



Biogeochemical characterization of a Mediterranean shallow lake using stable isotopes: Laguna del Cristo (NW Iberian Peninsula)

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Abstract

The present multi-isotopic study ($\delta^{18}\text{O}-\delta\text{D}_{\text{water}}$, $\delta^{34}\text{S}-\delta^{18}\text{O}_{\text{dissolved-sulphate}}$, $\delta^{13}\text{C}_{\text{dissolved-inorganic-carbon}}$, $\delta^{13}\text{C}-\delta^{18}\text{O}_{\text{shells-modern-gastropods}}$, $\delta^{13}\text{C}_{\text{plants}}$, and $\delta^{13}\text{C}_{\text{sedimentary-organic-matter}}$) is aimed at assessing the hydrogeochemical changes and biogeochemical dynamics in a Mediterranean shallow lake fed by a Quaternary–Tertiary aquifer, the “Laguna del Cristo” (NW Iberian Peninsula), a system sensitive to climate fluctuations, between 2010 and 2011. Lake water is of the bicarbonate type, and there are no major pollutants. $\delta^{18}\text{O}-\delta\text{D}_{\text{water}}$ values plot on a local evaporation line ($\delta\text{D}=5.29\delta^{18}\text{O}-12.29$) indicating that evaporative enrichment had a significant impact on lake water isotopic features. Periods of high water levels are characterized by lower $\delta^{34}\text{S}-\delta^{18}\text{O}_{\text{dissolved-sulphate}}$ and $\delta^{13}\text{C}_{\text{dissolved-inorganic-carbon}}$ values and suggest sulphate derived from weathering of sulphides in the catchment area, delivered to the lake by surface run-off or via groundwater, and in situ decay of organic matter. During lower water levels, sulphate reduction and enhanced primary productivity lead to higher $\delta^{34}\text{S}_{\text{dissolved-sulphate}}$ and $\delta^{13}\text{C}_{\text{dissolved-inorganic-carbon}}$ values. Evaporation induced enrichment in $^{18}\text{O}_{\text{sulphate}}$, $^{13}\text{C}_{\text{dissolved-inorganic-carbon}}$ and $^{13}\text{C}-^{18}\text{O}_{\text{shells-Galba-Gyraulus}}$. $\delta^{13}\text{C}_{\text{plant}}$ confirms the C3 photosynthetic pathway. Enrichment in ^{13}C submerged aquatic plants indicates that HCO_3^- is the main carbon source, except for ^{13}C -depleted *Potamogeton*. The TOC, $\delta^{13}\text{C}_{\text{org}}$ values, and TC/TN ratios in sediments all confirm the autochthonous character of organic matter contribution. This study provides a baseline for isotopic research into shallow, flow-through lakes fed by siliciclastic aquifers, and stresses the importance of evaporation and refilling (direct precipitation and groundwater discharge) in controlling the solute chemistry and stable isotopic composition in temperate regions with contrasting seasonal climates. The results also provide a snapshot of modern lake isotope variability that can be applied to paleoenvironmental reconstructions.

Keywords $^{18}\text{O}/^{16}\text{O}$ · D/H · DIC · Sulphur cycling · Freshwater gastropods · $\delta^{13}\text{C}_{\text{plants}}$

Introduction

Wetlands and shallow lakes are known to be highly sensitive to climate and human forcings. Human uses and climate-driven changes on wetlands affect temperature, water level fluctuations, and nutrient loading, with potential implications on their hydrologic regimes and biogeochemical cycles (Groffman and Taylor 1996). In temperate regions with contrasting seasons, where this type of lakes are abundant, fluctuations in water levels are determined not only by precipitation and run-off but also by groundwater (Rey Benayas 1990). As a consequence, water chemical composition will be the product of climatic, lithological, hydrogeological, biological, and/or human factors, with additional seasonal changes resulting from the physicochemical processes occurring in the free water body (Kehew 2001).

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Stable isotopes are an excellent tool to investigate the biogeochemical cycles and hydrological changes in lacustrine environments, and help to constrain the sources and relative contribution of material to the ecosystem, as well as its dynamics (Leng and Marshall 2004; Cole et al. 2007). The isotopic composition of lake water strongly depends on climatic parameters and reflects the meteorological conditions of the region. Variations of $\delta^{18}\text{O}_{\text{lake}}$ and $\delta\text{D}_{\text{lake}}$ will be especially sensitive to the balance between inputs (precipitation, surface and stream inflows, groundwater) and outputs (evaporation, surface and stream outflows, groundwater loss) (Leng et al. 2005; Darling et al. 2005). The stable carbon isotope composition of endogenic carbonates, dissolved organic carbon (DOC), and dissolved inorganic carbon (DIC) has been widely used to evaluate the main parameters controlling carbon cycles in lakes (Leng and Marshall 2004; Cole et al. 2007). Variations of $\delta^{13}\text{C}_{\text{DIC}}$ are related to the dissolution of carbonate minerals, ground- and surface water inputs, equilibration with atmospheric CO_2 , and ^{12}C -selective uptake by aquatic plants during photosynthesis (Mook 2002; Leng and Marshall 2004; Myrbo and Shapley 2006). $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of biogenic carbonates generally reflect the isotopic composition of lake water and allow the estimation of water temperature and precipitation patterns (Fritz and Poplawski 1974; Leng et al. 1999). $\delta^{13}\text{C}$ of plants is also determined by ambient climatic parameters such as temperature, humidity, precipitation, and the physiology of the plant (O’Leary 1988; Farquhar et al. 1989). $\delta^{13}\text{C}_{\text{org}}$ values are commonly used to distinguish between allochthonous and autochthonous sources of organic carbon. Depending on the photosynthetic cycle, $\delta^{13}\text{C}$ values in organic matter (OM) range between -33 and -20% for C3-type plants (Calvin–Benson photosynthetic pathway) and between -15 and -9% for C4-type plants (Hatch–Slack photosynthetic pathway) (Deines 1980; O’Leary 1988). Furthermore, $\delta^{13}\text{C}$ values on OM are preserved in lake sediments and can be used to infer past environmental changes in aquatic ecosystems (Meyers 1994). Sulphur isotope ratios have been used to identify sources and processes controlling the chemistry of S in natural waters. They are particularly valuable tools to investigate weathering and biogeochemical reactions, such as organic matter degradation, bacterial sulphate reduction, or sulphide mineral oxidation (Krouse and Grinenko 1992; Hoefs 2004).

