DEPARTAMENTO DE MICROBIOLOGÍA Y ECOLOGÍA

PALEOLIMNOLOGICAL STUDY IN TWO KARSTIC LAKES : CLIMATE SIGNAL IN VARVED SEDIMENT AND PHOTOTROPHIC ORGANISMS VARIABILITY

LIDIA ROMERO VIANA

UNIVERSITAT DE VALENCIA
Servei de Publicacions
2007

Aquesta Tesi Doctoral va ser presentada a Valencia el dia 20 de Juliol de 2007 davant un tribunal format per:

- D. Ramón Juliá Brugués
- D. Rutger de Wit
- D. Brendan J. Keely
- D. Blas Valero Garcés
- D. Francesc Mezquita Juanes

Va ser dirigida per:
D^a. Rosa Miracle Solé
D. Antonio Camacho González
D. Eduardo Vicente Pedros

Depòsit legal:
I.S.B.N.:978-84-370-6929-6
Edita: Universitat de València
Servei de Publicacions
C/ Artes Gráficas, 13 bajo
46010 València
Spain
Telèfon: 963864115

Paleolimnological study in two karstic lakes:

climate signal in varved sediment and phototrophic organisms variability

Estudio paleolimnológico en dos lagos cársticos:
señal climática en el sedimento laminado y variabilidad de los organismos fotosintéticos

Tesis doctoral

Lidia Romero Viana

Universitat de València

VNIVERSITAT
DE VALÈNCIA

FACULTAT DE BIOLOGIA

DEPARTAMENT DE MICROBIOLOGIA I ECOLOGIA

PALEOLIMNOLOGICAL STUDY IN TWO KARSTIC LAKES: CLIMATE SIGNAL IN VARVED SEDIMENT AND PHOTOTROPHIC ORGANISMS VARIABILITY

Estudio paleolimnológico en dos lagos cársticos:
señal climática en el sedimento laminado y
variabilidad de los organismos fotosintéticos

Tesis Doctoral presentada
para optar al grado de doctor por

Lidia Romero Viana

Dirigida por

Dra. Maria Rosa Miracle
Dr. Antonio Camacho
Dr. Eduardo Vicente

Agradecimientos

Hace un tiempo no veía el momento de escribir esta líneas, pero por fin ha llegado. La tesis ya está “cocinada”. No queda más que poner por escrito mi agradecimiento a todos los que se vieron implicados de una forma y otra en el desarrollo de este trabajo. Sin ellos, sin su colaboración, sin su ayuda, sin su tiempo no habría tesis que presentar.

Agraceder en primer lugar a mis directores por su esfuerzo, estímulo y respaldo. Gracias a la Dra. Miracle por sus apreciaciones y aportaciones teóricas en numerosas conversaciones que fueron estímulo a lo largo del desarrollo de esta tesis. Al Dr. Camacho debo agradecerle su esfuerzo, su paciencia y especialmente su maestría. Gracias al “rojo sangre” de sus correcciones durante estos años he aprendido y comprendido las pautas del pensamiento metódico y la necesidad de éste en el desarrollo del trabajo científico. Gracias al Dr. Vicente por sus consejos y atención en todo momento.

Me gustaría darle las gracias con especial cariño al Dr. Julià. Me siento muy orgullosa de haber podido trabajar con un reconocido y querido Ramón. En mis sucesivos viajes a Barcelona hemos tenido ocasión de hablar y conocernos y me he encontrado con un excelente científico y lo que es más importante, una excelente persona. Mi agradecimiento se hace extensivo a su mujer MªAngeles y su hija Anna. Gracias por todo lo que he aprendido y disfrutado con vosotros.

I am indebted to Brendan Keely who kindly offered himself and invited me to work with him and his group at York. He introduce me to the complex world of photosynthetic pigments. Wihtout his help, probably this work would not be concluded. Moreover I am very grateful to Angela Squier and Deborah Mawson who friendly helped me in the laboratory with the HPLC and LC-MS techniques. Me gustaría darles las gracias con cariño a Laura, Ernesto y Lourdes. Sin ellos mi estancia en York no habría sido tan productiva. Con ellos compartí la morriña por estar lejos de casa en el verano de 2003 pero juntos hicimos que York no fuera tan aburrida. Gracias compañeros!

Igualmente quiero agradecer la colaboración en este trabajo de Jesús García-Gil y Carles Borrego. Sin su ofrecimiento y ayuda para utilizar el material y las técnicas analíticas de la Unidad de Ecología Acuática de la Universidad de Girona todavía estaríamos empezando. Gracias a todos los compañeros de Girona con los que compartí trabajo pero también cafés y cenas, Noemí, Laia, Xavi, Marta y los demás.

Agradecer también las facilidades técnicas que nos ofrecieron Joan Grimalt y Joan Villanueva del Departamento de Química Medioambiental CID-CSIC de Barcelona.

Gracias a mis compañeros de la Unidad de Ecología Microbiana y Limnología; Sara, Maria, Carmen, Nieves, MariLoli, Loles, Dani, Antonio, Javi y Carlos. Con ellos he pasado la mayor parte del tiempo durante estos últimos años en Siberia y Microscopía. Juntos hemos compartido mucho trabajo; muestreos en Cañada, días de análisis interminables en Siberia, veranos desérticos en el campus y jornadas maratonianas pero también risas, cenas, cafés, confidencias... Gracias a David, Yolanda y Ester, los técnicos de laboratorio que estuvieron en Siberia, por su ayuda. Gracias también a los compañeros de secretaría del Departamento de Microbiología y Ecología.

Por último me gustaría agraceder con especial cariño a mis amigos y familia su constante apoyo y calidas palabras de aliento en todo momento difícil.

El trabajo presentado en esta tesis doctoral ha sido financiado por la Generalitat Valencia mediante una beca predoctoral.

“Antes no podía entender porque
mi pregunta no tenía respuesta; hoy no entiendo
como pude creer que la pregunta era posible.
De todos modos, yo no creía, solo preguntaba”.

F. Kafka, Informe para una Academia.

Abstract

This paleolimnological research work was focused on the analysis of the temporal variability of the primary producers community of two close karstic lakes, La Cruz and Lagunillo del Tejo (Cuenca, Spain). Photosynthetic pigments preserved in lake sediments were considered as the most suitable biomarker because of their taxonomic specifity and the accurate understanding of the transference from the water column into sedimentary signal by means of an experimental study about the sedimentation processes carried out over three annual cycles in Lake La Cruz. The multidimensional statistical analysis of the pigments stratigraphic profiles showed the site specific responses to different forcing factors over the last centuries. The detailed analysis of the sedimentary signal indicates that the lake-level changes associated to regional hydroclimatic variability have played a relevant role on the composition of the primary producers commnunity in Lagunillo del Tejo, whereas solar variability has pointed out as forcing factor of the inferred changes of Lake La Cruz primary production, which was characterised by a pigment compositional stability. On the other hand the annually laminated sediment of Lake La Cruz have been shown as an excellent quantitative climatic proxy; calibration analysis of calcite laminae thickness indicated a highly significant correlation with winter rainfall. Winter rainfall anomalies are, in turn, highly correlated with the phase of North Atlantic Oscillation (NAO). Given this strong climatic relationship, an annual winter rainfall reconstruction for the period 1589 A.D. to present was performed. The dominance of nonstationary component at high frecuencies of the climate signal over the last 420 years indicates that the connection between winter rainfall and the NAO is not stable over time and suggest that different patterns, not only NAO, have played a role in determining rainfall variability.

Resumen

Este estudio paleolimnológico se centra en el análisis de la variabilidad temporal de la comunidad de productores primarios de dos lagos próximos, la Laguna de La Cruz y el Lagunillo del Tejo (Cuenca, España). Se consideró que los pigmentos fotosintéticos conservados en el sedimento eran los biomarcadores más adecuados para trazar la varibilidad de los organismos fotosintéticos debido a la especificidad taxonómica que presentan y al conocimiento sobre su transferencia de la columna de agua al sedimento, obtenido en un estudio experimental de los procesos de sedimentación durante tres ciclos anuales en la Laguna de La Cruz. El análisis estadístico multidimensional de los perfiles estratigráficos de los diferentes pigmentos evidenció la respuesta específica de cada sistema a diferentes factores ambientales durante los últimos siglos. El análisis en detalle de la señal sedimentaria indicó que en el Lagunillo del Tejo las fluctuaciones de nivel de agua, asociadas a variabilidad hidroclimática, han influido notablemente en la composición de la comunidad de productores primarios. Sin embargo en la Laguna de La Cruz, caracterizada por una estabilidad en la composición de la comunidad de organismos fotosintéticos, los cambios inferidos en la producción primaria parecen haber sido condicionados por la variabilidad solar. Por otro lado este estudio ha demostrado que el sedimento laminado de la Laguna de La Cruz es un excelente indicador climático cuantitativo. Los resultados del análisis de calibración del espesor de las láminas de calcita indican que dichos espesores estan principalmente relacionados con las precipitaciones acumuladas durante los meses de invierno presentando una correlación altamente significativa. Las precipitaciones invernales, a su vez, mostraron una correlación altamente significativa con el índice atmosférico de Oscilación del Atlántico Norte (NAO). Dada esta significativa relación climática, utilizamos el sedimento laminado de la Laguna de La Cruz para reconstruir las precipitaciones invernales anuales desde 1589 d.C. hasta el presente. El análisis de la señal climática mostró un dominio de procesos no estacionarios en las altas frecuencias lo que sugiere que la conexión entre las lluvias regionales y el patrón NAO no ha sido estable durante los últimos siglos y que probablemente otros patrones de circulación hayan ejercido su influencia en la región.

Contents

1. Introduction

1a. Paleolimnology and recent challenges.

Lake sediments are made up of two basic components: allochthonous material, originating from outside the lake basin; and autochthonous material, produced within the lake itself (Margalef 1983). Allochtonous material is transported to lakes by rivers and streams, overland flow, aeolian activity, and subsurface drainage. It is made up of varying amounts of fluvial or eolian clastic sediments, dissolved salts, terrestrial macrofossils, and pollen. Autochthonous material is either biogenic in origin or it may result from inorganic precipitation within water column. Thus, lake sediment store in the successive stratigraphic levels an important amount of environmental information of catchment and lake itself during the past with both allochthonous and autochthonous sources of proxy data.

Paleolimnological approaches attempt the reconstruction of lake ontogeny, past existing communities, and physical and chemical lake features changes, based on the analysis of biological remains and geochemical features of lake sediments. The interpretation of fossil records depends on the understanding of present-day lake processes and current autoecology descriptions of the biota. Although early paleolimnological reconstructions were mainly qualitative, much technical and theoretical efforts have permitted a progressive development to more quantitative reconstructions. Three main factors have lead to the rapid development of paleolimnology over the last decades; the advances made on dating techniques; the increase of quantity and quality of information that we could learn from sedimentary record; and new statistical approaches which allowed quantitative inferences of environmental variables based on biotic remains after surface sediment calibrations sets.

In fact, the data requirements about past climatic features have been determinant for these advances in paleolimnology. With recent concerns about anthropogenically enhanced global warming, the need for reliable long-term paleoclimatic data is widely recognized because these records provide the background information against which the recent changes can be assessed. Paleoclimatology is a diverse and rapidly expanding science that encompasses a wide variety of proxy sources; ice cores, tree rings, corals, speleotherms and among others, marine and lacustrine sediments, that are capable of providing information on past climatic conditions (Bradley 1999). Lake sediments preserve within a relative untapped source of past climate data, since whole lacustrine processes and biota respond directly to climate variability. Two different approaches have been attempted to infer climatic variables; one based on the link between proxy and climatic variable and the other by means of the inference of the limnological variable that is related with climate. Paleolimnological approaches are broadly similar across climatic regions, however the environmental gradients that paleolimnologists track can be very different (Smol and Cumming 2000). For example, climatic inferences in polar regions have focussed on past lake ice conditions, whereas in closed-basin lakes in arid and semiarid regions past lakewater salinity which can be reconstructed from fossil assemblages, is closely tied to the balance of evaporation and precipitation. Nowadays the challenge for paleolimnologists is to recognize the individual lake response in order to improve paleoclimatic inferences. When studying responses of lake ecosystem to climate variability, the character and intensity of an individual response can vary considerably related with the catchment characteristics, lake morphometry, lake history and/or abiotic and biotic interactions (Blenckner 2005).

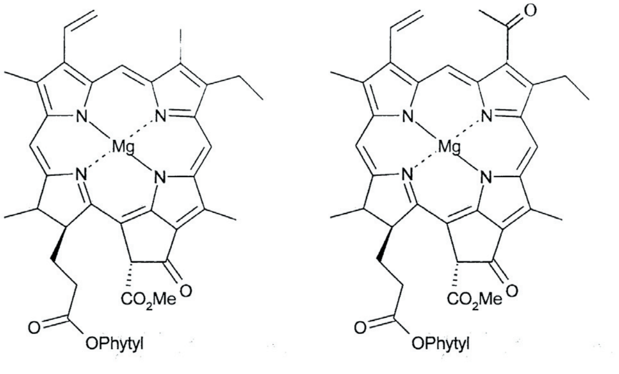
On the other hand, paleolimnology offers the long-term perspective which is not generally available to most “neolimnologists”. The importance of this temporal perspective to assist in the understanding of ecosystem disturbance and communities trajectories is now generally accepted by limnologists and has meant that lake sediment records are increasingly being used to provide information about recent rates of change, natural background conditions in lakes and natural variability (Anderson 1995b). Moreover lake sediment data may be used also deductively, to test models or hypothesis of contemporary limnology (Smol 1990). Although it is actually very difficult to approach sediment-based studies in any other way but inductively, the potential of paleolimnological studies could increase significantly. Nevertheless many possibilities of paleolimnological data remain relative unfulfilled as a result of the sporadic interaction between "neo" and paleolimnologists.

1b. Photosynthetic pigments as paleolimnological proxy.

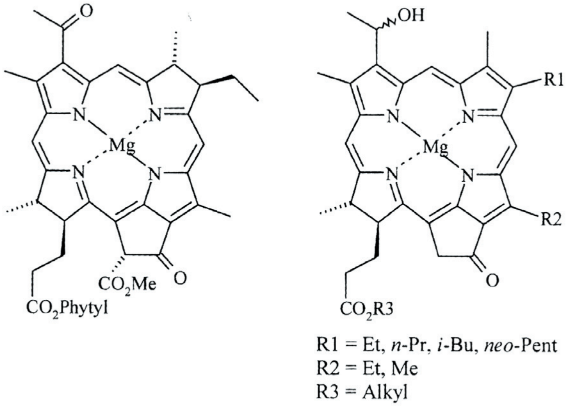
A widespread range of organic compounds are preserved in lake sediments; i.e. fatty acids, isoprenoid compounds, alcohols and ketones, steranes and triterpanes, hydrocarbons, sterols, carbohydrates, aminoacids, purines and pyrimidines, among them, photosynthetic pigments including chlorophylls and carotenoids. These compounds participate in the photosynthesis process whereby light energy is absorbed by pigments, for transduction to chemical energy. The chlorophylls are a group of magnesium coordination complexes of cyclic tetrapyrroles (Figure 1) which display a wide range of structural variations which relate to their different biological sources and to environmental conditions. Likewise carotenoids show a high structural diversity (Figure 2), although they have one structural feature in common: a system of regularly alternating single and double bonds in the central part of the molecule which are responsible for the ability of the molecules to absorb light.

Chlorophylls, carotenoids and their derivatives have been isolated and identified from aquatic sediments for over 50 years. Despite the fact that the earlier sedimentary pigment research was constrained by the relative coarse analytical techniques and the understanding of pigment biogeochemistry (Fox 1944; Vallentyne 1956), promising results were obtained using sedimentary pigments in paleolimnological studies as biochemical markers for the presence of former populations of phototrophic prokaryotes (Brown 1968) or for estimates of historic changes in lake productivity (Vallentyne 1957; Fogg and Belcher 1961). Furthermore the pioneer investigators described some of the factors that influence the deposition and preservation of pigments and recognised many of the problems that would follow in future investigations (Sanger 1988). Afterwards considerable efforts have gone into development of more sophisticated methods of chemical identification, better determinations of compounds distribution and taxonomic studies and detailed studies of pigment taphonomy.

In general, pigments have five propierties which make them useful chemosystematic compounds; common initial biosynthetic pathways within each class of molecule; diverse chemical structures arising from specific terminal steps during biosynthesis; ubiquitous distribution among phototrophic organism, with some taxonomic specificity; functions essential to survival, and; relative easier



Chlorophyll a *Chlorophyll b*



R1 = Et, *n*-Pr, *i*-Bu, *neo*-Pent
R2 = Et, Me
R3 = Alkyl

bacteriochlorophyll a *bacteriochlorophyll d*

Figure 1. Molecular structures of some chlorins. Chlorophyll_a is present in all photosynthetic algae and high plants. Also chlorophyll_b is present in high plants, green algae (chlorophyceae) and simbiotic prochlorophytes. Moreover chlorophylls_c (not showed) are present in dinophyta, bacillariophyta and crysophyta. Bacteriochlorophyll_a (bchl_a) is the main photosynthetic compound of Purple Sulfur Bacteria. Instead of phytyl, bchl_a macrocycle could be esterified by other alcohol such as geranyl-geraniol. In bacteriochlorophyll_d main photosynthetic compound of green sulfur bacteria, the R1, R2 and R3 substituents are highly variable. Moreover bacteriochlorophylls_c, _e, _b and _g are also present in photosynthetic bacteria.

Figura 1. Estructuras moleculares de varias clorofilas. La clorofila_ a está presente en todas las algas y plantas superiores. Además la clorofila_b es común en plantas superiores, el grupo de algas verdes (cloroficeas) y las proclorofitas simbiontes. Además de estas, las clorofilas_c (no se muestran) estan presentes en dinófitas, bacillariofitas y crisófitas. La bacterioclorofila_a es el principal compuesto fotosintético en el grupo de las bacterias rojas del azufre. En lugar del fitol, el macrociclo puede estar esterificado con otro alcohol como el geranil-geraniol. En el macrociclo de la bacterioclorofila_d, principal compuesto fotosintético de las bacterias verdes del azufre, los sustituyentes R1, R2 y R3 son altamente variables, dando lugar a múltiples homólogos. Además de estas dos bacterioclorofilas, existen bacterioclorofilas_c, _e, _b y _g presentes en los fotosistemas de las bacterias fotosintéticas.

analytical procedures (Leavitt and Hodgson 2001). Overall, the taxonomic specificity results highly useful in sedimentary pigments interpretations, reflecting the major distribution of the respective phytoplankton groups in the past (table 1). Unlike chlorophylls, the carotenoids are very numerous. Since the 1960s the number of isolated and identified carotenoids have increased non-stop, expanding on chemotaxonomic features of the different phytoplankton groups (Liaaen-Jensen 1979).

Most of this late development was due to the improvement of chromatographic techniques. High performance liquid chromatography (HPLC) is the latest in a long series of techniques developed to isolate component pigments from a whole algal, plant or sedimentary extracts. Since 1980, HPLC analyses have become the method of choice for rapid, quantitative determinations of carotenoids, chlorins and derivative content in aquatic ecosystem and their sediment (Millie et al. 1993; Jeffrey et al. 1997). A wide spectrum of chromatographic methods adapted to achieve compounds separation have being proposed including those of Mantoura and Llewellyn (1983) and the Scientific Committee of Oceanographic Research SCOR (Wright et al. 1991), both widely cited. Specific methods have been developed for sediment extracts which usually exhibited highly complex mixtures and display a broad range in polarity (Airs et al. 2001). HPLC eases identification and quantification of carotenoids and chlorins by their different retention time and UV/visible-light spectra obtained with in-line detectors. However these two properties alone are generally not considered sufficient for a secure identification of organic compounds. For rigorous assignment, however, it is necessary to obtain molecular mass and structural information by Mass Spectrometry. MS techniques permit the detection of specific mass ions, their fragmentation patterns, and the presence of key functional groups which were used to verify the identity of pigments and their derivatives. Over the last ten years, the considerable advances in analytical techniques and their application to the identification of photosynthetic pigment compounds have allowed greater certainty in structure assignments (Hodgson et al. 1997) and have revealed a number of novel derivate components (i.e. Eckardt et al. 1991; Wilson et al. 2004). Therefore the combined application of HPLC and MS techniques results in more accurate identification of pigments and their derivatives enhancing the potential value of fossil pigments as biomarkers.

On the other hand the interpretation of pigment stratigraphy in sediment columns depends on a clear understanding of the factors that promote preservation or degradation of the molecules (Figure 3). Much effort has been

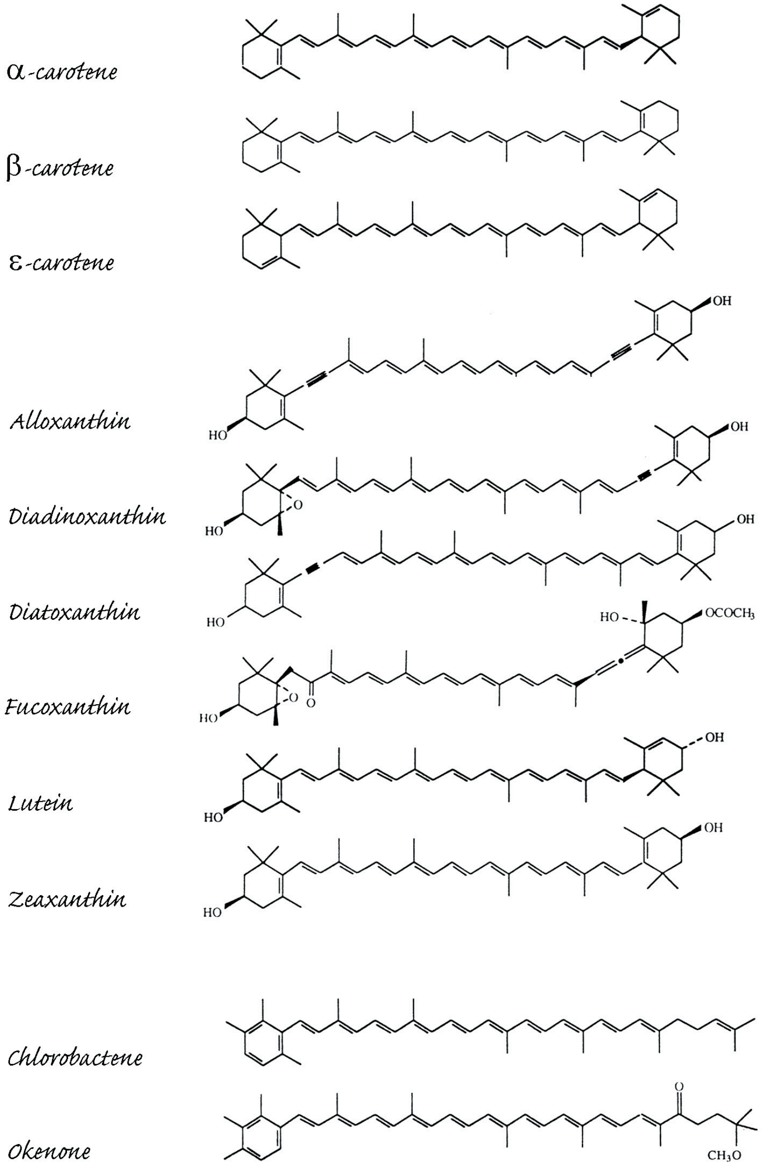


Figure 2. Some exemples of carotenoids diversity. The hydrocarbon carotenoids (i.e. α-carotene, β-carotene and ε-carotene) are known collectively as carotenes. The mayority of natural carotenoids, however, contain at least one oxygen function, and are knwon as xanthophylls (i.e. common algae xanthophylls; alloxanthin, diadinoxanthin, diatoxanthin, fucoxanthin, lutein and zeaxanthin). Also bacterial carotenoids chlorobactene and okenone of green and purple sulfur bacteria, respectively.

Figura 2. Algunos ejemplos de la diversidad de los carotenos. Las cadenas de hidrocarbono se conocen como carotenos (por ejemplo, α-caroteno, β-caroteno y ε-caroteno). Sin embargo, la mayoría de carotenos naturales contiene al menos un grupo funcional con oxígeno y son conocidos como xantofilas (por ejemplo, algunas xantofilas algales, aloxantina, diadinoxantina, diatoxantina, fucoxantina, luteina y zeaxantina). Además carotenos bacterianos como el clorobacteno y la okenona, propios de bacterias verdes y rojas del azufre respectivamente.

	Algal Divission/Class									
Pigments	**Cyanobacteria**	**Cryptophyta**	**Chlorophyceae**	**Prasinophyceae**	**Euglenophyta**	**Eustigmatophyta**	**Bacillariophyta**	**Dinophyta**	**Prymnesiophyceae**	**Chrysophyceae**
Chlorophylls										
a	H	H	H	H	H	H	H	H	H	H
b			H	H	H					
c1							H		H	
c2							H	H	H	H
c3									H	H
Carotenes										
α		L	t	t						
β	L		L	L	L	L	t	t	t	t
ε		t								t
Xantophylls										
Alloxanthin		H								
Antheraxanthin			t	t	t					
Astaxanthin			t			t		t		
Cantaxanthin	L					t				
Crocoxanthin		t								
Cryptoxanthin			H							
Diadinoxanthin					H		H	H	H	H
Diatoxanthin					t		t	t	t	t
Dinoxanthin								L		
Echinenone	L									
Fucoxanthin							H		H	H
Lutein			H	L						
Monadoxanthin		t								
Myxoxanthophyll	L									
Neoxanthin			H	H	t					
Oscillaxanthin	L									
Peridinin								H		
Prasinoxanthin				H						
Violaxanthin			H	H		H				
Zeaxanthin	H									

Code: H=major pigment, L=minor pigment, t=trace pigment

Table 1. Distribution of major and taxonomically significant pigments in algal divisions/ classes. (Adapted from Rowan 1989; Jeffrey and Vesk 1997).

Tabla 1. Distribución de los pigmentos más abundantes y taxonómicamente significativos en las divisiones/clases algales. (Adaptado de Rowan 1989; *Jeffrey y Vesk 1997).*

expended on quantifying pigment degradation in the water column and the sediment in both marine and fresh waters. Field studies of carotenoid and chlorophylls degradation were based on comparison of pigments from plankton, sediment trap and surface sediments or experimental determinations of decay (Leavitt 1993). Despite of the wide range of methods, results of *in situ* studies have proved uniform - most pigments in detrital material are degraded before permanent incorporation into lake and ocean sediments (Carpenter et al. 1986; Hurley and Amstrong 1990, Steenbergen et al. 1994). Degradation within the water column includes chemically or microbial-mediated oxidation (Carpenter et al. 1986; Louda et al. 1998), grazing by invertebrates (Shuman and Lorenzen 1975; Leavitt and Carpenter 1990b; Head and Harris 1992), bacterial degradation (Daley 1973; Spooner et al. 1994a), and cell lysis and enzymatic metabolism during senescence (Spooner et al. 1994a and b; Louda et al. 1998). Once deposited, pigments can be further degraded by chemical or biological mediated processes. All these processes interact in complex manner to regulate the quantity and composition of pigments assemblages in surface sediments. However, the magnitude and relative importance of variation in these loss factors may change with the transport process (fecal pellets and cell-sinking) (Reynolds et al. 1982; Lorenzen and Welschmeyer 1983), specific lake depth, the penetration depth of light and oxygen relative to lake detph (Leavitt and Carpenter 1990a) and the mean depth of producer populations (Hurley and Garrison 1993; Baines and Pace 1994; Cuddington and Leavitt 1999). Moreover the loss extend is influenced by pigment type since rates of degradation differ greatly among pigments depending of the molecular structure (Leavitt and Carpenter 1990a). In general compounds with complex functional groups and structures degrade more rapid than less complex compounds. However substantial variability in pigment loss rate also occurs because of differences in susceptibility of source populations to senescence and decomposition, either because of inherent biochemical characteristics of cells or because of differences in species habitat preferences, selective grazing by invertebrates or other ecological factors (Swain 1985). In spite of the problems outlined, rather than deterrent to paleoecological applications, improved definitions of pigment biogeochemistry allows investigators to identify more clearly the scope, limitations and applications of pigment analyses.

Development of more sophisticated analytical techniques and understanding of the patterns of pigment production, deposition and degradation has significantly improved the reliability of historical reconstructions based on fossil pigment analyses. Photosynthetic pigments have been used as indicators of algal and

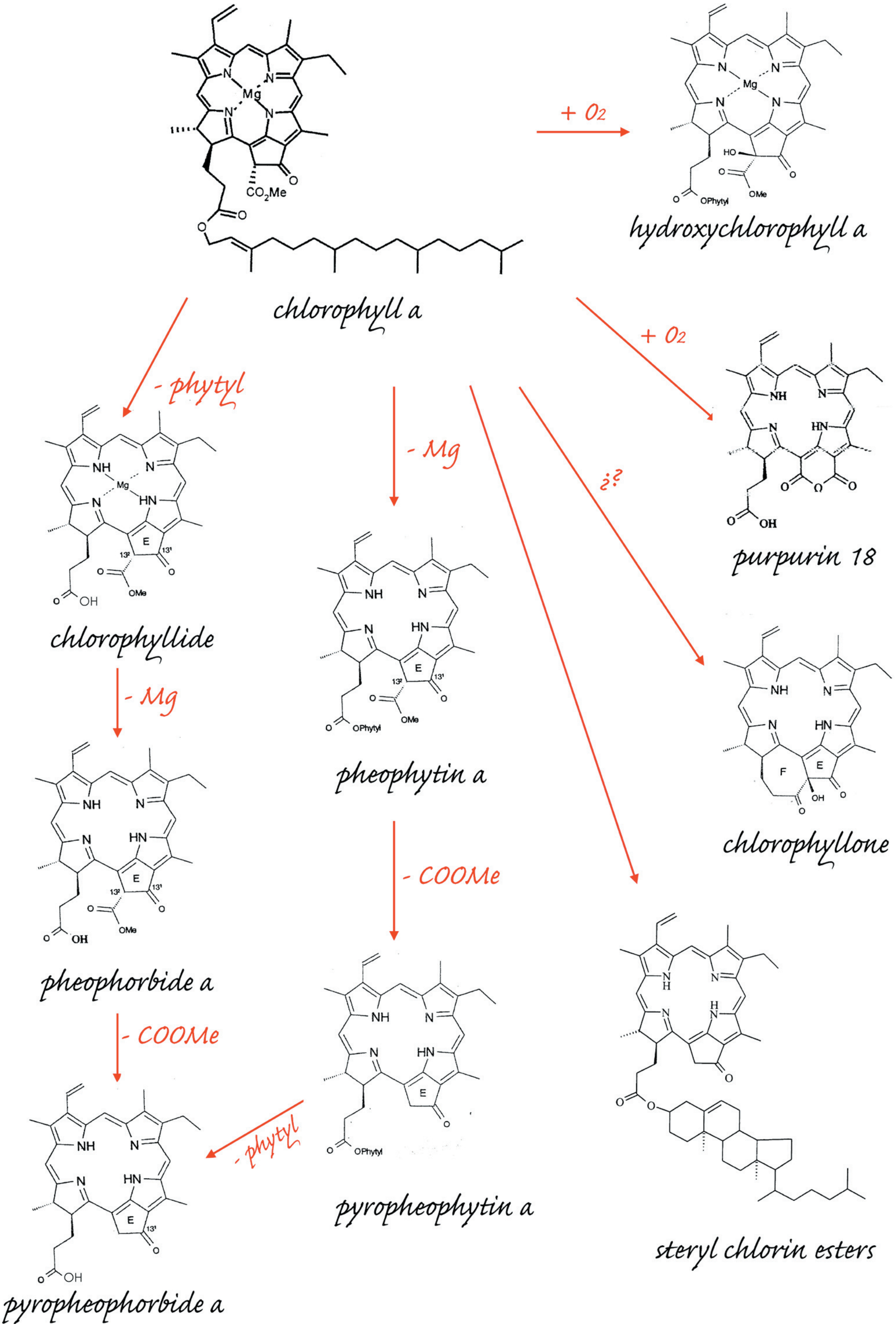


Figure 3. Simplified representation of main transformation reactions of chlorophyll_a.

Figura 3. Representación simplificada de las principales reacciones de transformación de las moléculas de clorofila_a.

bacterial community composition (Züllig 1981; Brown et al. 1984; Overman et al. 1993), food-web interactions (Carpenter et al. 1988), anthropogenic impacts on aquatic ecosystems including eutrophication, acidification, fisheries management, and land-use practiques (Guilizzoni et al. 1992; Hall et al. 1999), changes in the physical structure of lakes (Hodgson et al. 1998), mass flux within lakes (Ostrovsky and Yacobi 1999), and past UV radiation environments (Leavitt et al. 1997; Squier et al. 2004; Verleyen et al. 2005).

1c. Aims of the study

At eastern Spain, in a karstic area of the Iberian Ranges (Cuenca, Spain) there are several small flooded dolines. Over the last twenty years some of them have focused the research interest of the members from the Ecology Department of the University of Valencia. Numerous studies have reported a wide range of their ecological features improving the knowledge about these particular mediterranean ecosystems (Vicente and Miracle 1988; Dasi and Miracle 1991; Esparcia et al. 1991; Miracle et al. 1992; Rodrigo et al. 1993; Armengol-Diaz et al. 1993; Camacho and Vicente 1998; Camacho et al. 2000; Miracle et al. 2000; Rodrigo et al. 2000; Rodrigo et al. 2001; Camacho et al. 2003a; Camacho et al. 2003b; Morata et al. 2003). Framed in the ecological characterization of these lake-dolines of the Cañada del Hoyo karstic system the present study is a paleolimnological approach undertaken in two of these lacustrine ecosystems, Lake La Cruz and Lagunillo del Tejo.

The first aim of this study is the reconstruction of historical changes of phototrophic communities from Lake La Cruz and Lagunillo del Tejo over the last centuries, selecting photosynthetic pigments as the most useful proxy to trace quantitative and qualitative changes in the primary producers community, and attempt to determine which factors have conditioned them. To reach this objective we considered essential the characterization of the sedimentation patterns of planktonic populations. Hence, material collected by sediment traps which were used to study the sedimentation processes during a previous intensive survey over three years in Lake La Cruz, was analysed to determine the transfer of proxy from water column into lake sediment.

On the other hand, a sediment core recovered one decade ago from Lake La Cruz provided the first approximation to lake ontogeny (Julia et al. 1998). However the origin and the evolution of the current meromictic condition had remained unexplored. So the second purpose of this study was to achieve an understanding of meromixis in Lake La Cruz, focused in the biotic response.

Given the observed presence in Lake La Cruz of an undisturbed varved sediment in the uppermost 40 cm, the third objective was to explore the relationship between climatic factors and the calcite laminations since the features of the laminated sediments (i.e. varve thickness, micro-facies features, chemical composition) have been shown to preserve the climate signal (solar variability, temperature, rainfall, cumulative melting degree days, etc) which in general sense controls the varve formation.

1d. Structure of the study.

The results and conclusions of this study are exposed and developed in seven sections. After the present introduction (section 1) and the study site description (section 2), we present the sedimentation patterns of photosynthetic organisms from Lake La Cruz (section 3), the analysis of the climatic signal recorded in calcite laminated sediment (section 4) and the winter rainfall reconstruction performed over the last 430 years in basis of the previous results (section 5). Finally the paleolimnological study of Lake La Cruz and Lagunillo del Tejo over the last centuries are described in section 6 and 7, respectively. All these sections are developed similar; each chapter contains an introduction, methodology, results and a discussion section. In section 8 the main results of the different sections are summarized, being exposed in an integrated manner and finally the concluding remarks are exposed in section 9.

2. Study site

The Lake La Cruz and Lagunillo del Tejo are two close doline lakes, both in karstic system of Guadazaon river in Cañada del Hoyo village. They are located in the south of Iberian Ranges near Cuenca (UTM 30SWK95983 27029) at an altitude of around 1000 m a.s.l. (Figure 1A-B). The study area is characterized by a Mediterranean climate with a typical seasonal rhythm of hot and dry summers and cool, rainy winters (Figure 2). Total annual rainfall is around 525 mm (± 123 mm). Mean monthly temperature ranges from 5.6 ºC in the coldest month (January) to 25ºC in the warmest (July). Monthly temperatures variations could be quite extreme and differences between day and night are also very important, especially in summer, indicating the continental feature of the regional Mediterranean climate-type. The vegetation in the lake area is characterised by typical associations of calcareous karstic soils, corresponding to a continental dry supra-Mediterranean bioclimatic domain consisting of *Pinus nigra ssp salzmanii* and *Juniperus thurifera* populations. This arboreum stratum coexists with shurbs of other species of *Juniperus*, xerophylous bushes and steppic plants. In wetter and shady sites, a mixed forest with deciduous trees and several *Taxus baccata* which give its comon name to the Lagunillo, could be found as well.

The karstic complex of Cañada del Hoyo is composed by 34 sinkholes and the polje which is crossed by the Guadazaon river. Among the sinkholes located in the river's left side (Figure 1C), only seven have water permanently. These dolines are formed by dolomites from the Cenomanian stage of the Cretaceous period. The lakes have no surface runoff and are mainly fed by groundwater sources. Therefore the lake-level undergoes fluctuations in response to seasonal and long-term variations in groundwater levels.

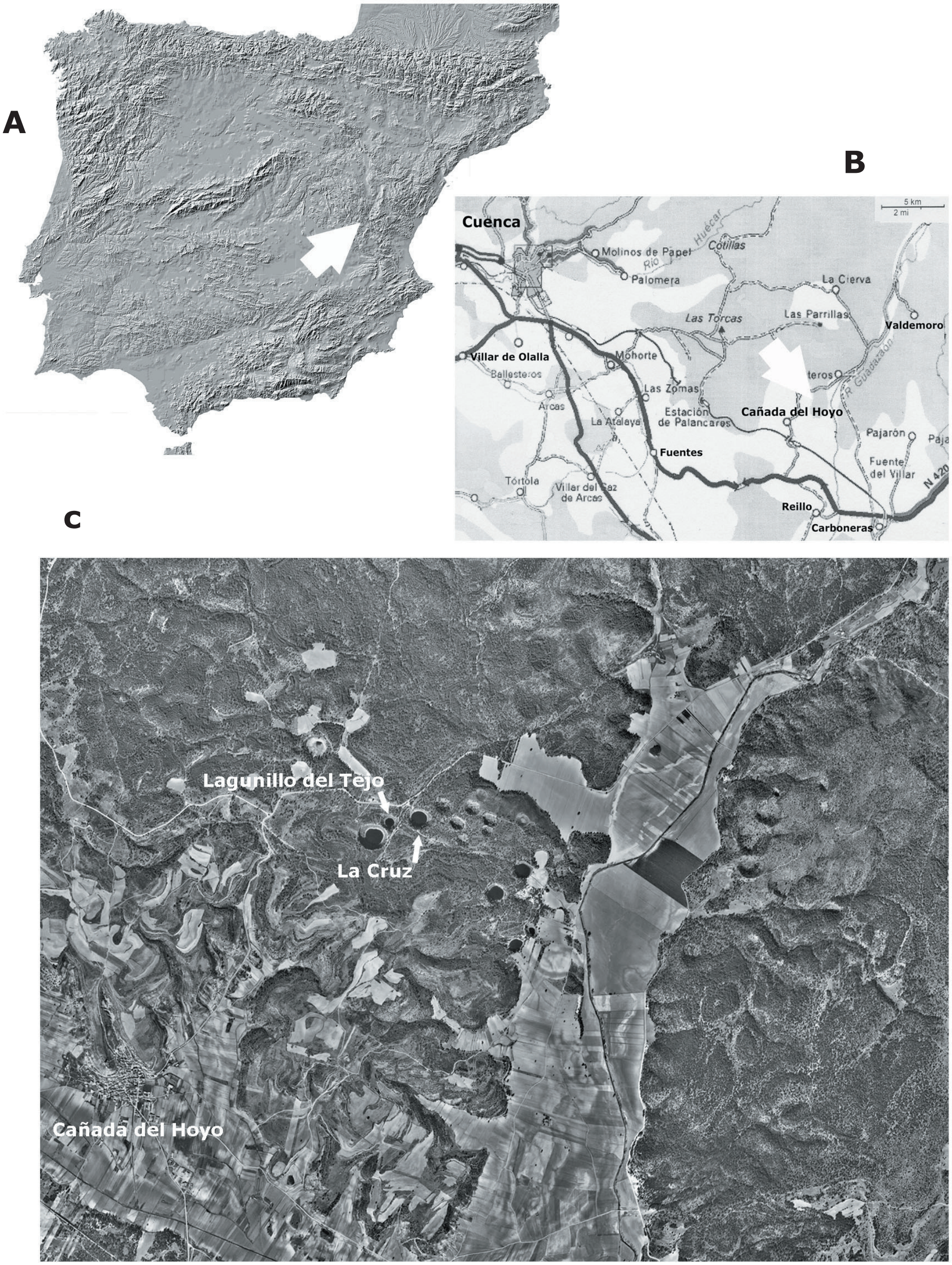


Figure 1. Location of the study site. The white arrow indicates situation of lacustrine system of Cañada del Hoyo in Iberian Peninsula (A) and at regional scale (B). Aerial photograph of the lacustrine system (C).

Figura 1. Localización de los sistemas lacustres estudiados. La flecha blanca indica la situación del sistema de Cañada del Hoyo en la Peninsula Iberica (A) y a escala regional (B). Fotografía aérea de la zona (C).

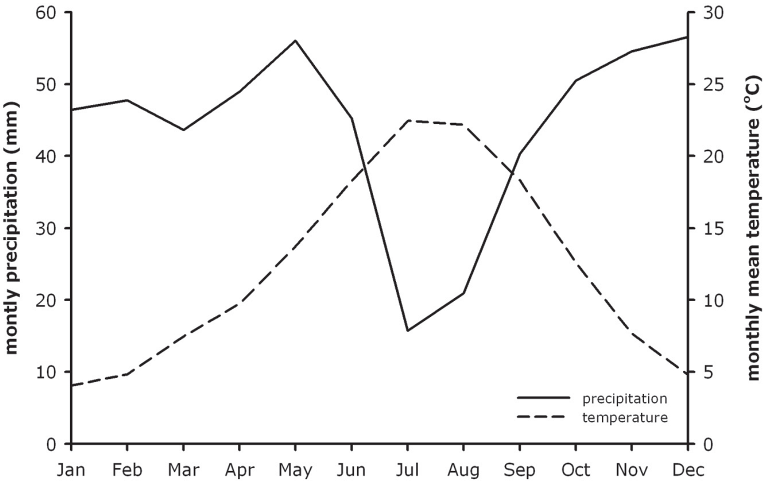


Figure 2. Regional climatic patterns, monthly precipitation (solid line) and mean temperature (dashed line). Meteorological data record from Cuenca (1950-2003).

Figura 2. Patrón climático regional, precipitaciones mensuales (línea continua) y temperaturas medias mensuales (línea discontinua). Registro meteorológico de Cuenca (1950-2003).

2a. Lake La Cruz

Lake La Cruz (Figure 3) has a circular surface with a mean diameter of 122 m and a maximun detph of 21 m. Water fills the bottom of a sinkhole of greater dimensions (170 m mean diameter with walls standing 16-25 m above the water level). The morphometry of the lake (small surface to depth ratio) and its location inside a sink solution basin, having steep vertical walls (Vicente and Miracle 1988), afford shelter from wind action. Lake La Cruz is currently meromictic with a permanently anoxic monimolimnion below 18 m which represents an 8% of total water volume. Meromixis is established by a density gradient which reachs four-fold increment of conductivity in the monimolimnion. The meromictic condition is mainly maintained by the chemical stratification of dissolved bicarbonate and calcium which are responsible of the conductivity gradient (Rodrigo et al. 2001). The lake thermally stratifies from early spring to late autumn. During the thermal stratification period the oxycline rise from the monimolimnion boundary to 11-12 m depth. At the end of November the overturn of the mixolimnion occurs and the oxic-anoxic boundary drops, the monimolimnion being the only anoxic zone remaining.

Figure 3. Lake La Cruz (May 2006).

Figura 3. La Laguna de La Cruz (Mayo 2006).

The ionic features of water in Lake La Cruz, characterised by a high bicarbonate concentration, are related with the chemical composition of the dolomites substrate. Surface waters usually had a pH around 8.5 and conductivity of about 500 μS cm^{-1} whereas in anoxic bottom waters, pH and conductivity varied to 6.5 and 1500 μS cm^{-1} respectively. The alkalinity is usually high (around 6 meq l^{-1} in mixolimnion), mainly due to high concentrations of bicarbonate. Magnesium was the most abundant cation in the oxic waters and also in the hypolimnion, whereras calcium predominated in the monimolimnion. The sulphate concentrations were low through the water column (< 0.1 meq l^{-1} in the mixolimnion).

One peculiarity of this lake is the annual summer whiting by the occurrence of a short-term massive $CaCO_3$ precipitation process (Rodrigo et al. 1993; Miracle et al. 2000). The water turns suddenly turbid and just few days after surface becomes clearer, with whiting ending in one or two weeks. Two main mechanisms can induce precipitation of calcite either as a result of seasonal temperature changes or as result of CO_2 uptake by photosynthetic organisms (Rodrigo et al. 1993). Although temperature could control the abrupt precipitation, the high amount of picocyanobacterial cells collected by the sediment traps after whiting

(Camacho et al. 2003a) suggest that biogenic processes might be also involved in the precipitation. Seasonal pulses of calcite crystals are responsible for varve sediment formation. Laminated sediment of Lake La Cruz which is formed by alternated light laminae, mainly composed by calcite crystals, and brownish ones, is clearly visible until 43 cm depth within the sediment cores.

The temporal and vertical distribution of phototrophic community through the water column in Lake La Cruz is conditioned by the physical and chemical gradients stablished (Dasi and Miracle 1991). The centric diatoms such as *Cyclotella glomerata* which is the most abundant diatom, show the highest relative abundance among the planktonic organisms during the vernal period. At the begining of thermal stratification populations of chlorophyceae, mainly *Crucigenia rectangularis,* started to grow reaching its maximal development in the epilimnion and metalimnion during summer. Cryptophyceae populations are present near the oxicline depth through the year. Dinoflagellate populations were relative significant at late spring and late summer, at the begining and at the end of the stratification period. The picocyanobacteria (Pcy) populations reach a high proportion of total phototrophic biomass in Lake La Cruz (Camacho et al. 2003a). Pcy developed dense populations through the oxic waters in spring, but a strong decrease in Pcy abundance in the epilimnion occurred in the late spring and early summer linked to nitrogen exhaustion in the epilimnetic waters. Anoxygenic bacterial populations establish just below the oxycline with a vertical microstratification (Rodrigo et al. 2000). A purple sulfur bacteria population of *Amoebobacter purpureus* develops their population maxima above those of the green sulfur bacterium *Pelodictyon chlatratiforme*. Although small spots of *Myriophyllum spicatum*, *Potamogeton lucens, Chara aspera and Chara vulgaris* (Cirujano 1995) could be found in the shoreline, the littoral comunity of Lake La Cruz is limited since the subtratum has no a slow depth gradation.

2b. Lagunillo del Tejo

Lagunillo del Tejo (Figure 4) is one of the dolines of the Cañada del Hoyo complex, close to Lake La Cruz and separated from Lake El Tejo by a rock cornice. At the time of the coring (May 2003), the water body had a diameter of 70 m (area = 3421 m^2) and a maximum depth of 8 m (mean depth = 3.75 m). However lake-level fluctuations are more evident than in the deeper lake La Cruz. As photographs show (Figure 5), water level in the pond has decresed notably since May 2003 to May 2006. At this late date maximun depth was only 4 m. According to a previous limnologycal survey (Vicente and Miracle 1984),

Figure 4. Lagunillo del Tejo (May 2006).

Figura 4. Lagunillo del Tejo (Mayo 2006).

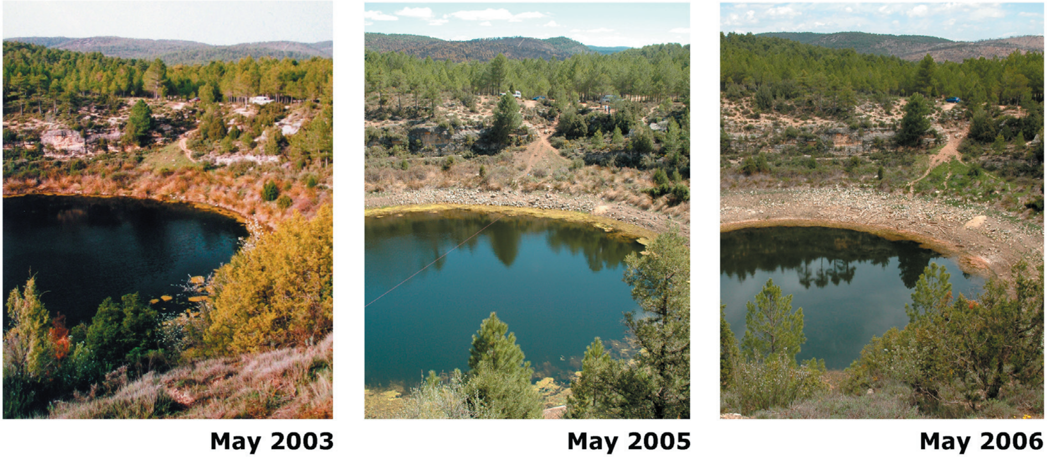


Figure 5. Lagunillo del Tejo lake level evolution since May 2003 to May 2006.

Figura 5. Evolución del nivel de agua en el Lagunillo del Tejo desde Mayo del 2003 a Mayo del 2006.

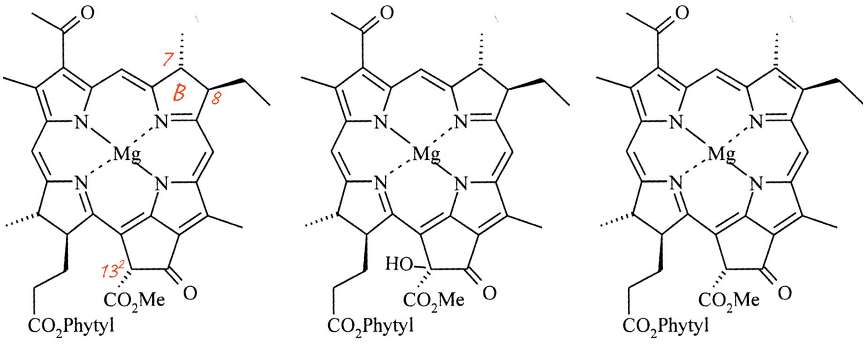
the lake is monomictic, with a thermal stratification from May to November which favours the development of anoxia in the deepest water layer. Moreover, the density gradient in the bottom contributes to water column stratification. Green and purple sulfur bacteria populations have been reported in the anoxic layer. Besides of diatoms and chlorophytes growing in the epilimnion and metalimnion, dense cyanobacterial and cryptophyta populations located in oxic-anoxic interface contribute to phototrophic biomass. The littoral zone of Lagunillo del Tejo is colonized by a wide macrophytic vegetation ring, principally by *Potamogeton pectinatus*, *Myriophyllum spicatum*, *Polygonum amphibium, Chara fragilis, Chara muscosa* and also *Chara desmacantha* (Cirujano 1995).

3. Sedimentation patterns of phototrophic organisms based on pigment markers in meromictic Lake La Cruz

3a. Introduction

An accurate understanding of factors controlling sedimentation processes is required to confidently use the photosynthetic pigments and their derivatives preserved in lacustrine sediments for paleolimnological reconstructions. Studies on photosynthetic pigment deposition in marine and lacustrine environments have typically focused on the phytoplankton community (e.g., Carpenter et al. 1986; Hurley and Armstrong 1990; Leavitt 1993; Cuddington and Leavitt 1999; Bianchi et al. 2002), whereas references on bacterial derivative products present in the water column and fluxes towards sediment (Hurley and Garrison 1993; Villanueva et al. 1994; Steenbergen et al. 1994) are scarce. Among sedimentary chlorins and carotenoids, which are useful tracers of past changes in productivity and planktonic community composition (Züllig 1981; Guilizzoni et al. 1986; Hodgson et al. 1998; Lami et al. 2000; Waters et al. 2005), those derived from anoxygenic bacterial photoautotrophs could add information about prevailing conditions in lacustrine systems since they are specific proxies for anoxic conditions in the water column (e.g., Repeta 1993; Squier et al. 2002; Itoh et al. 2003). Our aim is to characterize the phototosynthetic pigments transfer from source photosynthetic organisms, focused on the anoxygenic bacterial community, into the sedimentary signal, and to identify lacustrine factors involved in sedimentation processes of sulfur bacteria with regard to future paleolimnological interpretations of sedimentary remains.

Allomerization, an early stage reaction in the oxidative transformation of chlorophylls and bacteriochlorophylls in the natural environment, plays an important role on the formation of purpurins and ultimately, through fossilization, in the formation and preservation of aetioporphyrins isolated from sedimentary organic matter (Naylor and Keely 1998; Airs et al. 2001; Walker et al. 2002; Ocampo and Repeta 2002). As with hydroxy derivatives or allomers of chlorophylls, bacterioviridin (bvir) is an oxidised product readily formed in the presence of oxygen from unstable bacteriochlorophyll_a (bchl_a) (Figure 1). Woolley et al. (1998) observed that the macrocycle configuration of bchl_a, contrary to chlorophyll_a (chl_a), easily allows dehydrogenation by triplet oxygen into 3-devinyl-3-acetyl-Chl_a (bvir), because the hydrogen atoms at the position C-7 and C-8 are weakly bonded. First described in 1966 (Lindsay Smith and Calvin 1966), bvir was recently reported to be presented in a variety of lake sediments and it has been proposed as paleoindicator of the degree of oxygen to which the bacterial community has been exposed (Wilson et al. 2004a). In this paper, we present the first report of bacterioviridin in sinking water column particulate material, whose presence is related with environmental conditions of the lacustrine system, thus contributing to the knowledge of its usefulness as a paleolimnological biomarker.



1 2 3

Figure 1. Molecular structure of bacteriochlorophyll_a (1), hydroxybacteriochlorophyll_a (2) and bacterioviridin-Mg link (3).

Figure 1. Estructuras moleculares de bacterioclorofila_a (1), hidroxibacterioclorofila_a (2) y bacterioviridina (unida a Mg) (3).

