

Is there a bias between contemporary and subfossil cladoceran assemblages?

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ABSTRACT

Is there a bias between contemporary and subfossil cladoceran assemblages?

Sediment and water samples differ in both temporal and spatial resolution. In this study, we compare a set of contemporary cladoceran samples covering the past 30 years with a subfossil sedimentary sequence from a surficial core spanning the same period. More species were found in the sediment than in the water samples. This result reflects the accumulative and integrative consequences of sedimentation processes. For example, *Alona quadrangularis*, *Alona affinis*, *Pleuroxus truncatus* and *Tretocephala ambigua* were only found in the sedimentary sequence. In contrast, species occurring frequently in contemporary samples, such as *Simocephalus vetulus*, were not found in the sediment because their remains are poorly preserved. Ordination analyses (a CA for contemporary samples and a PCA for sediment samples) were performed to investigate the patterns and variation of cladoceran species abundances in both data sets. The application of a Procrustes rotation and PROTEST permutation test to the ordinations (CA and PCA) of these two sets of samples demonstrated that the two sets were significantly similar. However, the number of samples analysed to obtain the same integrating characterisation of the lake community must be much greater if we use live collections rather than sediment samples. This study evidenced the role of lake sediment as an important library for species information and highlighted the potential role of palaeolimnological approaches in biodiversity studies.

Key words: Cladocera subfossils, Chydoridae, Palaeolimnology, Procrustes rotation, PROTEST.

RESUMEN

¿Hay algún sesgo entre las muestras contemporáneas y los subfósiles de cladóceros?

Las muestras de poblaciones vivientes y de restos sedimentarios tienen diferente resolución temporal y espacial. En este trabajo se compara una colección de muestras contemporáneas de cladóceros, recogidas durante los últimos treinta años, con la secuencia de restos sedimentarios de un registro superficial que abarca el mismo tiempo. Las muestras de sedimento contenían un número de especies mayor que las tomadas de las poblaciones vivientes, lo que denota el valor acumulativo e integrativo de los procesos sedimentarios. Por ejemplo *Alona quadrangularis*, *Alona affinis*, *Pleuroxus truncatus* y *Tretocephala ambigua* sólo se encontraron en las muestras de sedimento. Por otra parte, especies frecuentes en las muestras contemporáneas, como *Simocephalus vetulus*, no se encontraron en el sedimento, ya que sus restos se conservan mal. Se realizaron análisis de ordenación (un CA en las muestras contemporáneas y un PCA en las muestras de sedimento) para investigar los patrones y las variaciones en las abundancias de las especies de cladóceros en ambos conjuntos. La aplicación de un test de permutación PROTEST y una rotación Procrustes a los resultados de las ordenaciones del CA y del PCA evidenció la existencia de una significativa similitud entre ambos conjuntos. Sin embargo, el esfuerzo de muestreo en términos del número de muestras que deben analizarse para llegar a una misma caracterización integrativa de la comunidad lacustre es mucho mayor si se analizan muestras contemporáneas que con la utilización de muestras de sedimento. Este trabajo evidencia el papel de los sedimentos de los lagos como un importante archivo de información y la utilidad de la Paleolimnología en los estudios de biodiversidad.

Palabras clave: Subfósiles de cladóceros, quidóridos, Paleolimnología, rotación Procrustes, PROTEST.

INTRODUCTION

Palaeolimnological studies attempt to reconstruct past environmental conditions using lake sediments. Of the indicators that can be used, microfossils provide some of the most detailed sources of information (Cohen, 2003). Biotic remains are transported and accumulated in sediments after death. However, before they collect in the sedimentary strata, they can be affected by dissolution processes, transport, physical abrasion and/or compaction, which can produce a bias between the living community and the fossil record. Additionally, microfossils of different species have a distinct taphonomic history, which needs to be understood to assure an accurate interpretation of the palaeoecological data.

Despite the increasing use of these palaeoecological data for climatic reconstructions, relatively few studies have investigated taphonomic processes in lacustrine biota, whereas marine or terrestrial fossils have been studied more extensively from this perspective (Kattell, 2009). Most of the investigations in freshwater ecosystems have focused on molluscs (e.g., Helama & Valovirta, 2007) and fishes (e.g., Zohar *et al.*, 2008), whereas remains from other groups have attracted less attention. In the case of Cladocera, we have only found four recent previous studies on the relationships between sediment records and living populations. Davidson *et al.* (2007) have compared zooplankton samples with contemporary top sediments in 39 lakes in Denmark. Kattell *et al.* (2007 and 2009) have linked assemblages obtained with sediment traps with those in top sediments and also with assemblages of living animals in different habitats. Only Nykänen *et al.* (2009) used assemblages in varved sediments covering a short time sequence and identified the correspondence between annual lamina and zooplankton records in contemporary samples. All of these previous investigators have outlined the difficulties of comparing these different sample types. Kattell (2009) has argued that, due to species-specific responses of cladocerans to habitat types and seasonality, these problems are particularly outstanding in small mountain lakes in temperate

and alpine zones because these animals are strongly influenced by macrophyte growth.

