South Spain between ~1850 and ~1880 is slightly reflected in P_{aq} and ACL (Fig. 12). The main difference between the P_{aq} and ACL values is observed during this period, 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 almost muted response of P_{aq} and ACL can be read as indicating relatively wetter environment compared to the rest of Stage One, likely induced by the combination of persistent high precipitation coeval with milder temperature.

Stage Two: period from ~1920s to the present

Warmer and drier climate conditions during Stage Two produced substantial changes in biological and organic geochemical proxies. The increasing values of the sedimentary chlorophyll-*a* record 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 (Figs. 13

and 2.1). An increase in aquatic primary production in remote lakes by warming has been reported (e.g. Michelutti *et al.*, 2010; Smol 2008; Reuss *et al.*, 2010). The strong and positive relationship between sedimentary chlorophyll-*a* record and MAAT Madrid in Río Seco Lake and in other five lakes of Sierra Nevada Mountains is presumably a consequence of the direct influence of regional air temperature on primary production (Chapter 3), and not a consequence of other potential factors such as possible human disturbances in Río Seco Lake (Chapter 1). This effect may have been enhanced by atmospheric deposition of nutrients from Saharan dust to these low-productivity lakes. For example, the high delivery of atmospheric P-rich Saharan dust during the last 50 years may partially explain the trends in the sedimentary chlorophyll-*a* record in Río Seco Lake, a phenomenon that has been widely demonstrated in Sierra Nevada lakes (Pulido-Villena, 2004; Morales-Baquero *et al.* 2006a; Pulido-Villena *et al.*, 2008). 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 Río Seco Lake. These observations are consistent with the appearance of the green alga *Pediastrum* from ~1950 onwards, also suggesting an increase of primary production (Reynolds *et al.*, 2002; Sarmaja-Korjonen *et al.*, 2006, Weckström *et al.*, 2010). Among *Pediastrum* species, *P. boryanum* var. *cornutum* (Sánchez-Castillo, 1988), *P. braunii* and *P. tetras* (personal communication) are the current species registered in Río Seco Lake. With the exception of *P. braunii*, only distributed in high mountain lakes, *P. boryanum* var. *cornutum* and *P. tetras* are both common species occurring in meso- to eutrophic lakes (Komárek & Jankovská, 2001; Weckström *et al.*, 2010). Hence, the appearance of *Pediastrum* is also consistent with a water temperature rise, triggering a longer open-water season (growing season), and an increased supply of nutrients to the lake due to accelerated melting of snow patches and ice.

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. 12 and 2.1). 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 providing more net water availability. This combination of a longer growing season and greater water availability due to an enhanced ice melting in the catchment basin, triggered the development of larger wetland areas, as shown by higher C/P ratio values recorded for the recent climatic stage. This is in concordance with Pérez-Palazón *et al.* (2015) who showed a trend towards decreasing extent and persistence of the ice and snow covered area over the Sierra Nevada from the 1960s onwards.

Warmer conditions during this period 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 *et al.*, 2002; Antoniades *et al.*, 2005; Karst-Riddoch *et al.*, 2005; Keatley *et al.*, 2006). Over the last ~60 years the most notable changes in lacustrine biota is shown by the trend of PCA axis 1 sample scores coincident with major shift in MAAT Madrid and AP San Fernando (Fig. 2.4). Lacustrine biota apparently exhibited a delayed response to changes in air temperatures, and significant responses to climate change are observed when the climatic shift intensified for the last ~60 years. Hence, it is likely the intensification of temperature and precipitation changes since the last decades crossed ecological thresholds for biota communities. The delayed response of biotic assemblages to 20th century warming may also reflect that water temperatures showed a delayed warming relative to air temperature trends, e.g. due to melting of snow and ice in the lake catchment.

Temperature is particularly important in determining shifts in chironomid assemblage composition (Heiri *et al.*, 2003; Bigler *et al.*, 2006; Catalan *et al.*, 2009). 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. Among these latter species, the taxon *C. plumosus*-type includes a number of species and is generally considered to be thermophilic and indicative of relatively warm lakes (Brooks & Heiri, 2013), although it can also occur in lakes in the subalpine vegetation belt at low abundances (Heiri & Lotter 2010). Hence, the new arrival and increase of *C. plumosus*-type in Río Seco Lake is probably related to water temperature rise. This represents the first occurrence of the genus *Chironomus* in alpine lakes of Sierra Nevada (Laville & Vílchez-Quero, 1986; Real *et al.*, 2000), yet it does occur in subalpine and lowland lakes of Central Europe (Heiri *et al.*, 2003; Heiri & Lotter 2010). Similarly, *M. insignilobus*-type has its maximum abundances at lower altitudes in the Alps (Bigler *et al.*, 2006; Heiri & Lotter 2010). Overall, the timing of appearance and major contribution to change of these two taxa in Río Seco Lake suggest warmer summer water temperature, which is also reflected in a warming of chironomid-inferred mean July air temperatures by about 2ºC from ~1950s onwards. This warming in climatic conditions is consistent with changes in cladoceran and diatom community composition at Río Seco Lake (Pérez-Martínez *et al.*, 2012; Pérez-Martínez, 2016; see Chapter 3). 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 (Pérez-Martínez, 2016; see Chapter 3), 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 other studies that showed changes in the aquatic community structure in remote alpine (Luoto & Nevalainen, 2012) and arctic lakes (Smol *et al.*, 2005; Rühland *et al.*, 2014), coinciding with the onset of recent warming.

From ca. the 1970s to the present, there was a decrease in the P_{aq} values (simultaneous with ACL values increase), and therefore apparently a decrease in the water availability in the catchment basin, agreeing with an unprecedented high temperatures and decrease in precipitation (Fig. 12). We suspect this suggests lesser water availability induced by greater evaporation rates, enhanced by higher frequency of intense summers droughts influenced by the Mediterranean climate under a scenario of warming. These changes also are coeval with maximum values in the C/N ratio and decreasing $\delta^{13}C_{org}$ values from mid-1970s onwards. This might be interpreted as a major vascular land plant contribution to bulk organic matter. Even though this warming promoted a longer growing season and increased aquatic productivity (more abundant *Pediastrum,* higher sedimentary chlorophyll-*a* and LOI_{550}) the atomic C/N ratio from the bulk sediment suggests a previous decrease in the relative algae content in the sediment (around 1978), which is coeval with the abrupt decrease in P_{aq} after the 1960s. Hence, the vascular land plant contribution to the bulk organic matter apparently was much higher than the algae contribution during this period. Further, the maximum and permanent high level of sand/(clay+silk) ratio and the major input of allochthonous material from the catchment area during the last 30-years, related to the mountain hut situated in the shoreline of Río Seco Lake (Chapter 1), are consistent with these observations.

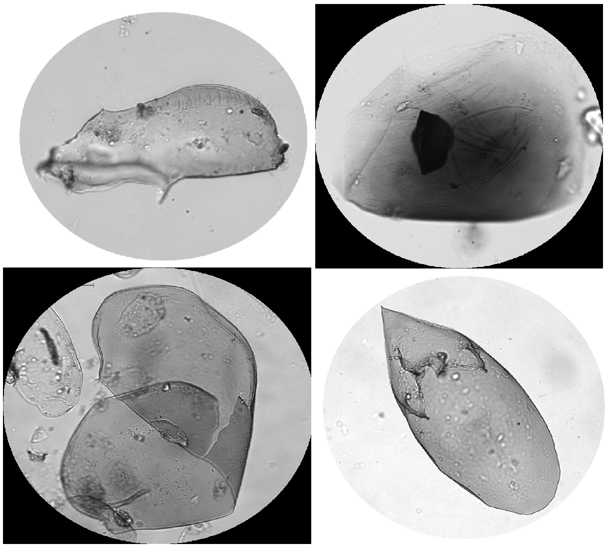
After ~1988, CPI and ACL values sharply increase and P_{aq} decreases which agrees with the extreme droughts of the early 1990s in the Mediterranean region, and is in concordance with the increased values in the MAAT Madrid record and decreases in AP San Fernando. These observations point towards an increase in terrestrial vegetation in the catchment, probably related to the regional precipitation decrease, consistent with the highest values of the atomic C/N ratio.

Concluding remarks

The short-core multiproxy study provide a valuable high-resolution record of paleoenvironmental and paleolimnological changes for the last ~180 years. Two general climatic stages, a cold and wet stage (1820 – ~1920s), and warmer and drier stage (~1920s onwards), are clearly reflected in biological and organic geochemical proxies. The climate-driven changes –shorter duration of ice-cover, higher summer water temperature and greater water availability for catchment plants – are responsible for the most noticeable changes in lake and catchment history. These climate-driven changes, either directly or indirectly, influenced by the seasonal character of the Mediterranean climate under a scenario of warming altered the ecological status of Río Seco Lake. Little is know concerning effects of recent warming in these ecosystems at short-time perspective. This is the first study based on multiples proxies that provides an integrated view of how these alpine ecosystems function. Hence, further investigations on similar lakes in the region are needed to provide better understanding of the effect of climate change on Mediterranean high altitude lake ecosystems.

Chapter 3

Climate change and Saharan dust drive recent cladoceran and primary production changes in remote alpine lakes of Sierra Nevada, Spain



ABSTRACT

Recent anthropogenic climate change and the exponential increase over the past few decades of Saharan dust deposition, containing ecologically important inputs of phosphorus (P) and calcium (Ca), are potentially affecting remote aquatic ecosystems. In this study, we examine changes in cladoceran assemblage composition and chlorophyll-*a* concentrations over the past ~150 years from high-resolution, well-dated sediment cores retrieved from six remote high mountain lakes in the Sierra Nevada Mountains of Southern Spain, a region affected by Saharan dust deposition. In each lake, marked shifts in cladoceran assemblages and chlorophyll-*a* concentrations in recent decades indicate a regional-scale response to climate and Saharan dust deposition. Chlorophyll-*a* production has increased since the 1970s, consistent with a response to rising air temperatures and the intensification of atmospheric deposition of Saharan P. Similar shifts in cladoceran taxa across lakes began over a century ago, but have intensified over the past ~50 years, concurrent with trends in regional air temperature, precipitation, and increased Saharan dust deposition. An abrupt increase in the relative abundance of the benthic cladoceran *Alona quadrangularis* at the expense of *Chydorus sphaericus*, and a significant increase in *Daphnia pulex* gr. was a common trend in these softwater lakes. Differences in the magnitude and timing of these changes are likely due to catchment and lake-specific differences. In contrast with other alpine lakes that are often affected by acid deposition, atmospheric Ca deposition appears to be a significant explanatory factor, amongst others, for the changes in the lake biota of Sierra Nevada that has not been previously considered. The effects observed in Sierra Nevada are likely occurring in other Mediterranean lake districts, especially in soft water, oligotrophic lakes. The predicted increases in global temperature and Saharan dust deposition in the future will further impact the ecological condition of these ecosystems.

Keywords: Saharan dust, High mountain lakes, Sierra Nevada, Warming, Cladocera, *Daphnia*, Chlorophyll-*a*

INTRODUCTION

Mountainous areas are amongst the most sensitive environments to anthropogenic climatic change, as their high relief and steep gradients make them especially vulnerable to even modest meteorological changes (Diaz *et al.,* 2003). In particular, Mediterranean high mountain ecosystems have been repeatedly identified as vulnerable to accelerated anthropogenic warming (Lionello, 2012; Pauli *et al.*, 2012). This is due in part to increased summer mean air temperature, and reduced annual precipitation heightening risk of summer drought in the Mediterranean region (Nogués-Bravo *et al.,* 2012).

In addition to climate change, remote alpine ecosystems are also affected by many other environmental stressors, particularly anthropogenic aerial deposition. The southernmost regions of the Mediterranean are predominantly influenced by substantial atmospheric deposits of Saharan dust (Lequy *et al.*, 2012; Pey *et al.*, 2013), accounting for ~ 50% of global dust production (Schütz *et al.,* 1981). Aerosols from the Sahara are transported mainly across the Atlantic (Carlson & Prospero, 1972; Swap *et al.,* 1992), but also to the Mediterranean region, with an estimated 80 – $120 \cdot 10^6$ t yr^{-1} of Saharan dust transported northward to Europe (D'Almeida, 1986). The amount of Saharan dust exported to the atmosphere has increased exponentially in recent decades as a consequence of droughts in North Africa (Prospero & Lamb, 2003), human-induced desertification (Moulin & Chiapello, 2006), and the development of commercial agriculture in the Sahel region (Mulitza *et al*., 2010). Saharan dust contains high amounts of phosphorus (P) and calcium (Ca), amongst other elements (Loÿe-Pilot *et al*., 1986; Morales-Baquero *et al*., 2013), and dust deposition may contribute significant inputs of P and Ca to a variety of ecosystems (Ridame & Guieu, 2002; Morales-Baquero *et al*., 2006a, Camarero & Catalan, 2012), and the ecological effects remain poorly understood.

Remote lakes are sensitive to both natural and anthropogenic factors (Smol, 2008; Adrian *et al*., 2009), and are often considered sentinels of environmental change (Williamson *et al.*, 2009). Sierra Nevada, located in the southeastern part of the Iberian Peninsula (Spain), is the southernmost mountain range of Europe (Figure 3.1). There are ~ 50 small alpine lakes situated between ~2800 and 3040 m asl and many of these have been analyzed in numerous limnological studies. Unlike many European mountain lakes that have a history of acidification (Alewell *et al.*, 2000; van Breemen & Wright, 2004), Sierra Nevada lakes receive little acid deposition (Camarero *et al.*, 1995), but these low productivity, softwater lakes are influenced by the deposition of nutrient-laden dust from the Sahara (particularly P and Ca fertilization). For example, the Ca input to the Río Seco Lake area from Saharan dust has been estimated at 12.4 kg Ca per year, an amount sufficient to account for the lake Ca content (Pulido-Villena *et al.*, 2006). The effect of Saharan dust deposition on lake biogeochemistry and chlorophyll content (Morales-Baquero *et al.*, 2006a) on the pool of dissolved organic matter (Mladenov *et al.*, 2011) and on bacterial activity (Reche *et al.*, 2009) has been found to be statistically significant. Interannual climate differences have been reported to affect water level and temperature in Sierra Nevada lakes (Villar-Argaiz *et al.*, 2001; García-Jurado *et al.*, 2011) and also modify phytoplankton and zooplankton biomass, nutrient availability (Barea-Arco *et al.*, 2001; Morales-Baquero *et al.*, 2006b; Pérez-Martínez *et al.*, 2013), and epilithic diatom communities (Sánchez-Castillo *et al.*, 2008).

Despite ongoing limnological studies in Sierra Nevada lakes, the lack of continuous monitoring data makes it difficult to place current limnological surveys within a long-term context. For such perspectives, a paleolimnological approach using the archive of material preserved in lake sediments is required (Smol, 2008). Previous sedimentary studies in the Sierra Nevada have focused on millennial-scale trends in physical and geochemical proxies, highlighting the potential role of Saharan dust deposition on lake production during the mid-Holocene (Jiménez-Espejo *et al.*, 2014), as well as the effect of increasing temperatures on the disappearance of glacial ice from mountain cirques with post-Little Ice Age (LIA) warming (Oliva & Gómez-Ortiz, 2012). To date, the only paleolimnological investigation of biological changes over the past two centuries within Sierra Nevada lakes is a pigment analysis of Río Seco Lake (see Chapter 1). Little is known regarding the potential interactions between climate change and the deposition of Saharan dust on the biological communities of these remote lakes.

The Cladocera are an order of aquatic invertebrates whose remains preserve well in lake sediments and have been used extensively in a variety of paleolimnological analyses to study topics such as trophic dynamics (Manca *et al*., 2007; Perga *et al*., 2010), water temperature (Fischer *et al*., 2011; Nováková *et al*., 2013), and lake-water levels (Nevalainen *et al*., 2013). Both the direct influence of climate warming, and indirect climate-driven limnological changes (e.g. length of the ice-free period and growing season, changes in oxygen availability, thermal stability or changes in chemical properties, etc.) may affect species distribution and dominance among cladoceran assemblages in alpine lakes (Kamenik *et al*., 2007; Luoto & Nevalainen, 2016). Acidification-induced declines in the lakewater Ca concentration of many Canadian softwater lakes have been associated with declines in the abundance of *Daphnia* spp. (Jeziorski *et al.,* 2008). Ca minerals are an important structural component for development of cladoceran exoskeletons (Greenaway, 1985), and as members of *Daphnia* spp. have higher Ca content (% dry weight) than many of their competitors they are more vulnerable to changes in ambient Ca concentrations (Jeziorski & Yan, 2006; Jeziorski *et al*., 2015). Although Saharan dust deposition affects large regions of the world (dubbed the "global dust belt" by Prospero *et al*. (2002)), the potential role of atmospheric Saharan Ca inputs on cladoceran populations, particularly *Daphnia* abundance, has not been investigated.

Here, we explore changes in chlorophyll-*a* concentrations and in the relative abundances of cladoceran microfossils in lake sediments to determine whether regional warming and Saharan dust deposition over the past ~150 years have affected Sierra Nevada lakes. We hypothesize that both of these environmental factors have had significant effects on these remote, softwater alpine lakes. Due to their geographic location, the lakes are exposed to large amounts of dust transported from Saharan Africa, but otherwise have naturally low Ca concentrations that may be limiting for some cladocerans, particularly certain *Daphnia* spp. (Ashforth & Yan, 2008). Warmer water temperatures associated with recent climate warming may have lengthened growing seasons and increased primary production in these lakes, conditions that can favor large, warm water cladoceran taxa over cold-tolerant, smaller taxa. The Ca-rich Saharan dust inputs and evapoconcentration of lake Ca together with warming-induced increases in water residence time is expected to favor Ca-rich cladoceran taxa such as *Daphnia* spp.

To provide a regional assessment of the effects of recent warming and Saharan dust deposition, we strategically selected six alpine lakes to represent the range of lake types and environments present within Sierra Nevada. Our objective was to analyze changes within the sedimentary cladoceran assemblages and sedimentary chlorophyll-*a* concentrations over the past ~150 years to test: (1) if the nature, magnitude, direction and timing of cladoceran assemblages changes are consistent with recent warming; (2) if *Daphnia* relative abundance changes can be linked to variations in Saharan Ca deposition; and (3) if changes in primary production (inferred through sedimentary chlorophyll-*a*) can be linked to warming and increased delivery of P-laden Saharan dust.

MATERIALS & METHODS

Site description

In the Sierra Nevada mountains of SE Spain (36º 55'-37º 15' N, 2º 31'-3º 40' W; maximum altitude 3482 m asl), there are ~50 small lakes of glacial origin at an elevation of ~2800-3100 m asl (Fig. 3.1) (Castillo, 2009).

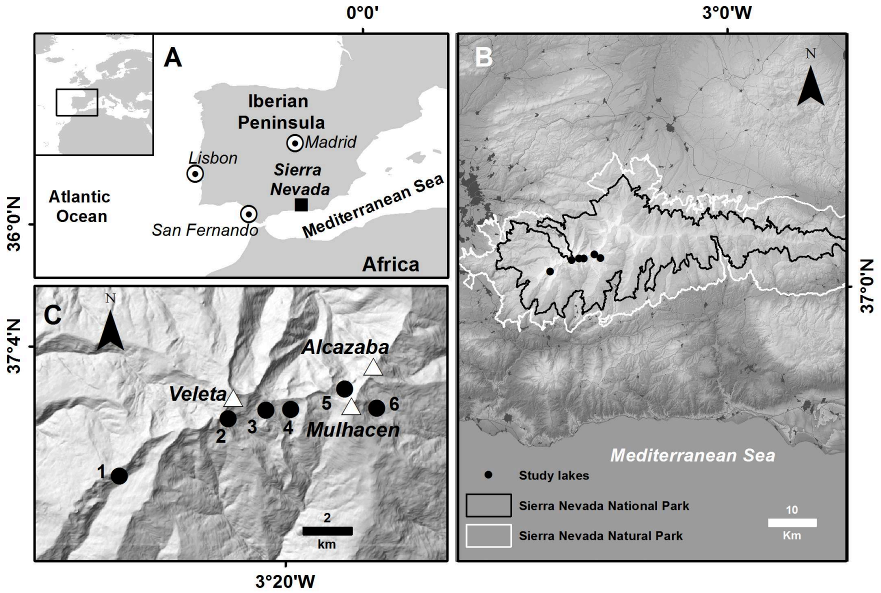


Figure 3. 1. A) The inset map and Iberian Peninsula map showing the location of the study area. B) Map of the Sierra Nevada mountain range showing geographical locations of the six study lakes. Boundaries of the Natural and National Park are shown by white and black continuous lines, respectively. C) Geographic locations of the six study lakes (circles; see Table 3.1 for further information). 1: Cuadrada (CD); 2: Aguas Verdes (AV); 3: Río Seco Superior (RSS); 4: Río Seco (RS); 5: Mosca (MC); 6: Borreguil (BG). Triangles represent the highest mountain peaks in the Sierra Nevada mountain range.

The predominant geologic substrate in these catchment basins is slow-weathering, metamorphosed siliceous rock, mainly mica-schist with graphite and mica-schist with feldspar (Puga *et al.*, 2007). The lakes are remote, located above the tree line, on poorly developed soils that do not support agriculture or forestry, and the vegetation surrounding some of the lakes is restricted to sparse meadows (alpine tundra). These glacial mountain lakes are typically shallow (maximum depth < 10 m) and small (surface area< 1 ha). During summer, some of the lakes may become dry while others may experience variable reductions in their water level. This water level reduction becomes greater and longer-lasting during warm and dry years relative to cold and wet years. Most lakes in this region have an open basin, usually with temporary surface inlets or outlets. 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 m asl) reports a mean annual temperature of 4.4 ºC and total precipitation of 700 mm, with 80% occurring as snow between October and April.