“Laguna del Cristo” (El Cristo lake), a representative wetland of the “dehesa¹” environment, is one of the relatively frequent shallow lakes, nourished by siliciclastic aquifers, that can be found in the NW Iberian Peninsula, particularly

so under the Mediterranean climatic conditions of the Duero Basin. Its existence is linked to the discharge of the aquifer hosted by the Plio-Pleistocene quartzitic conglomeratic deposits known as “raña” unit in the Spanish Cenozoic basins. This singular geological setting controls the position of the local groundwater level, raised over the river plains. Several studies have focused on the influence of hydrological processes within the aquifer on water chemistry (Rey Benayas 1990; Bernáldez et al. 1993), environmental parameters and crustacean assemblages (Alonso 1998), and, more recently, on the presence of pesticides and PAHs (Hijosa-Valsero et al. 2016). For this study, we have determined the physicochemical parameters and stable isotope ratios of lake water and its solutes, in order to understand the dominant hydrogeological and biogeochemical processes, which is useful for developing and implementing good environmental and conservational practices on wetlands.

Previous hydrogeochemical studies have demonstrated that chemical changes of water in similar shallow lakes settings are influenced by precipitation and the residence time of groundwater deeper regional flows (Fernández Aláez et al. 2006; Fernández Aláez and Fernández Aláez 2010). Earlier work by the authors concluded that El Cristo lake was formed by a combination of morphotectonic and chemical weathering processes and showed the influence of groundwater flow in the chemical composition of its water (Jambrina et al. 2013). However, little information is available regarding the variations of isotopic composition in Mediterranean shallow lakes that can help understanding the links between hydrological and ecological processes, as these may trigger transformations on these sensitive aquatic ecosystems. To that end, we have studied El Cristo lake on an annual basis (2010–2011), to assess the seasonal variations and evaluate the changes caused by evaporative concentration and water level change, determining physicochemical parameters, $\delta^{18}\text{O}_{\text{water}}$ and $\delta\text{D}_{\text{water}}$, $\delta^{34}\text{S}$ and $\delta^{18}\text{O}$ isotopic ratios in the sulphate ion in order to understand sulphate sources and to evaluate possible factors controlling S biogeochemistry and $^{13}\text{C}/^{12}\text{C}$ ratios in DIC, terrestrial and aquatic plants, gastropod shells and sedimentary organic matter, with the aim of constraining the relative contribution of inorganic and organic carbon pools and changes therein.

Site description

El Cristo lake (40°40′55″ N, 6°14′17″ W, 845 m a.s.l.) lies in a shallow depression on the Ciudad Rodrigo sub-basin, within the Duero Basin (NW Spain) (Fig. 1a), is 720 m long \times 300 m wide (23.94 ha) and has, at high water, a maximum depth of 1.8 m. The depression hosting the lake resulted from the morphotectonic control of NW–SE and NE–SW faults, combined with the loss of fine-grained

¹ The “Dehesa” ecosystem is a managed Mediterranean forest, populated by *Quercus evergreens*, that was cleared for pastures, extending for some 2×10^5 Km² in the Iberian Peninsula.

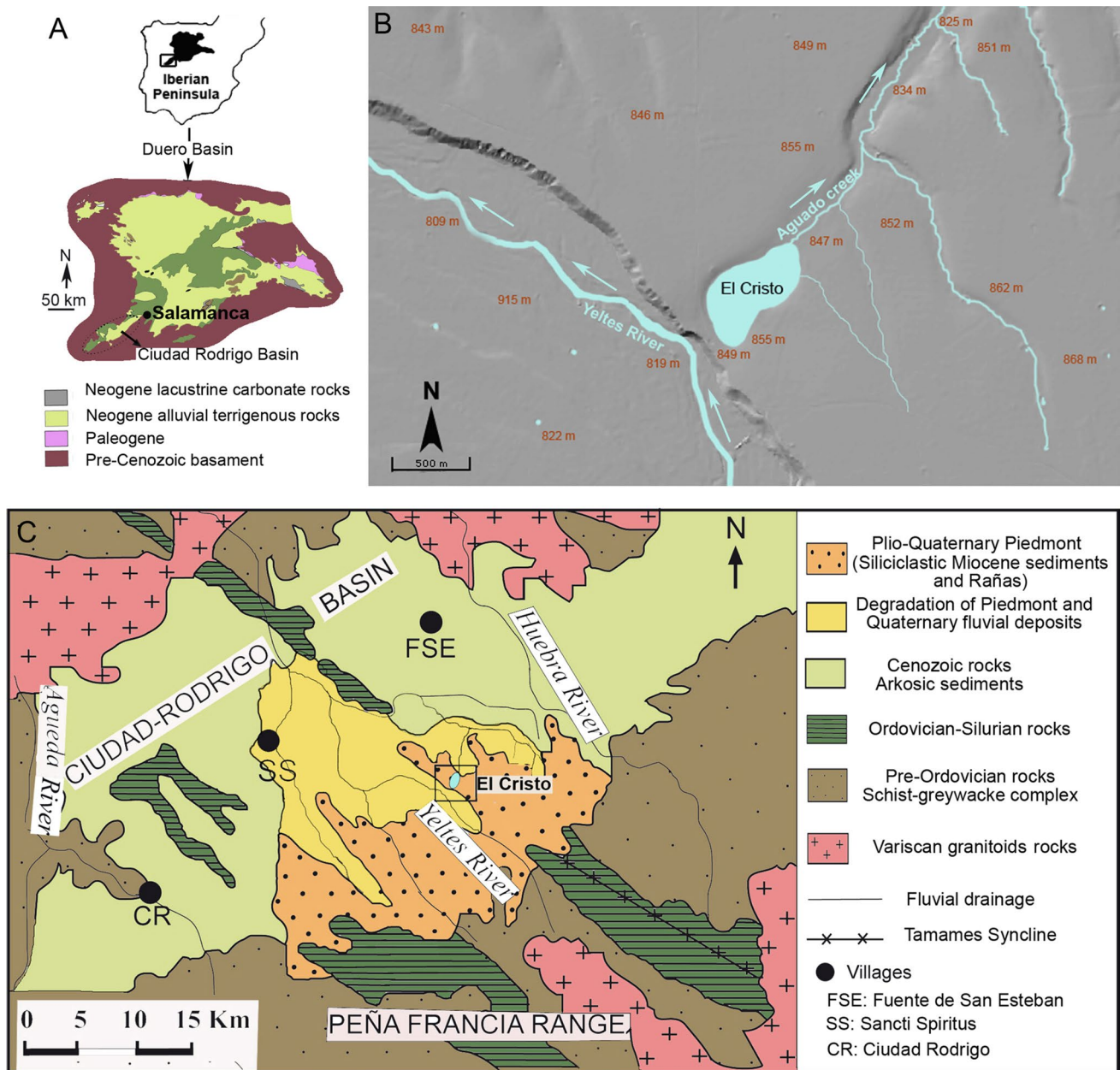


Fig. 1 **a** Location map of the study area in the Iberian Peninsula and geological map of the Duero Basin, **b** drainage area of El Cristo lake (modified from www.idecyl.jcyl.es; arrows indicate river-flow direc-

