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

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ABSTRACT

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

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 rainfall 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.

Key words: Paleolimnology, sedimentary pigments, HPLC, alpine lake, Sierra Nevada.

RESUMEN

Pigmentos fotosintéticos sedimentarios como indicadores de perturbaciones climáticas y de la cuenca en un lago alpino del sudeste de España

Se tomó un testigo corto de sedimento de la laguna de Río Seco, un lago alpino y oligotrófico localizado en Sierra Nevada (sudeste de España) para determinar los cambios en la comunidad algal de los últimos 200 años. Se analizaron pigmentos fósiles y sus derivados, variables geoquímicas (índice C/N, análisis granulométrico e isótopos de datación) y variables climáticas (valores de temperatura y precipitación procedentes de series instrumentales extensas). Los principales pigmentos analizados fueron carotenoides indicadores de cianobacterias (zeaxantina, equinona y mixoxantofila), diatomeas y crisofíceas (fucoxantina y diadinoxantina) y algas verdes (luteína). Los cambios en la abundancia de pigmentos se explicaron principalmente por la temperatura. La zeaxantina presentó un marcado descenso desde el siglo XIX hasta la actualidad, y se atribuye a las cianobacterias picoplanctónicas en la laguna de Río Seco. Este descenso puede producirse por factores dependientes del clima que afectan a la presión de herbivoría y al tiempo de residencia del agua. La 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.

Palabras clave: *Paleolimnología, pigmentos sedimentarios, HPLC, lago alpino, 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, 2013). 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 analysing 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/rainfall 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 analysing the pigment record and identifying signs of catchment perturbation by analysing 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 (37°03′N, 3°20′W) is a small (0.4 ha surface and 9.9 ha catchment area), oligotrophic, and shallow (Z_{max} = 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). 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 mmol/L, 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. It is a fishless lake, the chlorophyll concentration is approximately 0.5-2 μg/L (Morales-Baquero *et al*., 2006a), and the phytoplankton biomass is less than 20 μgC/L (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.

Sediment coring and sampling

A sediment core was collected from the deepest part of the lake in September 2008 using a slide-hammer gravity corer (Aquatic Research Instruments, Hope, Idaho, USA) with an inner diameter of 6.8 cm. The core (16 cm), which was extracted in a methacrylate cylinder, was immediately wrapped in a dark bag to keep it protected from the light, sectioned into 0.5 cm slices, and

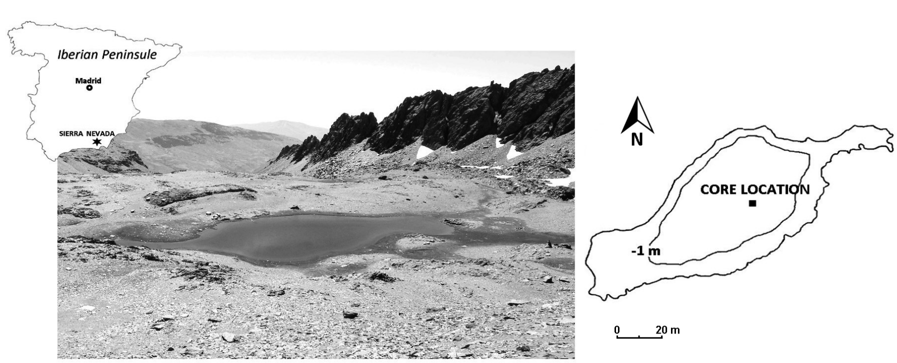

Figure 1. The location of Río Seco Lake in the Sierra Nevada (southern Spain). Left: a photo of the sampling zone; right: a core site. *Localización de la laguna de Río Seco de Sierra Nevada (sur de España). A la izquierda, foto de la zona de muestreo. A la derecha, localización del testigo de sedimento.*

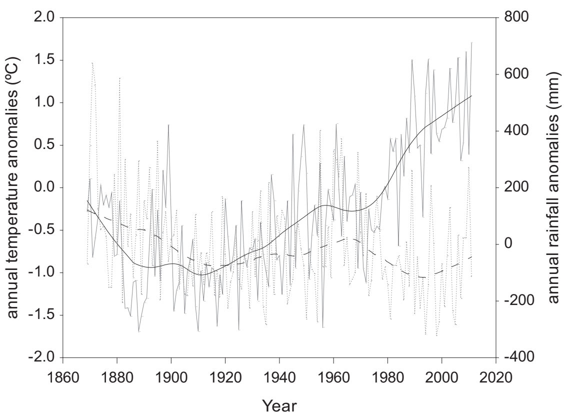

Figure 2. The mean annual air temperature anomalies from the Madrid climate station from 1869 to 2011 (continuous line); anomalies are relative to the 1961-1990 period. The annual rainfall anomalies from the San Fernando climate station from 1839 to 2011 (dashed line); anomalies are relative to the whole period. A LOWESS smoother (span = 0.25) was applied to the climate data to improve the clarity of the figure and highlight trends. *Anomalías de temperatura media anual del aire procedente de la estación climática de Madrid desde 1869 a 2011 (línea continua). La anomalías son relativas al período 1961-90. Las anomalías de la precipitación anual son de la estación climática de San Fernando desde 1839 a 2011 (línea discontinua); las anomalías son relativas al período entero. Para mejorar la claridad de la figura, se ha aplicado un suavizado LOWESS (span = 0.25) para resaltar las tendencias climáticas.*