Six lakes were strategically selected (Fig 3.1) for retrieving high-resolution paleolimnological sedimentary records using the following criteria: 1) they are permanent water bodies with maximum depths > 2.0 m; 2) they have a non-rocky bottom; and 3) they are located in five different glacial valleys close to the mountain ridge of the Sierra Nevada to better characterize regional heterogeneity. We selected permanent lakes to ensure that their sediments had not suffered disturbances owing to air exposure or extreme reduction of their maximum depth as has been found to occur in some of the deeper Sierra Nevada lakes (e.g. La Caldera; Villar-Argaiz *et al.*, 2001; Sánchez-Castillo *et al.*, 2008). Short-term studies and observations indicate that these six study lakes experienced only minor changes in water level over the past 20 years. CD and RSS lakes are currently closed basin systems, while the remaining lakes show surface inlets or outlets that frequently became dry at the end of summer. Although the six study lakes represent different lake types and environmental settings, they are all shallow, clear and well-mixed water lakes that are characterized by littoral habitat (i.e. no clearly differentiated profundal zone). The lakes are circumneutral to slightly acidic, and low in alkalinity and productivity (Table 3.1).

	Lake (lake code)					
	Río Seco (RS)	**Río S. Superior (RSS)**	**Aguas Verdes (AV)**	**Borreguil (BG)**	**Mosca (MC)**	**Cuadrada (CD)**
Latitude	37º03’07.63”N	37º03’06.69”N	37º02’54.79”N	37º03’09.53”N	37º03’35.03”N	37º01’37.18”N
Longitude	3º20’43.92”W	3º20’53.04”W	3º22’06.16”W	3º17’59.03”W	3º18’53.03”W	3º25’06.64”W
Altitude (m asl)	3020	3040	3050	2980	2920	2840
Lake Area (ha)[a]	0.42	0.07	0.19	0.18	0.44	0.24
Catchment area (ha)[a]	9.9	4.7	12.8	50.9	39.7	4.0
Maximum depth (m)	2.9	2.6	2.8	2.0	2.8	4.8
Maximum volume (m^3)[b]	4772	447	1262	2070	7044	-
Catchment area/surface area[a]	21.5	78.3	67.4	282.8	82.7	16.7
pH	6.0-7.6 (6.9)	6.4-7.8 (7.2)	6.2-7.2 (6.7)	6.3	7.5-7.8 (7.7)	7.7
Conductivity (µS cm^{-1})	10-77 (24)	14-17 (15)	25-30 (27)	13-15 (14)	27-37 (32)	6-9 (7)
Alkalinity (meq L^{-1})	0.05-0.16 (0.11)	0.14-0.17 (0.16)	0.07-0.23 (0.16)	0.07-0.1 (0.09)	0.27-0.31 (0.29)	0.09-0.20 (0.14)
TP (µg L^{-1})	7-27 (16)	13-17 (15)	12-28 (20)	13-27 (18)	11-28 (17)	8-11 (9)
TN (µg L^{-1})	99-732 (403)	133-435 (284)	216-251 (236)	180-380 (280)	268-308 (288)	41-126 (83)
Chl *a* (µg L^{-1})	0.3-1.1 (0.6)	0.6-2.1 (1.2)	0.6-1.1 (0.8)	1.4-1.7 (1.5)	0.04-2.1 (1.1)	0.5-1.8 (1.1)
DOC (mg L^{-1})	0.7-2.7 (1.8)	0.9-1.3 (1.1)	0.7-1.2 (0.9)	0.6-1.1 (0.9)	1.1-1.4 (1.2)	0.3-1.3 (0.7)
Calcium (mg L^{-1})	0.5-2.1 (1.2)	0.5-2.8 (1.9)	1.9-2.1 (2.0)	0.8-1.1 (1.0)	3.0-6.6 (5.0)	0.3-1.1 (0.6)

[a] Data from Morales-Baquero *et al.* (1999).
[b] Data from Egmasa S.A.

Chemical and biological characteristics are from Sánchez-Castillo *et al.* (1989), Morales-Baquero *et al.* (1999), Reche *et al.* (2005) as well as from water column samples taken during each core sampling day and for punctual subsequent samples. Data are derived from a monitoring study over the past 10 years in RS Lake and forpunctual samplings inRSS, AV, BG, MC and CD lakes. Range and mean values (in brackets) are from a minimum of 4 samplesfor RSS and AV, 3 for BG, 2 for MC and CD. Abbreviations: TP, Total phosphorus; TN, Total nitrogen; Chl *a*, Chlorophyll-*a;* DOC, Dissolved Organic Carbon

Table 3. 1. Location and environmental characteristics of the six study lakes in Sierra Nevada Mountains. Range and mean values or single of the chemical and biological parameters from w ater measurements are shown.

The ice-cover season for lakes in the Sierra Nevada region typically lasts from November to June; however, large interannual differences in the duration of ice cover have been linked to annual variations in climatic conditions (Barea-Arco *et al.*, 2001; Morales-Baquero *et al.*, 2006b; Pérez-Martínez *et al.*, 2007). These shallow study lakes are fishless and do not thermally stratify during the summer. The six lakes, Río Seco (RS), Río Seco Superior (RSS), Aguas Verdes (AV), Borreguil (BG), Mosca (MC) and Cuadrada (CD), are within an 8 km radius, and with the exception of MC, are located on the south face of Sierra Nevada. The lakes are relatively remote, with local human activity currently limited to some sheep and cattle herding within the surrounding meadows during summer months. For ~30 years prior to the establishment of the Sierra Nevada National Park in 1999, there was also a mountain hut situated close to RS, and a dirt road passing near AV, RS and RSS that experienced minor summer traffic activity. Additional chemical and biological details for the study lakes can be found elsewhere (Morales-Baquero *et al.*, 1999, Morales-Baquero & Conde-Porcuna, 2000; Reche *et al.*, 2001; Reche *et al.*, 2005; Pérez-Martínez *et al.*, 2007).

Sediment coring and field measurements

Sediment cores were collected during the summer of 2011 (except for RS, which was sampled in 2008) from the deepest area of each lake using a slide-hammer gravity corer (Aquatic Research Instruments, Hope, Idaho, USA) with an inner core-tube diameter of 6.8 cm. For further details of sediment coring see p. 83.

Tube samplers (6.7 cm diameter) of different lengths were used to collect the whole water column in an integrated sample from the deepest point of each lake and analyzed for a suite of limnological variables following the techniques detailed in Barea-Arco *et al.* (2001) and Morales-Baquero *et al.* (2006b). Specific conductivity and pH were measured on site with a Waterproof PC 300 meter.

Sediment chronology

Sediment cores were dated using gamma spectroscopy to measure the activities of radioisotopes and establish a chronology for the past ~150 years. The methods is described in section "Sediment chronology- Analytical methods", see p. 75.

Climate data

For further details of intrumental climate data (temperature and precipitation series) see pp. 81-84. We use MAAT Madrid (mean annual air temperature series from Madrid station) and AP San Fernando (annual precipitation series from San Fernando station) as representatives of air temperature and precipitation tendencies of the Sierra Nevada region throughout the analyses. The average of temperature and precipitation data was calculated for each dating interval of the RS, RSS, AV, BG, MC y CD Lakes core (see Appendix A and B).

Chlorophyll-*a*

For each core, sedimentary chlorophyll-*a* concentrations were 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 *et al.* (2005, 2010). The chlorophyll-*a* concentrations include chlorophyll-*a*, as well as all chlorophyll isomers and its major derivatives (pheophytin and pheophorbide), and therefore accounts for the major diagenetic products (Michelutti *et al.*, 2010; Michelutti & Smol, 2016).

Subfossil Cladocera

Subfossil cladoceran samples were analyzed and identified using the methods described in Szeroczyńska & Sarmaja-Korjonen (2007). For each sediment core interval, ~1 cm^3 of fresh sediment was heated for 20 minutes in

10% KOH to remove humic matter, and then washed and sieved through a 38 μm-mesh under tap water. The samples were centrifuged (5 min, 3000 rpm) to concentrate the fossil cladoceran remains, and mounted onto microscope slides using glycerol gelatin dyed with safranin. A minimum of 200 remains (carapaces, headshields, postabdomens, postabdominal claws, valves, shells and ephippia) were identified and enumerated from each sediment interval under a light microscope at 200x magnification (Kurek *et al*., 2010). The most abundant remain from each taxon was used to calculate the number of individuals present in the sample (Frey, 1986). For *Daphnia* taxa, the abundance of individuals was principally determined from ephippia and postabdominal claws, as these were the only remains encountered in most samples. Due to the difficulties associated with identifying individual daphniid remains to the species level, daphniid taxa were grouped into the same groups, *Daphnia pulex* gr. (represented by *Daphnia pulicaria* in RSS, RS and CD, and *Daphnia pulex* in BG). The presence of two *Daphnia* species (*D. pulicaria* and *D. pulex*) from *D. pulex* species complex was determined by recent genetic analyses of daphniid ephippial eggs in Sierra Nevada lakes from the last 60 years (Conde-Porcuna *et al*., in preparation). Counts of individual cladocerans are presented as relative abundances and all the cladoceran taxa from each site are included in the stratigraphic plots. Biostratigraphic zones based on the cladoceran sedimentary assemblages over time were identified through cluster analysis using constrained incremental sum of squares (CONISS), on square root transformed relative abundance data with chord distance as the dissimilarity coefficient using the program Tiliagraph View (TGView), version 2.02 (Grimm, 2004), zones were determined via the broken stick model (Bennett, 1996).

Saharan dust deposition data and drivers

To determine whether past *Daphnia* abundance may be linked to Saharan atmospheric dust input, we inferred past trends in Ca deposition using a selection of proxies. First, we measured the zirconium aluminum (Zr/Al) ratio in sediment core intervals from one of our study lakes (RS) to represent dust deposition for this region. This approach was chosen because Saharan dust is rich in Zr (Guieu & Thomas, 1996) and this ratio has been successfully used as a proxy of Saharan input in the Mediterranean and other areas (e.g. Wehausen & Brumsack, 1999, Jiménez-Espejo *et al.*, 2007). Saharan deposition has been demonstrated to be synchronous among three sites (intersite distance of 40 km) close to the Sierra Nevada (Morales-Baquero & Pérez-Martínez, 2016), thus the temporal trends in the Zr/Al ratio measured in one of our lakes must be the same for the rest of the study lakes. Al was measured by atomic absorption spectrometry (AAS) using a Perkin-Elmer 5100 spectrometer and Zr was performed using Inductively Coupled Plasma–Mass Spectrometry (ICP–MS) after digestion in HNO_3 (65%) + HF (48%) of 0.1 g of sample powder in a Teflon-lined vessel for 150 min at high pressure and temperature, evaporated to dryness, and subsequent dissolved in 100 ml of 4 vol.% HNO3. Instrumental measurements were carried out in triplicate with a NexION 300d (Perkin Elmer) spectrometer. We then used ice core data obtained from the French Alps glacier by Preunkert & Legrand (2013), who chemically identified Saharan dust events and quantified the Ca concentrations. Their data set spans almost 100 years from 1906 to 2004, with an 18-year gap between 1953 and 1970. Collectively, the available literature on Saharan aerosols shows that a significant amount of African dust is transported to the Mediterranean region annually (especially in spring and summer), with Saharan annual dust deposition being higher in southern Mediterranean areas than at more northern sites (reviewed in Pey *et al.*, 2013). Hence, Saharan dust deposition in Sierra Nevada is likely higher than in the French Alps, although the long-term trend of Saharan dust deposition should be similar in both regions.

Within Europe, Sierra Nevada summits are ideally situated to receive a high concentration of atmospheric dust deposition due to their proximity to the Sahara (70% of Saharan dust is deposited within 2000 km), high altitude

(Saharan dust circulates between 1500 and 4000 m asl), and because they are in the path of the Saharan dust particles' movement towards the western Mediterranean (Morales-Baquero *et al.*, 2013; Pey *et al.*, 2013). As the Sierra Nevada lakes have naturally low P and Ca concentrations (Table 3.1), we expect changes in the deposition of dust laden with P and Ca to affect primary production and local biota, especially Ca-rich cladoceran species. To test this hypothesis we compared the sedimentary Zr/Al ratio and the Ca concentration record derived from the Alps ice core with our paleolimnological cladoceran records. However, the Zr/Al ratio is only usable for RS sediment intervals and the available ice core Ca record has an 18-year gap and is shorter than the paleolimnological cladoceran records. For this reason, to make comparisons with our cladoceran assemblage trends, we used the wNAO (winter North Atlantic Oscillation) index and the Sahel precipitation index records (both series longer than ice core Ca record) as representatives of Saharan and Ca deposition tendencies in Sierra Nevada because the intensity of Saharan dust emission and transport is influenced by the wNAO in winter and strongly controlled by the Sahel drought in summer and winter (Moulin *et al.*, 1997; Chiapello *et al.*, 2005). The wNAO index (DJFM) defined by Hurrell (1995) is based on the difference of normalized sea level pressure between the Azores High (Lisbon, Portugal) and the Iceland Low (Stykkisholmur, Iceland) stations. Hurrell's wNAO index data set extends back to 1864 on a monthly mean basis (http://climatedataguide.ucar.edu/guidance/hurrell-north-atlantic-oscillation-nao-index-station-based). The Sahel precipitation index (SPI), extending back to 1900, provided by the University of Washington and the Joint Institute for the Study of the Atmosphere and Ocean (http://jisao.washington.edu/data/sahel/), provided a standardized rainfall index data for the Sahelian zone of northern Africa. The stations are situated within 20-10N, 20W-10E, and the data set was obtained from the NOAA Global Historical Climatology Network (see Appendix C and D).

Statistical analyses

Relationships between climate and dust metrics (MAAT Madrid, AP San Fernando, the ice core Ca record, Zr/Al ratio, the wNAO index and the SPI) and between these metrics and sedimentary proxies (individual cladoceran taxa for species-scale trends, chlorophyll-*a* concentrations for trends in primary production) were examined for each study lake using Pearson correlation analysis, after assessing each variable for normality using the Kolmogorov–Smirnov test. The cladoceran relative abundance data were square root transformed prior to the analyses to equalize variance among taxa. The annually resolved climate and dust metrics were averaged over the period of accumulation for each dated interval, thereby integrating the instrumental data with the paleolimnological data (Sorvari *et al.*, 2002). To correct for possible trends in the variables and avoid spurious correlation we follow the procedure used by Gerten & Adrian (2000) whereby the Mann-Kendall trend test (PAST software; Hammer *et al.*, 2001) was applied to each correlating variable to detect trends over time. Those variables for which the Mann-Kendall test indicated a significant linear trend were detrended by fitting a linear regression versus time and the residuals were then used for the subsequent correlation analysis.

Breakpoint analyses using a two-segment piecewise linear regression were applied to MAAT Madrid and AP San Fernando series data to estimate the timing of largest change in each series.

To explore potential drivers of chlorophyll-*a*, model selection analyses (Burnham & Anderson, 2002) were performed with climate and atmospheric variables as independent variables. To identify the explanatory variables of cladoceran assemblage changes, two complementary techniques were chosen: 1) model selection analyses with chlorophyll-*a*, climate and atmospheric variables as independent variables and axis 1 scores of the principal component analysis (PCA) of cladoceran assemblages as the dependent variable, and 2) partial redundancy analysis (pRDA) with the same explanatory variables for the entire cladoceran assemblages. The PCA axis 1 scores summarizes the main variation in cladoceran assemblage and therefore the results of the hierarchical regression indicate the drivers of the main shift in Cladocera, while the pRDA explores the drivers of changes among the entire cladoceran assemblages. The pRDA (with forward/backward-selection) was performed in order to identify the statistical independence and relative strength of each of the explanatory variables of the cladoceran assemblage changes, using Monte Carlo permutation tests (999 unrestricted permutations) with a significance level of $p < 0.05$. Finally, model selection analyses with chlorophyll-*a*, climate and atmospheric variables as independent variables were performed to explore the explanatory variables of *Daphnia* changes. To normalize the variance, explanatory variables were z-score transformed prior to the analyses. Ordination and model selection analyses were performed using the vegan (Oksanen *et al.*, 2015) and the MuMIn (Multi-Model Inference; Bartoń, 2014) packages for the R software environment (R Development Core Team, 2015), respectively. Akaike Information Criterion adjusted for sample size (AICc) (Burnham & Anderson, 2002) was used to select the optimum model. Models with a difference of ΔAICc less than 2 compared to the lowest AICc were considered the best models and statistically equivalent. The significance and the percentage of variation explained for each variable was determined in order to define the contribution to the final model. Residuals of the final models were examined to check for normality of data and absence of over-dispersion. In order to avoid spurious relationships in multiple regression results, multicollinearity among the explanatory variables were explored by analyzing the Variance Inflation Factors (VIFs). All the explanatory variables yielded VIFs < 5 and therefore were kept in the analysis due to the low degree of collinearity.

For the chlorophyll-*a* records, the uppermost sedimentary interval (0-0.5 cm for RS and 0-0.25 for the other five lakes) was excluded from the statistical analyses because it could not be reliably identified as exclusively representing sedimentary chlorophyll-*a* due to the presence of algal mat material.

RESULTS

Sediment chronology

The total ^{210}Pb activity profiles for most cores (RS, AV, MC and BG) exhibited a characteristic exponential decline with sediment depth, while in RSS and CD the decline was more gradual. The supported ^{210}Pb levels (background) were reached between 7 and 13 cm in all cores (Fig. 3.2). Clear peaks in ^{137}Cs activity were observed in four of the sediment cores, while the peaks observed in RSS and CD were less distinct (Fig. 3.2); but still correspond to an estimated ^{210}Pb age of 1960-1970, consistent with the 1963 peak in atmospheric radioisotopic fallout (Fig 3.2). The average sediment accumulation rate for the past 50 years ranged from 0.01-0.03 g cm^{-2} yr^{-1}, which is common in alpine lakes situated above tree line where sedimentation rates are typically low (Saros *et al.*, 2003). The temporal resolution of the sediment cores ranged between 2 and 5 years per interval for the past 50 years (at 0.25 cm intervals for all the lakes, except for RS at 0.5 cm intervals).

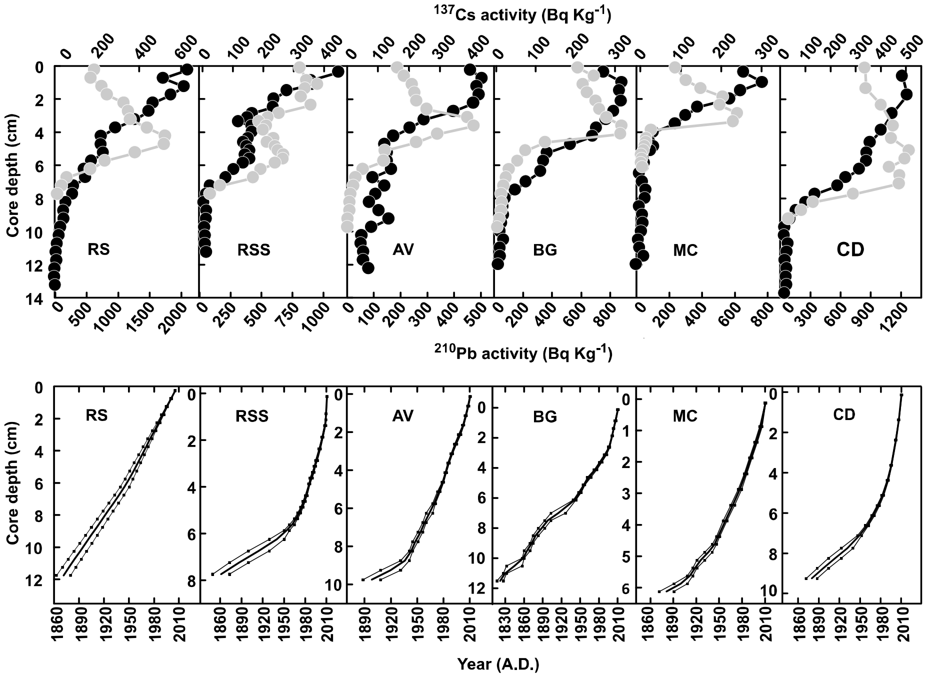


Figure 3. 2. Radiometric chronology showing ^{210}Pb (black circle) and ^{137}Cs (grey circle) activity (Bq Kg^{-1} dried sediment) for the six study lakes in the Sierra Nevada region. Below, A. D. year for each lake; the dating errors (1 SD in sediment age) associated with each dated interval are represented by continuous lines and symbols (■).

Climate data

Mean annual air temperature (MAAT) from the Madrid climate station (1869-2011) indicates a warming trend began at the turn of the 20th century (Fig. 3.3). Over the 143-year record, MAAT increased by 1.76 ºC, and a two-segment, piecewise linear regression applied to the MAAT Madrid series identified a threshold change to significantly higher mean temperatures in the early 1970s (breakpoint = 1972 ± 4.7 years, $p < 0.0001$). The temperature increased by 0.52 ºC over the period 1869 to 1972 and by 1.68 ºC over the period 1972 to 2011.Total annual precipitation (AP) from the San Fernando climate station indicates that the second half of the 19th century was wetter than the rest of the precipitation series, 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. 3.3). The last 40 years of the AP San Fernando record exhibit persistent low precipitation values, that were particularly low from 1985-1995. No significant breakpoint was identified by piecewise linear regression on the precipitation data.