tion), **c** geological map of El Cristo lake surrounding area (Ciudad Rodrigo sub-basin) (based on Martín-Serrano et al. 1998)

material of the substrate by chemical weathering processes (Jambrina et al. 2013). It is located on a Pleistocene alluvial piedmont with a regional north-western slope (5.3%) from the Peña Francia range (Tamames syncline, 1000 m a.s.l.) to the lake (845 m a.s.l.), but the drainage outlet of the lake follows a local north-east slope conditioned by tilted block faulting (Jambrina et al. 2013) (Fig. 1b, c). The catchment area consists of siliciclastic Miocene gravel deposits with thinner intercalations of muddy sandstones and mudstones, and Plio-Quaternary deposits made up of quartzite gravels

in a sandy clay matrix (“rañas” and alluvial terraces) which superficially cover the Tertiary detrital deposits (Martín Serrano et al. 1998; Molina and Cantano 2002) (Fig. 1c). Overall, the piedmont is poorly drained, as a result of the impermeable character of the clay matrix of the conglomeratic deposits of the raña substratum.

The climate is Mediterranean with marked dry summers and cool, wetter winters (mean annual temperature is 12.2 °C, and the mean annual precipitation is 415 mm; data from the Matacán-Salamanca weather station,

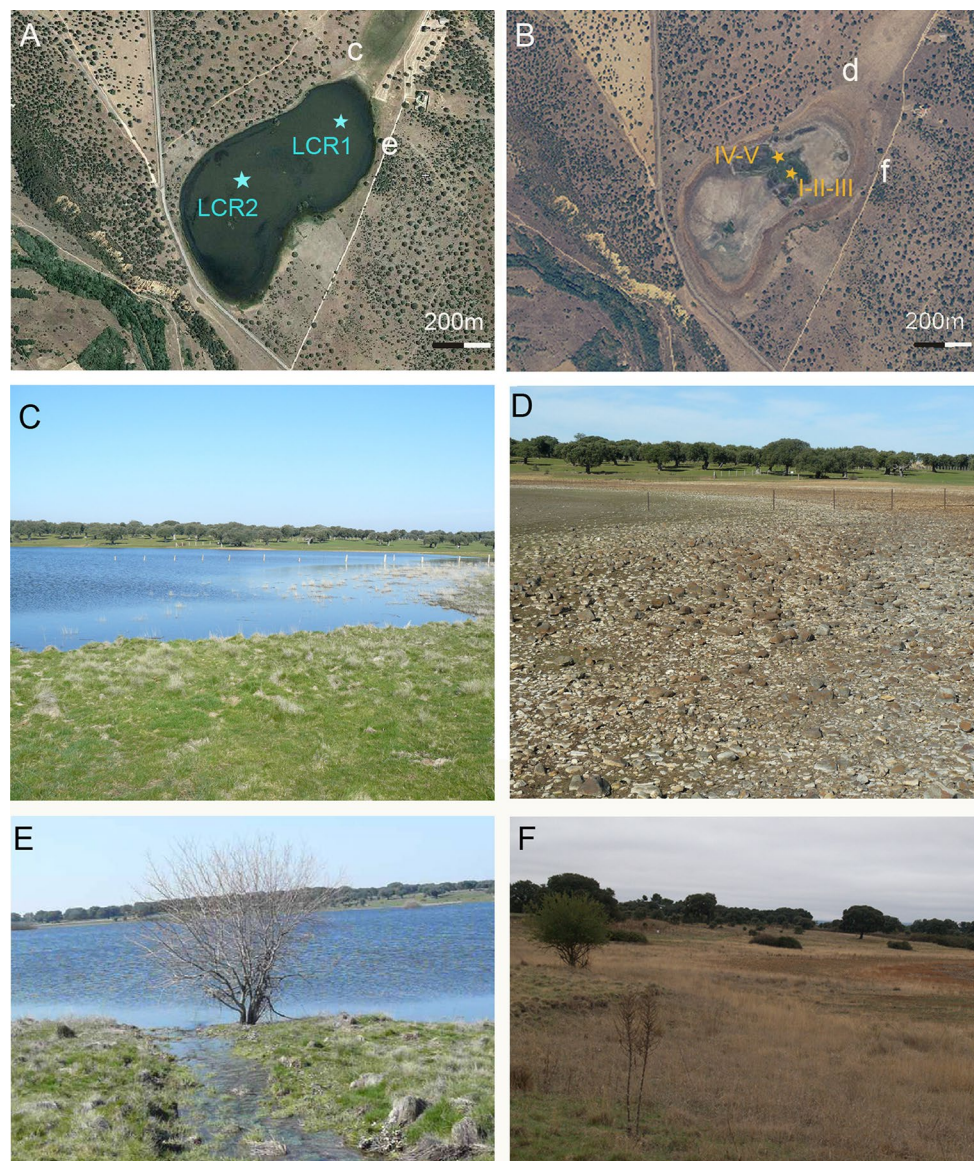


Fig. 2 Location of the sampling sites. **a** El Cristo lake in 2010, during a period of high water level and location of water sampling sites (LCR1 and LCR2). **c** and **e** correspond to the wetland outlet and inlet respectively. **b** The lake in 2009, during a dry period when the

lake almost completely dried up, and location of sediment sampling sites (I–V). **d** and **f** correspond to the lake outlet and inlet at the time, respectively

40°57'34"N – 5°29'54"W; AEMET.) The lake water balance is largely controlled by direct precipitation and the regional groundwater inflow of a surficial, unconfined aquifer within Tertiary detrital deposits, which may extend into the shallower unit (“raña” deposits). The lake is shallow and surface inlet and outlets are intermittent: during the rainy season, there is an ephemeral inlet, but an outlet is only active in exceptionally rainy years (Fig. 2). The lake waters are diluted (main ions are Na^+ , Ca^+ and HCO_3^- , Cl^-) but with a marked increase in SO_4^{2-} and Cl^- during periods of intense evaporation (Jambrina et al. 2013).