3b. Material and methods

The study of sedimentation processes in Lake La Cruz was performed from March 1996 to December 1998, at monthly sampling intervals. Vertical profiles of dissolved oxygen and photosynthetically-active radiation (PAR, 400-700 nm) were measured in situ with a WTW Oxi 91 oximeter and a 4-π scalar irradiance sensor (Li-Cor), respectively. Photosynthetic pigments of planktonic populations were extracted with acetone over 24 h at 4ºC from water samples filtrated through a glass fibre filter (Whatman GF/F) and the absorbance of the extract was determined using a Beckman DU-7 spectrophotometer. Pigment concentrations were calculated according to Parkin and Brock (1981).

The sediment traps used here (Figure 2), similar to those described by Pedros-Alió et al. (1989), were designed following the recommendations outlined by Bloesch and Burns (1980) and Blomqvist and Håkanson (1981) in order to study microbial sedimentation. The simplest and best shape for the trap seems to be a cylinder with an aspect ratio >5 and a mouth diameter >20mm (Bloesch and Burns 1980; Blomqvist and Håkanson 1981). Sediment traps used in this study, had eight tubes of 16 mm diameter and 200 mm length (aspect ratio 12.5). The high aspect ratio avoid resuspension of the material and the mouth diameter seems enough for planktonic microbial-sized particles from Lake La Cruz. The material collected in two of these tubes was used for pigment extraction. The traps were submerged near lake bottom at the monimolimnion, always remaining under anoxic conditions and sampled monthly. After collection from the lake, the tubes were immediately placed in the dark and stored at -30ºC until pigment extraction. Fixatives or preservatives were not added.

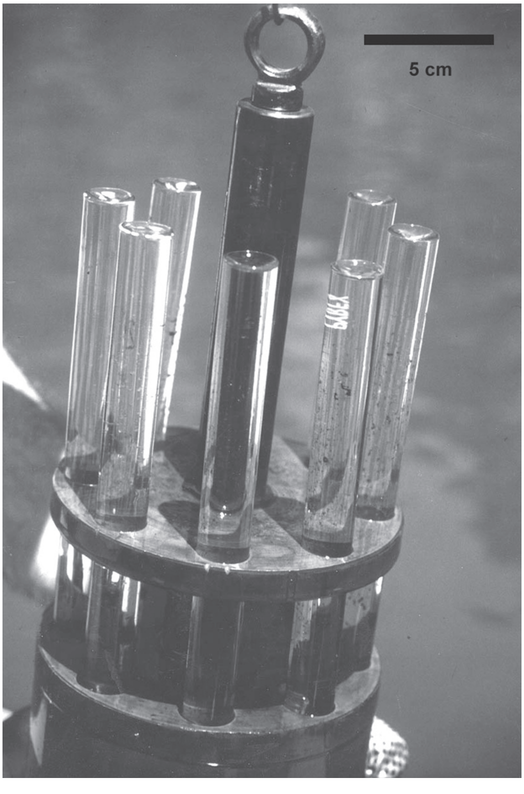


Figure 2. Picture of the sediment trap used in this study.

Figure 2. Fotografía de las trampas de sedimentación utilizadas en este estudio

Settled material from sediment traps was centrifuged and the overlying water was discarded, then pigments were extracted with acetone for 24 h at 4ºC following a three step procedure (Camacho and De Wit 2003). We used 100% acetone for pigment extractions, since the high water content of the settled material lowered the acetone concentration to c.a. 90%, a grade that extracts more efficiently pigments (Leavitt and Hodgson 2001). Pigment extracts were dried under N_2 and stored at -30ºC until HPLC analyses. A Waters 510 liquid chromatograph equipped with a photo-diode array detector (Waters 996) was used to separate and identify individual compounds. Separation was performed on a HYPERSIL®ODS chromatography column (5 μm particle diameter, 250 x 4.6 mm) using a modification of the method described by Villanueva et al. (1994). The mobile phase was a mixture of two solvents (A and B) comprising methanol: ammonium acetate 0.1M (8:2) and methanol:acetone (1:1), respectively. The gradient consisted of an initial solvent of 70% A and 30% B with a 20 min linear gradient to 0% A and 100% B, and a hold at 100% of solvent B for 20 min. The re-equilibration of the column takes 15 min. The volume injected was 100 μl of acetone: ammonium acetate (0.1M) (7:3) of rediluted extracts. The solvent flow was 1 ml min^{-1}. Peaks were identified according to their absorption spectra measured by means the photodiode-array detector and confirmed by mass spectrometry (MS). MS provided molecular mass data expressed as mass-to-charge-ratio (m/z), value equal to the addition of the atomic masses of the most abundant isotope of each element that comprises the molecule (assuming that the ion is a single-charge ion) and a hydrogenion from the ionisation process. The MS system comprised an Esquire 3000plus with Agilent 1100 ChemStation auto-sampler and APCI ion source. Separation conditions were as described above for HPLC. LC-MS settings were as follows: capillary temperature 150ºC, APCI vaporised temperature 450ºC, discharge current 5 μA, sheath gas flow 60 (arbitrary units). Absorption coefficients used for carotenoids and chlorophyll-derivative pigments quantification were those given by Villanueva et al. (1994). Bacterioviridin fluxes were expressed as absorbance units per $m^{-2}day^{-1}$, enough for comparative analysis within the samples, because the specific absorption coefficient for this bacteriochlorophyll_a derivative is not available and the isolation of the molecule was not achieved.

3c. Results

Water column scenario

To better understand the results from the sediment traps, we briefly summarize the distribution of photosynthetic pigments in the water column (Figure 3) and the annual patterns of stratification (Figure 4) over the three years of the sedimentation study. The lake thermally stratifies from early spring to late autumn and the thermocline in mid summer extends between 5 and 10 m depth. Chlorophyll_a concentration in the water column (Figure 3a), used as an indicator of the biomass abundance by eukaryotic algae and picocyanobacteria, started to increase at the beginning of spring. During the thermal stratification period the oxycline rose from the monimolimnion boundary to 11-12 m depth and defined two distinct environments in Lake La Cruz, the oxic and the anoxic parts of the water column. Light availability at the oxycline level is usually lower than 1% of surface irradiance, although more than 0.1% penetrates to the upper part of the anoxic layer occupied by sulfur bacteria. Anoxygenic bacterial populations establish just below the oxycline with a vertical microstratification (Rodrigo et al. 2000). A purple sulfur bacteria population of *Amoebobacter purpureus* (Figure 3b), with bacteriochlorophyll_a (bchl_a) as its main photosynthetic pigment, develops their population maxima above those of the green sulfur bacteria, *Pelodictyon chlatratiforme*, which in turn possess bacteriochlorophyll_d (bchl_d) (Figure 3c). The highest abundances of photosynthetic bacteria were reached in autumn when light availability at the oxycline (Figure 4) was maximized and the oxygenic phytoplankton community, traced by chl_a, declined after summer nutrient exhaustion in the epilimnion and co-precipitation with calcium carbonate particles (Camacho et al. 2003a). At the end of November the thermocline collapses, overturn of the mixolimnion occurs and the oxic-anoxic boundary drops (Figure 4), the monimolimnion being the only anoxic zone remaining. While in 1996 the maximum depth of the oxycline was 15 m, in 1997 and 1998 the oxic layer reached the monimolimnion upper limit. Scattered phototrophic bacterial populations found refuge in the permanently anoxic monimolimnion during the mixing period, as indicated by their signature pigments bchl_a and bchl_d (Figure 3b and 3c).

Photosynthetic pigments collected in sediment traps

Characteristic pigments and derivatives of purple and green sulfur bacteria populations, bchl_a and _d, were detected in the settled material collected by the sediment traps. Two representative HPLC-UV (300-800 nm) chromatograms plotted at λ_{max} for each peak (maxplot) of acetone extracts from these samples are shown in Figure 5. In these chromatograms we usually identified two peaks with the same absorbance spectrum, corresponding to hydroxybchl_a (OH-bchl_a) (a1) and native bchl_a (a2). In addition, just after a1 and a2

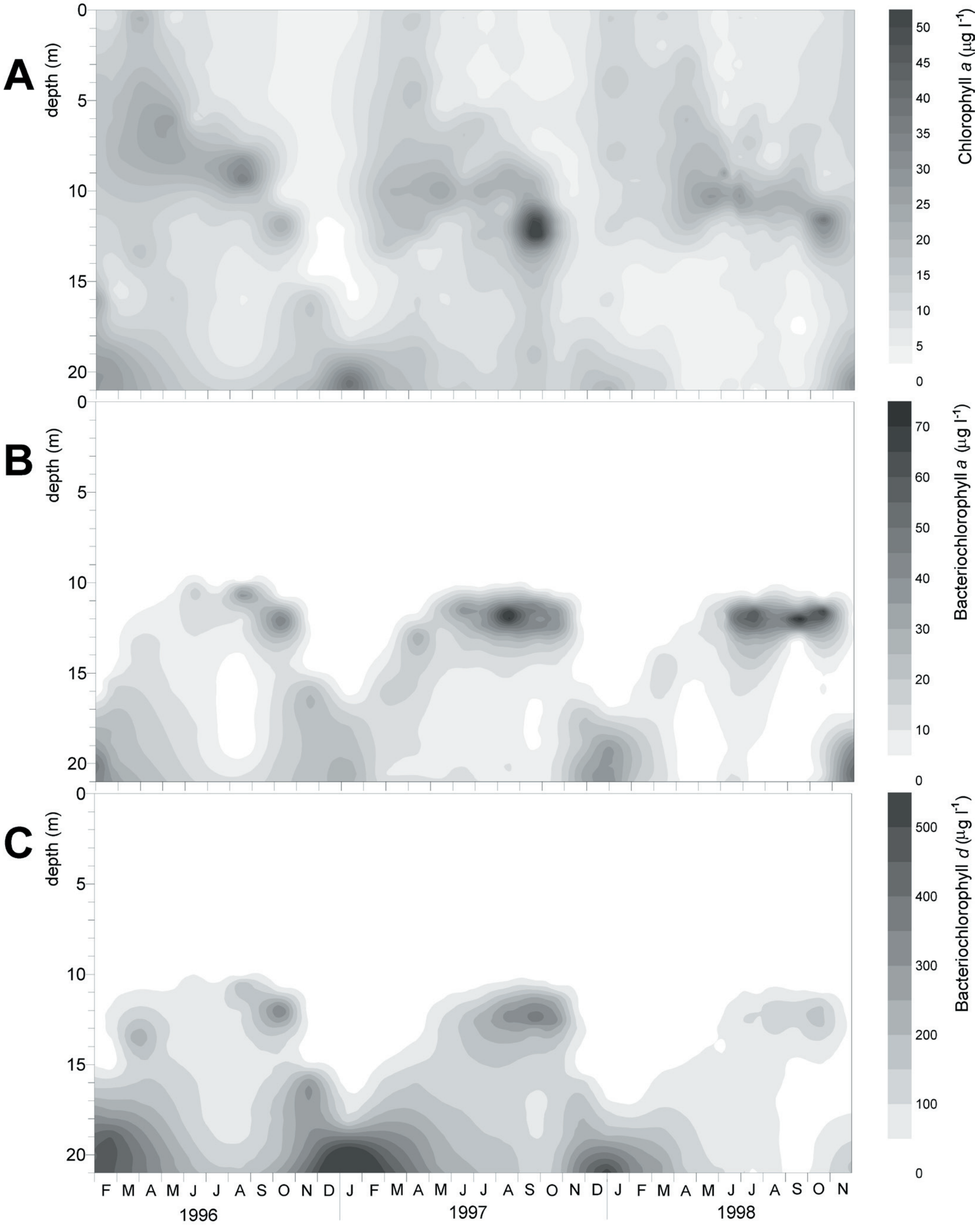


Figure 3. Isopleths of photosynthetic pigments (μg l-1) concentration in Lake La Cruz from 1996 to 1998; chlorophyll_a (A), bacteriochlorophyll_a (B) and bacteriochlorophyll_d (C).

Figura 3. Isolíneas de la concentración de pigmentos fotosinteticos (μg l-1) en la Laguna de La Cruz desde 1996 hasta 1998; chlorofila_a (A), bacterioclorofila_a (B) y bacterioclorofila_d (C).

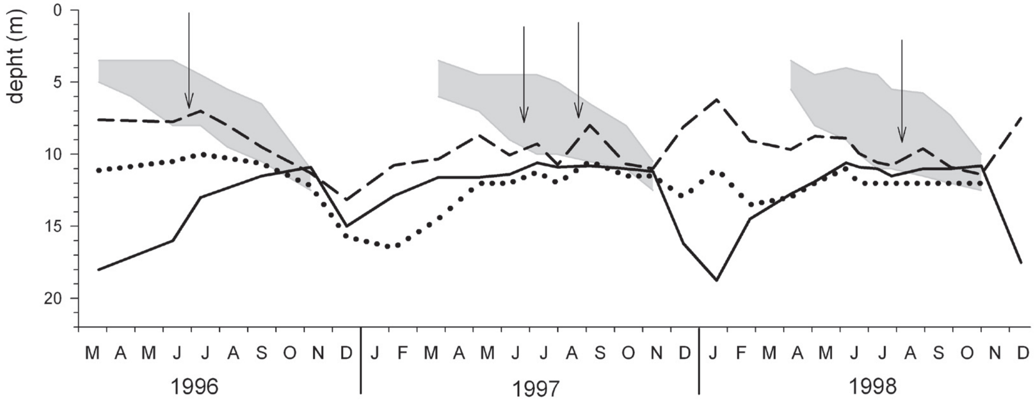


Figure 4. Position of the oxycline (solid line, oxygen extinction depth), the euphotic zone (dashed line and dotted line indicate the depth where light availability was 1% and 0.1% of surface irradiance, respectively) and the thermocline (grey area (temperature variation > 1ºC m^{-1})) in the vertical profile of Lake La Cruz during the studied period. Arrows indicate massive calcium precipitation (whiting events).

Figura 4. Posición de la oxiclina (línea continua, profundidad de extinción del oxígeno), de la zona eufótica (la línea discontinua y la punteada indican la profundidad en la que la disponibilidad de luz corresponde al 1% y el 0,1% de la radiación en superficie, respectivamente) y la termoclina (area gris (variación de la temperatura > de 1ºC m^{-1})) en el perfil vertical de la Laguna de La Cruz durante el periodo estudiado. La flechas indican la precipitación masiva de carbonato cálcico.

(Figure 5) two peaks (b1 and b2) appear corresponding to molecules that are not often described. The absorption spectra of b2 and b1 peaks (386-412-686 nm) correspond to the dehydrogenated derivative, bacterioviridin (bvir) from bchl_a and OH-bhl_a respectively (cf. Wilson et al. 2004). The appearance and position of Soret and Qy absorption bands of b1 and b2 indicates a Mg containing macrocycle. The full mass spectrum revealed that b2 gave a protonated molecule at m/z 909 (m/z 911 for bchl_a), consistent with the decrease of 2 Da expected from the oxidation of ring B of its bchl_a precursor. On the other hand, the b1 peak gave a protonated molecule at m/z 923 (m/z 925 for OH-bchl_a) probably corresponding to the bvir derivative of OH-bchl_a (OH-bacterioviridin). Three main homologues of bchl_d (d1,d2 and d3), together with several secondary peaks were also identified, bchl_d2 being the most abundant among the former. Among bchl_d and _a derivatives, bacteriophaeophytins (bph) usually occurred, although an important amount of bacteriophaeophorbide_d, a dephytylated derivative, was also observed, especially in December 1997 as confirmed by LC-MS.

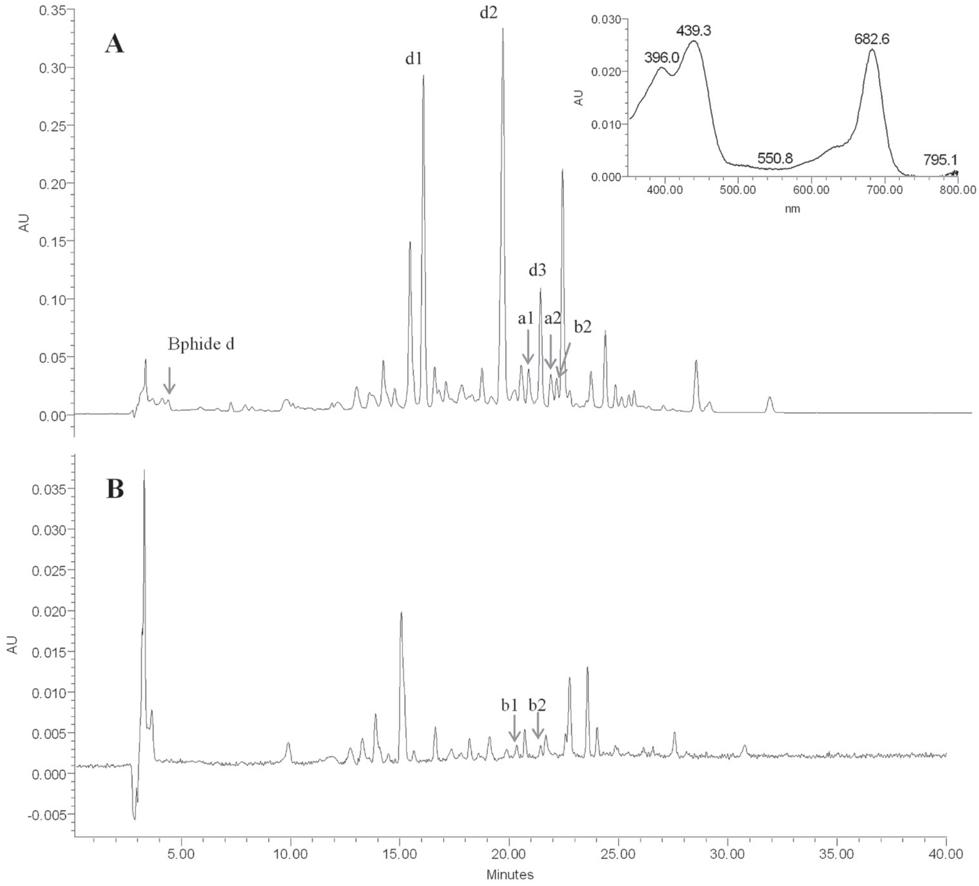


Figure 5. HPLC chromatograms of the extracts from settled material collected by sediment traps in Lake La Cruz in December 1996 (A) and June 1998 (B). Peak labels are explained in text. Inset:UV/vis spectrum of bacterioviridin (b2).

Figura 5. Cromatogramas de los extractos del material recogido por las trampas de sedimentación en la Laguna de La Cruz en Diciembre 1996 (A) y en Junio de 1998 (B). Las etiquetas de los picos estan explicadas en el texto. Inserto: espectro UV/visible de bacterioviridina (b2).

Fluxes of bacterial pigments to sediment traps reached the highest levels during December, just after thermal overturn. Both bchl_ a and OH-bchl_a (Figure 6a) contributed, in similar proportions in December of 1996 and 1998, whereas in December 1997 native bchl_a was not found and simultaneously OH-bchl_a was more abundant than in the other years. Maximum amounts of bvir were also recorded in December, coinciding with the bchls peak, although this derivative was also found during most of the year in sediment traps (Figure 6b) and showed a secondary peak of abundance consistently found in June. This

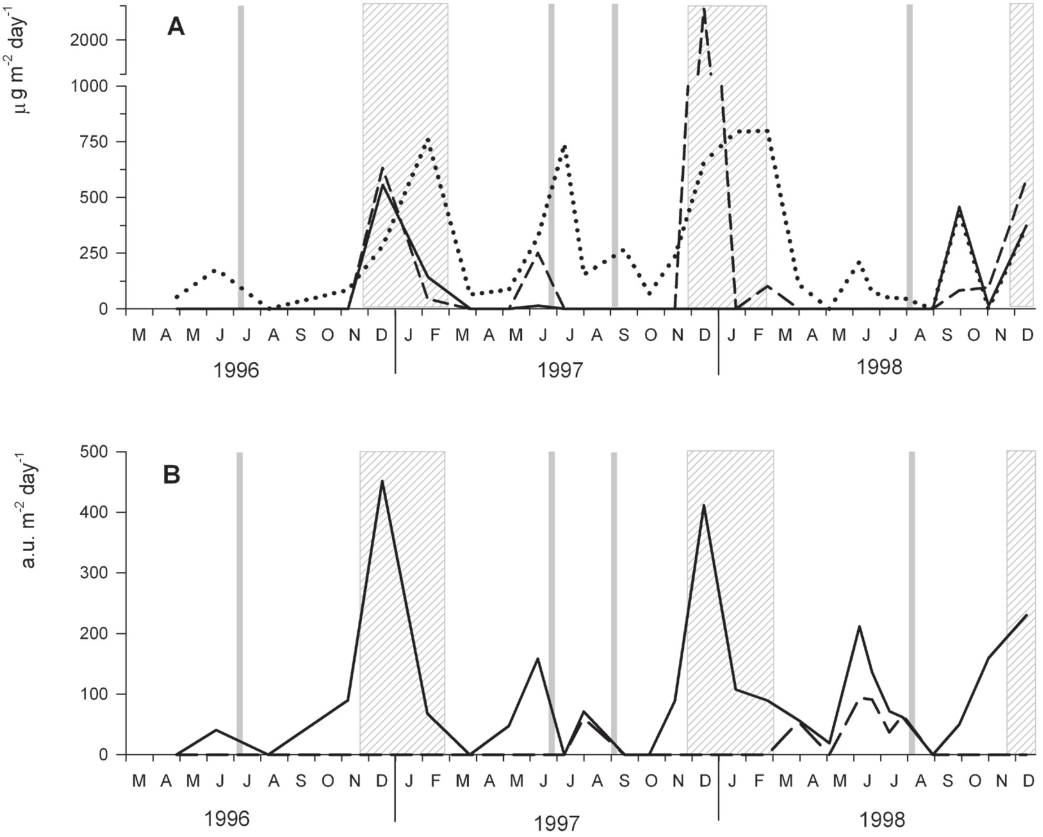


Figure 6. (A) Fluxes to the sediment of photosynthetic pigments from purple sulfur bacteria (μg m^{-2} day^{-1}); bacteriochlorophyll_a (solid line), bacteriophaeophytin_a (dotted line) and OH-bacteriochlorophyll_a (dashed line) and; (B) fluxes of bacterioviridin (solid line) and OH-bacterioviridin (dashed line) (a.u. m^{-2} day^{-1}). Vertical bars show the occurrence of whiting episodes during the studied period and shaded areas correspond to the mixing period in the mixolimnion.

Figura 6. (A) Flujos de sedimentación de los pigmentos fotosintéticos de las bacterias rojas del azufre (μg m^{-2} día^{-1}); bacterioclorofila_a (línea continua), bacteriofeofitina_a (línea punteada) y OH-bacterioclorofila_a (línea discontinua); (B) bacterioviridina (línea continua) y OH-bacterioviridina (línea discontinua) (u.a. m^{-2} día^{-1}). Líneas verticales indican la ocurrencia de los fenómenos de blanqueado y las áreas sombreadas los periodos de mezcla vertical.

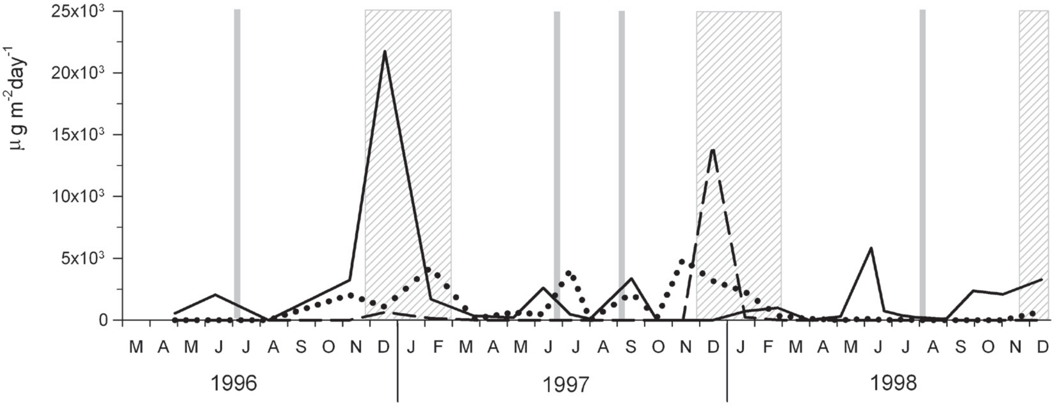


Figure 7. Fluxes of photosynthetic pigments from green sulfur bacteria (μg m^{-2} day^{-1}); bacteriochlorophyll_d (solid line), bacteriophaeophytin_d (dotted line) and bacteriophaeophorbide_d (dashed line).The occurrence of the whiting events is indicated by verticals bars and shaded areas correspond to the period of thermal overturn of the mixolimnion.

Figure 7. Flujos de sedimentación de los pigmentos fotosintéticos de las bacterias verdes del azufre (μg m^{-2} día^{-1}); bacterioclorofila_d (línea continua), bacteriofeofitina_d (línea punteada) y bacteriofeoforbido_d (línea discontinua). Líneas verticales indican la ocurrencia de los fenómenos de blanqueado y las áreas sombreadas los periodos de mezcla vertical.

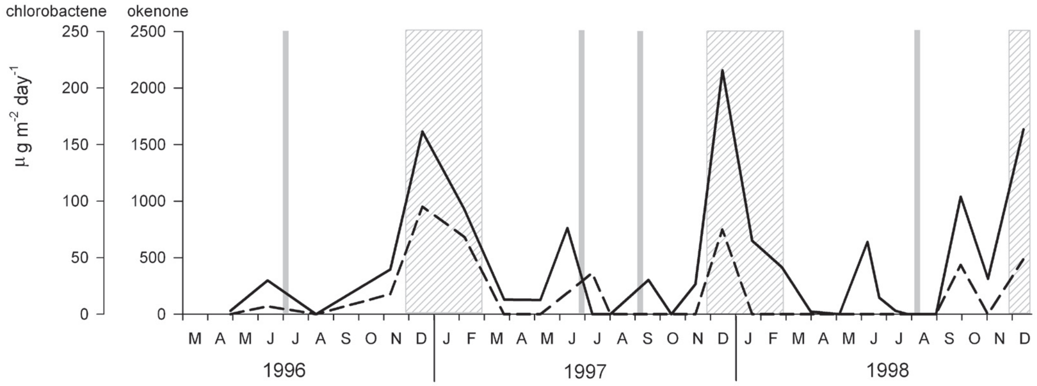


Figure 8. Fluxes of bacterial carotenoids, chlorobactene (dashed line) from green sulfur bacteria and okenone (solid line) from purple sulfur bacteria (μg m^{-2} day^{-1}). Whiting episodes are indicated by vertical bars and mixing periods by shaded area.

Figura 8. Flujos de los carotenos bacterianos; clorobacteno (línea discontinua) de las bacterias verdes del azufre y okenona (línea continua) de las bacterias rojas del azufre (μg m^{-2} día^{-1}). Líneas verticales indican la ocurrencia de los fenómenos de blanqueado y las áreas sombreadas los periodos de mezcla vertical.

secondary peak was not so commonly found for native or OH-bchl_a, the latter only being observed in 1997 and being replaced by a bacteriophaeophytin_a (bph_a) peak in the other years. The highest abundance of bph_a (Figure 6a) was displaced with repect to native bchl_a and other derivatives, overcoming them in the settled material in January and February.

Figure 7 shows the sedimentation pattern of bchl_d and their derivatives. As for bchl_a, there is a peak of abundance in the settled material just after autumn overturn. Native bchl_d accounted for the main contribution to the settled material in December 1996. In December 1997 an unusually high bacteriophaeophorbide_d concentration was observed and native compounds were not found. In 1998 minimum values of bchl_d and derivatives were found, consistent with the much lower abundance of green bacteria during this year (Figure 3c). Bacterial carotenoids, chlorobactene and okenone (Figure 8), as specific markers for green and purple sulfur bacteria respectively, corroborate the bchls annual sedimentation patterns, showing a main peak after fall overturn and small secondary peaks, the most important found in early summer for purple bacteria pigments.

By contrast, sedimentation patterns of chl_a and their derivatives were different to those of the bacterial pigments. Fluxes of chlorin derivatives from algae and picocyanobacteria reached the highest level just after the whiting

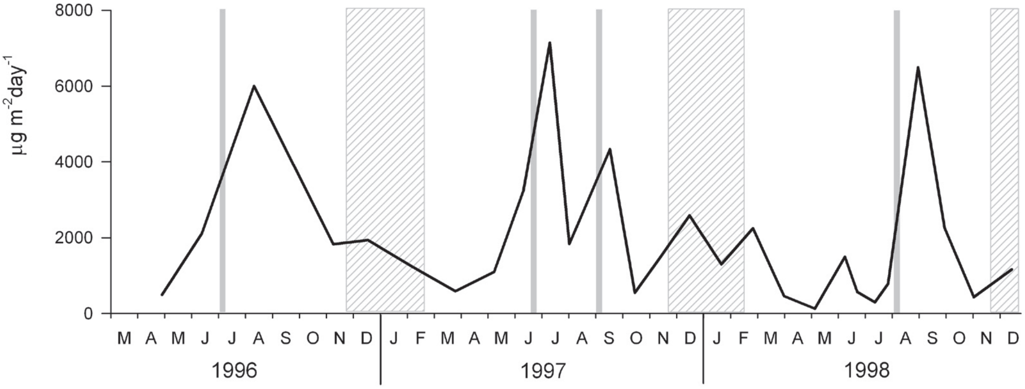


Figure 9. Fluxes of total chlorophyll_a and derivatives collected by sediment trap (μg m^{-2} day^{-1}). Whiting events are indicated by vertical bars and mixing periods by shaded area.

Figure 9. Flujo total de clorofila_a y sus derivados registrado por las trampas de sedimentación (μg m^{-2} $día^{-1}$). Líneas verticales indican la ocurrencia de los fenómenos de blanqueado y las áreas sombreadas los periodos de mezcla vertical.

event in summer (Figure 9), while the massive calcium precipitation had no sinking effect on bacterial pigments. Moreover, in summer 1997, when two whiting events happened, two chlorin derivative maxima were subsequently detected in the sediment traps. Conversely, fluxes of pigment compounds from phytoplankton and picocyanobacteria sources were not important at the end of autumn after thermal destratification.

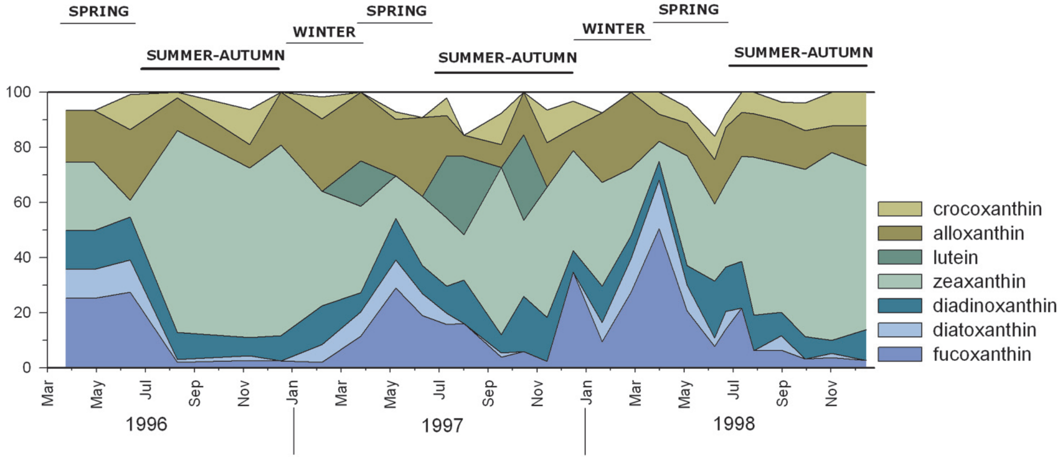


Figure 10. Percentage contribution of specific carotenoids to settled material.

Figura 10. Contribución relativa de los carotenos específicos al material sedimentado.

Total fluxes of algal and pycocianobacterial carotenoids showed a similar pattern as total sum of chlorin derivatives. However in figure 10 differences in the tempotal patterns of sedimentation among the different planktonic populations could be observed. Zeaxanthin, main carotenoid derived from pycocianobacterial population, is the most abundand in the settled material during summer and autumn whereas carotenoids derived from diatoms and crysophytes such as diatoxanthin and fucoxanthin reached higher fluxes during winter and spring. Sedimentation fluxes of other common carotenoids such as diadinoxanthin, common pigment from dinoflagellate populations and those derived from cryptophyceae, alloxanthin and crocoxanthin, were similar through the year.

3d. Discussion

Our results provide further information on how physical and chemical features of the lacustrine system conditioned sedimentation patterns of the planktonic community whose photoautotrophic component was traced by the abundance of photosynthetic pigments in both water column and in the sedimentary record. Fluxes of bacteriochlorin derivatives and bacterial carotenoid from purple and green anoxygenic bacteria to the sediment reached an annual maximum in late autumn after thermocline destruction. This followed the maximal bchls concentrations in the vertical profile, which were detected one month before. The decrease in the abundance of the overlying oxic phototrophs as stratification advanced, as traced by chlorophyll concentrations in the vertical profile, allowed increased light penetration into the anoxic waters, leading to a growth pulse of the bacterial populations at the end of stratification period. Thus, the main bacteria sedimentation pulse, whose intensity is related with the previous density of planktonic populations, is expected to be registered in the sediment, confirming that annual bacterial biomass contributes considerably to the sedimentary pigment record in Lake La Cruz.

Collapse of thermal stratification causes oxygen exposure of anoxygenic bacteria and mass mortality of these populations. The maximum concentrations of bacterial pigments (Figures 3b and 3c), corresponding to growing populations, are located at the chemocline and later, after overturnm, a new maximum appears near the bottom trapped, in the density gradient of the anoxic monimolimnion. Most probably, this relates to senescent or non active cells, since at this depth light availability is almost zero. Similar sedimentation peaks of purple bacteria have also been found in other lakes after fall overturn (Dickman 1987; Overmann et al. 1994). Regarding the relative abundance of the different pigments and their derivatives associated with the main purple bacteria settling pulse, the higher relative content of native bchl a just after mixing, whose presence during the annual cycle was important in the traps only at that time, indicated lesser degradation of the settled pigments. This may be due to fast cell sinking, since *Amoebobacter* has been described to increase their sedimentation rates by several orders of magnitude when cells aggregate rapidly in contact with oxygen (Overmann and Pfennig 1992; Van Gemerden and Mas 1995). In contrast, low sinking velocity accelerates pre-depositional breakdown of native photosynthetic pigments, thus increasing the relative abundance of pigment derivatives with respect to native molecules as observed in January. At this time, most pigment recovered from the sedimentation trap was bacteriophaeophytin, derived from senescent bacteria populations trapped in the aphotic deepest layers of the water column. This is also reflected in the relative distributions of bacterial pigments in the water column between November and December.

The relative abundance of oxidised bchl a derivatives in winter traps showed several interannual differences. Although mixing during the 1998 overturn period was greater than the other years, even eroding the upper part of the monimolimnion, the sedimentation features of OH-bchl_a were similar to those of 1996, in which mixing was limited to the upper 15 m. However, in December 1997 native bchl_a was not found and OH-bchl_a dominated. Bchl_a concentrations measured in November 1997 at the microaerobic layer of the lake (< 1 mg l^{-1} O_2) were higher than in 1996 and 1998. This might have led to the most active cells, located at the upper part of the bacterial layer where light availability is higher, to experience the strongest oxidative conditions and contribute a strong sedimentary signal during this period. Additionally, longer residence of bacteria cells in the water column during the 1997 overturn could also explain the increase in the relative importance of oxidised bchl a derivatives observed in the sedimentation trap.

The presence of bacteriophaeophorbide_d has rarely been reported in previous studies as a degradation compound of bchl_d in water samples (Le Bris et al. 1998). Among other possible factors involved in the formation of dephytylated chlorin derivatives, the abundance of phaeophorbides has been related with grazing processes ocurring in the water column (Louda et al. 1998). The observed presence of dense populations of anaerobic ciliates in the anoxic waters of Lake La Cruz and their possible grazing on phototrophic bacteria might contribute to the breakdown of bchl, although grazing pressure on the bacterial community was not studied here. However, the presence of bacteriophaeophorbide_d in the settled material in 1997 suggests that this compound might also be present in buried lacustrine sediment from Lake La Cruz, although so far only bacteriophaeophorbide_a has been identified in lacustrine sediments (Ocampo and Repeta 2004).

By studying sedimentary fluxes of bacterial pigments in Lake La Cruz, bacterioviridin was found in sediment traps. As indicated by its continuous presence through the year in sedimentation traps together with bacteriophaeophytin a, bvir seems to be a common derivative of bchl_a in settled bacterial remains, although the highest abundances were registered just after overturn. Its presence in the sediment traps, which were accurately located in permanently anoxic waters, indicates that bvir is a pre-depositional derivative of bchl_a, being partly formed in the water column. Moreover, after these findings we looked for the presence of bvir in water samples in order to confirm that the oxidative transformation could happen in the water column. Bvir was also found during the thermal stratification period in water samples from Lagunillo del Tejo, a smaller holomictic karstic lake located close to Lake La Cruz (Vicente and Miracle 1984). Recently, Wilson et al. (2004a) proposed that bvir can be seen as a paleolimnological tracer of the disruption of the chemocline by oxygenated waters. In this paper we report for the first time the presence of bvir in settling material from the water column, thus relating its presence with physical and chemical environmental conditions. Our results provide support for Wilson et al (2004) hypothesis on the origin of bvir after exposure of bchl_a to oxygen, in our case caused by the disruption of the thermocline, although differences in the fluxes of the allomer and the bvir indicate that the formation of OH-bchl_a was favoured when strong oxidation conditions occurred.

Our results show that sedimentation processes of phototrophic plankton in Lake La Cruz, traced by photosynthetic pigments, ocurred in discrete pulses. A first pulse, mainly affecting to the oxygenic phototrophs from epilimnetic and metalimnetic waters, was associated with the annual massive calcium precipitation in summer which produces a whiting phenomenom. Whiting has been previously reported as the main sink vector for phytoplanktonic biomass in this lake (Miracle et al. 2000) where settling materials, such as organic and mineral fractions, were analysed. Camacho et al. (2003a) performed picocyanobacterial (Pcy) counts on the settled material collected in the sediment traps deployed in this study and concluded that settling was generally not an important loss factor for the Pcy population of Lake La Cruz, except during the whiting event where they largely contribute to the chlorin maxima observed in the settled material after whiting. Seasonal pulses of calcite deposition are responsible for varve formation in Lake La Cruz sediment, producing fine laminated white and black sediments (Julià et al. 1998).

Contrary to the oxygenic phototrophs, the annual sinking pattern of anoxygenic bacteria derived organic matter is strongly related to thermal overturn and vertical oxygenation. In spite of this delay with respect to the main sedimentation pulse, the anoxygenic bacterial community contributes to the dark layer of the laminated sediment of Lake La Cruz. Thus, as shown by the work described here, these signature compounds could provide accurate information for future paleolimnological studies in Lake La Cruz and similar lacustrine systems, not only about the community composition but also concerning anoxygenic primary production and related stratification conditions of the water column during past times.

4. Climate signal in varve thickness; Lake La Cruz, a case study.

4a. Introduction

Annually laminated sediments are preserved in lakes in different environmental settings at a wide spatial distribution and for many time windows (Bauer 2004). Althought the term varve was defined for glacio-lacustrine annual laminated sediment (De Geer 1912), nowadays it is commonly applied also to different types of non-clastic laminated sediments in which the seasonal nature of the layering has been proved. In solution lakes originated in carbonaceous bedrock, calcite deposition varves are commonly observed and reported (Kelts and Hsü 1978). This type of laminations appear as couplets of light and dark layers resulting from spring-summer sedimentation of calcite crystals and deposition of organic matter throughout the year (Nipkow 1920). Two main mechanisms have been usually argued to explain the calcite precipitation, being either a result of seasonal temperature changes or resulting from increased CO_2 uptake by photosynthetic organisms (Megard 1968; Brunskill 1969). Moreover in lakes where picocyanobacteria are also abundant they have been shown to promote epicellular precipitation (Thompson et al. 1997; Dittrich et al. 2004).

Over the last years varved sediments have become relevant paleoclimate and paleoenvironmental archives because they allow the highest time-resolution (season to years) in proxy-data time series in lake sediment studies (Lotter et al. 1997). Studies on glacial-fed lake systems have shown that the features of the laminated sediments such as the varve thickness and the micro-facies structure, record a climate signal corresponding to the climatic factors like summer temperatures and cumulative melting degree days which control the varve formation (i.e. Leeman and Niessen 1994; Itkonen and Salonen 1994;

Zolitschka 1996; O'Sullivan et al. 2002; Lamoureux and Gilbert 2004). Despite different environmental conditions involved in non-clastic varve formation, the analysis of the calcite laminated sediments in different lakes have provided evidence of how climatic factors may contol its formation (Lotter and Birks 1997; Livington and Hajdas 2001; Dean et al. 2002).

Lake La Cruz (Spain) is a karstic meromictic lake that shows annually laminated sediment due to a short-term tumultuous calcium carbonate precipitation at summer (Rodrigo et al. 1993). This precipitation is very conspicuous producing an annual whiting event very known by the people of the area, which originates white micrite microlaminae in the sediment that are maintained due to the permanent anoxic monimolimnion, since probably the Little Ice Age (Julia et al. 1998). The extensive knowledge of the biological community acquired over the last twenty years (Vicente and Miracle 1988; Rodrigo et al 2001; Camacho et al 2003) as well as the understanding of the sedimentation processes in Lake La Cruz (Miracle et al. 2000; Romero et al. 2006) enable us to evaluate the possible pathways of climatic "input" signals into the sediment of this lake. This study was undertaken to explore the potential use of this non-clastic varves as quantitative climate proxy, by calibrating varve thickness against instrumental climatic data. The results confirm the link between hydroclimatic variability and calcite laminae thickness and thus enhance the perspectives for future paleoclimate reconstruction using calcite laminated sediments.

Background

In Lake La Cruz, whiting process is one of the shortest reported (Rodrigo et al. 1993). At summer season, the water turns suddenly turbid and just few days after that surface water becomes clearer, with whiting ending in one or two weeks. According to our data from a survey covering several years (1996-1998), the mixolimnetic water of Lake La Cruz is permanently saturated or oversaturated for calcite, independently of phytoplankton succession and the photosynthetic rates (Figure 1). Even calcite oversaturation was sometimes higher in other periods than during the periods immediately before whiting events. Although temperature could enhance the sudden calcite precipitation, the high amount of picocyanobacterial cells collected by the sediment traps after whiting (Camacho et al. 2003) suggest that biogenic processes are also involved in the precipitation (e.g. induced nucleation by the highly abundant picocyanobacterial cells).

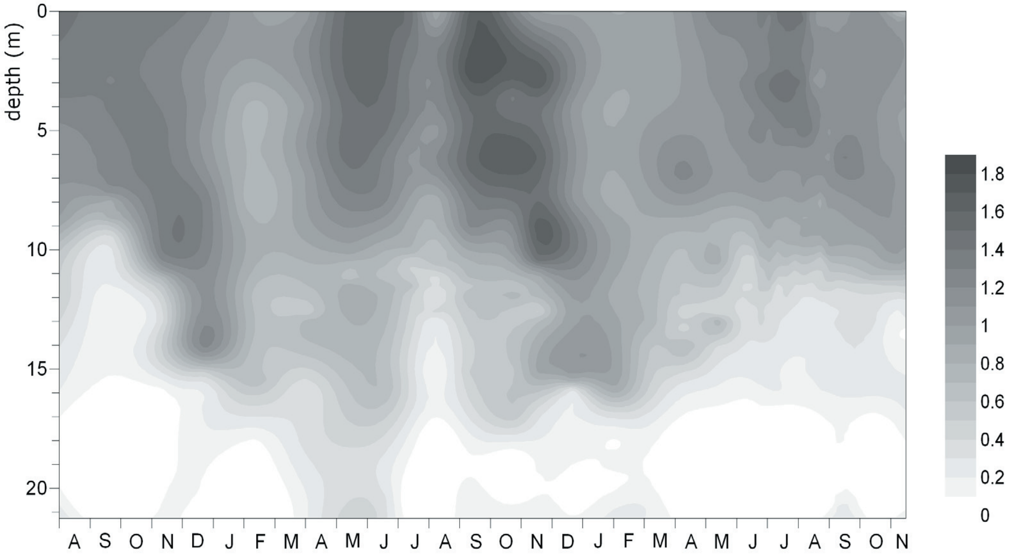


Figure 1. Isopleths of calcite saturation index (1=saturation) from August 1996 to November 1998 in Lake La Cruz.

Figura 1. Isolíneas del índice de saturación de calcita (1=saturación) desde Agosto de 1996 hasta Noviembre 1998.

Sediment traps (Figure 2A), which were collected monthly in Lake La Cruz during a period of three years (Romero et al. 2006), demonstrate that the white laminations in the sediment of this lake are formed by the seasonal pulses of calcite deposition after summer whitings. Just after the whiting events, the mineral material recovered by traps was mainly composed by Mg-calcite crystals (Miracle et al. 2000), which were the usual crystalline phase that could be found in the water and sediment samples (Rodrigo et al. 1993). The dark lamination of the varved sediment is formed by organic and mineral material deposited during the time elapsed between annual summer calcite precipitation (figure 2A). However the mineral contribution to the sediments increased significantly during December and January. During these periods detrital grains of quartz and dolomite were mostly collected in the sediment traps.

The seasonal time-course of epilimnetic calcium concentrations in Lake La Cruz reflects the lacustrine events associated to calcium carbonate sedimentation processes. Decrease in epilimnetic calcium concentrations (Figure 2B) can usually be observed after whiting episodes (Rodrigo et al. 1993; Miracle et al. 2000). This decalcification (about 22%) of the water was due to the sedimentation of calcium carbonate crystals into the deeper waters. However

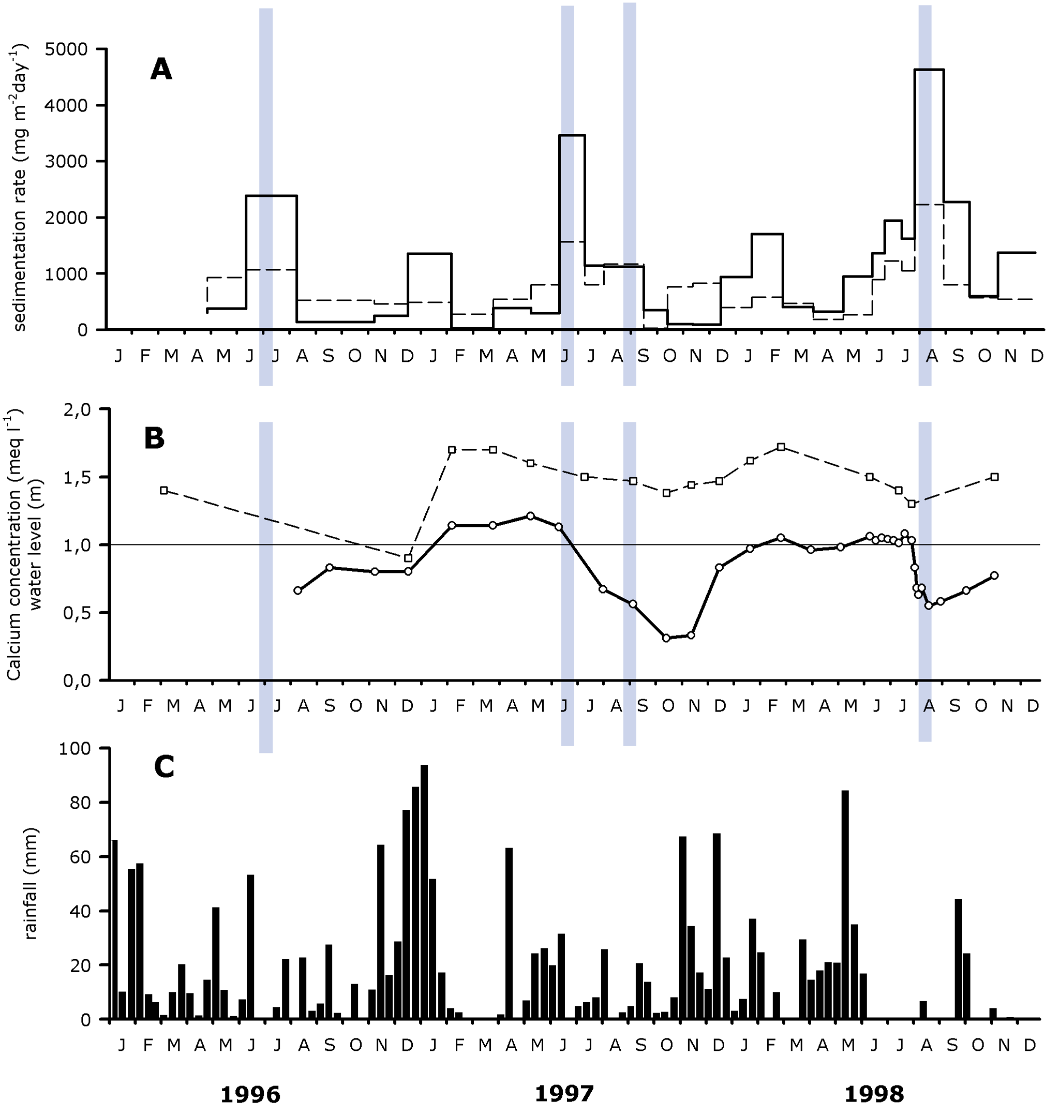


Figure 2. A: Sedimentation rates of mineral matter (solid line) and organic matter (dashed line) in Lake La Cruz; B: epilimnetic calcium concentration (solid line) and water level fluctuations (dashed line) over a reference level and C: accumulated ten days rainfall for the period in which sedimentation patterns were studied. Vertical grey bars indicate the occurrence of the whiting episodes.

Figura 2. A: Tasas de sedimentación del material mineral (línea continua) y materia orgánica (línea discontinua) en la Laguna de La Cruz; B: concentración de calcio (línea continua) y fluctuaciones de nivel (línea discontinua) sobre el nivel de referencia y C: lluvia acumulada durante diez dias consecutivos durante el periodo en que fueron estudiados los procesos de sedimentación. Las líneas grises verticales indican la ocurrencia de los procesos de precipitación masiva de carbonato cálcico.

calcium concentrations recovered after winter rainfall when groundwater increased the discharge and lake level rose (Figure 2C).

4b. Methods

Thin sections were obtained from sediment core collected using a gravity corer in the central and deepest point of Lake La Cruz in May 2003. In the laboratory, the core pipe was cut lengthwise and the sediment was split into halves. Overlapping sediment slabs were sampled using aluminium trays (1x2x18 cm). Thin sections (30 microns in thickness) of sediment were obtained after freeze-drying followed by saturation with Spurr's epoxy resin hardening sediment slabs (Lamoureux, 2001). The thin sections were scanned at 1600 dpi and the high resolution images obtained were analysed with the UTHSCSA Image tool program (ftp://maxrad6.uthscsa.edu). The laminae number and thickness were determined between visually discernible marked horizons. Measurements of laminae thickness were resolvable to 15 μm and documented using the measuring tools in the software. We report the mean dark and light laminae thickness of three separate measurements within each laminae. Statistical analyses were performed with SPSS 12.0S software (2003).

Meteorological dataset

Cuenca is the most important town in the region and its located 20 km from the study site (section 2, Figure 1). This meteorological series was chosen for analysis because is the longest and most complete available (1909-2002) in the region. However the 1909-1949 instrumental record was discarded due to the lack of reliability of the dataset and the high number of gaps in it (more frequently during 1915-1917, 1919-1921, 1925-1926, and 1936-1941, the latter caused by the Spanish Civil War). The remaining gaps in the Cuenca's rainfall dataset (1950-2002) were filled by inferred values from the nearest meteorological stations (section 2, Figure 1) after testing that a high statistical correlation existed between the meteorological series (Cuenca-Carboneras, r^2=0.74, n=874; Cuenca–Fuentes, r^2=0.74, n=1081, Cuenca-Cañada del Hoyo, r^2= 0.865, n=293).

Seasonal rainfall data (1950-2003) were calculated as annual and seasonal cumulative amounts; autumn (September to November, SON), winter (December to February, DJF), spring (March to May, MAM) and summer (June to August, JJA). Temperature data series from Cuenca included seasonal maximum means, minimum means and overalls means: SON (1953-2002), DJF (1954-2002), MAM (1950-2002, Tmin 1952-2002) and JJA (1952-2002, Tmin 1951-2002). Annual data corresponds to the hydrological year starting from September (previous year) and finishing in August. Rainfall and temperature variables were standardized for the time period 1950-2002.

The North Atlantic Oscillation (NAO) winter (December to March) index as the normalised sea level-pressure differences between Lisboa (Portugal) and Reykjavik (Iceland) was obtained from the Climate Analysis Section, NCAR, Boulder, USA, at http://www.cgd.ukar.edu/kas/jhurell/indices.html. Southern oscillation index (SOI) was obtained from University of East Anglia at http://daac.gsfc.nasa.gov/.