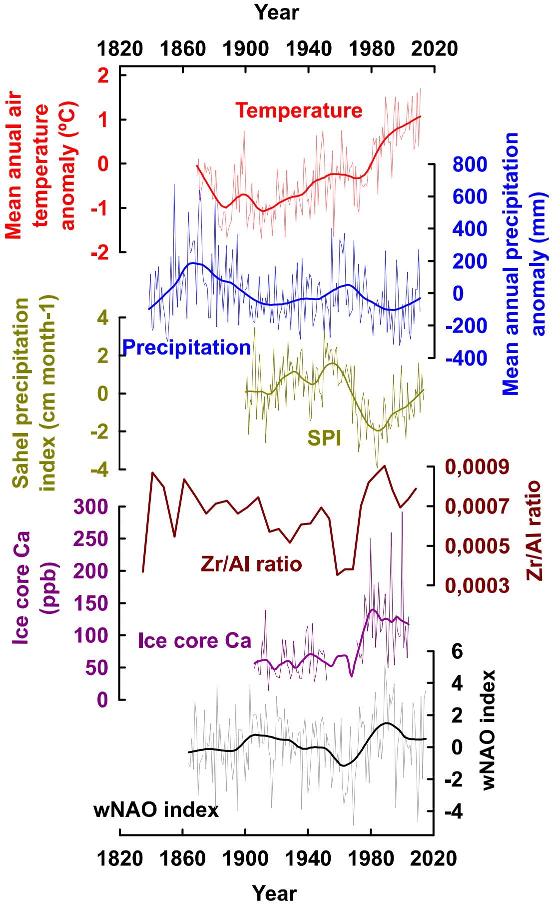


Figure 3. 3. The mean annual air temperature anomaly from Madrid climate station (MAAT Madrid) and annual precipitation anomaly from San Fernando climate station (AP San Fernando) since 1860 and 1840, respectively, and wNAO index and the Sahel precipitation index (SPI) and Saharan calcium values (ppb) from an ice core from the French Alps (Preunkert & Legrand, 2013) and Zr/Al ratio (proxy for regional Ca inputs) from the RS sediment core are presented. Temperature anomalies are related to the period 1961-1990 and precipitation anomalies are related to the whole period. The anomalies of the Sahel precipitation index (SPI) are with respect to 1900 and 2013, and based on June through October averages for each year. A LOESS smoother (span = 0.2) was applied to all the variables to improve the clarity of the figure and highlight trends.

Sedimentary chlorophyll-*a*

All six lakes have experienced notable increases in sedimentary chlorophyll-*a* concentrations beginning between ~1960 and ~1970 that are especially prominent since the ~1990s (Fig. 3.4). For each lake, strong and significant correlations exist (all $r > 0.40$, $p < 0.05$) between chlorophyll-*a* trends and the MAAT Madrid trends (variables detrended when needed), after integrating air temperature to the period represented for each dated interval (*sensu* Sorvari *et al.*, 2002). Chlorophyll-*a* is also significantly correlated with SPI in RS, AV and BG ($p<0.05$) and with AP San Fernando in RSS ($p<0.05$). Model selection analyses indicate that air temperature is the main driver of chlorophyll-*a* in all the lakes with SPI and AP San Fernando as secondary drivers in some of the lakes (Table 3.2).

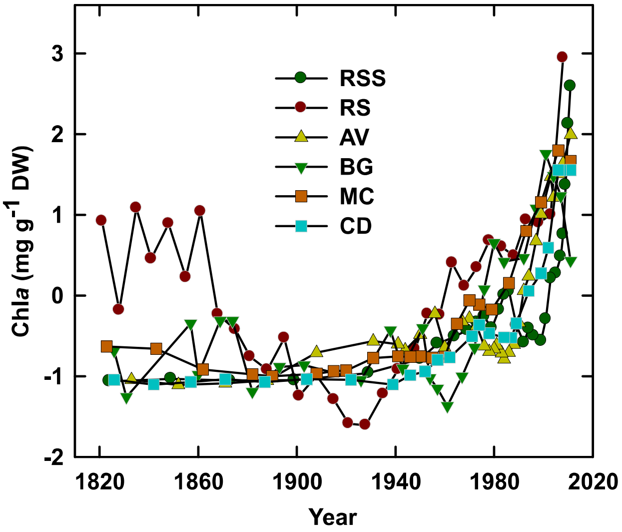


Figure 3. 4. Concentration of sedimentary chlorophyll-a and derivatives (standardized to mean variance using z-scores) for the six study lakes. Lake name abbreviations: Río Seco (RS), Río Seco Superior (RSS), Aguas Verdes (AV), Borreguil (BG), Mosca (MC) and Cuadrada (CD)

	Response Variable	Regression Model	Adj R^2	F	AICc
RS Lake					
1	Chl	-0.25 + 0. 74***Temp	0.624	37.6	30.5
RSS Lake					
1	Chl	0.09 + 0.64***Temp - $0.13^{\S}$SPI	0.772	36.5	23.9
		0.14 + 0.63***Temp	0.701	54.6	25.4
AV Lake					
1	Chl	0.04 +0.65***Temp	0.520	30.3	55.9
BG Lake					
1	Chl	0.03 + 0.53**Temp - 0.29*SPI - 0.45*Precip	0.840	30.6	32.2
MC Lake					
1	Chl	-0.08 + 0.83***Temp - $0.17^{\S}$SPI	0.808	32.5	26.5
2		0.05 + 0.89***Temp	0.745	53.6	26.9
CD Lake					
1	Chl	0.03 + 0.74** Temp	0.708	44.6	29.7
2		-0.03 + 0.72**Temp- 0.12^{ns}SPI	0.682	17.1	31.5

Table 3. 2. Results of the best models for predicting sedimentary chlorophyll-*a* for the six study lakes, based on Akaike Information Criterion (AICc) values. Temp = Madrid air temperature; Precip = San Fernando precipitation; SPI = Sahel precipitation index; wNAO = winter NAO index. Explanatory variables were standardized to mean variance using z-scores prior analyses. Adj R^2= Adjusted R^2. Significant level: *** $P < 0.001$; ** $0.001 < P < 0.01$; * $0.01 < P < 0.05$; § $0.05 < P < 0.1$; ^{ns} $P < 0.1$.

Subfossil Cladocera

The paleolimnological cladoceran records share similarities in timing and direction in taxon-specific changes across all of the Sierra Nevada study lakes (Figs. 3.5 and 3.6). The most striking cladoceran assemblage shift within the past ~150 years is the decline in relative abundance of *Chydorus sphaericus* and the concurrent increase in *Alona quadrangularis* (or *Daphnia pulex* gr. in CD) (Fig. 3.5). In three of the lakes (MC, RS and RSS), *A. quadrangularis* is either a new arrival or was present in only trace abundances in the deeper sedimentary intervals. With the exception of AV, this taxon-specific cladoceran change started gradually at the turn of the century, but was most striking in all lakes post-1980s, as highlighted by PCA z-scores (Fig. 3.6). Biostratigraphic zones of change in the cladoceran records were identified by the broken-stick model at ~1990 in RS, RSS, BG and MC, ~2000 in CD, whereas no important zonation was identified in AV (Fig. 3.5).

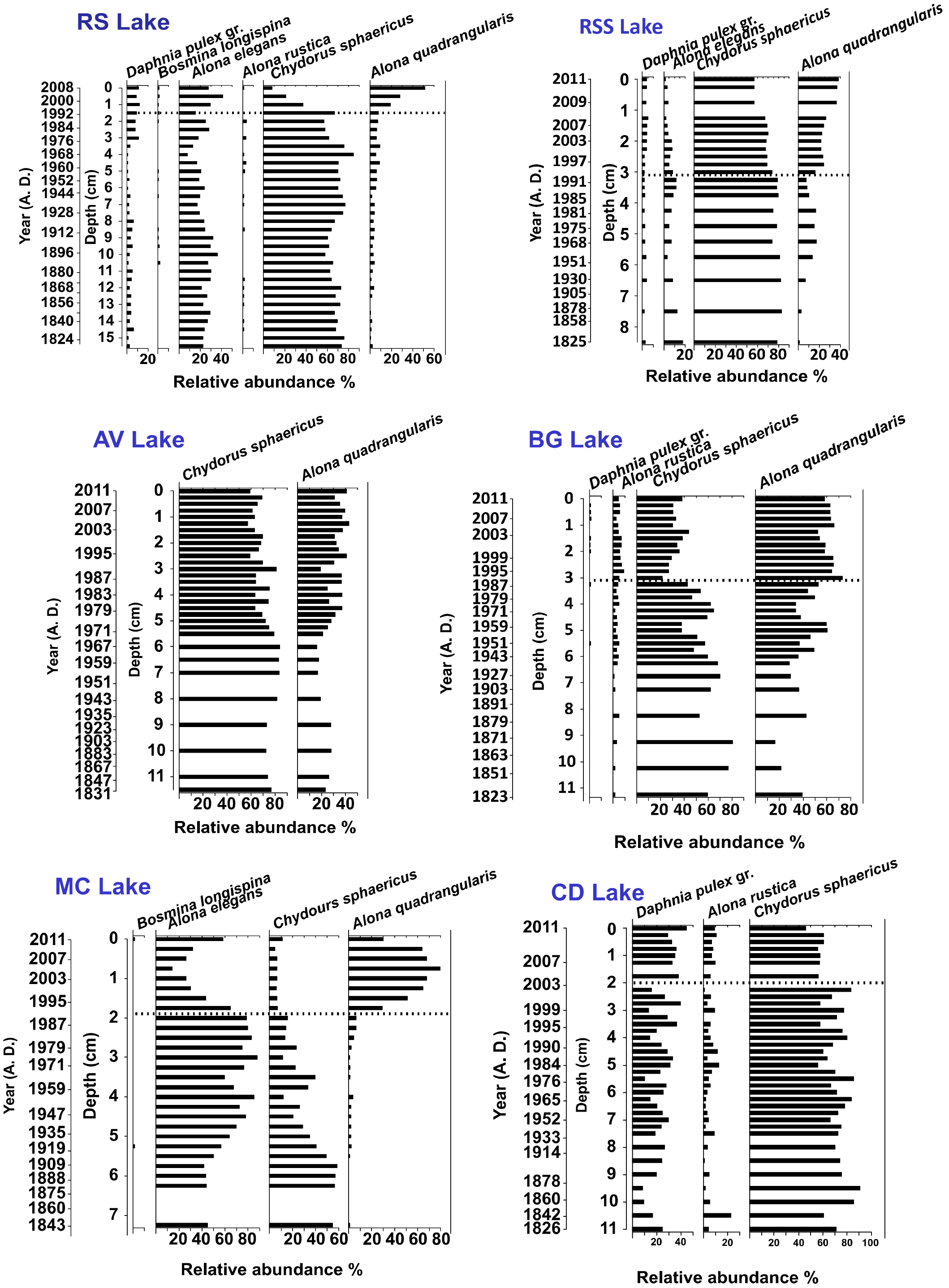


Figure 3. 5. Relative frequency diagrams of the most common cladoceran taxa recorded in the sediment cores from the six study lakes. The broken line represents the main zonation identified by the broken-stick model.

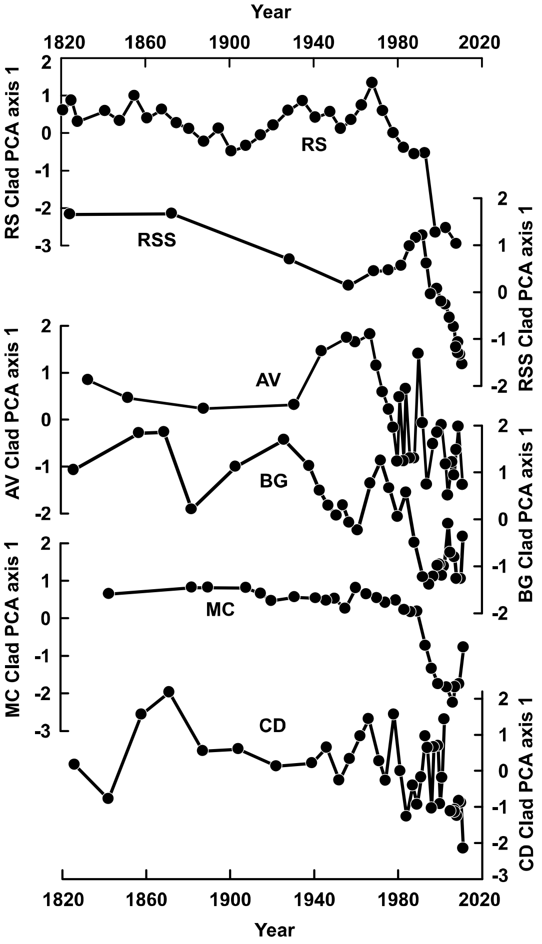


Figure 3. 6. Comparison of Cladocera PCA axis 1 sample scores (standardized to mean variance using z-scores) for the six study lakes. Lake name abbreviations: Río Seco (RS), Río Seco Superior (RSS), Aguas Verdes (AV), Borreguil (BG), Mosca (MC) and Cuadrada (CD).

Changes in the cladoceran sedimentary assemblages coincide with trends in the MAAT Madrid and AP San Fernando data (Figs. 3.3 and 3.6). Increases in air temperature and relatively low precipitation values following the wet period at the end of the 19th century are concurrent with subtle changes in the cladoceran record. The highest air temperatures on record occurred during a period of low precipitation post-1980 that was accompanied by the most pronounced cladoceran changes in the study lakes (Figs. 3.3 and 3.6). Increased primary production was observed after the 1970s in all lakes and is consistent with increased temperatures and with the period of maximum Saharan dust deposition (Figs. 3.3 and 3.4), delivering P-rich dust to the lakes. *D. pulex* gr. was observed in four of the six study lakes (RS, RSS, BG and CD) with relative abundances that were generally greatest in the recent sediments (Fig. 3.5), although the magnitude of this increase varies among lakes (Fig. 3.7). For example, a striking increase in *Daphnia* has occurred in RS since the early 1970s, while in CD, increases in *Daphnia* abundance follow a gradual increase since the early 1900s that is particularly pronounced after ~1980. In RSS, *Daphnia* occurs in low relative abundance and exhibits minimal changes with a slight increase since ~2000 (Figs. 3.5 and 3.7). In BG, *D. pulex* gr. is represented by only a few sedimentary ephippia and postabdominal claws over the last fifty years.

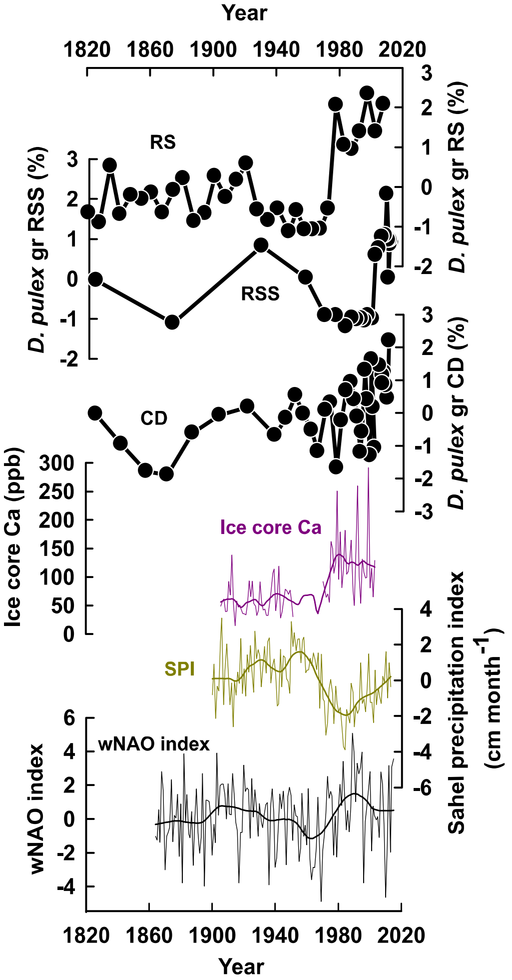


Figure 3. 7. Comparison of *Daphnia pulex* gr. percent relative abundance (standardized to mean variance using z-scores) for RS, RSS and CD lakes, and ice core Ca record (ppb), SPI (Sahel precipitation index, cm month^{-1}) and wNAO index. A LOESS smoother (span=0.2) was applied to the climate indices and the Ca record to improve the clarity of the figure and highlight trends. Lake name abbreviations: Río Seco (RS), Río Seco Superior (RSS) and Cuadrada (CD).

Saharan dust deposition data and drivers

The Zr/Al ratio from RS and the Ca record derived from the Alps ice core (adapted to RS intervals) show a highly significant correlation (r = 0.785, $p = 8.8 \cdot 10^{-4}$). Both variables were considerably higher in the last ~50 years than in the early 20th century (Fig. 3.3), coinciding with the observed changes in SPI and the wNAO index, that respectively experienced their lowest and highest values in recent decades. As both indexes control Saharan dust emission and transport, we can consider them to be predictors of the transport and intensity of Saharan dust events in Sierra Nevada and representative of P and Ca deposition trends in Sierra Nevada. The significant correlations found between Zr/Al ratio and both indices (SPI: $r = -0.862$, $p = 2.0 \cdot 10^{-6}$;wNAO: $r = 0.361$, $p = 0.049$) and between Ca ice core and SPI ($r = -0.448$, $p = 2.8 \cdot 10^{-5}$) support this assumption. The wNAO index was consistently high (positive index values) from the mid-1970s onwards, reaching a maximum during the 1980-90s (Fig. 3.3). A predominantly wet period (positive values) in the SPI record occurred from 1900 to ~1970, followed by a relatively stable and dry period (negative values) from ~1970 to the 1980s onwards with lowest values observed during the 1980-1990s (Fig. 3.3).

The wNAO index and AP San Fernando and wNAO index and SPI are negatively correlated ($r = -0.454$, $p = 4 \cdot 10^{-10}$ and $r = -0.250$, $p = 0.007$, respectively), as are the SPI and MAAT Madrid ($r = -0.278$, $p = 0.003$).

Relationships between proxy data and instrumental records

Results of RDA indicate temperature to be the main driver of the cladoceran sedimentary assemblages of all the lakes except MC and CD where chlorophyll-*a* is the main explanatory variable (Table 3.3). SPI is a secondary explanatory variable in some of the lakes. The percentage of explained variance ranged from 11% in CD to 53% in MC.

		Df	Variance	F	*p*-values
RS	Temp	1	1.691	1.691	**_0.007***_**
	Residual	16	6.917		
	Adj R^2	0.146			
RSS	Temp	1	0.885	7.512	**_0.007**_**
	SPI	1	0.456	3.872	**_0.033*_**
	Residual	17			
	Adj R^2	0.299			
AV	Temp	1	0.457	9.343	**_0.010**_**
	SPI	1	0.253	5.163	**_0.039*_**
	Residual	24	1.175		
	Adj R^2	0.386			
BG	Temp	1	0.918	8.204	**_0.001***_**
	Residual	22	2.462		
	Adj R^2	0.239			
MC	Chl	1	1.892	25.106	**_0.001***_**
	Residual	20	1.507		
	Adj R^2	0.534			
CD	Chl	1	0.307	4.306	**_0.029*_**
	Residual	25	1.786		
	Adj R^2	0.113			

Table 3. 3. Summary of partial redundancy analyses (pRDA) for the six study lakes with cladoceran assemblages as response variables and predictor variables including Temp = Madrid air temperature; Precip = San Fernando precipitation; SPI = Sahel precipitation index; Chl = chlorophyll-a record; wNAO = winter NAO index. Forward/backward-selection was used to select the explanatory variables and were z-score transformed to standardize to mean variance. Only selected predictor variables are shown in table. Adj R^2= Adjusted R^2. Significance levels: *** $P < 0.001$; ** $0.001 < P < 0.01$; * $0.01 < P < 0.05$.

PCA axis 1 sample scores explained 38% (RS), 75% (RSS), 99% (AV), 66% (BG), 53% (MC) and 69% (CD) of the variance of the cladoceran assemblage data, while PCA axis 2 sample scores explained less than 29% in all the lakes.

PCA axis 1 sample scores track the main cladoceran assemblage changes, particularly the replacement of *C. sphaericus* by either *A. quadrangularis* or *D. pulex* gr. The model selection analyses indicate temperature and/or chlorophyll-*a* to be the main predictor variables of PCA axis 1 sample scores for all the study lakes (Table 3.4), with the amount of variance explained by these variables ranging from 34% in CD to 88% in MC. Secondary explanatory variables in all the lakes are SPI and wNAO.

	Response Variable	Regression Model	Adj R^2	F	AICc
RS Lake					
1	PCA 1	-0.05 - 0. 51**Temp - 0.31*wNAO	0.440	9.65	47.8
2		-0.11 - 0.55**Temp + 0.21^{ns}Precip	0.365	7.33	47.9
RSS Lake					
1	PCA 1	-0.15 - 0.42**Temp - 0.37*Chl - 0.33*SPI	0.562	9.13	43.9
AV Lake					
1	PCA 1	0.32 - 0.58***Chl + 0.41***SPI	0.477	12.9	66.9
BG Lake					
1	PCA 1	-0.00 - 0.41***Temp - 0.12**wNAO	0.660	27.2	16.7
MC Lake					
1	PCA 1	-0.06 - 1.08***Chl - 0.18*SPI + $0.20^{\S}$Precip	0.885	54.97	26.2
2		-0.08 - 0.99***Chl	0.876	176.9	26.7
CD Lake					
1	PCA 1	0.08 - 0.60**Chl - 0.04^{ns}Precip - 0.12^{ns}wNAO	0.335	6.377	72.6

Table 3. 4. Results of the model selection analyses predicting PCA axis 1 score of Cladocera for the six study lakes. The best models according to the Akaike Information Criterion (AICc) values are shown. Explained variables were z-score transformed prior to analyses. Temp = Madrid air temperature; Precip = San Fernando precipitation; SPI = Sahel precipitation index; Chl = chlorophyll-a record; wNAO = winter NAO index. Adj R^2= Adjusted R^2;. Significance levels: *** P < 0.001; ** 0.001 < P < 0.01; * 0.01 < P < 0.05; § 0.05 < P < 0.1; ^{ns} P < 0.1.