Vegetation around the lake consists on an open forest of *Quercus ilex*. The littoral zone is colonized by a well-developed aquatic plant community (*Phragmites*, *Eleocharis palustris*, *Chara fragifera*, *Nitella opaca*, *Ranunculus*, *Myriophyllum*, *Ceratophyllum* and *Potamogeton* sp.), and *Spyrogyra* is found at about 1.5 m depth water (Alonso 1998).

Materials and methods

Materials

Water samples (LCR1 and LCR2, Fig. 2) for chemical, physical, and isotope analysis were collected every 3 months between March 2010 and February 2011, with one additional sample collected in October 2011. During the wet season, additional samples were taken from the inlet to the lake. No surface inflows to the lake have been observed for the rest of the year (Fig. 2). Lake water was sampled at 30 cm depth using high-density polyethylene bottles (10 L), previously washed with 2.5 N HCl and distilled water, and 50-mL narrow-necked bottles with stopper and screw cap to avoid evaporation. To minimize changes in chemical composition due to bacterial activity, samples were kept refrigerated at 4 °C before laboratory analysis.

Well-preserved shells of two species of gastropods, *Planorbis (Gyraulus)* sp. and *Lymnaea (Galba)* sp. (pulmonate freshwater snails), were collected in June 2010 and September 2010 from aquatic grasses consisting of *Myriophyllum* and *Baldellia ranunculoides*. Vegetation associated with the gastropods was also collected for isotopic analysis. *Galba* shells were collected again in October 2011 from the lake bottom when the lake partially dried.

Two species of terrestrial plants (*Glyceria* and *Juncaceae*) and five species of aquatic plants (*Myriophyllum*, *Ranunculus sect. batrachium*, *Baldellia ranunculoides*, *Scirpus lacustris* and *Potamogeton*) were sampled in March, June, and September 2010.

Five sediment cores (20 cm long) were retrieved with a 5-cm ID corer at two points at the centre of the lake in October 2009 (Fig. 2).

Methods

In situ measurements of temperature (°C) and pH were made using a Hanna HI 9023 pH meter and a CRISON CDTM 523 electronic meter for conductivity ($\mu\text{S cm}^{-1}$). Cl^- , SO_4^{2-} and PO_4^{3-} (mg L^{-1}) were measured at the field site with a PF-11 Macherey-Nagel photometer. Alkalinity ($\text{CO}_3^{2-} + \text{HCO}_3^-$) was determined by acid titration with methyl orange and is expressed in mg L^{-1} as CaCO_3 . Major and minor cations (Al, Ca, Fe, K, Mg, Na, Si, Cu, Zn; ppm) were analysed on acidified water samples (HNO_3 to $\text{pH} \approx 1$) using ICP-OES (Ultima II from Jobin Yvon) and minor and trace elements (Li, Be, B, Ti, V, Cr, Co, Ni, As, Se, Rb, Sr, Cd, Sb, Ba, Hg, Pb; ppb) with ICP-MS (Elan 6000 from Perkin-Elmer) at the Servicio General de Análisis Químico Aplicado, Universidad de Salamanca, Spain.

Oxygen isotope analyses on water ($\delta^{18}\text{O}_{\text{water}}$) were done by equilibration with CO_2 , using a Multiflow (Micromass) equilibrium device. D/H ratios were determined on H_2 gas obtained by the Cr-reduction method, using an EuroVector EA3000 elemental analyser. Isotopic ratios were measured on a continuous flow Isoprime (Micromass) mass spectrometer coupled online to the preparation devices. Water samples for $\delta^{34}\text{S}_{\text{SO}_4^{2-}}$, $\delta^{18}\text{O}_{\text{SO}_4^{2-}}$ and $\delta^{13}\text{C}_{\text{DIC}}$ analyses were filtered through 0.45- μm nylon filters. Water-soluble sulphate was precipitated by addition of 10% BaCl_2 solution to the previously acidified (to $\text{pH} \approx 2$) water. The BaSO_4 precipitate was separated from the solution by filtering through Whatman #42 filter (125 mm diameter), washed with distilled water, and air-dried. Sulphur isotope analyses ($\delta^{34}\text{S}$) were done on SO_2 produced offline following the method of Robinson and Kusakabe (1975), with modifications for sulphates by Coleman and Moore (1978). Isotopic ratios were determined on a dedicated dual-inlet SIRA-II (VG-Isotech) mass spectrometer. The oxygen isotopic composition of the BaSO_4 ($\delta^{18}\text{O}_{\text{SO}_4^{2-}}$) was measured on CO obtained by pyrolysis on a EuroVector EA3000 elemental analyser, coupled online to an Isoprime continuous flow mass spectrometer. $\delta^{13}\text{C}$ of HCO_3^- was determined on SrCO_3 precipitated from the previously basified water (to $\text{pH} > 9$, using 15% NaOH) by addition of 45% SrCl_2 . Shell samples were rinsed with deionized water, grounded using an agate mortar and pretreated for 30 min in a low-temperature oxygen plasma to remove any remaining organic matter or other volatile contaminant. $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ ratios were determined on CO_2 obtained offline by reaction with 103% H_3PO_4 (McCrea 1950; Craig 1957), measured on a second dual-inlet SIRA-II mass spectrometer. Carbon isotope ratios on plant samples (previously rinsed with deionised water, dried, and powdered using a cryogenic mill) and on five decarbonated sediment samples (II and IV cores) were measured on CO_2 produced by combustion in an EuroVector elemental analyser interfaced to a CF Isoprime mass spectrometer. Stable isotope measurements were performed at the Laboratorio de Isótopos Estables, Universidad de Salamanca, Spain. All stable isotope results are expressed as “ δ ” values (such that δ sample = $1000[(R_{\text{sample}}/R_{\text{standard}}) - 1]$, where R is the $^{18}\text{O}/^{16}\text{O}$, D/H, $^{34}\text{S}/^{32}\text{S}$ or $^{13}\text{C}/^{12}\text{C}$ ratio in sample and standard), representing deviations in per mil (‰) from VSMOW for oxygen and deuterium, CDT for sulphur and PDB for carbon. Overall precision, determined by repeated analysis on both internal and international reference materials, is better than $\pm 0.2\text{‰}$ for $\delta^{18}\text{O}$, $\pm 0.5\text{‰}$ for δD , $\pm 0.2\text{‰}$ for $\delta^{34}\text{S}$ and $\pm 0.1\text{‰}$ for $\delta^{13}\text{C}$.