In this study, we compare contemporary samples of cladocerans with a short sediment core from Lagunillo del Tejo spanning the last 30 years, using the approach and methodology followed by Davidson *et al.* (2007). Lagunillo del Tejo is a small lake situated in the Iberian Ranges (Cuenca, Spain). The site's cladoceran populations are primarily controlled by the configuration of the lake's two rings of macrophytes (López-Blanco *et al.*, 2012). Moreover, this small doline has been the target of many field investigations serving to monitor this freshwater ecosystem during the past 30 years (Miracle & Vicente 1983, Vicente & Miracle 1984, Armengol & Miracle 1999, Boronat *et al.*, 2001). The living and non-living communities of a small temperate mountain lake have not previously been compared. The principal aims of our study are to (1) assess the degree of concordance between water and sediment samples by comparing cladoceran assemblages in both sets and (2) evaluate the relative role of water samples and sediment samples in identifying the species composition of cladocerans in a given lake.

STUDY SITE

Lagunillo del Tejo (39°59'15.45" N, 1°52' W; 1000 m.a.s.l.) is a small sinkhole situated in the Iberian Ranges (Spain) within the karstic complex of the Guadazaón River. This complex is formed by a polje comprising the river valley and many sinkholes, 7 of them holding water, in the Cañada del Hoyo area (Fig. 1). Lagunillo del Tejo is a hard-water lake with conductivities of approximately 400-600 $\mu\text{S cm}^{-1}$. The pH in the epilimnetic zone ranges between 8 and 9 (Miracle & Vicente, 1992). The lake diameter and depth vary interannually. The reported minimum diameter and depth are 45 m and 4 m, respectively, both occurring in March 2008 (López-Blanco *et al.*, 2011), and the reported maxima are 80 m in diameter and 11 m in depth, recorded in 1980 (Vicente & Miracle, 1984). At high water levels, the lake has a long strati-

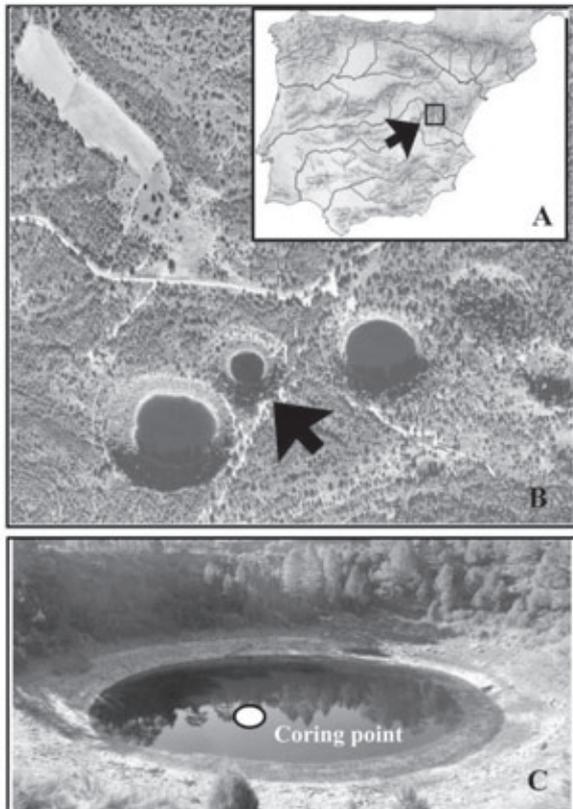


Figure 1. Lagunillo del Tejo: (A) Location of the Guadazaón Complex in the Iberian Peninsula (B) Aerial photograph and (C) Lake photograph with the coring point indicated by a white circle. *Lagunillo del Tejo: (A) localización del complejo Guadazaón en la Península Ibérica (B) Fotografía aérea y (C) fotografía del lago con el punto de muestreo señalado con un círculo blanco.*

fication period (March-October) and an anoxic bottom layer (Vicente & Miracle 1984; Miracle & Vicente, 1983). During the past 30 years, an approximately 11 year cycle of water level oscillations has been observed, with minima in 1983, 1995 and 2007 (Romero-Viana *et al.*, 2009; Lopez-Blanco *et al.*, 2011). The phytoplankton production in the open waters of the lake is low (generally, surface chlorophyll *a* < 5 μgL^{-1} ; a sharp peak of higher concentration may occur at the oxic-anoxic interface), but production by littoral plants is very high, and the waters are heavily stained by humic compounds. The littoral zone is composed of two concentric rings of macrophytes (Fig. 1 C), whose configuration changes as a function of the lake level and has

a strong influence on the cladoceran populations (López-Blanco *et al.*, 2012).