The timing of the main changes of *D. pulex* gr. were generally coherent with climate metrics (MAAT Madrid and AP San Fernando) and chlorophyll-*a* changes (Figs. 3.3, 3.4 and 3.7). The period of *Daphnia* increase in RS, BG and CD is also concurrent with the period of maximum Ca deposition, and the timing of maximum change in the wNAO index and SPI (Fig. 3.7). *Daphnia* relative abundances are significantly and negatively correlated to the SPI record in RS and CD (r > -0.45, p < 0.01) and marginally significant in RSS (r = -0.42, p = 0.057). Moreover, *Daphnia* is significantly and positively correlated to chlorophyll-*a* (r = 0.38, p = 0.036) and wNAO index in RS (r = 0.68, p = 0.001). A marginally significant correlation between *Daphnia* and MAAT Madrid was found in RS (r = 0.40, p = 0.058) and with AP San Fernando in CD (r = -0.29, p = 0.081) (Model selection analyses indicate that the main predictor variables of *D. pulex* gr. in RS is the SPI, with wNAO index and AP San Fernando as secondary explanatory variables (all models explained ~ 60% of the observed variation) and chlorophyll-*a* in RSS and CD with more than 20% of explained variance (Table 3.5).

	Response Variable	Regression Model	Adj R^2	F	AICc
RS Lake					
1	*D. pulex* gr.	-0.00 - 0.57**SPI + 0.34§wNAO	0.620	14.9	46.5
2		-0.06 - 0.59**SPI - 0.48§Precip	0.598	13.7	47.5
RSS Lake					
1	*D. pulex* gr.	-0.03 + 0.93*Chl	0.261	7.02	52.61
CD Lake					
1	*D. pulex* gr.	-0.03 + 0.50**Chl + 0.06^{ns}wNAO	0.223	5.74	74.2

Table 3. 5. Results of model selection analyses predicting D. pulex gr. abundance in RS, RSS and CD lakes. The best models as determined by the Akaike Information Criterion (AICc) values are shown. Explained variables were z-score transformed prior to analyses.. Precip = San Fernando precipitation; SPI = Sahel precipitation index; Chl = chlorophyll-a record; wNAO = winter NAO index. Adj R^2= Adjusted R^2; . Only significant models are shown in table. Significance levels: *** $P < 0.001$; ** $0.001 < P < 0.01$; * $0.01 < P < 0.05$; § $0.05 < P < 0.1$; ^{ns} $P < 0.1$.

DISCUSSION

We identified air temperature and SPI (proxy of Saharan dust deposition) or wNAO as the best predictor variables for changes in primary production (chlorophyll-*a*) in all six study lakes. In some lakes, precipitation and SPI were deemed to be significant predictors of chlorophyll-*a*. The relationship between warming and increases in primary production in alpine lakes has been frequently attributed to decreased ice cover, warmer water temperatures and a longer growing season (Michelutti *et al.,* 2005, 2010). There is growing evidence from annual to multi-year (>10) studies that P-rich dust input exerts a notable influence on primary production of oligotrophic lakes (e.g. Brahney *et al*., 2015a and b) including Mediterranean alpine lakes such as lakes in the Pyrenees and the Sierra Nevada (Morales-Baquero *et al*., 2006a; Camarero & Catalan, 2012). Our centennial-scale study supports previous work in the Sierra Nevada. Thus, the intensification of warming and of delivery of P-rich Saharan input since the 1970s has resulted in a combined effect on the primary production of Sierra Nevada lakes.

The main shift within the cladoceran sedimentary assemblages of each study lake occurred during the latter half of the 20th century, and is coincident with trends in regional air temperature (MAAT Madrid) and sedimentary chlorophyll-*a* concentrations. These two independent measurements were deemed to be the best predictor variables of the cladoceran assemblage changes for all lakes (Table 3.3 and 3.4). The principal cladoceran shift also occurs concurrently with the increase in atmospheric Ca deposition since ~1980 onwards as well as with the period of maximum change in the two proxies for atmospheric Ca deposition (wNAO index and SPI).

The combined effect of rising temperatures and prolonged drought beginning in the 1970s (Fig. 3.3) have led to a scarcity of snow in the Sierra Nevada region (Bonet *et al*., 2016, Pérez-Luque *et al*., 2016), and likely also to shorter ice-cover periods, warmer waters and water level decrease in most of the study lakes (Morales-Baquero *et al*., 2006b; Pérez-Martínez *et al*., 2007). Moreover, Southern Spain suffered acute periods of drought during the late-1980s and 1990s, as warmer and drier climatic conditions became more pronounced (Udelhoven *et al*., 2009). These climatic changes may have compounded the cladoceran assemblage shifts that have occurred in each study lake since the 1990s (Figs. 3.5 and 3.6). Indeed, the onset of cladoceran assemblage changes in most lakes occurs at the end of a wet period, during the last decades of the 19th century and the rise in temperature at the beginning of the 20th century (Fig. 3.3). Warming-related changes in Sierra Nevada lakes suggest a process that began over a century ago, that has intensified in the past ~50 years.

Several mechanisms associated with climate change may explain the observed cladoceran assemblage shifts. For example, *A. quadrangularis* and *C. sphaericus* are both well represented in the sedimentary assemblages of the study lakes, and have different habitat preferences. *C. sphaericus* is principally associated with shallow or littoral habitats (Korhola, 1999), but frequently enter the planktonic zone (de Eyto & Irvine, 2001; Walseng *et al.*, 2006; Davidson *et al.*, 2010), while *A. quadrangularis* is principally associated with bottom mud substrata and/or benthic habitats (Whiteside *et al.*, 1978; Tremel *et al.*, 2000). In other regions, water level changes have been reported to significantly affect lake zones and therefore habitat quality and quantity (Nevalainen *et al.*, 2013; Levi *et al.*, 2016). However, our Sierra Nevada study lakes are small, clear water, shallow and littoral-dominated systems with no clear differentiation between littoral and profundal zones. Moreover, based on the sedimentary biological assemblages, these lakes have likely always been shallow and have experienced little change in water level (de Vicente *et al.* 2010). This is in stark contrast with the extensively studied deep, closed basin, La Caldera Lake (Villar-Argaiz *et al.*, 2001). In our study, the shift from *C. sphaericus* to *A. quadrangularis* may be due to climate change related factors rather than habitat preference. For instance, *C. sphaericus* is associated with lakes with longer periods of ice-cover and low water temperatures and is therefore typified as a cold-tolerant species, whereas *A. quadrangularis* is associated with less harsh climatic conditions (Bigler *et al.*, 2006; Catalan *et al.*, 2009; Nováková *et al.*, 2013). The shorter ice-cover periods and warmer waters in Sierra Nevada following the 1970s likely favored *A. quadrangularis* to the detriment of *C. sphaericus.* In addition, lake water residence time likely increased with the onset of a warmer and dryer climate resulting in reduced snowpack and runoff, potentially favoring taxa with larger body size and lower growth rates, such as *A. quadrangularis*.

The timing of cladoceran assemblage changes is generally consistent with regional air temperature increases, precipitation decreases, and dust deposition increases (Figs. 3.3, 3.5 and 3.6). However, discrepancies in the timing may be attributed to lake-specific factors. For example, the cladoceran assemblage shift at ~1900 was evident in all lakes with the exception of AV. The lack of an earlier biological change in AV may be due to its geographical setting, as it is the highest elevation study lake and is proximal to the largest, most persistent snow patches in Sierra Nevada, that deliver cold water to AV throughout the ice-free period. Therefore, AV has a relatively high water renewal time, experiences very little change in water level, and has lower water temperatures than the other study lakes (i.e. nearby RS is located at a similar altitude). These features can explain this lake's delayed response to regional air temperature warming.

In two of our study lakes, chlorophyll-*a* concentrations were identified as an important explanatory variable of cladoceran changes (Table 3.3). The strong relationship between cladoceran assemblages and chlorophyll-*a* in these Sierra Nevada lakes may be a consequence of the strong influence of temperature, rather than a direct effect of the chlorophyll-*a* increase. Therefore, the increase (or arrival) of *A. quadrangularis* in each study lake may signal the onset of more favorable environmental conditions in terms of water temperature and ice cover, relative to *C. sphaericus* which is tolerant of harsher conditions (e.g. colder, ultraoligotrophic waters) in alpine lakes (Harmsworth, 1968; Whiteside, 1970, Lotter *et al*., 1997; Bigler *et al.,* 2006). In central European lakes, *C. sphaericus* has been found in sediment records from the Late-Glacial (Frey, 1958) and was a dominant taxon during the Pleniglacial (Hofmann, 1991), with its highest abundance prior to the rapid warming following the last glacial period (Nováková *et al*., 2013). The decrease of *C. sphaericus* may be due to stronger competition with taxa less well-adapted to cold conditions such as *A. quadrangularis*, that increased in relative abundance with the onset of recent warming. It is plausible that warmer water temperatures, longer growing seasons and increased primary production allowed more specialized taxa such as *A. quadrangularis* to thrive, and may have also favored open water filter feeders such as *Daphnia* spp.

Daphnia is a keystone species in many freshwater ecosystems due to its intermediate trophic position and its high efficiency at filtering phytoplankton (Brooks & Dodson, 1965, Persson *et al.*, 2007). Although *Daphnia* spp. were not a dominant component of the Sierra Nevada assemblages, with the exception of CD, their presence and recent increases in relative abundance are nevertheless ecologically important. The high values of Saharan Ca deposition, high air temperatures, and low precipitation over the past few decades coincide with the significant *Daphnia* increase in RS (since 1970) and in CD (since 1900 and most pronounced after ~1980) (Figs. 3.3, 3.5 and 3.7).

Significant correlations between *Daphnia* and Saharan input drivers SPI and wNAO (Table 3.5) and the identification of SPI as the best predictor variable of *Daphnia* abundance in RS support the hypothesis that increased Saharan dust deposition throughout the 20th century are partially responsible for increases in *Daphnia*. Low lake water Ca concentrations can impair *Daphnia* growth and reproduction, and the naturally low Ca concentrations of Sierra Nevada lakes (Table 3.1), have likely been limiting for daphniids (Hessen *et al.*, 2000; Ashforth & Yan, 2008). Furthermore, those lakes that have experienced recent *Daphnia* increases (RS, BG and CD) are particularly low in Ca (< 1.5 mg L^{-1}) and potentially sensitive to Ca enrichment (Table 3.1). In contrast, RSS with a higher Ca concentration, recorded minimal changes in *Daphnia* relative abundances through time. Lake Ca concentration can be significantly affected by Saharan Ca input in Sierra Nevada lakes (Pulido-Villena *et al.*, 2006), thus *Daphnia* is significantly correlated to Saharan input drivers in the three lakes and SPI is the best *Daphnia* predictor in RS. In addition to the increase of atmospheric input, increased Ca concentrations due to evapoconcentration (Pulido-Villena *et al.*, 2006), and also longer exposure to Saharan dust with increased water residence time and a longer ice-free period, have all likely occurred in recent decades.

Increased relative abundances of *Daphnia* in the low alkalinity lakes of Sierra Nevada represents a distinct ecological signal of recent environmental changes in this region, contrasting with the recent declines of *Daphnia* linked to the declines in Ca concentration that are a legacy of acid deposition in many Canadian softwater lakes (Jeziorski *et al*., 2008; 2012). Sierra Nevada is not particularly affected by acid deposition; instead the Ca-rich Saharan deposition is a major contributing factor to the aquatic ecosystem dynamics in this region (Pey *et al*., 2013, Morales-Baquero & Pérez-Martínez, 2016).

In addition to Saharan dust metrics, *Daphnia* increases coincide with increases in chlorophyll-*a.* This proxy for primary production was identified as the best driver of *Daphnia* changes in RSS and CD. As previously mentioned, the relationship between *Daphnia* and chlorophyll-*a* in Sierra Nevada lakes can be a consequence of the strong influence of temperature on chlorophyll-*a* (Table 3.2). In this case, there are several mechanisms to explain the positive influence of warming on *Daphnia* development. Warmer waters and longer growing seasons has likely been advantageous to relatively large cladoceran species such as *D. pulicaria* in our study lakes, consistent with the conclusions of previous studies in Sierra Nevada lakes (Morales-Baquero *et al*., 2006b; Pérez-Martínez *et al*., 2013) and with long-term trends of *Daphnia* increases associated with warming reported in several studies (Luoto & Nevalainen, 2013b, Jeziorski *et al*., 2015; Luoto *et al*., 2015). In Sierra Nevada, hydraulic washout can be an important process for *Daphnia* loss in these lakes. However, the warmer and drier climate of the past decades could favor *Daphnia* population growth through an increase in lake water residence time.

It is also possible that an increase in food availability (i.e. increased primary production) favored the herbivorous *D. pulex* gr. in these lakes as other studies have identified increases in *Daphnia* related to increased algal production (Jeziorski *et al.*, 2015, Korhola & Rautio, 2001). Villar-Argaiz *et al.* (2002, 2012) indicate food quantity and high seston C:P values to limit *Daphnia* development in La Caldera Lake of Sierra Nevada and observed an enhancement of *Daphnia* population in exceptionally high atmospheric load years. If this is the case, both warming and Saharan P input would be the ultimate drivers of cladoceran change; however, this is not possible to determine based solely on sedimentary chlorophyll-*a* trends.

In addition to climate and Saharan P inputs, there are a variety of other factors that may have contributed to increases in chlorophyll-*a*, including both human activities and atmospheric nitrogen (N) inputs. Although our Sierra Nevada study lakes differ in the amount of anthropogenic disturbance they experience, in general they are minimally affected by human activity. Despite the low and different levels of disturbance, all lakes exhibited similarly increased sedimentary chlorophyll-*a* in recent decades suggesting that increased primary production is not directly associated with human disturbances. Another potential explanation may be an increased atmospheric N input that has been reported as a mechanism of fertilization in remote lakes (Bergström & Jansson, 2006). However, in the Sierra Nevada mountain region, N deposition is very low, relative to other Mediterranean zones (Morales-Baquero *et al.*, 2006a) and heavily industrialized areas in central Europe (Holland *et al.*, 2005). Furthermore, N deposition mainly occurs as wet deposition (i.e., during the ice-cover period in Sierra Nevada lakes, and therefore less likely affecting lake biota (Morales-Baquero & Pérez-Martínez, 2016). It is therefore unlikely that atmospheric N-deposition has contributed much to the increase in chlorophyll-*a*, and the cladoceran assemblage changes recorded in these lakes. In contrast, the high delivery of P-rich Saharan dust during the last 50 years, mainly occurring in spring-summer time, can better explain (together with higher air temperatures and longer ice-free periods) the increasing trends in chlorophyll-*a*. This corroborates the short-term study of

Morales-Baquero *et al.* (2006a) who reported that chlorophyll-*a* concentrations were significantly influenced by Saharan dust deposition in Sierra Nevada lakes. Camarero & Catalan (2012) also highlight the role played by African atmospheric P deposition in the N:P stoichiometry and the nutrient limitation status of phytoplankton in the Pyrenean lake district over the last two decades. It is likely that warmer temperatures and longer growing seasons, together with increased delivery of P-laden dust, has resulted in notable increases in chlorophyll-*a* across the Sierra Nevada lakes.

Based on our data, we conclude that Sierra Nevada lakes have recently undergone changes that are consistent with a regional-scale response to a warmer and drier climate together with increases in Saharan dust inputs (Ca and P). Increases in atmospheric P and Ca deposition have likely affected primary production and cladoceran assemblage composition, specifically the arrival of *Daphnia* in remote, shallow Sierra Nevada lakes. Saharan dust deposition has important ecological implication on aquatic systems for many regions of the world and warrants further exploration.

Chapter 4

Cladoceran assemblage distribution in alpine lakes of Sierra Nevada (Spain) and its relationship to environmental variables

ABSTRACT

Surface sediments from 17 high-mountain lakes in the Sierra Nevada (Spain) were analyzed for subfossil cladoceran assemblages in order to evaluate the relationships between species assemblages and measured environmental variables. According to multivariate techniques, distribution of cladoceran assemblage is mainly associated to morphometric variables (e.g. extent of the catchment vegetation in the basin, lake depth and size and the presence of inlets and/or outlets), while the influence of chemical parameters, seems to play a secondary role as explanatory variables, being TN the most important variable. Among cladoceran taxa, *Alona quadrangularis* was strongly associated to lakes with inlets and/or outlets and surrounded by meadows, both lake features which may implies a higher input of material within the lake from its catchment. *A. quadrangularis* and *C. sphaericus* relative abundance showed a significant negative relationship. Higher abundance of *C. sphaericus* was found in closed or open/closed basins with less or no meadows surrounding the lake, mainly associated to their advantage to inhabiting littoral and water pelagic column, particularly in these relatively small Sierra Nevada lakes. On the other hand, *Alona elegans* may be linked to open and shallow lakes with a high nutrient and ion concentration, likely attributed to the development of vegetation. *Daphnia pulex* gr. were most clearly associated to low TN lakes and closed and open/closed basin. Supplemental analysis further support a strong influence of two morphometric variables (maximum depth and presence of inlets and outlets) on *Daphnia* presence. The absence of *Daphnia* in open systems may be related to the negative effect of dilution on *Daphnia* population development associated to a high water renewal in these lakes.

Keywords: Cladocera, Paleolimnology, high-mountain lakes, environmental variables

INTRODUCTION

There has been increasing evidence documenting the effect of recent climate warming on high mountain ecosystems (e.g., Lotter *et al.*, 1997; Rühland *et al.*, 2006; Hobbs *et al.*, 2010). Among direct and indirect consequence of the global warming, the increase in lake-water temperatures, melting of snow and ice in the lake catchment, earlier seasonal ice break-up, large variation of lake water level or increasing weathering rates are expected to affect the aquatic ecosystems in high mountain areas (Livingstone & Lotter, 1998, Gobiet *et al.*, 2014). As climatic forcing alter many of the physical, chemical and biological aspects of lake ecosystems (Battarbee, 2000; Adrian *et al.*, 2009), the combined effects of the multiple stressors could cause a cascading effects on ecosystems functioning thought the influence on the physical and chemical structure, and as a consequence, on the aquatic biota in these alpine lake ecosystems (Catalan *et al.*, 2009). Actually, major changes in biota communities have already reflected through substantial ecological changes to 20th century warming in alpine lakes (Battarbee *et al.*, 2009; Weckström *et al.*, 2016).

Although lakes will respond to climate effects that will be higher in amplitude in the sensitive, high elevation areas (Pepin *et al.*, 2015), there are other regional and local effects, such as lake size, catchment area, depth, etc. that need to be considered (Baron & Caine, 2000; Adrian *et al.*, 2009; Smol, 2010). Collectively these characteristics may play an important role in determine the direct and indirect forcing drivers controlling current biota distributions. In fact, catchment and lake-specific differences, such us the present or absence of vegetation in the catchment has explained differences in the nutrient limitation in similar alpine lakes in Sierra Nevada area (Morales-Baquero *et al.*, 2006b; Mladenov *et al.*, 2008), or factors such us water renewal time have partially explained differences in magnitude and timing of cladoceran changes in six alpine lakes in Sierra Nevada over the past ~150 years (see Chapter 3).

Among biota communities, the Cladocera (Crustacea: Branchiopoda) is a key group of organisms in many high mountain lakes, recognized as useful environmental indicators because inhabit a wide range of habitats, and they are sensitive indicators lake water chemistry, such as pH (Whiteside, 1970; Marmorek & Korman, 1993) and calcium (Hessen *et al.*, 1995; Jeziorski *et al.,* 2008); trophic dynamics (Jeppesen *et al.*, 2003; Perga *et al.*, 2010), lake water temperatures (Korhola, 1999; Fischer *et al.*, 2011), lake-water level (Nevalainen *et al.*, 2011; Sittonen *et al.*, 2011) or climate (Korponai *et al.*, 2011). Moreover, cladocerans are a fundamental component of the zooplankton and benthos, being the last group scarcely studied in intensive monitoring programs. Despite their usefulness, little is known concerning the cladoceran communities in the Sierra Nevada region, or the factors regulating their abundance and occurrence.

Routine monitoring researches of biotic and abiotic changes are not always available due the remoteness location and difficult access to these high mountain regions (Battarbee *et al.*, 2002). Although, Sierra Nevada region constitute a key monitoring points for natural climate variability (Zamora *et al.*, 2016), a complete long time datasets are almost inexistent, despite their current ongoing limnological studies. An alternative to intensive monitoring programs could be the palaeolimnological techniques, providing a more comprehensive overview of the distribution of cladoceran taxa within the surface sediments in relation with the existing environmental factors of each lake. These approaches could help to estimate the relationship between the species distributions and environmental condition, and therefore, to better understand their interpretation. The use of subfossil cladoceran remains to assess their relationships with environmental factors over many spatial and temporal scales have been used successfully in other remote regions including arctic lakes (Sweetman *et al*, 2010; DeSellas *et al.*, 2008) and alpine and subalpine lakes (Bigler *et al.*, 2006; Kamenik *et al.*, 2009).