Sediment cores were sub-sampled for total organic carbon (TOC) and total nitrogen analysis (TN) every 5 cm. Up to 10 mg of dried sediment samples were homogenized using an agate mortar and acidified before analysis by the addition

of 10% HCl at 80 °C to remove carbonates. TOC and TN determinations were measured with a FlashEA1112 (ThermoFinnigan) elemental analyser at Servicios de Apoyo a la Investigación (SAI), Universidad de La Coruña, Spain. C/N values are expressed as atomic ratios.

Hydrogen and oxygen isotopic values of rainfall are derived from interpolation algorithms and depending on the density of data in the region (latitude 40°, longitude - 6°, altitude 845 m, online isotopes in precipitation calculator-OIPC, <http://www.waterisotopes.org>).

Results

Physical and chemical parameters

The chemical analyses of water samples are presented in Table 1. The pH ranged from 5.9 to 9.9, and the water temperature varied between 4.1 °C and 24.3 °C. The NO₃⁻ and PO₄³⁻ concentrations, although tested for, were below the instrumental detection limit (< 2 and < 4 mg L⁻¹, respectively) and are therefore not included in Table 1. The SO₄²⁻ concentration ranged from 20 to 48 mg L⁻¹, whereas Cl⁻ was between 8 and 40 mg L⁻¹. The HCO₃⁻ contents showed a minimum value of 18.3 and a maximum of 134.2 mg L⁻¹. Electric conductivity (EC) ranged from 35.3 to 317.0 μS cm⁻¹, and the total dissolved solids (TDS) ranged from 23.7 to 212.4 mg L⁻¹. All major cations (Na⁺, Ca²⁺, Mg²⁺, K⁺) and anions (Cl⁻, SO₄²⁻) were higher in summer (June 2010, September 2010) and mid-autumn (November 2010 and October 2011), than in spring (March 2010) and winter (February 2011). Whatever the parameter or season, inlet creek waters are always the most diluted.

Values for selected minor and trace elements are shown in Table 2. Among them, Si recorded higher values in winter and spring (0.2–1.4 mg L⁻¹) and Fe in summer and mid-autumn (0.2–1.1 mg L⁻¹). The average levels of metals were below the allowable concentrations established by the World Health Organization—WHO (2011).

Stable isotopes (water, plants, and modern gastropods)

The isotope analyses of water samples are presented in Table 1. δ¹⁸O_{water} ranged from - 7.6 to + 5.5‰ and the δD_{water} varied from - 51.9 to + 14.9‰. The d-excess is higher (+ 5.0 and + 8.9‰, in March 2010, to - 6.3‰, in February 2011) in the more humid months than in the dry season (- 6.4‰, in November 2010, to - 29.1‰, in October 2011). The δ³⁴S_{SO₄} ranged between + 7.1 and + 20.0‰, and δ¹⁸O_{SO₄} fluctuated between + 5.5 and + 14.4‰. The δ¹³C_{DIC} data varied from - 14.0 to - 26.7‰. The lowest isotopic water values (δ¹⁸O_{water},

Table 1 Physicochemical and isotopic data measured on water samples collected from El Cristo lake (LCR) and its only surface inlet (Creek)

| Sample | T°C | pH | Ca ²⁺ (mg L ⁻¹) | Mg ²⁺ (mg L ⁻¹) | Na ⁺ (mg L ⁻¹) | K ⁺ (mg L ⁻¹) | HCO ₃ ⁻ (mg L ⁻¹) | Cl ⁻ (mg L ⁻¹) | SO ₄ ²⁻ (mg L ⁻¹) | EC (μS cm ⁻¹) | TDS (mg L ⁻¹) | δ ¹⁸ O _{water} (‰ VSMOW) | δD _{water} (‰ VSMOW) | d-excess | δ ³⁴ S _{sulfate} (‰ CDT) | δ ¹⁸ O _{sulfate} (‰ VSMOW) | δ ¹³ C _{DIC} (‰ PDB) |
|--------|---------|--------|---|---|--|---|--|--|--|------------------------------|------------------------------|---|----------------------------------|----------|---|---|---|
| Creek | mar-10 | 10.5 | 6.0 | 4.3 | 3.6 | 0.3 | 18.3 | 8 | 21 | 64.1 | 42.9 | - 6.5 | - 47.1 | 4.9 | 8.1 | 5.5 | - 26.4 |
| Creek | feb-11 | 4.3 | 6.3 | 4.5 | 3.6 | 0.3 | 21.0 | 8 | 20 | 35.3 | 23.7 | - 7.6 | - 51.9 | 8.9 | 9.3 | 7.1 | - 26.7 |
| Winter | LCR-1 | feb-11 | 4.2 | 6.8 | 14.9 | 5.7 | 10.1 | 1.9 | 23 | 88.8 | 59.5 | - 3.0 | - 30.3 | - 6.3 | 16.8 | 14.4 | - 21.1 |
| LCR-2 | feb-11 | 4.1 | 6.8 | 15.0 | 5.4 | 10.0 | 1.9 | 23 | 23 | 81.5 | 54.6 | - 3.5 | - 31.1 | - 3.1 | 16.6 | 13.8 | - 20.9 |
| Spring | LCR-1 | mar-10 | 10.1 | 5.9 | 15.2 | 5.0 | 8.0 | 3.0 | 48 | 147.4 | 98.8 | - 6.3 | - 45.4 | 5.0 | 7.1 | 7.5 | - 25.8 |
| Summer | LCR-1 | jun-10 | 22.2 | 8.0 | 26.3 | 8.7 | 12.4 | 6.6 | 44 | 245.3 | 164.4 | - 1.8 | - 23.1 | - 8.7 | 10.6 | 6.4 | - 21.1 |
| LCR-2 | jun-10 | 24.3 | 7.9 | 26.6 | 8.8 | 12.5 | 6.2 | 39 | 214.2 | 143.5 | - 2.3 | - 22.8 | - 4.4 | 10.4 | 8.7 | - 14.6 | |
| LCR-1 | sept-10 | 21.9 | 8.8 | 26.4 | 11.1 | 16.9 | 6.7 | 30 | 316.0 | 211.7 | 2.3 | - | - | - | 16.0 | 11.3 | - 18.5 |
| LCR-2 | sept-10 | 23.1 | 9.5 | 29.0 | 11.4 | 16.5 | 7.1 | 29 | 317.0 | 212.4 | 3.4 | - | - | - | 15.9 | 9.1 | - 16.2 |
| Autumn | LCR-1 | nov-10 | 13.0 | 9.9 | 24.8 | 10.7 | 18.6 | 5.4 | 24 | 227.0 | 152.1 | 2.5 | 4.5 | - 15.5 | 18.3 | 13.9 | - |
| LCR-2 | nov-10 | 12.3 | 9.9 | 23.6 | 9.6 | 18.2 | 5.2 | 26 | 207.6 | 139.1 | 2.2 | 1.2 | - 16.4 | 18.2 | 13.6 | - | |
| LCR-1 | oct-11 | 17.0 | 8.9 | 18.8 | 10.3 | 21.9 | 0.9 | 35 | 304.0 | 203.7 | 5.2 | 14.4 | - 27.2 | 19.4 | - | - | - 14.2 |
| LCR-2 | oct-11 | 17.1 | 8.7 | 18.7 | 10.8 | 23.3 | 1.0 | 25 | 248.5 | 166.5 | 5.5 | 14.9 | - 29.1 | 20.0 | - | - | - 14.0 |