sealed in sterile "Whirlpack" bags, which were stored and transported in a cold box. In the laboratory, the subsamples were collected at each interval and frozen (−80 °C) until ready for pigment analysis. Subsamples for loss-on-ignition (LOI), C, N, and grain size analysis and for dating and subfossil Cladocera were kept in a cold (4 °C) and dark room until ready for analysis.

Analytical methods

The sediment was dated by gamma spectroscopy (measuring radionuclide ^{210}Pb, ^{137}Cs, and ^{226}Ra) and alpha spectroscopy (^{210}Pb in deepest part of core) according to the method of Eakins & Morrison (1978). The dating and sedimentation rate were calculated by using the constant flux: the constant sedimentation (cf:cs) model (Appleby & Oldfield, 1983). All of these analyses were performed by the Radioisotope Service of the University of Seville (Spain).

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 °C 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 % $AcNH_4$ 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: $AcNNH_4$ 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 Szeroczyńska and Sarmaja-Korjonen (2007). In brief, 1 cm^3 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 20× 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. 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 $r = 0.78$, $p < 0.001$, $n = 35$). 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. There are no useful series of rainfall data at the Sierra Nevada summit. Therefore, we analysed the correlation between a short homogenized series of total annual rainfall 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 rainfall is similar among the different areas in the south of Spain. The average of temperature and rainfall data was calculated for each dating interval of the Río Seco Lake core.

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, rainfall 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

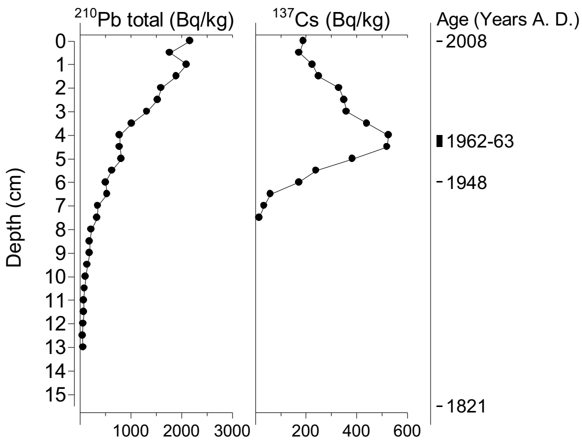

Figure 3. Depth distribution of total ^{210}Pb and ^{137}Cs (Bq/kg). The depth of stratigraphic sediment, expressed in cm, is represented on the left y-axis. On the right y-axis, the estimated ages (A. D.) of the sediment are shown. *Distribución en profundidad del ^{210}Pb total y ^{137}Cs (Bq/kg). La profundidad del sedimento estratigráfico, expresado en cm, está representado en el eje Y izquierdo. En el eje Y derecho, la edad estimada (A. D.) del sedimento.*

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, rainfall has progressively decreased, except during the 1960s, and the last 40 years have been especially dry (Fig. 2).

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}

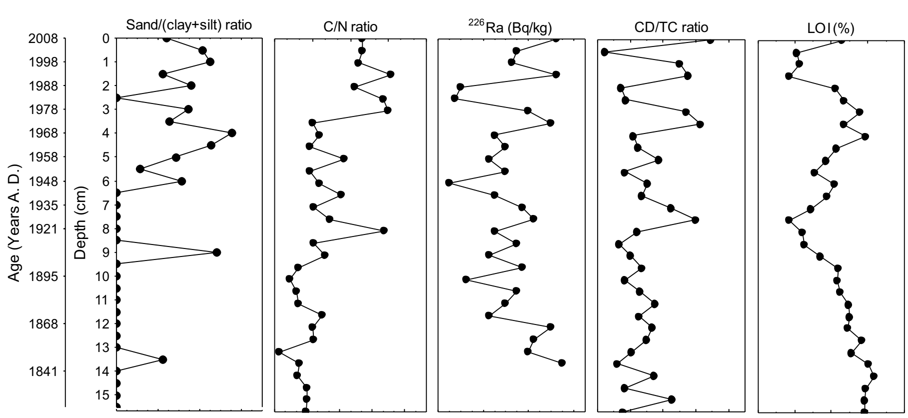

Figure 4. The Sand/(clay + silt) ratio, C/N ratio, ^{226}Ra activity, CD/TC ratio and LOI (%) in the sediment of Río Seco Lake. *El índice arena/limo + arcilla, índice C/N, actividad de ^{226}Ra, índice CD/TC y LOI (%) en el sedimento de la laguna de Río Seco.*