METHODS

Sediment samples

A sediment core 5.5 cm in length (CN-JK-2009) was recovered from Lagunillo del Tejo in June 2009 using a Jenkins surficial sediment corer (68 mm inner diameter) to obtain undisturbed soft sediment from the top layers. This core was taken at the centre of the lake, approximately the same site as that of the CN-3 and CN-4 cores described in López-Blanco *et al.* (2011, 2012). Slices 0.5 cm in thickness were cut for cladoceran analysis, with 1 cm^3 of sediment separated from each slice for the analysis of water content, density and organic matter.

Water content was measured by oven-drying aliquots of wet sediment for 2 h at 105 °C. Density was calculated as wet sediment weight normalised by the known volume of wet sediment aliquots. Organic matter content was determined from dried samples by loss-on-ignition (LOI) for 6 h at 460 °C and was expressed as the percentage of dry matter (APHA-AWWA-WEF, 1992).

Analyses of cladocerans were performed according to Szerczynska & Sarmaja-Korjonen (2007) with minor modifications. Samples of 1 cm^3 of sediment were heated in 10 % KOH solution (< 100 °C) using a YKA thermostatic heating plate on an YKA Basic KS260 orbital shaker for 30 minutes. After this treatment, ultrasonic waves were applied for 30 seconds to enhance the cleaning process. The samples were then sieved through 40 μm mesh. The residue was transferred to a beaker, and several drops of glycerol-safranin were added. The samples were counted in a Petri dish under a Nikon Eclipse Ti-U inverted microscope at 100-600 magnification. More than 200 remains of the most abundant species were counted per sample. The total number of individuals was estimated as the maximum count of head shields, post-abdomens or carapaces. Cladoceran species identifications and ecological characteristics

were obtained primarily from Szerczynska & Sarmaja-Korjonen (2007) and Alonso (1996).

Water samples

Zooplankton net samples collected from January 1980 through June 2009 were identified and counted to compare with the sediment results. We collected three types of samples: planktonic samples (P) were collected in the pelagic zone very close to the centre of the lake; littoral samples (L) were sampled near the lake shore, among the vegetation; and planktonic-littoral samples (P-L) were recovered by throwing a hand net from the shore towards the middle of the lake and then pulling it to the littoral. The P-L samples were considered to contain species from both environments. The P and P-L samples were recovered with a 45 μm plankton net, whereas the L samples were collected with a 90 μm hand net. Table 1 shows the sampling dates, types of

samples and numbers in terms of relative abundance. The samples were collected primarily in spring (April-June) at the time of the highest diversity of plant-associated cladocerans. The samples were fixed in the field and counted in the laboratory using a Petri dish and an inverted microscope (Nikon Eclipse Ti-U). A minimum of 300 individuals of the most abundant species were counted to obtain the relative abundances of the cladoceran species.

Data analysis

The major stratigraphic zones for cladocerans were identified using a CONISS constrained cluster analysis performed with TILIA computer software, version 2.02 (Grimm, 1987). A total sum of squares (dissimilarity measures obtained from the CONISS analysis on the total record of cladoceran remains) of 0.20 was chosen for use in identifying the major zones.