- No data
d-excess value (d = δD-8δ¹⁸O)

Table 2 Selected minor and trace elements in water samples and comparison with international standards (World Health Organization—WHO, 2011)

| | Sample | Li | Be | B | Ti | V | Cr | Co | Ni | As | Se | Rb | Sr | Cd | Sb | Ba | Hg | Pb | Al | Cu | Fe | Si | Zn |
|----------|---------------|--------------------|----|------|-----|-----|-----|-----|-----|-----|-----|-----|-------|-----|-----|------|-----|-----|----|-----|-----|-----|-----|
| | | μg L ⁻¹ | | | | | | | | | | | | | | | | | | | | | |
| | | mg L ⁻¹ | | | | | | | | | | | | | | | | | | | | | |
| Winter | Creek -mar-10 | 1.1 | * | * | * | 1.2 | 0.7 | * | 0.9 | 0.2 | 0.4 | 1.2 | * | * | * | 36.1 | * | 2.5 | * | * | * | 0.5 | * |
| | Creek-feb- 11 | 1.0 | * | * | * | 1.2 | * | * | 0.4 | 0.2 | * | 0.3 | 22.4 | * | * | 32.5 | * | * | * | * | * | 1.4 | * |
| | LCR-1 feb-11 | 1.5 | * | * | 1.2 | 3.3 | * | * | 1.1 | 1.0 | | 1.3 | 72.0 | * | * | 45.5 | * | * | * | * | 0.1 | 0.2 | * |
| | LCR-2 feb-11 | 1.5 | * | * | 1.5 | 3.6 | * | * | 1.3 | 1.0 | * | 1.4 | 74.5 | * | * | 44.7 | * | * | * | * | 0.1 | 0.3 | * |
| Spring | LCR-1 mar-10 | 2.1 | * | * | * | 1.3 | 0.9 | * | 1.6 | 0.6 | 0.6 | 2.2 | * | * | * | 34.7 | * | 3.9 | * | * | * | 0.6 | * |
| Summer | LCR-1 jun-10 | 2.6 | * | * | 1.8 | 1.6 | 1.9 | 0.5 | 3.9 | 2.2 | 0.8 | 5.0 | * | * | * | * | 1.5 | * | * | 0.2 | * | * | |
| | LCR-2 jun-10 | 2.7 | * | * | * | 1.5 | 0.9 | * | 2.6 | 1.6 | 0.7 | 4.4 | * | * | * | 0.9 | * | 1.2 | * | * | 1.1 | * | * |
| | LCR-1 sep-10 | 2.9 | * | * | * | 1.6 | 1.0 | 0.5 | 2.6 | 3.5 | 1.2 | 6.1 | * | * | * | 1.0 | * | 0.5 | * | * | 0.8 | * | * |
| Autumn | LCR-2 sep-10 | 3.5 | * | * | * | 1.5 | 0.9 | 0.5 | 3.6 | 3.3 | 1.3 | 6.1 | * | * | * | 1.0 | * | 0.4 | * | * | 0.8 | * | * |
| | LCR-1 nov-10 | 2.3 | * | 14.6 | 0.9 | * | * | * | 1.9 | 2.2 | * | 3.3 | 0.2 | * | * | 54.5 | * | * | * | * | 0.3 | * | * |
| | LCR-2 nov-10 | 2.3 | * | 7.2 | * | * | * | * | 2.0 | 2.0 | * | 3.2 | 0.3 | * | * | 42.3 | * | * | * | * | * | * | * |
| | LCR-1 oct-11 | 0.9 | * | 22.1 | * | 1.6 | 2.6 | 0.5 | 1.6 | 2.6 | 0.9 | * | 129.5 | 0.1 | 1.3 | 72.9 | * | 0.6 | * | * | 0.5 | 0.1 | * |
| WHO-2011 | | – | – | 2400 | – | – | 50 | 40 | 70 | 10 | 40 | – | – | 3 | 20 | 700 | 6 | 10 | – | 2.0 | – | – | 3.0 |

* < 0.1 μg L⁻¹; – no data

Table 3 Carbon isotope composition of vegetation in El Cristo

| | Species of plants | Date | δ ¹³ C (‰PDB) |
|--------------------------------|------------------------------------|----------------|--------------------------|
| Submerged/floating macrophytes | <i>Ranunculus sect. batrachium</i> | March 2010 | – 18.3 |
| | | September 2010 | – 20.7 |
| | <i>Myriophyllum</i> | June 2010 | – 18.7 |
| | | September 2010 | – 21.8 |
| Emergent macrophytes | <i>Potamogeton</i> | September 2010 | – 26.7 |
| | <i>Baldellia ranunculoides</i> | March 2010 | – 27.1 |
| | | June 2010 | – 24.1 |
| Herbs | | September 2010 | – 25.1 |
| | <i>Scirpus lacustris</i> | June 2010 | – 25.5 |
| | <i>Glyceria</i> | March 2010 | – 28.7 |
| | <i>Juncaceae</i> | June 2010 | – 30.3 |
| | | September 2010 | – 28.2 |

δD_{water}, δ¹⁸O_{SO₄=}, δ³⁴S_{SO₄=} and δ¹³C_{DIC}) were detected in springtime (March 2010), whereas the highest values were detected in the mid-autumn (November 2010 and October 2011).

The weighted stable isotope composition of precipitation at Laguna del Cristo region (OIPC, <http://www.waterisotopes.org>) ranges from – 7.7 to – 4.2‰, with a mean value of – 6.3‰ (n = 12) for δ¹⁸O and from – 48 to – 25‰, with a mean value of – 39‰ (n = 12) for δD. As expected, the δ¹⁸O and δD values for the precipitation in the period May–October are higher than corresponding values in the wet (colder) season.