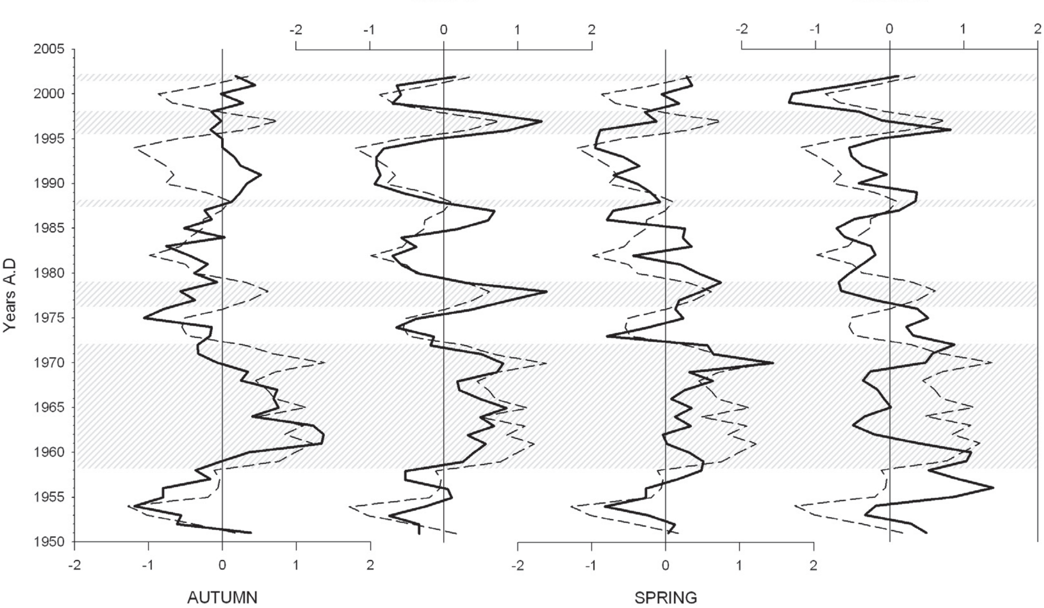
4c. Results

Meteorological variables

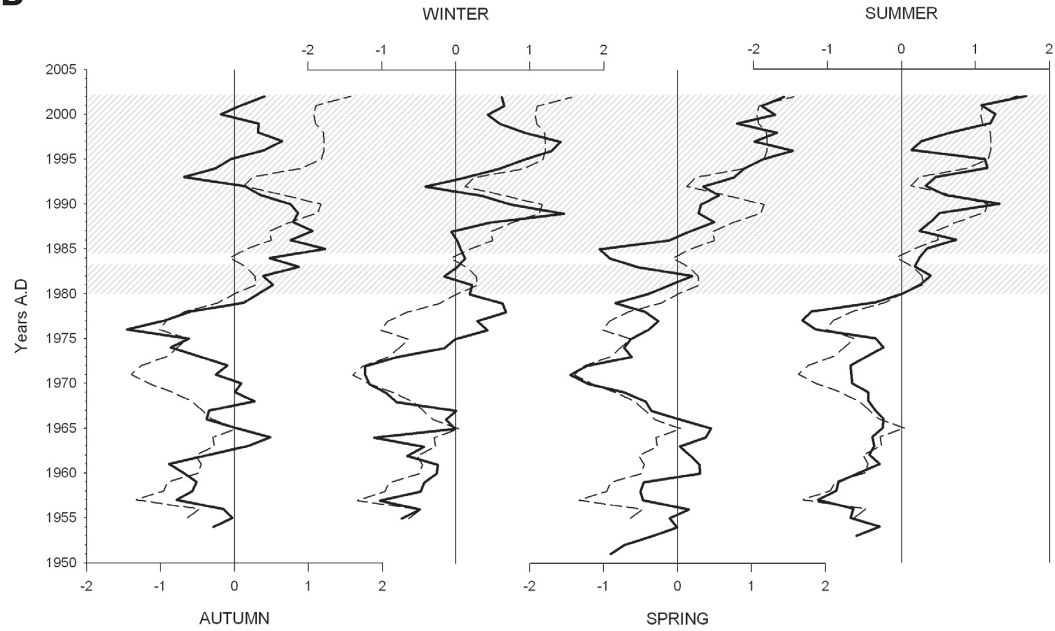
The seasonal rainfall and temperature in the region shows significant interdecadal variability from 1950 to 2002 (Figure 3) and strikingly an increasing trend in temperature since the end of eighties. The analysis of correlation between rainfall and temperature (Table 1) confirms their linkage. Their seasonal relationships were significant in all seasons when maximun and mean temperatures were correlated with rainfall. However minimum temperatures were only correlated with rainfall in winter, indicating that dry winter are relatively colder than wet winters.

Correlations are also significant with general circulation indexes. NAO winter (DJFM) index shows significant correlation with regional rainfall from December through March (r=-0,824; p< 0.01; n=52) (Figure 4, table 1 shows also the correlation of this index with accumulated rainfall from December to February). The SOI (from September to November, SON) is significantly correlated with autumn and spring rainfall (Table 1). A delay between SOI onset and when it affects the climate of the region ranging between 3 and 21 months has been suggested by Rodo et al (1997). This delay was also observed with our data, since a displaced correlation between SOI (SON) and spring plus autumn rainfall of next year was also significant (r=0.406; p<0.01; n=51). Nevertheless this ENSO effect seems weaker than that of NAO in the Cuenca rainfall record.

A


WINTER
SUMMER
-2
-1
0
1
2
Years A.D
2005
2000
1995
1990
1985
1980
1975
1970
1965
1960
1955
1950
AUTUMN
SPRING

B


WINTER
SUMMER
-2
-1
0
1
2
Years A.D
2005
2000
1995
1990
1985
1980
1975
1970
1965
1960
1955
1950
AUTUMN
SPRING

rainfall		Temperature		NAO (DJFM)	SOI (SON)
	Mean	Minimum	Maximum		
SON	-0,357(*) *50*	ns	-0,614(**) *50*	ns	-0,399(**) *52*
DEF	ns	0,628(**) *49*	-0,399(**) *49*	-0,746(**) *52*	ns
MAM	-0,474(**) *53*	ns	-0,639(**) *53*	ns	0,304(*) *52*
JJA	-0,422(**) *51*	ns	-0,540(**) *51*	ns	ns
ANNUAL	ns	ns	-0,409(**) *48*	-0,623(**) *52*	ns

Table 1 Correlations between seasonal rainfall and temperature, NAO and SOI (1950-2002): r, ((*) p<0.05 and (**)p<0.01), n in italic. (ns: non significant correlation).

Tabla 1. Correlaciones entre las precipitaciones estacionales y las temperaturas, NAO y SOI (1950-2002): r,((*)p<0.05 y (**)p<0.01), n en cursiva. (ns: correlacion no significativa).

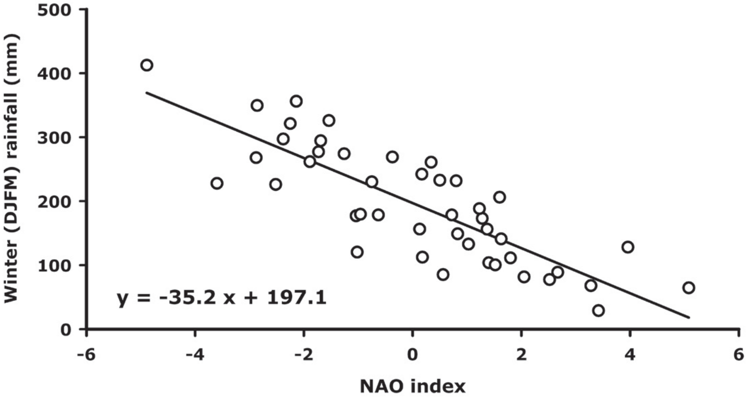


Figure 4. Relationship between NAO index and regional winter (DJFM) rainfall (1950-2002).

Figura 4. Relación entre el índice NAO y los valores regionales de lluvia acumulada durante el periodo invernal (DEFM) (1950-2002).

Previous page. Figure 3. (A) Seasonal (solid line) and annual (dashed line) rainfall. (B) seasonal (solid line) and annual (dashed line) temperature. Values were normalized by mean and standard deviation of the period 1950-2002 and filtered with a centered three years moving average. Grey areas indicate time periods of annual positive anomalies.

Página anterior. *Figura 3. (A) Lluvias estacionales (línea continua) y anuales (línea discontinua). (B)Temperaturas medias estacionales (línea continua) y anuales (línea discontinua). Valores normalizados por la media y la desviación estándar para el periodo 1950-2002 y filtrados por una media movil de tres años centrada. Areas grises indican las anomalias positivas durante los periodos anuales.*

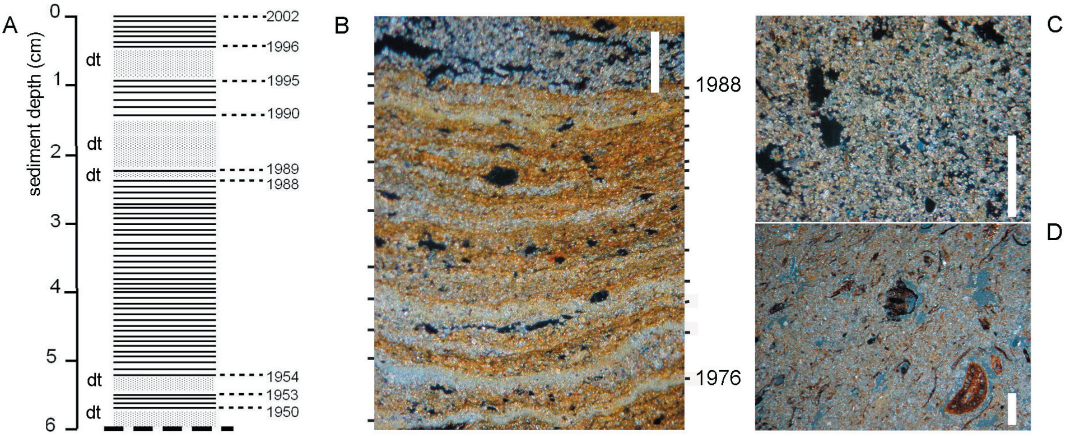


Figure 5. Sedimentary varves of Lake La Cruz. (A) Schematic log of the topmost 6 cm laminated sequence indicating the varves and detrital layers (dt). Microphotographs of (B) varves (1976-1988), and (C)(D) detail of a detrital layer formed by equigranular of ehuedral dolomite rhombohedrons, showing charcoal particles, charophyte remains and siliciclastic grains. Scale bar 1 mm. B and C with crossed polars.

Figura 5. Sedimento varvado de la Laguna de La Cruz. A: representación esquemática de los 6 cm superficiales de la secuencia laminada indicando la presencia de las varvas y las capas detríticas (dt). Microfotografias de (B) varvas (1976-1988) y (C)(D) detalle de una capa detrítica formada por granos rombohédricos de dolomía de similar tamaño y con la presencia de partículas carbonosas, restos de carófitas y granos siliciclásticos. Escala 1 mm.

Laminated sediment

The topmost 43 cm of the studied sediment core of Lake La Cruz showed a laminated sequence, however this study is focused in the uppermost 6 cm because our aim was to compare these laminations with instrumental meteorological data-series. The sequence of this part of the core (Figure 5) is formed by couplets of light and dark (brownish) laminae (Figure 5B), interrupted by thick discrete layers of unsorted coarse material, mainly dolomite which clearly differs from regular endogenic varve formation. The light laminae are composed by calcite crystals deposited after the whitings as we mentioned above and the darker ones consist mainly of organic-rich silts and fine mineral clasts. The total number of calcite laminae in the topmost 6 cm of the studied core are 53. Given the annual nature of calcite laminations in Lake La Cruz which was demonstrated by a monthly resolution study of sedimentation process by means of sediment traps (Miracle et al. 2000; Romero et al. 2006) and the recorded number of varves in the sediment core recovered in May 2003 the recent laminations correspond to the time period 2002-1950.

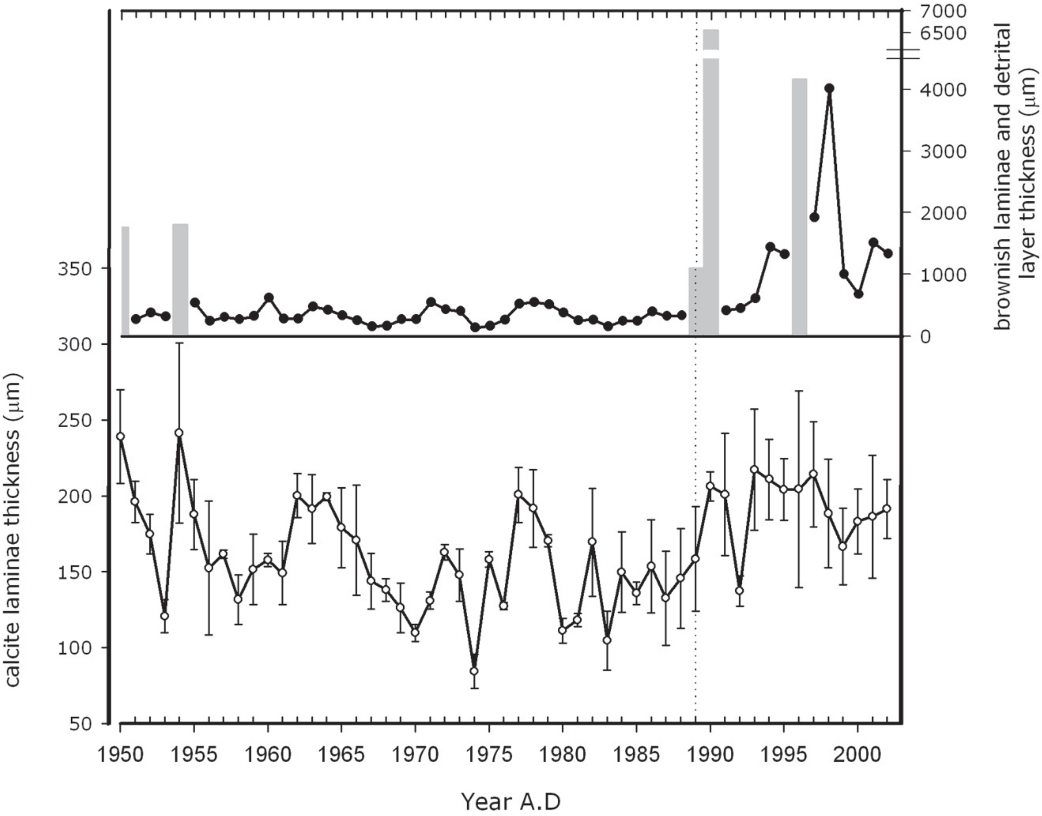


Figure 6. In upper panel brownish laminae thickness (black dots) and detrital layer thickness (grey vertical bars). Lower panel calcite laminae thickness (white dots). Vertical bars indicate the standard deviation of mean measurement for calcite laminae.

Figura 6. En la gráfica superior espesores de las láminas oscuras (puntos negros) y las capas detríticas (barras gris verticales). En el gráfico inferior, espesores de las láminas de calcita (puntos blancos). Las barras verticales indican la desviación estandard de las medidas de espesor de las láminas de calcita.

Varve thickness shows considerable interannual variability (Figure 6). Moreover two periods could be clearly differentiated based on subtantial change in laminae thickness, 1950-1988 and 1989-2002. The brownish laminae contribution to the total varve thickness is around 69% over the first period (1950-1988), however its contribution significantly increased until 87% between 1989 to 2002 (Figure 6). The difference in calcite laminae thickness between these periods is not so apparent, however the calcite laminae thickness in the last period (1989-2002) increased also relative to observed values from 1950-1988. Calcite and dark laminae show a similar temporal pattern of variability, with a significant correlation between these two structural components of varves (r=0.442; p<0.01; n= 37) for the period 1950-1988 and (r=0.445; p<0.01; n=48) for the whole period 1950-2002. However the correlation was not significant between light and brownish laminae during the period 1989-2002.

Sediment-climate relationships

In order to evaluate the climatic signal contained in Lake La Cruz sediment, a correlation analysis was performed between light or brownish laminae with regional climate series. The results of the analysis showed relationships between laminae thickness and temperature variables (Table 2). However both laminae series showed a substantial change in thickness at 1989 that coincides with the onset of an increasing trend in regional temperature variables (Figure 3). The high correlations are produced by the increased temperatures due to general warming in parallel with the varve thickness in recent times due to a less compactation of the sediment and higher trophic level and detrital input. For this reason the correlation with the 1950-1988 series is a better indicator of interannual variability.

When the varved series are split into two periods based on thickness significant change at 1989, winter rainfall is the best predictor of both calcite and brownish laminae thickness for the time period 1950-1988, althought the correlation of brownish laminae thickness with rainfall time series is weaker than those calcite laminae (Table 2). Moreover the climate linkage increase its significantce if rainfall accumulated from December through March is considered (rainfall DJFM-calcite laminae thickness; r=0.725, p<0.01, n=35). This correlation was obtained after removing four outliers (1950, 1954, 1969,1970) whose values were above two standard deviations from mean calcite laminae thickness (156±34 mm), such as 1950 (238 mm) and 1954 (241 mm) and winter rainfall (DJFM) such as 1969 (412 mm). In addition we considered 1970 as outliers since the January rainfall contribution to the total winter amount was around 63% and its value (166 mm) was above two standard deviations from the mean January rainfall for the entire period studied. By contrast, consistent relationship between air temperatures and calcite laminae thickness was not observed in the period 1950-1988 since the relationship between rainfall and temperature suggests that the correlations observed are result of collineality (Table 1).

			calcite laminae	brownish laminae
DJF	P	**1950-1988**	0,495(*37*)	0,426(*37*)
		1989-2002		0,679(*10*)
	TM	1950-2002	0,496(*48*)	0,456(*44*)
		1989-2002		0,820(*10*)
	TMIN	1950-2002	0,498(*48*)	
		1950-1988	0,482(*34*)	
		1989-2002		0,819(*10*)
	TMAX	**1950-1988**		-0,443(*34*)
MAM	TM	1950-2002	0,369(*51*)	0,637(*47*)
		1989-2002		0,773(*10*)
	TMIN	1950-2002	0,460(*50*)	0,605(*46*)
	TMAX	1950-2002		0,505(*47*)
		1989-2002		0,746(*10*)
JJA	TM	1950-2002		0,369(*46*)
	TMIN	1950-2002		0,505(*46*)
NAO (DJFM)		**1950-1988**	-0,419(*37*)	

Tmin: minimun mean temperature, Tmax : maximun mean temperature, Tm: mean temperature, P: precipitation

Table 2. Correlations between laminae thickness and climate variables: r ($p<0.01$), n in italics.

Tabla 2. Correlaciones entre los espesores de las laminas y las variables climáticas: r (p<0.01), n en cursiva.

The uppermost sediment recorded several events of high sediment accumulation (1949-1950, 1953-1954, 1989-1990 and 1994-1995). Among the different triggering mechanisms that could generate detrital layers in non-glacial lacustrine sediment, flash-floods (Mangili et al 2005), earthquakes (Shiki et al 2000), landslides, and droughts are the most commonly argued. Extreme precipitation events are frequent in this Mediterranean region reaching heavy and violent class (32-64 mm/day and 64-128 mm/day, respectively, according to Alpert et al. (2002) classification). The meteorological record of Cañada del Hoyo, the nearest village to the lake, begins in 1956, and the strongest rainstorm registered during the second half of the 20th century (52 mm/day in August 1974), was not followed by an increase in sedimentation. Earthquakes have been also reported for the same years when these detrital layers formed (i.e.1951 and 1988) within a 70 km distance from the Lake La Cruz (Spanish Seismic

Catalogue, Martinez-Solares and Mezcua 2002). However both earthquakes and flash-floods normally generate well-sorted turbidites (Rodriguez-Pascua et al. 2000). The dominance of unsorted grains in these detrital layers suggests that other mechanisms could be involved in their formation. This fact, together with the occurrence of charcoal particles and macrophyte remains, indicates the input of reworked littoral sediments. Extreme negative anomalies in previous years and the synergic effect of drought and common wildfires during dry periods (Johnson and Larsen 1991; Johnson and Gutsell 1994) suggest local slope inestabilities and slumping during lake level drops. Doline slopes are formed by dolostones with crevices which have caused rupture in huge fragments. In lake bottom and specially in the shore there are fallen stones of huge dimensions and also trees which have produced important landslips as well as resuspension and redeposition of sediment.

4c. Discussion

Relationship between varve thickness and climate variables.

The analysis of correlation has showed that the calcite laminated sediment from Lake La Cruz records a climate signal. The strongest correlation between calcite laminae thickness and the meteorological dataset is through winter (DJFM) rainfall. The winter rainfall recorded in the region explains 52% of the variance contained in the calcite laminae from 1950 to 1988. These results indicate that winter rainfall variability could be the main factor controling the extent of summer whitings and/or the total amount of calcite crystals precipitated. The seasonal evolution of epilimnetic calcium concentrations in Lake La Cruz suggest the linkage between calcite laminated record and climate variability. Calcium water column concentrations significantly decrease after whintings due to the sedimentation of calcite crystals (Rodrigo et al. 1993; Miracle et al. 2000). However calcium concentrations were restored after winter rainfall. In closed Lake La Cruz, the interannual rainfall variability controls the groundwater discharge and therefore the lake level fluctuations. The seasonal water renewal contributes significantly to the restoration of water column calcium concentrations before summer calcite precipitation process. Winter rainfall also determines the brownish laminae thickness. According to our previous results of sediment traps, the flux of mineral material during December and January appears to be associated with density currents and groundwater discharge after winter rainfall.

Some other studies have attempted to link climate variables and variations in the thickness of calcareous varves. Lotter and Birks (1997) observed that rainfall explained more variance than other explanatory variables (both climatic and limnological) in the thickness of the both types of lamina (light and dark) in varved sediment of Baldeggersee (Switzerland). Likewise this relationship with calcite lamina was related with the input of carbonate and additional nutrients from the catchment thus allowing further biogenic calcite precipitation pulses during summer. Seibold (1958) also made a detailed comparison between climatic variables and variations in the thickness of calcareous varves from the bottom of a closed bay, Malo Jesero, on the island of Mljet in the Adriatic Sea. The clearest correlation existed between varve thickness and high summer precipitation records from Rome, which goes back to A.D. 1782. On the other hand, Kempe and Degens (1979) observed a correlation in varved sequence of Lake Van (Turkey) between calcite varve thickness and high water level and wetter periods.

Interestingly the thickness of calcite laminae in Lake La Cruz over the calibration time period was not correlated with summer temperature variables, although temperature has been argued as a main triggering factor of calcite precipitation (Brunskill 1969). A similar lack of correlation was also observed in a previous extensive study in Lake Zurich, where no correlations were found between calcite varve thickness and temperature or plankton production (Kelts and Hsü 1978). By contrast, in the varved sediments from Baldeggersee, also located in Switzerland, some relationship of varve thickness with summer temperature was observed, although the correlation was more clear for the dark layer than for the calcite layer (Lotter and Birks 1997). Nevertheless, the isotopic composition of autogenic calcite of Lake Baldeggersee showed that the $\delta^{18}O$ of calcite is a reliable proxy for temperature and $\delta^{18}O$ of the water (Teranes et al. 1999). Although temperature is undoubtedly a trigger of massive calcite precipitation, in Lake La Cruz the amount of calcite crystals settled mainly depends of calcium concentrations in water. Therefore, a minor relationship between temperature and calcite laminae thickness would be expected.

However the relationship between Lake La Cruz calcite laminae and climatic variability was weaker during the last 14 years. Both calcite and brownish laminae show significant thicker laminae from 1989 to 2002. Although these thicker varves are mainly related with the lower compactation in the unconsolidate nature of the topmost centimetres of the sediment, lacustrine changes observed since the begining of eighties could have contributed significantly. Since 1982 Lake La

Cruz level has decreased progressively from 25 m of maximun depth (Vicente and Miracle 1988) to 20 m at present. As commented above the interannual fluctuations of lake level are due to hydroclimatic variability, however the long-term decrease observed during the last years could be additionally explained by the increased aquifer exploitation in the area. Therefore the increase of brownish laminae thickness is related to the detrital input derived from the littoral erosion during long-term lowering as during drastic lake level drops forming the detrital layers. Further analysis in recent calcite laminae about mineral ratios (calcite/dolomite) and surface morphology and shapes of carbonate grains shoud be undertake in orden to distinguishing primary lacustrine carbonates from a likely detrital contribution to white laminae thickness. On the other hand, a slight increasing trend in trophic status of Lake La Cruz have been reported by the limnological surveys over the last twenty years. Althought significant changes of ortophosphates concentrations have not been detected, integrated chlorophyll *a* contentrations in the oxic water column during spring blooms have increase from less than 100 mg m^{-2} at 1981-1982 (Vicente and Miracle 1988) to more than 150 mg m^{-2} during late nineties (Camacho et al. 2003a). This trophic level change, accompained by increased erosion in Lake La Cruz due to recent tourist pressure and heavier sheep uses of the lake as drinking point, may be reflected in this recent change observed in light as well as dark laminae thickness. In Baldegersee (Switzerland), the increase in calcite laminae thickness from 1950-1980 was related with an eutrophication process (Lotter et al. 1997). Moreover others studies have shown that calcites are sensitive indicators of seasonal productivity variations (Brunskill 1969; Kelts and Hsü 1978; Lotter 1989).

Paleoclimatic reconstructions

The results of this study has shown the potential of Lake La Cruz varved sediment as high-resolution paleoclimate archive which will enable future reconstruction of past regional rainfall. Figure 7 shows the inferred winter rainfall based on white laminae thickness and the winter rainfall data from 1950 to 1988. The linear regression model ($y=0.045x^{1.662}$; $r^2=0.52$) developed will enable the estimation of winter rainfall for a given calcite thickness observed value. Although extreme events, such as heavy rains (i.e. 1969) are not exactly mirrored in the laminated sediment, our model infers the interannual hydrological variability. Since brownish laminae results from different sedimentary processes (eolian, surface runoff, wave erosion, pelagic primary production, etc) during longer period than calcite laminae, the last seem to be better proxy for paleoclimate studies.

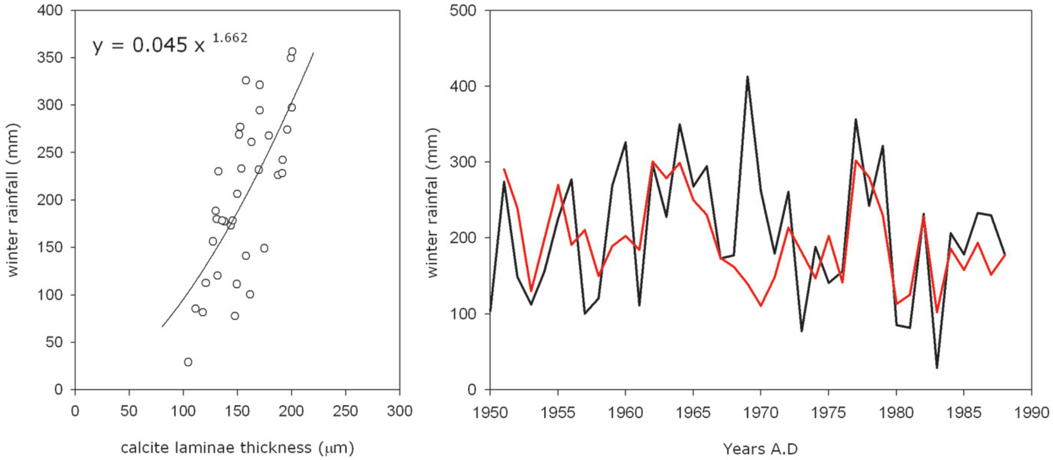


Figure 7. Comparison based on regresion model (A) between winter (DJFM) rainfall instrumental values (black solid line) and inferred winter rainfall from calcite laminae thickness of Lake La Cruz sediments (red solid line).

Figura 7. Comparación basada en el modelo de regresión (A) entre los valores instrumentales de la lluvia invernal (DEFM) (línea continua) y los valores inferidos de lluvia invernal (línea roja continua) a partir de los espesores de las láminas de calcita del sedimento de la Laguna de La Cruz.

Regional winter precipitations, in Lake La Cruz area are highly correlated with the phase of the North Atlantic Oscillation (NAO). The coupling between NAO and Cuenca precipitation was expected based on previous results (Rodo et al. 1997; Rodriguez-Puebla et al. 1998; Esteban-Parra et al. 1998) which demonstrated the significance of NAO for the long-range prediction of Iberian Peninsula precipitation, specially over the center-southwestern Spain where the responses become more intense. However regional interannual variability is explained by other large-scale atmospheric circulation modes. In the Iberian Peninsula, specially in the south-east, seasonal rainfall during spring and autumn is also related with the Southern Oscillation (ENSO) (Rodo et al. 1997; Rodriguez-Puebla et al. 1998). However Cuenca record (1950-2002) shows a weaker relationship with SOI. The patterns of both NAO and ENSO interplay with the spatial variability of the annual precipitations over the Iberian Peninsula, although some studies reported the instability of teleconnection over time, and an intensification of the ENSO signal in the region during the second half of the 20th century (Janicot 1996; Rodo et al. 1997; Rocha 1999). Our results show that among these two different large scale circulation modes, annual precipitation anomalies in Cuenca (1950-1998) were mainly associated by NAO influence.

The results of our study in Lake La Cruz highlight new directions for paleoenvironmental research using calcite varve sediment records. Since winter rainfall explains a high percentage of the variance for calcite laminae thickness, annually laminated sediment could be use to reconstruc past hydroclimatic variability in the region. Nevertheless the understanding of local deposition processes is essential before calcite laminated sediment in any particular lake can be used as climate signal record since other environmental factors could have conditioned the formation and hence the calcite laminae thickness. The potential of varved lake sediments has not been fully exploited and further studies on calcite laminated records can significantly contribute to paleoclimatic reconstructions as quantitative proxies.

5. Reconstruction of winter rainfall over the last centuries from calcite laminated sediment of Lake La Cruz.

5a. Introduction

A step towards the better understanding of anthropogenic impacts on the climate system whitin the global change debate, is the assessment of the characteristics of natural variability. The time window recorded by the recent instrumental data, however, is too short for the study of decade to century climatic varibility and to differentiate the anthropogenic effects. Therefore, further work is needed on the systematic collection of long-term instrumental and proxy observations of climate system variables at high-temporal and spatial resolution, to evaluate the general circulation models under development which could reduce uncertain in predicting and detecting future climate changes.

Because of the many indirect and direct linkages that exist between climate, lakes and the organisms contained therein, an increasingly important source of proxy data comes from the sedimentary records of lakes (Smol and Cumming 2000, and references therein). Preserved in lake sediments, either organic and inorganic components have enabled direct and indirect reconstructions of wide range of climate-related limnological variables such as ice-cover, river discharge, DOC, pH, temperature, conductivity and water column stability. In particular, in semiarid and semihumid regions the link between hydroclimatic variablity and lake level is presumed to work through the balance of precipitation and evapotranspiration acting upon the lake surface and groundwater (Dearing 1997). In these areas, closed-basin systems which lack inlets and outlets, have been shown to produce regionally consistent sedimentary records of climate-controlled lake-level fluctuations (Verschuren 1999; Rodó et al. 2002; Giralt et al. 2004).

Lake sediment proxies provide a wide range of paleoclimatic data at different temporal resolutions. Annually laminated (varved) lake sediments, which are formed by seasonal changes in sediment deposition, are capable of preserving information at higher resolution, annual and even subannual. Since annual laminations are present in different environmental settings, both freshwater and marine environments (i.g Cariaco Basin, Santa Barbara Basin, Saanich Inlet), at wide spatial distributions and for many time windows, these archives could provide an unfilled source of proxy data.

Numerous hydrological and sedimentological process studies in glacial-fed lakes, which are common in boreal and alpine regions, have demonstrated that the fluxes of runoff and suspended sediment vary directly as a function of average summer and snowmelt seasonal temperature and therefore clastic laminae thickness are high resolution proxy of seasonal temperature (Leeman and Niessen 1994; Itkonen and Salonen 1994; Zolitschka 1996; Lamoureux and Gilbert 2004). In other areas, however varved sediments are linked to other origins such as biogenic and physical-chemical processes (i.e. evaporitic varves) (Bauer 2004). In particular, in carbonaceus bedrock basins, calcite laminations are common due to the seasonal autogenic precipitation of calcium carbonate in the water column and sedimentation of calcite crystals (Kelts and Hsü 1978). Some approaches have assesed the analysis of climatic signal recorded in calcite laminated sediments using periodicities in laminations thickness as an exploratory technique (Livingston and Hajdas 2001; Dean et al. 2002; Muñoz et al. 2002). The persistent periodicities observed at sub- and multicentenial scale, which were similar to those in spectra of radiocarbon production, have been interpreted as indicating an association between solar forcing and varve thickness.

At eastern Spain, the karstic Lake La Cruz shows an excellent preserved annually calcite laminated sediment (Julià et al. 1998). In order to explore the potential use of laminations as quantitative climate indicator, calibration analysis between calcite laminations thickness and rainfall/temperature variables during the regional instrumental record, 1950 onwards, has been performed (section 4). The results have shown that accumulated winter rainfall, from December to March, is the best predictor of annual calcite laminations thickness. The monitoring study carried out in the lake during three years has confirmed that calcium availability during summer and then, the total amount of crystals precipitated during summer whiting, depends on annual calcium concentration renewal controlled by the aquifer discharge after winter rainfall. Based in this previous work, this study attemps the winter rainfall reconstruction using calcite laminae thickness as rainfall proxy for the first time.

The results of our study highlight the potential of calcite varved sediment records in paleoclimatic research supporting the use of calcite laminae thickness as a consistent quantitative proxy of past hydro-climatic variability. Moreover this regional winter rainfall reconstruction increases both temporal and spatial resolution of past rainfall data from Iberian Peninsula contributing to the work that have been carried out over the last decades about paleoclimatic characterization in western mediterranean region. There is a relatively low number of rainfall proxies available (Pauling et al. 2005), hence mostly regional rainfall anomalies during the last centuries have been estimated by historical indexes based on documentary sources (Martin-Vide and Barriendos 1995, Rodrigo et al. 1999; García-Herrera et al. 2003) and natural proxies as tree-rings (Creus-Novau et al. 1997). At this point regional and large-scale reconstructions of changes in precipitation become essential because complement the surface temperature series and improve our understanding of the forcings that have contributed to the climate variability (such as the 11-year sunspot cycle or the volcanic activity injecting sulfate aerosols into the stratosphere during major volcanic eruptions).

5b. Methods

Three sediment cores were used to construct the varve chronology. All of them were obtained from the central and deepest point of Lake La Cruz in different data. The first sediment core (CV-94) was extracted in 1994 with a Wright (1980) corer and the other two (CV-98 and CV-03) using a gravity corer in December 1998 and May 2003, repectively. The CV-94 core was processed in the laboratory after it was recovered, and the other two were processed in a similar way during 2004. An extra sediment core was processed for dating by isotopic methodology.

In the laboratory, the core pipes were cut lengthwise and the sediment was split into halves. Overlapping sediment slabs were sampled using aluminium trays (1x2x18 cm). Thin sections (30 μm in thickness) of sediment were obtained after freeze-drying and Spurr’s epoxy resin hardening sediment slabs (Lamoureux, 2001). In CV-94 thin sections, the light and brownish laminae were counted and the thickness of each lamina was measured under a petrographic microscope with an ocular micrometer. The thin sections of CV-98 and CV-

03 were scanned at 1600 dpi and the high resolution images obtained were analysed with the UTHSCSA Image tool program (ftp://maxrad6.uthscsa.edu). The laminae number and thickness were determined between visually discernible marked horizons. Measurements of laminae thickness were resolvable to 15 μm and documented using the measuring tools in the software. We report the mean dark and light laminae thickness of three separate measurements within each laminae.

The three sequences were crossmatched following as sedimentological criteria, (1) the occurrence of detrital events and (2) the number of varves between detrital layers. The method used to expres the similarity between series is the Student's t-test proposed by Baillie and Pilcher (1973) which is reported by Maddy and Brew (1995). The degree of correlation between the two series (x and y) is measured by r, the product moment correlation coefficient, defined as

$$r = (\sum xy - N\bar{x}\,\bar{y}) / (\sqrt{((\sum x^2) - (N\bar{x}^2))((\sum y^2) - (N\bar{y}^2))})$$

where $\bar{x}$ and $\bar{y}$ are the means of all x and y values respectively. The Student's t-test is then performed to provide a measurement of the probability of the calculated value of r having arisen by chance. The value of the Student's t-test is given by

$$t = (r\sqrt{(N-2)})/(\sqrt{(1-r2)}$$

where N is the number of samples, which here means the number of overlapping calcite laminae. Before r was calculated, the data were transformed so that the sets of values (x and y) became bivariate-normal. Each calcite lamina was converted to a percentage respect to the mean of the five calcite laminae of which it is the central value. Log to base e of these percentages is then calculated.

Once the samples were cross-dated, the laminae thickness from each individual core were averaged year by year to produce a master chronology for Lake La Cruz sediment. Averaging the series also increases the ratio climate signal to noise. This is because climatically related variance, common in all records, is not lost by averaging, whereas non-climatic noise, which varies from core to core, will be partially canceled in the averaging processes.The trend of the master series due to lower sediment compactation in the more recent part was removed by fitting a second order function. The functions ($y=0.0012x^2$-

0.3x+214, $r^2=0.217$) and ($y=0.004x^2-0.9x+397$, $r^2=0.284$) were fitted to the calcite laminae thickness and brownish laminae thickness master series, respectively. The master index series were calculated as the ratio between observed value and function fitted value. Based in the previous work of calcite laminae calibration againts climatic variables (section 4), reconstrucction of winter rainfall was performed using the regression model described, but changing the calcite laminae thickness for its index value ($y=171x^{1.82}$, y; (DJFM) rainfall (mm) and x; calcite laminae thickness (index)).

Finally, the paleoclimatic reconstruction was verified by comparing the reconstructed rainfall values for the period 1860 to 1950 with the instrumental data from Cuenca. Instrumental dataset for Cuenca station covers from 1909 to nowadays. Unfortunately gaps in the dataset appear especially for the first half of the 20th century, more frequently during 1915-1917, 1919-1921, 1925-1926, and 1936-1941, the latter caused by the Spanish Civil War. A high statistical correlation was found between the rainfall record of Cuenca and Madrid ($r^2=0.7384;p<0.001;\ n=76$). The instrumental climatic data from Madrid, (1860 onwards), were used to reconstruct Cuenca winter (DJFM) rainfall between 1860 and 1909, and to fill the remaining gaps in the Cuenca's dataset. The skill of reconstruction has been estimated using the reduction error (RE) measure (Cook et al. 1994), which is defined as

$$RE= 1- [(\Sigma(y_i - \hat{y}_i)^2)/(\Sigma(y_i - y)^2)]$$

Where y_i are the observed values over the verification period, $\hat{y}_i$ are the reconstructed values over the verification period and y is the mean of the observed values over the calibration period. RE values of 1 indicate a perfect reconstruction (no difference between the reconstructed and the predictand during the verification period), a value of 0 means that the reconstruction is as good as climatology (mean over the verification period) and –1 is equivalent a random guess. This test is passed if the RE value is positive.

Since it is expected that the spectral content of the data evolves in time as response to the complex non-stationary assemble of ambient sources, we therefore employ a time-frequency analysis which decomposes the time series into local spectra and which permits to track non-stationary signals in time. In the following we shortly introduce and review the S-transform which we then employ to analyse the data. There exist many approaches to study the time-frequency evolution of signals. The short time Fourier transform (STFT) based on Gabor (1946) and the continuous wavelet transform (CWT) (e.g. Daubechies 1990) are among the most common methods. The STFT determines the local spectra by applying the Fourier transform on overlapping data segments of constant width while the CWT permits a frequency-dependent time resolution by using a scaled replica of a mother wavelet for data segmentation. The constant time resolution of the STFT limits the time resolution of high-frequency signals while causing a poor frequency resolution at periods which are larger than the window length. The variable time-frequency resolution of the CWT is therefore an advantage over the STFT.

Here, we employ the S transform (Stockwell et al. 1996) which has elements of the STFT and CWT. It can be considered as an extension of the STFT with frequency-dependent resolution. That is, moving windows are scaled inversely by frequency similar to a mother wavelet in the CWT. Consequently, the windows are short at high frequencies and long at low frequencies to allow the detection of high frequency bursts and to permit a good frequency resolution at the lower frequencies. The windows can have arbitrary shape (McFadden et al. 1999), but we use the originally proposed Gaussian-shaped windows since they are the most compact in time and frequency. The standard deviation of the Gaussian window is chosen as a constant factor of each period to be analysed. As long as this factor is not too large the corresponding time-frequency spectra are in a good approximation analytic (Schimmel and Gallart 2006) at a fixed frequency. This is an attractive property which enables the determination of attributes like the instantaneous frequencies.

The S-transform carries over most of the properties of Fourier analysis and its application is easy and intuitive. Further, the averaging of the local spectra over time gives the Fourier spectrum of the complete time series (Stockwell et al. 1996). The S transform is therefore indeed a representation of the local spectrum. This permits to move freely between the time, frequency and time-frequency domain which invites to attenuate undesired signals in the time-frequency domain for further analyses in the time domain (Schimmel and Gallart 2005; Simon et al. 2006). In the following, we name the time-frequency spectra obtained through the S-transform, S-spectra for brevity.

5c. Results

Sediment sequences

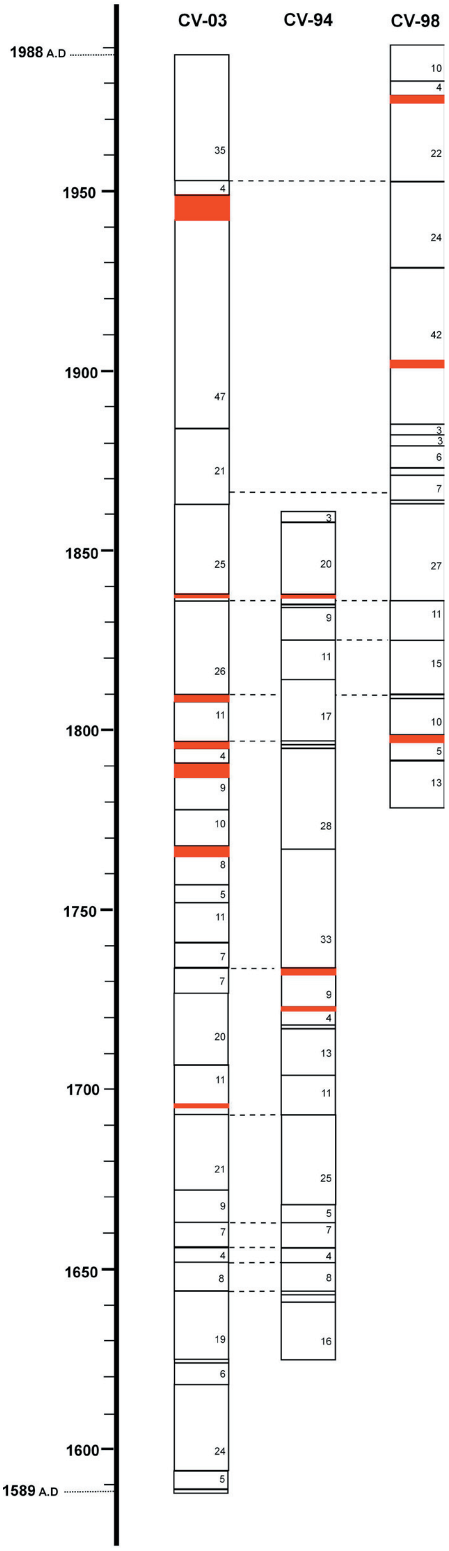


Figure 1. Crossmatching of the three studied laminated sequences. The scale correspond to the varve-year unit. The black lines indicate detrital events and the red lines indicate varve loss. The number of varves between detrital horizons are indicated trought the sediment sequences.

Figura 1. Cruce estratigráfico de las tres secuencias laminadas estudiadas. La escala corresponde a la unidad varva-año. Las líneas negras indican la ocurrencia de eventos detríticos y las líneas rojas la perdida de varvas. El numero de varvas viene indicado en las secuencias sedimentarias.

The laminated section of Lake La Cruz, which is formed by couplets of alternated white and brownish laminations, extends approximately the uppermost 43 cm.The laminated sequence is interrupted at some depths by events of high detrital input. The presence of these detrital layers and the number of total laminations between these events were used as sedimentological criteria to assess the cross-matching of the studied sedimentary sequences (Figure 1). Among the three sedimentary sequences available, only CV-03 recorded the total extension of the laminated section. Although the first core recovered, CV-94, was the longest (173 cm long), the most recent laminated structure was damaged during the transportation because the uppermost centimeters of the sediment were extramately wet. By contrast, CV-98 lengthed only 30 cm long, being too short to recover the oldest varves.

During the matching we noted the absence of varves, offently less than two annual laminations and just before some detrital layers. The occurrence of lost varves before the detrital inputs suggest a possible erosive effect on delicated laminations. However the availability of more than only one sedimentary sequence enable the confidently assessment of a Lake La Cruz chronology. Therefore after crossmatching the sequences, a total number of 417 annual laminations have been identified; consequently the onset of laminated sediment is expected to be at A.D. 1589.

Annual laminations provide a better chronological data than radioisotopic methods, however laminations chronology should be tested by an independent dating methology. Figure 2 shows the results of ^{210}Pb activity measurements. The ^{210}Pb profile in Lake La Cruz sediment showed a decreasing curve from 188 Bq kg^{-1} at 8.5 cm depth to 35 Bq kg^{-1} at 18 cm depth. The lowest most samples, at 20 cm depth, have a ^{210}Pb content of 35 Bq kg^{-1}. This lowest value was considered as supported ^{210}Pb, and thus the background for Lake La Cruz. The ^{210}Pb activity fluctuations at the topmost may be attributed to the dilution effect related with the observed detrital content. Despite these ^{210}Pb values fluctuations, a sedimentation rate model can be constructed fitting an exponential curve resulting in a mean sedimentation rate in Lake La Cruz of 1 mm y^{-1} until 6 cm depth. This chronological model matched with the varve dating. The equilibrium between total ^{210}Pb activity and the supporting activity is effectively achieved after a maximun of about 6-7 ^{210}Pb half-lives, 130-150 years (Appleby 2001). The varve-years between 1880-1850 A.D. coincided at 18-20 cm depth with the lowest values considered supported activity, discarting the erosive effects of detrital layers as an alteration of the chronology.

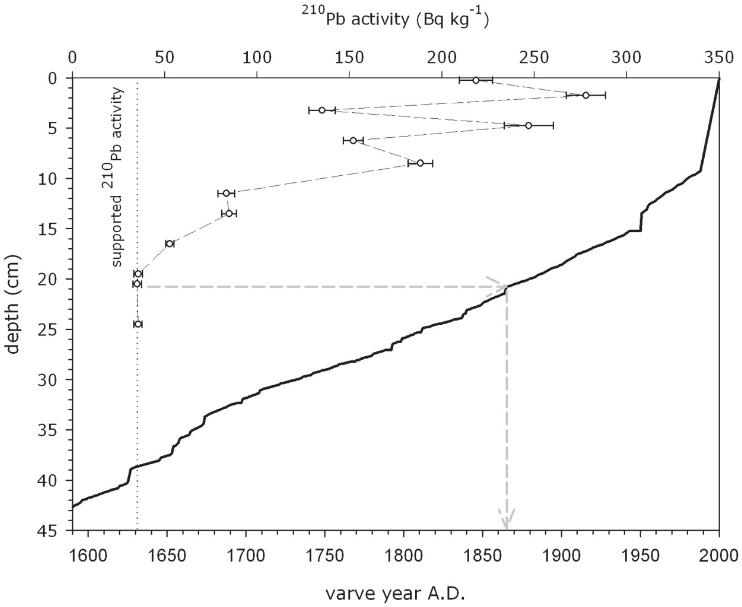


Figure 2. Age-depth relationship by varve counting (solid line) and ^{210}Pb activity (dashed line). Supported ^{210}Pb activity is indicated by the vertical dashed line.

Figura 2. Relación profundidad-edad mediante el contaje de las varvas (línea continua) y la actividad del ^{210}Pb (línea discontinua). El nivel base en la actividad del ^{210}Pb se indica mediante la línea vertical discontinua.

Detrital layers occur with no obvious primary textural organization. They consist of detrital equigranular of ehuedral dolomite rhombohedrons. Commonly small plant fragments and charcoal particles are present. Figure 3 shows the occurrence of these layers within the three sequences and the thickness values. Although some of them occur in all the sequences, other detrital layers occur only in one or two of the three cores. Moreover the thickness of these layers is high variable. For instance, the thickest detrital layer (1.9 cm thick) was registered by CV-94 at 1798 A.D. while in CV-03 was only 3.5 mm. In spite of this inter-sequences variability, it is possible to identified periods with an increase of detrital deposition frecuency (i.e. 1642-1673 and 1859-1886) and others such as during 18th century and 1875-1940 which present thickness values below the mean (table 1).

The annual varve is formed by a calcite and a brownish lamina which is composed by organic matter mixed with detrital material. The mean value of varve thickness is 0.6 mm and the standard deviation around 0.2 mm (table 1). Commonly the mean contribution of the brownish laminae to the total varve thickness is higher than calcite laminae, around 65% (table 1). Figure 4 shows the master series of brownish laminae thickness index (see methods). Notably, during lenghty (1650-1750 and 1780-1805) as well as shorter time periods (around 1625, 1840 and 1900) the brownish laminae thickness showed values above the mean. Some of them correspond to periods of higher frecuency detrital inputs (1625, 1780-1805, 1840).

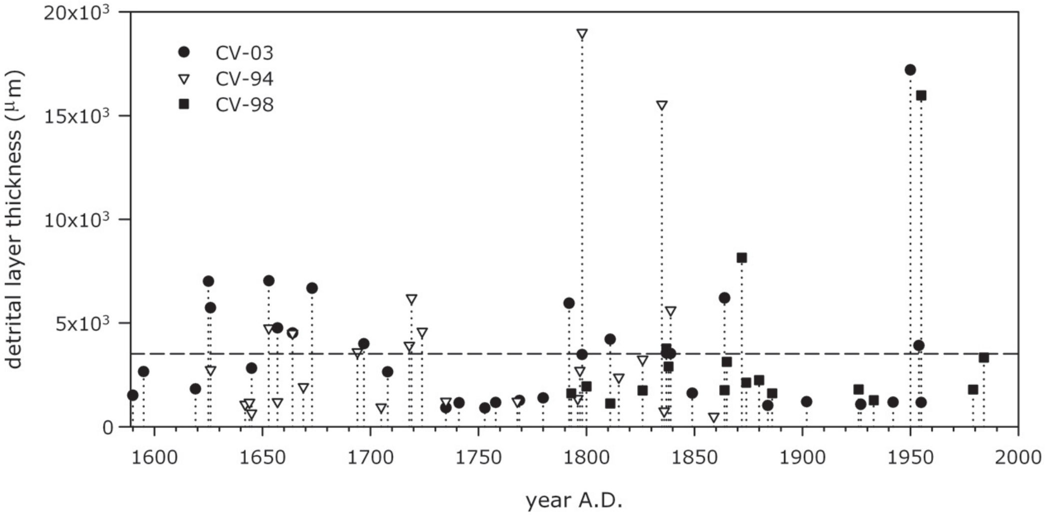


Figure 3. Detrital layers thickness (mm) in the three sedimentary sequences.

Figure 3. Espesores de las capas detríticas (mm) en las tres sequencias sedimentarias.

	detrital layers	varves	brownish laminae	calcite laminae
mean	3.441	0.635	0.414	0.219
standard deviation	3.667	0.196	0.164	0.058
maximun	19.000	1.599	1.185	0.472
minimun	0.500	0.126	0.052	0.089

thickness unit mm

Table 1. Thickness features of detrital layers, varves, brownish and cacite laminae.

Tabla 1. Características de los espesores de las capas detríticas, varvas y láminas oscuras y de calcita.

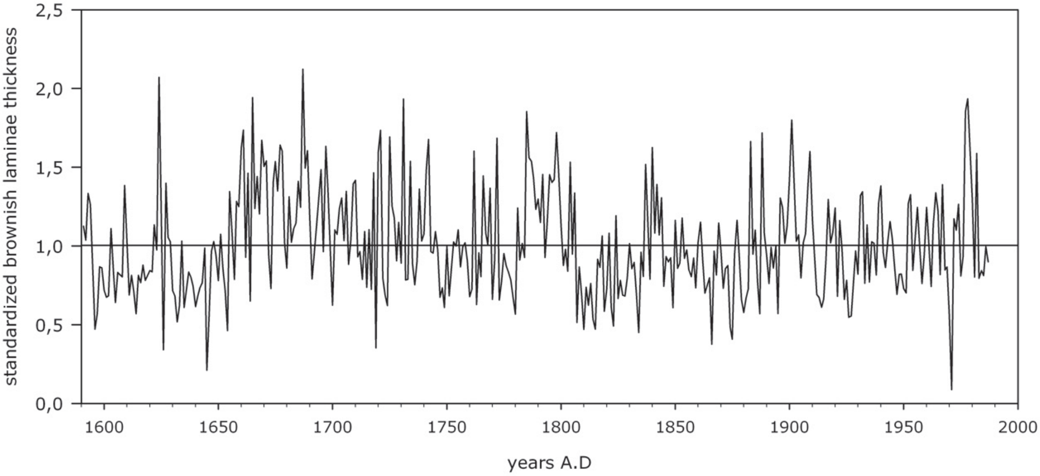


Figure 4. Master time series of brownish laminae thickness index.

Figure 4. Serie temporal del índice del espesor de las láminas oscuras.

Time series of calcite laminae and rainfall reconstruction

The measurements of calcite laminae thickness of the three sedimentary sequences studied showed high statistically significant correlation. The results of the Student's t-test correlation between CV-03 to CV-94 (t= 5.11, df= 179, p<0.0001) and CV-03 to CV-98 (t=7.22, df= 177 p<0.0001) expres the similarity between series. Both CV-03 and CV-98 showed an increasing trend during the last century related with the lower compression in the upper depth of the sediment column (Figure 5). The three series were averaged year by year resulting in a master series with a mean value of calcite laminae thickness of 635 μm and a standard deviation of 196 μm. The standard deviation of annual lamina thickness reached higher values in past periods when detrital sedimentation increased, as it is indicated by the occurrence of detrital layers and brownish laminae high index values. Before attempting the reconstruction the master series of calcite laminae thickness was detrended by fitting a second grade function (Figure 5A).

Once the index series was obtained and given the relationship between calcite laminae thickness and regional winter rainfall (from December to March, DJFM) the calibration linear function defined (see methods) is employed to reconstruct rainfall until the beginning of the laminated sediment, 1589 A.D (Figure 5B). While the mean of rainfall inferred is similar to the mean winter rainfall for the calibration period (1950-1988) around 200 mm, the standard deviation is slightly lower; 92 mm and 86 mm for the calibration period and for the reconstructed period respectively. The empirical threshold for a dry year was stablished between one and two standard deviation, this means seasonal precipitation between 150 and 100 mm. Lengthy dry periods, as could be seen in the figure 5C, were; 1590-1600, 1640-1675, around 1690, and 1740, 1755-1770, 1810-1840, 1860-1930, and around 1970. In addition high occurrence of dry years were observed between 1630 and 1665 and between 1675 and 1700. By contrast, during the first and last part of the 18th century and around 1850 and 1950 the winter rainfall amount was relatively above the mean.