Here, we examine the composition of sedimentary cladoceran assemblages based on their remains from surface sediment of a set of 17 alpine lakes ranging in elevation from 2825 to 3050 m asl. Our analyses provide a detailed description of cladoceran assemblages (including pelagic and littoral taxa) and their relationships to morphometric and chemical parameters, thus complimenting existing monitoring surveys that mainly focus on the pelagic environment. Hence, the objectives of this study are to relate the cladoceran assemblages in surface-sediment samples to morphometric and chemical measured parameters in 17 lakes across the Sierra Nevada region and to determine which subset of environmental parameters contribute statistically most significantly to the distributions of cladocerans. Moreover, we are particularly interested in *Daphnia* distribution and associated parameters in these lakes because the risk attached to the detection of an invasive *Daphnia* taxon in the last decades in Sierra Nevada lakes (see Chapter 3).

MATERIALS & METHODS

Study site and study lakes

In the Sierra Nevada Mountains of SE Spain, there are ~50 small and remote lakes of glacial origin at an elevation of ~2800-3100 m asl, all of them located above the tree line (Fig. 4.1) (Castillo, 2009). The catchment bedrock is siliceous and largely comprised of mica-schist with graphite and/or feldspar (Puga *et al.*, 2007). The soil is poorly developed and does not support agriculture or forestry. 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 m asl) reports a mean annual temperature of 4.4 ºC and total precipitation of 700 mm, with 80% occurring as snow between October and April (Castillo *et al.*, 1996). The ice-cover season in the Sierra Nevada region typically lasts from November to June; however, large interannual differences in the duration of ice cover have been linked to annual variations in climatic conditions (Barea-

Arco *et al*., 2001; Morales-Baquero *et al*., 2006a; Pérez-Martínez *et al*., 2007).

Seventeen lakes located in five different glacial valleys close to the mountain ridge of the Sierra Nevada were strategically selected to better characterize regional lake heterogeneity (Fig. 4.1). The seventeen lakes, Caballo (CD); Nájera (NJ); Cuadrada (CD), Lanjarón (LJ), Ermita Baja (EB), Ermita Media (EM), Virgen Superior (VS), Virgen Media (VM), Aguas Verdes (AV), Río Seco Superior (RSS), Río Seco (RS), Majano (MJ), Mosca (MC), Borreguil (BG), Culo Perro (CP), Hondera (HD), Peñón Negro (PN) are within a 13 km radius, and with the exception of EB, EM, VM, VS and MC, are located on the south face of Sierra Nevada (Figure 4.1). The seventeen study lakes are fishless and do not thermally stratify during the summer. Moreover, they are all clear and well-mixed water lakes. The lakes are circumneutral to slightly acidic, and low in alkalinity and productivity. The phytoplankton and zooplankton communities are extremely simple. However, some different environmental settings and lake types are represented (Table 4.1).

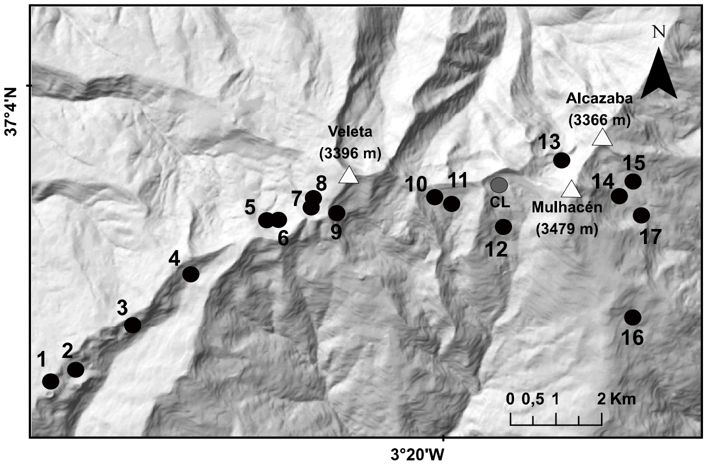


Figure 4. 1. Geographic locations of the seventeen study lakes (circles; see Table 4.1 for further information). 1: Caballo (CB); 2: Nájera (NJ); 3: Cuadrada (CD); 4: Lanjarón (LJ); 5: Ermita Baja (EB); 6: Ermita Media (EM); 7: Virgen Superior (VS); 8: Virgen Media (VM); 9: Aguas Verdes (AV); 10: Río Seco Superior (RSS); 11: Río Seco (RS); 12: Majano (MJ); 13: Mosca (MC); 14: Borreguil (BG); 15: Culo Perro (CP); 16: Peñón Negro (PN); 17: Hondera (HD). La Caldera lake (CL) is also shown (grey circle). Triangles represent the highest mountain peaks in the Sierra Nevada.

These study lakes are typically small and shallow (Table 4. 1) with no clearly differentiated littoral and profundal zone. Differences in catchment area and alpine meadow extent are noticeable. As the summer progresses, some of the study lakes may become dry (i.e. NJ, MJ and PN lakes), whereas others may experience variable reductions in their water level (i.e. CB, CD, LJ, RSS, RS CP and BG lakes) and others suffer minor changes in water level (i.e. EB, EM, VS, VM, AV, MC and HD lakes). However, the water level reduction becomes greater and longer-lasting during warm and dry years relative to cold and wet years. Most lakes in this region have an open basin, usually with surface inlets or outlets that frequently became dry at the end of summer such us NJ, LJ, RS, MJ, CP, BG and PN lakes. Chemical parameter values of the lakes are shown in Table 4. 1.

Lake (code)	Environmental characteristics and physico-chemical parameters													
	Altitude (m)	Lake area (ha)	Catchment area (ha)	Lake area/ Catchment area	Maximum depth (m)	Presence of inlets and/or outlets	Shoreline in contact with meadows (%)	Meadows area/ Catchment area	pH	Alkalinity (meq L^{-1})	Conductivity (uS cm^{-1})	TP (µg L^{-1})	TN (µg L^{-1})	DOC (mg L^{-1})	Ca (mg L^{-1})
Caballo (CB)	2850	0,48	10,5	0,0457	4	0/0	0	0	6,64	0,068	9,585	11,78	127,16	1,07	0,667
Nájera (NJ)	2838	0,22	14,38	0,0153	0,5	1/0	7,2	0,020	6,92	0,066	12,16	32,17	591,79	3,04	0,785
Cuadrada (CD)	2910	0,24	4	0,0600	5	0/0	4	0,013	7,65	0,144	7,39	9,32	83,26	0,71	0,62
Lanjarón (LJ)	2978	0,29	35,8	0,0081	3,2	1/0	14,7	0,014	6,75	0,022	7,93	17,91	132,1	0,645	0,927
Ermita Baja (EB)	2844	0,01	71,3	0,0001	0,4	1/1	100	0,001	6,28	0,038	43	12,31	172,61	14,64	4,464
Ermita Media (EM)	2863	0,02	71,3	0,0003	0,9	1/1	100	0,003	6,30	0,056	37,5	4,09	344,8	3,03	2,712
Virgen Superior (VS)	2940	0,08	25,1	0,0032	1,4	1/1	100	0,005	7,15	0,355	60,4	7,22	256,64	1,07	6,814
Virgen Media (VM)	2945	0,01	21,2	0,0005	0,8	1/1	100	0,002	6,83	0,197	37,6	23,81	518	3,472	4,727
Aguas Verdes (AV)	3050	0,19	12,8	0,0148	2,8	1/1	100	0,024	6,71	0,158	27,3	19,72	233,5	0,93	1,982
Río Seco Superior (RSS)	3040	0,06	4,7	0,0128	2,6	0/0	53	0,004	7,15	0,156	15,39	15,17	284	1,12	1,91
Río Seco (RS)	3020	0,46	9,9	0,0465	2,9	1/0	100	0,092	6,87	0,114	23,80	15,99	402,52	1,78	1,178
Majano (MJ)	2900	0,27	72,2	0,0037	0,8	1/0	100	0,007	7,61	0,302	33,4	49,33	632,39	5,76	3,783
Mosca (MC)	2896	0,48	39,7	0,0121	3,4	1/1	100	0,006	7,80	0,292	31,55	16,80	288	1,23	4,949
Borreguil (BG)	3020	0,18	50,9	0,0035	2,5	1/0	100	0,011	6,25	0,086	13,63	17,85	280	1,575	0,992
Culo Perro (CP)	2974	0,19	57,1	0,0033	0,5	1/0	100	0,014	7,01	0,158	27	6,71	249,13	4,41	3,477
Peñón Negro (PN)	2825	0,40	28,2	0,0140	3,3	1/0	16,3	0,011	7,07	0,120	17,73	30,28	367,13	2,266	1,527
Hondera (HD)	2890	0,57	154,6	0,0037	0,7	1/1	100	0,019	7,03	0,085	22	10,47	217,63	1,638	2,638
Caldera (CL)	3050	2,10	23,5	0,0894	12	0/0	0	0	7,34	0,320	47,34	5,215	330,76	4,953	4,115

Chemical and biological characteristics are from Sánchez-Castillo *et al*. (1989), Morales-Baquero *et al*. (1999), Reche *et al*. (2005) as well as from water column samples taken during each cor e sampling day and for punctualsubsequent samples. Abbreviations: TP, Total phosphorus; TN, Total nitrogen; DOC, Dissolved Organic Carbon; Ca, calcium.Presence of inlets and outlets during both periods (earlier ice -free period/later ice-free period). 0, lakes without inlets or; 1= lakes with in lets or outlets.

Table 4. 1 . Location and environmental characteristics of the seventeen study lakes in the Sierra Nevada (La Caldera lake is also included). Mean values of the physico-chemical and biological parameters from water column measurements are sh own.

	Latitude (N)	Longitude (W)
Caballo (CB)	37º 00’ 52.51”	3º 26’ 15.97”
Nájera (NJ)	37º 01’ 00.14”	3º 25’ 54.99”
Cuadrada (CD)	37º 01’ 37.18”	3º 25’ 06.64”
Lanjarón (LJ)	37º 02’ 15.55”	3º 24’ 01.81”
Ermita Baja (EB)	37º 02’ 58.38”	3º 23’ 09.01”
Ermita Media (EM)	37º 02’ 59.71”	3º 23’ 06.04”
Virgen Superior (VS)	37º 03’ 02.91”	3º 22’ 47.87”
Virgen Media (VM)	37º 03’ 06.90”	3º 22’ 46.82”
Aguas Verdes (AV)	37º 02’ 54.79”	3º 22’ 06.16”
Río Seco Superior (RSS)	37º 03’ 06.69”	3º 20’ 53.04”
Río Seco (RS)	37º 03’ 07.63”	3º 20’ 43.92”
Majano (MJ)	37º 02’ 46.71”	3º 19’ 32.45”
Mosca (MC)	37º 03’ 35.03”	3º 18’ 53.03”
Borreguil (BG)	37º 03’ 09.53”	3º 17’ 59.03”
Culo Perro (CP)	37º 03’ 16.85”	3º 17’ 47.79”
Peñón Negro (PN)	37º 01’ 42.97”	3º 17’ 47.22”
Hondera (HD)	37º 02’ 53.10”	3º 17’ 40.14”
Caldera (CL)	37º 03’ 16.89”	3º 19’ 45.28”

Table 4. 1 .Continued. Latitude (N) and Longitude (W) of the study lakes

Dissolved organic carbon (DOC) values range from 0.65 to 14.64 mg/L (values from LJ and EB lakes, respectively), conductivity values range from 7.34 to 43 µS cm^{-1} (values from CD and EB lakes, respectively), pH values range from 6.25 to 7.8 (BG and MC lakes, respectively). Additional physicochemical and biological features for the study lakes lake have been published in several papers (Morales-Baquero *et al*., 1999, Morales-Baquero & Conde-Porcuna, 2000; Reche *et al*., 2001; Reche *et al*., 2005; Pérez-Martínez *et al*., 2007).

Sediment coring and field measurements

Sediment cores were collected during the summer of 2008 (for RS), 2011 (for RSS, AV, BG, MC and CD) and 2013 (for CB, NJ, LJ, EB, EM, VS, VM, MJ, CP, HN, PN) from the deepest basin of each lake using a slide-hammer gravity corer, with an inner core-tube diameter of 6.8 cm. The top 1-cm of sediment (representing approximately 2–5 years of accumulated material) was collected from each lake, except for RS, RSS, AV, BG, MC and CD, which average values (from 0 to 1 cm-4 first samples) were calculated. (see dating of Chapter 3 and Appendix E). The sediment samples were immediately returned to the University of Granada (Spain) and stored in a cold room at 4 ºC until cladocerans remain analyses was performed.

Tube samplers (6.7 cm diameter) of different lengths were used to collect the whole water column in an integrated sample from the deepest point of each lake during each core sampling. For nutrient concentration analyses and, alkalinity and calcium determination, water samples were collected in acid-washed polyethylene bottles and immediately cooled.

For dissolved organic carbon (DOC) analyses, samples were stored in precombusted amber glass bottles at approximately 4ºC in the dark until laboratory analysis. Specific conductivity, water temperature and pH were measured on site with a multiparameter probe (Oakton PC300). For pH and conductivity, equipment was calibrated at each lake prior to measurements.

Sediment samples for loss-on-ignition (LOI) and subfossil Cladocera analysis were kept in a cold (4 ºC) and dark room until analysis. Water for nutrient analyses was frozen until analysis

Data analyses

Sedimentary proxy record

Subfossil cladoceran surface sample from each of the 17 Sierra Nevada lakes were analyzed and identified using the methods described in Szeroczyńska & Sarmaja-Korjonen (2007). For the surface sediment core interval, ~1 cm^3 of fresh sediment was heated for 20 minutes in 10% KOH to remove humic matter, and then washed and sieved through a 38 µm-mesh under tap water. The samples were centrifuged (5 min, 3000 rpm) to concentrate the fossil cladoceran remains, and mounted onto microscope slides using glycerol gelatin dyed with safranin. A minimum of 200 remains (carapaces, headshields, postabdomens, postabdominal claws, valves, shells and ephippia) were identified and enumerated under a light microscope at 200x magnification (Kurek *et al.*, 2010). The most abundant remain from each taxon was used to calculate the number of individuals present in the sample (Frey, 1986). Cladoceran data are expressed as a percent relative abundance of the total number of individuals counted for each study lakes.

Loss on ignition (LOI) was measured to calculate the organic matter content in the sediments. Samples were dried in an oven at 105 ºC for 24 h and weighed. The content of the organic matter was analyzed by incinerating the samples at 550ºC for 4 h using a muffle furnace (Dean, 1974; Heiri *et al.*, 2001).

Water chemistry analyses

Dissolved calcium (Ca) concentration was analyzed by atomic absorption at Center of scientific instrumentation at University of Granada. Dissolved organic carbon (DOC) concentrations were carried out by thermal oxidation with a ShimadzuTOC-5000 equipped with a Shimadzu platinised-quartz catalyst for high sensitivity analysis following the method described in Mladenov *et al* (2008). Previous to the analyses Ca and DOC samples of all the study lakes where filtered through precombusted (2 h at 500 ºC) Whatman GF/F glass fiber filters (20µm).

Concentrations of silicon oxide (SiO2), total nitrogen (TN) and total phosphorus (TP) were measured according to standard methods [American Public Health Association (APHA), 1995]. TN and TP measurement were measured in unfiltered water samples after acidic digestion in an autoclave for 2h at 120ºC. Herein we refer to the sum of dissolved organic and inorganic N and P plus particulate forms as total nitrogen and total phosphorus, respectively. The total phosphorus: total nitrogen (TP:TN) ratio was calculated from the mass data and expressed as mass ratio.

Chemical characteristics are from water column samples taken during each core sampling day except for DOC and Ca (data derived from water column samples taken during each core sampling day and for punctual subsequent samples, and from Morales-Baquero *et al.* (1999), Reche *et al.* (2005)). Total alkalinity was measured according to titration method (APHA, 1995).

Lake-morphometric data

Catchment, meadow and lake surface area for each lake were obtained from Morales-Baquero *et al.* (1999) and also by analyzing images captured from Google Maps with a microscope imaging software (Leica Application Suite).

Statistical analyses

Statistical analyses were performed using R 3.4.0 (R Foundation for Statistical Computing) program. Ordination analyses and model selection analyses were performed using the *vegan*: Community Ecology package (Oksanen *et al.*, 2015) and the *MuMIn:* Multi-Model Inference package (Bartoń, 2014) for the R software environment.

All the cladocera taxa occurring in at least 2 of the lakes with a relative proportion of > 1% in at least one of these lakes were included in ordination analysis. Multivariate analysis and inference models were performed on square-root transformed species percentage values in order to stabilize the variance. In all ordinations, rare taxa were downweighted in proportion to their frequency (ter Braak 1988).

Potential explanatory variables of cladoceran assemblage were transformed and standarized prior analyses. All quantitative environmental variables were tested for skewness and, if necessary, ln $(x + 1)$ transformed (or square root transformed in the case of percentage variables), prior to data analyses (Birks, 2012). Redundancy analyses (RDA) was used to transform multistate qualitative variables, previously recoded as dummy variables, into quantitative variables (Legendre and Legendre, 1998). All the variables were subsequently standardized by z-score transformation. Prior to RDA analysis, a detrended correspondence analysis (DCA) was undertaken on the cladoceran taxa in order to determine the gradient length of the DCA axis and, based on the results (gradient length axis 1= 1.86), we resolved that linear ordination methods would be appropriate.

Pearson correlation matrix was used to identify the strengths of relationships between the 16 environmental variables. Significantly correlated ($p < 0.05$) groups of environmental variables were identified. Moreover, principal components analysis (PCA) of the environmental variables was performed to identify group of correlated variables.

A series of constrained RDAs with associated Monte Carlo permutation tests (999 unrestricted permutations, $p < 0.05$) were run to identify a subset of the 15 environmental variables collected that would statistically significantly explain the variation in the species data (Table 4. 1). Two methods were carried out for this selection. In the first method (Hadley *et al*., 2013) a series of initial RDAs starting with all environmental variables were performed. To remove variables with high multicollinearity (Variance Inflation Factors, VIFs > 5), we performed a first RDA, and the variable with the highest VIF was eliminated, then, the RDA was subsequently repeated until all environmental variable VIFs were < 5. This procedure eliminated a total of seven variables, and selected nine. Finally, the reduced environmental data set and the cladoceran assemblage set was subsequently used for a series of RDAs in which variable selection was made by backward elimination of those less significant until a final set of 6 variables, which gave the highest percentage of explained variance of cladoceran data. Of these, only significant environmental variables were included in the final RDA in order to identify the statistical independence and relative strength of each of the explanatory variables of the cladoceran assemblage changes, using Monte Carlo permutation tests (999 unrestricted permutations) with a significance level of $p < 0.05$.

A second method (Korhola, 1999, Bigler *et al*., 2006) was used for variable selection. We performed a series of RDAs with each environmental variable as sole constraining variable to assess its explanatory power and the nine most powerful variables explaining the highest amount of variance of the cladoceran data set were selected. From that point we proceeded as the method described above and only significant ($p < 0.05$) environmental variables were included in the final RDA.

Additionally, partial redundancy analyses (pRDA), with forward/backward-selection, with the nine preselected explanatory variables from the method 1 and the nine preselected explanatory variables obtained by method 2 were performed to identify the significant variables explaining the cladoceran assemblage's variation.

The purpose of using different analyses is to compare the results and to ensure that the most explanatory variables of cladoceran assemblages in our set of lakes were selected.

We analyzed the occurrence of *D. pulex* gr. in the Sierra Nevada study lakes using generalized linear models with a binomial distribution, being the dependent variable the occurrence of *Daphnia* in each lake considered as a binary variable (0 = absence, 1 = presence), and as predictor variables we considered the set of the 16 environmental variables (Table 4. 1). We included in these analyses La Caldera Lake, a system in which *Daphnia* is present (Villar-Argaiz *et al*., 2012) although no data of cladoceran assemblage is available. As explained above, the environmental variables were transformed and standardized by z-score transformation, being the qualitative variables recoded as dummy variables. Similarly to the first method used to explain the variation in the cladoceran assemblages, a series of initial models starting with all environmental variables were performed. Akaike Information Criterion adjusted for sample size (AICc) (Burnham & Anderson, 2002) was used to select the best models. Models with a difference of ΔAICc less than 2 compared to the lowest AICc were considered the best models and statistically equivalent and those variables selected in any of the best models but with VIF > 5 were eliminated one by one for the subsequent analyses of logistic regression with model selection. With this procedure we obtained a set of 6 variables yielding VIFs < 5 that therefore were kept in the analysis due to the low degree of collinearity. The significance of environmental variables (categorical) was tested with the function "Anova" in *car* package (Fox & Weisberg, 2011). Only significant ($p < 0.05$) environmental variables were included in the final model.

RESULTS

In general, cladoceran richness was extremely low in the studied lakes, ranging from 2 to 8 species per lake, the mean value being 3 species. Six of the 8 cladoceran taxa identified in the sampled lakes met the requirements to be used in the analyses (Fig. 4. 2).

