



## Research paper

# Subfossil Cladocera from surface sediment reflect contemporary assemblages and their environmental controls in Iberian flatland ponds

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## ABSTRACT

Cladoceran subfossils from surface sediments have been recognized as useful and reliable ecological indicators in freshwater ecosystems. Nevertheless, investigations concerning the relationships between contemporary and sedimentary assemblages are scarce in the Mediterranean region and no studies are available from Iberian flatland ponds. Here, we explore the concordance between present-day Cladocera assemblages and their sedimentary remains from 25 ponds distributed across a vast heterogeneous region in the Iberian Plateau. Overall, this study aims at identifying sediment biological proxies with good capacity to track long-term changes in Iberian flatland ponds. We found a total of 15 and 18 taxa in the contemporary and sedimentary samples, respectively, with daphniids dominating water column samples whereas the majority of the remains recovered from surface sediments were from chydorids. In accordance with previous work, we found that subfossil Cladocera properly reflect the contemporary assemblages from which they are derived. Similarly, our results showed not only analogous environmental controls structuring contemporary and sedimentary assemblages (mainly pH and soluble reactive phosphorous), but also a similar proportion of explained variance in the two. Although there were several taxa occurring only in contemporary samples (*Diaphanosoma brachyurum*, *Moina rosea*, *Macrothrix hirsuticornis*, *Scapholeberis* sp. and *Simocephalus* sp.), we found that subfossil assemblages were significantly more rich, diverse, equitable and heterogeneous, indicating that an integrated sample of surface sediment from each pond may supply enough information for assessing cladoceran composition and the numerous drivers controlling their assemblage structure.

## 1. Introduction

Zooplankters play a key ecological role in freshwaters because their communities reflect a combination of physical, chemical and biological characteristics of the systems they occupy (Davidson et al., 2007). They are also an important component of the trophic web occupying an intermediate position between primary producers and consumers (Jeppesen et al., 2001). Surface sediments usually contain natural archive of zooplankton remains which offer an excellent opportunity to study their potential as a proxy of the contemporary communities from which they are derived (Battarbee, 2000). Several taxa of zooplankton assemblages, particularly copepods and rotifers, are very poorly represented by sedimentary subfossils (Rautio et al., 2000). On the contrary, cladoceran remains (Crustacea, Branchiopoda) represent one of the most valuable ecological proxies that can be analyzed for palaeolimnological reconstructions (Korhola and Rautio, 2001) because they are widespread in both pelagic and littoral zones of lakes and ponds and they often represent the dominant taxa of zooplankton communities in terms of biomass (Frey, 1960). Additionally, chitinous remains of

cladocerans (typically Bosminidae, Chydoridae and Daphniidae) such as carapaces, post-abdomens, claws, mandibles, sections of antennae and ephippia are generally abundant in surface sediments (Frey, 1960; Hann, 1988) and they have been used in a large number of investigations addressing effects of climate change on aquatic ecosystems, chydorid diversity, relation with aquatic macrophyte coverage, past water chemical conditions, lake depth, salinity and changes in food web structure (Brodersen et al., 1998; Lotter et al., 2000; Kattel et al., 2007; Jeziorski et al., 2008; Davidson et al., 2011; Korosi et al., 2013). However, cladoceran subfossils are fragmentary and incomplete by their nature and exhibit a differential preservation (Frey, 1958; Hann, 1988; Korhola and Rautio, 2001). Accordingly, the chitinous and large species such as chydorids and *Bosmina* spp. have been found to be well preserved in sediment, providing an accurate view of their contemporary assemblages, while species with thinner exoskeletons (*Daphnia* spp., *Ceriodaphnia* spp. and *Simocephalus* spp.) tend to be under-represented since their remains usually consist of claws and ephippia which are not as abundant as other subfossil materials (Korhola and Rautio, 2001).

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Much work in the palaeoecology of crustacean Cladocera needs to be done since the analysis of the sedimentary record is not always straightforward and cladoceran research has historically lagged behind other ecological proxies such as diatoms and chironomids (Eggermont and Martens, 2011). To this regard, there is an urgent need to realize whether cladoceran subfossils are sufficiently representative of their present-day communities in order to avoid biases and to provide a reliable relationship between modern and sedimentary assemblages (Kattel et al., 2007; Davidson et al., 2011). Additionally, many factors still restrict the interpretation and exploitation of cladoceran subfossil records since published studies about the distribution and environmental drivers of cladocerans are relatively scarce, hindering their use in palaeoecological reconstructions (Manca and Comoli, 1995; Davidson et al., 2007; Eggermont and Martens, 2011). Therefore, efforts to enhance the knowledge of the agreement between present-day and sedimentary cladoceran assemblages, and with their environmental controls are still needed nowadays (Tolotti et al., 2016). Furthermore, much of the published literature on subfossil cladocerans comes from temperate and deep lakes and only a few studies exist in the Mediterranean Region, specifically in semi-arid environments (Çakiroğlu et al., 2014). To the best of our knowledge, few investigations examining the correspondence between contemporary data and subfossil record and their relationships to a set of environmental drivers originate from the Iberian Plateau. Only Romero-Viana et al. (2010) and López-Blanco et al. (2011, 2013); analyzed the biases between contemporary and sedimentary cladoceran assemblages in a small karstic sinkhole located in the South of Spain.