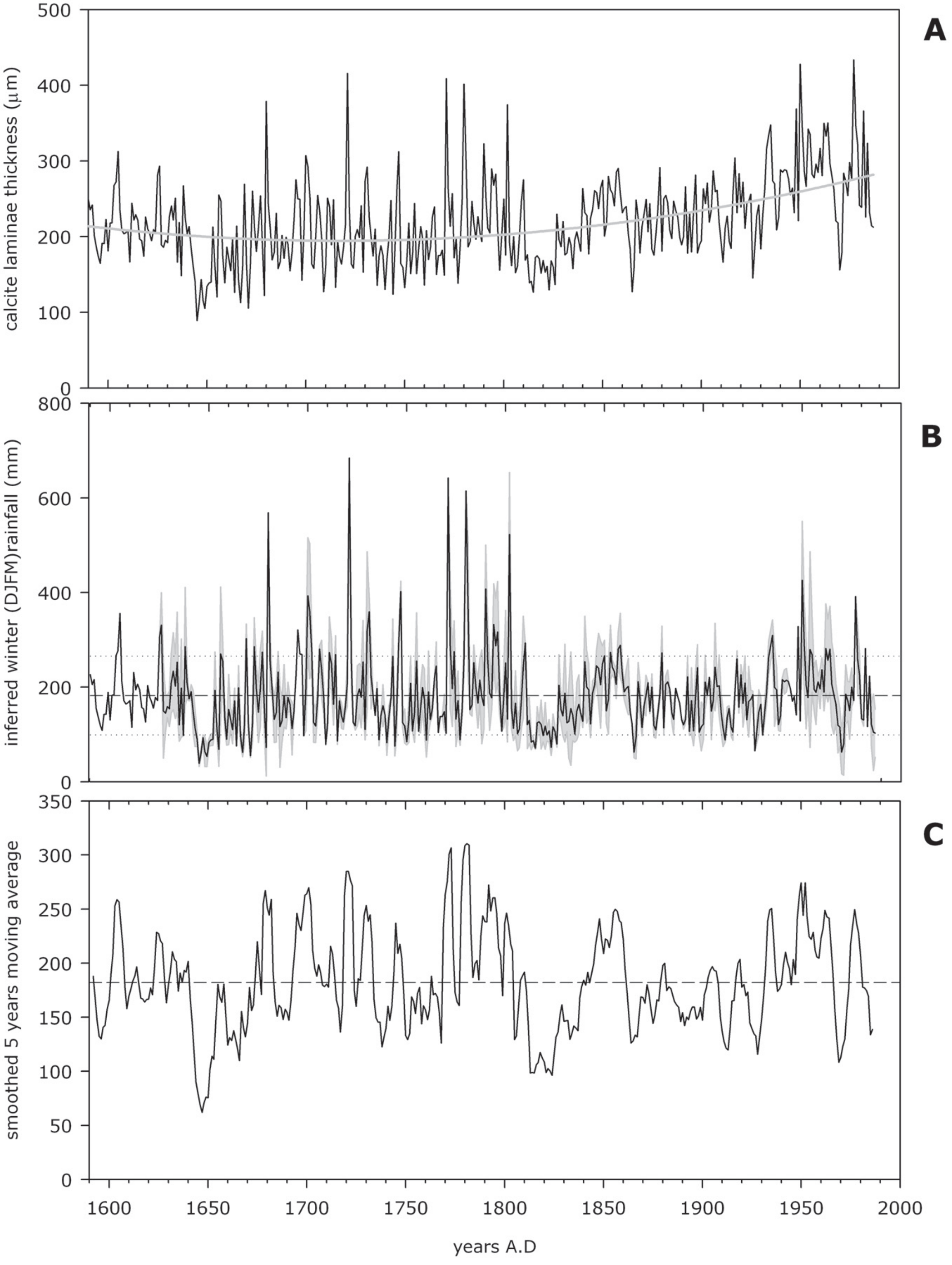
Verification process have confirmed that the winter (DJFM) rainfall varibility estimation skill is highly significant. The reduction of error (RE) was accounted with the inferred instrumental data of Cuenca and Madrid rainfall registered until 1860 (Figure 6). The reduction error obtained between the winter estimated value and the corresponding year instrumental value averaged with the winter rainfall of the previous year was 0.42.

Analysis of the climate signal

The measured values for the thickness of the calcite bands (Figure 7) are plotted versus time in Figure 7a. The black and grey line mark the raw and the locally smoothed data, respectively. Our smoothing algorithm detects the outlying measurements and replace them by a local mean value. This is motivated since outlying amplitudes can obscure the frequency components of small amplitude signals (Schimmel 2001). We detect the outliers through a moving window analysis with windows which are 15 years long and which are centered at each measurement. A measurement is detected as outlier whenever its amplitude lies outside of the 2-sigma interval of a Gaussian function with

Figure 5. Facing page. A. Calcite laminae thickness (μm) and detrented function (grey solid line). B. Inferred winter (DJFM) rainfall values (mm), grey area corresponds to the deviation from mean value. Dashed black line is mean value of winter rainfall reconstruction and dotted lines standard deviation. C. Smoothed winter rainfall reconstruction by five years moving average. Dashed line is the mean value of winter rainfall reconstruction.

Figura 5. Página siguiente. A. Espesores de las lámina de calcita (μm) y función de tendencia (línea continua gris). B. Valores inferidos de lluvia invernal (DJFM), el área gris corresponde a la desviación con respecto al valor medio de cada lámina. La línea continua indica el valor medio de la reconstrucción y las líneas discontinuas la desviacion estandard. C. Suavizado de la reconstrucción por una media movil de 5 años. La línea discontinua indica el valor medio de los valores.


A
B
C
calcite laminae thickness (μm)
inferred winter (DJFM)rainfall (mm)
smoothed 5 years moving average
0
100
200
300
400
500
0
200
400
600
800
0
50
100
150
200
250
300
350
1600
1650
1700
1750
1800
1850
1900
1950
2000
years A.D

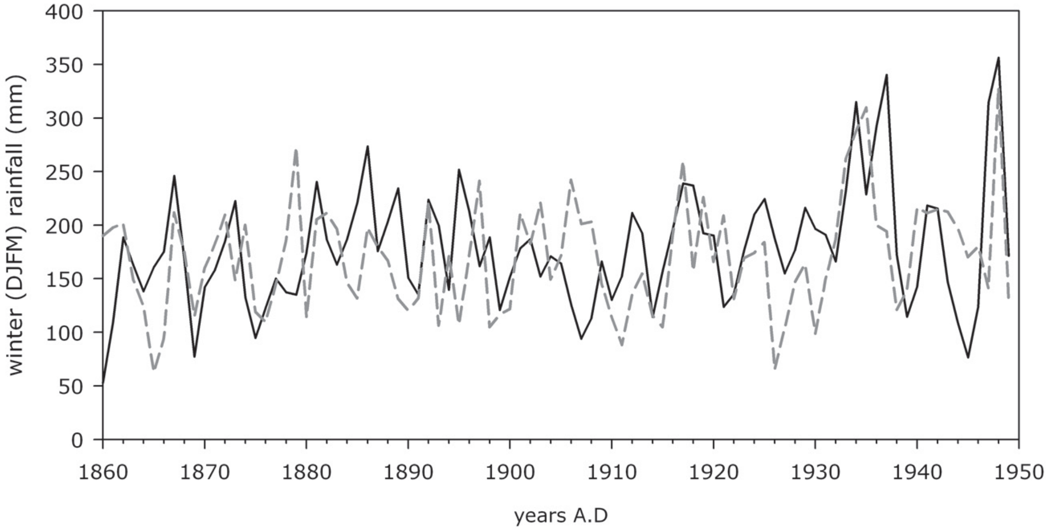


Figure 6. Verification process. Instrumental winter rainfall (annual record averaged by the previous year) (black solid line) and annual inferred winter rainfall values (grey dashed line) (mm).

Figura 6. Verificación. Valores instrumentales de la lluvia invernal (valores anuales promediados con el año anterior) (línea continua) y valores de lluvia inferidos (línea gris discontinua) (mm).

standard deviation (sigma) and mean determined for the windowed data. This guarantees a minimum manipulation of the raw data that is, we maintain the main data characteristics by localized interventions for isolated measurements. A total of 17 measurements have been replaced by their local mean. 50 % of these measurements have been detected for the years 1720 to 1810.

Figure 7b shows the Fourier amplitude spectra of both time series (raw data in black and processed data in grey). The inlet contains the spectra at the high frequencies with a linear frequency scale for visual purposes. It can be seen that the outlying measurements mainly influence the high frequency part of the amplitude spectrum. For instance, features at frequencies of about 0.2, 0.27, 0.36, and 0.42 1/year have been caused by the outlying measurements. At the lower frequencies, a significant signal is observed at a period of about 84 years (frequency 0.0117 1/year). Our frequency resolution is limited by the length of the time series which determines the fundamental frequency.

The Fourier transform assumes that the data characteristics are stationary, but as mentioned before, it is expected that there are non-stationary

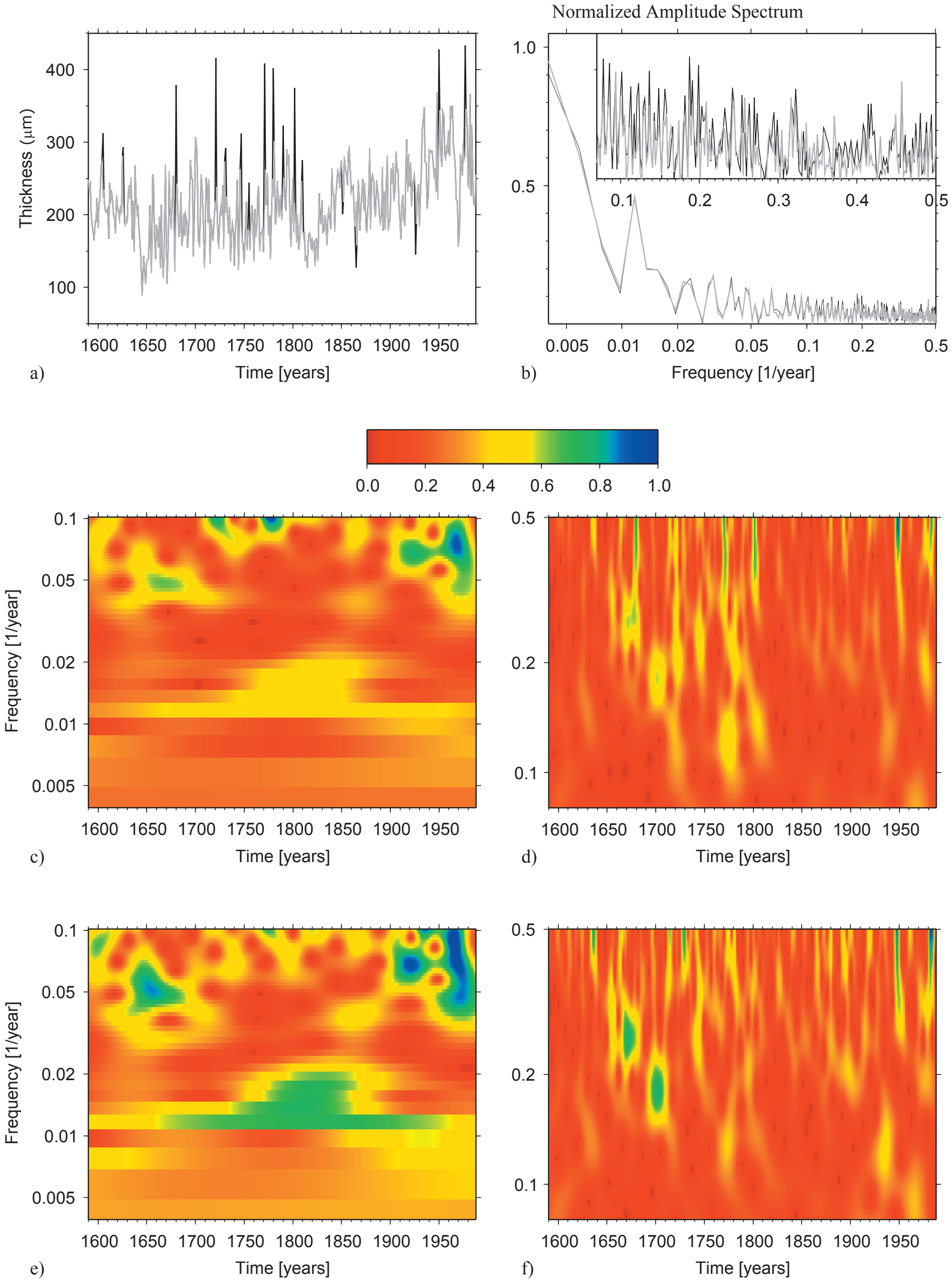


Figura 7. A. Calcite laminae thickness, raw data (black line) and smoothed data (grey line). B. Fourier amplitude spectra of raw data (black line) and smoother data (grey). C-D. Amplitude S-spectra for the raw data. E-F same as C-D for smoothed data.

figura 7. A. Espesores de las láminas de calcita, datos no tratados (línea negra) y suavizados (línea gris). B. Espectro de amplitud de Fourier de los datos no tratados (línea negra) y los suavizados (línea gris). C-D Espectro-S de amplitud de los datos no tratados. E-F lo mismo que C-D pero para los datos suavizados.

components in the data. We therefore decompose the data into a time-frequency representation. Figures 7c,d and 7e,f show the amplitude S-spectra for the raw and smoothed data (black and grey line in Figure 7a), respectively. We show the low and high frequencies in separate figures for visual purposes. The amplitudes have been normalized to one for each figure to improve the visibility of possible signals. One observes the good time resolution for high frequency components (Figure 7d and f) which is due to the usage of shorter data windows at the higher frequencies (Figure 7c and e). The lower frequency signals are less well resolved in time due to the longer windows at the larger periods. The S-spectra of both time series are roughly similar. The S-spectra of the smoothed data set, however, seem to show some more signals which, in fact, have been enhanced due to the attenuation of strong amplitude components. No new signals have been revealed with the processed data. The 84 years signal seems to be localized to the years 1700 to 1900. This signal corresponds to the low frequency oscillation which can be recognized by eye in the data from Figure 7a. It can further be observed that there are no signals with frequencies ranging from 0.02 to 0.05 1/year. Altogether, the S-spectra reveals that the data is “governed” mainly by non-stationary events.

5d. Discussion

In this study we propose calcite laminae thickness as an indicator of past rainfall. The direct link between sediment features and climatic variability described previously (section 4) has allowed the reconstruction of regional winter precipitation over the last centuries, using a novel climate proxy. However some methodological difficulties and inherent limitations must be considered in order to exploit in future its ussefullness as climatic proxy. Firstly a relative variability of thickness within each calcite lamina was observed. Althought the resin impregnation could alter the laminated structure, the variability was due to the calcite crystals sedimentation process. Instead of a continuous and homogeneus layer, it is common to observe a wavy border between calcite and the upper brownish laminae. In order to reduce this variability at least three measurements were obtained from each lamina along the thin section. On the other hand the crossmatching of the studied sequences showed the high inter-variability of sedimentary columns even recovered in the vicinity, in particular the absence or presence as well as thickness of detrital layers. In spite of this intersequences variability, the correlation between calcite laminae thickness series was statistically significant. Moreover the crossmatching have significantly reduced the uncertainity in producing a time series since it is possible to detect the lost varves, mainly before detrital horizons. The erosion effect on this delicate sedimentary structure due to events of detrital input could alter the chronology leading to errors in the further analysis of the climate signal (Livingston and Hajdas 2001). Therefore the attemp of a climatic reconstruction should not be based only in one sediment sequence, the averaging of more chronologies is essential to reduce the site noise relative to climate signal and then increase the confidence in producing a time serie.

On the other hand no differences were observed between the mean rainfall value for the reconstruction period and the calibration period whereas the standard deviation of estimated values was significantly lower than the standard deviation of instrumental data (1950-1988). This probably indicates that the extremely dry or wet years were not mirrored in La Cruz sediment. This smoothing effect of the lacustrine system was detected previously during the calibration study. The maximun rainfall amount usually has lower incidence in aquifer because is lost by runoff. Moreover during the extremely dry periods the lake has a remanent calcium concentration which anyway results in a background amount of calcite crystals formation.

Despite inherent limitations and methodological difficulties described above, the verification process confirmed the reconstruction skill, providing high degree of confidence in the rainfall estimation. The reconstruction performed has showed the incidence of climate stress periods in the region, specially those of drought. The most prevailing negative anomalies were observed during 1590-1600, 1640-1675, around 1690 and 1740, 1755-1775, 1805-1840, 1860-1930, and around 1970. In fact during these drier periods the ocurrence of detrital inputs could be observed. In the previous section we discarded the occurence of detrital layers related to storms, earthquakes and other eventual factors, by contrast, these layers seem to be related with drastic water level drops during drier years and favoured by lower temperatures (section 4). However detrital layers were also present during the 18th century, althought this century was characterised by estimated rainfall values above the mean value, but from 1650 to 1805 the interannual varibility is significantly higher than for the previous and latter years, including the Maunder Minimun (A.D 1645-1715) and the Maldá Anomaly described in the western Mediterraneran basin (AD 1760-1800) (Barriendos and Llasat 2003). These time periods were characterised by considerable interannual irregularity, positive and negative episodes succeding each other within short periods of time and reaching extreme values. Some of the clastic events, for instance around 1627, corresponded to drastic reductions in annual reconstructed winter rainfall amount but not always the driest years corresponded to a detrital sedimentation event because of the physical and hydrodynamic factors which control localy clastic deposition in Lake La Cruz.

Regarding the brownish laminae thickness, the other sedimentary feature studied, the fluctuations observed over the reconstruction period showed a different pattern from calcite laminae thickness, except during the dry period 1805-1840 when thinner brownish laminae were measured. Although the brownish laminae thickness was related with the aquifer discharge in winter season, however a secondary linkage with temperature was observed during the calibration period. It is possible that the temperature relationship was underestimated since during past periods temperature values were more extreme than over the last fifty years (Luterbacher et al. 2004). In particular values of brownish laminae above mean were observed during the documened cold periods of the Maunder Minimun (1645-1715), a period inside de Little Ice Age in which the solar activity decrease notably.

Although spatial heterogeneities limited comparisons within the Iberian Peninsula, the regional rainfall reconstruction is consistent in general with historical observations from other regions in Spain based on documentary proxy data. In the Iberian Peninsula the study of grape harvest dates in Valladolid (Castille) during the the 16th and 17th centuries (Benassar 1967) indicates a maximun in rainfall around 1620-1630 and a important dry period in 1675-1690. The analysis of rogations *pro pluviam* in Catalonia (northeast of the Iberian Peninsula) suggest a dry period in 1630-1640 and significantly around 1680, with wetter conditions at the begining of the 18th century (Barriendos 1994). In Andalusia rainfall anomalies reconstrution based on documentary sources suggest a wet period during the begining of the 16th and extending until mid-17th century and a positive anomaly reaching its maximun around 1850 (Rodrigo et al. 1999). By constrast the 18th century in Andalusia was defined as a dry period with a minimun around 1750. However the regional rainfall reconstruction performed using the calcite laminae thickness indicated that the 18th century was significantly wetter period in the region although around 1750 a negative anomaly was inferred by Lake La Cruz record. Moreover Lake La Cruz sediment has recorded the intense droughts betweeen 1880 and 1935 documented by Font-Tullot (1988) in Spain. Unfortunally comparisons with dendroclimatic reconstructions are not possible. Although there are many long chronologies around the Iberian Peninsula, in a general sense, correlation with precipitation variables are weaker than with the temperature variables (Manrique and Fernandez-Cancio 2000). In the Iberian Range area, the calibration studies suggest a tree-ring response to spring-summer precipitation and temperature but there is not a clear response to winter precipitation (Fernandez-Cancio et al. 1993).

The variability of the climatic signal contained in Lake La Cruz varved sediment has shown a strong multi-decadal component. In fact, changes in winter precipitation variability over the large Mediterranean area reveals a similar low frequency component which is centered between 1750 to 1850 (Luterbacher et al. 2005). In Lake La Cruz's climate signal, this period of about 84 years (frequency 0.0117 1/year) coincided to the Gleissberg solar cycle which is commonly observed in sunspot data (Schatten 1988; Hoyt and Schatten, 1997; Peristykh and Damon, 2003). However interpretations have to be done with caution because of the temporal limitation of the time serie. In addition, on decadal to centenial time scales, a variety of climate forcing are at play, including changes in solar activity.

At the interannual up to decadal time scale regional winter rainfall seems to be govened by a non-stationary component, according to previous studies which have report non-stationarities in the European/Noth Atlantic climate system (Casty et al. 2005; Pauling et al. 2005; Touchan et al. 2005). Nevertheless the correlation analysis between Cuenca regional winter precipitation during the last 50 years and NAO index (from December to March) have shown a strong correlation with this large-scale pattern (section 4). Moreover previous studies have confirmed that the North Atlanthic Oscillation pattern is the most important atmospheric phenomenon in the Atlantic area associated with the iberian winter rainfall (Zorita et al. 1992; Rodo et al. 1997;Esteban-Parra et al. 1998; Rodriguez–Puebla et al. 1998). However our results show that the connection of winter rainfall to the NAO is not stable over time and suggest that different patterns, not only NAO, have played a role in determining precipitation variability. Indeed the Empirical Orthogonal Function (EOF) analysis of the reconstructed Eastern North Atlantic/European sea level pressure (SLP) fields (Lutherbacher et al. 2002) has indicated the inestabilitites on regional winter Mediterranean climate. The first EOF of winter (December-Frebuary) SLP for the period 1764-2002 explains 44% of the total winter SLP variability and reveals the well-known dipole pattern which resembles the NAO. The second EOF of winter SLP which explains around 24% of variability, is featured by one centre of action west of Ireland. Pauling et al. (2005) found highly significant correlation between the first EOF and reconstruction of winter precipitation variability over southern Spain and Morrocco during the late 17th century as well as during most of the 19th and 20th century, whereas second EOF was equally important to the NAO-pattern in terms of explaining winter precipitation towards the end of the 17th, 18th, 19th and 20th century. Furthermore smaller scale processes also influence reginal rainfall variability. As Xoplaki et al. (2004) noted, land-sea effects and interaction, the influence of sea surface temperature connected with latent and sensible heat flux, orographical features and thermodynamical aspects interact with each other on different time scales and are superimposed on the quasi-stationary planetary waves which control large-scale advection.

6. Paleolimnological reconstruction of the variability among the phototrophic community over the last centuries in meromictic Lake La Cruz

6a. Introduction

Meromictic lakes are chemically stratified ecosystems with an incomplete vertical circulation of their waters, since mixing can only occur up to a depth where the mixing forces, thermal convection and wind action, are stronger than the stabilizing forces, that can result from changes in water temperature or electrolyte concentration. Either external factors such as saline water intrusion (ectogenic meromixis) or submerged mineralized inflow (crenogenic meromixis), which bring a continuous supply of denser water into the lower stratum of the lake, and internal forcing (biogenic meromixis; when permanent stratification is derived from increasing electrolyte concentration in the lower strata due to the decomposition of organic material) could start the meromictic condition of such a lake.

Nevertheless, irrespectively of the process by which a meromixis has developed, the permanent stratification results in anaerobic conditions and then electrolyte concentration in the monimolimnion increases due to decomposition of organic material. According to Hakala (2004) biogenic meromixis is a secondary cause and left-over category for lake cases where the primary cause is difficult to determine. Paleolimnological research, however, has underlined the relationship between climate perturbation effects and stratification patterns. For instance, time ago Loffler (1975) showed that the onset of meromixis in Austrian lakes is closely related to climate warming during the glacial or early Holocene. Another example was reported by Schmidt et al. (1998), which found different anoxic levels in Längsee sediment with respect to climate changes during the late glacial and Holocene, and have proposed a conceptual model of meromixis in Längsee where climate forcing determines natural transitions between holomixis and meromixis (Schmidt et al. 2002).

On the other hand meromixis may have an overriding effect on lake biogeochemical processes since chemocline segregates the whole water column into two layers; the mixolimnion and the permanently anoxic monimolimnion. But, what is the response of the biotic component to the onset and development of meromictic condition and the derived effects on nutrient cycles? Only long-term perspectives could add information about community trajectory over time. For example, in Mono Lake a saline lake which may periodically change between different mixing regimes (meromixis and monomixis) as a response to water level fluctuations, long-term measurements of plankton populations and their physical and chemical environment offers an excellent opportunity to contrast monomictic and meromictic conditions (Melack and Jellison 1998). Although long-term monitoring approaches have assessed holomixis/meromixis fluctuations in lakes (e.g. Tyler and Bowling 1990; Hammer 1994), only paleolimnological studies could improve the knowledge about forcing mechanisms involved in the origin of meromixis and the characterization of its development providing a longer temporal perspective. (Ryback and Dickman 1988; Lami et al. 1994; Tracey et al. 1996)

In the karstic lacustrine system of Cañada del Hoyo (Cuenca, Spain) there are several small flooded dolines located very close to each other in the same substrate, with Lake La Cruz being the only meromictic lake in this area (Vicente and Miracle 1988). The presence of a density gradient at 3-4 m above the bottom due to the high concentration of dissolved bicarbonate and calcium avoids the complete turnover in autumn. Moreover, the location of the lake inside a circular depression, sheltered from the wind, its morphometric features, and the lack of a relevant subterranean inflow directly to the bottom of the basin which could destabilize monimolimnion, favour the maintenance of the meromictic condition (Rodrigo et al. 2001). As other meromictic lakes, Lake La Cruz has an annual laminated sediment (Julià et al. 1998). This type of sediment offers an exceptional advantage; biogenic laminations preserve an accurate and continuous chronology and provide a record of sediment accumulation with high resolution.

Hence, we undertook a paleolimnological approach to assess the changes in the phototrophic community in Lake La Cruz. This study attempts to cope with the timing and characterization of lake’s primary productivity evolution and with the evaluation of forcing factors involved, especially the indirect effects of chemocline development on planktonic populations. For this purpose, photosynthetic pigments present in the sediment have been used as a sensitive proxy of biological response since they have shown to be useful biomarkers that can be used to reconstruct historical changes in the community of primary producers, both in abundance and composition (Leavitt and Hodgson 2001). Moreover our study, more focused in the laminated section of Lake La Cruz sediment than the previous report (Julià et al. 1998), is expected to offer new data about meromixis origin.

6b. Methods

A 44 cm sediment core of 2.5 cm diameter was recovered from the lake at its deepest point in May 2003, using a gravity corer. The core was extracted with a metacrylate cylinder ballasted by a fixed piston and was quickly protected from light by opaque wrapped and stored in a cold chamber. The core was transported to the laboratory, where it was sectioned. The core was sliced into 2-3 mm sections, sealed in sterile "Whirlpack" bags and conserved frozen at -20ºC in darkness until analysis. Water content in the sediment was determined by drying 2-3 g of wet sediment at a temperature of 105 ºC for 2 hours. Organic matter was obtained by the loss-on-ignition of dried sediment samples at 450ºC for 4 hours (APHA 1992).

Annually laminated sediment in Lake La Cruz provides an accurate chronological model by varve counting. However varve chronology was independently verified by radioisotopic methodology. Sediment dating was performed by ^{210}Pb detection by gamma spectrometry at the analytical service of the Department of Physics of the Autonomous University of Barcelona.

Photosynthetic pigment analysis

Regarding to photosynthetic pigments, analytical procedures were performed as described previously by Airs et al. (2001) and Squier et al. (2002). Samples were extracted into acetone (100 %) using sonication. After centrifugation (5 min at 3500 rpm) the supernatant was filtered trough a cotton wool plug. This extraction process was repeated until the supernatant was colourless. The supernatants from each extraction were combined and any water present was removed in vacuum. Extracts were treated with diazomethane to methylate free acid groups and then dried with N_2 atmosphere and stored at 4ºC until immediate HPLC analyses.

High performance liquid chromatography (HPLC) was carried out using a Waters (Milford, MA, USA) system consisting of a 600MS system controlled, 717 autosampler and 996 photodiode array (PDA) detector recording between 300 and 800 nm. Instrument control, data processing and analysis were carried out using Waters Millenium 32 software. Separations were performed in the reverse-phase using two Waters Spherisorb 3μ ODS2 columns (4.6 x150 mm) coupled in series. The mobile phase was a mixture of four solvents; ammonium acetate (0.01 M), methanol, acetonitrile and ethyl acetate. The gradient employed is detailed in Table 1. 10 μl of acetone extracts were injected into the system. The solvent flow was 0.7 ml min^{-1}. Peaks were identified according to their absorption spectra measured by means of a photodiode-array detector and confirmed by mass spectrometry (MS). MS provided molecular weight data expressed as mass-to-charge-ratio (m/z), value equal to the sum of the atomic masses of the most abundant isotope of each element that comprises the molecule (assuming the ion is a single-charge ion). The MS system included an Esquier 3000plus with Agilent 1100 ChemStation auto-sampler and APCI ion source. Separation conditions were as described above for HPLC. Liquid Chromatography-MS settings were as follows: capillary temperature 150ºC, APCI vaporised temperature 450 ºC, discharge current 5 μA, sheath gas flow 60 (arbitrary units). Specific extinction coefficients were obtained from literature (Villanueva et al. 199; Jeffrey 1997). Pigment concentrations were expressed in micrograms per gram of organic matter (μg g_{om}^{-1}). If specific extinction coefficients for compounds are not available, concentrations are expressed as absorbance units per grams of organic matter.

Statistical analysis

A Principal Component analysis was performed using the SPSS 12 package for Windows. For treatment, samples were taken as cases and photosynthetic pigments as variables. The main carotenoids and chlorin derivatives identified were included in the multivariate analysis (table 1, compounds marked with i). Epimers' values were added to their counterparts. The PCA data sets consisted of squared root transformed percentages contribution of variables to each sample. Zonation was performed with the software TILIA v.2.0.2 (2004), which uses CONISS (Grimm 1987) for the constrained cluster analysis with the Edwards and Cavalli-Sforza distance.

Time (min)	%Ammonium acetate (0.01M)	%Methanol	%Acetonitrile	%Ethyl acetate
0	5	80	15	0
5	5	80	15	0
100	0	20	15	65
105	0	1	1	98
110	0	1	1	98

Table 1. HPLC gradient employed inthis study.

Tabla 1. Gradiente de HPLC utilizado en este estudio.

6c. Results

Lake La Cruz sediment shows a laminated sequence in the uppermost 43 cm, although some detrital layers interrupted it. The sedimentary sequence recovered and analyzed in our study covers this calcite laminated section. The annual laminations allow an accurate chronology (Figure 2, section 5). Nevertheless the dating estimated by varves was confirmed also by means of ^{210}Pb radionuclide analysis. The sedimentation rates and dry mass accumulation rates at different depths obtained by means of varve-chronological model (see details of the chronological model in section 5) were fairly constant ca 1 mm y^{-1} and 0.1 g cm^{-2} y^{-1} (Figure 1). Both sedimentation rate and dry mass accumulation rates show an increase, to 5 mm y^{-1} and 0.5 g cm^{-2} y^{-1} respectively in the uppermost 6 cm, due to the higher influx of littoral material and the lack of compactation. Figure 2 shows the stratigraphic variations in loss-on-ignition (LOI). LOI is used as an indicator of the amount of total organic matter in the sediment. When calculated as per unit of dry weight, organic material is present at a relatively constant percentage (10-15 %) throughout the older sediment sequence up to 20 cm depth, and slightly increases upwards to 15-20%. Higher values of dry density indicated relative increase of mineral matter in the older part of the sediment sequence (44-35 cm depth).

Identification of photosynthetic pigments and their transformation products.

HPLC analysis of acetone extracts of Lake La Cruz sediment revealed a wide variety of algal and bacterial pigments (Table 2). Photosynthetic pigments were identified on the basis of chromatographic retention times, on-line UV/vis spectra and on-line MSn spectra. By this way it was possible to identify native chlorophyll_a and _b (peak 30 and 21) and their commonly observed derivatives

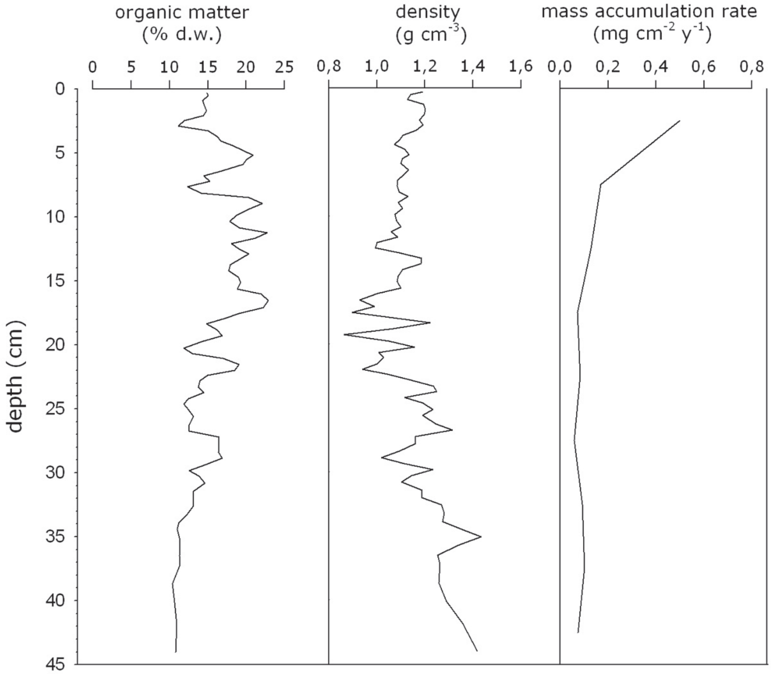


Figure 1. Stratigraphic variations in loss-on-ignition expressed as percent of dry weight, dry density (g cm^{-3}) and mass accumulation rate (mg cm^{-2}y^{-1}).

Figura 1. Variaciones en la columna sedimentaria de la materia orgánica expesada como portentaje del peso seco, la densidad en seco (g cm^{-3}) y la tasa de acumulacion (mg cm^{-2}y^{-1}).

phaeophytins (peak 53 and 46) and pyrophaeophytins (peak 58 and 55). Also, other minor chlorin_a derivatives were detected in some sediment samples and identified as dephytillated compounds from chlorophyll_a (phaeophorbide and pyrophaeophorbide, peaks 14 and 18) and oxidation products (purpurins and chlorophyllone; peaks 57, 6 and 8) according with their MSn spectra. In addition, late eluting clusters of peaks (59-62) with absorption spectra matching that of phaeophytin_a, were observed in almost all sections of the core. The MSn spectra of the protonated molecules confirmed these components as steryl chlorin esters (SCE´s) which are sterol esters of pyropheophorbide_a in which the phytyl moiety of pyropheophytin is replaced by sterol moieties. SCE`s have been shown as biotransformation products formed by zooplankton herbivory (Harris et al. 1995; Harradine et al. 1996; King and Wakeham 1996) and thus are accepted as direct and specific biomarkers of grazing activity.

The large number of structural isomers, similarities in UV/Vis spectra, and the lower ionisation efficiencies difficult an accurate carotenoid identification. Nevertheless major carotenoids were identified as alloxanthin (peak 5), lutein

peak	abs. max. (nm)	[M+H]$^+$ (m/z)	fragment ions	component			
1		484		mixoxanthophyll		mix	i
2	359/746	625	585-479	bacteriophorb a Me ester			
3	450			fucoxanthin-like		fucox-like	i
4	448			fucoxanthin-like		fucox-like	i
5	452/481	565	547	alloxanthin		Allox	i
6	410/664	533	515	chlorophyllone		phyllone	i
7	450/480	567	549	diatoxanthin		diatox	i
8	410/671	533		chlorophyllone		phyllone	i
9	445/473	569		lutein		Lut	i
10	450/478	569		zeaxanthin		Zeax	i
11	442/471	485		lutein-5,6-epoxy		lut-epox	a,i
12	436/463	569		lactucaxanthin		lac	i
13	447/476			diadinoxanthin		diadinox	i
14	409/663	607	547-461	phaeophorbide a		pheoph a	i
15	409/663	607	547-461	phaeophorbide a epimer			i
16	482	565		cantaxanthin		cantax	i
17	466			adinorubin		adoni	i
18	409/666	549	521	pyrophaeophorbide a		pyropheoph	i
19	386/686	887	609	bacterioviridin	Mg-link	bvir	
20	405/660	771	567-523	bacteriophaeophytin d	d2-farnesol	d2F	i
21	465/650	885	607-547	chlorophyll b		chl b	i
22	445/473			cryptoxanthin		crypto	i
23	405/660	785	581-537	bacteriophaeophytin d	d3-farnesol	d3F	i
24	465/650			chlorophyll b epimer			i
25	445/475	551		crocoxanthin		crocox	i
26	431/466			chlorophyll a allomer		OH-chl a	i
27	487	579	547	okenone		ok	i
28	487			okenone isomer			i
29	487			okenone isomer			i
30	431/466	871	593	chlorophyll a		chl a	i
31	409/660	789	567-523	bacteriophaeophytin d	d2-C16:1	d2-C16:1	i
32	409/660	867	695-551	bacteriophaeophytin d	d4-GG	d4-GG	i
33	466	551		echinenone		echin	i
34	431/466			chlorophyll a epimer			i
35	409/660	777	567-523	bacteriophaeophytin d	d2-C15:0	d2-C15:0	i
36	357/750	906	627-554	hydroxybacteriophaeophytin a		OH-bph a	i
37	409/660	843	535	unidentified chlorin			
38	409/660	791	567-523	bacteriophaeophytin d	d2-C16:0	d2-C16:0	i
39	409/660	871	594	unidentified chlorin			i
40	436/650	901	623-550	hydroxypheophytin b			
41	409/660	845	567-523	bacteriophaeophytin d	d2-phytol	d2-phytol	i
42	357/750	889	611	bacteriophaeophytin a		bph a	i
43		887	609	bacterioviridin	Mg free	bvir	i
44	462/493			chlorobactene		chlorbac	i
45	357/750			bacteriophaeophytin a epimer			i
46	436/650	885	607-547	phaeophytin b		ph b	i
47	410/664	887	609-550	hydroxyphaeophytin a		OH-ph a	i
48	436/650			phaeophytin b epimer			i
49	410/665	887	609-550	hydroxyphaeophytin a epimer			i
50	357/750	831	552-526	pyrobacteriophaeophytin a		pyrobph a	i
51	441/470			ε-carotene		e-car	i
52	447/475			α-carotene		a-car	i
53	410/665	871	593-533	phaeophytin a		ph a	i
54	447/475	537		β-carotene		b-car	i
55	436/650	827	549-521	pyrophaeophytin b		pyroph b	i
56	410/665			phaeophityn a epimer			i
57	408/697	843	565-503	purpurin 18 phytyl ester			
58	410/665	813	535-507	pyrophaeophytin a		pyroph a	i
59	410/665	903	535	sce a	C27sterol	SCE	i
60	410/665	929	535	sce a	C29sterol		i
61	410/665	931	535	sce a	C29sterol		i
62	410/665	947	535	sce a	C30sterol		i

a=tentative assignment, i= included in PCA

Table 2. Assignment of pigments found in Lake La Cruz sediment core.

Tabla 2. Identificación de los pigmentos encontrados en el sedimento de la Laguna de La Cruz.

(peak 9), zeaxanthin (peak 10), lactucaxanthin (peak 12), diadinoxanthin (peak 13) and β-carotene (peak 54). Besides, some minor carotenoids (concentrations lower than 1 μg g_{om}^{-1}) were observed and identified as fucoxanthin-like (peak 3-4), myxoxanthophyll (peak 1), diatoxanthin (peak 7), cantaxanthin (peak 16), cryptoxanthin (peak 22), crocoxanthin (peak 25), echinenone (peak 33), α-carotene (peak 51) and ε-carotene (peak 52). These biomarkers can be assigned to specific groups of phototrophs (Rowan 1989; Young and Britton 1993). While zeaxanthin and echinenone becomes mainly from cyanobacteria, alloxanthin, crocoxanthin and α-carotene trace crytophyceae contribution. Both fucoxanthin and diatoxanthin are common in diatoms, however diadinoxanthin and ε-carotene are also present likewise in other minoritary lacustrine algal groups from Lake La Cruz such as crysophyceae and dinophyceae. Other minoritary carotenoids like cryptoxanthin and cantaxanthin occur usually in chlorophyceae and cyanobacteria, respectively. However feeding experiments have confirmed that many lacustrine crustaceans can absorb and metabolize simple plants carotenoids as β-carotene, into complex, animal-specific pigments such as astaxanthin and cantaxanthin (Kleppel and Lessard 1992; Leavitt 1993). On the other hand, although chlorophyceae usually share photosynthetic pigments with vascular plants, mainly lutein and chlorophyll_b, lactucaxanthin occurs additionally in the latter and can be used as an specific biomarker of these plants (Siefermannharms et al. 1981; Phillip and Young 1995).

In addition to algal photosynthetic pigments, specific pigments of phototrophic sulfur bacteria were detected in Lake La Cruz sediment, confirming their presence in the lake over the time period covered by the sediment core. Although intact bacteriochlorophyll molecules were not observed, bacteriophaeophytins_a and _d, from purple and green sulfur bacteria respectively were present. Okenone (peak 27) and chlorobactene (peak 44), specific carotenoids of purple and green bacterial populations respectively, were registered through the sediment core although chlorobactene was present only in trace amounts. Noteworthy, oxidative derivatives of bacteriochlorophyll_a, such as OH-bacteriophaeophytin_a (peak 36) and bacterioviridin (peak 19-43), were observed in some sections of the Lake La Cruz core. Bacteriophaephytins_d (bph_d) show a complex distribution of seven homologues which exhibited structural variations, with three different macrocycles (alkylation pattern) and six different sterified alcohols. Peaks 20 and 23, gave ions [M+H]+ at m/z 771 and 785. On MS/MS fragmentation the protonated molecule gave rise to ions at 567 and 581, resulting from the loss of 204 daltons corresponding to farnesyl. Bph_d macrocycle at m/z 567 (d2) corresponds to an alkylation pattern of [Et, Et] or [n-Pr, Me] at C8 and C12. The other bph_d macrocycle (m/z 581) (d3) corresponds to an alkylation pattern of [n-Pr, Et] or [i-Bu, Me]. Four homologues with the same macrocycle (m/z 567) sterifyed by different alcohols; C16:1 (peak 31), C15:0 (peak 35), C16:0 (peak 38) and fityl (peak 41), were also identified. Finally, peak 32 corresponds to different macrocycle alkylation patterns at C8 and C12. The residual macrocycle mass indicates either [i-Bu, Et] or [n-Pent, Me] (d4) in the extent of alkylation, sterifyed by geranylgeraniol.

Besides all these compounds, the methodology procedure allow the extraction of some compounds which eluted the earliest with maximum absorbance around 330 nm, 360nm and 386 nm. Although the identification has not been improved in our case, it is known that these compounds can be related with UV-photoprotectors of phytoplanktonic organisms (Sinha et al. 1998).

Stratigraphic profiles and relationships between photosynthetic pigments

Figure 2 shows the changes in pigment concentrations thought the laminated sediment of Lake La Cruz. Looking at the stratigraphic profiles of pigment concentration, a strikingly feature of the biological signal can be observed; a common increasing trend from the bottom to the top of all photosynthetic compounds. Expressed as accumulation rate (μg cm^{-2} y^{-1}) of photosynthetic pigments, the stratigraphic profiles maintain similar variations because mass accumulation rates were steady through laminated sediment except at the topmost part of the core (Figure 1). Among the algae specific group of carotenoids, zeaxanthin, derived from cyanobacteria populations, was the most abundant compound. Regarding photosynthetic bacteria, the bacteriochlorophyll_a derivatives from purple sulfur bacteria (PSB) were also present in higher concentrations than bacteriochlophyll_d derivatives.

The vertical zonation of the sediment core established by constrained cluster analysis differentiates six zones (1A, 1B, 2A, 2B, 2C and 3; Figure 2). The zone 1A, below 38 cm, showed the lowest concentration of photosynthetic compounds, however, between 37 cm to 31 cm depth (1B), steryl chlorin esters (SCE´s) were detected for the first time and PSB pigments reached high concentrations. At zones 2A and 2B algal chlorins and carotenoid concentrations, mainly zeaxanthin and lutein, increased significantly. Unlike PSB derivatives, those derived from green sulfur bacteria (GSB) showed trace amounts in both the 2A and 2B zones, but were detected continuously and in higher concentrations through 2C and

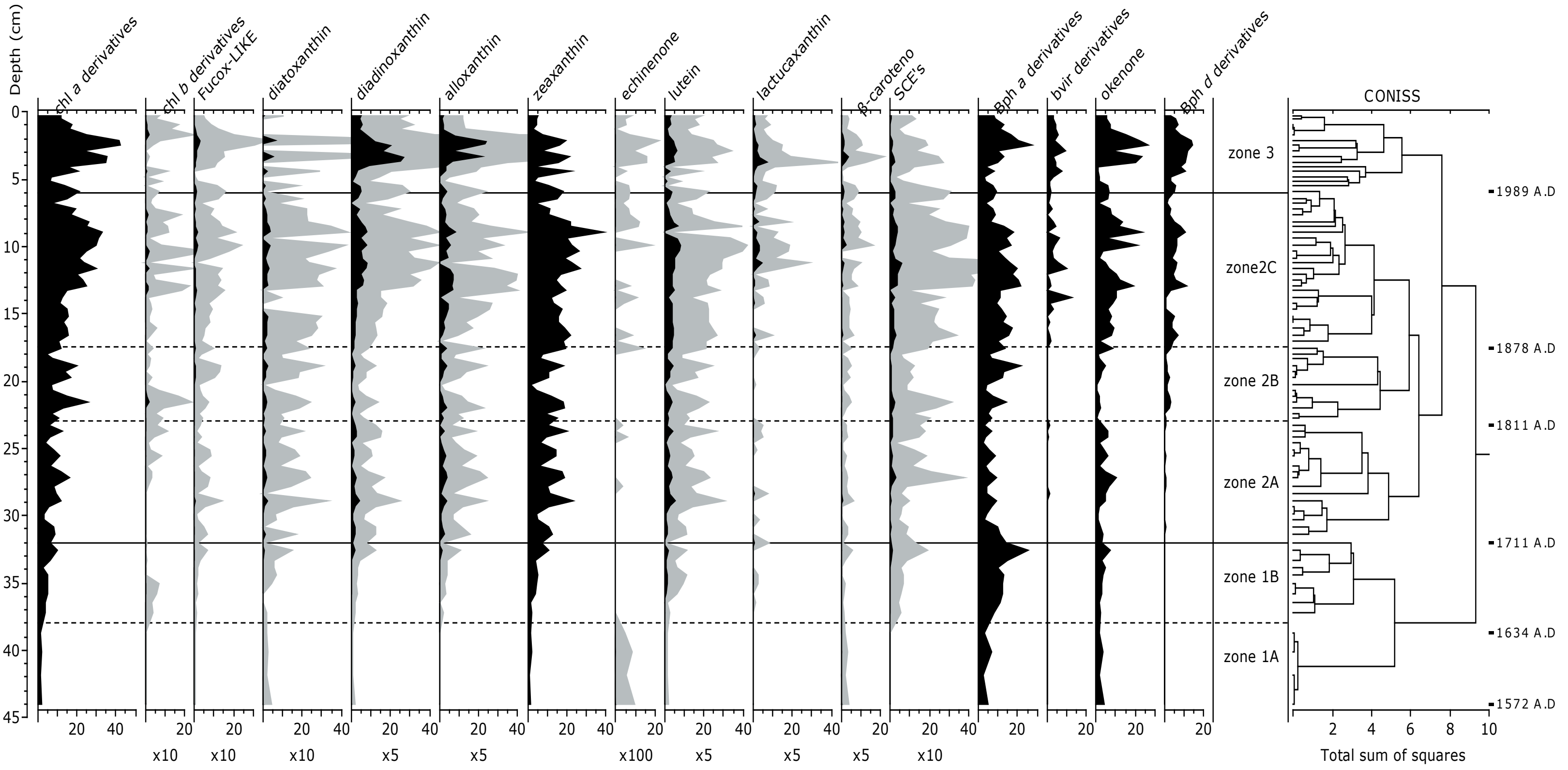


Figure 2. Stratigraphic profiles of photosynthetic pigments in a sediment core from Lake La Cruz. All compounds are expressed as μg pigment g^{-1} organic matter, except bvir derivatives (see methods). Exaggerated areas in grey. See text for taxonomic affinities.

Figura 2. Perfiles estratigráficos de los pigmentos fotosintéticos en el testigo sedimentario de la Laguna de la Cruz, expresados como μg pig. g^{-1} de materia orgánica, excepto bvir (ver métodos). areas exageradas en gris. Especificidades taxonómicas comentadas en el texto.

3 zones. The highest concentration of zeaxanthin was recorded in zone 2C, around 9 cm depth. However a general decrease of pigment concentration could be observed at the top of zone 2C. In the uppermost zone 3, from 6 cm depth to the top of the core the most abundant carotenoids are alloxanthin and diadinoxanthin, which are related with cryptophyceae and mostly dinoflagellate populations respectively.

Among the identified compounds, those derived by transformation reactions were analysed in detail. Chlorophyll_a undergoes three primary early diagenetic reactions; demetallation (pheophytins), loss of the C13-COOMe moiety (pyro derivatives), and hydrolysis of the phytyl chain (pheophorbides). In order to estimate the relative extent to which each of these degradative reactions has occurred, the percent of compounds that have resulted from each degradation process was calculated (figure 3). Among chlorophylls, demetallation is the main process affecting 80-90% of the molecules, although systematic down core change was not observed. Formation of pyroderivatives is relatively the second extensive transformation process. In this case remarkable higher percentages, indicating a relative increase of the importance of this transformation reaction, were observed in the oldest sediment zone. By contrast, dephytylated derivatives were highly correlated with specific biomarkers of grazing activity, SCE's, suggesting that in general they are derived from biotransformation processes occurring in water column.

Given the differences in chemical stabilities, the ratios between different pigments groups could add information related with the preservation conditions. For instance, laboratory and field studies have confirmed slower degradation rates and better preservation of carotenes against xanthophylls, which include oxygen in the molecule. In our case this ratio, xanthophylls to carotenes, showed significantly lower values in the bottom part of the core, indicating a probably loss of xantophylls relative to total carotenoids. By contrast, the ratio between chlorin derivatives to total carotenoids, which has been used as degradation proxy (although many factors could interfer), showed a slight trend and reached higher values at some defined depths coinciding with the occurrence of detrital layers observed in the sediment core.

The performed multidimensional statistical analyses based on relative pigments concentration resulted in two first principal components (Figure 4) which together explain 32.68% of the variance. Onto the first principal component (18.56% of the variance), cyanobacteria, chlorophyceae and

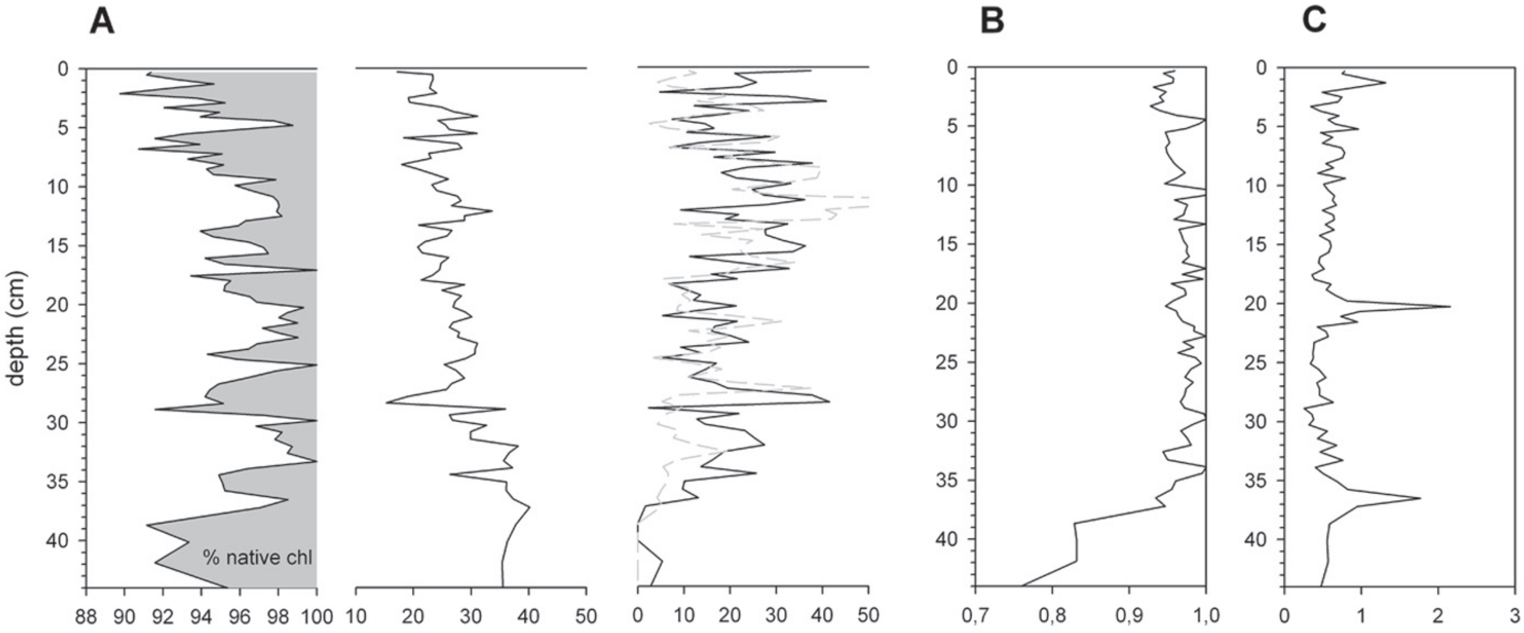


Figure 3. Stratigraphic profiles of diagenic proxies. A; percentage of chloropigments that have loss the central Mg atom (demetallation) and percentage of native chlorophyll in grey area, the percentage of pyroderivatives and the percentage of dephytyllated derivatives including phaeophorbides and chlorophyllones and SCE's concentrations ($\mu g\ g_{o.m.}^{-1}$, exaggerated one fold) in dashed line. B; xanthophyll: total carotenoids ratio, C; chlorin derivatives: total carotenoids ratio.