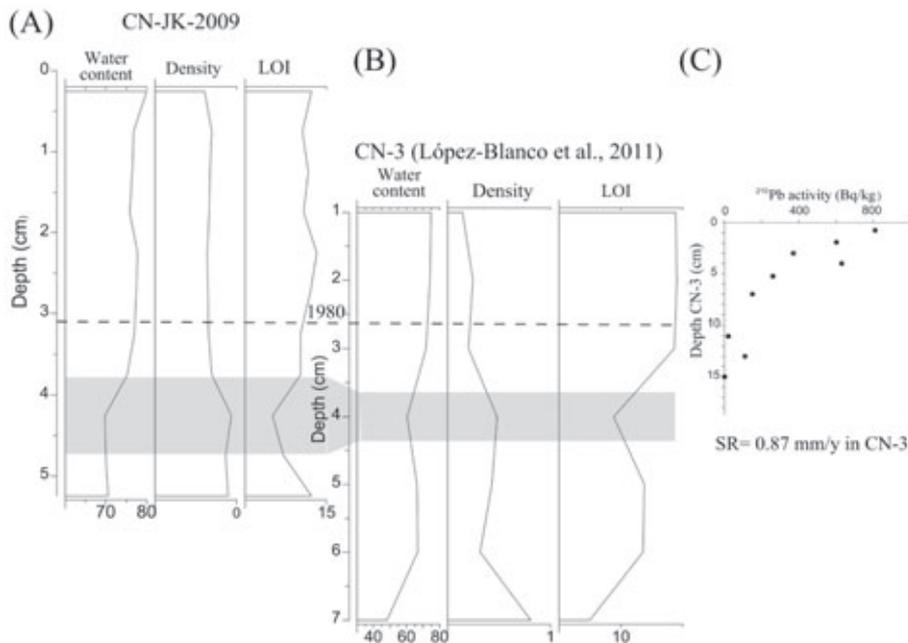


Figure 2. (A) and (B) Water content (%), density ($\text{g} \cdot \text{cm}^{-3}$) and LOI (loss on ignition, in % of dry sediment) of cores CN-JK-2009 and CN-3. The values shaded by the grey band were the basis used to establish the correlation between the cores. (C) Radionuclide activities (Bq/kg) of ^{210}Pb in core CN-3 (from López-Blanco *et al.*, 2011). *Contenido en agua (%)*, *densidad ($\text{g} \cdot \text{cm}^{-3}$)* y *LOI (pérdida por ignición, en % de sedimento seco)* de los registros CN-JK-2009 y CN-3, respectivamente. Los valores circunscritos por una banda gris han sido la base para establecer la correlación entre ambos. (C) *Actividad de los radionucleótidos (Bq/kg) de ^{210}Pb en el registro CN-3* (de López-Blanco *et al.* 2011).

An ordination analysis was performed to investigate the patterns and variations of species abundances in our dataset (TerBraak & Prentice, 1988). A Detrended Correspondence Analysis (DCA) was performed to explore the variability of community composition. Depending on the results, a Principal Component Analysis (PCA) or a Correspondence Analysis (CA) was conducted. Both ordination analyses were performed using CANOCO, version 4.5 (Ter Braak & Smlauer, 2002) on the square root of the relative abundances of species. All of the analyses (DCA, CA and PCA) included all of the species.

To explore the relationship between water and sediment samples and to test the significance of any link found, we applied a Procrustes rotation and a PROTEST permutation test to the results of the CA and PCA ordinations (Jackson, 1995; Peres-Neto & Jackson, 2001). PROTEST is a multivariate method for exploring the concordance between species data matrices by minimising the sum of the squared deviations between data values through matrix translation, reflection, rigid rotation and dilatation. The degree of concordance between the two matrices is given by m_{12} and an associated p value. The values of m_{12} vary from 0 to 1, with lower m_{12} values indicating a greater concordance.

RESULTS

Cladoceran assemblages in the surficial sediment sequence

The 5.5 cm core (CN-JK-2009) from Lagunillo del Tejo consisted of undisturbed and highly homogeneous surficial dark mud sediments. At certain depths, however, it showed an appreciable differentiation of LOI, density and water content (Fig. 2). In fact, the marked decrease in water content and organic matter as well as the density peak at approximately 4.25 cm in CN-JK-2009 (shown by a grey contour in Fig. 2) represented the basis used to correlate it with the CN-3 core. The chronology of CN-JK-2009 was then obtained by its correlation with CN-3, which had previously been dated with ^{210}Pb , ^{137}Cs and

five ^{14}C AMS dates (López-Blanco *et al.*, 2011). The past 30 years would correspond to the upper 3.1 cm of the CN-JK-2009 core (Fig. 2).

The cladoceran signal of the stratigraphic profiles of the 18 cladoceran species found in the sediment shows wave-shaped oscillations without clear discontinuities (Fig. 3). Nevertheless, the cluster analysis revealed the presence of two main zones divided into two subzones. Subzone CL1a is characterised by high relative abundances of the *Daphnia longispina* group, which shows a peak in the upper part of the zone at the same time that the relative abundance of *Chydorus sphaericus* decreases. Plant-associated species such as *Graptoleberis testudinaria* and *Acroporus angustatus* are also abundant in this subzone.

Subzone CL 1b is marked by a decrease in the *D. longispina* group and by an increase in the relative abundance of *C. sphaericus*, *Oxyurella tenuicaudis* and *Alona affinis*. *Leydigia leydigii* peaks at the bottom of this subzone, and *Alona quadrangularis* is only present in its middle portion.

Subzone CL 2a is characterised by an increase in *D. longispina* group remains in the bottom of the zone. The uppermost part of the sequence shows substantial differentiation. *Ceriodaphnia* remains are restricted to subzone CL 2b. In this subzone, the relative abundance of *Alona rectangularis* increases, whereas *G. testudinaria* decreases.

A gradient shorter than 3 SD was found with the trial DCA, indicating that linear methods such as PCA are the most appropriate for modelling species responses. The PCA performed with the sediment samples shows two main patterns of variation: (1) samples dominated by planktonic species are distinguished from samples dominated by littoral species; and (2) samples are ordered by age, with recent samples situated in the most positive region of axis 2, whereas older samples are located in the negative region of this axis (Fig. 4). The planktonic *D. longispina* is situated at the positive end of axis 1 and the negative end of axis 2, whereas the more euryoic and ubiquitous species are also situated at the positive end of axis 1 but at the positive end of axis 2. In contrast, the more specialised phytophilous species, such as *A. tuberculata*, *G. testudinaria* and *A. angustatus*, are situated on

the negative end of axis 1 and at an opposite extreme from the euryoic species. The planktonic species *Ceriodaphnia* is also at the right-hand extreme of axis 1 but became more abundant in recent times along with the euryoic species *A. rectangulara* and *Pleuroxus aduncus*.