Ponds have been recognized as important features of the landscape since they are the most widespread continental lentic ecosystems and harbor a large number of taxa, including rare and endangered species (Scheffer et al., 2006; Downing, 2010; Miracle et al., 2010). For this reason, there is growing awareness in the Mediterranean Region about the importance of ponds and increasing understating of their ecology and palaeoecology (Della Bella et al., 2008). Furthermore, the general lack of historical data in Iberian ponds makes subfossil Cladocera as the only source of information for reconstructing historical evolution of planktonic assemblages, as well as of the environmental variables driving their community composition (Levi et al., 2014). To this regard, analysis of subfossil Cladocera would provide an opportunity to assess if these sedimentary remains could be used as a biological proxy able to integrate both present-day assemblages and their major environmental controls.

In the present study, we investigate the relationships between contemporary and subfossil cladoceran assemblages of 25 Iberian flatland ponds. Cladocera were retrieved from the water column and as remains from surface sediment along with measurements of their environmental controls. First, we checked the differences in richness, diversity, equitability and heterogeneity between contemporary and subfossil assemblages. Finally, we explored the relationships between present-day and sedimentary datasets and identified which environmental drivers accounted for most variation observed in the distribution of cladoceran assemblages. This study, therefore, aims at identifying the value of subfossil Cladocera as sediment proxy in the poorly investigated Iberian ponds.

## 2. Material and methods

### 2.1. Study area

This study was performed on 25 ponds located in a vast heterogeneous Iberian region called Castilla y León, a relatively low and flat area located in northern and central Spain (700–1000 m.a.s.l.; Fig. 1). All of the ponds are immersed in an agricultural intensive landscape consisting mainly on cereal crops and scattered scrubs. The climate of the region is Mediterranean, with hot, dry summers and cold, rainy winters (400–600 mm of mean annual rainfall). The 25 ponds are permanent and present a broad gradient of conductivity, nutrient content, macrophyte coverage and fish density (Table 1). Macrophyte

communities consisted mainly of *Myriophyllum alterniflorum* (DC.), *Chara* (L.), *Polygonum amphibium* (L.) and *Potamogeton trichoides* (Cham. Schlecht.), while *Tinca tinca* (L.) and *Chondrostoma arcasii* (Steindachner) dominated fish assemblages.

### 2.2. Field sampling and laboratory analyses

Environmental variables, present-day cladoceran and surface sediment samples were collected in June and July 2004 and 2005; each pond was sampled only once. In each pond, several water samples were randomly collected at different depths along a shore-centre transect using a cylindrical tube (diameter = 6 cm and length = 100 cm) and were combined and mixed to form a single composite sample. The number of samples ranged between 3 and 15 depending on the pond area. Mean depth was also recorded for each pond by measuring depth at several sites along transects within each pond. In the final integrated sample pH, conductivity, temperature, dissolved oxygen and turbidity were measured using WTW field probes and a Portable Turbidimeter (Model HACH 2100P). The composite samples were analyzed in laboratory following APHA methods (APHA, 1989) to determine total phosphorous (TP), soluble reactive phosphorous (SRP), total nitrogen (TN), nitrate (NO<sub>3</sub>-N) and chlorophyll *a* concentrations (Chla). Nutrient samples were fixed with mercuric chloride and preserved at 4 °C until analyses were conducted.

Fish abundance (Catch per unit effort; CPUE; number net<sup>-1</sup>) for two size classes (10 cm < fish < 10 cm) was determined using gillnets. One or two nets, depending on the pond area, were set overnight and retrieved after 18 h, usually in the morning.

Plant Volume Inhabited (PVI) was estimated based on submerged species data sampled at varying intervals along profiles (Canfield et al., 1984). The number of profiles varied according to the area of the pond and the development of the shore (Jénsen, 1977). More details can be found in Fernández-Alález et al. (2016).

Samples for the analysis of contemporary cladoceran assemblages were obtained by filtering 3–7 L of the integrated water samples through first a 50 µm and then a 20 µm mesh size net. The samples were fixed with formalin with a final concentration of 4%. A minimum of 100 individuals of the dominant taxa were counted using a subsample of known volume from each fraction (50 and 20 µm). Cladocera were identified with reference to Alonso (1996) using a Nikon Eclipse TE300 inverted light microscope.

From each pond, three surface sediment (0–5 cm) samples were retrieved using a cylindrical corer (6 cm diameter). These three samples were mixed and preserved at 4 °C until taphonomic analysis. For the subfossil taphonomical analysis 10 g wet homogenized surface sediment were boiled in a 10% KOH solution for 20 min and were washed through 125 and 50 µm sieves (Frey, 1986). For the counting process, residue retained on the 125 and 50 µm sieves were separated and colored with a safranin dye. Finally, 50 µL aliquots of each fraction (125 and 50 µm) were pipetted on to quantitative slides and all remains (carapaces, head shields, post abdomens, post-abdominal claws and ephippia) were counted. As the different fragments of subfossil Cladocera are unequally preserved, the most abundant body part of them all was used in subsequent analysis (Amsinck et al., 2005; Çakiroğlu et al., 2014). Sedimentary remains were identified and counted using a Nikon Eclipse TE300 inverted light microscope. For taphonomical identification, the keys in Frey (1962, 1986, 1987) and Alonso (1996) were used. Daphniids were separated as belonging to *D. pulex* (L.) complex and *D. longispina* (Müller) complex in order to give a level of taphonomic resolution corresponding to that attainable with post-abdominal claws (Davidson et al., 2007; DeSellas et al., 2008; Korosi and Smol, 2011). For example, *D. parvula* (Fordyce), *D. rosea* (Sars), *D. galeata* (Sars) and *D. cucullata* (Sars) were grouped into *D. pulex*, whereas *D. obtusa* (Kurz) and *D. curvirostris* (Eylman) were amalgamated into the complex *D. longispina*.

For statistical analyses, present-day Cladocera are presented as individuals 100 cm<sup>-3</sup> of water and subfossil Cladocera as number of remains 100 cm<sup>-3</sup> of fresh sediment.

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