Figura 3. Perfiles de indicadores de procesos diagenéticos. A; porcentaje de clorinas que han perdido el átomo central de Mg, área gris porcentaje de clorofila nativa, porcentaje de piroderivados y porcentaje de derivados que han perdido la cadena fitol incluidos feoforbidos y clorofilonas, concentraciones de SCE ($\mu g\ g_{o.m.}^{-1}$, exagerado un orden de magnitud). B; relación xantofilas: total carotenos, C; relación derivados clorofila y carotenos totales.

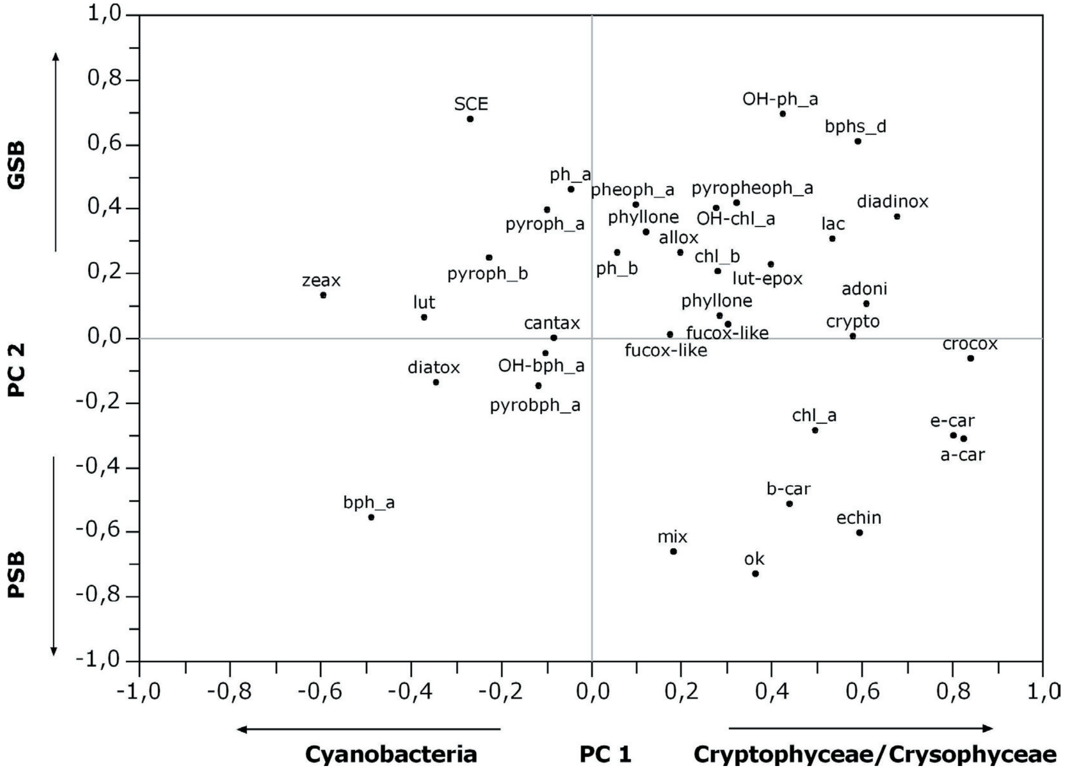


Figure 4. Distribution of the variables in the space defined by the two principal components resulting from the PCA. Arrows indicate the phototrophic group whose specific pigments have the highest loading onto PC1 and PC2.

Figura 4. Distribución de las variables en el espacio definido por los dos componentes principales resultado del análisis multivariante. Las flechas indican el grupo de organismos cuyos pigmentos específicos poseen valores más altos para cada componente.

diatoms carotenoids were negative loaded while cryptophyceae's secondary carotenoids such as crocoxanthin, and crysophyceae's carotenoids such as α- and ε-carotene, diadinoxanthin were positive correlated. On the other hand PC2, which explains 14.11% of the variance, clearly differentiates between anoxygenic sulfur bacteria derivatives from purple sulfur bacteria (PSB) and green sulfur bacteria (GSB); bacteriopheophytin_d homologs from GSB were positive correlated whereas the pigments of PSB, bactph_ a and okenone, were negative loaded onto PC2. Figure 5 shows the samples score for the two principal components. Sample scores for PC1 were positive in the lowermost section before 1630 A.D and afterward showed negative scores during approximately 250 years. However PC1 sample scores changed and returned to be positive since c.a 1900. By contrast samples were extremly negatively scored on PC2 at the bottom. However, a gradual increasing trend could be observed 1630 A.D onwards.

6d. Discussion

The annually laminated sediment of Lake La Cruz provides a temporal framework where the phototrophic community changes could be evaluated at high-resolution. The biological signal preserved in Lake La Cruz sediment over the last 430 years showed two main features, (1) a long-term increasing trend in photosynthetic pigment concentrations and (2) a stable composition of the phototrophic signature through the sedimentary sequence. The higher pigment concentrations towards the uppermost depth may indicate a progressive increase of the primary productivity over the last centuries. Nevertheless, interpretation of production changes based on pigment data should be done with caution. This long-term trend could additionally be related with systematic change of diagenic conditions involving breakdown and transformations of photosynthetic pigments and/or a change in the sedimentation patterns or mass accumulation rates. In the case of Lake La Cruz, however, a previous study of sedimentation process in Lake La Cruz (Miracle et al. 2000; Romero et al. 2006, section 3) concluded that nowadays sedimentation is mainly accomplished in two discrete pulses which determine the formation of the laminated sediment. Therefore the presence of calcite laminations implies the assumption that the sedimentation processes have been similar to present through the laminated sequence. Moreover, annual laminations offer a accurate chronological model which showed no drastic shifts in sediment accumulation rates. Other paleolimnological records have shown that an increase in the accumulation rate have a dilution effect on pigment concentrations underestimating lake productivity (Swain 1985; Engstrom et al. 1985). In Lake La Cruz the stratigraphic profiles of pigment concentrations are not misleading, since a steady mass accumulation rate was registered within laminated sediment.

Furthermore, the transformation products related with diagenetic processes did not show an evident trend down core within the sedimentary sequence. The most extensive transformation reaction, demetallation, fluctuates through stratigraphic profile from Lake La Cruz showing a lack of systematic down core change. The differences observed between relative contribution of bacterial native compounds in settled material recovered by sediment traps (Romero et al. 2006, section 3) and the lack of these compounds in the sediment core compared to the material collected by sediment traps confirms that photosynthetic pigment transformations follow extensively after sedimentation. However pheophytin, the dominant transformation product of native chlorines, is known to be very stable under anoxic conditions (Sun et al. 1993). Moreover, the ratio between

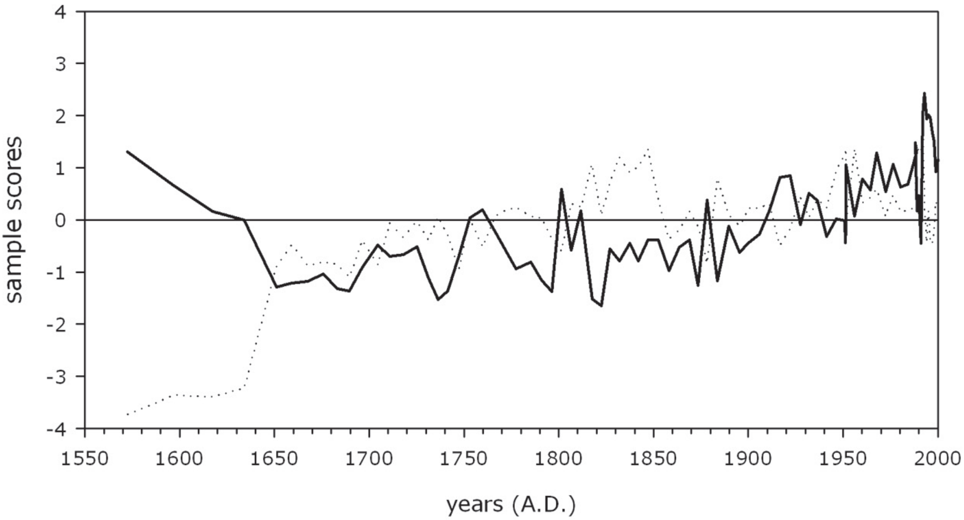


Figure 5. Temporal evolution of sample scores for PC1 (solid line) and PC2 (dotted line).

Figura 5. Evolución temporal de los valores de las muestras en el componente principal el 1 (línea continua) y 2 (línea punteada).

xanthophylls to carotenes (Leavitt and Carpenter 1990a; Steenbergen et al. 1994) share a similar lack of pattern through the sedimentary sequence except at the bottom (43 to 37 cm depth) where lower values of the xantophyll to carotene ratio were found, probably suggesting a not stable anoxic bottom water layer until approximately 1634 A.D. As this regard, there is not another direct evidence of this possible unstable bottom anoxia, although this initial stage is clearly differentiated by the cluster analysis (zone 1A). In addition it is important to consider that calcite laminated sediment occurs in several holomictic lakes, which have optimal conditions for preservation of annual laminations, such as periods of hypolimnetic summer anoxia (i.e. Gruber et al. 2000; Peck et al. 2002; De Vicente et al. 2006). This means that the onset of laminations could mark the onset of a dynamic process towards meromixis instead of permanently anoxic monimoliminion in agreement with pigment evidence. By contrast the ratio chlorin derivatives to total carotenoids, which was frequently used likewise as a degradation proxy, showed a weak steady trend as well as exceptionally high values at discrete layers. However many different factors could interfering in this ratio (Swain 1985) indicating in this case occasional allocthonous inputs and a progressive eutrophication process as reported for other lakes (Engstrom et al. 1985) rather than a diagenetic change. Consequently, a detailed analysis of chlorin and carotenoid derivatives suggest in our case that the variation of photosynthetic pigment concentration within the sedimentary sequence may be considered directly related with the primary production variability because there are substantial evidences of the lack of down-core changes in diagenetic/ preservation conditions.

Two phases of "response" by the phototrophic community could be differentiated over the last centuries. The first stage was characterised by the low photosynthetic pigments concentrations at the beginning of the supossed meromictic condition until 32 cm depth (c.a. 1711 A.D). This lower productivity period could be due to the reduced flux of nutrient-rich deep water into the mixolimnion when the monimolimnion is increasingly stable. A similar pattern of productivity reduction just after the chemical stability and thus isolation of deep water layer was also observed, for instance, in Mono Lake (California, USA) (Jellison and Melack 1993). The photosynthetic activity measured during a 8-yr period in Mono Lake, spanning the onset, persistence and breakdown of its ectogenic meromixis, indicates that algal biomass in spring and autumn decreased following the onset of the meromixis and that the annual photosynthetic production was reduced compared to nonmeromictic conditions. In Lake La Cruz, after this initial phase, pigment concentrations indicated a progressive increase of primary productivity since ca. 1711 A.D.

Nevertheless this long term increase of lake production and its fluctuations are not linked to shifts in sedimentary pigment assemblages. The pigment signature is characterized by the main contribution of zeaxanthin (around 50% of total carotenoid concentrations through the laminated sequence), a specific carotenoid of cyanobacteria which remains stable from 1600 A.D. to the second half of the 20th century. Although sedimentary pigment signature could not be immediately interpreted as the composition of the phytoplankton assemblage, the high contribution of zeaxanthin through the sedimentary sequence and the high densities reached by present *Synechococcus sp* population in Lake La Cruz (Camacho et al. 2003a) suggest a significant contribution of the cyanobacterial populations in the primary productivity of the whole system both during the period of relative low trophic status and the further progressive increase. Other cyanobacterial populations as *Anabaena sp*. and *Mycrocystis sp*. have been detected in Lake La Cruz (Dasi and Miracle 1988), although in lower concentrations. The absence of changes in the relative abundances of specific photosynthetic pigments of cyanobacteria populations such as zeaxanthin, echinenone and myxoxanthophyll suggests a stable biodiversity within the cyanobacterial group and the constant relative dominance of picocyanobacteria over the period studied which unlike other cyanobacterial populations usually

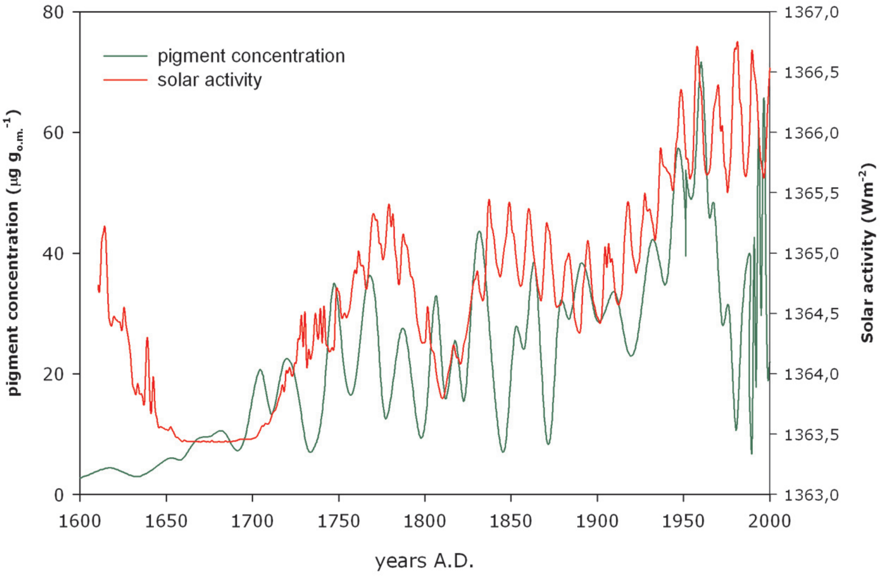


Figure 6. Comparision of pigment concentration (zeaxanthin and total chlorin a derivatives) (solid green line) and solar irradiance variations arising from de 11-year activity cicle (Lean 2000) (solid red line).

Figura 6. Comparación entre la concentración de pigmentos fotosintéticos (suma total de los derivados de la clorofila a y zeaxantina) (línea continua verde) y las variaciones en la radiación solar (Lean 2000) (línea continua roja).

contains high amounts of zeaxanthin.

Although a satisfactory explanation of the changes occurring on primary production is difficult to achieve in lacustrine ecosystems because of many factors interacting in a complex way, the temporal pattern of sedimentary signal in Lake La Cruz, in particular zeaxanthin plus total chlorin_a derivatives concentrations (around 45% of the total pigment concentration), has showed a significant relationship with the solar activity reconstruction (Lean 2000) over the last three centuries as given by the NOAA web page (Figure 6), suggesting that changes in underwater light climate could be a control factor for the assemblage of primary producers. Currently, a deep chlorophyll maximum can be observed in the vertical profile of Lake La Cruz during the water column stratification period (section 3). Likewise, deep chlorophyll maxima (DCM) have been reported for oligotrophic and mesotrophic temperate lakes of North America, Central and northern Europe where dense populations of metalimnetic phototrophs develop taking advantage of higher nutrient availability (Camacho 2006, and reference therein). In Lake La Cruz, the DCM is mainly formed by picocyanobacteria, although diatoms and cryptophytes, as well as other algae form deep chlorophyll layers (Camacho 2006). The adaptive features of picocyanobacteria seem to favour its population growth under Lake La Cruz's conditions (Camacho et al. 2003a, 2003b), and probably its relative dominance during thermal stratification periods over the last centuries as in the present. The advantage of picocyanobacteria is explained by the high surface-to-volume ratio, promoting a higher nutrient uptake respect to eukaryotic algae and the ability to harvest green light by means of phycoerythrin (Camacho et al. 2003b), a phycobylin(protein)-type photosynthetic pigment that due to its proteic nature does not preserve in the sediment. The ability to grow in the low yellow-green enriched field in the deep waters of the upper hypolimnion allows these cyanobacteria to cope with nutrient depletion in surface waters by growing in deeper layers where nutrient availability is higher. The *in situ* inorganic carbon assimilation measurements have confirmed that, in spite of commonly showing the absolute maxima of carbon photoassimilation, the populations of metalimnetic phototrophos forming DCM are usually light limited (Camacho 2006). Therefore, past fluctuations of light irradiance at lake water column due to solar variability could have significant effects of phototrophs, as remarked by other studies that have reported indirect evidences of solar variability on lake primary production (Livingstone and Hajdas 2001; Bradbury et al. 2002). Nevertheless the effects on lake production derived from other factors could no be ruled out, especially those related with watershed area (i.e. Ryback and Dickman 1988). In fact, a previous paleolimnological approach in Lake La Cruz at lower resolution has shown that the pollen sequence coinciding with the initiation of laminated sequence indicates a decrease in the extend of forests and reclamation of land for agricultural purposes (Julià et al. 1998). Consequently, increased soil erosion and associated nutrient input to the lake could also contribute to the eutrophication process.

In spite of the long-term compositional stability of the phototrophic community, the pigment signature has shifted as indicates the progressive higher positive sample scores for PC1 over the last two decades. This compositional change is due to the significant increase of dinophyta and cryptophyta populations biomarkers in relation to their past sedimentary concentrations. Since the end of the eighties the algae community of Lake La Cruz was extensively studied. The results of water column and sediment traps confirmed the development and stability of the cryptophyceae population through the year near the oxicline depth and its continuous sedimentation whereas dinoflagellates reached higher concentration during spring and late summer-autumn, thus settling mainly during late summer and before overturn (Dasi and Miracle 1989). However the last twenty years is a very short time-window to infer which factors could be operating behind the change in these two phytoplanktonic populations. Nevertheless we speculate that among several factors which can not be ruled out, the persistent positive anomalies registered in seasonal temperatures especially during spring and autumn over the last twenty years (Figure 3, section 4) could expand the thermal stratification period, delaying fall overturn and thus increasing dinoflagelletes growth period. This environmental change of preferential growth conditions of dinoflagellates which have been considered as k-strategists (Reynolds 1984), usually present in water column at the end of annual succession, could explain the relative increase of diadinoxanthin sediment contribution.

Regarding the anoxygenic productivity, the pigment signature indicates that, like nowadays (Rodrigo et al. 2000), two different populations of phototrophic bacteria, Green Sulfur Bacteria (GSB) and Purple Sulfur Bacteria (PSB) were present in Lake La Cruz during the last centuries. In meromictic lakes, the existence of sharp gradients offers a suitable environment for the development of photosynthetic anoxygenic bacteria (Repeta 1993; Overman et al. 1993; Hodgson et al. 1998) where usually green and purple populations coexist, although they have different requirements (Van Gemerden and Mas 1995). The changes observed in their relative dominance (PC 2) could be related with the differences in optimal conditions for each bacterial development, mainly differences in light quality and quantity at oxycline depth (Parkin and Brock 1980; Vila and Abella 1994) and sulphide availability. The sediment samples were negative scored for PC 2 at the beginning of the laminated sequence until 1711 A.D., due to the exclusive presence of purple bacterial derivatives. Afterward bph_d from GSB was detected indicating the coexistence of GSB and PSB populations, although the low concentrations of bchl_ d derivatives until 17 cm depth (1878 A.D.) suggest that the GSB growth was limited. In Lake La Cruz at present day the prevailing sulphide concentrations are low (Rodrigo et al. 2000), since sulphate concentrations are very low and the anaerobic degradation of organic matter is mainly following the methanogenic pathway, and consequently the production of sulfide becomes only from the degradation of –SH groups from proteins and not from sulphate acting as electron acceptor, which gives a much lower sulfide yield per unit of organic matter consumed. Both GSB and PSB use sulphide as an electron donor, but PSB could store intracellular sulfur granules that can also be used as electron donor for photosynthesis, thus being less dependent of sulphide concentration in the water column. Therefore

GSB population started to develop conspicuous populations in Lake La Cruz when the progressive increase of oxygenic productivity could provide enough sulphide derived by organic matter recycling.

Nevertheless higher deep oxygenic production had a limitation effect on PSB populations. Since the presence of oxidative derivatives such as bvir and OH-bph_a in settling material is related to the microaerobic conditions in the upper layer of the oxicline (Romero et al. 2006), the observed increase of their concentrations in zone 2C and 3, suggest that past PSB plate had to move up in the vicinity of microaerobic layers trying to avoid the shading and light intensity reduction. Rodrigo et al. (2000), by means of laboratory experiments, have confirmed that nowadays PSB from Lake La Cruz were much more sensitive to lower light conditions than GSB. The motile capacities of PSB together with the intracellular sulfur storing capacity allow them to actively adjust themselves to the light climate conditions (Clayton and Sistrom 1978). The wide absorption range provided by the okenone plus the Soret and Qx bands of the Bchl_a (up to 500-600 nm) gives Chromaticeae the capacity to develop in a high diversity of light quality conditions. However their green-yellow light harvesting adaptation like phycocyanin-phycoerythrin containing cyanobacteria makes them more dependent of light intensity than GSB populations. By contrast GSB are adapted to light climate limitation at oxicline by either synthesis of different grade of alkilatation homologues and sterification of higher alcohols than farnesol, which allow effective energy transfer of bacteriochlorophyll_d (bchl_d) (Borrego et al. 1998; Wilson et al. 2004b). Furthermore the specific contents in bchl_d are very high in present GSB natural populations of Lake La Cruz (Rodrigo et al. 2000), being one of the highest described in the literature (Van Gemerden and Mas 1995).

Even though the meromictic stratification pattern was established most probably short time after the onset of laminations as commented above, the analysis of photosynthetic pigments have not provided direct evidences about the timing of permanent chemical stratification. However, the onset of laminations at the end of the 16th century suggest that the climate forcing could be behind the meromixis origin in Lake La Cruz as firstly proposed Julià et al. (1998). According to Font-Tullot (1988), the historical records of very cold episodes begin in the 15th century, mainly in Europe, although the situation was extreme in the second half of the 16th century, with global extension. Either cold winters (extended ice-cover) and warm-dry summers (Ambrosetti and Barbanti 2005; Jankowski et al. 2006) could lead towards meromixis by different ways, as proposed Schmidt et al. (2002) for lake Längsee. In the case of Lake La Cruz, extreme cold winters at the end of 16th century likely could result in prolonged anoxia promoted by the ice cover on the lake lasting for longer periods, enhancing the accumulation of dissolved ions in bottom waters. Moreover other factors are considered to have a synergic effect. On one hand the possible increase of water level registered with the onset of laminations evidenced by other biological evidences (Mezquita and Miracle 1997; Julià et al. 1998) could enhance the stability effect derived from morphological lake features (wind sheltered basin and the relative depth). In addition CO_2 oversaturation under strong hypolimnetic anoxia could cause calcite dissolution and therefore accumulation of dissolved calcium and bicarbonate ions could enhanced chemical stratification in the earlier development of meromixis (Wüest et al. 1992). Furthermore the drastic drop in the temperatures during the end of the 17th century (Lutherbacher et al. 2004), known as Maunder Minimum (1675-1700 A.D.), probably reinforced this process.

As a conclusion, the sedimentary pigment signature has provided a valuable assistance in the characterization of long-term variability of Lake La Cruz productivity and the understanding of forcing factors involved. However, there are still many processes in the lacustrine response to climate forcing to be understood in more detail. The key to a better understanding is the combination of present limnological knowledge and the long-term perspective provided by the lake sediments. Predictions on lacustrine ecosystems trajectories within the global change debate will request future work to exploit the information contained in lake sediments.

7. Past phototrophic organisms assemblages as response to climate variability inferred from preserved pigments in the sediment of Lagunillo del Tejo

7a. Introduction

In closed basins where the catchment is relatively small, the link between hydroclimatic variablity and lake level is presumed to work through the balance of precipitation and evapotranspiration acting upon the lake surface and groundwater (Dearing 1997). Especially in semiarid and semihumid regions, changes in lake levels may be closely linked to climatic shifts (Smol and Cumming 2000). In these areas closed-basin systems which lack inlets and outlets, have been shown to produce regionally consistent sedimentary records of climate-controlled lake-level fluctuations (Verschuren 1999; Rodo et al. 2002). Closed lakes seems to act as low-pass filters of climatic variables (Mason et al. 1994).

Water level fluctuations may have an overriding effect on the lacustrine ecology, mainly due to a structuring role in spatial and temporal extension and functioning of planktonic-littoral and aquatic-terrestial transition zone (Coops et al. 2003). The effects of water levels are widespread, involving effects on nutrient retention capacity and indirect changes in the foodweb biotic interactions. However the role of water level fluctuations is far from being fully understood, since lakes respond in a non-linear fashion to disturbance. Indeed the character and intensity of a lake response can vary considerably conditioned by its local and regional landscape characteristics (geographic position, catchment characteristics and lake morphometry) and internal filter comprising lake history and biotic/abiotic interactions (Magnuson et al. 1990; Blenckner 2005). Therefore understanding the role of water level fluctuations in ecosystem functioning by long-term perspectives has become even more crucial especially with current concerns about global climate change (Coops et al. 2003).

This section presents a paleolimnological study over the last centuries from the closed-basin lake Lagunillo del Tejo located in the eastern Spain and exposed to the mediterranean climate dynamics. Lagunillo del Tejo has undergone fluctuations in water depth of several meters in response to interannual variations in ground water levels during the last twenty years. The aim of this study is to assess the changes in primary producers community over last centuries and elucidate if climatic perturbations conditioned autotrophic composition of the foodweb. Photosynthetic pigments and derivatives preserved in the sediment were used to perform the paleolimnological reconstruction, since these biomarkers are biosynthetised exclusively by phototrophic organisms and, as such, are ideal proxy for the primary producer community existing in the past (Züllig 1981).

7b. Methods

A 37 cm core of 2.5 cm diameter was recovered from the pond at its deepest point in May 2003, using a gravity corer. The core was extracted in a metacrylate cylinder and was quickly protected from light by opaque wrapped and stored in a cold chamber. The core was transported to the laboratory, where it was sectioned. The core was sliced into 2-3 mm sections, sealed in sterile "Whirlpack" bags and conserved at –20ºC in darkness until analysis. Water content and density were determined by drying 2-3 g of wet sediment at a temperature of 105 ºC for 2 hours. Organic matter was obtained by the loss-on-ignition of dried sediment samples at 460ºC for 6 hours (APHA 1992). Sediment dating was performed by ^{210}Pb, ^{137}Cs and ^{226}Ra detection by gamma spectrometry at the analytical service of the Department of Physics of the Autonomous University of Barcelona (Spain). Regarding to photosynthetic pigments, analytical procedures were performed as described previously in section 6.

Multivariate statistical analysis

Principal Component analysis was performed using the SPSS 12 package for Windows. The PCA data set consisted of normalised absolute abundances of photosynthetic pigment in the sedimentary column. The variables were normalised by calculating for each sample the percentage with respect to the total amount of each pigment in the whole core and then square root transformed. The variables included in the analysis were main carotenoids and chlorin derivatives identified in the sediment sequence (table 1, compounds marked with i). Epimer values were added to their counterparts.

7c. Results

Identification of photosynthetic pigments and transformation products.

HPLC analysis of acetone extracts of Lagunillo del Tejo sediment revealed a wide range of algal and bacterial pigments (table1) and changes in abundances throughout the core. Photosynthetic pigments were identified on the basis of chromatographic retention times, on-line UV/vis spectra and on-line MSn spectra. In this manner it was possible to identify native chlorophyll_a and _b (peak 32 and 23; chl_a and chl_b) and their commonly observed derivatives phaeophytins (peak 57 and 49; ph_a and ph_b) and pyrophaeophytins (peak 64 and 59; pyroph_a and pyroph_b). Also, other minor chlorin a derivatives were detected in some sediment samples and identified as dephytillated compounds from chlorophyll_a (phaeophorbide and pyrophaeophorbide, peaks 15 and 20; pheoph_a and pyropheoph_a) and oxidation products (purpurins and chlorophyllone, peaks 56, 62, 6 and 8 respectively) according with their MSn spectrums. In addition, late eluting clusters of peaks (64-73) with absortion spectra matching those of phaeophytins_a and _b, were observed in almost all sections of the core. The MSn spectra of the protonated molecules confirmed these components as steryl chlorin esters (SCE´s) which are sterol esters of pyropheophorbide in which the phytyl moiety of pyropheophytin is replaced by sterol moieties. Mass chromatography enable the identification of five saturated, mono and di unsaturated C29-C30-C31 sterols as esterifiying alcohols. SCE`s are biotransformation products formed by zooplankton herbivory (Harris et al. 1995; Harradine et al. 1996; King and Wakeham 1996) and thus are accepted as direct and specific biomarkers of grazing activity.

The large number of structural isomers, similarities in UV/Vis spectra and the lower ionisation efficiencies difficult accurately carotenoid identification. Nevertheless major carotenoids were identified as alloxanthin (peak 5, Allox), lutein (peak 9, Lut), zeaxanthin (peak 10, Zeax), lactucaxanthin (peak 12, Lac), diadinoxanthin (peak 13, Diadinox) and β-carotene (peak 58, b-car). Besides some minority carotenoids (concentrations lower than 1 $\mu g\ g_{om}^{-1}$) were observed and identified as fucoxanthin (peak 3, fucox), mixoxanthophyll (peak

peak no.	abs. max. (nm)	[M+H]+ (m/z)	fragment ions (m/z)	component			
1	484			mixoxanthophyll		mix	
2	359/746	625	585-479	bacteriophorb a Me ester		bpheoph a	i
3	450	659(581)		fucoxanthin-like		fucox-like	
4	407/433			aureoxanthin			
5	452/481	565	547	alloxanthin		Allox	i
6	410/664	533	515	chlorophyllone		phyllone	i
7	450/480	567	549	diatoxanthin		diatox	i
8	410/671	533		chlorophyllone		phyllone	i
9	445/473	569		lutein		Lut	i
10	450/478	569		zeaxanthin		Zeax	i
11	442/471	485		lutein-5,6-epoxy		lut-epox	a,i
12	436/463	569		lactucaxanthin		lac	i
13	447/476			diadinoxanthin		diadinox	i
14		623	595-503	OH-phaeophorbide a			
15	409/663	607	547-461	phaeophorbide a		pheoph a	i
16		607	547-461	phaeophorbide a epimer			i
17	407/664	713	535	unidentified chlorin			
18	482	565		cantaxanthin		cantax	i
19	466			adinorubin			
20	409/666	549	521	pyrophaeophorbide a		pyropheoph	i
21	386/686	887	609	bacterioviridin	Mg-link	bvir	i
22	405/660	771	567-523	bacteriophaeophytin d	d2-farnesol	d2F	i
23	465/650	885	607-547	chlorophyll b		chl b	i
24	445/473			cryptoxanthin			
25	405/660	785	581-537	bacteriophaeophytin d	d3-farnesol	d3F	i
26	465/650			chlorophyll b epimer			
27	445/475	551		crocoxanthin			
28	434/664			chlorophyll a allomer		OH-chl a	i
29	487	579	547	okenone		ok	i
30	487			okenone isomer			
31	487			okenone isomer			
32	431/466	871	593	chlorophyll a		chl a	i
33	409/660	789	567-523	bacteriophaeophytin d	d2-C16:1	d2-C16:1	i
34	409/660	867	695-551	bacteriophaeophytin d	d4-GG	d4-GG	i
35	466	551		echinenone			
36	431/664			chlorophyll a epimer			i
37	409/660	777	567-523	bacteriphaeophytin d	d2-C15:0	d2-C15:0	i
38	357/750	906	627-554	hydroxybacteriophaeophytin a		OH-bph a	i
39	409/660	841	535-507	unidentified chlorin			
40	409/660	843	535-507	unidentified chlorin			
41	409/660	791	567-523	bacteriophaeophytin d	d2-C16:0	d2-C16:0	i
42	409/660	871	594	unidentified chlorin			i
43	436/650	901	623-550	hydroxyphaeophytin b			
44	409/660	845	567-523	bacteriophaeophytin d	d2-phytol	d2-phytol	i
45	357/750	889	611	bacteriophaeophytin a		bph a	i
46		887	609	bacterioviridin	Mg free	bvir	i
47	462/493			chlorobactene		chlorbac	i
48	357/750	889	611	bacteriophaeophytin a epimer			i
49	436/650	885	607-547	phaeophytin b		ph b	i
50	410/664	887	609-550	hydroxyphaeophytin a		OH-ph a	i
51	436/650	885	607-547	phaeophytin b epimer			i
52	410/665	887	609-550	hydroxyphaeophytin a epimer			i
53	357/750	831	552-526	pyrobacteriophaeophytin a		pyrobph a	i
54	441/470			ε-carotene			
55	447/475			α-carotene			
56		917	639-552	purpurin 7 phytyl ester			
57	410/665	871	593-533	phaeophytin a		ph a	i
58	447/475	537		β-carotene		b-car	i
59	436/650	827	549-521	pyrophaeophytin b		pyroph b	i
60	410/665			phaeophytin a epimer			i
61	386/686	830	551-508	unidentified chlorin			
62	408/697	843	565-503	purpurin 18 phytyl ester		purpurin	i

a=tentative assignment, i= included in PCA

63	410/665	813	535-507	pyrophaeophytin a		pyroph a	i
64	437/650	918	549	sce b			
65	437/650	944	549	sce b			
66	410/665	903	535	sce a	C27sterol	SCE	i
67	410/665	929	535	sce a	C29sterol		i
68	410/665	917	535	sce a	C28sterol		i
69	410/665	931	535	sce a	C29sterol		i
70	410/665	919	535	sce a	C28sterol		i
71	410/665	945	535	sce a	C30sterol		i
72	410/665	933	535	sce a	C29sterol		i
73	410/665	947	535	sce a	C30sterol		i

Table 1. Assignment of pigments found in Lagunillo del Tejo sediment core.

Tabla 1. Identificación de los pigmentos encontrados en el sedimento del Lagunillo del Tejo.

1), auroxanthin (peak 4) (Mallorqui et al. 2005), diatoxanthin (peak 7, Diatox), cantaxanthin (peak 18, Cantax), cryptoxanthin (peak 24), crocoxanthin (peak 27), echinenone (peak 35), α-carotene (peak 55) and ε-carotene (peak 54). These biomarkers can be assigned to specific phototrophs groups (Rowan 1989; Young and Britton 1993). While zeaxanthin, echinenone and mixoxanthophyll are derived mainly from cyanobacteria, alloxanthin, crocoxanthin and α-carotene are biomarkers of cryptophyceae contribution. Fucoxanthin and diatoxanthin are common in diatoms, however diadinoxanthin and ε-carotene are present likewise in other minoritary lacustrine algal groups such as crysophyceae and haptophyceae. Other minoritary carotenoids like cryptoxanthin and cantaxanthin occur usually in clorophyceae and cyanobacteria, respectively. However feeding experiments have confirmed that many lacustrine crustaceans can absorb and metabolize simple plant carotenoids as β-carotene, into complex, animal-specific pigments such as astaxanthin and cantaxanthin (Kleppel and Lessard 1992; Leavitt 1993). Although chlorophyceae usually share photosynthetic pigments with vascular plants, mainly lutein and chlorophyll_b, lactucaxanthin occurs additionally in the latter (Siefermannharms et al. 1981; Phillip and Young 1995). On the other hand, in some sediment extracts a peak (peak 11, λ_{max}=442/471 nm; m/z 485) eluted between lutein and zeaxanthin was detected. The molecular similarity of these compounds prevents a clear chromatographic separation and dificulted mass spectrometry differentiation. In spite of this, the peak was tentatively assigned as lutein 5-6 epoxy (lut-epox), oxidised derivative of lutein and known as taraxanthin likewise. There are scarce references about this lutein derivative, however it was found in recent sediments and its occurrence related with lutein and chlorophyll_b could be associated to macrophyte debris (Brotas and Plante-Cuny 2003).

In addition to algal photosynthetic pigments, specific pigments of phototrophic sulfur bacteria were detected in Lagunillo del Tejo sediment, confirming their presence in the lake over the time period studied. Although intact bacteriochlorophylls were not observed, bacteriophaeophytins_ a and _d, from purple and green sulfur bacteria respectively were present. Okenone (peak 29, Ok) and chlorobactene (peak 47, chlorbact), specific carotenoids of purple and green sulfur bacteria respectively, were registed through the sediment core although chlorobactene was present in trace amounts. Noteworthy, oxidative derivatives of bacteriochlorophyll_a like OH-bacteriophaeophytin_ a (peak 38, OH-bph_a) and especially bacterioviridin (peak 21-46, bvir) were observed in some sections of the Lagunillo del Tejo core. Bacteriophaephytins_d show a complex distribution of seven homologues which exhibited structural variations; three different macrocycles (alkylation pattern) and six different sterified alcohols. Peaks 22 and 25 eluted before okenone, gave ions [M+H]+ at m/z 771 and 785. On MS/MS fragmentation the protonated molecule gave rise to ions at 567 and 581, resulting from the loss of 204 daltons corresponding to farnesyl. Bph_d macrocycle at m/z 567 (d2) corresponds to an alkylation pattern of [Et, Et] or [n-Pr, Me] at C8 and C12. The other bph_d macrocycle (m/z 581) (d3) corresponds to an alkylation pattern of [n-Pr, Et] or [i-Bu, Me]. Four homologs with the same macrocycle (m/z 567) sterifyed by different alcohols; C16:1 (peak 33), C15:0 (peak 37), C16:0 (peak 41) and fityl (peak 44), were found eluting between chlorophyll_a an bph_a. Finally peak 34 corresponds to different macrocycle alkylation patterns at C-8 and C-12 (d4), sterifyed by geranylgeraniol. The residual macrocycle mass indicates two posible alkylation paterns; [i-Bu, Et] and [n-Pent, Me].

Beside all these compounds, chromatograms have shown a high amount of different minoritary ones (peak 17, 39,40 or 42), probably bacterial derivatives (Gibbison et al. 1995) which remain unidentified. Furthermore the methology procedure allow the extraction of some compounds which eluyed the earliest with maximun absorbance around 330 nm, 360nm and 386 nm. Although the identification has not been completed, these compounds have been related with UV-photoprotectors of phytoplanktonic organisms (Sinha et al. 1998).

Sediment core lithology and chronology

Lagunillo del Tejo sediment core was colour banded throughout (Figure 1); dark mud layers (10YR 1.7/1 and 10YR 3/2, colour Munsell) alternated with brown ones (10YR 5/3). This lithological features suggested distinct depositional environments over time corresponding to a changing redox condition. Oxidized

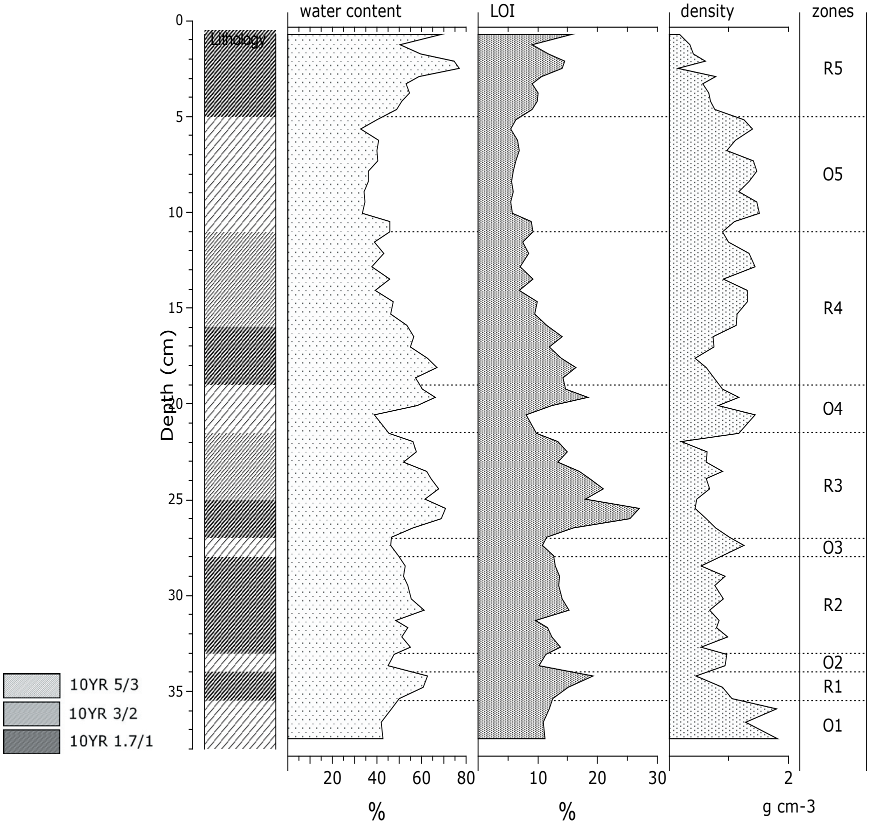


Figure 1. Lithology of Lagunillo del Tejo sediment core: sediment colour features (see leyend below), water content (%), LOI (%), and density (g cm^{-3}).

Figura 1. Litología del testigo sedimentario del Lagunillo del Tejo: caracteristicas de color (ver la leyenda), contenido en agua (%), LOI (%) y densidad (g cm^{-3}).

horizons (O zones) were characterised by the highest density values and the lowest LOI and pigment concentrations (Figure 1). Contrary reduced sediment units (R zones) showed higher organic matter and photosynthetic pigment concentrations.

^{210}Pb, ^{137}Cs, and ^{226}Ra activity profiles of Lagunillo del Tejo core are shown in Figure 2. The profile of ^{210}Pb in sediment core does not show a typical exponential decline. ^{210}Pb activity showed decreasing values from 249 Bq kg^{-1} at the topmost to 80 Bq kg^{-1} at 4.75 cm depth. In this interval the mass sedimentation rate is apparently 0.054±0.002 g cm^{-2} yr^{-1} (i.e. 0.071±0.002 cm yr^{-1}).

The presence of a 8 cm interval (between 5 and 12.5 cm depth) in sediment core, whose activity falls lower than the layer between 13.5 and

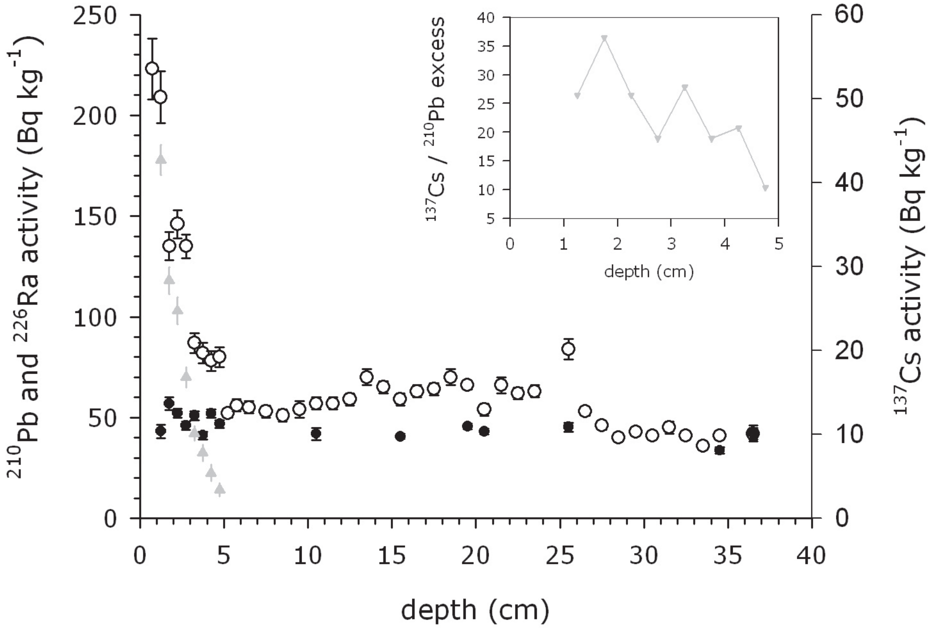


Figure 2. Radionuclide activities (Bq kg^{-1}); ^{210}Pb (white circles), ^{226} Ra (black circles) and ^{137} Cs (gray triangles up). Inlet: ^{137}Cs to ^{210} Pb excess ratio in the uppermost 5 cm depth.

Figura 2. Actividades de los radionucleotidos (Bq kg^{-1}); ^{210}Pb (circulos blancos), ^{226} Ra (circulos negros) and ^{137} Cs (triangulos grises). Dentro: relación entre ^{137}Cs y ^{210} Pb en exceso en los primeros 5 cm de profundidad del sedimento.

14.5 is not consistent with a model of radionuclide decay. It is assumed that unsupported ^{210}Pb, once deposited in the lacustrine sediment declines with age in accordance with the law of radiactive decay. However, the intervals trough the sediment sequence whose radionuclide activity falls and keeps lower than expected values according to a exponential function decay (i.e from 5 to 12.5 cm depth and at 20 cm depth, in figure 2) are consistent with the presence of oxidased layers with higher density and low organic matter at these depths. Lithological changes seem to play an important role shaping the radionuclide activity profiles. We suspect that non-linealities in the ^{210}Pb record may be due mostly to dilution of the atmospheric fallout by increased sedimentation rates or even to turbidite events.

Despite these ^{210}Pb anomalies, a reference data could be situated in the sedimentary profile (Figure 2) using the ^{137}Cs radionuclide activity. Since ^{137}Cs activity was restricted to the uppermost depth, probably the topmost 5 cm correspond to the last 50 years according to the onset of atmospheric testing

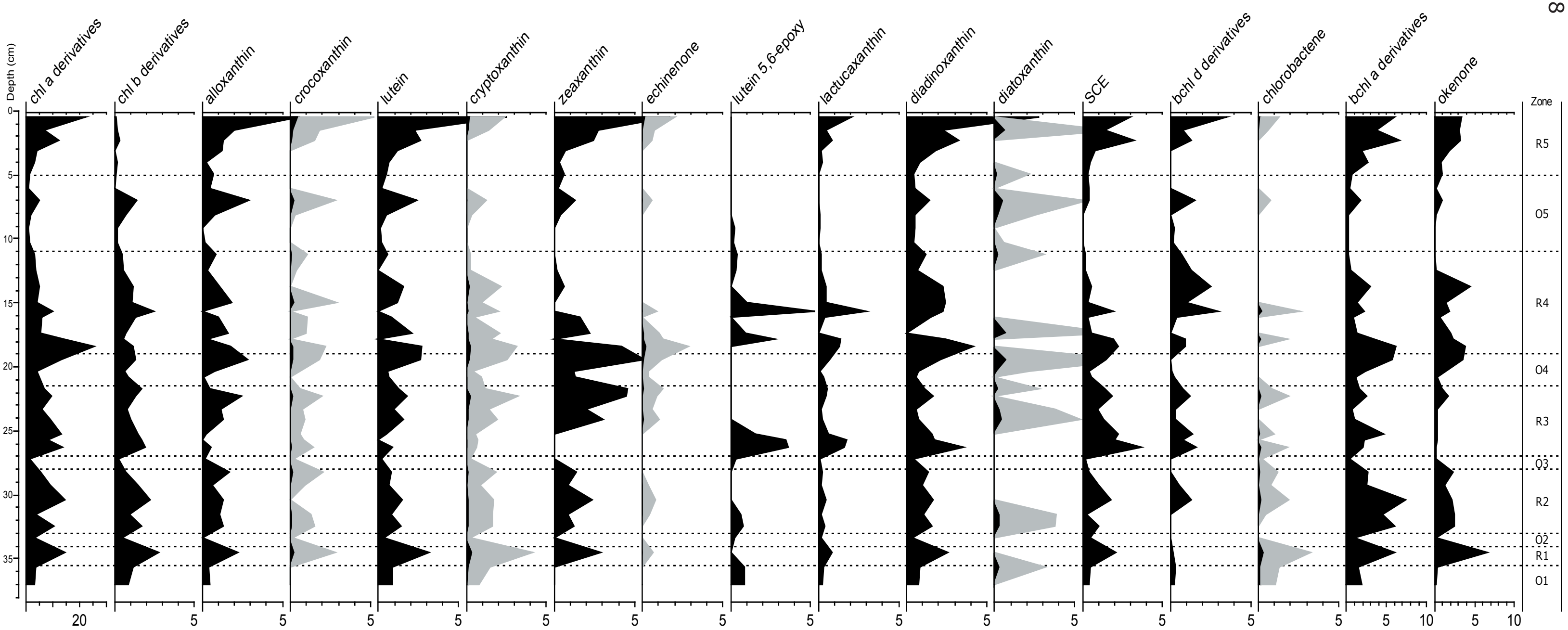


Figure 3. Stratigraphic profiles of main photosynthetic pigments ($\mu g\ g^{-1}{}_{om}$). Cryptophyceae markers; alloxanthin and crocoxanthin. Chlorophyceae markers; lutein and crytoxanthin. Cyanophyceae markers; zeaxanthin and echinenone. Chrysophyta markers; diadinoxanthin and diatoxanthin. Macrophytes markers; lutein-5,6-epoxy and lactucaxanthin and steryl chorin ester as biomarkers of grazing activity. Green sulfur bacteria markers; bchl_d homologs and chlorobactene. Purple sulfur bacteria markers; bchl_a derivatives and okenone. Grey areas correspond to exaggerated scale (x10).

Figura 3. Perfiles estratigráficos de los principales compuestos ($\mu g\ g^{-1}{}_{om}$). Marcadores de criptofíceas: alloxantina y crocoxantina. Clorofíceas: luteina y criptoxantina. Cianobacterias: zeaxantina y equinenona. Crisófitas: diadinoxantina y diatoxantina. Macrófitos: luteina-5,6-epoxy y lactucaxantiaina y SCE como biomarcadores de depredación. Bacterias verdes del azufre: Bchl_d homologos y clorobacteno. Bacterias rojas del azufre: Bchl_a derivados y okenona. Areas grises corresponden a escalas exageradas (x10).

of nuclear weapons at the beginning of fifties. The profile of ^{137}Cs in sediment core does not show any relevant peak, however, dilution events, such as that could be inferred in the ^{210}Pb profile, could distort the ^{137}Cs profile and in these circumstances the ^{137}Cs/^{210}Pb ratio may be a better guide to the 1953 depth than the raw ^{137}Cs profile. ^{137}Cs/^{210}Pb ratio in sediment core showed two maximum peaks at 3.25 and 1.75 cm depth which are consistent with the two main sources of artificial fallout radionuclides; the renewed testing nuclear weapons following the expiry of the moratorium in 1961 and the catastrophic Chernobyl reactor fire in April 1986, respectively.

Total ^{210}Pb activity appears to reach equilibrium with the supporting ^{226}Ra at a depth of around 27-28 cm where 42±4 Bq kg^{-1} is considered as supported ^{210}Pb. Then 27-28 cm depth could be dated approximately at 1850 A.D. because the equilibrium between total ^{210}Pb activity and the supporting activity is effectively achieved after a maximum of about 6-7 ^{210}Pb half-lives, 130-150 years (Appleby 2001). In summary the sediment accumulation in Lagunillo del Tejo appeared not be constant trough the sediment sequence. Unlike reduced horizons as the uppermost 5 cm with a sedimentation rate of c.a. 1 mm yr^{-1}, oxidased horizonts coud be turbidite events or even lengthy periods with higher erosion rates and then sediment accumulation.

Stratigraphic profiles of photosynthetic pigments

Figure 3 shows the stratigraphic profiles of photosynthetic pigments. The integrated signal of chl_a and _b derived compounds follow similar trend to each other, except around 18 cm depth and at the topmost 5 cm. Note that chl_b concentrations are always 5 fold less than chl_a. In figure 3, all derived compounds of ch_b are added together, but notably, until 27 cm depth from the bottom, pyroph_b and ph_b make similar contributions to the summed signals of total chl_b derived components. However from the top to 27 cm depth, peaks of chl_b derivatives are due exclusively to pyroph_b, and they are coincidident with peaks of the carotenoid biomarkers of littoral vegetation: lactucaxanthin and lutein epoxy. Grazing biomarkers (SCE's), are present thought the core. Nevertheless abundance trend follow chl_b derivatives and the just mencioned carotenoids. On the other hand, the depth profiles of carotenoids derived from cryptophyta, chlorophyta and cyanobacteria, follow similar patterns, although cyanobacteria abundance increased significantly at horizons R3 and R4. Regarding other carotenoids, diatoxanthin profile was similar to planktonic biomarkers while diadinoxanthin which probably has more than one principal source (diatoms, crisophyceae, dinoflagellate), seems to change like compounds related with littoral vegetation. Pigment concentrations of purple sulfur bacteria were higher than green sulfur bacteria pigments, indicating the relative domain of purple bacteria over the anoxic productivity. Increases in bacterichlorophyll_a derivatives were coincident with increases of planktonic productivity which might be inferred by chlorins and algal carotenes, only with the exception at 21-23 cm depth, when the abundance of purple bacteria decreased whereas zeaxanthin had high amount. Noteworthy, significant variations in relative abundance of chlorobactene to bacteriochlorophyll_d, specifc carotenoid from green sulfur bacteria, were detected thought the sediment core.