Contemporary cladoceran assemblages

A total of 12 cladoceran species were identified in the water samples from Lagunillo del Tejo. *D. longispina*, *A. rectangulara* and *C. sphaericus* were the most common species in the plankton samples. *S. vetulus*, *P. aduncus* and, again, *A. rectangulara* were the most frequent in the littoral samples. Table 1 shows the percentages of each species in the plankton and littoral samples.

The gradient of the exploratory DCA performed with the data from the water samples was 3.35 SD, indicating that unimodal methods such as CA are the most suitable for exploring the relationship between the species and their

environment. The CA performed with contemporary samples indicates a marked separation between the pelagic and littoral samples and, consequently, a remarkable difference in the distribution of the planktonic and littoral species. *D. longispina* is located in the most positive region of axis 1, whereas the group *S. vetulus*-*L. leydigi* -*P. aduncus* is located at the opposite extreme of this axis (Fig. 5). *G. testudinaria* and *A. angustatus* central position. Thus, the first CA may indicate specific functional conditions occurring during littoral development, with the group containing *P. aduncus* occurring in the first stages of littoral plant development. *G. testudinaria* occurs when a well-developed ring of macrophytes is established and *D. longispina* is found when planktonic conditions dominate the lake. The second axis, as found in the sediment samples, appears to be a function of the sample age. Older samples are situated at the bottom of the graph, whereas the most recent samples are located at the upper part of the graph. *A. rectan-*

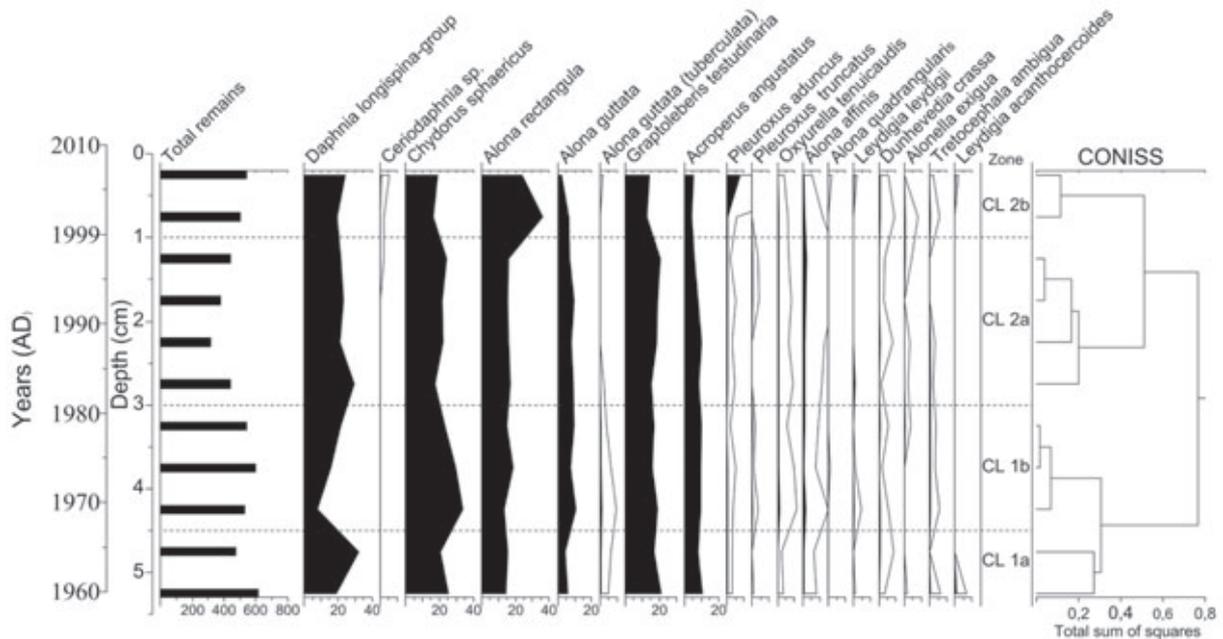


Figure 3. Total number of cladoceran sub-fossils (remains \cdot cm $^{-3}$) and relative abundance of each cladoceran species (%) in core CN-JK-2009. Zones (CL) are based on the constrained cluster analysis outlined at right. *Número total de sub-fósiles de cladóceros (restos/cm 3) y abundancias relativas (%) de cada especie en el registro sedimentario CN- JK-2009. Las zonas (CL) están basadas en el análisis cluster indicado a la derecha.*

gula and *Dunhevedia crassa* play a major role in older samples, whereas *Alona guttata* occupies a more prominent role in recent samples.