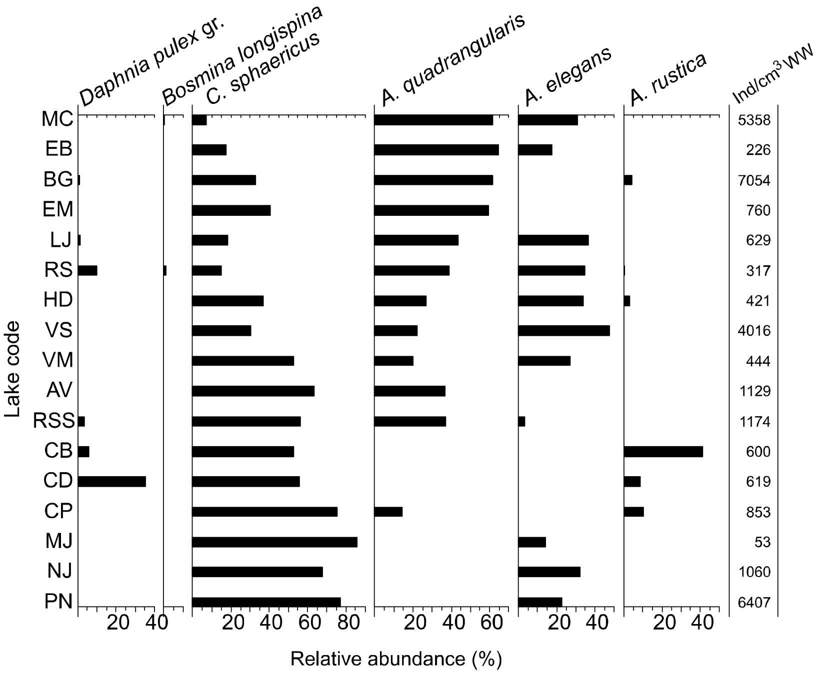


Figure 4. 2. Distribution of Cladocera in surface sediments of the sampled lakes in Sierra Nevada region. Lake code: Mosca (MC); Ermita Baja (EB); Borreguil (BG); Ermita Media (EM); Lanjarón (LJ); Río Seco (RS); Hondera (HD); Virgen Superior (VS); Virgen Media (VM); Aguas Verdes (AV); Río Seco Superior (RSS); Caballo (CB); Cuadrada (CD); Culo Perro (CP); Majano (MJ); Nájera (NJ); Peñón Negro (PN). Number of cladoceran individuals per cm^3 of wet weight is also shown (last column).

The cladoceran assemblages were dominated by chydorids, being the most abundant species *Chydorus sphaericus, Alona quadrangularis* and *Alona elegans*. *C. sphaericus* was the most abundant taxon in the data-set, occurring in all the study lakes with a mean relative proportion of 46.2 % (23.7 %, s.d.) per lake and showing more than 50% of relative abundance in 9 of the 17 lakes. *A. quadrangularis* and *A. elegans* are present in 12 and 11 lakes with a mean relative proportion of 28.7 % (24.1%, s.d.) and 17.7 % (16.6 %, s.d.) per lake, respectively. *C. sphaericus* and *A. quadrangularis* relative abundances show a negative correlation in the study lakes (Pearson correlation; r = -0.75, $p < 0.001$) Of the identified cladocerans only two are considered strictly planktonic species, *Daphnia pulex* gr. and *Bosmina longispina*, and they were only found in the cladoceran assemblages of relatively deep lakes ($z_{max} > 2.5$ m). *B. longispina* was only found in two lakes and with low relative abundance.

Pearson correlation also show a high positive correlation ($r > 0.60$, $p < 0.01$) between open/closed basin, meadows area/lake area, catchment area, % shoreline with meadows, and ion concentration (conductivity and calcium) and all of them negative correlated to maximum depth and lake area/catchment area. Other variables positive correlated to the first group of variables although with less strength are pH and TN:TP.

The first two axis of the PCA analysis accounted for 61 % of the variation (43% axis 1, 18% axis 2) in the environmental data (Fig. 4. 3).

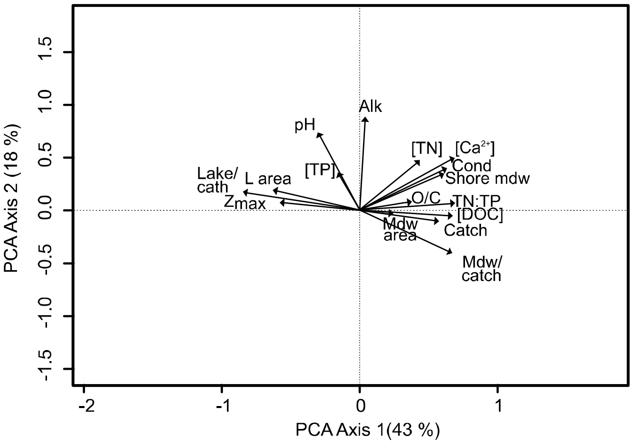


Figure 4. 3. Principal components analysis (PCA) biplot of all 15 measured environmental variables from 17 study lakes in the Sierra Nevada. Abbreviations: Z_{max}, Maximum depth (m); Lake/catch, Lake area/Lake catchment; L area, Lake area (ha); TP, Total phosphorus; Alk, Alkalinity (meq L^{-1}); Mdw area, Meadows areas; Mdw/catch, Meadows area/Catchment area; O/C, Open/Closed basin (Presence of inlets and outlets); TN, Total nitrogen; Ca^{2+}, calcium (mg L^{-1}); Cond, Conductivity (µS cm^{-1}); Shore mdw, Shoreline in contact with meadows (%); TN:TP; ratio total nitrogen: total phosphorus; DOC, Dissolved Organic Carbon (mg L^{-1}); Catch, Catchment area (ha).

The PCA was described by a primary axis of catchment features and lake morphometry (variables related to meadows extent, catchment area and presence of inlets/outlets with positive values and lake area and depth with negative values) and ion concentrations also with positive values (calcium and conductivity) and by a secondary axis of pH, alkalinity and TN. Thus, those lakes with open or open/closed basin are also lakes with a higher catchment area, higher meadows area in the catchment and higher extent of surrounding meadows as well as higher ion content whereas the opposite features are found in deeper and bigger lakes.

In the initial RDAs, highly collinear variables were deleting on the basis of their high VIFs (see Methods) and resulted in a preselection of a set of nine variables in the first method used for variable selection (lake area, Open/Closed basin, shoreline in contact with meadows, pH, TN, calcium, Lake area/Catchment area, maximum depth, and conductivity) and also nine variables in the second method used (meadows area, meadow/lake area, Open/Closed basin, shoreline in contact with meadows, TN, calcium, Lake area/Catchment area, maximum depth and conductivity). Both set of ten variables only differed in two variables.

The relationship between both reduced set of environmental variables and the cladoceran distribution was assessed by means of RDAs. Backwards elimination in subsequently RDAs performed on these two set of variables separately identified a final set of 6 variables (lake area, Open/Closed basin, shoreline in contact with meadows, pH, TN and calcium), which gave the highest percentage of explained variance of cladoceran data and finally backwards elimination in subsequently RDAs identified three environmental variables, presence of inlets and outlets (Open/Closed basin), shoreline in contact with meadows and TN, which explained significantly ($p < 0.05$) the variation in the cladoceran data (Table 4.2). The pRDA with forward/backward-selection also selected the same three significant variables.

	Df	Variance	F	*p*-values
O/C basin	1	8.687	12.975	0.001***
Shore mdw	1	2.360	2.360	0.020*
TN	1	3.574	5.338	0.001***
Residual	13	8.704		
Adjusted $R^2 = 0.604$				

Table 4. 2. Summary of partial redundancy analyses (pRDA) for the seventeen study lakes with cladoceran assemblages as response variables and predictor variables including variables showed in Table 4. 1. Abbreviations: O/C, presence of inlets and outlets (Open/Closed basin); Shore mdw, shoreline in contact with meadows and TN, total nitrogen. Forward/backward-selection was used to select the explanatory variables and were z-score transformed to standardize to mean variance. Only selected predictor variables are shown in table. Adj R^2= Adjusted R^2. Significance levels: *** $P < 0.001$; ** $0.001 < P < 0.01$; * $0.01 < P < 0.05$.

RDA axis 1 ($\lambda_1 = 0.14$) and axis 2 ($\lambda_2 = 0.04$) were both significant ($p < 0.01$) and explained 64 % of the cumulative variance in the cladoceran data (50 % axis 1 and 14 % axis 2), a larger proportion of the variance than is explained by PCA. The axis 3 only accounted for 4.3% of the species variance. The amount of explained variance is 86% than that of the RDA obtaining the highest amount of explained variance, i.e. the RDA using six variables (lake area, pH, TN, Open/Closed basin, calcium shoreline in contact with meadows) of the preselected set of nine variables, suggesting that the three most important variables provide a good representation of the overall ecological patterns within the data.

The presence of inlets and outlets (Open/Closed basin) was the strongest environmental variable associated with RDA axis 1 (Fig. 4. 4), and % shoreline covered by meadows was also associated with this first axis although in less extent. The second axis was mainly related to TN. The first axis separates the lakes with outlets and a high percentage of shoreline cover by meadows (e.g. EB, VS, VM, MC, HD), located at the right hand of the plot, from closed basin and with less o no perimeter covered by meadows (e.g. CB, NJ) on the left (Fig. 4. 4). Moreover, axis 2 separates lakes with high TN content in the upper part from low TN lakes in the bottom. The similarity in the structure of the axes in the PCA of environmental data and the RDA denotes that these three environmental variables provide a reasonable interpretation of the ecological gradients structuring cladoceran assemblages.

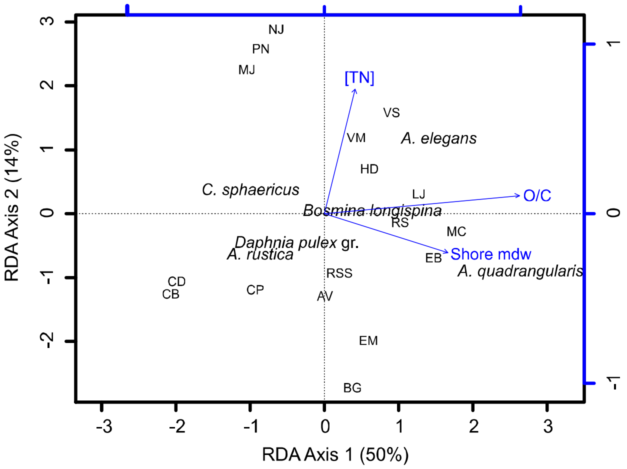


Figure 4. 4. Redundancy analysis (RDA) ordination plot showing cladoceran assemblages data and the 17 study lakes in relation to selected environmental variables. Abbreviations: O/C, (Open/Closed basin) Presence of inlets and outlets; TN, Total nitrogen; Shore mdw, Shoreline in contact with meadows (%). Lake code and taxon are found in Table 4.1 and Figure 4.2, respectively.

Cladoceran taxa (Fig. 4. 4) were clearly separated along RDA axes 1 and 2. *A. quadrangularis* was strongly and positively associated to axis 1. It was found at higher abundances in lakes with outlets and surrounded by meadows and also negatively associated to axis 2, implying that this species is preferentially occurring in lakes with low TN concentration. *A. quadrangularis* and *C. sphaericus* relative abundance show a negative correlation and RDA plot separated both species along axis 1. Thus, although *C. sphaericus* is occurring with relatively high percentages in all the lakes, higher abundances was found in closed or open/closed basins with less or no meadows surrounding the lake perimeter. As *A. quadrangularis, A. elegans* is also associated positively to axis 1 and moreover this species show the highest species scores to axis 2, this means that show higher abundances in high TN concentration lakes. The remaining taxa, *D. pulex* gr. and *A. rustica* are negatively associated to both axis although with lower scores than the above species, both seem to be taxa most clearly associated to low TN lakes and closed and open/closed basin.

Results of logistic regression with model selection analysis indicate that two morphometric variables, maximum depth and presence of inlets and outlets, were the main drivers of *Daphnia* presence in our set of lakes (see Table 4.1). Thus, in Sierra Nevada *Daphnia* is occurring in those deep lakes ($Z_{max} > 2.5$ m) with closed or open/closed basins (Open/Close basin lakes $X^2 = 7.719$, $df = 2$, p = 0.02; Maximum depth $X^2 = 5.062$, $df = 1$, p = 0.02).

DISCUSSION

The studied lakes are representative of the different type of lakes in Sierra Nevada except those in which their rocky substrate hinders paleolimnological studies (e.g. La Caldera Lake). The lakes are located in a narrow altitude range and, in general they are small, shallow and with low ion and nutrient concentration. The range of altitude of Sierra Nevada lakes is narrow and, therefore the air temperature they experience. The ranges of most of the chemical variables are also narrow. However, differences in the lake basin and catchment features seem to account for most of the variation among lakes.

Two groups of environmental variables, morphometric and chemical, were used in this study, and between them morphometric variables (open/closed basins and % shoreline surrounded by meadows) had greater contribution to the total explained variance respect chemical variables (TN). This result emphasize the role of the morphometric variables in controlling the cladoceran distribution in Sierra Nevada lakes. The extent of meadows area respect to lake area and the presence of inlets and outlets are the most explanatory morphometric variables. Both highly correlated with each other and negatively correlated to lake maximum depth and surface area. Our data indicate that lakes with higher catchment area and meadows area/lake area also show a higher ionic content (Conductivity, calcium) and DOC values which can be explained by the higher runoff input entering into the lakes, because of the high watershed but also because of the meadows.

Korhola (1999) also emphasize that are physical variables as maximum lake depth, sediment organic content, epilimnetic summer temperature, lake perimeter, and lake catchment instead of chemical variables which explained most of the total variance of cladoceran assemblage in small, shallow and oligotrophic subartic lakes of Fennoscandia. Several authors have indicated altitude-related variables as the most explanatory variables for cladoceran assemblage data (Lotter *et al*., 1997, Rautio, 1998; Bigler *et al*., 2006). Sierra Nevada lakes are located in a narrow altitude range and all above the tree line, on inhabitant areas with no agricultural uses. Therefore altitude-related variables as temperature or land uses which have been indicated as explanatory variables in wide altitudinal gradient lake sets are not enforced in our study. The range of altitude of Sierra Nevada lakes is narrow and, therefore the air temperature they experience. However, temperature may be result of other factors as lake depth and surface area. We measured temperature data of our lakes on each sampling day but since water temperature can high fluctuate considerably both within a giving month as well as throughout the ice-free period in the shallow and small Sierra Nevada lakes, single spot values give only a poor approximation of mean water temperature values (Livingstone *et al*., 1999; Bigler *et al*., 2006).

Morales-Baquero *et al* (1999) analyzed influence of lake morphometric and chemical variables on nutrient content and limitation in 30 Sierra Nevada lakes and they found a positive relationship between lake catchment area and DIN:SRP ratio explained by the higher TN:TP ratio of runoff from natural, undisturbed watersheds which export much less P than N (Downing & McCauley 1992). Their results suggest than lakes with larger catchment areas in Sierra Nevada are more likely to be P-limited because of greater runoff inputs (N enriched) than lakes with smaller catchment areas. We do not find a direct correlation between catchment area and TN:TP neither a higher P limitation in larger catchment area lakes. However we found a positive correlation between TN:TP ratio and meadows area/Lake area ratio and % shoreline covered by meadows and also deeper lakes show lower TN:TP ratios. This likely means a higher N input (particulate and/or soluble) from the catchment with meadows then from the catchments with no o poor developed meadow area. Moreover since the lakes with high meadows area/Lake area are also the shallower and smaller lakes and with higher catchment areas this high N:P runoff input is diluted in a low water. In fact, in previous studies we indicate the important input of organic matter from the catchment in one of our lakes (see Chapter 1 and 3). Respect to nutrient limitation, Guilford & Hecky (2000) establish TN:TP values lesser than 20 (molar) for N-deficient algal growth whereas P-deficient growth occurred at TN:TP > 50 (molar) and at intermediate TN:TP ratios, either N or P can become deficient. According to TN:TP ratio, most Sierra Nevada lakes are P-deficient for algal growth or show a N and/or P limitation (Guilford & Hecky, 2000). Only one lake (LJ) was categorized as a N limiting lake.

The extremely low cladoceran diversity in the sediment analysis of all 17 lakes is typical of high altitude lakes in Europe (Hofmann *et al*., 2003; Brancelj *et al*., 2009). The cladoceran communities were mainly characterized by cold-tolerant planktonic (Bosminidae, Daphniidae) and littoral and benthic (Chydoridae) Cladocera. Most of our Sierra Nevada study lakes are small, shallow littoral-dominated systems with no clear differentiation between littoral and profundal zones and it is not expectable to found high abundance and richness of planktonic species except in the deepest lakes. Studies on Sierra Nevada lakes show *D. pulicaria* is the dominant planktonic cladoceran species (Morales-Baquero *et al*., 1992; Pérez-Martínez *et al*., 2013)

The most important benthic/littoral cladoceran occurring in all the lakes were *A. quadrangularis* and *C. sphaericus.* This latter species is a generalist species, known to occur over a wide range of environmental conditions (Korhola, 1999; Duigan & Birks, 2000; Miroslaw-Grabowska & Niska, 2005). It has been described as tolerant of harsher conditions (e.g. colder, ultraoligotrophic waters) in alpine lakes (Harmsworth, 1968;

Whiteside, 1970, Lotter *et al.*, 1997; Bigler *et al.,* 2006) and also occur regularly in the late glacial assemblages in different European lakes (Frey, 1958, Whiteside 1970, Hofmann, 1991, Nováková *et al.*, 2013). On the contrary *A. quadrangularis* is usually not a dominant species in alpine lakes and therefore less information on his ecology is found. Bigler *et al* (2006) indicate this species, among others, characterizes lower altitude lakes (1500-2500 m asl) than *C. sphaericus* does (> 2500 m asl). There is no a wide altitude range (225 m in our study lakes) in Sierra Nevada lakes and altitude was not a influential parameter of cladoceran assemblage (based in previous analyses, altitude was not considered in the set of explanatory variables for the sake of reducing number of variables). However, the negative relationship between both taxa and their opposite position in the RDA plot suggest these species adapted to diverging conditions in Sierra Nevada lakes. In our set of lakes, *A. quadrangularis* is strongly associated to lakes with inlets and/or outlets and surrounded by meadows, lake features that are positively correlated with catchment area and meadows area/lake area ratio. All these features probably implies a higher input of material (particulate and soluble) within the lake from his catchment. Moreover these open and surrounded by meadows lakes are also small and shallow lakes which means warmer water lakes than in deeper and bigger lakes. Therefore the lakes with highest abundance of *A. quadrangularis* likely have less harsh living conditions than those with *C. sphaericus* high abundance and this is according with the above description of this latter species. Hence, *C. sphaericus* could obtain advantage under tough conditions because the difficulties of other taxa to thrive. In this respect, we found an abrupt increase in the relative abundance of *A. quadrangularis* at the expense of *C. sphaericus* in the last 50 years in the sediment record of several Sierra Nevada lakes, including some of the studied lakes in the present study (see Chapter 3). This species shift was related to the more favorable habitat conditions in terms of water temperature, ice-cover period and food availability arising from the recent warming which favored *A. quadrangularis* against *C. sphaericus*.

A. quadrangularis is a benthic species associated to well oxygenated bottom mud substrata habitat (Whiteside *et al*., 1978; Tremel *et al*., 2000, Nevalainen & Luoto, 2012) which is more frequent in shallow and small lakes with large catchment and meadows area. This species inhabit bottom area whereas *C. sphaericus* is known to be a facultative pelagic species, a species inhabiting littoral and water pelagic column, particularly in these relatively small Sierra Nevada lakes. Thus Korhola (1999) indicates *A. quadrangularis* preference for large-catchment area lakes whereas *Chydorus* cf. *sphaericus* for lakes with small drainage areas. Although *C. sphaericus* is usually considered as littoral species, it can utilize pelagic resources particularly when lake productivity increases (Hofmann, 1987; Frey, 1988) or when other taxa decline (Manca & Comoli, 1995; Korhola, 1999).

In our systems, *A. elegans* is the only species clearly positively associated to the RDA axis 2 and located in the upper right quadrant which is linked to open, high TN and conductivity-calcium lakes. TN and TP are highly and positively correlated in our study lakes, whereas TN is negatively correlated with maximum depth. Therefore this species in Sierra Nevada can be linked to open and shallow lakes with a high nutrient and ion concentration. However the highest values of conductivity in Sierra Nevada lakes (60 $\mu S\ cm^{-1}$) are not comparable with those of temporary lowland pond of semiarid region where *A. elegans* has been mainly cited (Margaritora 1985, Alonso, 1996; Marrone *et al*., 2006; Van Damme & Dumont, 2008). This taxon occurred in 11 of the 17 studied lakes and has been reported in 22 of the 30 Sierra Nevada lakes studied by Morales-Baquero *et al*. (1992). However, it has never been cited in alpine and permanent lakes in Spain, not even in Europe, at the time. Its high relative abundance and presence in Sierra Nevada mountain lakes indicates the need for a better understanding of this cladoceran group. *A. elegans* is a true littoral species with preference for environment rich vegetation (Aoujdad *et al*., 2014) and his abundance in shallow lakes may result from the greater abundances of strict littoral habitat and macrophyte coverage in such lakes, providing favorable habitat for littoral cladocerans (Tremel *et al.*, 2000; Sakuma *et al.*, 2004; Jeppesen *et al.* 1996; DeSellas *et al.*, 2008). There are no macrophytes “sensu stricto” in our lakes but the lake shore of shallow lakes are covered by bryophytes than eventually fall down to the lake and, moreover, the presence of filamentous Zygnemataceae and Cyanobacteria in the bottom is common. This vegetation could favor the development of *A. elegans* in this lakes.

The only abundant pelagic cladoceran is *D. pulex* gr., with negative scores to both RDA axis 1 and RDA axis 2 and therefore more abundant in closed or semiclosed lakes and low nutrient content. These lakes are moreover deep lakes, given the negative and significant correlation of depth with the three selected variables in RDA. A positive relationship between lake depth and the relative abundance of pelagic cladocerans has been documented in other studies (Hofmann, 1998; Korhola *et al.*, 2005; Bigler *et al.*, 2006; DeSellas *et al.*, 2008) and it is expectable this results in our selected lakes. In fact *D. pulex* gr. is absent of any lake having less of 2.5 m of maximum depth, and depth is one of the variables selected to explain the presence of *Daphnia* in Sierra Nevada lakes. The other explanatory variable of *Daphnia* presence and also abundance is the presence of inlets and/or outlets in the lake system. Indeed, within the ten lakes with maximum depth > 2.5 m, *Daphnia* is present in those deep lakes with closed or semiclosed system with the exception of PN Lake, probably because this system suffers a substantial lake water level reduction as summer progresses and even became dry in some years, and it is lacking in the two open lakes, MC and AV. The absence of *Daphnia* in open systems may be related to the negative effect of dilution on *Daphnia* population development associated to a high water renewal in these lakes. This washout effect has been widely reported as an important factor of *Daphnia* population successful (Threlkeld, 1982; Rellstab, 2007). In Sierra Nevada, hydraulic washout can be an important process for *Daphnia* loss in these lakes. Hence, *Daphnia pulex* gr. is a big species that can have difficulties to maintain his population given the generally low temperatures of our alpine lakes (affecting his growth rate) and the short time of the ice-free period. Therefore, the limited growth rate of the *Daphnia* population could not compensate the washout losses in open lakes with high water renewal rate.