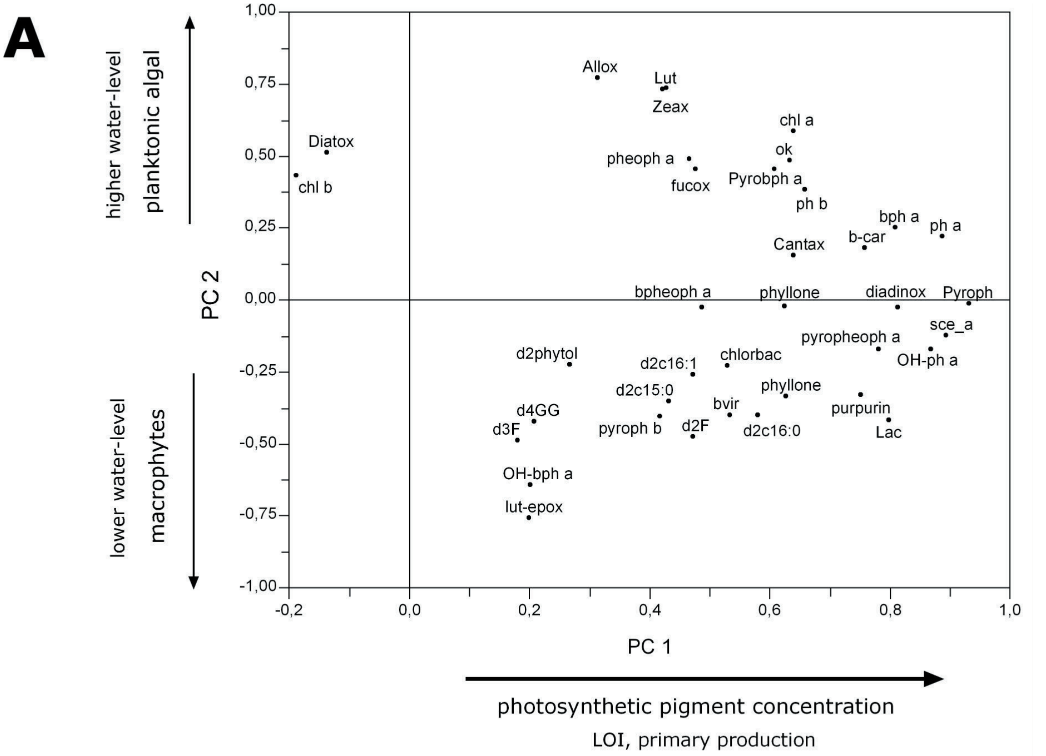
Relationships between photosynthetic pigment compounds.

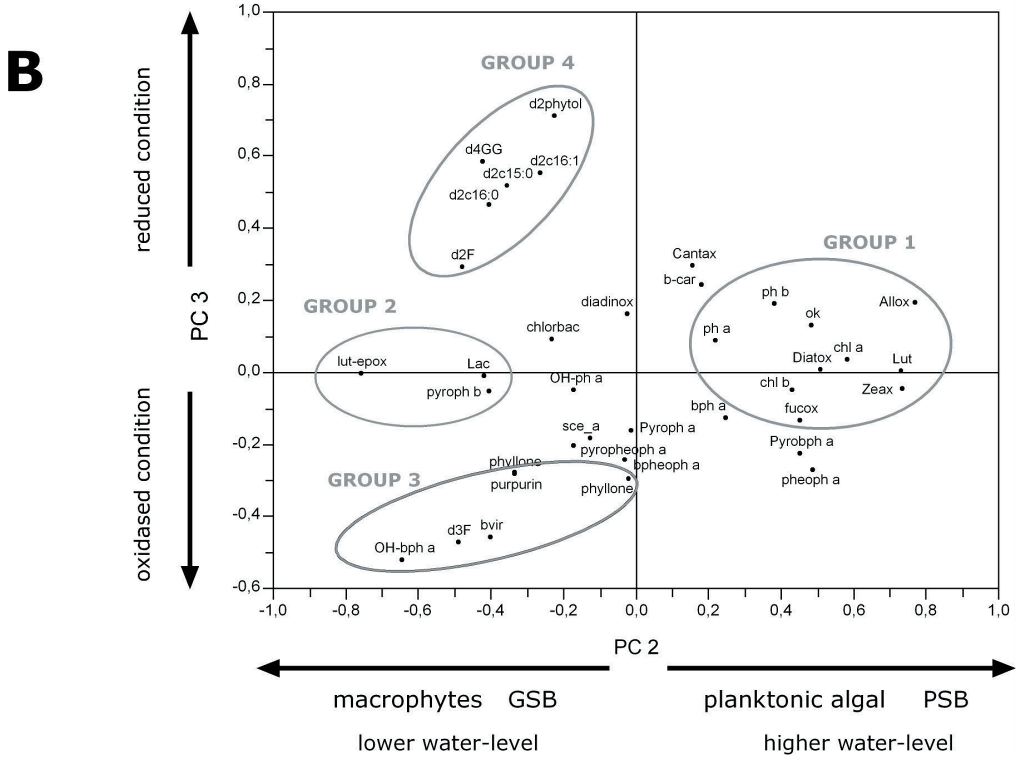
In order to explore the relationships between photosynthetic pigments and derivatives through the Lagunillo del Tejo sequence, a principal component analysis of the data was performed. The first PC explained 34.78 % of the variability, the second and the third accounted for 17.67% and 8.87%, respectively. Figure 4 (A and B) show the factor loadings for the photosynthetic pigments with respect to the first three components. As figure 4A shows, the variables positively loaded onto axis 1 were the phosynthetic pigments with higher concentration extracted from the sediment, which contribuyed in large proportion to the total sample pigment amount. The compounds with negative loadings were the least frequent and with lowest concentration. The sample scores for PC1 and total pigment profile (Figure 5) share the same pattern. Thus, this axis is mainly a function of the abundances of pigment compounds. Sample scores for first factor show a high positive correlation with sediment organic content and a significant negative correlation with density.

In the arrangement of variables according to second principal component (figure 4B), major algal carotenoids (group 1) have the greatest positive correlations, these include cyanobacterial (zeaxanthin), cryptophyta (alloxanthin) and clorophyta (lutein) major carotenoids. Native chl_a and _b share also similar high positive correlations. However their oxidative derivatives as allomers or purpurins were in the negative side of this axis. Contrarily to

Figure 4. Facing page. Distribution of the variables in the space defined by the three principal components resulting from the PCA. 3A, PC1 and PC2. 3B, PC2 and PC3.

Figura 4. Página siguiente. Distribución de las variables en el espacio definido por los tres componentes principales resultado del análisis multidimensional. 4A, componente 1 frente a 2. 4B, componente 2 frente al 3.


A
higher water-level
planktonic algal
lower water-level
macrophytes
PC 2
PC 1
photosynthetic pigment concentration
LOI, primary production
1,00
0,75
0,50
0,25
0,00
-0,25
-0,50
-0,75
-1,00
-0,2
0,0
0,2
0,4
0,6
0,8
1,0
Allox
Lut
Zeax
chl a
ok
Diatox
chl b
pheoph a
fucox
Pyrobph a
ph b
bph a
ph a
b-car
Cantax
bpheoph a
phyllone
diadinox
Pyroph
sce_a
pyropheoph a
OH-ph a
d2phytol
d2c16:1
chlorbac
d2c15:0
phyllone
d4GG
bvir
purpurin
d3F
pyroph b
d2F
d2c16:0
Lac
OH-bph a
lut-epox


B
reduced condition
oxidased condition
PC 3
PC 2
macrophytes GSB
lower water-level
planktonic algal PSB
higher water-level
1,0
0,8
0,6
0,4
0,2
0,0
-0,2
-0,4
-0,6
-1,0
-0,8
-0,6
-0,4
-0,2
0,0
0,2
0,4
0,6
0,8
1,0
GROUP 4
d2phytol
d4GG
d2c16:1
d2c15:0
d2c16:0
d2F
GROUP 1
Cantax
b-car
ph b
Allox
ok
ph a
chl a
Diatox
Lut
chl b
Zeax
fucox
bph a
Pyrobph a
pheoph a
GROUP 2
diadinox
chlorbac
lut-epox
Lac
pyroph b
OH-ph a
sce_a
Pyroph a
pyropheoph a
bpheoph a
GROUP 3
phyllone
purpurin
phyllone
d3F
bvir
OH-bph a

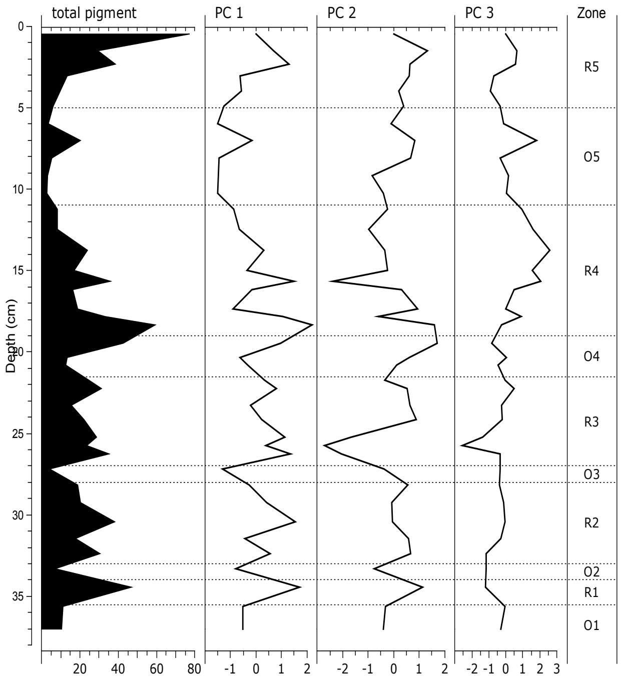


Figure 5. Total pigment concentration (μg pig $g^{-1}{}_{mo}$), sample scores for PC1, PC2, and PC3.

Figura 5. Concentración total de compuestos fotosintéticos (μg pig $g^{-1}{}_{mo}$), valores de las muestras para componente 1, 2 y 3.

algal derivatives, the plant biomarkers carotenoids lactucaxanthin and lutein-epoxy were placed in negative end of PC2 axis (group 2). Regarding bacterial populations fingerprinting, purple sulfur bacteria pigments (bph_a, pyrobph_a and okenone) were situated positive loaded onto PC2, near algal carotenoids (group 1). However bacterial oxidation products, as bvir and OH-bph_a, were negative correlated with PC2 and have significant negative loadings on PC 3 (group 3). Bph_d homologs formed a closed cluster (group 4) which was negative correlated with PC2 but positive correlated with PC3.

Notably the identified groups of biochemical compounds showed differential loading onto third axis according to the relative stabilities among them (Figure 6). While oxidated chlorin and bph_a derivatives have the highest negative scores for PC3, bph_d were loaded onto the positive axis. In addition, the chlorin derivatives show higher negative scores than the group of carotenoids.

Figure 5 also includes PC2 and PC3 sample scores. Most frecuently oxidised horizons were negatively scored PC2 while samples of reduced zones were

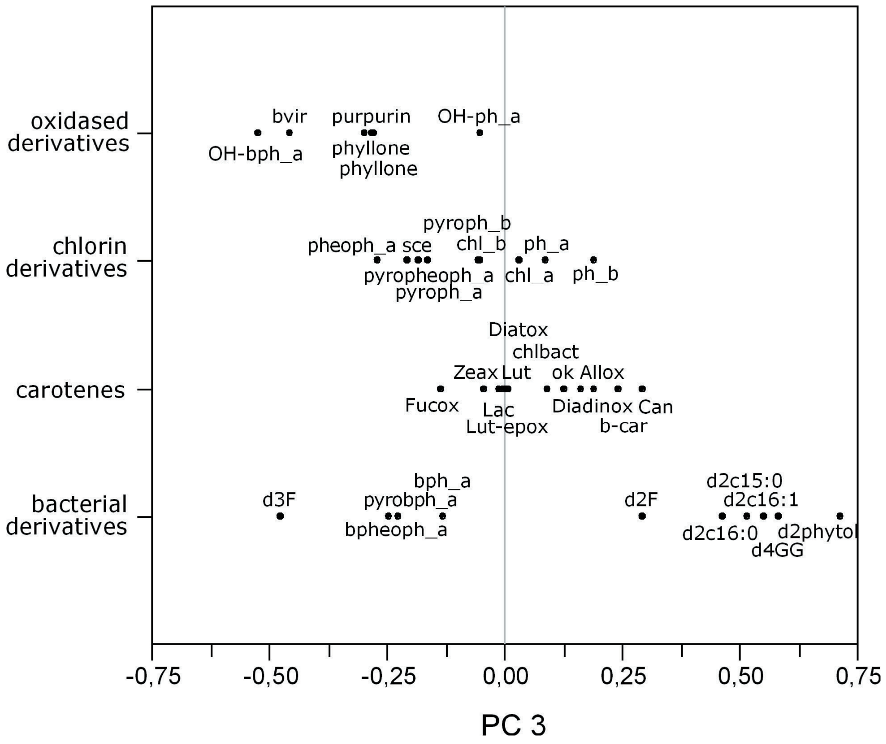


Figure. 6 Photosynthetic pigments loading onto PC3 by compound type.

Figura 6. Valores con respecto al componente principal 3 de los diferentes pigmentos fotosintéticos según el tipo de compuesto.

positively loaded onto PC2, except at the bottom of R3 and the top of R5 zone. Strikingly sample scores for PC3 corresponding to depths from the bottom to 18 cm had negative values, and positive values corresponded to horizons between 18 and 6 cm depth.

7d. Discussion

The Lagunillo del Tejo responds with lake level fluctuations to regional hydroclimatic variability, being a highly sensitive ecosystem to short-term rainfall variability. Moreover, its sediment recorded the hydroclimatic fluctuations over the past and the changes of the biological community linked to climate driver.

The abundances of photosynthetic compounds showed wide changes through the sedimentary sequence. The concentrations of photosynthetic pigments derived from phototrophic comunity are linked to autogenic productivity. However shifts of compounds accumulations could not be interpreted simply as a variations in autocthonous production because significant decreases were recorded paralelly to higher sediment density values. These higher density values are due to increases of mineral matter content related with alteration of sedimentation processes, with periods of high sedimentation of allocthonous material. Furthermore the non-linealities in the ^{210}Pb profile suggests changes in the sedimentation rate over the past in Lagunillo del Tejo.

Lake sedimentation processes are complex and controlled by differents forms of energy input (Hakansson and Jansson 1983). However Lagunillo del Tejo lies in the bottom of a wind-shelted sinkhole without surficial inflow and likely remains most of the year thermaly stratified as suggested by the continuos presence of anoxic bacteria remains trought the sedimentary column, only changes in water level may condition sedimentation dynamics by changes in the extension of different zones of erosion, transport and sediment accumulation (Dearing 1997). If lake depth at lowering remains greater than a critical depth which prevents non-deposition or erosion of previosly deposited matterial, sediment accumulation may increase significantly because peripheral wave action or intermittent deep mixing will transfer relatively greater amount of shallow-water sediment into a relatively smaller zone of accumulation (Verschuren 1999). Periodic lake–level decline in the pond over the past first caused increased focussing of low-organic shallow water sediment to offshore profundal areas, which dilutes the organic matter and therefore the biological signal derived from pelagic algal production that has settled locally. The organic-carbon proportion of offshore lake sediments is usually not a function of organic production, but reflects the dilution of organic matter by mineral sediment input, and/or exposure to oxidation before its permanent burial (Rowan et al. 1992). Classical works warned about the care that must be taken to not ascribe changes in pigment concentrations and productivity to what really represents changes in lake morphometry with time (Gorham and Sanger 1972; Sanger 1988). Nervertheless, others factors could have conditioned the sedimentation processes. In the case of Lagunillo del Tejo, there is a documented agricultural activity during the first half of 20th century in the lake doline where agricultural terraces were built in the steep walls, to take advantage of the moisture of substrate. After the Civil War in Spain during the fourties a strong negative rainfall anomaly was recorded. The anthropic activity and drought, together could have a synergic effect on the increases of sedimentation rates during this period (11 to 5 cm depth). In this lacustrine system the bottom dynamics and sedimentation processes which in turn are mainly related with the lake-level fluctuations, control the quantitative changes of the biological signal in sediment (factor 1 from PCA).

On the other hand hydroclimatic variability seems to have overriding effects on phototroph organisms assemblages of Lagunillo del Tejo. The second factor of biological signal variance (factor 2 from PCA) is interpreted as a gradient of inshore-offshore biological community influence. Phototrophs assemblages recorded in Lagunillo del Tejo sediment corresponded to two different communities; a planktonic (group 1) mostly composed by cyanobacteria, chlorophytes and cryptophytes againts a phototroph community associated to macrophytes, which switched over the past.

Fluctuations in water level, up or down and dependent upon the catchment and lake basin contours may result in changes of macrophyte vegetation (Dearing 1997). In this pond lowering results in an inward spread of macrophytes and littoral vegetation. During periods of low water level, productivity was basically derived from macrophytes and associated phototrophic populations, mostly epiphytic diatoms, crysophyceae or dinoflagellates, which contain diadinoxanthin, a non specific carotenoid. Further conclusions about phototrophic populations associated to macrophyte development are limited due to the lack of specific carotenoids. Traced by biomarkers of grazing activity (sce and pheophorbides), zooplankton populations seems to be associated to this shift in phototrophic community. Both zooplankters and plant associated microinvertebrates take advantage (refuge and food resources) of aquatic vegetation development and their density is higher than in the open water. Moreover littoral development is assumed to influence species richness of limnetic zooplankton communities by means of providing new ecological niches and by shelter against predation (Hobaek et al. 2002). By contrast the relative abundances of main specific carotenoids suggests that phytoplanktonic community development during macrophyte dominance is rather limited. In macrophyte-dominated habitats phytoplankton plays a peripheral role in the biotic movement of carbon (Padisak and Reynolds 2003). Drop in the lake level is an allogenic perturbation which induced the temporal dissaperance of a developed planktonic community of phototrophic organisms. When water level recovered to highstand, however, mainly populations of chlorophytes, cryptophytes and cyanobacteria returns to previous similar relative abundances. Through the sedimentary sequence substantial changes of relative abundances of main phytoplankton classes during wetter periods were not observed. Therefore, the composition of the biological community of Lagunillo del Tejo seems to be controled by climatic forcing factor, switching two different ecological status.

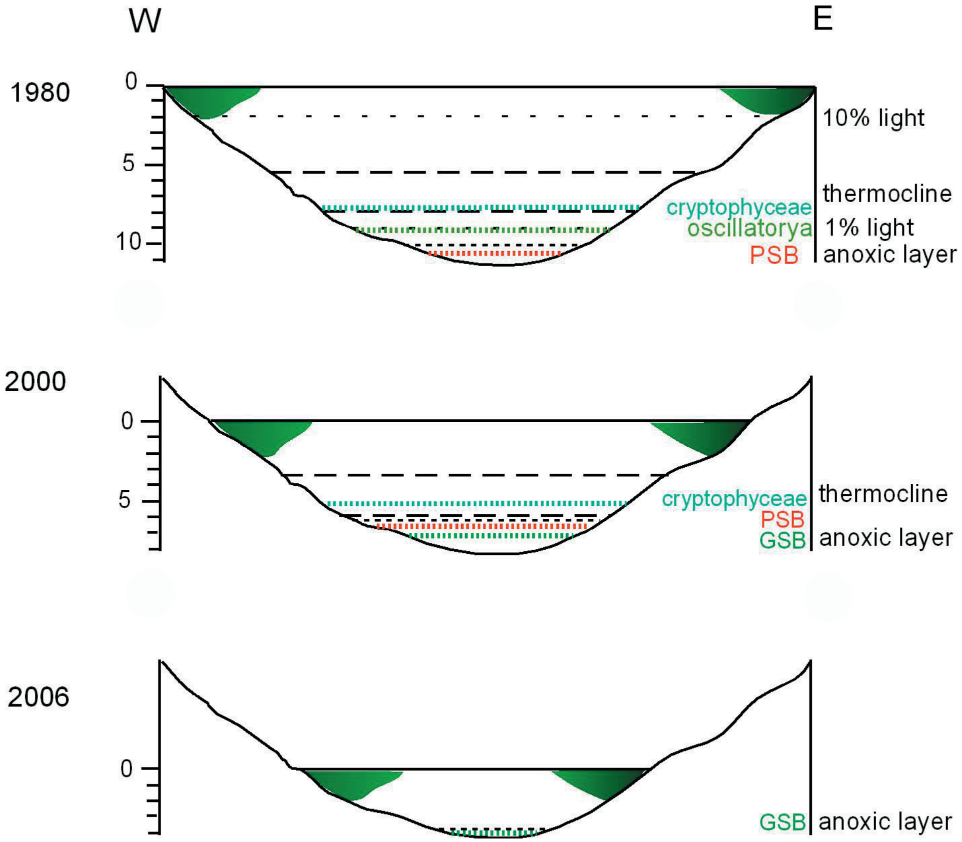


Figure 7. Schematic representation of the phototrophs organism assemblages and physical and chemical parameters corresponding to water level fluctuations as response to hydroclimatic variability.

Figura 7. Representación esquemática del ensamblaje de los organismos fotosintéticos y parametros fisico-químicos correspondientes a las fluctuaciones de nivel en repuesta a la variabilidad hidroclimática.

Likewise the composition of anoxic bacterial community also respond to lake level fluctuations. The relative dominance of purple bacteria in high level periods alternated with green bacteria dominance at lowering. This changes in relative abundances of phototrophic anoxic bacterial populations could be associated with differences in light quality at oxicline depth. Indeed the effect of light quality on the selection of natural population of GSB is considered to be a classical factor in determination of their ecological niches (Parkin and Brock 1980; Vila and Abella 1994). The transmission of solar radiation through water column is attenuated by absorption and scattering. Water absorption effects accumulated with depth resulting in a relative increase in photons of the central part of the spectrum (500-600 nm). The green-yellow light harvesting adaptation of chromatiaceae favours development of purple sulfur bacteria in deeper depths versus green sulfur bacteria whose photoadaptive response to light limitation is based on bacteriochlorophylls at red-NIR absorption bands. Either synthesis of different grade of akilatation homologs and sterification of higher alcohols than farnesol such as geranyl-genariol and fityl allow effective energy transfer of bacteriochlorophyll_d (Borrego et al. 1998; Wilson et al. 2004b). The long bands (red light) are usually attenuated in the first meters depth, thus in shallow environments contrary to deep depths, the proportion of red and NIR light increased favouring green sulfur bacteria population development. In different lacustrine systems with bacterial anoxic productivity, changes in the relative dominace of bacterial populations has been porposed as indicators of oxicline depth fluctuations (Repeta 1993; Itho et al. 2003). In this case, due the shallowness of Lagunillo del Tejo, changes in relative abundance of bacteria population are related with water level fluctuations and consecuently with oxicline depth in relation to water surface.

These two alternative ecological patterns in Lagunillo del Tejo during the past inferred from pigment signature could be confirmed by the sporadic limnological samplings during the last twenty years (Figure 7). Since 1980 oscillations of maximun depth between 3.5 m and 11 m were recorded, meaning variations of more than 50 % of volume due to morphological features of this doline lake. At the beginning of the eighties, when the maximun depth was 11 m, Vicente and Miracle (1984) reported dense population of cyanobacteria (*Oscillatoria annae*) and purple sulfur bacteria. However during the eighties and the beggining of nineties the maximun depth was lower due to and extremly dry anomaly. At this time green sulfur bacteria populations were relatively higher than purple sulfur bacteria and cyanobacteria almost not detected. Macrophytes colonise the shore line until 3 m detph, forming a littoral ring of 4-5 m width. A decrease in water detph results in an inward spread of macrophytes as we could observed recently during the last 3 years (2003- 2006) (Figure 4, section 2). Furthermore three rings of *Scirpus lacustris*, mediterranean vegetation linked to the littoral environment, could be observed at different heights above shore line suggesting past water levels higher than the observed maximun 11 m depth during last twenty years.

On the other hand, pigment transformations and degradation within the water column and early stages of sedimentation could influence the photosynthetic derivatives sedimentary record. Factors belived to enhance preservation include low oxygen concentration, low temperature, low light penetration and sediment resuspension, high sedimentation rates, absence of benthic organisms, and increased levels of eutrophy (Sanger 1988; Leavitt 1993). Although preservation-degradation conditions were discarted as the main factor controlling sedimentary signal, the variables scores for third factor of PCA suggest the effect of post-depositional transformations related with oxygen presence at water-sediment interface. The oxidised derivatives such as purpurin, bvir and chlorophyllone, which have the highest negative scores, have been observed in environments with highly oxygenated waters during long time periods (Naylor and Keely 1998; Walker et al. 2002; Chen et al. 2003; Wilson et al. 2004a), derived either from pre-depositional transformations occured within water column (i.e. bvir; Romero et al. 2006), and partially from post-depositional oxidation. Moreover the detailed analysis of pigment scores on PC3 indicates that chlorin derivatives has more negative scores than carotenoids, suggesting differential stability against oxidizing conditions. In fact it has been reported that chlorophyll pigments are less well preserved in anoxic conditions than are carotenoids, whereas the opposite is true for oxic conditions (Gorham and Sanger 1972; Swain 1985). Among carotenoids, decay rate constants differ greatly, ubiquitous β-carotene is the most stable while xanthophylls are more labile. However carotenoid relationships with factor 3 from PCA do not show a clear pattern, according to decay rate of carotenoids reported in others studies (i.e. Leavitt and Carpenter 1990a; Hurley and Armstrong 1991; Steenbergen et al. 1994). Therefore the positive trend of sample scores from past to present for third factor from PCA could be related with a progresive enhancement of anoxic conditions at the bottom water layer. Although the presence of bacterial derivatives and native chl a trought the sedimentary sequence suggest temporal anoxia at water sediment interface, this trend could also suggest a long-term increase of the thermal stratification period associated with an increase of temperature mainly during spring and autumn, the seasons of the stablishment of thermocline and the onset of overturn. Nevertheless this biological signal could no be directly assigned to a climate forcing factor because other factors such as photochemical conditicions or changes in the specific composition of producers among others could be interferring in the post-depositional sedimentary pigment composition (Sanger 1988; Leavitt and Carpenter 1990b).

Changing water level will influence many aspects of a lake's physical, chemical and biological characteristics. Only long term perspectives or paleolimnological studies could provide information about the biological response to water level fluctuation climaticaly derived. Some reports show that biotic community do not respond in a straighforward way to lake level fluctuations (Vinebrooke et al.

1998; Versuchren et al. 1999). Many factors could be exerting a direct control on the biological community. However in this close basin, the phototrophic community is highly sensitive to hydrological variability, switching between planktonic and littoral communities during wet and dry periods in the region. Photosynthetic pigments and derivatives have been shown useful biomarkers of past phototrophs assemblages in Lagunillo del Tejo. An accurate methodology for pigments analysis improves the whole system inferences from these biological proxies.

8. General discussion

Actually, the point of departure of a paleolimnological study is an exciting event. After an usual exhausting coring one could hold a sediment core. Then one faces up to a witness from the past being ready to tell us what we are ready to understand. Over the last decades the advances reached by the paleolimnologist community have overcome some of earlier problems of handling sediment data. Many new approaches and techniques, developed at fast rate, are now available to paleolimnologists. Nevertheless, the amount of information stored in sediments is staggering (Smol et al 2001) and the full content of lake sediments is still unexplored as considerable amount of information is destroyed when the analytical methodology is applied to process the sediment samples (Anderson 1993). I share the opinion that these are exciting times for paleoenvironmental research (Smol and Cumming 2000, Cohen 2003). Long term data demanding related with the response of the ecosystems to environmental variability within the global change debate, will require new common efforts to improve both, the capabilities to learn information from lake sediments and the spatial characterization of ecosystems hotspots in remote regions or tropical areas.

Nowadays nobody doubt about the suitability of sediment data to infer environmental change and lake ontogeny. However upcoming progress can only be achieved if paleolimnologists are fully aware of the limitations of the sediment record and hence their data, and continue to approach the subject in a rigorous and quantitative way. Evaluating the environmental information available from lake sediment firstly requires an overview of possible links of environmental variability and lake response as well as lake dynamics and processes involved in the transference of limnological variables into the sedimentary signal based on actual limnological data (Bauer 2004). Furthermore the character and intensity of a lake response can vary considerably conditioned by its local and regional landscape characteristics (geographic position, catchment characteristics and lake morphometry) and internal filter comprising lake history and biotic/abiotic interactions (Blenckner, 2005). Therefore the knowledge of specific lake processes operating at present time ease and guarantee the paleolimnological reconstruction and consequently a monitoring study or at least some data recompilation is essential as for down-core studies. When physical, chemical and biotic lake data are not available mostly in remote regions, lake monitoring programs should be planned (e.g. Bradley et at 1996, Peck et al 2002) to provide the basis for the interpretation of proxy stratrigraphies. In the case of the lakes of Cañada del Hoyo karstic system, the biotic/abiotic relationships have been studied since the end of the eighties and also the sedimentation processes by means of sedimentation traps during three years of monthly survey (1996-1998). These limnological studies of the lacustrine processes have been necessary in order to asses the paleolimnological reconstructions presented in this study. In particular, the monitoring time period in this lake have enabled the establishment of the linkage between climatic variability, limnological dynamics and sedimentary signal, being decisive in the proposal of calcite laminae thickness to be used as a quantitative proxy of winter regional rainfall.

On the other hand the analysis of photosynthetic pigments recovered by the sediment traps in Lake La Cruz has been essential to consider and interpret the photosynthetic pigment stratigraphies. The sedimentation pattern of phytoplanktonic groups and particularly the bacterial plates has been conditioned by the lacustrine processes, in particular summer whiting and autumn overturn, which are the factors expected to control the sedimentary signature at least through the calcite laminated sequence, over the last 420 years. In the light of numerous empirical studies describing the patterns of pigment sedimentation (review in Leavitt 1993) and experimentation to identify possible regulatory factors (e.g. Hurley and Armstrong 1990, Steenbergen et al. 1994), the pigment deposition is regulated by complex interactions among primary production, lake morphology, pigment oxidation, vertical structure of plankton communities, and grazing by herbivores, among others. Since there have been few attemps to synthesize these studies or to develop a conceptual framework for studying pigment sedimentation (Cuddington and Leavitt 1999), the conservative approach should be based on the specific examination of biological processes and physical characteristics of the ecosystem studied. Nevertheless future studies in paleolimnology should include modelling approaches to quantify past lake production and food web structure in sedimentary deposits, although further research is required before predictive models can be use confidently (Cuddington and Leavitt 1999).

Moreover this study has presented the first report of bacterioviridin, a bacterial derivative (Wilson et al. 2004a), in sinking water column particulate material contributing to the knowledge of its usefulness as a paleolimnological biomarker. As other pigment compounds such as SCE or transformation products, bvir seems to be a tracer of limnological processes, such as in this case, a circumstantial indicator of competition between phototrophs. Since its presence in settling material is related to the microaerobic conditions in the upper layer of the oxicline, the increase of its concentration over the last century in laminated sediment from Lake La Cruz related to the sedimentary signal of algae carotenoids and chlorins may be due to the shading effect and then the competition pressure exerted by the deep cyanobacterial populations at the oxicline depth on phototrophic purple bacteria. In a general sense the presence of this bacterial oxidation product in sedimentary sequences may be related to desiccation events or disruptions of the water column stratification (Wilson et al 2004a), however the specific site features may be conditioning the signal provided by the sedimentary proxies. Again, the understanding of specific sedimentation factors is essential to attempt a paleolimnological interpretation.

On the other hand, due to the many indirect and direct linkages that exist between climate, lakes and the organisms contained therein, sediment records of lakes are an increasingly important source of paleoclimatic data. Preserved in lake sediments, either organic (Smol and Cumming 2000 and references therein) and inorganic components have enable direct and indirect reconstructions of wide range of climate-related limnological features such as ice-cover, river discharge, DOC, pH, temperature, conductivity, water column stability, and lake-level fluctuations. Both, Lake La Cruz and Lagunillo del Tejo sediments have recorded the response of the lacustrine systems to the hydroclimatic variability, although by different proxies and temporal resolutions. In particular calcite laminated sediment from Lake La Cruz has allowed a quantitative estimation of annual winter rainfall in the region over the last 420 years using for the first time the thickness of calcite laminae as climate proxy. This result contributes significantly to paleoclimatic archives from Iberian Peninsula since few natural proxies (Pauling et al 2005) as well as documentary data (Barriendos 1994, Rodrigo 1994) have been showed as valuable indicators of rainfall anomalies in this western Mediterranean area. The regional reconstructions have a great importance since local, together with large-scale reconstructions of changes in precipitation could complement the surface temperature series and improve our understanding of the forcing factors that have contributed to the climate variability. Moreover the results of our study on the Lake La Cruz climatic signal highlight new directions for paleoclimatic research using calcite varve sediment records. Although local hydrological features and deposition processes could condition the varve signal, laminated sediments located in carbonaceus bedrock around the world could store unexplored information about past hydro-climatic events.

The study of the temporal variability of phototrophic community assessed by the analysis of sedimentary photosynthetic pigments in both, Lake La Cruz and Lagunillo del Tejo, has underlined the site specific responses even under the influence of the same environmental variables. The Lagunillo del Tejo pigment record have been shown highly sensitive to hydroclimatic variability whereas pigment stratigraphies in Lake La Cruz showed a different pattern governed mainly by other factors. Clearly some lakes will be more responsive to climatic change than others. In this case the specific response is a function of lake morphometry. An increase of water flow into these lakes results in a drastic change of surface area to depth ratios in Lagunillo del Tejo, whereas in Lake La Cruz this ratio changes much less because of its higher relative depth and volume. Changing water levels have strongly determined the structure and composition of the primary producers community in Lagunillo del Tejo. This study as others before (Vinebrooke et al. 1998) has showed that water level changes can be tracked using photosynthetic pigments as other traditional paleophycological approaches such as diatoms assemblages, chrysophycean cysts, and macrophyte remains (i.e. Cumming et al. 1993; Hannon and Gaillard 1997; Wolin and Duthie 1999). However the unequivocal interpretation of the data extracted from lake records sensitive to lake-level faces up to a main outstanding problem. There is an inherent conflict between lake sensitivity to hydroclimatic variability and its persistence and integrity as an archive of climate variability (Verschuren 2003). On one hand it is difficult that the lacustrine system survive without desiccation or erosion during the most arid events preserving a continuous record. On the other age models based on radioisotopes capture with difficulties the distortions of the age-depth relations caused by the changes in sediment-accumulation rate that usually accompany lake-level fluctuations (Verschuren 1999). Indeed the limitations in ^{210}Pb chronology due to the alteration of sediment deposition in Lagunillo del Tejo over the time window studied have difficulted making conclusions about fluctuations in lake production and increased the uncertainities in the timing of moisture changes.

By contrast the pigment signature in Lake La Cruz sediment showed an increasing trend of compounds concentration but also a compositional stability. Thanks to the annually resolved sediment by the annual calcite laminations, the quantitative fluctuations could be adscribed to changes in primary production discarding the dilution effects due to changes in the sedimentation rates (Swain 1985, Sanger 1988). Once characterized the trophic evolution of Lake La Cruz, the open question to be solved is what are the forcing factors driving long-term changes in primary production in Lake La Cruz. Possible factors involved have been discussed in the previous sections, however solar variability is suspected to lead both the long-term trend and decennial variability of primary productivity inferred by photosynthetic pigments because of the striking match we found with the solar activity reconstruction over the last centuries (Lean 2000). Moreover solar irradiance varies at all wavelengths in ways that reflect the different solar origins of the emissions from a range of temperatures and structures within the solar atmosphere, however maximum energy changes occurs at wavelengths from 400 to 500 nm whereas at UV wavelengths the energy changes are considerably smaller (Frölich and Lean 2004). Changes in this region of PAR can be especially significant for phototrophic organisms, since the higher absorbance of photosynthetic pigments, such as chlorophylls and carotenoids is found within this range of the electromagnetic spectrum. The relationship between the solar irradiance variability and the lake La Cruz production based in a logic premise *a priori*, however open many new questions about the environmental forcings impact on lake production.

Recently some long-term processes have been postulated as forcing mechanisms of productivity, with most of these studies based on time serie analysis of data recovered from varved sediments. For instance, in the estuarine annually laminated sediment of the Saanich Inlet (Canada) Villanueva and Hastings (2000) observed 18.9 years periodicity in the pigment record which could be the result of changes in the basin production as modulated by the lunar nodal tidal cycle of 18.6 years because of the tidal mixing effects on nutrient inputs. The high resolution study of these laminations based on fabric and diatom assemblages allowed the construction of time series data for spectral analysis, and the comparison of spectral analysis results with modern analogues suggests that some link may exist between seasonal diatoms blooms and some large-scale atmospheric patterns active in the region such as Pacific Decadal Oscillation, El Niño Southern Oscillation and Quasi-Biennial Oscillation (Dean and Kemp 2004). In our best knowledge there are scarce references, some of them indirect, about the solar activity as forcing of primary productivity. The presence of pronounced cycles in the thickness of biogenic carbonate varves in Soppenseen (Switzerland) agrees with the results of other studies on varve thickness and $\delta^{14}C$ which have been interpreted as indicating an association between solar forcing and varve thickness; because the Soppenseen varves are of biogenic rather than clastic origin any influence fluctuations in solar irradiance is suspected to have an effect exerted via primary production (Livingston and Hajdas 2001). Moreover the periodicities that appear to be persistent in many proxy variables in the Elk Lake (USA) record such as varve thickness and diatom data suggest that there may be a solar connection (Dean et al 2002). However the physical connection may be a solar-geomagnetic connection affecting atmospheric circulation as suggested by Anderson (1992) or through a solar insolation control on lake productivity as suggested by Bradbury et al (2002).

The lake productivity is a complex question frequently treated by limnologists. A debate has accompanied the recent growth of information about phytoplankton as to what are the dominant processes controlling their abundance and the community structure in lakes (Anderson, 1995). Equilibrium theories, such as the role of nutrient ratios and interspecific competition (Sommer 1988), contrast with non-equilibrium approaches that consider the fluctuations of the natural (physical) environment to be more important (Harris 1985). And example of the later is the Hutchinson's Paradox of the Plankton. On the other hand, the relative roles of the predation (top-down) and nutrient supply (bottom-up) processes in controlling phytoplankton biomass and community structure are also controversial (McQueen 1990, Carpenter and Kitchell, 1993). To solve these questions the temporal perspective becoming from paleolimnological studies is relevant and added to knowledge of present functioning could improve our understanding of forcing factors controlling the primary producers community.

Concerning the future advances of paleolimnology, this study as others before has confirmed the valuable assistance of photosynthetic pigments. Fossil pigments can be used in any paleoecological application in which historical changes in lake production or primary producers composition are key responses (e.g. eutrophication, acidification, food-web interactions, human impacts) (Leavitt and Hodgson 2001). Furthermore it is important to take into consideration that photosynthetic pigment is a "multidimensional" proxy. Using an adequate statistical methodology is possible to define the most important components of variance of the different pigment compounds stratigraphies and address the environmental factors related with both quantitative and qualitative fluctuations observed through the sedimentary sequence based in the understanding of the derived organisms autoecology (i.e. ecological requirements, optimal thresholds, niches). These target compounds offer an overview of the past primary producers community and then an unfulfilled potential to infer a wide range of limnological variables related to different past environmental conditions. Surely future requirements of paleoenvironmental data will stimulate new developments and approaches about the photosynthetic pigments proxy which will reach similar quantitative potential as other current proxies such as diatoms (Battarbee et al. 2001).

In summary, this study has enabled a long-term perspective of Lake La Cruz and Lagunillo del Tejo trophic and hydrographic evolution, describing the natural parameters of lake variability and complementing the ecological characterization developed over the last years for these lakes. Clearly pigment stratigraphy could be most meaningful if other paleoecological and limnological indicators were included in this study, or in the case of Lagunillo del Tejo if the pigment signatures were fitted in a well dated time-window. However this end dot should be the beginning of future work. The possibilities of paleolimnological contribution to burning environmental questions, mainly the ecological effects of global environmental change encourage us to ask ourselves new questions and attempt to look for responses.

9. Conclusions

1. Lacustrine dynamics and processes conditioned sedimentation patterns of phototrophic planktonic components. Our results from sediment traps showed that sedimentation processes of planktonic phototrophs traced by photosynthetic pigments in Lake La Cruz are accomplished in two discrete pulses of deposition:(1. late autumn pulse) the flux of bacteriochlorin derivatives from phototrophic bacteria towards the sediment reached an annual maximun in late autumn after thermocline destruction. The collapse of thermal stratification causes oxygen exposure of the layers of anoxygenic bacteria and a mass mortality of purple and green sulfur bacteria populations. (2. early-mid summer pulse) The annual massive calcium carbonate precipitation occuring in summer affects the oxygenic phototrophs, mainly cyanobacteria. The high amounts of zeaxanthin just after whiting events indicates that calcite crystals are vectors of plankton (picocyanobacteria) sedimentation.

2. Among the identified compounds recovered by sediment traps, bacterioviridin, an oxidation product of bacteriochlorophyll a, is a useful paleoindicator of the degree of oxygen exposure experienced by the purple sulfur bacteria population. This compound was detected at high relative abundance in the settled material. The increased flux of bacterioviridin after thermal overturn, associated with the mass mortality of the sulfur bacterium, *Amoebobacter purpureus,* confirms the suitability of this molecule as a proxy of oxygenic conditions.

3. Although the organic matter input to the sediment is mainly accomplished in the above mentioned two pulses, the algal carotenoids identified in the settling material during an annual period reflect the plankton succession in Lake La

Cruz. Photosynthetic pigments derived from diatoms such as fucoxanthin and diatoxanthin were mostly observed in the material collected by sediment traps over winter and spring seasons whereas over summer and before overturn the carotenoid zeaxanthin, derived from picocyanobacteria, was the dominant pigment. Moreover alloxanthin, the specific carotenoid of the perennial cryptophytes populations growing near oxicline, was present over the year in the settled material.

4. The study of the climate signal in the varved sediment of Lake La Cruz has confirmed the potential of laminated sediment as a powerful tool documenting hydroclimatic variability. The calibration analysis performed between calcite laminae thickness and synchronous instrumental record of rainfall and temperature variables in the region showed that accumulated winter rainfall, from December to March, is the best predictor of calcite laminae thickness ($r=0.725$, $p<0.01$, $n= 35$). The interannual variability of groundwater discharge which depends directly on rainfall variability, controls the seasonal time-course of calcium concentrations in Lake La Cruz water column and so the extend of summer whiting and annual calcite lamina thickness.

5. The winter rainfall reconstruction from A.D.1589 to present based on the calibration function developed for calcite laminae thickness indicates the occurrence of lengthy drier and wetter periods in the region. The most prevailing negative rainfall anomalies were observed during the second half on the 17th century, around 1750-1770, the first half and the end of the 19th century and the begining of the 20th century. However estimated winter rainfall values were above the mean during the 18th century and around 1850 and 1950.

6. The analysis of the instrumental precipitation records from the meteorological stations located in Cuenca over the last 50 years of the 20th century showed a marked influence of the North Atlantic Oscillation, NAO, (from December to March, DJFM) in the winter (DJFM) Cuenca rainfall anomalies ($r = -0.824$, $p<0.01$, $n= 52$). However the statistical analysis of the climate signal over the last 430 years showed the dominance of nonstationary component at high frequencies which suggest that different patterns, not only NAO, have played a role in determining precipitation variability.

7. The onset of calcite laminations at the end of the 16th century is coincident with extreme cold conditions (Little Ice Age) reported for the north hemisphere. This suggests that climate forcing is behing the development of the meromictic condition in Lake La Cruz. Most probably the meromictic stratification pattern was established because of the prolonged anoxia promoted by the ice cover on the lake lasting for longer periods allowing a dimictic condition and then the accumulation of dissolved ions in bottom waters. Moreover factors such as higher lake level, CO_2 oversaturation under strong hypolimnetic anoxia and derived calcite dissolution and therefore accumulation of dissolved calcium and bicarbonate ions, as well as the drastic drop in the temperatures during the end of 17th century known as Maunder Minimum (1675-1700 A.D.) could reinforce the meromictic process.

8. The analyses of photosynthetic pigments in Lake La Cruz showed a progressive increase of their concentration trough the laminated sequence since c.a. 1711 A.D. In this case the variability in the sedimentary pigment concentration is due to past changes in lake productivity because firstly, drastic changes in sedimentation rates which could mislead sedimentary signal were not observed, and secondly, we did not observe evidence of degradation process affecting the pigment signature over the last three centuries.

9. Solar variability could be the forcing factor being behind changes in Lake La Cruz primary production since the establishment of the meromictic stratification pattern. The high contribution of zeaxanthin through the sedimentary sequence and the high *Synechococcus* densities occurring now in Lake La Cruz suggest a significant contribution of the cyanobacterial populations to the primary productivity of the whole system over the studied period. Despite multiple factors interacting in a complex way, the fluctuations of light intensity could have been a limiting factor for Lake La Cruz primary production which in part was due to deep populations as metalimnetic cyanobacteria. Nevertheless the effects on lake production derived from other factors could no be ruled out, especially those related with watershed area.

10. The photosynthetic bacteria have occurred in the oxicline of Lake La Cruz during past periods as currently. However the increase of green sulfur bacteria (GSB) derivatives when pigment concentration derived from oxygenic planktonic populations reached higher levels, suggests that GSB population started to develop conspicuously in Lake La Cruz when the progressive algal production increase could provide enough sulphide derived from recycling of organic matter. On the other hand the oxidative derivatives such as bvir and OH-bph_a observed in the upper part of sediment sequence, indicate the shading effect on PSB layer when higher algal production was inferred.

11. The pigment record of Lagunillo del Tejo have shown to be highly sensitive to hydroclimatic variability because lake level fluctuations in response to groundwater influx results in drastic change of ratios of surface area to depth due to the morphometry of the basin. Unlike Lake La Cruz the quantitative variability of biochemical compounds through the sedimentary column of Lagunilo del Tejo was related with changes in the sedimentation dynamics, in this case controlled by lake level fluctuations, instead of describing lake production variability.

12. In Lagunillo del Tejo level fluctuations appear to control shifts of sedimentary pigments assemblages. The pigment signature suggests that two different communities, (1) the planktonic composed by cyanobacteria, chlorophytes, cryptophytes and purple sulfur bacteria and (2) another community of charophytes, vascular submerger macrophytes and algae related with the littoral environment, alternated their dominance as a biotic response to lake level fluctuations during wet and dry periods, respectively.

13. Likewise the composition of anoxic bacterial community of Lagunillo del Tejo also responses to lake level fluctuations. The relative dominance of purple bacteria in high level periods alternated with green bacteria dominance at lowering. These changes in relative abundances of phototrophic anoxic bacterial populations could be associated with differences in light quality at oxicline depth conditioned by differences in light attenuation with depth.

10. Resumen

Introducción

A nivel global el modelo de desarrollo económico de las sociedades actuales está modificando sustancialmente las características de la superficie terrestre así como el contenido de aerosoles y gases de efecto invernadero en la atmósfera. Desde los años ochenta se vienen acumulando evidencias que confirman el efecto perturbador de la actividad antrópica en la dinámica de los ecosistemas naturales y en el clima. Cada vez con más fuerza se discuten las consecuencias del impacto antrópico y la preocupación de la comunidad científica ante esta nueva era, el "antropoceno", ha traspasado los límites del ámbito científico y en la actualidad comienza a ser una cuestión de carácter político. Sin embargo, la evaluación del impacto antrópico en todas sus dimensiones es difícil de determinar si no se conocen desde una perspectiva temporal las dinámicas y los complejos mecanismos de la variabilidad natural tanto ecológica como climática.

En este contexto cobran importancia los sedimentos lacustres como archivos donde quedan registrados en sucesivos niveles estratigráficos una valiosa información ambiental sobre la cuenca y el mismo lago. Mediante el análisis de los restos de origen biológico y las características geoquímicas del sedimento, los estudios paleolimnológicos ofrecen una inestimable perspectiva temporal para conocer la variabilidad natural y entender las dinámicas de los sistemas lacustres frente a la perturbación, así como la trayectoria de las comunidades biológicas. Además, los sedimentos lacustres, junto con otros archivos naturales como los anillos de crecimiento de árboles, los corales y los testigos de hielo recuperados en las zonas polares y de hielos perpetuos, conservan una amplia variedad de indicadores climáticos que han permitido la reconstrucción de diferentes variables como la temperatura y las precipitaciones con una elevada resolución temporal. Finalmente podemos decir que pocos registros paleoambientales, si no ninguno, contiene una información tan precisa de la relación entre el ambiente y las sociedades humanas como los registros lacustres.

El trabajo que se presenta a continuación es un estudio paleolimnológico de dos lagunas próximas, la Laguna de La Cruz y el Lagunillo del Tejo, localizadas en la provincia de Cuenca a unos 1000 m. de altura sobre el nivel del mar y pertenecientes al sistema cárstico de Cañada del Hoyo. Numerosos estudios llevados a cabo por la linea de investigación de Limnología y Ecología Microbiana de la Universidad de Valencia durante los últimos 20 años, han contribuido a la caracterización ecológica y mejorado notablemente el conocimiento de este particular ecosistema mediterráneo. Ambas lagunas poseen una superficie circular de la lámina de agua y un fondo tronco-cónico. Sin embargo las dimensiones de la Laguna de la Cruz (122 m de diámetro y una profundidad máxima de 21 m) superan a las del Lagunillo del Tejo (70 m de diámetro y 8 m de profundidad máxima). Puesto que en las lagunas no desemboca ningún afluente superficial, el nivel del agua fluctúa dependiendo del patrón de carga y descarga del acuífero.

En ambas lagunas el periodo de estratificación térmica y por consiguiente, la condición de anoxia en las capas de agua más profundas comienza a primeros de primavera manteniéndose hasta bien entrado el otoño. Justo por debajo de la oxiclina se desarrollan poblaciones de bacterias fotosintéticas. A mediados de Noviembre cuando las temperaturas comienzan a ser más frescas, se destruye la termoclina y se mezcla la columna de agua. Sin embargo, en la Laguna de La Cruz el proceso de mezcla de la columna de agua no supera los 17 m de profundidad, la profundidad donde se encuentra una quimioclina permanente. Este gradiente químico debido a la solubilización y acumulación de sales supone un gradiente de densidad que condiciona la interaccion entre mixolimnion y monimolimnion. La Laguna de La Cruz es por tanto un lago de tipo meromíctico lo que implica que la capa de agua más profunda, el monimolimnion, al no mezclarse con el resto de la columna de agua y dominar procesos de remineralización de la materia orgánica sedimentada, permanece anóxica de forma estable.

En la Laguna de La Cruz anualmente se observa todos los veranos el fenómeno de blanqueado debido a la precipitación masiva de carbonato cálcico. Esta precipitación acontece en un lapso de tiempo corto y queda registrada en el sedimento dando lugar a una capa de micrita. En el sedimento de la Laguna de La Cruz estas capas claras constituidas mayoritariamente por cristales de cálcita alternan con capas oscuras formadas por materia orgánica y materiral detritico sedimentado durante el resto del año. Dos de estas láminas, una oscura y una clara, corresponden a un ciclo anual de sedimentación. Este tipo de acumulación cíclica de materiales autóctonos conocido como sedimento varvado supone un conservado y continuo paleoregistro de alta resolución temporal.

El primer objetivo de este estudio es determinar el rango de variabilidad natural de la comunidad de productores primarios y analizar la posible incidencia de factores naturales o antrópicos condicionantes. En este sentido se han analizado los cambios cuantitativos y cualitativos de los pigmentos fotosintéticos presentes en secuencias sedimentarias de La Laguna de La Cruz y del Lagunillo del Tejo como indicadores de las diferentes poblaciones de productores primarios durante los últimos siglos. Por otro lado, el segundo objetivo de este trabajo se centra en el análisis de la condición meromíctica en La Laguna de La Cruz. Un estudio paleolimnologico previo sobre la Laguna de La Cruz (Julia et al 1998) nos permite tener un conocimiento sobre la ontogenía de lago y concretamente sobre la meromixis en la laguna. Sin embargo los factores implicados en el origen y desarrollo, así como, la respuesta de la biota permanecían inexplorados. Finalmente consideramos importante analizar con detalle la secuencia varvada de La Cruz y determinar si existe una relación entre alguna variable climática y las laminaciones que se observan en los 40 cm más superficiales del sedimento de la Laguna de La Cruz, puesto que los sedimentos laminados tienen una relación entre sus características y la variable climática que condiciona su formación.

Con el objeto de alcanzar los objetivos propuestos planteamos el trabajo en cinco bloques. En primer lugar consideramos necesario para la interpretación de los perfiles estratigráficos de los pigmentos fósiles determinar la transferencia del indicador elegido, en este caso los pigmentos fotosintéticos, de la columna de agua en señal sedimentaria. Con tal fin analizamos el material recogido por unas trampas de sedimentación que situadas en el monimolimnion de la Laguna de La Cruz fueron muestreadas de forma mensual durante tres años de estudio limnológico intensivo desde 1996 hasta 1998.

Una vez conocidos los factores que controlan la sedimentación de los organismos fotosintéticos planctónicos, analizamos el contenido de pigmentos fotosintéticos en sendos testigos sedimentarios recuperados en el Lagunillo de Tejo y La Laguna de La Cruz. El análisis de estos compuestos, clorofilas y carotenos tanto de origen algal como bacteriano, se realizó mediante técnicas de cromatografía líquida (HPLC) y espectrometría de masas (LC-MS).

Finalmente abordamos el análisis de las laminaciones de calcita en los 40 cm superficiales del sedimento de La Laguna de La Cruz. Después de hacer una impregnación con resina obtuvimos láminas finas de la secuencia varvada. Mediante técnicas de análisis de imagen medimos los espesores de las varvas, de las dos láminas que las componen y de los niveles detríticos que eventualmente interrumpen las laminaciones. Recopilamos los datos meteorológicos disponibles de las estaciones cercanas y calibramos la señal de espesor de las láminas con los parámetros instrumentales de temperaturas y precipitaciones registrados en los últimos cincuenta años en la región. Una vez calibrada la señal realizamos una reconstrucción anual al parámetro climático sensible hasta el comienzo de la secuencia laminada, 1589 d.C.

Conclusiones

El análisis de los pigmentos fotosintéticos extraídos del material recogido por las trampas de sedimentación ha proporcionado una valiosa información sobre como las dinámicas y procesos lacustres condicionan los patrones de sedimentación de los organismos fotosintéticos. Los resultados de este estudio muestran que la sedimentación de los organismos planctónicos, trazada mediante sus respectivos pigmentos específicos, se produce en dos pulsos de sedimentación. (1. Pulso de finales de otoño) Los compuestos derivados de las bacterias fotosintéticas alcanzan un máximo anual a finales de otoño después de la destrucción de la termoclina. El colapso de la estratificación térmica causa una exposición al oxígeno y por consiguiente la mortalidad de las poblaciones bacterianas. (2. Pulso de principios-mediados de verano) Por otro lado la precipitación masiva de carbonato cálcico durante el verano afecta mayoritariamente a los organismos fotosintéticos oxigénicos. Los altos valores de zeaxantina justo después de los fenómenos de blanqueado indican que los cristales de calcita son vectores de sedimentación para los organismos planctónicos, mayoritariamente cianobacterias unicelulares.