Comparison of sample sets

As expected, we found more cladoceran species (18) in the sediment than in the water samples (12). Only *S. vetulus*, which was frequent in the water samples, was absent from the sediment samples because it does not leave long-lasting remains. In contrast, species that tend to form small populations, such as *A. guttata* (*tuberculata*), *Pleuroxus truncatus*, *O. tenuicaudis* and *Alonella exigua*, and species that live in the water-sediment interphase, such as *A. affinis*, *A.*

quadrangularis and *Leydigia acanthocercoides*, were found in the sediment samples but not in the water samples. However, the results of the PCA with the sediment samples and the CA with the water samples show that samples and species show similar alignments in both analyses as a function of the sample age and the littoral or planktonic conditions. The small differences between sample sets disappear if a Procrustes analysis is applied to the PCA and CA matrices. The results of Procrustes rotation and PROTEST diagnostics performed on the results of PCA and CA analyses were as follows:

Procrustes rotation sum of squares: 0.4396
PROTEST m12: 0.3913 p value: 0.0230

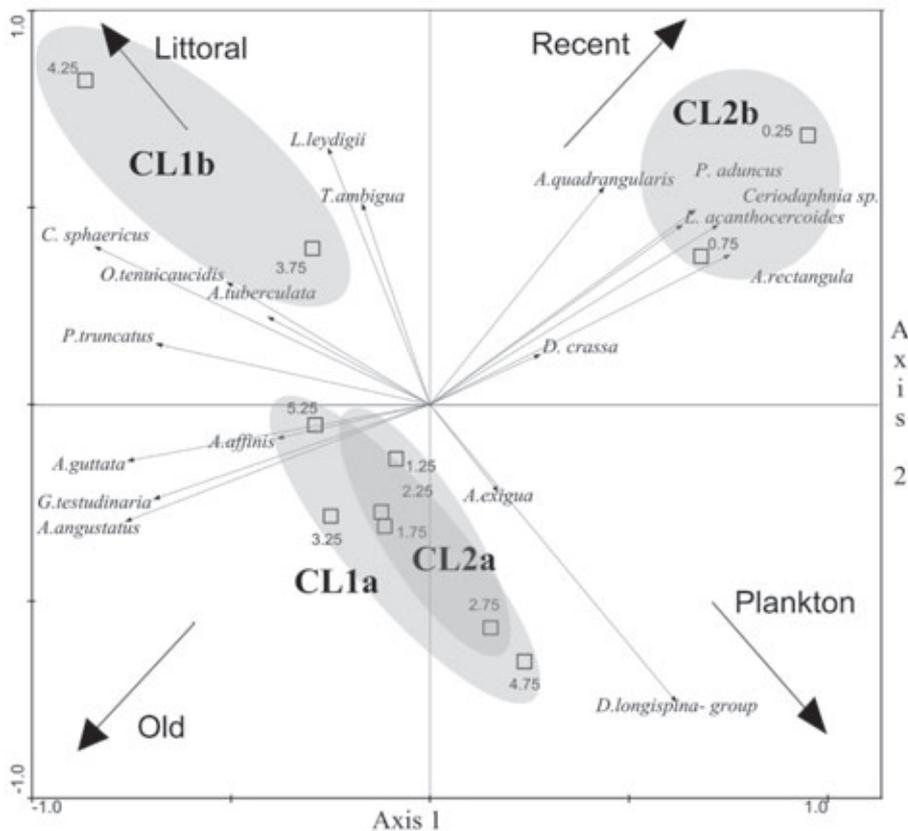


Figure 4. The distribution of the variables (species names) and samples (squares with corresponding core depths, cm) in the space defined by the two principal components resulting from the PCA. Grey areas join the samples belonging to the groups from the cluster analysis of Figure 3. The first two axes explain 69.6 % of the total variability. *Distribución de las variables (nombre de las especies) y las muestras (cuadrados con la profundidad en cm) en el espacio definido por los dos componentes principales resultantes del PCA. Los dos primeros ejes explican el 69.6 % de la variabilidad. Las áreas grises corresponden a los grupos del análisis de la figura 3.*

These results indicate that the two data sets show statistically significant similarities. The matrices from the CA and the PCA were slightly reflected, rotated or dilated to obtain an optimal mutual fit. The lower the rotation sum of the squares and the root mean square error (RMSE), the better the agreement between the ordination results in their optimal fit position (Davidson *et al.*, 2007). However, a more robust assessment of the correlation can be observed relative to the m_{12} values and their associated P value (p value < 0.05), which show a remarkably good outcome in our results.