δ¹³C_{plant} ranged from – 30.3 to – 18.3‰ (Table 3) and were higher in submerged and floating macrophytes

Table 4 δ¹³C and δ¹⁸O of gastropod shells, and δ¹⁸O_{water} and δ¹³C_{DIC} of lake water at the time they were sampled

| Species | Date | δ ¹³ C _{Shell} (‰PDB) | δ ¹⁸ O _{Shell} (‰PDB) | δ ¹⁸ O _{water} (‰VSMOW) | δ ¹³ C _{DIC} (‰PDB) |
|-----------------|---------|---|---|---|---|
| <i>Galba</i> | Jun-10 | – 8.6 | 5.6 | – 2.1 | – 21.1 |
| <i>Gyraulus</i> | Jun-10 | – 10.5 | 4.0 | – 2.1 | – 21.1 |
| <i>Galba</i> | Sept-10 | – 10.1 | 3.1 | 2.9 | – 18.5 |
| <i>Galba</i> | Oct-11 | – 0.2 | 12.7 | 5.4 | – 14.2 |

(*Myriophyllum* and *Ranunculus sect. batrachium*; – 21.8 to – 18.7‰ and – 20.7 to – 18.3‰, respectively) than on emergent macrophytes (*Baldellia ranunculoides*, δ¹³C = – 27.1 to – 24.1‰; *Scirpus*

Table 5 TOC and TN of sedimentary organic matter, and $\delta^{13}\text{C}_{\text{Org}}$. Location of cores in Fig. 2b

| Core ID | Sample ID | TN Atom % | TOC Atom % | C/N | $\delta^{13}\text{C}_{\text{Org}}$ ‰ versus PDB |
|---------|-----------|--------------|---------------|------|---|
| I | LCR1A-t | 0.5 | 4.5 | 10.9 | – |
| | LCR1A-b | 0.2 | 1.3 | 10.1 | – |
| II | LCR1B-t | 3.1 | 25.0 | 9.3 | – 25.0 |
| | LCR1B-b | 0.2 | 1.9 | 9.5 | – 28.6 |
| III | LCR1C-t | 1.3 | 10.9 | 10.1 | – |
| | LCR1C-b | 0.3 | 2.8 | 1.9 | – |
| IV | LCR2A-t | 1.1 | 8.8 | 9.3 | – 20.6 |
| | LCR2A-b1 | 1.0 | 8.2 | 9.7 | – 29.9 |
| | LCR2A-b2 | 0.4 | 2.2 | 7.4 | – 30.0 |
| V | LCR2B-t | 2.4 | 18.2 | 9.0 | – |
| | LCR2B-b | 0.6 | 5.1 | 9.3 | – |

– No data

lacustris, $\delta^{13}\text{C} = -25.5\text{‰}$), except submerged *Potamogeton* ($\delta^{13}\text{C} = -26.7\text{‰}$). All $\delta^{13}\text{C}_{\text{plant}}$ values on land plants (grasses) were within the range expected for the C3 photosynthetic pathway (*Glyceria*, $\delta^{13}\text{C} = -28.7\text{‰}$; Juncaceae, $\delta^{13}\text{C} = -28.2$ to -30.3‰). $\delta^{13}\text{C}_{\text{plant}}$ values of submerged, and floating macrophytes were 3‰ lower in

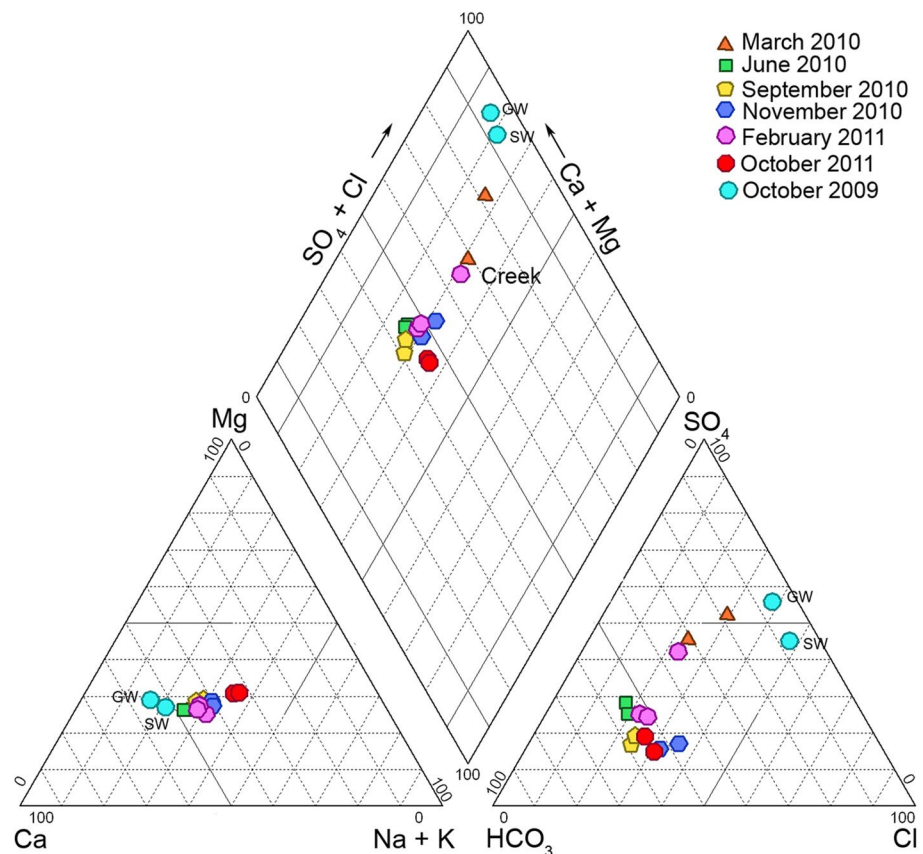
September than in March or June 2010, whereas emergent macrophytes and land plants were 2‰ higher in September than in March and June 2010.

Gastropod shells of *Galba* and *Gyraulus* (Table 4) are composed of aragonite. $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of *Galba* collected in October 2011 ($-0.2 / +12.7\text{‰}$, respectively) were higher than the same species collected in June 2010 ($-8.6 / +5.6\text{‰}$) and in September 2010 ($-10.1 / +3.1\text{‰}$). Carbon and oxygen isotope values of *Gyraulus* ($-10.5 / +4.0\text{‰}$), only sampled in June 2010, are lower than those of *Galba* collected at the same time.