Entre los compuestos recogidos por las trampas de sedimentación, se identificó la bacterioviridina, un compuesto oxidado derivado de la bacterioclorofila_a. Este estudio es la primera evidencia descrita sobre la formación de este derivado en el material sedimentado antes de su incorporación al sedimento lacustre. El aumento de la tasa de sedimentación de bacterioviridina justo después de la mezcla otoñal, asociada a la mortalidad masiva de bacterias del azufre, concretamente *Amoebobacter purpureus,* confirma la viabilidad de utilizar esta molécula con indicador sedimentario del grado de exposición al oxígeno experimentado por la población bacteriana.

Aunque el mayor flujo de materia orgánica se produce durante estos dos pulsos de sedimentación, los carotenos de origen algal identificados en el material sedimentado durante un periodo anual reflejan la sucesión del fitoplancton que ocurre en la Laguna de La Cruz. Los pigmentos fotosintéticos derivados de la diatomeas como la fucoxantina y diatoxantina fueron observados mayoritariamente en el material sedimentado recogido durante el invierno y la primavera, mientras que durante el verano y antes de la mezcla otoñal la zeaxantina, derivado de la poblaciones de cianobacterias, aumentó notablemente su importancia relativa. Además la aloxantina y crocoxantina, carotenos específicos de las poblaciones perennes de criptofíceas que crecen en las proximidades de la oxiclina, estuvieron presentes durante todo el periodo anual en el material recogido por las trampas de sedimentación.

Por otro lado los resultados del análisis del sedimento varvado de La Cruz han confirmado el potencial del este tipo de sedimento como archivo natural de la variabilidad climática. La calibración realizada entre los espesores de las laminas de calcita y el registro sincrónico de temperaturas y precipitaciones en la región (1950- 2002) ha mostrado que la lluvia acumulada durante el periodo invernal desde diciembre hasta marzo es la mejor variable predictora del espesor de las láminas anuales de calcita ($r=0.725$, $p<0.01$, $n=35$). Durante el periodo de estudio en la Laguna de La Cruz (1996-1998) se registró una intensa descalcificación en la columna de agua justo después de la precipitación de carbonato calcico. Las concentraciones de calcio solo se recuperaban en la columna de agua después de las lluvias invernales cuando el nivel del lago subía. Por tanto concluimos que la variabilidad interanual de descarga del acuífero condicionada directamente por las precipitaciones invernales, controla las concentraciones de calcio en la columna de agua y por extensión la formación de cristales durante los fenómenos de precipitación masiva de carbonato cálcico y en definitiva del espesor de la lámina anual de calcita.

La reconstrucción de los valores anuales de lluvias invernales desde 1589 d.C., utilizando la función de calibración desarrollada para el espesor de las láminas de calcita, muestra la ocurrencia de periodos secos y húmedos en la región. Las más intensas anomalías negativas se observaron durante la segunda mitad del siglo XVII, alrededor de 1750-1770, la primera mitad del siglo XIX y hacia finales de este mismo siglo y principios del siglo XX. Sin embargo los valores estimados de lluvias invernales durante el siglo XVIII y alrededor de 1850 y 1950, estuvieron por encima de la media para todo el periodo reconstruido.

El análisis de los valores instrumentales de lluvias registrados en la región de Cuenca durante los últimos 50 años mostró una marcada influencia del patrón de circulación del Atlántico Norte (NAO) en las lluvias invernales, desde diciembre a marzo ($r=-0.824$, $p<0.01$, $n= 52$). Sin embargo el análisis estadístico de la señal climática registrada en el sedimento de la Cruz durante los últimos 430 años tan solo muestra como significativa una periodicidad de 84 años y un dominio del componente no estacionario para las altas frecuencias. Estos resultados sugieren que otros patrones de circulación atmosférica, no solo la NAO, pudieron ejercer un dominio sobre la variabilidad interanual en las precipitaciones regionales.

El inicio de las laminaciones de calcita al final del siglo XV coincide con condiciones extremadamente frías conocidas como la Pequeña Edad del Hielo registradas en todo el hemisferio norte, lo que sugiere que el factor climático podría estar implicado en el desarrollo de la condición de meromixis en la Laguna de La Cruz. Probablemente el patrón de estratificación meromíctico se estableció debido a la progresiva acumulación de sustancias disueltas en las capas profundas durante periodos prolongados de anoxia no solo en verano sino también en las épocas invernales, cuando la superficie del lago se congelaba. Otros factores como el aumento del nivel de agua, la disolución de los cristales de calcita en la zona hipolimnética así como la drástica caída de las temperaturas a finales del siglo XVII, periodo conocido como el Mínimo de Maunder, pudieron reforzar el desarrollo de la meromixis

El análisis de los pigmentos fotosintéticos en la secuencia laminada de la Laguna de La Cruz mostró un progresivo aumento en sus concentraciones desde aproximadamente 1711 d.C. En este caso la variabilidad en las concentraciones de pigmentos en el sedimento se debe a cambios en la productividad porque no se detectaron cambios drásticos en las tasas de sedimentación ni se observaron evidencias de diferencias en los procesos de degradación de los compuestos pigmentarios durante los últimos tres siglos. La variabilidad solar ha sido identificada como el principal factor condicionante de producción primaria desde el establecimiento de la meromixis. Las altas concentraciones de zeaxantina en la secuencia sedimentaria estudiada y las altas densidades poblacionales de picocianobacterias en la actualidad sugieren una considerable contribución de las cianobacterias a la productividad primaria del sistema durante el periodo de tiempo estudiado. A pesar de los múltiples factores que interaccionan de forma compleja, las fluctuaciones en la intensidad de luz parecen haber sido el factor limitante en la productividad de La Laguna de La Cruz, en gran parte debida a poblaciones de organismos fotosintéticos que se desarrollan en zonas profundas del metalimnion como las cianobacterias. No obstante los efectos en la producción primaria debidos a otros factores concretamente los relacionados con la cuenca no deben ser descartados.

Las bacterias fotosintéticas han estado presentes en la Laguna de la Cruz durante periodos de tiempo pasados al igual que lo están en la actualidad. Sin embargo el aumento de los pigmentos fotosintéticos característicos de las bacterias verdes del azufre cuando las concentraciones de derivados algales alcanzaron los valores más elevados, sugiere que estas poblaciones bacterianas se desarrollaron cuando el aumento de la productividad oxigénica permitía una suficiente concentración de sulfhídrico por el reciclado de la materia orgánica. El aumento en paralelo de los derivados oxidados como la bacterioviridina y OH-bacteriofeofitina_a cuando se infieren los valores más elevados de producción algal, sugieren una exposición de las bacterias rojas del azufre a condiciones oxidantes debido a la necesidad de compensar la limitación en la disponibilidad de luz, alcanzando posiciones más someras en el límite de la oxiclina y por tanto expuestas a condiciones microaerobias.

El registro sedimentario del Lagunillo del Tejo resultó ser sensible a la variabilidad hidroclimática puesto que las fluctuaciones de nivel en respuesta al patrón de descarga del acuífero suponen cambios drásticos en las relaciones entre superficie y profundidad debido a las características de la cubeta. A diferencia de la Laguna de La Cruz, las variaciones cuantitativas de las concentraciones de pigmentos en la columna sedimentaria estan condicionadas por los cambios en las dinámicas y tasas de sedimentación, controlados por las fluctuaciones de nivel en lugar de ser adscritos a variabilidad en la producción primaria.

En el Lagunillo del Tejo las fluctuaciones de nivel controlan los cambios en las concentraciones relativas de los diferentes pigmentos fotosintéticos que se observaron en la secuencia sedimentaria. La señal sedimentaria sugiere que dos comunidades diferentes; (1) una comunidad planctónica formada por cianobacterias, clorofíceas, criptófitas y bacterias rojas del azufre y otra (2) una comunidad formada por carófitas y macrófitos sumergidos y algas ligadas a ambientes litorales, alternaron su dominancia relativa como respuesta de la comunidad biótica a los cambios de nivel durante los periodos húmedos y secos en la región. La composición de la comunidad bacteriana también responde a las fluctuaciones de nivel en el Lagunillo del Tejo. El dominio relativo de las bacterias rojas del azufre durante los periodos con un mayor nivel de agua alterna con el dominio de las bacterias verdes del azufre cuando este se reduce. Estos cambios en las abundancias relativas de las bacterias fotosintéticas anoxigénicas están relacionados con las diferencias en la calidad de la luz que llega a la oxiclina debido al efecto de absorción de la luz que se da en función de la profundidad en este lago.

References

Airs RL, Atkinson JE and Keely BJ. 2001. Development and application of a high resolution chromatographic method for the analysis of complex pigment distributions. J Cromatogr. A. 917: 167-177.

Alpert P, Ben-Gai T, Baharad A, Benjamini Y, Yekutieli D, Colacino M, Diodato L, Ramis C, Homar V, Romero R, Michaelides S and Manes A. 2002. The paradoxical increase of Mediterranean extreme daily rainfall in spite of decrease in total values. Geophys Res Lett. 29: 31-1.

Anderson NJ. 1993. Natural versus anthropogenic change in lakes: the role of the sediment record. TREE. 8: 356-361.

Anderson NJ. 1995a. Temporal scale, phytoplankton ecology and paleolimnology. Freshwater Biol. 34:367-378.

Anderson NJ. 1995b. Using the past to predict the future: lake sediments and the modelling of limnological disturbance. Ecol Model. 78: 149-172.

Anderson RY. 1992. Possible connection between surface winds, solar activity and the Earth’s magnetic field. Nature. 358: 51-52.

Ambrosetti W and Barbanti L. 2005. Evolution towards meromixis of Lake Iseo (Northern Italy) as revealed by its stability trend. J Limnol. 64: 1-11.

APHA. 1992. Standard methods for the examination of water and wastewater. 18th edition. American Public Health Association. Washington D.C., 1100 pp.

Appleby PG. 2001. Chronostratigraphic techniques in recent sediments. In: Tracking Environmental Change Using Lake Sediments: Basin Analysis, Coring and Chronological Techniques, vol 1. Last WM, Smol JP (eds). Kluwer Academic Plublishers, Dordrecht: pp 171-203.

Armengol-Diaz J, Esparcia A, Vicente E and Miracle MR. 1993. Vertical distribution of planktonic rotifers in a karstic meromictic lake. Hydrobiologia. 255/256: 381-388.

Baillie MGL and Pilcher JR. 1973. A simple cross-dating program for tree-ring timescale. Tree-ring Bull. 38: 35-43.

Baines SB and Pace ML. 1994. Relationships between suspended particulate matter and sinking flux along a trophic gradient and implications for the fate of planktonic primary production. Can. J. Fish. Aquat. Sci. 51: 25-36.

Barriendos M. 1994. El clima historico de Catalunya. Aproximaciones a sus caracteristicas genrales (ss XV-XIX), PhD thesis, University of Barcelona, Barcelona.

Barriendos M and Llasat M.C. 2003. The case of the “Maldá” Anomaly in the western Mediterranean Basin (AD 1760-1800): An exemple of a strong climatic variability. Climatic Change. 61: 191-216.

Barnston AG and Livezey RE. 1987. Classification, seasonality and persistence of low frecuency atmospheric circulation patterns. Mon Weather Rev. 115: 1825-1850.

Battarbee RW, Jones VJ, Flower RJ, Cameron NG, Bennion H, Carvalho L and Juggins S. 2001. Diatoms. In:Tracking environmental change using lake sediments. Terrestial,algal and siliceous indicators (vol. 3). Smol JP, Birks JB and Last WM. Kluwer Academic Publishers, Dordrecht, pp: 155-202

Bauer A. 2004. Annually laminated lake sediments and their paleoclimatic relevance. In: The climate in historical times. Towards a synthesis of Holocene proxy data and climate models. Fisher, H., Kumke, T., Lohmann, G., Flöser, G., Miller, H., von Storch, H. and Negendank J.F.W. (eds). Springer, Berlin: pp 109-129.

Benassar B. 1967. Valladolid au Siècle D'or, Flammarion, Paris.

Bianchi TS, Rolff C, Widbom B and Elmgren R. 2002. Phytoplankton pigments in Baltic Sea seston and sediments: seasonal variability, fluxes, and transformations. Estuar Coast Shelf Sci. 55: 369-383.

Blenckner T. 2005. a conceptual model of climate-related effects on lake ecosystem. Hydrobiologia. 533:1-14.

Bloesch J and Burns NM. 1980. A critical review of sediment traps technique. Schweiz. Z. Hydrol. 42: 15-55.

Blomqvist S and Håkanson L. 1981. A review on sediment traps in aquatic environments. Arch Hydrobiol. 91: 101-132.

Borrego CM, Garcia-Gil J, Cristina XP, Vila X and Abella CA. 1998. Occurrence of new bacteriorophyll d forms in natural populations of green sulphur bacteria. FEMS Microbiol Ecol. 26: 257-267.

Bradbury JP, Cumming BE and Laird KA. 2002. A 1500-year record of climatic and environmental change in Elk Lake, Minnesota III: Measures of past primary productivity. J Paleolimnol. 27: 287-299.

Bradley RS, Retelle MJ, Ludlam SD, Hardy DR, Zolitschka B, Lamoureux SF and Douglas MSV. 1996. The Taconite Inlet Lakes Project: a systems apprach to paleoclimatic reconstruction. J Paleolimnol. 16: 97-110.

Bradley RS. 1999. Paleoclimatology. Academic Press, San Diego, 613 pp.

Brotas V and Plante-Cuny M-R. 2003. The use of HPLC pigment analysis to study microphytobenthos communities. Acta Oecologica. 24: S109-S115.

Brown SR. 1968. Bacterial carotenoids from freshwater sediments. Limnol Oceanogr. 13: 233-241.

Brown SR, McIntosh HJ and Smol JP. 1984. Recent paleolimnology of a meromictic lake: fossil pigments of photosynthetic bacteria. Int Ver Theor Angew Limnol Verh. 22: 1357-1360.

Brunskill GJ. 1969. Fayetteville Green Lake, New Tork.II: Precipitation and sedimentation of calcite in a meromictic lake with laminated sediments. Limnol Oceanogr. 14: 830-847.

Camacho A.2006. On the occurrence and ecological features of deep chlorophyll maxima (DCM) in spanish stratified lakes. Limnética 25: 453-478.

Camacho A and Vicente E. 1998. Carbon photoassimilation by sharply stratified phototrophic communities at the chemocline of Lake Arcas (Spain). FEMS Microbiol Ecol. 25: 11-22.

Camacho A, Vicente E and Miracle MR. 2000. Ecology of a deep–living Oscillatoria (= Planktothrix) population in the sulphide-rich waters of a Spanish karstic lake. Arch Hydrobiol. 148: 333-355.

Camacho A, Picazo A, Miracle MR and vicente E. 2003a. Spatial distribution and temporal dynamics of picocyanobacteria in a meromictic karstic lake. Arch Hydrobiol Suppl. (Algol. Stud. 109). 148: 171-184.

Camacho A, Wurtsbaugh WA, Miracle MR, Armengol X and Vicente E. 2003b. Nitrogen limitation of phytoplankton in a spanish karst lake with a deep chlorophyll maximum: a nutrient enrichment bioassay approach. J Plankton Res. 25: 397-404.

Camacho A. and de Wit R. 2003. Effect of nitrogen and phosphorus additions on a benthic microbial mat from a hypersaline lake. Aquat Microb Ecol. 32: 261-273.

Carpenter SR, Elser MM and Elser JJ. 1986. Chlorophyll production, degradation, and sedimentation: Implications for paleolimnology. Limnol Oceanogr.31: 112-124.

Carpenter SR, Leavitt PR, Elser JJ and Elser MM. 1988. Chlorophyll budges: response to food web manipulations. Biogeochemistry 6: 79-90.

Carpenter SR and Kitchell JF (eds). 1993. The trophic cascade in Lakes. Cambridge University Press, Cambridge.

Casty C, Handorf D, Raible CC, González-Rouco JF, Weisheimer A, Xoplaki E, Luterbacher J, Dethloff K and Wanner H. 2005. Recurrent climate winter regimes in reconstructed and modelled 500 hPa geopotential height fields over the North Atlantic/European sector 1659-1990. Clim Dynam. doi: 10.1007/s00382-004-0496-8.

Chen N, Bianchi TS and Bland JM. 2003. Implications for the role of pre- versus post-deposional transformations of chlorophyll-a in the Lower Mississippi River and Louisiana shelf. Marine Chemistry 81: 37-55.

Cirujano S. 1995. Flora y vegetacion de las lagunas y humedales de la provincial de Cuenca. CSIC, Madrid.

Clayton RK and Sistrom WR. 1978. The photosynthetic bacteria. Plenum Press, New York: pp 7-8

Cohen AS. 2003. Paleolimnology. The history and Evolution of Lake Systems. University Press. Oxford.

Cook ER, Briffa KR and Jones PD. 1994. Spatial regression methods in dendroclimatology-a review and comparison of two techniques. Int J Climatol. 14: 379-402.

Coops H, Beklioglu M and Crisman TL. 2003. The role of water-level fluctuations in shallow ecosystems- workshop conclusions. Hydrobiologia. 506-509: 23-27.

Creus-Novau J, Fernandez A and Manrique E. 1997. Dendrocronología y clima del último milenio en España. Aspectos metodológicos y avance de resultados. En: El paisaje mediterraneo a traves del espacio y tiempo. Implicaciones en la desertificacion. Ibanez, Valero-Garces and Machado (eds). Logroño,Geoforma.

Cuddington K and Leavitt PR. 1999. An individual-based model of pigment flux in lakes: Implications for organic biogeochemistry and paleoecology. Can J Fish Aquat Sci. 56: 1964-1977.

Cumming BF, Wilson SE and Smol JP. 1993. Paleolimnological potential of chrysophyte cysts and scales and of sponge spicules as indicators of lake salinity. Int J Salt Lake Res. 2: 87-92.

Daley RJ. 1973. Experimental characterization of lacustrine chlorophyll diagenesis. II: Bacterial, viral, and herbivore grazing effects. Arch Hydrobiol. 72: 409-439.

Dasí MJ and Miracle MR. 1991. Distribucion vertical y variacion estacional del fitoplancton de una laguna karstica meromictica, la Laguna de La Cruz (Cuenca, España). Limnética. 7: 37-59.

Daubechies I. 1990. The wavelet transform, time-frequency localization and signal analysis. IEEE Trans. on Information Theory, vol. 35 (5) 1990.

De Geer G. 1912. A geochronology of the last 12000 years. Proc. 11th Internat. Geological Congress 1910: 241-253.

Dean JM and Kemp AES. 2004. A 2100 year BP record of the Pacific Decadal Oscillation, El Niño Southern Oscillation and Quasi-Biennial Oscillation in marine production and fluvial input from Saanich Inlet, British Columbia. Palaeogeogr Palaeoclimatol Palaeoecol. 213: 207-229.

Dean W, Anderson R, Bradbury JP and Anderson D. 2002. A 1500-year record of climate and environmental change in Elk Lake, Minnesota I: Varve thickness and gray-scale density. J. Paleolimnol. 27: 287-299.

Dearing JA. 1997. Sedimentary indicators of lake-level changes in the humide temperate zone: a critical review. J. Paleolimnol. 18:1-14.

De Vicente I, Cattaneo K, Cruz-Pizarro L, Brauer A and Guilizzoni P. 2006. Sedimentary phosphate fractions related to calcite precipitation in an eutrophic hardwater lake (Lake Alserio, northern Italy). J Paleolimnol 35: 55-64

Dickman M. 1987. Lake sediment microlaminae and annual mortalities of photosynthetic bacteria in a oligomictic lake. Freshwater. Biol. 18: 151-164.

Dittrich M, Kurz P and Wherli B. 2004. The role of autotrophic picocyanobacteria in calcite precipitation in an oligothrophic Lake. Geomicrobiol J. 21:45-53.

Eckardt CB, Keely BJ and Maxwell JR. 1991. Identification of chlorophyll transformation products in a lake sediment by combined liquid chromatography- mass spectrometry. J. Chromatogr. 19: 217-227.

Engstrom DR, Swain EB, Kingston JC. 1985. A paleolimnological record of human disturbance from Harvey´s Lake, Vermont: geochemistry, pigments and diatoms. Freshwater Biol. 15: 261-288.

Esparcia A, Armengol J, Vicente E and Miracle MR. 1991. Vertical distribution of Anuraeopsis species as related to oxygen depletion in two stratified lakes. Verh Int Ver Limnol. 24: 2745-2749.

Esteban-Parra MJ, Rodrigo FS and Castro-Diez Y. 1998. Spatial and temporal patterns of precipitation in Spain for the period 1880-1992. Int J Climatol 18: 1557-1574.

Fernandez-Cancio A, Manrique-Menendez E, Genova-Fuster M and Creus-Novau J. 1993. Estudio fitoclimatico de la Serrania de Cuenca en los ultimos 300 años. Congreso Forestal Español. Lourizan, Ponencias y comunicaciones Tomo I, pp 93-98.

Fogg GE and Belcher JH. 1961. Pigments from the bottom deposits of and English lake. New Phytol. 60: 129-138.

Font-Tullot I. 1988. Historia del clima de España. Cambios climáticos y sus causas.

Instituto Nacional de Meteorología, Madrid.

Fox DL. 1944. Biochemical fossils. Science 100: 111-113.

Frölich C and Lean J. 2004. Solar radiative output and its variability: evidence and mechanism. The Astron Astrophys Rev. 12: 273-320.

Gabor D. 1946. Theory of communication. J. Inst. Elect. Eng. vol. 93 (3): 429-457.

Garcia-Herrera R, Macias A, Gallego D, Hernandez E, Prieto MR, Gimeno L and Diaz H. 2003. Reconstruction of the precipitation in the Canary Islands for the period 1595-1836. B Am Metorol Soc. 84: 1037

Gibbison R, Peakman TM and Maxwell JR. 1995. Novel porphyrins as molecular fossils for anoxygenic photosynthesis. Tetrahedron Lett. 36: 9057-9060.

Giralt Romeu S and Julià Brugués R. 2004. Water level reconstruction in closed lakes based on the mineralogical composition of sediments. Limnogelogy in Spain: a tribute to Kerry Kelts. In: B. Valero-Garcés (Ed.), Departamento de Publicaciones del CSIC, Madrid, pp: 305-326

Gorham E and Sanger JE. 1972. Fossil pigment in the surface sediments of a meromictic lake. Limnol Oceanogr. 17: 618-622.

Grimm EC.1987. CONISS: a FORTRAN 77 program for statigraphically constrained cluster analysis by the method of incremental sum of squares. Computers and Geosciences. 13:13-35

Gruber N, Wehrli B and Wüest A. 2000. The role of biogeochemical cycling for the formation and preservation of varved sediment in Soppensee (Switzerland). J Paleolimnol. 24: 277-291.

Guilizzoni P, Lami A, Ruggiu D and Bonomi G. 1986. Stratigraphy of specific algal and bacterial carotenoids in the sediment of Lake Varese (N. Italy). Hydrobiologia 143: 321-325.

Guilizzoni P, Lami A and Marchetto A. 1992. Plant pigment ratios from lake-sediments as indicators of recent acidification in alpine lakes. Limnol. Oceanogr. 37: 1565-1569.

Hakala A. 2004. Meromixis as a part of lake evolution- Observations and a revised classification of true meromictic lakes in Finland. Boreal Environm Res. 9: 37-53

Håkanson L and Jansson M. 1983. Principles of lake sedimentology. Springer, Berlin.

Hall RI, Leavitt PR, Quinlan R, Dixit AS and Smol JP. 1999. Effects of agriculture, urbanization and climate on water quality in northern Great Plains. Limnol. Oceanogr. 43: 739-756.

Hammer UT. 1994. Life and times of five Saskatchewan Saline Meromictic Lakes. Int Revue ges Hydrobiol. 79: 235-248

Hannon GE and Gaillard MJ. 1997. The plant-macrofossil record of past lake-level changes. J Paleolimnol. 18: 15-28.

Harradine PJ, Harris PG, Head RN, Harris RP and Maxwell JR. 1996. Steryl chlorin esters are formed by zooplankton herbivory. Geochim. Cosmochim. Acta. 60:2265-2270.

Harris GP. 1985. Phytoplankton Ecology. Chapman and Hall, London

Harris PG, Carter JF, Head RN, Harris RP, Eglington G and Maxwell JR. 1995. Identification of chlorophyll transformation products in zooplankton faecal pellets and marine sediment extracts by liquid chromatography/mass spectrometry atmospheric pressure chemical ionization. Rap Comm Mass Spec. 9: 177-183.

Head EJH and Harris LR. 1992. Chlorophyll and carotenoid transformation and destruction by *Calanus spp* grazing on diatoms. Mar Ecol Prog Ser. 86: 229-238.

Hilton J, Lishman JP and Allen PV. 1986. The dominant processes of sediment distribution and focusing in a small, eutrophic, monomictic lake. Limnol Oceanogr. 31: 125-133.

Hobaek A, Manca M and Andersen T. 2002. Factors influencing species richness in lacustrine zooplankton. Acta Oecologica. 23: 155-163.

Hodgson DA, Wright SW and Davies N. 1997. Mass Spectrometry and reverse phase HPLC techniques for the identification of degraded fossil pigments in lake sediments and their application in paleolimnology. J Paleolimnol. 18: 335-350.

Hodgson DA, Wright SW, Tyler PA and Davies N. 1998. Analyses of fossil pigments from algae and bacteria in meromictic Lake Fidler, Tasmania, and its application to lake management. J Paleolimnol. 19: 1-22.

Hoyt DV and Schatten KH. (eds) 1997. The role of the Sun in Climate Change. Oxford Univ Press. New York

Hurley JP and Armstrong DE. 1990. Fluxes and transformations of aquatic pigments in Lake Mendota, Wisconsin. Limnol Oceanogr. 35: 384-398.

Hurley JP and Armstrong DE. 1991. Pigment Preservation in lake sediments: A comparision of sedimentary environment in Trout Lake, Wisconsin. Can J Fish Aquat Sci 48: 472-486.

Hurley JP and Garrison PJ. 1993. Composition and sedimentation of aquatic pigments associated with deep plakton in lakes. Can J Fish Aquat Sci. 50: 2713-2722.

Itkonen A and Salonen VP. 1994. The response of sedimentation in three varved lacustrine sequences to air temperature, precipitation and human impact. J Paleolimno.l 11: 323-332.

Itoh N, Tani Y, NagataniT and Soma M. 2003. Phototrophic activity and redox condition in Lake Hamana, Japan, indicated by sedimentary photosynthetic pigments and molybdenum over the last 250 years. J Paleolimnol. 29: 403-422.

Janicot S. 1996. Sahel droughts and ENSO dynamics. Geophys Res Lett 23:515-518.

Jankowski T, Livingstone DM, Buhrer H, Forster R, Niederhauser P. 2006. Consequences of the 2003 European heat wave for lake temperature profiles, thermal stability, and hypolimnetic oxygen depletion: Implications for a warmer world. Limnol Oceanogr 51: 815-819.

Jeffrey SW. 1997. Chlorophyll and carotenoid extinction coefficients. In Jeffrey SW, Mantoura RFC and Wright SW (eds) Phytoplankton pigments in Oceanography. Unesco Publishing, Paris, pp 595-596.

Jeffrey SW and Vesk M. 1997. Introduction to marine phytoplankton and their pigments signature.In Jeffrey SW, Mantoura RFC and Wright SW (eds) Phytoplankton pigments in Oceanography. Unesco Publishing, Paris, pp 37-84.

Jellison R and Melack JM. 1993. Algal photosynthetic activity and its response to meromixis in hypersaline Mono-Lake, California. Limnol Oceanogr. 38: 818-837

Johnson EA and Larsen CPS. 1991. Climatically induced change in fire frequency in Southern Canadian Rockies. Ecology. 72: 194-201.

Johnson EA and Gutsell SL. 1994. Fire frecuency models, methods and interpretation. Adv Ecol Res 25: 239-287.

Julià R, Burjachs F, Dasí MJ, Mezquita F, Miracle MR, Roca JR, Seret G and Vicente E. 1998. Meromixis origin and recent trophic evolution in the spanish mountain lake La Cruz. Aquat Sci. 60:279-299.

Kelts K and Hsü K J. 1978. Freshwater carbonate sedimentation. In: Lakes. Geology, Chemistry, Physics. A Lerman (ed). Springer Verlag, New York: pp 295-323.

Kempe S and Degens EG. 1979. Varves in the Black Sea and Lake Van (Turkey). In: Morains and Varves. C Schlüchter (ed). Balkema, Rotterdam: pp. 309-318.

Kleppel GS and Lessard EJ. 1992. Carotenoid pigments in microzooplancton. Mar Ecol Prog Ser. 84: 211-218.

King LL and Wakeham SC. 1996. Phorbin steryl ester formation by macrozooplankton in the Sargasso Sea. Org Geochem.. 24: 455-465.

Lami A, Niessen F, Guilizzoni P, Masaferro J and Belis CA. 1994. Paleolimnological studies of the eutrophication of volcanic Lake Albano (Central Italy). J Paleolimnol. 10: 181-197.

Lami A, Guilizzoni P and Marchetto A. 2000. High resolution analysis of fossil pigments, carbon, nitrogen and sulfur in the sediment of eight European Alpine lakes: the MOLAR project. J Limnol. 59: 15-28.

Lamoureux SF. 2001. Varve chronology techniques. In: Last WM and Smol JP (eds), Paleoenvironmental Research (DPER) Volume 2, Tracking Environmental Change Using Lake Sediment: Physical and Geochemical Techniques. Kluwer, pp.247-260.

Lamoureux SF and Gilbert R. 2004. A 750-yr record of autumn snowfall and winter storminess recorded in varved sediments of Bear Lake, Devon Island, Artic Canada. Quaternary Res. 61: 134-147.

Lean J. 2000. Evolution of the Sun’s spectral Irradiance since the Maunder Minimun. Geophys Res Lett. 27:2425-2428

Leavitt PR. 1993. A review of factor that regulate carotenoid and chlorophyll deposition and fossil abundance. J Paleolimnol. 9: 109-127.

Leavitt PR and Carpenter SR. 1990a. Aphotic degradation in the hypolimnion: Implications for sedimentation studies and paleolimnology. Limnol Ocenogr. 35: 520-534.

Leavitt PR and Carpenter SR. 1990b. Regulation of pigment sedimentation by photo-oxidation and herbivore grazing. Can J Fish Aquat Sci. 47: 1166-1176.

Leavitt PR and Hodgson DA. 2001. Practical methods for analysis of sedimentary pigment. In: Developments in Paleoenvironmental Research, Tracking Environmental Changes Using Lake Sediments, Terrestrial, Algal and Siliceous Indicators, Vol 3. Smol JP, Birks HJB and Last WM (eds). Kluwer Academic Plublishers, Dordrecht: pp 295-325.

Leavitt PR, Vinebrooke RD, Donald DB. Smol JP and Schindler DW. 1997. Past ultrviolet radiation environments in lakes derived from fossil pigments. Nature. 388: 457-459.

Leemann A and Niessen F. 1994. Varve formation and climatic record in an Alpine proglacial lake: calibrating annually-laminated sediments against hydological and meteorological data. The Holocene. 4: 1-8.

Le Bris S, Plante-Cuny MR and Vacelet E. 1998. Characterisation of bacterial and algal pigments and breakdown products by HPLC in mixed freshwater planktonic populations. Arch Hydrobiol. 143: 409-434.

Liaaen-Jensen S. 1979. Carotenoids: a chemosystemic approach. Pure Appl Chem. 51: 661-675.

Lindsay Smith JR and Calvin M. 1966. Studies on the chemical and photochemical oxidation of bacteriochlorophyll. J Am Chem Soc. 88: 4500-4506.

Livingston DM and Hajdas I. 2001. Climatically relevant periodicities in the thickness of biogenic carbonate varves in Soppensee, Switzerland (9740-6870 calendar yr BP). J Paleolimnol. 25: 17-24.

Löffler H. 1975. The onset of meromictic conditions in Alpine lakes. In: Suggate Rp and Cresswell MM (eds), R. Soc. N.Z. Wellington, 211-214

Lotter AF and Birks HJB. 1997. The separation of the influence of nutrients and climate on the varve time-series of Baldeggersee, Switzerland. Aquatic Sci 59: 362-375.

Lotter AF, Sturm M, Teranes JL and Wehrli B. 1997. Varve formation since 1885 and high-resolution varve analyses in hypertrophic Baldeggersee (Switzerland). Aquat Sci. 59: 304-325.

Lorenzen CJ and Welschmeyer NA. 1983. The in situ sinking rates of herbivore fecal pellets. J Plankton Res. 5: 929-933.

Louda JW, Li J, Liu L, Winfree MN and Baker EW. 1998. Chlorophyll a degradation during cellular senescence and death. Org Geochem. 29: 1233-1251.

Luterbacher J, Xoplaki E, Dietrich D, Rickli R, Jacobeit J, Beck C, Gyalistras D, Schmutz C and Wanner H. 2002. Extending North Atlantic Oscillation reconstruction back to 1500. Atmos Sci Lett. 2: 114-124.

Luterbacher J, Dietrich D, Xoplaki E, Grosjean M and Wanner H. 2004. European seasonal and annual temperature variability, trends, and extremes since 1500. Science. 303: 1499- 1503.

Luterbacher J, Xoplaki E, Casty C, Wanner H, Pauling A, Küttel M, Rutishauser T, Brönnimann S, Fischer E, fleitmann D, González-Rouco FJ, Garcia-Herrera R, Barriendos M, Rodrigo F, Gonzalez-Hidalgo JC, Saz MA, Gimeno L, Ribera P, Brunet M, Paeth H, Rimbu N, Felis T, Jacobeit J, Dünkeloh A, Zorita E, Guiot J, Türkes M, Alcoforado MJ, Trigo R, Wheeler D, Tett S, Mann ME, Touchan R, Shindell DT, Silenzi S, Montagna P, Camuffo D, Mariott A, Nanni T, Brunetti M, Maugori M, Zerefos C, De Zolt S and Lionello P. 2005. Mediterranean climate variability over the last centuries: A review. In: The Mediterranean climate: An overview of the main characteristics and issues. Lionello P, Malanotte-Rizzoli P and Boscolo R (eds). Elsevier.

Maddy D and Brew JS. 1995. Stadistical modelling of Quaternary science data. Technical guide 5, Quaternary Research Association, Cambridge, p. 271.

Magnuson JJ, Meissner JD and Kill DK. 1990. Potential changes in thermal habitat of Great Lakes fish after global climatic warming. Trans Am Fish Soc 119:254-264.

Mallorquí N, Arellano JB, Borrego CM and García-Gil LJ. 2005. Signatura pigments of green sulfur bacteria in lower Pleistocene deposits from the Banyotes lacustrine area (Spain). J. Paleolimnol. 34: 271-280.

Mangili C, Brauer A, Moscariello A and Naumann R. 2005. Microfacies of detrital event layers deposited in Quaternary varved lake sediment of the Piànico-Sèllere Basin (Northern Italy). Sedimentology 52: 927-943.

Manrique E and Fernandez-Cancio A. 2000. Extreme climatic events in dendroclimatic reconstructions from Spain. Climate Change. 44: 123-138.

Mantoura RFC and Llewellyn CA. 1983. The rapid determination of algal chorophyll and carotenoids pigments and their breakdown products in natural waters by reverse-phase high performance liquid chromatography. Anal Chim Acta. 151: 297-314.

Margalef R. 1983. Limnologia. Ed. Omega, Barcelona.

Martin-Vide J and Barriendos M. 1995. The use of the rogations ceremony records in climatic reconstruction: A case study from Catalonia (Spain). Climate Change. 30: 201-221.

Martínez-Solares JM and Mezcua J. 2002. Catálogo sísmico de la Península Ibérica (880 a.c.-1900). Monografía Num. 18. Instituto Geográfico Nacional. Ministerio de Fomento

Mason LM, Gruzkowska MA and Rapley CG. 1994. The response of lake levels and areas to climate change. Climate Change. 27: 161-197.

McFadden PD, Cook JG, Forster LM. 1999. Decomposition of gear vibration signals by the generalized S-transform. Mech Sys Sig Process. 13: 691-707.

McQueen DJ. 1990. Manipulating lake community structure: where do we go from here?. Freshwater Biol. 23: 613-620.

Megard RO. 1968. Planktonic photosynthesis and the environment of calcite carbonate deposition in lakes. Interim Rept. 2. Limnol Res Center U Minn.

Melack JM and Jellison R. 1998. Limnological conditions in Mono Lake: contrasting monomixis and meromixis in the 1990s. Hydrobiologia. 384: 21-39

Mezquita F and Miracle MR. 1997. Chydorid assemblages in the sedimentary sequence of Lake La Cruz (Spain) subject to water level changes. Hydrobiologia 360: 277-285.

Millie DF, Paerl HW and Hurley JP. 1993. Microalgal pigment assessments using high-performance liquid chromatography: A synopsis of organismal and ecological applications. Can J Fish Aquat Sci. 50: 2513-2527.

Miracle MR, Vicente E and Pedrós Alió C. 1992. Biological studies of Spanish meromictic and stratified lakes. Limnética. 8: 59-77.

Miracle MR, Camacho A, Julià R and Vicente E. 2000. Sinking process and their effect on the sedimentary record in the meromictic Lake La Cruz (Spain). Verh Int Ver Limnol. 27: 1209-1213.

Morata SM, Camacho A, Miracle MR and Vicente E. 2003. Asociaciones fitoplanctónicas y su periodicidad en un lago marcadamente estratificado. Limnética. 22: 35-52.

Muñoz A, Ojeda J and Sanchez-Valverde B. 2002. Sunspot-like and ENSO/NAO-like periodicities in lacustrine laminated sediment of the Pliocene Villarroya Basin (La Rioja, Spain). J Paleolimnol. 27: 453-463.

Naylor CC and Keely BJ. 1998. Sedimentary purpurins: oxidative transformation products of chlorophylls. Org Geochem. 28: 417-422.

Nipkow F. 1920. Vorläufige Mitteilungen über Untersuchungen des Schlammabsatzes in Zürichsee. Z Hydrol. 1: 1-27.

Ocampo R and Repeta DJ. 2002. Isolation and structure determination of two novel C(132)-OH bacteriopheophytin a allomers from a coastal salt pond sediment. Org Geochem. 33: 849-854.

Ocampo R and Repeta DJ. 2004. 132(S)-OH methyl bacteriopheophorbide a allomer in sedimentary organic matter. Org Geochem. 35: 209-214.

Ostrovsky I and Yacobi YZ. 1999. Organic matter and pigments in surface sediments: Possible mechanisms of their horizontal distributions in a stratified lake. Can J Fish Aquat Sci. 56: 1001-1010.

O’Sullivan PE, Moyeed R, Cooper MC and Nicholson MJ. 2002. Comparision between instrumental observational and high resolution proxy sedimentary records of Late Holocene climate change- a discussion of possibilities. Quaternary Int. 88: 27-44.

Overmann J and Pfennig N. 1992. Buoyancy regulation and aggregate formation in Amoebobacter purpureus from Mahoney Lake. FEMS Microbiol Ecol. 10: 67-79.

Overmann J, Sandmann G, Hall KJ and Northcote TG. 1993. Fossil carotenoids and paleolimnology of meromictic Mahoney Lake, British Columbia. Aquat Sci. 55: 31-39.

Overmann J, Beatty JT and May KI. 1994. Photosynthetic activity and population dynamics of Amoebobacter purpureus in a meromictic saline lake. FEMS Microbiol Ecol. 15: 309-320.

Padisak J and Reynolds CS. 2003. Shallow lakes: the absolute, the relative, the functional and the pragmatic. Hydrobiologia. 506-509: 1-11.

Parkin TB and Brock TD. 1980. The effects of light quality on the growth of phototrophic bacteria in lakes. Arch Microbiol. 125: 19-27.

Parkin TB and Brock TD. 1981. The role of phototrophic bacteria in the sulfur cycle of a meromictic lake. Limnol Oceanogr. 26: 880-890.

Pauling A, Luterbacher J, Casty C and Wanner H. 2005. 500 years of gridded high resolution precipitation reconstructions over Europe and the connection to large-scale circulation. Clim. Dinam.DOI 10.1007/s 00382-005-0090-8

Peck JA, Khosbayar P, Fowell SJ, Pearce RB, Ariunbileg S, Hansen BCS and Soninkhishig N. 2002. Mid to Late Holocene climate change in north central Mongolia as recorded in the sediment of Lake Telmen. Palaeogr Palaeoclim Palaeoecol. 183: 153-153.

Pedrós-Alió C, Mas J, Gasol JM and Guerrero R. 1989. Sinking speeds of free-living phototrophic bacteria determined with covered and uncovered traps. J Plankton Res. 11: 887-905.

Peristykh AN and Damon PE. 2003. Persistence of the Gleissberg 88 yr solar cycle over the last 12.000 years. J Geophys Res. 108.1003; doi:10.1029/2002JA009390

Phillip D and Young AJ. 1995. Occurrence of the carotenoid lactucaxanthin in higher-plants LHC-II. Photosynth Res. 43:273-282.

Repeta DJ. 1993. A high resolution historical record of Holocene anoxygenic primary production in the Black Sea. Geochim Cosmochim Acta 57: 4337-4342.

Reynolds CS, Morison HR and Butterwick C. 1982. The sedimentary flux of phytoplankton in the south basin of Windermere. Limnol Oceanogr. 27: 1162-1175.

Reynolds CS. 1984. Phytoplankton periodicity: the interactions of form, function and environmental variability. Freshwater Biol. 14: 111-142.

Riera S, Wansard G and Julia R. 2004. 2000-year environmental history of a karstic lake in the Mediterranean Pre-Pyrenees: the Estanya lakes (Spain). Catena. 55: 293-324.

Rocha A. 1999 Low-frecuency variability of seasonal rainfall over the Iberian Peninsula and ENSO. Int J Climatol. 19: 889-901.

Rodo X, Baert E and Comin FA. 1997. Variations in seasonal rainfall in Southern Europe during the present century: relationships with the North Atlantic Oscillation and the El Niño-Southern Oscillation. Cim Dynam. 13: 275-284.

Rodo X, Giralt S, Brujachs F, Comin FA, Tenorio RG and Julia R. 2002.High-resolution saline lake sediments as enhanced tools for relating proxy paleolake records to recent climatic data series. Sed Geol. 148: 203-220.

Rodrigo FS, Esteban-Parra MJ, Pozo-Vazquez D and Castro-Diez Y. 1999. A 500-year precipitation record in southern Spain. Int J Climatol. 19:1233-1253.

Rodrigo MA, Vicente E and Miracle MR. 1993. Short term calcite precipitation in the karstic meromictic Lake La Cruz (Cuenca, Spain). Verh Int Ver Limnol. 25: 711-719.

Rogrido MA, Vicente E and Miracle MR. 2000. The role of light and concentration gradients in the vertical stratification and seasonal development of phototrophic bacteria in a meromictic lake. Arch Hydrobiol. 148: 533-548.

Rodrigo MA, Vicente E and Miracle MR. 2001. The meromictic Lake La Cruz (Central Spain). Patterns of stratification. Aquat Sci. 63: 406-416.

Rodriguez-Pascua MA, Calvo JP, de Vicente G and Gomez-Gras D. 2000. Soft-sediment deformation structures interpreted as seismites in lacustrine sediments of the Pre-Betic Zone, SE Spain, and their poteintial use as indicators of earthquake magnitudes during the Late Miocene. Sed Geol. 135: 117-135.

Rodriguez-Puebla C, Encinas AH, Nieto S and Garmendia J. 1998. Spatial and temporal patterns of annual precipitation variability over the Iberian Peninsula. Int J Climatol. 18: 299-316.

Romero L, Camacho A, Vicente E and Miracle MR. 2006. Sedimentation patterns of photosynthetic bacteria based on pigment markers in meromictic Lake La Cruz (Spain): Paleolimnological implications. J Paleolimnol. 35: 167-177.

Rowan KS. 1989. Photosynthetic pigments of algae. Cambridge University Press. 334pp.

Rybak M and Dickman M. 1988. Paleoecological reconstruction of changes in the productivity of a small, meromictic lake in Southern Ontario, Canada. Hydrobiologia. 169:293-306.

Sanger JE. 1988. Fossil pigments in paleoecology and paleolimnology. Palaeogeogr Palaeoclimatol Palaeoecol. 62: 343-359.

Schatten KH. 1988. A model for solar constant secular changes. Geophys Res Lett. 15: 121-124.

Schimmel M. 2001. Emphasizing difficulties in the detection of rhythms with Lomb-Scargle periodograms. Biol Rhythm Res. 32: 341-345.

Schimmel M and Gallart J. 2005. The inverse S-transform in filters with time-frequency localization. IEEE Trans. Signal Process. 53: 4417-4422.

Schimmel M and Gallart J. 2006. Frequency-dependent phase coherence for noise suppression in seismic array data. J Geophys Res. (accepted)

Schmidt R, Wunsam S, Brosch U, Fott J, Lami A, Löffler H, Marchetto A, Müller HW, Prazákova M and Schwaighofer B. 1998. Late and post-glacial history of meromictic Längsee (Austria), in respect to climate change and anthropogenic impact. Aquat Sci. 60: 56-88.

Schmidt R, Psenner R, Müller J, Indinger P and Kamenik C. 2002. Impact of late glacial climate variations on stratification and trophic state of the meromictic lake Längsee (Austria): validation of a conceptual model by multiproxy studies. J Limnol. 6: 49-60.

Seibold E. 1958. Jahreslagen in Sedimenten der Mittleren Adria. Geol Rundschan 47: 100-117.

Shiki T, Kumon F, Inouchi Y, Kontani Y, Sakamoto T, Tateishi M, Matsubara H and Fukuyama K. 2000. Sedimentary features of the seismo-turbidites, Lake Biwa, Japan. Sed Geol. 135:37-50

Shuman FR and Lorenzen CJ. 1975. Quantitative degradation of chlorophyll by marine herbivore. Limnol Oceanogr. 20: 580-586.

Siefermannharms D, Hertzberg S, Borch G and Liaaenjensen S. 1981. Carotenoids of higher-plants. 13. Lactucaxanthin, an epsilon-caoten-3,3-diol from lactuca-sativa. Phytochemistry. 20:85-88.

Simon C, Ventosa S, Schimmel M, Dañobeitia JJ, Gallart J, Manuel A. 2006. The S-transform and its inverses: side effects of discretising and filtering, IEEE Trans. Signal Process.(accepted).

Sinha RP, Klisch M, Gröniger A and Häder DP. 1998. Ultraviolet-absorbing/screening substances in cyanobacteria, phytoplankton and macroalgae. J Photochem Photobiol B Biol. 47: 83-94.

Smol JP. 1990. Are we building enough bridges between paleolimnology and aquatic ecology?. Hydrobiologia. 214: 201-206.

Smol JP and Cumming BF. 2000. Tracking long-term changes in climate using algal indicators in lake sediments. J Phycol. 36: 986-1011.

Smol JP, Birks HJB and Last WM. 2001. Using biology to study long-term environmental change. In: Tracking Environmental Change using Lake Sediments, vol 3: Terrestrial, Algal, and siliceous Indicators. JP Smol, HJB Birks and WM Last (eds). Kluwer Academic Publishers, Dordrecht, pp:1-3

Sommer U. 1988. Growth and survival strategies of planktonic diatoms. Growth and reproductive strategies of freshwater phytoplankton. CD Sandgren (eds). Cambridge University Press, Cambridge pp 227-260.

Spooner N, Harvey HR, Pearce GES, Eckardt CB and Maxwell JR. 1994a. Biological defunctionalisation of chlorophyll in aquatic environment. 2. action of endogenous algal enzymes and aerobic-bacteria. Org Geochem. 22: 773-780

Spooner N, Keely BJ and Maxwell JR. 1994b. Biologically mediated defunctionalisation of chlorophyll in the aquatic environment. 1. senescence/decay of the diatom *Phaeodactylum tricornutum*. Org Geochem. 21: 509-516.

Squier AH, Hodgson DA and Keely BJ. 2002. Sedimentary pigments as markers for environmental change in an Antartic Lake. Org Geochem. 33: 1655-1665.

Squier AH, Hodgson DA and Keely BJ. 2004. A critical assessment of the analysis and distributions of scytonemin and related UV-screening pigments in sediments. Org Geochem. 35: 1221-1228.

Steenbergen CLM, Korthals HJ and Dobrynin EJ. 1994. Algal and bacterial pigments in non-laminated lacustrine sediment: studies of their sedimentation, degradation and stratigraphy. FEMS Microbiol Ecol. 13: 335-352.

Stockwell RG, Mansinha L, Lowe RP. 1996, Localization of the complex spectrum: the S Transform, IEEE Trans. Signal Process. 44: 998-1001.

Sun MY, Lee C and Aller RC. 1993. Anoxic and oxic degradation of 14C-labeled chloropigments and 14C-labeled diatom in Long Island sediments. Limnol Oceanogr. 38: 1438-1451.

Swain EB. 1985. Measurement and interpretation of sedimentary pigments. Freshwater Biol. 15: 53-75

Teranes JN, McKenzie JA, Bernasconi SM, Lotter AF and Sturm M. 1999. A study of oxygen isotopic fractionation during bio-induced calcite precipitation in eutrophic Baldeggersee, Switzerland. Geochim Cosmochim Acta 63: 1981-1999.

Thompson JB, Schultze-Lam B, Beveridge TJ and Des-Marais DJ. 1997. Whiting events: biogenic origen due to the photosynthetic activity of the cyanobacteral picoplankton. Limnol Oceanogr 42: 133-141.

Touchan R, Xoplaki E, Funkhouser G, Luterbacher J, Hughes MK, Erkan N, Akkemik Ü and Stephan j. 2005. Reconstructions of spring /summer precipitation for the Eastern Mediterranean from tree-ring widths and its connection to large-scale atmospheric circulation. Clim Dynam. 25: 75-98.

Tracey B, Lee N and Card V. 1996. Sediment indicators of meromixis: comparison of laminations, diatoms, and sediment chemistry in Brownie Lake, Minneapolis, USA. J Paleolimnol. 15: 129-132.

Tyler PA and Bowling LC. 1990. The wax and wane of meromixis in estuarine lakes in Tasmania. Verh Int Verein Limnol. 24: 117-121.

Vallentyne JR. 1956. Epiphasic carotenoids in postglacial lake sediments. Limnol Oceanogr. 1: 252-262.

Vallentyne JR. 1957. Carotenoids in 20,000-year old sediment from Searles Lake. California. Arch Biochem Biophys. 70: 29-34.

Van Gemerden H and Mas J. 1995. Ecology of Phototrophic Sulfur Bacteria. In: Anoxygenic photosynthetic bacteria. Blankenship, R.E., Madigan, M.T. and Bauer, C.E. (eds). Kluwer Acad. Publ., Dordrecht, pp. 49-85.

Verleyen E, Hodgson DA, Sabbe K and Vyverman W. 2005. Late Holocene changes in the ultraviolet penetration recorded in an East Antartic lake. J Paleolimnol. 34: 191-202.

Verschuren D. 1999. Sedimentary controls on the preservation and time resolution of climate-proxy records from shallow fluctuating lakes. Quaternary Sci Rev. 18: 821-837.

Verschuren D. 2003. Lake-based climate reconstruction in Africa: progress and challenges. Hydrobiologia. 500: 315-330.

Verschuren D, Cocquyt C, Tibby J, Roberts CN and Leavitt PR. 1999. Long-term dynamics of algal and invertebrate communities in a small, fluctuating tropical soda lake. Limnol Oceanogr. 44:1216-1231

Vicente E and Miracle MR. 1984. Distribution of photosynthetic organism in a temporal stratified karstic pond near Cuenca, Spain. Verh Int Verein Limnol. 22: 1704-1710.

Vicente E and Miracle MR. 1988. Physicochemical and microbial stratification in a meromictic lake of Spain. Verh Int Ver Limnol. 23: 522-529.

Vila X and Abella CA. 1994. Effects of light quality on the physiology and the ecology of planktonic sulfur bacteria in lakes. Photosyn Res. 41: 53-65.

Vila X and Abella CA. 2001. Light-harvesting adaptations of planktonic phototrophic micro-organism to different light quality conditions. Hydrobiologia 452: 15-30.

Vila X, Dokulil M, Garcia-Gil J, Abella CA, Borrego CM and Baneras L. 1996. Composition and distribution of Phototrophic bacterioplankton in deep communities of several central european lakes: the role of light quality. Aquat Microb Ecol.48:183-196.

Villanueva J, Grimalt JO, de Wit R, Keely BJ and Maxwell JR. 1994. Sources and transformations of chlorophylls and carotenoids in a monomictic sulphate-rich karstic lake environment. Org Geochem. 22: 739-757.

Villanueva J and Hastings DW. 2000. A century-scale record of the preservation of chlorophyll and its transformation products in anoxic sediment. Geochim Cosmochim Acta 64: 2281-2294.

Vinebrooke RD, Hall RI, Leavitt PR and Cumming BF. 1998. Fossil pigments as indicators of phototrophic response to salinity and climatic change in lakes of western Canada. Can J Fish Aquat Sci. 55 :668-681.

Walker JS, Squier AH, Hodgson D A and Keely BJ. 2002. Origin and significance of 132-hydroxychlorophyll derivatives in sediments. Org Geochem. 33: 1667-1674.

Waters MN, Schelske CL, Kenney WF and Chapman AD. 2005. The use of sedimentary algal pigments to infer historic algal communities in Lake Apopka, Florida. J Paleolimnol. 33: 53-71.

Wilson MA, Airs RL, Atkinson JE and Keely BJ. 2004a. Bacterioviridins: novel sedimentary chlorins providing evidence for oxidative processes affecting palaeobacterial communities.

Org Geochem. 35: 199-202.

Wilson MA, Hodgson DA and Keely BJ. 2004b. Structural variations in derivatives of the bacteriochlorophylls of Chlorobiaceae: impact of stratigraphic resolution on depth profiles as revealed by methanolysis. Org Geochem 35: 1299-1307.

Wolin JA and Duthie HC. 1999. Diatoms as indicator of water level change in freshwater lakes. In; diatoms: Applications for the environmental and earth sciences. Stoermer EF and Smol JP (eds). Cambridge University Press, Cambridge, pp: 183-204.

Woolley PS, Moir AJ, Hester RE and Keely BJ. 1998. A comparative study of allomerization reaction of chlorophyll a and bacteriochlorophyll_a. J Chem Soc Perkin Trans. 2: 1833-1839.