DISCUSSION

Sediment and water samples differ in both temporal and spatial resolution. Water samples are snapshots of a distinct period and habitat, whereas sediment samples integrate the variation of the entire community throughout space and time. Similarities between neo- and palaeo-communities may also be affected by the degree of preservation of the remains. For this reason, comparisons between living and sediment communities have not always been easy and straightforward. The principal factors that have been shown to cause biases in

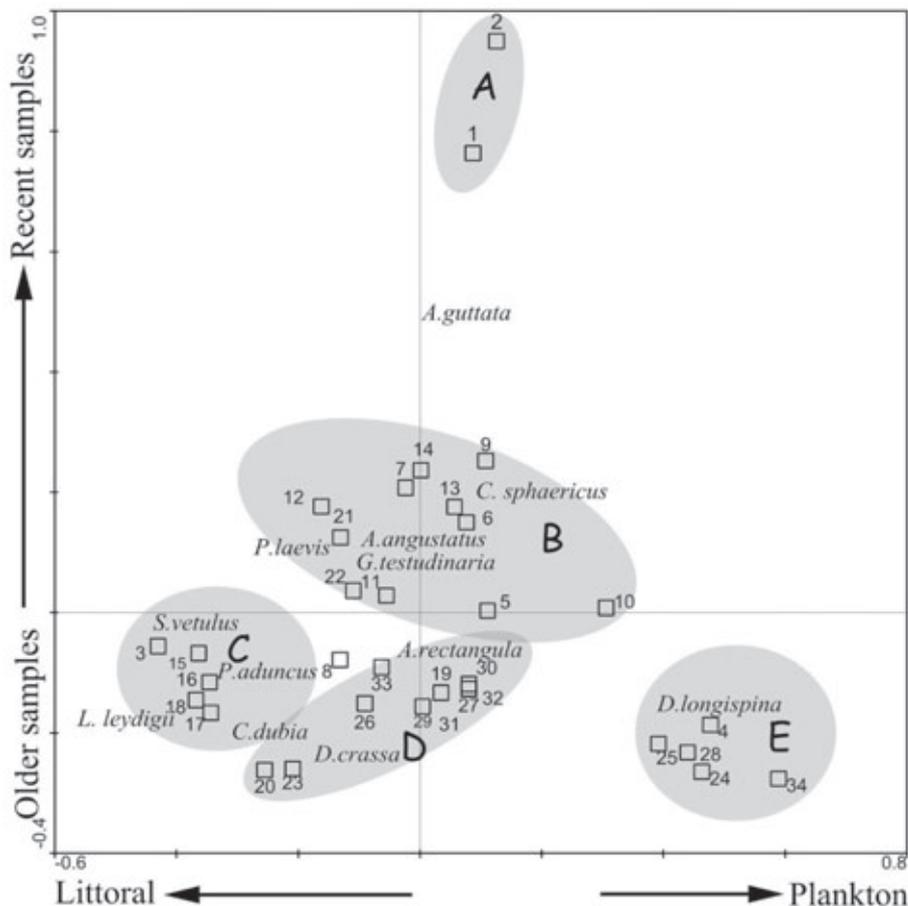


Figure 5. The distribution of the species (names) and the samples (squares with a number indicating the ascending order of sampling years) in the space defined by the first and third components resulting from the CA analysis. These axes explain 38.6 % of the total variability. Grey circles join the samples from consecutive years and allow the tracking of their time trajectory. *Distribución de las especies (nombres) y las muestras (cuadrados con el número de orden ascendiente del año de muestreo) en el espacio definido por la primera y tercera componentes resultantes de análisis CA. Estos ejes explican el 38.6 % de la variabilidad. Las áreas grises reúnen muestras de años consecutivos y permiten seguir su trayectoria en el tiempo.*

stratigraphic studies are sieving (Nykänen *et al.*, 2009), the vertical habitat preferences of species (Nykänen *et al.*, 2009), the presence of zooplanktivorous fish (Davidson *et al.*, 2007; Nykänen *et al.*, 2009) and/or an overestimation of planktonic species in central cores (Kattel *et al.*, 2007).

In our work, we chose a randomisation test based on Procrustes analysis as a measure of the concordance between matrices of contemporary and subfossil Cladocera. The degree of fit between these two matrices was greater than expected ($m_{12} = 0.3912$, $p = 0.023$), indicating that the contemporary and fossil assemblages in Lagunillo del Tejo show a relatively strong concordance. Despite this statistical similarity, several differences involving the number of species, the gradient of the analyses and the configuration of the matrices remain and must be considered. First, as previously stated, the number of species was higher in the sediment (18 species) than in the contemporary samples (12 species). Second, the length of the exploratory DCA in each set of samples was also distinct, reflecting the higher degree of variability in the water samples than in the sediment. Lastly, the positioning of species in the ordination analyses is slightly different because the species composition of each group of samples was not exactly the same. For example, *S. vetulus* occurred only in the water samples so the PCA based on the sediment samples cannot show this species in the diagram. However, these slight disagreements are eliminated by the Procrustes analysis because of the similar global alignment of samples and species in both the CA and the PCA as a function of the sample age and the littoral-planktonic conditions (Figs. 4 and 5). These similar orderings of both sets of samples are, most likely, related to similar factors that control cladoceran populations in freshwater and sediment samples. Romero-Viana *et al.* (2009) has studied a longer core from this lake and has suggested that lake depth and littoral configuration are the principal drivers of population trends in this microcrustacean group. In the present 5.5 cm core, we found the same species found by Romero-Viana *et al.* (2009) and a greater number of species than that found in the contemporary samples for the same period. Nevertheless,