In addition, *Daphnia* growth rate can be certainly affected by the low food availability of the low productive Sierra Nevada lakes (Villar-Argaiz *et al*. 2002; 2012) and by the water calcium contents which are likely limiting values for *Daphnia* growth and reproduction in our study lakes (Ashforth & Yan (2008), see Chapter 3). Nevertheless, our results do not show a clear relationship between *Daphnia* abundance and occurrence and these variables for our set of lakes. Thus, while Sierra Nevada lakes show low nutrient and Ca values in general, these vary considerably among lakes and *Daphnia* is found in both low calcium and high calcium lakes (e.g. CD and CDR respectively, Table 4.1) and the same is true for different TN and TP lake water concentration. *Daphnia* individual and population growth is likely affected by calcium concentration and food availability within each Sierra Nevada lakes and previous studies on this lakes support it (see Chapter 3) but it seems morphometric variables as depth and water renewal time are critical variables for *Daphnia* population development and persistence.

In summary, cladoceran assemblage variation in Sierra Nevada lakes is mainly regulated by morphometric variables as the extent of the catchment vegetation in the basin, lake depth and size and the presence of inlets and/or outlets. The influence of chemical parameters seems to play a secondary role as explanatory variables of the cladoceran variation and among them, TN significant and partially explained the cladoceran assemblage. The predicted changes derived from current warming will probably affect many of the lake basin features (e.g. water temperature, maximum depth, catchment runoff input) and the significant regulating variables of cladoceran assemblages. Hence, future changes in cladoceran assemblage distribution as we have already demonstrated occurred in the past, might occur in Sierra Nevada lakes. Nevertheless, unmeasured factors such as mixing regimes and oxygen concentrations, increasing UV radiation with altitude, length of ice cover, competition, grazing, and predation may also strongly influence the distribution and abundance of the organisms studied.

General discussion

General Discussion

Remote areas are amongst the most sensitive environments to anthropogenic climate change (IPCC 2013), and many freshwater ecosystems located in these areas have already responded to warming, some beginning in the early 20th century. These changes have mainly been evidenced by major shifts in lake biota assessed by examining changes in sedimentary assemblages (e.g. Sorvari *et al*., 2002; Smol *et al*., 2005; Luoto & Nevalainen, 2012; Rühland *et al*., 2014).

Throughout this thesis we have explore distinct potential drivers of local to regional-scale variability in the climate response of biological and geochemical variables. Firstly, we analyzed the interaction between climate-driven factors as the dominant driver of algal communities changes (Chapter 1), and the strong cascading effects of recent warming with special focus in the role of ice cover period in the ecology and environment in Río Seco Lake and its catchment (Chapter 2). Secondly, we analyzed the interaction between climate and nutrient inputs as the main driver of sedimentary cladoceran assemblages and primary production assessed by sedimentary chlorophyll-*a* (Chapter 3), and the role of limnological and watershed characteristics in the distribution of surface sedimentary cladoceran within a context of global change across Sierra Nevada lakes (Chapter 4).

The research presented mainly in Chapter 1 and 2 contributes to the developing narrative of recent anthropogenic climate change in Río Seco Lake by providing a paleolimnological records that track a coherent overview, with other paleoenvironmental records, of cooler, wetter conditions from 1820 to the 1920s, followed by a drier, warmer conditions from ~1920s to the present. The most significant contribution of these studies are the insight provided into how the climate-driven changes (e.g. duration of ice-cover period, changes in water temperature, water residence time or water availability in catchment) may influence the in-lake and catchment related processes in this alpine ecosystem.

During a colder and wetter condition, the high abundant of the picoplanktonic cyanobacteria *Synechococcus nidulans* in Río Seco Lake would be controlled by a reduced water residence time and nutrient limitation, that may have favoured these type of species with elevated growth rates and high competitive ability for nutrients (Chapter 1). The later seasonal ice-off dates and larger accumulation of snow in the catchment basin may also hinder any nutrient input and promote a slow disappearance of snow patches. This condition produce a water runoff through all the ice-free season that result in a longer presence of inlet and outlet, that favour a water renewal time. The large accumulation of snow in the catchment is also supported by the reduced catchment surface, less water availability and growing season for wetland plants (lower P_{aq} and C/P ratio and highest ACL values than the most recent climatic stage) (Chapter 2) and a higher contribution of algae to the bulk organic matter (lower values of atomic C/N ratio) (Chapter 1 and 2). Apart from abiotic factors, the in-lake processes may be partially responsible of the high abundant of cyanobacteria, supported by the low herbivorous grazing pressure assessed by the low relative abundance of *Daphnia pulex* gr. mainly attributed to the efficient grazer *D. pulicaria* in Río Seco Lake (Chapter 1 and 3). Hence, the weaker top-down control of phytoplankton biomass during cold years appears more prevalence.

The colder condition predominant during this stage also determine the characterization of lacustrine biota in the Sierra Nevada. PCA axis 1 sample scores of cladocera, diatoms and chironomids remains unchanging indicating a stable stage (cold and wetter condition) for the dominance species abundance at that moment (Chapter 2). These muted response are mainly related to long ice-cover period (predominance of the cladoceran *Chydorus sphaericus*) (Chapter 3), to water turbulence and wetter condition (high abundance of the picoplanktonic diatoms *Aulacoseira alpigena) (*Pérez-Martínez *et al*., 2012), and to cold water temperature (high contribution of cold-tolerant taxa to chironomids assemblage, such a *Micropsectra radialis* and *Psectrocladius sordidellus*-type) in Río Seco Lake (Chapter 2).

Our studies highlights the second stage (~1920s to the present) as a climatic period that requires special attention in the Sierra Nevada, given the significant similarities in timing and nature of the biotic response in our cores. The most notable changes in lacustrine biota response to climate started gradually at the turn of the 20th century, but intensified over the past ~ 60 years. The climatic inference from the subfossil cladoceran assemblages and sedimentary chlorophyll-*a* changes across Sierra Nevada lakes (a regional perspective) (Chapter 3) were largely consistent with the previous studies conducted on Río Seco Lake, assessed by sedimentary photosynthesis pigments (Chapter 1), subfossil chironomids assemblages and leaf wave biomarkers (Chapter 2) (a local perspective).

These changes from both regional and local perspective are concurrent with trends in regional air temperature, precipitation, and increased Saharan dust deposition. Hence, these similarities in timing and nature of changes given an idea of how the climate forcing mechanism may act in others similar alpine lakes of the region, despite the relative contribution of catchment and lake-specific differences to changes, that merits further studies. The lake-specific characteristics are partially responsible for the observed discrepancies in the magnitude and timing of biological changes across lakes (Chapter 3), and the distribution of subfossil cladoceran assemblages for recent years (Chapter 4). Hence, the assessment of the relative contribution of local environmental conditions and regional climate influences in Sierra Nevada lakes are particularly important to better understanding the complexity of environmental stressors.

The influence of warming and Saharan dust input on subfossil cladoceran assemblages and sedimentary chlorophyll-*a*, were specially examined over the second stage (~1920s to the present) with particular attention since the 1970s onwards. The main cladoceran assemblage shift within the past ~150 years was the decline in relative abundance of *Chydorus sphaericus* and increase in *Alona quadrangularis* (or *Daphnia pulex* gr. in Cuadrada Lake), mainly associated to longer growing seasons and increased primary production allowing more specialized (*A. quadrangularis)* to thrive, and also favoring open water filter feeders (*Daphnia* spp). Thus, the trends in regional air temperature and sedimentary chlorophyll-*a* concentrations were the best predictor variables of the cladoceran assemblage changes (Chapter 3). However, apart from previous mentioned climate-driven factors, subfossil cladoceran communities were also influenced by a warmer water temperature, the major climatic factor responsible of the increase of chironomids taxa better adapted to warmer condition (*C. plumosus*-type, *H. marcidus*-type, *M. insignilobus*-type) in Río Seco Lake from ~1940-50s onwards (Chapter 2).

A few decades later, since the 1970s the intensification of warming and of delivery of P-rich Saharan input resulted in a combined effect on the primary production of Sierra Nevada lakes (Chapter 3). Apart from aquatic primary production, the signal of increased terrestrial primary production assessed by increased atomic C/N ratio and *n*-alkanes indices (higher P_{aq} and lower ACL values) and higher C/P ratio in Río Seco Lake were also determined by the combined effect of rising temperatures enhancing the melting season that led in a shorter ice-cover periods and greater water availability in the catchment during the 1960s that shows high precipitation coeval with higher temperatures (Chapter 2). Hence the duration of ice cover can induce the establishment of greater habitats for wetland plants.

The high air temperatures and low precipitation together with the high values of Saharan Ca deposition, over the past few decades were also consistent with the significant *Daphnia* increase in Río Seco Lake (since 1970) and in Cuadrada Lake (since 1900 and most pronounced after ~1980). Significant relationship between *Daphnia* and Saharan input drivers SPI and wNAO, supported the hypothesis that increased Saharan dust deposition throughout the 20th century were partially responsible for increases in

Daphnia (Chapter 3). Particularly, the increase in *Daphnia* in Río Seco Lake was responsible of the pronounced decrease in cyanobacteria pigments indicator (mainly zeaxanthin) since the 1980s partially attributed to an increase in herbivorous grazing pressure during warm years, enhancing the top-down control of phytoplankton biomass (Chapter 1). During this climatic stage (warmer and drier climate of the past decades), the increase of lake water residence time was partially responsible of decrease cyanobacteria community and the increase of relative abundance of *Daphnia pulex* gr (Chapter 1 and 3). The increase of atmospheric input, increased Ca concentrations due to evapoconcentration (Pulido-Villena *et al.*, 2006), and also longer exposure to Saharan dust with increased water residence time and a longer ice-free period, have all likely occurred in recent decades.

On the other hand, a higher sand/(clay + silt) ratio and sedimentation rate since the 1940s, and the increase of sedimentary signal of CD/TC ratio, atomic C/N ratio, and ^{226}Ra activity, and a decrease of LOI_{550} in Río Seco Lake core profile is explained by a considerable alteration of the shoreline and the surrounding area during the last decades, specially intensified by the human disturbances in the watershed since the 1960s, resulting in an erosion of the catchment, and consequent dilution of chlorophylls and labile carotenoids (Chapter 1). These discrepancies allowed to discern a period of human pressure in its catchment. However, these changes seem not to obscure the climate signal over time, allowing us to use these high mountain lakes as ideal ecosystems for exploring long-term effects of natural climate variability.

Additionally, using multiproxy studies that enhance the coherent level between independent environmental proxies (each associated to distinct mechanistic linkages to climatic shifts) in a common response towards a recent warming (Chapter 2), together with lake surveys in a extensive geographic scale (Chapter 3 and 4) contribute to a better understanding of a regional and local influence to biogeochemical response from centennial (Chapter 3) and decades and/or years time-scales (Chapter 4). Collectively, these studies providing an integrated view of how these alpine Mediterranean environments functions, and, ultimately, enhance the reliability of paleolimnological record in these area. More specifically, the Chapter 4 incorporate valuable information about the ecological interpretation on the distribution of subfossil cladoceran assemblages across Sierra Nevada lakes. Actually, the current cladoceran distribution were mainly associated to morphometric variables (e.g. extent of the catchment vegetation in the basin, lake depth and size and the presence of inlets and/or outlets), followed by chemical parameters as secondary explanatory variables (e.g. TN). Throughout this thesis, we have detected substantial changes in limnological and environmental variables from a long-term scale, that will most likely lead to a more pronounced changes derived from current and continued warming. Hence, the studies of direct and indirect forcing drivers regulating current biota distributions are essential for any site with high local heterogeneity as Sierra Nevada lakes.

On the other hand, the research presented in this thesis contributes to our knowledge of how cladocerans, chironomids (variations in subfossil assemblages) and diatoms (shifts in PCA axis 1) in lakes at high altitudes respond to changes in climate by identifying and investigating multiple stressors over a 150-year time-scale that can modulate the biotic response to warming. Besides, these studies also served to elucidate how the catchment-relate processes are affected by climate-driven factors. Collectively these studies demonstrate the importance of understanding the environmental and ecological context when interpreting species changes and distribution, and shifts in geochemical variables in paleolimnological records. These are the first high-resolution paleoclimatic studies for Sierra Nevada lakes, providing important long-term context for the more recent climate changes (past 60 years) experienced in the region. Moreover, these research should result in better criterion for more informed site selection for paleoclimate investigations using cladocerans, and better interpretation of paleoenvironmental records, and potentially improved a more comprehensive adaptation and mitigation plans to counteract the effects of climate change on the Sierra Nevada, a region of considerable interest for environmental change research.

Climate is the overriding stressor in high-altitude ecosystems and its impacts could be seen in every lake examined in this thesis. Continued warming at the magnitude and rate currently will lead to more pronounced, directional ecosystem responses with subsequent cascading effects throughout this high mountain region.

Future perspectives

Base on our data, lakes from the Sierra Nevada continue to be ideal systems for exploring the long-term impacts of global change (anthropogenic climate change, dust deposition, human pressure, etc). Further investigations on similar lakes in the region are therefore needed to provide better understanding of these effects. In particular, studies based on fossil biota (e.g. high resolution taxonomy and reproductive traits of subfossil cladocerans or analysis of diatoms assemblages) may provide potentially interesting findings for future research.

Recent advance in paleolimnological techniques offer the opportunity of use subfossil cladocerans in many ways. Therefore, the resolution of questions concerning of cladoceran remains (taxonomic resolution or different applicability of specific remains) allow to make us a coherent interpretation of how the global change affect these ecosystems. This thesis provided much needed information on the long-term changes of Cladocera in the Sierra Nevada using lake sediments, but there is still a need for better identification of particular species. Due to the difficulties associated with identifying individual daphniid remains to the species level, daphniid taxa must be placed into species complexes. Here, using genetic analyses on *Daphnia* resting eggs recovered from lake sediments to reconstruct shifts in *Daphnia* species composition in the Sierra Nevada over decades-centennial scales may help to identified the daphniids present in the study lakes. This could be particularly useful for Cuadrada, Río Seco and Río Seco lakes, which have shown large increases in *Daphnia* taxa since the last decades; and for Borreguil lake, where *Daphnia* are only presented over the last ~50 years (Chapter 3). Hence, genetic analysis allow us to discern which species were present over time, and whether the new arrival in Borreguil Lake is the result of a recent process of invasion and colonization from other Sierra Nevada lakes or, even, other different regions. On the other hand, in lacustrine sediments only some fragments of *Daphnia* such us postabdominal claws and ephippia are well preserved (Korhola 1999, Sarmaja-Korjonen 2002). Therefore, an alternative less expensive to genetic analyses in order to differentiate between species within complexes may be the identification of morphological features of postabdominal claws of *Daphnia*. This technique has been already applied in other regions (Korosi *et al*., 2011), and its applicability in Sierra Nevada lakes may be a first approach for trying to address taxonomic resolution problems in this specific region.

Among other subfossil Cladocera analysis, the use of total chydorid ephippia (TCE) has been used in paleolimnological studies by offering some valuable information about the relationship between climatic environmental changes and chydorid reproduction patterns (Kultti *et al*., 2011). Among cladocera, *Daphnia* reproduction has been widely analysed in Sierra Nevada lakes (Pérez-Martínez *et al*., 2013), however little is know concerning chydorids reproduction based in neo- and paleolimnological approaches. For example, TCE values has been used as proxy to indicate climate variables, such as temperature and length of open-water season (Sarmaja-Korjonen, 2007; Nevalainen, 2008). The idea behind using TCE curve as a proxy for past changes in climate (temperature, open-water season) is that in northern or high-altitude lakes chydorids reproduce most of the open-water season asexually (indicated by carapaces in sediment layers) and an environmental stress, mainly the oncoming winter, triggers sexual reproduction, which produces resting eggs enclosed by ephippia (indicated by ephippia in sediments). However, the non-climatic events sometimes can generate intense gamogenesis, which can biases the climate signal. Both non-climatic and climatic signal have been identified by some independent proxies in this thesis. Hence, these findings could already avoid probably misinterpretation of TCE values in this mountain region.

Among biological proxies, diatoms are ideal biological indicators in paleolimnological studies (Stoermer & Smol, 1999). They are often well preserved in lacustrine sediments, as well as abundant and ecologically diverse. Diatoms respond quickly to indirect effects of warming such as an increase in the ice-free season, diversification and changes in aquatic habitat availability, changes in thermal structure, pH, conductivity or alkalinity, etc. Thus, analysis of diatoms assemblages could served to elucidate how these alpine lakes responds to multiple stressors over a 150-year time-scale, and reinforce regional interpretation assessed by cladocerans assemblages and sedimentary chlorophyll-*a* records in six study lakes in Sierra Nevada region (Chapter 3).

There is still a need for a long-term monitoring data of biological, physical and chemical characteristics in some Sierra Nevada lakes. While RS Lake has been widely studied derived from a monitoring study, during the ice-free period over the past 14 years (unpublished data), many others study lakes presented in this thesis have been scarcely studied, except for some punctual samples. The catchment and lake-specific differences have been used to explained a partial variability of the magnitude and timing in cladocerans and sedimentary chlorophyll-*a* (Chapter 3), as well as, the human disturbances in the watershed of Río Seco Lake over 30 years period have been enough to explain some biological and geochemical changes (Chapter 1 and 2). Hence, valuable monitoring datasets in these ecosystems are useful data for evaluating environmental variability under a scenario of global warming, that may result from multiple environmental stressors at current time, but also to better understand the sensitivity and degree of the lakes from an individual perspective. Moreover, although the extension of these valuable datasets are not prior to the onset of major environmental disturbances, comparison of contemporary monitoring data together with previous palaeolimnological data, could help to provide a holistic approach to interpreting the observed changes, and even to develop future model projections.

Overall, paleolimnological short-term studies were inexistent at Sierra Nevada Mountains prior to this thesis. Hence, I consider this first approach to be an introduction to paleolimnological applications of biogeochemical proxies in the Sierra Nevada, with many questions and concerns still to be answered.

Articles in preparation

- Genetic diversity of the *Daphnia pulex-pulicaria* in Sierra Nevada: spatio-temporal differences
- Morphological diversity of the *Daphnia pulex-pulicaria* complex in Sierra Nevada: spatio-temporal differences and relation to genetic diversity
- Sedimentary chydorid ephippia in relation to lake climate condition in remote alpine lakes of Sierra Nevada, Spain
- Climate change and atmospheric deposition drive diatoms assemblage changes over the past ~150 years in remote alpine lakes from Sierra Nevada, Spain
- Exploring plankton trophic mismatching in an alpine lake during the ice-free season over the last 14 years

Conclusions

Conclusions

1.- Changes in biota of Sierra Nevada lakes began at the turn of the 20th century, but intensified over the past ~ 60 years. Biological shifts were concurrent with trends in regional air temperature, precipitation, and increased Saharan dust deposition. These shifts were similar in nature and direction for subfossil cladoceran assemblages, sedimentary chlorophyll-*a* record, sedimentary photosynthesis pigments, subfossil chironomids assemblages and leaf wave biomarkers.

2.- Climate-driven ecological thresholds were crossed for biota communities. Shifts in subfossil cladoceran assemblage since ~1980s onwards were attributable to increase of nutrient inputs and recent warming. Concretely, the increase of the primary production and *Daphnia* relative abundance since the 1970s were partially explained by the delivery of P and Ca-rich Saharan input, respectively. Algal communities exhibited shifts more attributable to zooplankton grazing pressure and reduce of water residence time since ~1950s, while changes in subfossil chironomid assemblage were associated to warmer water temperature since ~1940-50s.

3.- Ice-cover period played an important role in determining the degree of wet conditions in Río Seco Lake catchment basin over the past ~200 years. A greater water availability and longer growing season for wetland plants were attributed to a reduce ice-cover period from the first half of the 20th century to present time.

4.- The catchment and lake-specific characteristics partially explained the differences in magnitude and timing of subfossil cladoceran assemblages. The assessment of the relative contribution of local environmental conditions together with regional climate influences on biota communities in Sierra Nevada lakes is necessary to better interpretation of biological proxy record.

5.- Shifts in surface sediment cladoceran assemblages were regulated by morphometric variables related to the extent of the catchment vegetation in the basin, lake depth and size, and the presence of lake inlets and outlets. The influence of chemical parameters played a secondary role in controlling distribution of cladocerans.

6.- High mountain lakes were ecosystems especially vulnerable to a global change scenario. They may be considered as a powerful tool for paleoenvironmental reconstruction and evaluating impacts of global change.

Conclusiones

1.- Los cambios en las comunidades biológicas de los lagos de Sierra Nevada comenzaron a principios del siglo XX, pero se intensificaron en los últimos 60 años. Estos cambios son simultáneos con los cambios en las tendencias de la temperatura regional del aire, la precipitación y el aumento de la deposición del polvo sahariano. Similitudes tanto en la naturaleza como en la dirección de dichos cambios se observaron en las comunidades de cladóceros fósiles, clorofila-*a* sedimentaria, pigmentos fotosíntesis fósiles, comunidad de quironómidos fósiles y biomarcadores foliares.