Wright HE. 1980. Cores of soft lake sediments. Boreas. 9: 107-114.

Wright SW, Jeffrey SW, Mantoura RFC, Llewellyn CA, Bjornland T, Repeta D, Welschmeyer NA. 1991. Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. Mar Ecol Progr Ser. 77: 183-196.

Wüest A, Aeschbacchherting W, Braur H, Hofer M, Kipfer R and Schurter M. 1992. Density structure and tritium-helium age of deep hypolimnion water in the northern basin of Lake Lugano. Aquat Sci. 54: 205-218.

Xoplaki E, Gonzalez-Rouco JF, Lüterbacher J and Wanner H. 2004. Wet season Mediterranean precipitation variability: influence of large-scale dynamics and trends. Clim Dynam 23: 63-78.

Young A and Britton G. 1993. Carotenoids in photosynthesis. Chapman and Hall, London,498pp.

Zolitschka B. 1996. Processes of recent sedimentation in a High Artic lake, northern Ellesmere Island, Canada. J Paleolimnol 16: 169-186.

Zorita E, Kharin V and von Storch H. 1992. The atmospheric circulation and sea surface temperature in the North Atlantic area in winter: their interaction and relevance for Iberian precipitation. J Clim. 5 : 1097-1108.

Züllig H. 1981. On the use of carotenoid stratigraphy in lake sediments for detecting past developments of phytoplankton. Limnol Oceanogr. 26: 970-976.

Appendix 1.

Summary of pigments characteristics determined by HPLC and LC-MS methodologies.

a. Modification of the method described by Villanueva et al. (1994).

Peak number	Retention time (min)	UV/vis absorption bands (nm)	Pigment compound	$[M+H]^+$ (m/z)
1	4.035	429/660	Bacteriophaeophorbide_d	
2	4.289	429/661	Bacteriophaeophorbide_d	
3	9.553	410/663	Phaeophorbide_a	
4	9.836	450	Fucoxanthin	
5	13.848	453/482	Alloxanthin	
6	14.105	444/471	Antheraxanthin	
7	14.5	451/480	Diatoxanthin	
8	15.042	452/480	Zeaxanthin	
9	15.2	447/475	Lutein	
10	15.629	428/650	Bacteriochlorophyll_d	
11	16.62	449/478	Diadinoxanthin	
12	17.333	428/650	Bacteriochlorophyll_d epimer	
13	18.647	446//473	Crocoxanthin	
14	19.159	427/650	Bacteriochlorophyll_d	
15	19.971	428/650	Bacteriochlorophyll_d epimer	
16	20.277	406/660	Bacteriophaeophytin_d	
17	20.299	362/770	OH-Bacteriochlorophyll_a	925
18	20.4	396/439/682	OH-Bacterioviridin	923
19	20.502	362/770	OH-Bacteriochlorophyll_a epimer	
20	20.698	464/648	OH-Chlorophyll_b	
21	20.837	427/650	Bacteriochlorophyll_d	
22	21.287	362/770	Bacteriochlorophyll_a	911
23	21.547	396/439/682	Bacteriviridin	909
24	21.666	465/650	Chlorophyll_b	
25	21.812	485	Okenone	
26	21.98	465/650	Chlorophyll_b epimer	
27	22.875	432/664	OH-Chlorophyll_a	
28	23.067	406/660	Bacteriophaeophytin_d	
29	23.71	432/664	Chlorophyll_a	
30	2.163	432/664	Chlorophyll_ a epimer	
31	24.429	406/660	Bacteriophaeophytin_d	
32	24.751	452/481	Chlorobactene	
33	24.99	357/747	Bacteriophaeophytin_a	
34	24.333	357/747	Bacteriophaeophytin_a epimer	
35	25.632	435/650	Phaeophytin_b	
36	25.9	435/650	Phaeophytin_b epimer	
37	26.285	409/665	OH-Phaeophytin_a	
38	26.553	409/665	OH-Phaeophytin_a epimer	
39	26.666	356/750	Pyrobacteriophaeophytin_a	

cont.

40	27.744	409/665	Phaeophytin_a
41	27. 808	446/473	α-carotene
42	27. 902	436/650	Pyrophaeophytin_b
43	28.211	452/476	β-carotene
44	28.329	409/665	Phaeophytin_a epimer
45	31.011	409/665	Pyrophaeophytin_a

b. Method described by Airs et al. (2001)

peak number	Retention time (min)	UV/vis absorption bands (nm)	[M+H]+ (m/z)	fragment ions (m/z)	Pigment compound	
1	14.524	484			mixoxanthophyll	
2	15.47	359/746	625	585-479	bacteriopheophorbide a	
3	16.636	448(450)	659(581)		fucoxanthin-like	
4	17.862	407/433			aureoxanthin	
5	19.063	452/481	565	547	alloxanthin	
6	19.708	410/664	533	515	chlorophyllone	
7	20.22	450/480	567	549	diatoxanthin	
8	21.134	410/671	533		chlorophyllone	
9	22.049	445/473	569		lutein	
10	22.708	450/478	569		zeaxanthin	
11	22.775	442/471	485		lutein-5,6-epoxy	
12	24.018	436/463	569		lactucaxanthin	
13	24.682	447/476			diadinoxanthin	
14	25.163		623	595-503	OH-phaeophorbide a	
15	26.393	409/663	607	547-461	phaeophorbide a	
16	27.653		607	547-461	phaeophorbide a epim	
17	31.010	407/664	713	535-507	unidentified chlorin	
18	31.505	482	565		cantaxanthin	
19	34.155	466			adinorubin	
20	35.755	409/666	549	521	pyrophaeophorbide a	
21	36.657	386/686	887	609	bacterioviridin	Mg-link
22	37.691	405/660	771	567-523	bacteriophaeophytin d	d2-farnesol
23	38.109	465/650	885	607-547	chlorophyll b	
24	38.751	445/473			cryptoxanthin	
25	39.378	405/660	785	581-537	bacteriophaeophytin d	d3-farnesol
26	40.343	465/650			chlorophyll b epimer	
27	42.580	445/475	551		crocoxanthin	
28	43.82	434/664			chlorophyll a allomer	
29	46.048	487	579	547	okenone	
30	46.78	487			okenone isomer	
31	47.447	487			okenone isomer	
32	48.281	431/664	871	593	chlorophyll a	
33	49.307	409/660	789	567-523	bacteriophaeophytin d	d2-C16:1
34	49.733	409/660	867	695-551	bacteriophaeophytin d	d4-GG
35	50.014	466	551		echinenone	
36	50.981	431/664			chlorophyll a epimer	
37	52.296	409/660	777	567-523	bacteriophaeophytin d	d2-C15:0
38	52.637	357/750	906	627-554	hydroxybacteriophaeophytin a	
39	52.807	409/660	841	535-507	unidentified chlorin	

cont.

40	54.643	409/660	843	535-507	unidentified chlorin	
41	55.265	409/660	791	567-523	bacteriophaeophytin d	d2-C16:0
42	56.4	409/660	871	593-533	unidentified chlorin	
43	56.9	436/650	901	623-550	hydroxyphaeophytin b	
44	57.515	409/660	845	567-523	bacteriophaeophytin d	d2-phytol
45	58.086	357/750	889	611	bacteriophaeophytin a	
46	58.577		887	609	bacterioviridin	Mg free
47	60.050	462/493			chlorobactene	
48	61.365	357/750	889	611	bacteriophaeophytin a epimer	
49	62.237	436/650	885	607-547	phaeophytin b	
50	64.018	410/664	887	609-550	hydroxyphaeophytin a	
51	64.527	436/650			phaeophytin b epimer	
52	65.64	410/665	887	609-550	hydroxyphaeophytin a epimer	
53	66.086	357/750	831	552-526	pyrobacteriophaeophytin a	
54	66.426	441/470			ε-carotene	
55	67.722	447/475			α-carotene	
56	68.428		917	639-552	purpurin 7 phytyl ester	
57	69.141	410/665	871	593-533	phaeophytin a	
58	69.586	447/475	537		β-carotene	
59	70.017	436/650	827	549-521	pyrophaeophytin b	
60	71.462	410/665			phaeophytin a epimer	
61	75.816	386/686	830	551-508	unidentified chlorin	
62	76.542	408/697	843	565-503	purpurin 18 phytyl ester	
63	77.583	410/665	813	535-507	pyrophaeophytin a	
64	84.823	437/650	918	549	sce b	
65	86.093	437/650	944	549	sce b	
66	91.705	410/665	903	535	sce a	C27sterol
67	92.536	410/665	929	535	sce a	C29sterol
68	93.293	410/665	917	535	sce a	C28sterol
69	94.263	410/665	931	535	sce a	C29sterol
70	94.908	410/665	919	535	sce a	C28sterol
71	95.456	410/665	945	535	sce a	C30sterol
72	95.901	410/665	933	535	sce a	C29sterol
73	97.849	410/665	947	535	sce a	C30sterol

Appendix 2.

Diazomethane preparation

We routinely methylate free acids using diazomethane. The addition of diazomethane to the pigment extracts prevents peak tailing and stabilises the molecules, allowing safety storage of extract for future analysis

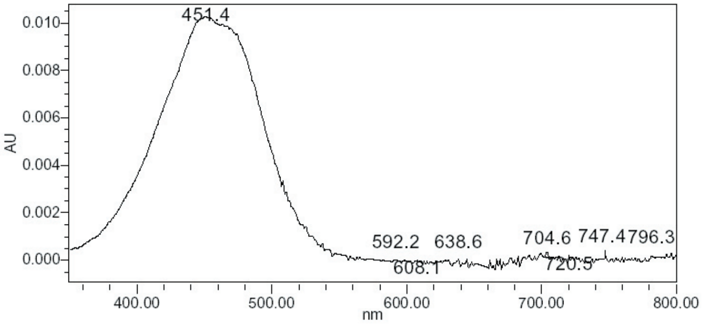
Warning: diazomethane is explosive. Potential sources of detonation are scratched the glassware, heat and impact. Preparation should be carried out in a fume hood to prevent inhalation. Gloves should be worn to prevent contact with the skin.

Method:

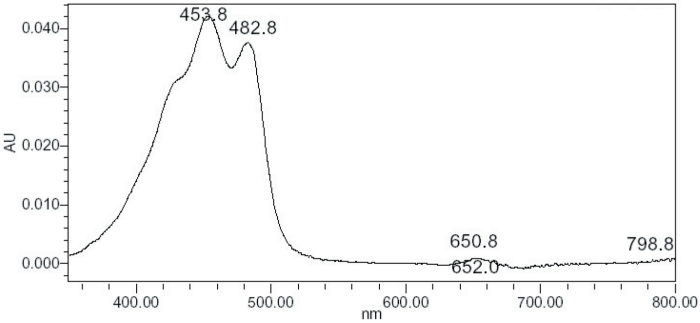
-dissolve KOH in deionised water (37% solution) in a flask over ice.
-stand the diazomethane generator in a ice bath. Add diathyl ether (2 ml) to the outer tube of the apparatus and screw the red collar.
-Take a pre-weighed sample tube containing 0.3 g diazald. Add diethyl ether (1 ml) and ethanol (1 ml) to the sample tube and mix thoroughly. Transfer the solution to the inner tube of the apparatus and screw on the small cap.
-Using a syringe, add 37% KOH (1.5 ml) dropwise to the inner tube through the septum.
-Leave the apparatus for several hours, shaking gently every 10 minutes. A yellow solution of diazomethane in ether is produced in the outer tube.
-After using the required amount of diazomethane solution, the remainder can be destroyed by addition of dilute HCl (0.1M), which should turn the solution colourless.

Appendix 3.

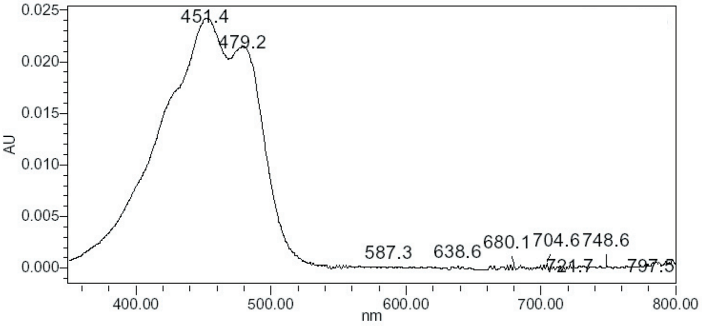
UV/vis absorption spectrum in eluant (modification of method describped by Villanueva et al. (1994)) for main photosynthetic pigments.



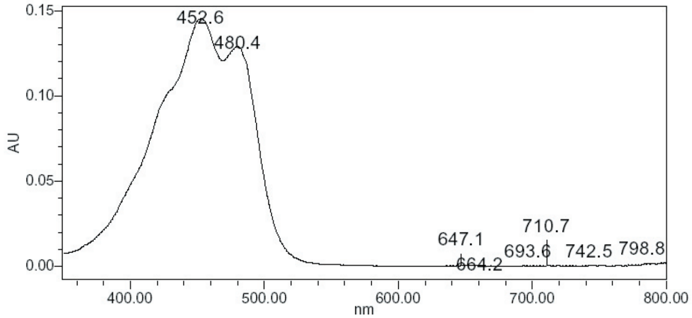
Fucoxanthin



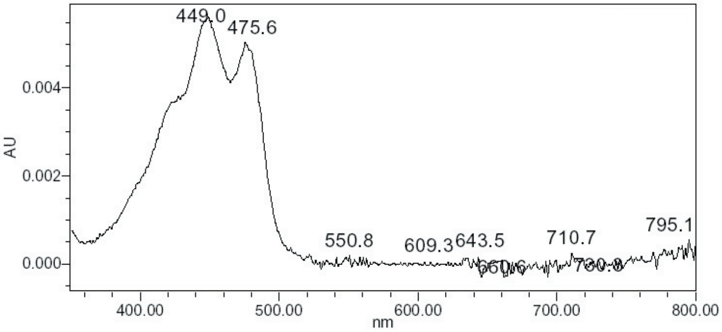
Alloxanthin



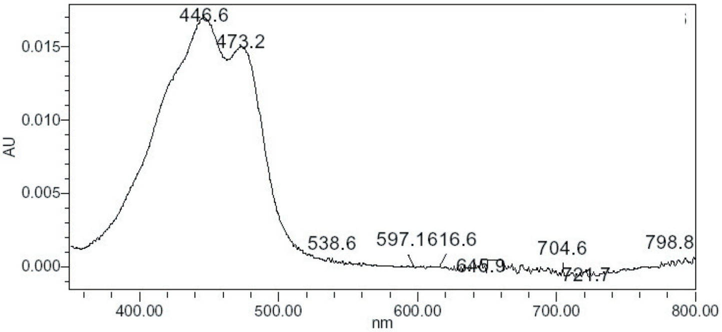
Diatoxanthin



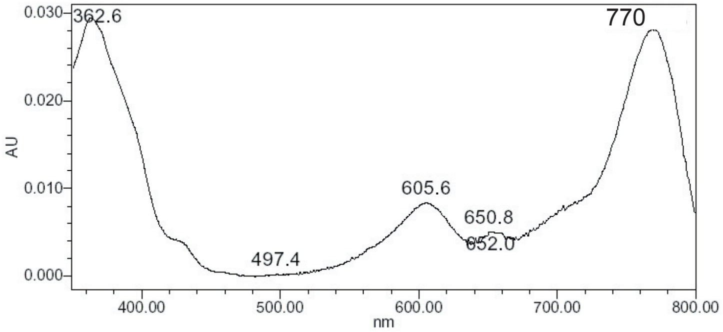
Zeaxanthin



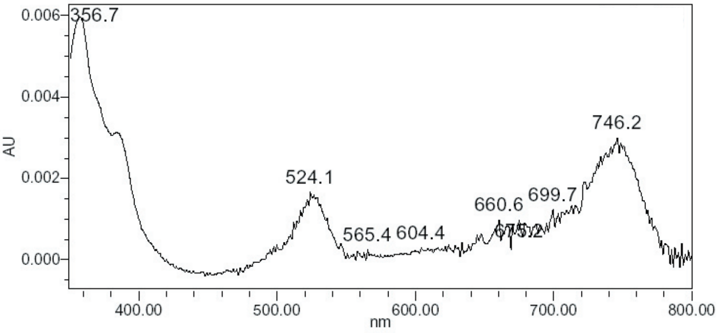
Diadinoxanthin



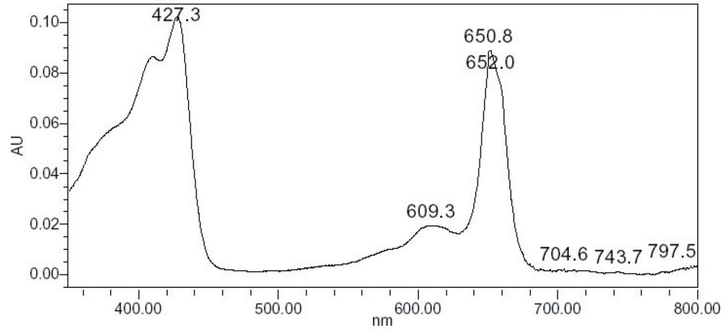
Crocoxanthin



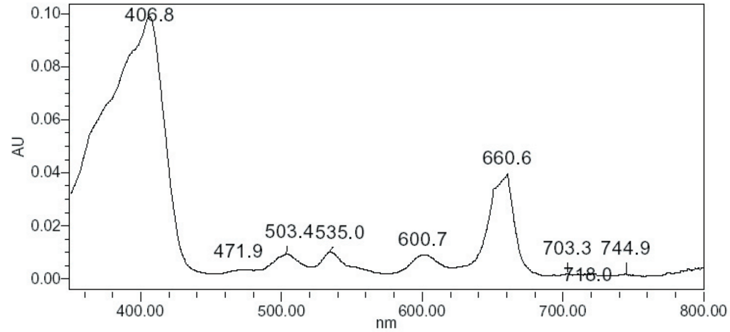
Bacteriochlorophyll_a



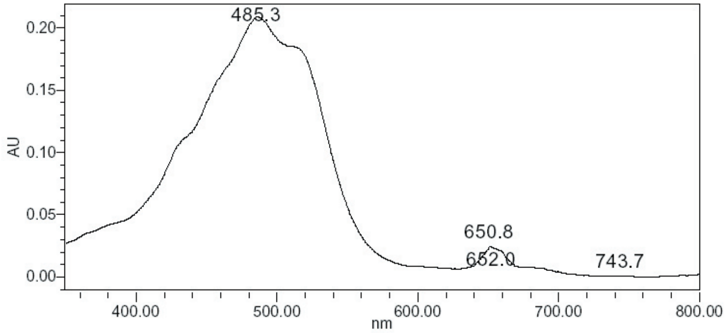
Bacteriophaeophytin_a



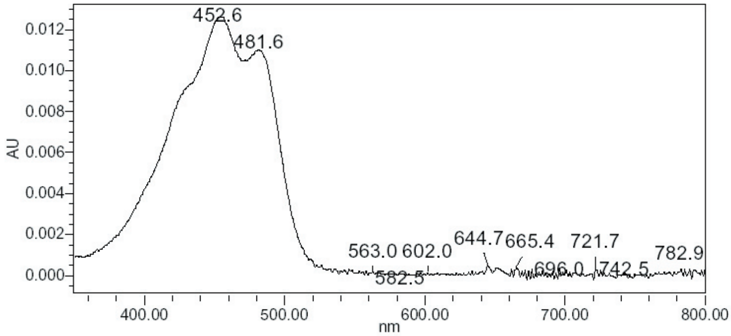
Bacteriochlorophyll_d



Bacteriophaeophytin_d



Okenone

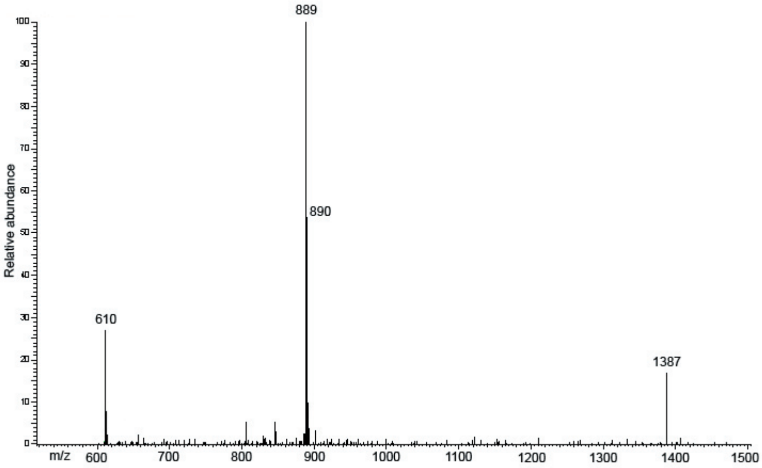


Chlorobactene

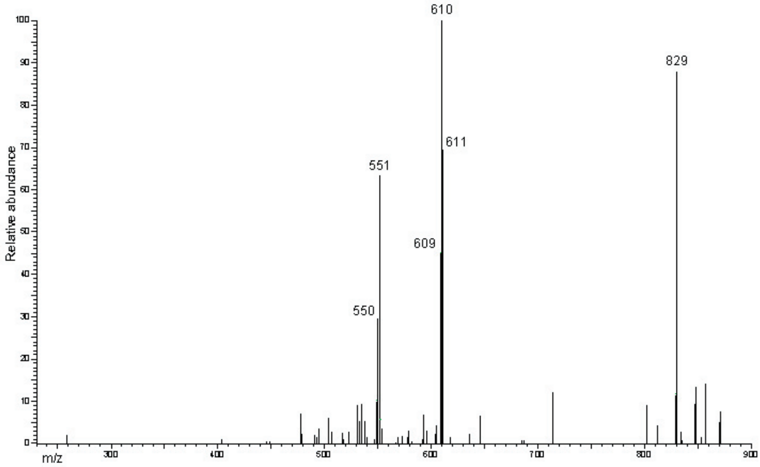
Appendix 4.

Mass spectra for bacterial photosynthetic pigments and derivatives.

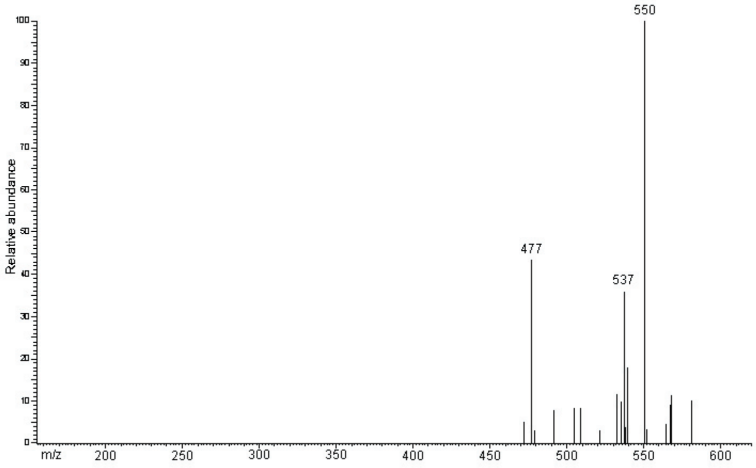
Bacteriophaeophytin_a



a. Full MS spectrum

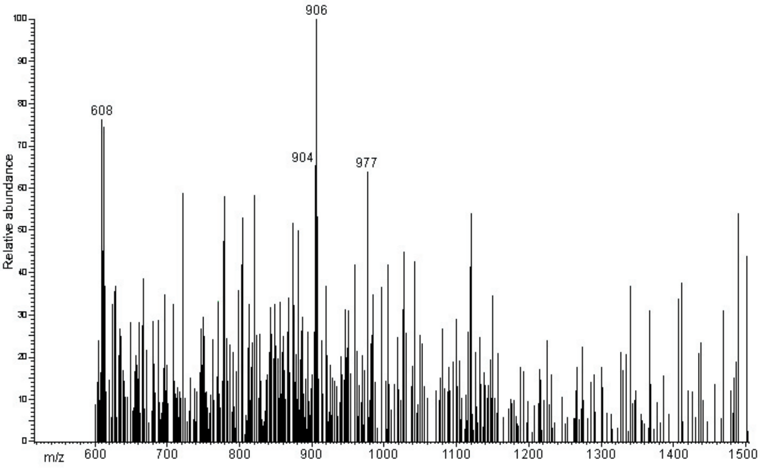


b. MS² spectrum

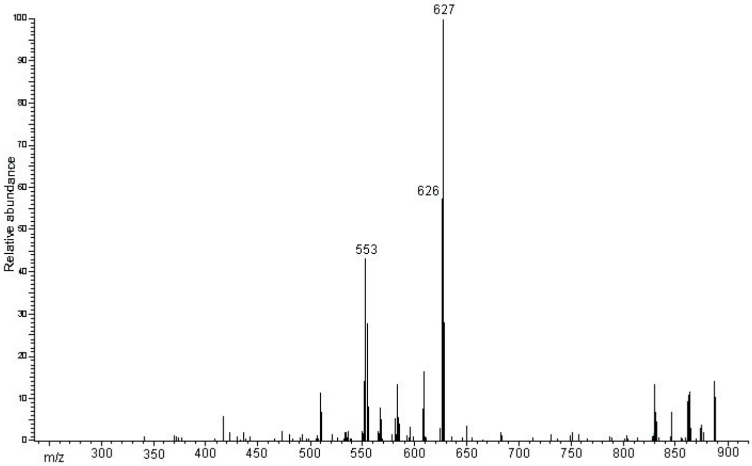


c. MS³ (precursor m/z 610) spectrum

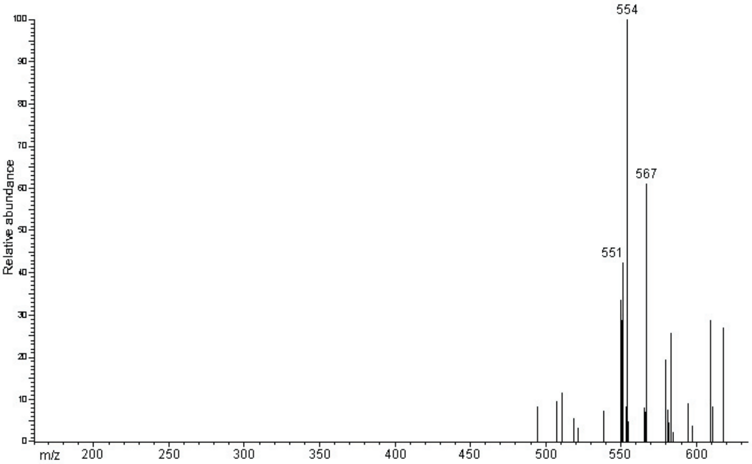
Hydroxybacteriophaeophytin_a



a. Full MS spectrum

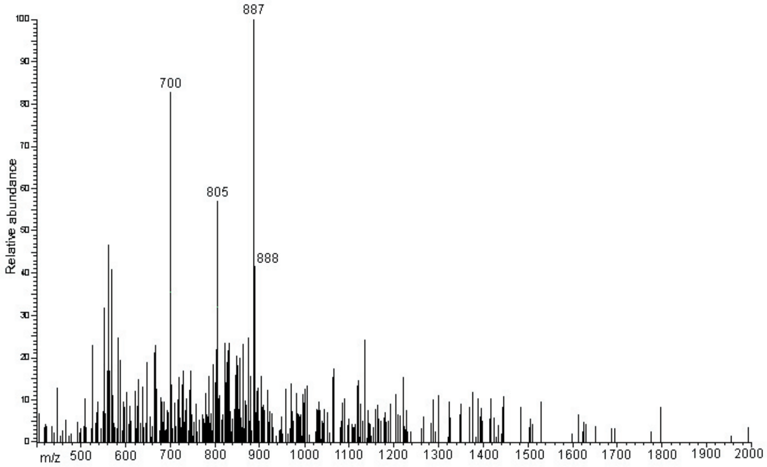


b. MS² spectrum

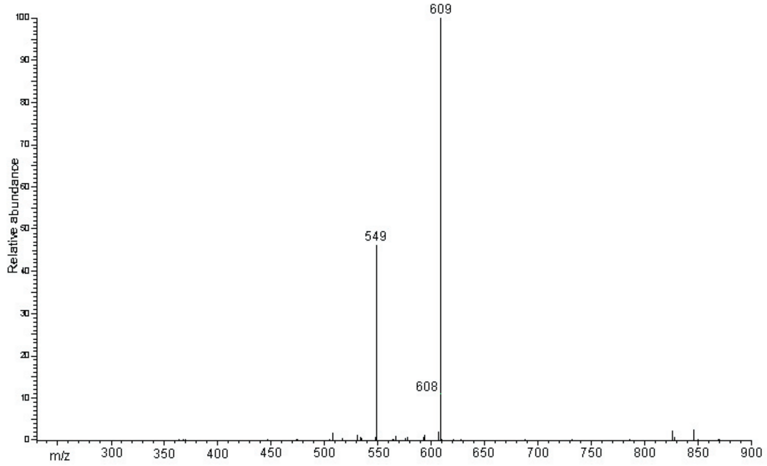


c. MS³ (precursor m/z 627) spectrum

Bacterioviridin

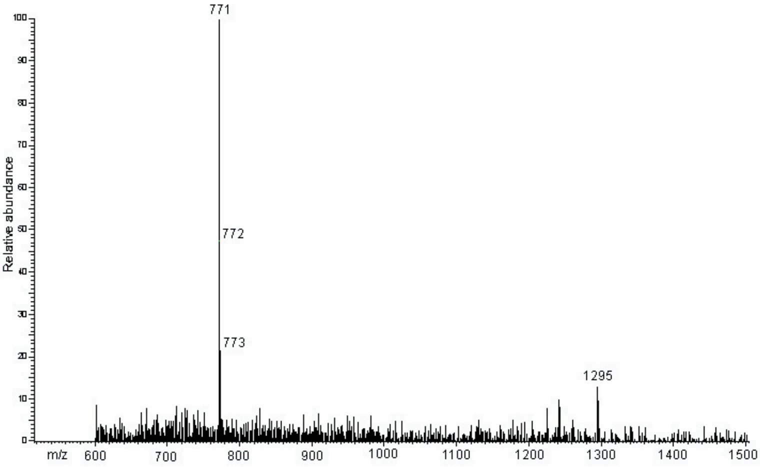


a. Full MS spectrum

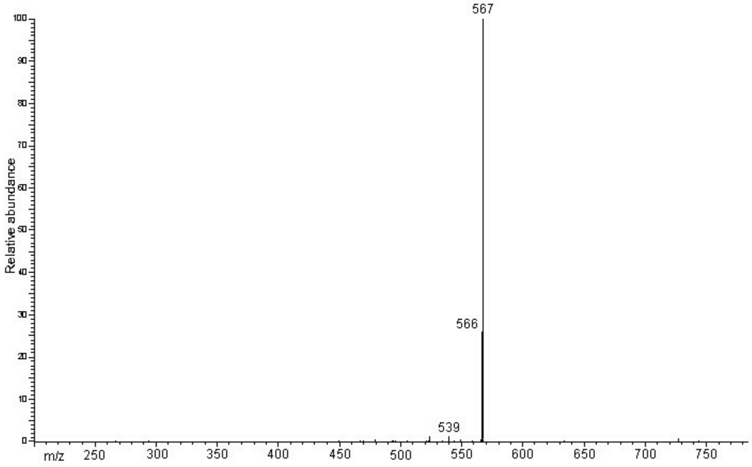


b. MS^2 spectrum

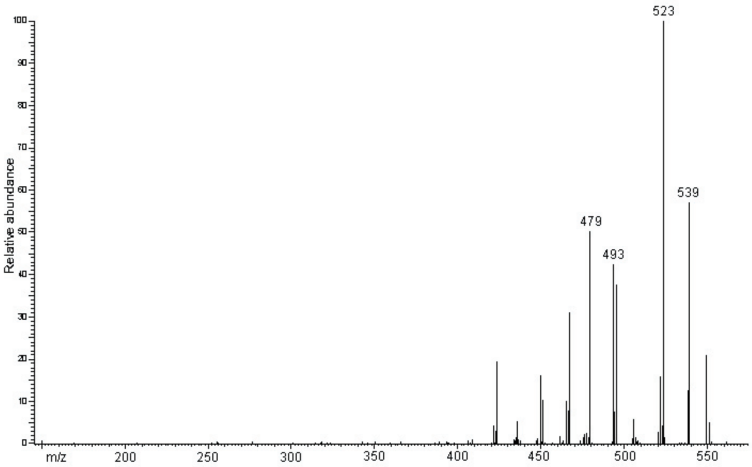
Bacteriophaeophytin_d (d2-farnesol)



a. Full MS spectrum

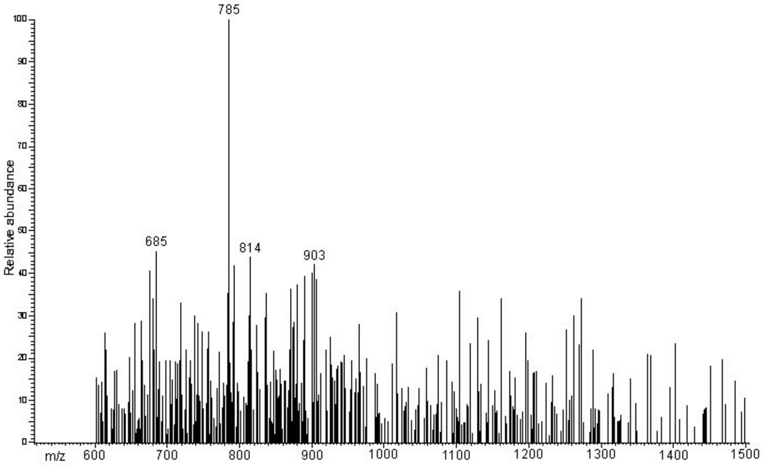


b. MS² spectrum

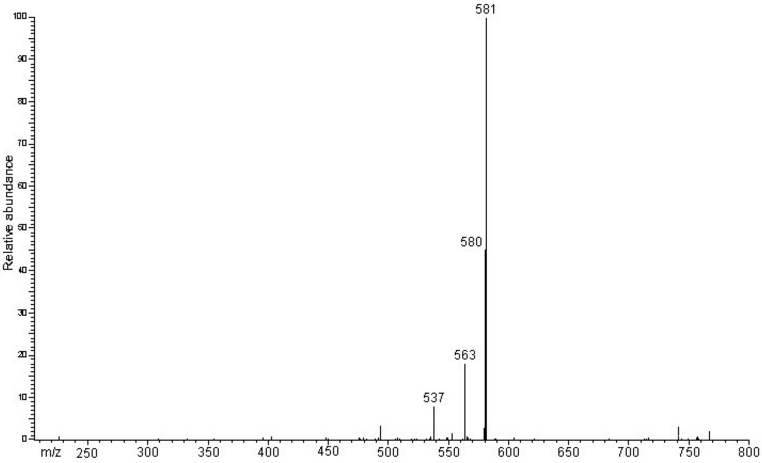


c. MS³ (precursor m/z 567) spectrum

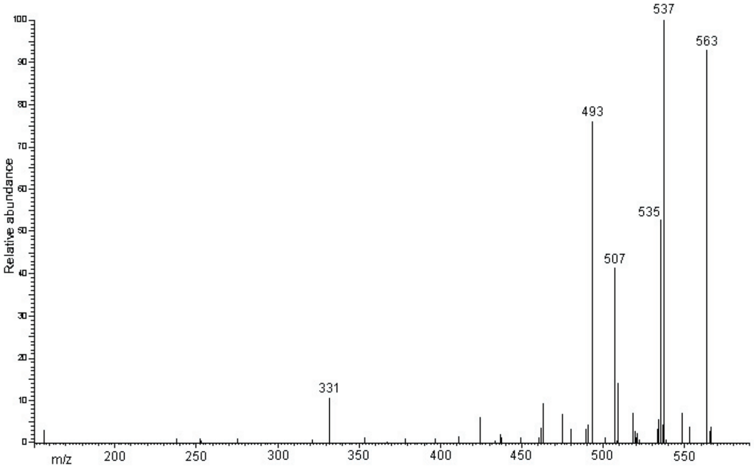
Bacteriophaeophytin_d (d3-farnesol)



a. Full MS spectrum

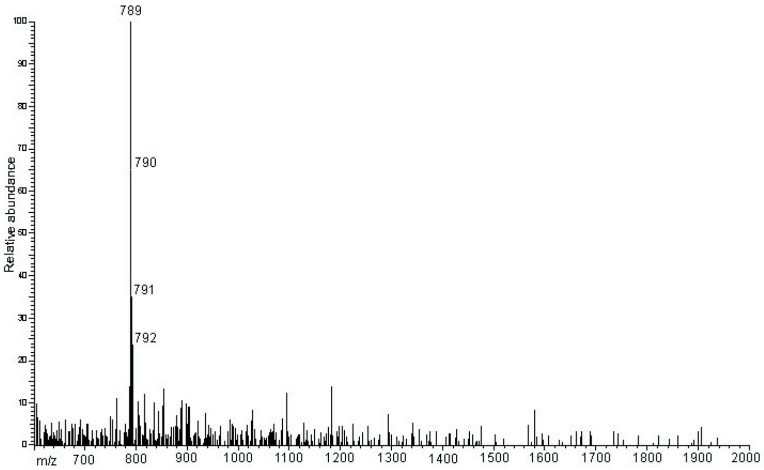


b. MS² spectrum

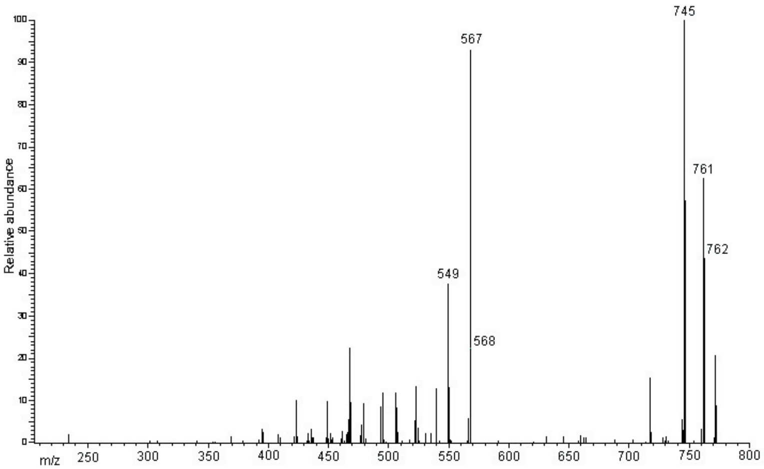


c. MS³ (precursor m/z 581) spectrum

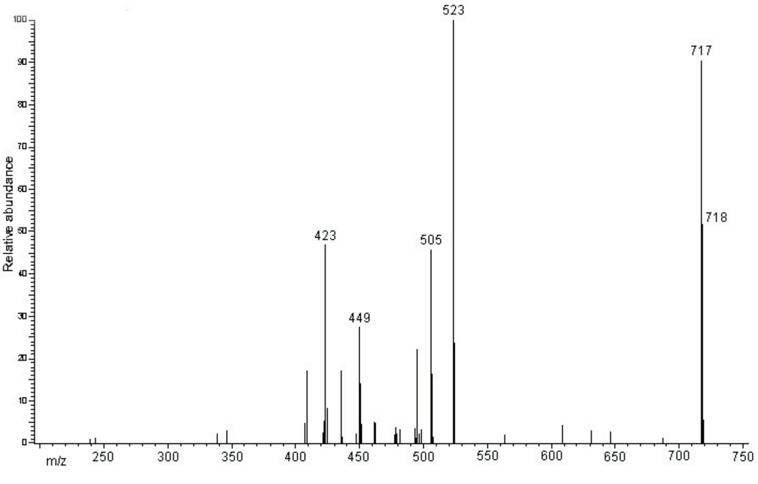
Bacteriophaeophytin_d (d2-C16:1)



a. Full MS spectrum

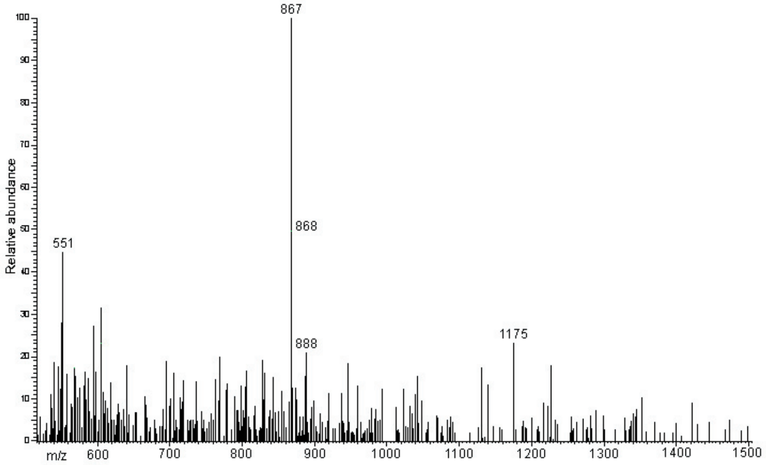


b. MS² spectrum

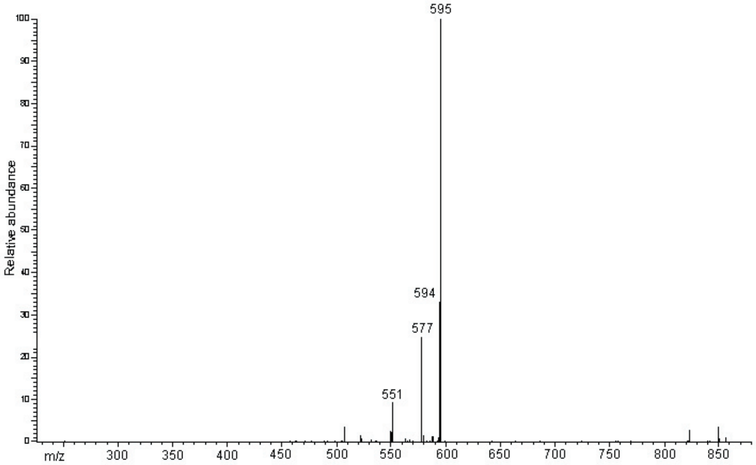


c. MS³ (precursor m/z 567) spectrum

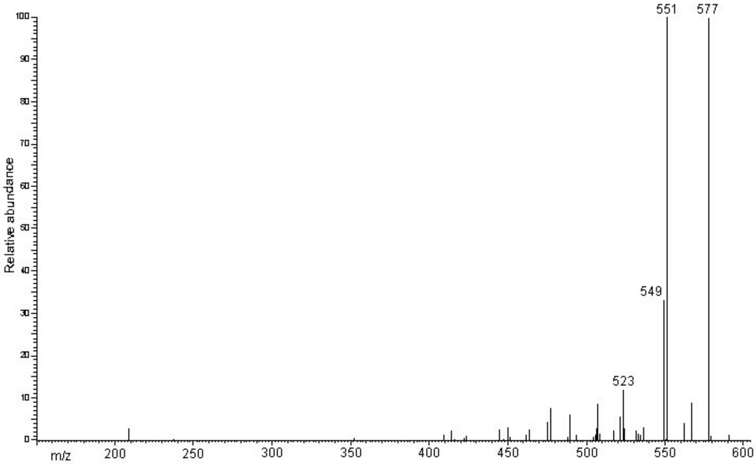
Bacteriophaeophytin_d (d4-GG)



a. Full MS spectrum

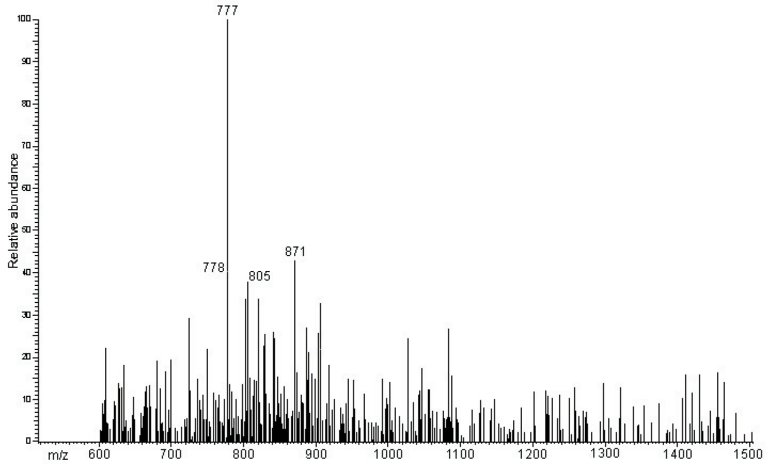


b. MS² spectrum

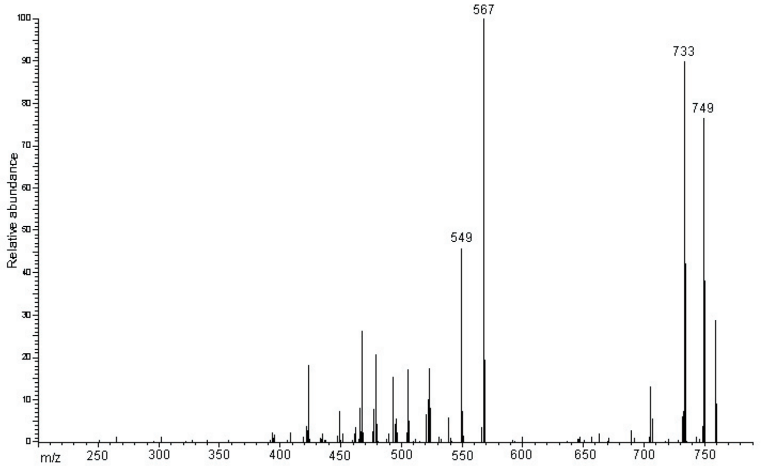


c. MS³ (precursor m/z 595) spectrum

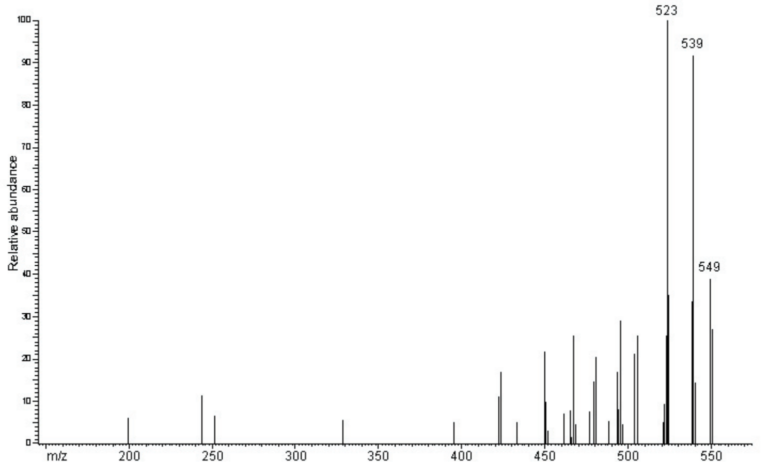
Bacteriophaeophytin_d (d2-C15:0)



a. Full MS spectrum

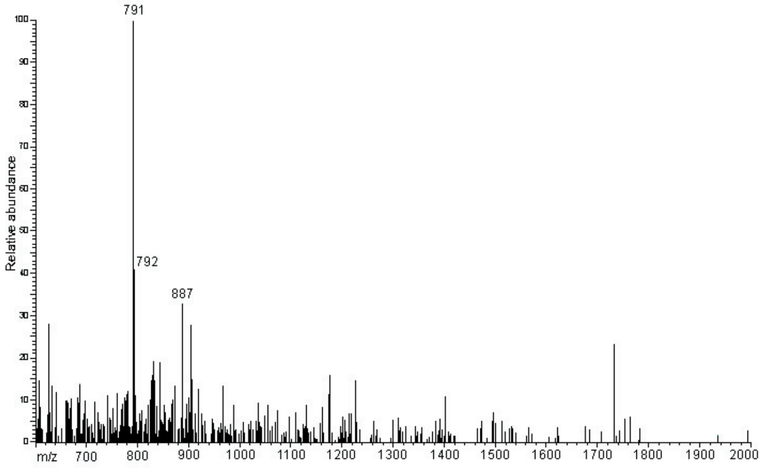


b. MS² spectrum

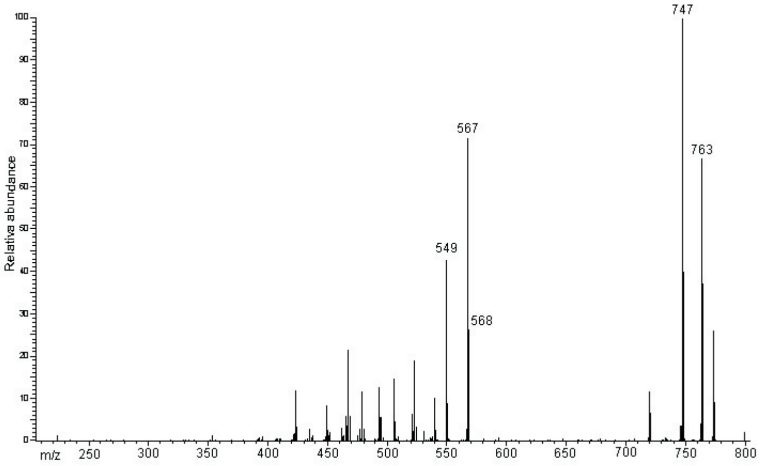


c. MS³ (precursor m/z 567) spectrum

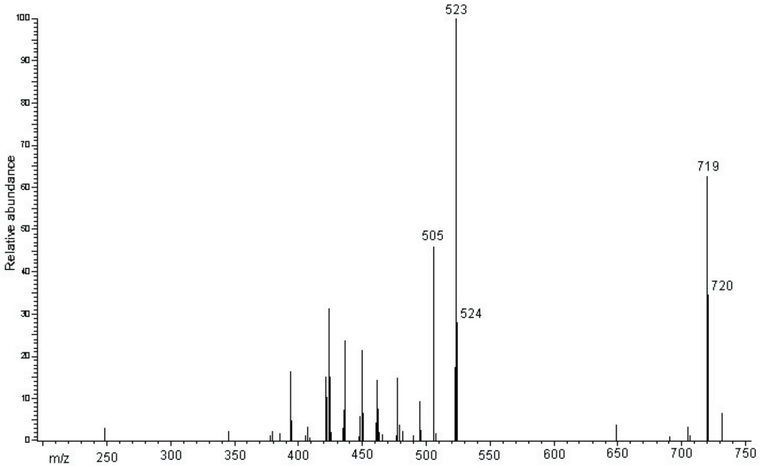
Bacteriophaeophytin_d (d2-C16:0)



a. Full MS spectrum

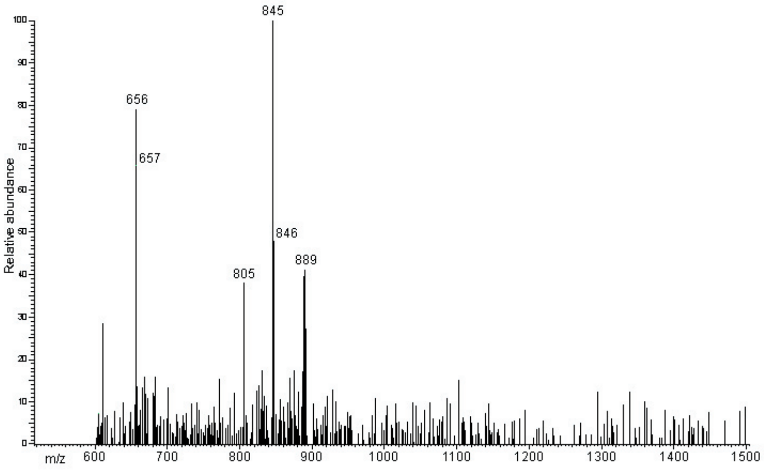


b. MS² spectrum

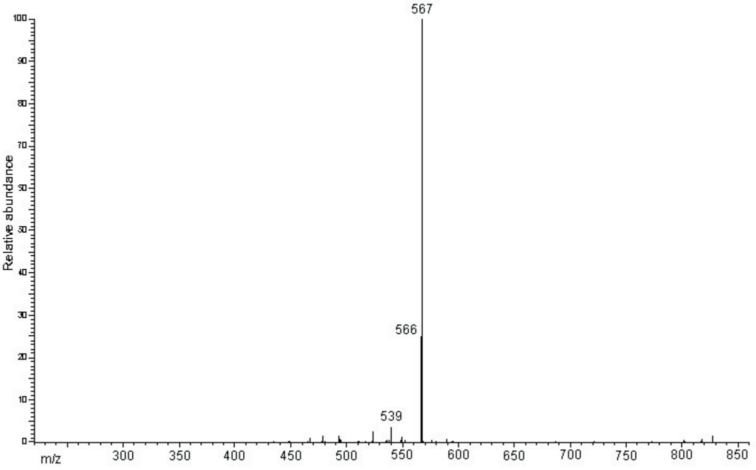


c. MS³ (precursor m/z 567) spectrum

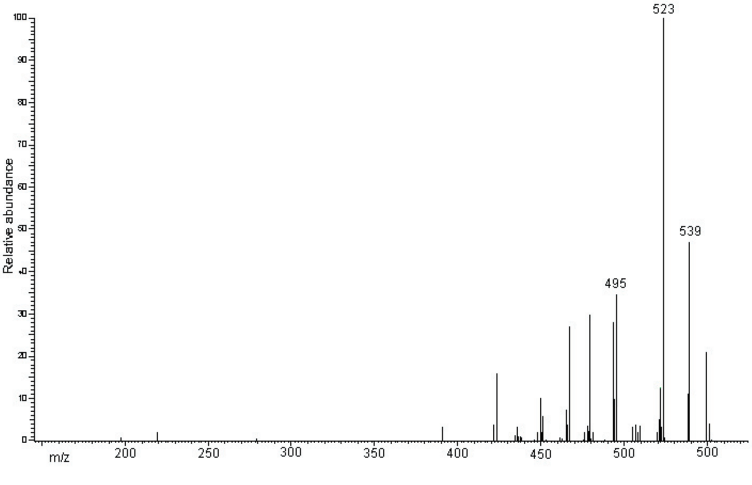
Bacteriophaeophytin_d (d2-phytol)



a. Full MS spectrum



b. MS² spectrum



c. MS³ (precursor m/z 567) spectrum