Table 1. The results of the redundancy analyses (RDA) with temperature, rainfall and the relative abundance of *Daphnia pulex* gr. as predictor variables and pigment data as response variables. *Resultados del análisis de redundancia (RDA) con la temperatura, precipitación y abundancia relativa de* Daphnia pulex *gr. como variables predictoras; y el conjunto de pigmentos como variables respuesta.*

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

Adjusted R^2 = 0.148

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. 3).

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. 4).

The Río Seco Lake sediment sample exhibited a narrow variety of sedimentary photosynthetic pigments (Fig. 5). 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

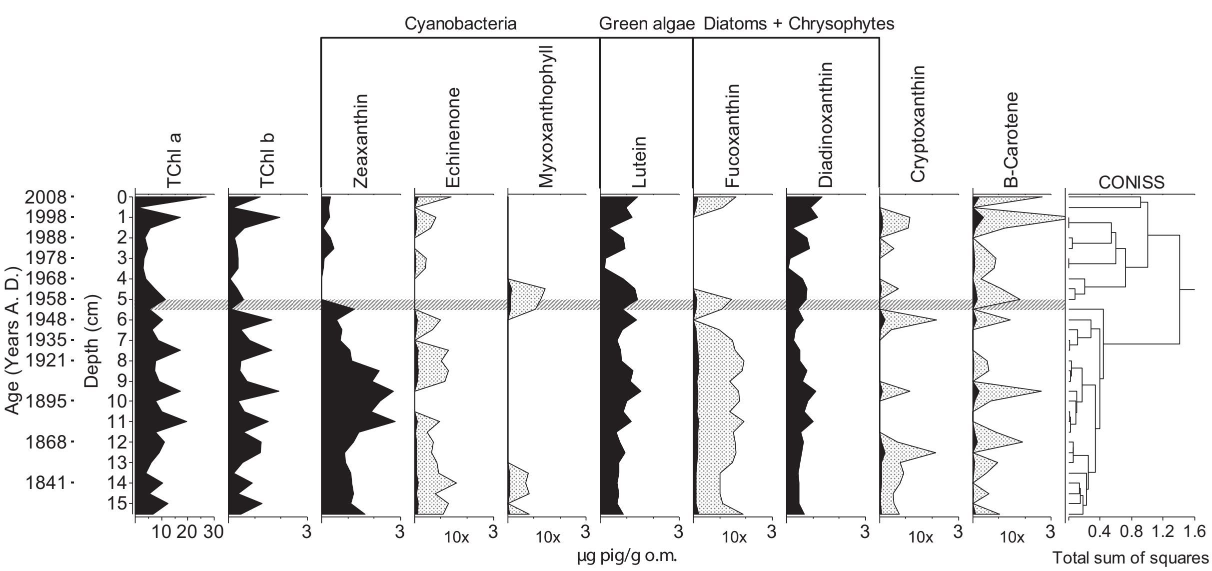

Figure 5. A stratigraphic profile of specific pigments (TChl a, TChl b, zeaxanthin, echinenone, myxoxanthophyll, lutein, fucoxanthin, diadinoxanthin, cryptoxanthin and β-carotene). All of the compounds are expressed as µg pigment/g of organic matter. The exaggerated areas are dotted (each exaggeration 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. *Perfil estratigráfico de pigmentos específicos (TChl a, TChl b, zeaxantina, equinenona, mixoxantofila, luteína, fucoxantina, diadinoxantina, criptoxantina y β-caroteno). Todos los compuestos están expresados en microgramos de pigmentos por gramos de materia orgánica. Las áreas de exageración están indicadas con un patrón de puntos (la escala de exageración está indicada debajo de cada columna). La línea horizontal delimita diferentes zonas establecidas por el análisis cluster usando el análisis de agrupamiento (CONISS). La muestra superficial (0-0.5 cm) se presenta en la gráfica, pero no es considerado exclusivamente como pigmentos sedimentarios.*

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). The stratigraphic pigment signal in Rio Seco Lake shows a vertical profile marked by two distinct zones (Fig. 5). 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. 6). The concentration of zeaxanthin was higher during wet and cold periods than during dry and warm periods over the past few decades (Fig. 7). 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. 4). The TC values showed a significant negative correlation with the C/N ratio ($r = -0.46$, $p < 0.05$).