the characterisation of the lake community based on live samples requires a much greater sampling effort than the characterisation based on sediment samples. The number of live samples required for an analysis to cover all of the habitats and every period of the year is much greater than the number of sediment samples required for characterisation. This difference emphasises the role of the sediment as an important library for species information. Most frequently, lake biodiversity is assessed using contemporary survey data, which, as mentioned before, only provide a “snapshot” measure and involve a large number of samples. In contrast, a palaeoecological approach offers an alternative method of species quantification. Additionally, it provides information on changes in biodiversity over time. Such information is crucial for understanding, predicting and managing the consequences of extremely rapid declines in biodiversity (Millennium Assessment, 2005).

Our results agree with those obtained by Davidson *et al.* (2007), who also used Procrustes analysis to compare living cladoceran populations and their remains in the top sediment (0-1 cm) and found statistically significant similarities between the two data sets. However, although their study covered a great number of lakes in a shorter period of time, they also found distinct species compositions in the raw data sets, with 40 species in the water samples and 44 in the sediment samples. Species such as *Diaphanosoma brachyurum* were only present in water samples (Davidson *et al.*, 2007; Kattel *et al.*, 2007), whereas, e.g., *Alonella excisa* or *L. acanthocercoides* were found in the sediment but not in the water samples. The case of *D. brachyurum* in Davidson *et al.* (2007) and Kattel *et al.* (2007) is comparable to *S. vetulus* in our data. Both species are large sized and have highly labile tissue that may have been affected by predation or may have decayed before subfossilisation. The factors responsible for the underrepresentation of *Leydigia leydigi* and *A. affinis* (in our samples, Table 1) or *A. excisa* or *L. acanthocercoides* (in Davidson *et al.*, 2007) are, most likely, the same. These species are either poor swimmers that generally live attached to the substrate or are under-represented in water sam-

ples because they occur during a brief portion of the annual cycle (Vandekerckhove *et al.*, 2005).

Nykänen *et al.* (2009) have stated that the under-representation of *Daphnia*, *Diaphanosoma* and *Ceriodaphnia* in sediment samples is most likely due to the small size of the remains left by this species (the post-abdominal claw), which pass through the 50 µm sieve used to prepare the samples. Kattel *et al.* (2007) have also noted the absence of *Ceriodaphnia* and *D. brachyurum* in water samples processed with a 38 µm sieve. In this study, we also noted the distinct characteristics of *Ceriodaphnia* and *Daphnia* remains. Post-abdominal claws of the *Daphnia longispina* group were very common both in the recent core (CN-JK-2009) of Lagunillo del Tejo presented here and in the longer core presented by Romero-Viana *et al.* (2009). However, *Ceriodaphnia* remains have only been found in the uppermost part of the recent core (CN-JK-2009) and are absent from the longest core (Romero-Viana *et al.*, 2009). Although these results may suggest that *Ceriodaphnia* remains were softer than *Daphnia* remains, a detailed experimental study that incorporates abundances, ecological conditions, predation and taphonomic processes would be necessary to verify this suggestion.

However, a direct link between our study and these studies might appear highly audacious because of the differing contemporary species composition and the differing methodologies. A standardised methodology to assess the bias that can affect comparisons of cladoceran assemblages between living and sediment samples is needed to compare results from different regions. The complexity of the studied ecosystems and the presence of many microenvironments are closely related to the presence of vegetation in the lake. Vegetation plays a multidimensional role (Stefanidis & Papastergiadou, 2010), providing diverse surfaces for colonisation, feeding and refuge to cladoceran species (Geraldés & Boavida, 2004). Lakes with a high PVI (percentage of volume infested), such as Lagunillo del Tejo, are more susceptible to this bias, as shown by Davidson *et al.* (2007).

Because our study could have been influenced by the incompleteness of the available con-

temporary sample collection, we chose Procrustes methodology to link the two sets of ensemble data in preference to direct comparisons between the sets of samples. We found that the two sets of samples showed statistically significant similarities, although a greater number of species was found in the sediment. This result highlights the potential role of palaeoecology in biodiversity studies. In comparison with previous studies, which had compared samples only from the top layer of the sediment, this work provides a wider temporal framework. Such a framework is crucial because a changeable environment is involved, and this approach furnishes an interface with future palaeolimnological studies. Additionally, this study provides new raw data from southern Europe and from a lake with a high PVI. These data can be incorporated in future models of environmental change. Nevertheless, future studies should focus on improving the methodology for comparing these types of samples and on determining the influence that physical and chemical conditions in latitudinal gradients exert on taphonomy.

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