2.- Las comunidades biológicas han cruzado umbrales ecológicos impulsados por el clima. Los cambios en el conjunto de cladóceros fósiles a partir de 1980, están relacionados con un aumento de entrada de nutrientes y al reciente cambio climático. Concretamente, el aumento de la producción primaria y la abundancia relativa de *Daphnia* desde los años 70 del siglo XX, se explicaron parcialmente por la entrada de nutrientes procedentes del polvo sahariano, P y Ca, respectivamente. Las comunidades de algas exhibieron cambios más relacionados con la presión de herbivoría del zooplancton y una disminución en el tiempo de residencia del agua desde la década de los 50 del siglo XX, mientras que los cambios en la comunidad de quironómidos fósiles se relacionó con un aumento de la temperatura del agua desde 1940-50 hasta la actualidad.

3.- El período de cubierta de hielo desempeñó un papel importante en la determinación del grado de humedad en la cuenca de la laguna de Río Seco durante los últimos 200 años. Una mayor disponibilidad de agua y un mayor período de crecimiento para la vegetación de la cuenca se asoció a un período de reducción de la cubierta de hielo desde la primera mitad del siglo XX hasta la actualidad.

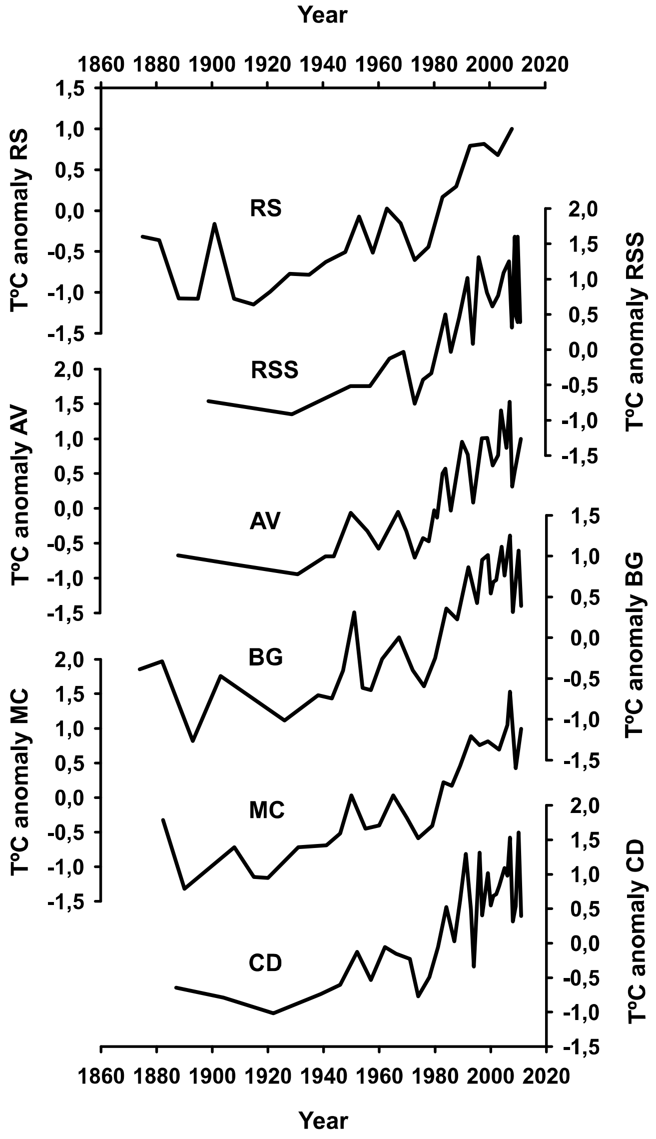
4.- Las características específicas de las cuencas de captación y de las lagunas explicaron las diferencias en la magnitud y el momento del cambio de las comunidades de cladóceros fósiles. La evaluación de la contribución relativa de las condiciones ambientales locales junto con las influencias climáticas regionales en las comunidades biológicas de las lagunas de Sierra Nevada es necesario para una mejor interpretación del registro de variables biológicas sedimentarias.

5.- Los cambios de las comunidades de cladóceros fósiles del sedimento superficial se atribuyeron a variables morfométricas relacionadas con la extensión de la vegetación de cuenca de captación, la profundidad y tamaño del lago así como la presencia de afluentes y efluentes. Los parámetros químicos desempeñaron un papel secundario en dichos cambios.

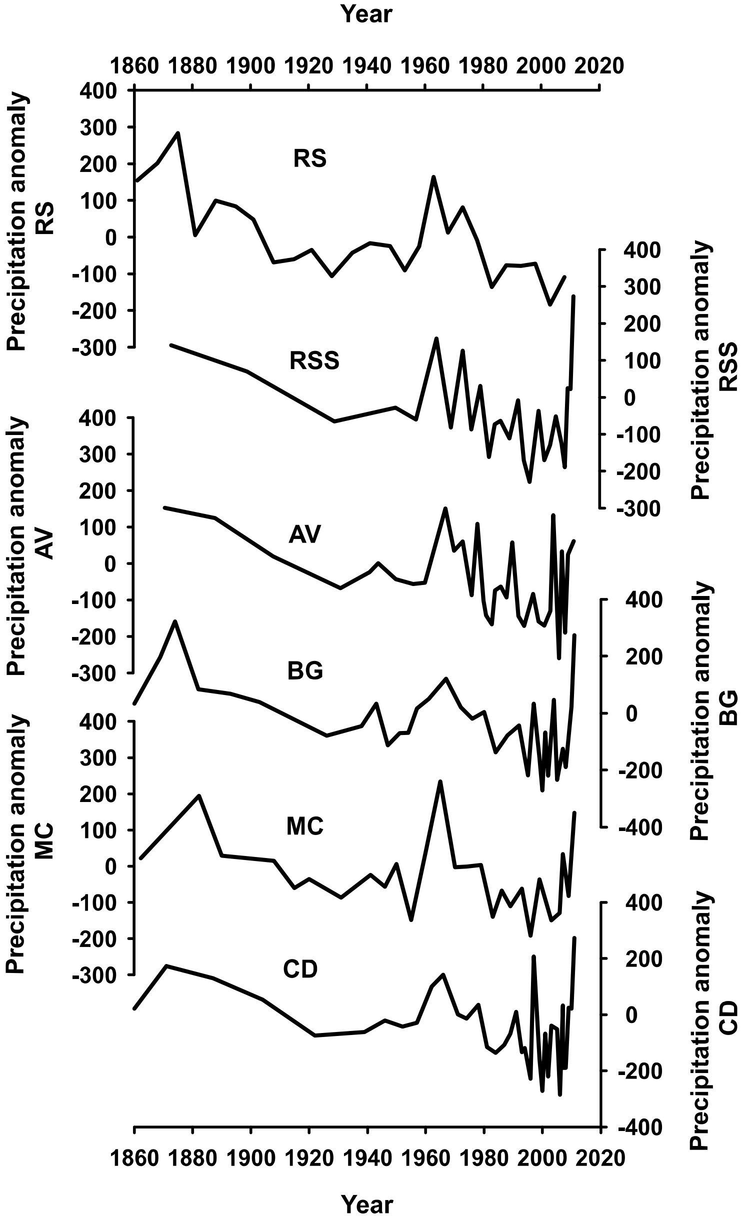
6.- Los lagos de alta montaña son ecosistemas especialmente vulnerables a un escenario de cambio global. Estos lagos pueden ser considerados como una poderosa herramienta para la reconstrucción de cambios ambientales pasados, así como para la evaluación del impacto del cambio global.

Appendices

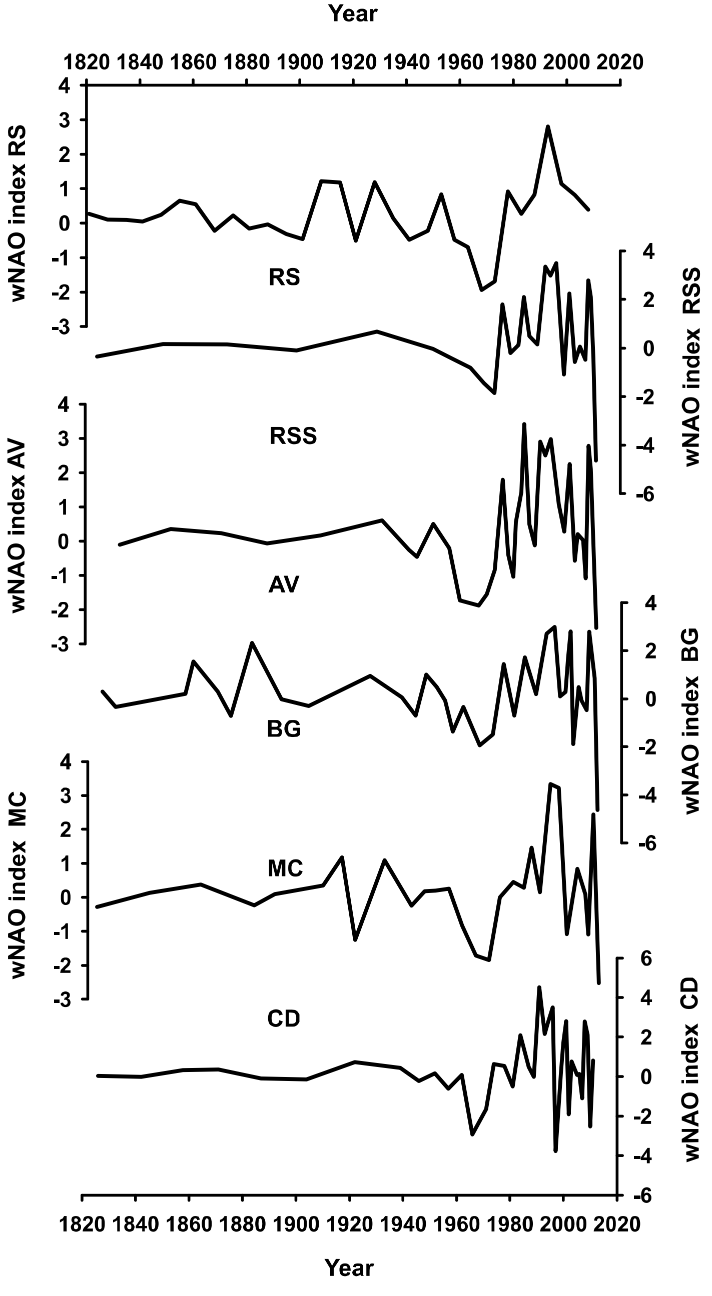
APPENDIX A. The mean annual air temperature anomaly from Madrid climate station (MAAT Madrid) from 1869 to 2011; anomalies are relative to the 1961-1990 period. The annually resolved temperature was averaged over the period of accumulation for each dated interval for all study lake (RS, RSS, AV, BG, MC and CD). Data use in Chapter 3 (for all the lakes) and Chapter 1 and 2 (for RS Lake).



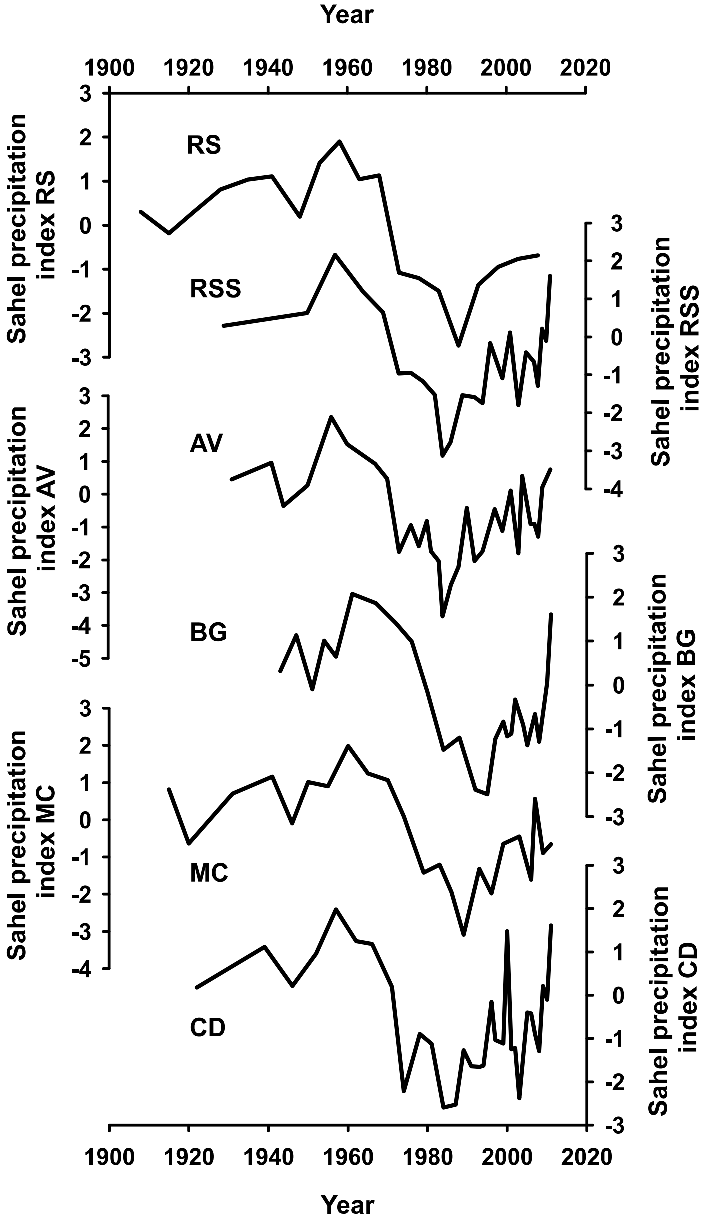
APPENDIX B. The annual precipitation anomaly from San Fernando climate station (AP San Fernando) from 1839 to 2011; anomalies are relative to the whole period. The annually resolved precipitation was averaged over the period of accumulation for each dated interval for all study lake (RS, RSS, AV, BG, MC and CD). Data use in Chapter 3 (for all the lakes) and Chapter 1 and 2 (for RS Lake).



APPENDIX C. The wNAO index is presented. The annually resolved wNAO index was averaged over the period of accumulation for each dated interval for all the study lake (RS, RSS, AV, BG, MC and CD). Data use in Chapter 3.

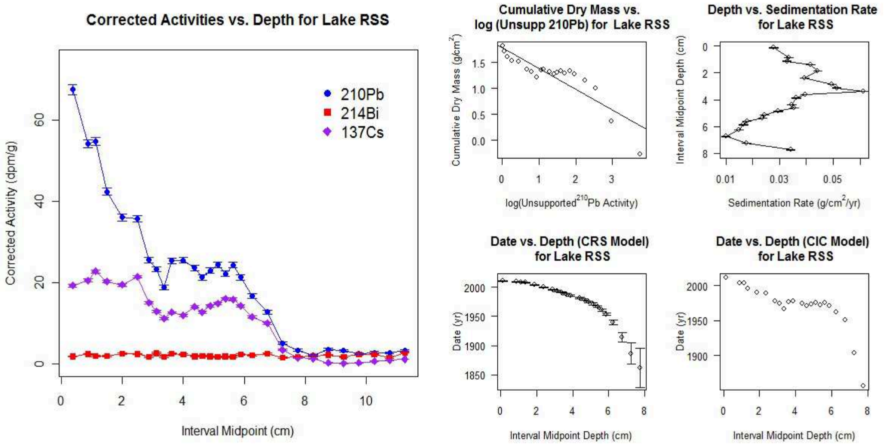


APPENDIX D. The Sahel precipitation index (SPI) is presented. The anomalies of the Sahel precipitation index (SPI) are with respect to 1900 and 2013, and based on June through October averages for each year. The annually resolved SPI was averaged over the period of accumulation for each dated interval for all the study lake (RS, RSS, AV, BG, MC and CD). Data use in Chapter 3.

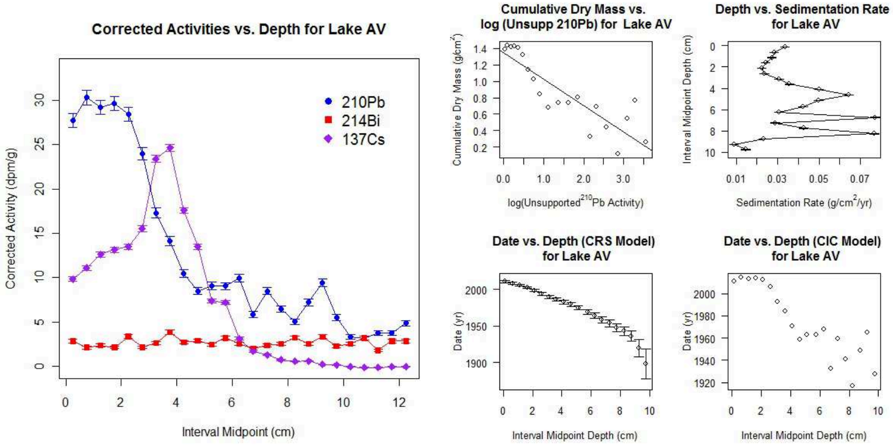


APPENDIX E: Results of ^{210}Pb gamma dating for Chapter 3 (with the exception of RS Lake)

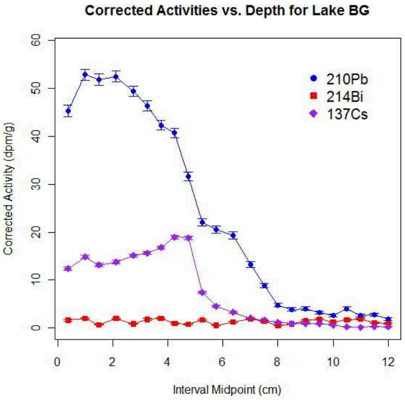
E.1 RSS Lake

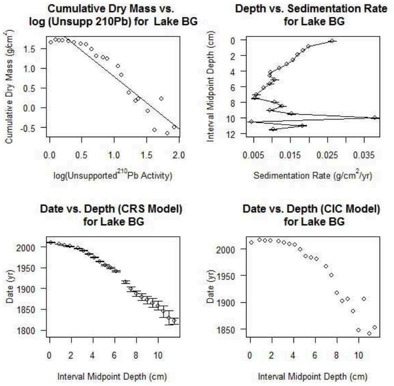


E.2 AV Lake

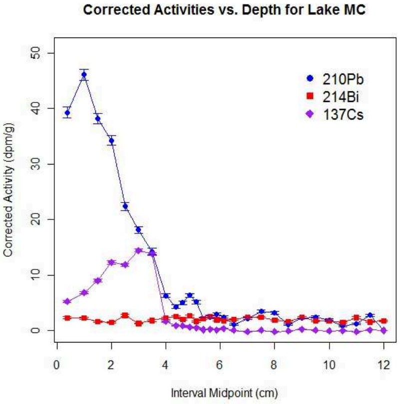


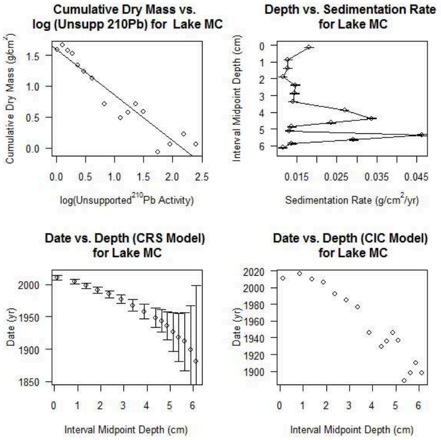
E.3 BG Lake



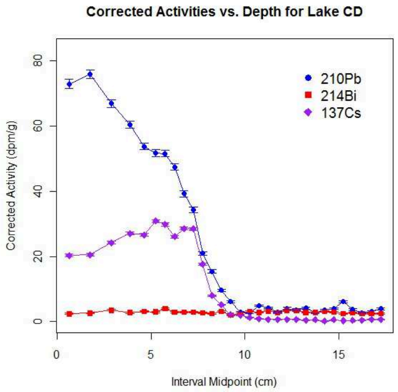


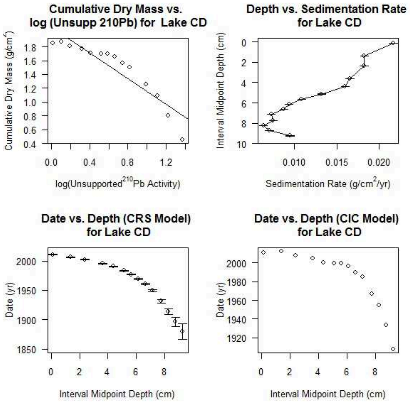
E.4 MC Lake





E.5 CD Lake


Corrected Activities vs. Depth for Lake CD
Corrected Activity (dpm/g)
0
20
40
60
80
● 210Pb
■ 214Bi
◆ 137Cs
0
5
10
15
Interval Midpoint (cm)


Cumulative Dry Mass vs. log (Unsupp 210Pb) for Lake CD
Cumulative Dry Mass (g/cm²)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.0
0.4
0.8
1.2
log(Unsupported 210Pb Activity)
Depth vs. Sedimentation Rate for Lake CD
Interval Midpoint Depth (cm)
0
2
4
6
8
10
0.010
0.015
0.020
Sedimentation Rate (g/cm²/yr)
Date vs. Depth (CRS Model) for Lake CD
Date (yr)
2000
1950
1900
1850
0
2
4
6
8
Interval Midpoint Depth (cm)
Date vs. Depth (CIC Model) for Lake CD
Date (yr)
2000
1980
1960
1940
1920
0
2
4
6
8
Interval Midpoint Depth (cm)

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