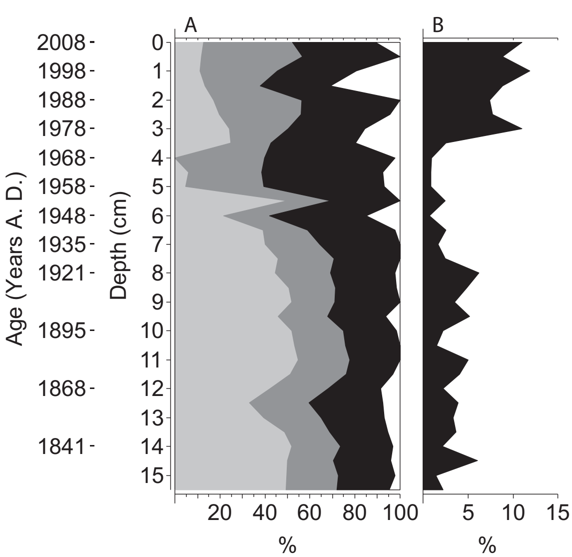

Figure 6. (A) The percentage contribution of the sum of the main algal carotenoids; from left to right: zeaxanthin, echinone and myxoxanthophyll (light grey), diadinoxanthin and fucoxanthin (medium grey), lutein (black) and other carotenoids (white). (B) The relative abundance, expressed as a percentage, of *Daphnia pulex* gr. in the sediment of Río Seco Lake. *(A) Porcentaje de los principales carotenoides, de izquierda a derecha: zeaxantina, echinenona y mixoxantofila (gris claro), diadinoxantina y fucoxantina (gris oscuro), luteina (negro) y otros carotenoides (blanco). (B) Abundancia relativa, expresada en porcentajes, de* Daphnia pulex *gr. en el sedimento de la laguna de Río Seco.*

DISCUSSION

The low variety and concentration of sedimentary pigments in the Rio 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

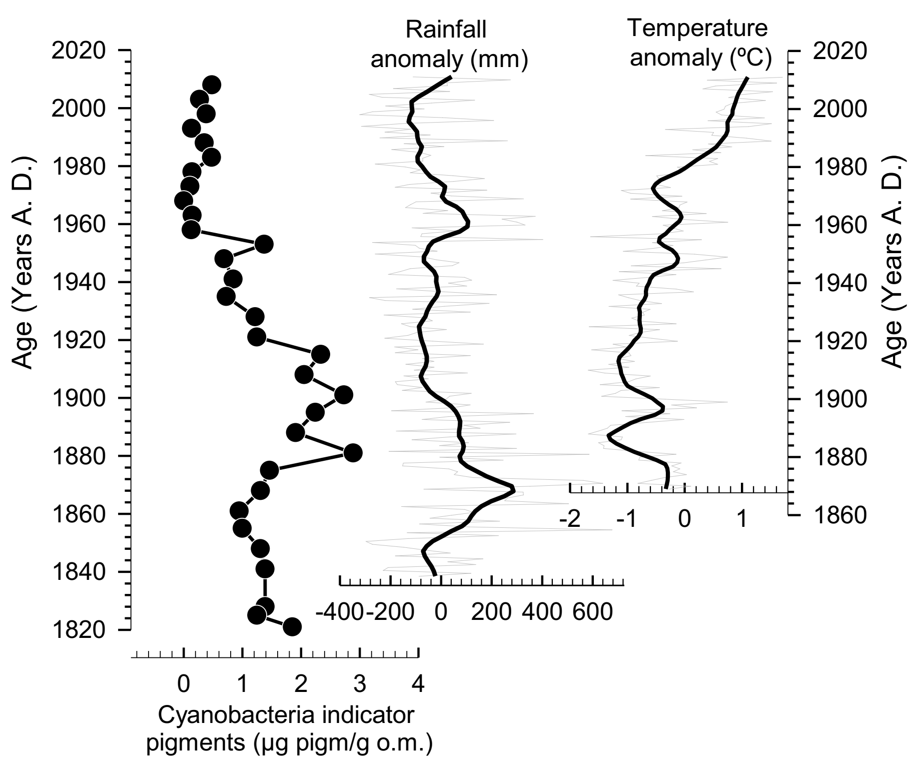

Figure 7. A sedimentary profile of cyanobacteria indicator pigments and climate data representing the mean temperature and rainfall anomalies for the dating intervals of Río Seco Lake. A LOESS smoother (span = 0.1) was applied to the climate data. *Perfil estratigráfico de pigmentos indicadores de cianobacterias junto con los datos climáticos. Se representa la media de las anomalías de temperatura y precipitación ajustados a los intervalos de datación de la laguna de Río Seco. Se ha aplicado un suavizado LOESS (span = 0.1) a las series climáticas.*

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 Rio 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 Rio 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.

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