Surface lacustrine sediments

Contents of TOC and TN in surface sediments (20-cm-long core; Table 5) were highly variable and decreased with depth. Higher TOC (25.0–4.5%) and TN (3.1–0.5%) contents and higher C/N ratios (10.9–9.0) were found at the top of the cores (to 5 cm depth) and decreased towards the bottom (10 and 15 cm in depth) of the cores (TOC=5.1–1.3%; TN=0.6–0.2%; C/N=10.1–7.4). $\delta^{13}\text{C}_{\text{org}}$ values range from -20.6 to -30.0‰ . Lower values (-30.0 to -28.6‰) were measured at the bottom of the cores, increasing higher upward (-20.6 to -25.0‰).

Fig. 3 Piper diagram showing the chemical composition of the lake and inlet creek water samples. Surface (SF) and groundwater (GW) samples during 2009 were also included for comparison (Jambrina et al. 2013)



Discussion

Hydrochemical characteristics

El Cristo lake waters (Table 1; Fig. 3) are diluted ($<5 \text{ meq L}^{-1}$) based on the concentration of major ions (Cl–HCO₃–SO₄). HCO₃[–] is the main anion, followed by Cl[–] and SO₄^{2–}. Ca²⁺ is the most abundant cation, followed by Na⁺ and Mg²⁺. K⁺ was the scarcest cation. Cations increased as lake level went down, and the Na⁺ became the most abundant of them during the dry season (October 2011). Mineralization is lower during the wet stage (spring and winter; 1.9 meq L^{-1}) than in the dry season (summer and autumn; from 3.2 to 3.7 meq L^{-1}). Total dissolved solids (TDS = 139 – 212 mg L^{-1}), electric conductivity (EC = 208 – $317 \text{ } \mu\text{S cm}^{-1}$), and pH (8–10) values were higher in summer and mid-autumn, and lower in winter and spring (TDS = 55 – 99 mg L^{-1} , EC = 82 – $147 \text{ } \mu\text{S cm}^{-1}$, pH = 6–7) (Table 1), due to increased temperature and evaporation during the dry season. Higher pH values are associated with the periods of greater photosynthetic activity (Wurts and Durborow 1992). Creek waters (wet season only) showed the lowest ionic contents (1 meq L^{-1}).

Water composition is calcium–(sodium–magnesium)–bicarbonate type [Ca–(Na⁺–Mg²⁺)–HCO₃] that matches well with the dominance of shales, sandstones, and quartzites in the source areas. The lower amount of salts is correlated with low values of EC (Table 1; March 2010 to October 2011; $n = 13$; Fig. 3). It is evident a positive correlation of alkalinity with pH; when pH is > 8 , most DIC is as HCO₃[–] and very little as CO₃^{2–}. Furthermore, alkalinity can change by processes that yield or consume H⁺ and OH[–] (Stumm and Morgan 1996). In this lake, the alkalinity increase may be due to the photosynthetic activity of algae and sulphate reduction. The decrease in concentration of Ca²⁺ and SO₄^{2–} in early autumn is due to both gypsum precipitation because of intense evaporation and due to biogeochemical processes at/below the sediment–water interface, such as the decomposition of OM associated with bacterial sulphate reduction.

Chemical composition of creek and lake water samples in spring 2010 showed more sulphates than any other major anions (SO₄ \geq HCO₃). This could be due to solubilization of minerals from the substrate via groundwater flows and resolubilization of salts precipitated during the dry season. Although there are no sulphate minerals in the substratum, during episodes of intense drought, such as recorded in October 2009, the lake dried up, and a gypsum crust precipitated on the exposed floor (Jambrina et al. 2013).

Regarding minor and trace element concentrations, the mean contents in summer and mid-autumn were in the

order Fe $>$ Si, while in spring and winter the trend was different: Si $>$ Fe (Table 2). Weathering (hydrolysis) of aluminium silicate minerals in rocks and biological processes (photosynthesis, remineralization) of diatoms, siliceous algae, and siliceous flagellates contribute to supply and remove dissolved silica from the water. Iron variations are related to the redox conditions and precipitation processes from the oxic zone into the sediment and the release of Fe under anoxic conditions (Taillefert and Gaillard 2002). Average concentrations of selected metals were compared with water quality guidelines set by international authorities. The maximum concentrations measured for Cd, Co, Cr, Ni, and Pb were below the maximum permitted concentrations established by the WHO (2011) (Table 2). This is not surprising given the fact that there are no industries or other anthropogenic sources of metals, such as fertilizers and road run-off, which could impact the waters. Fe concentrations were higher in summer (0.8 – 1.1 mg L^{-1}), but still at the low end permitted by the WHO (from 0.5 to 50 mg L^{-1} ; WHO 2011). Persistent organic pollutants (POP: PAHs, pesticides, and insecticides) recorded at El Cristo lake are also negligible (Hijosa-Valsero et al. 2016). B, Sr, and Ba show a significant increase during the final summer dryness of 2011.

Seasonal isotopic variations of lake water ($\delta^{18}\text{O}_{\text{water}}$ and $\delta\text{D}_{\text{water}}$)

A Local Meteoric Water Line (LMWL; $\delta\text{D} = 6.18\delta^{18}\text{O} - 2.27$; $r^2 = 0.97$; $n = 12$; Fig. 4a) was determined by linear regression of the $\delta^{18}\text{O}$ and δD data in precipitation (OIPC, <http://www.waterisotopes.org>). Its slope and d-excess are lower than those of the Global Meteoric Water Line (GMWL; $\delta\text{D} = 8.13\delta^{18}\text{O} + 10.8$). Overall, lake waters plot off and to the right of both the LMWL and GMWL, on a Local Evaporative Line (LEL) with a slope of 5.29 ($r^2 = 0.99$) and intersect the GMWL at $\delta^{18}\text{O} = -12.29\text{‰}$ (Fig. 4a), suggesting that lake waters are significantly affected by evaporation (Kendall and Doctor 2003). The most positive values of $\delta^{18}\text{O}$ and δD (extreme right of the LEL) correspond to samples taken during the drought of November 2010 (monthly precipitation 18.5 mm , average water temperature $12.7 \text{ }^\circ\text{C}$) and October 2011 (precipitation of 23.7 mm , average water temperature $17.0 \text{ }^\circ\text{C}$) (Table 1; Fig. 4a). Creek waters, at the origin of the LEL, have values that are not very different from those of rainfall at the time, and therefore plot close to LMWL and GMWL (Fig. 4a), indicating that lake water results from fractionated (enriched) creek and precipitation water (Fig. 4a). Winter 2011 was dry (total monthly precipitation in February = 21.4 mm ; water temperature of $4.2 \text{ }^\circ\text{C}$), and the lake water was inherited from the previous summer and autumn, enriched in the heavy isotopes.

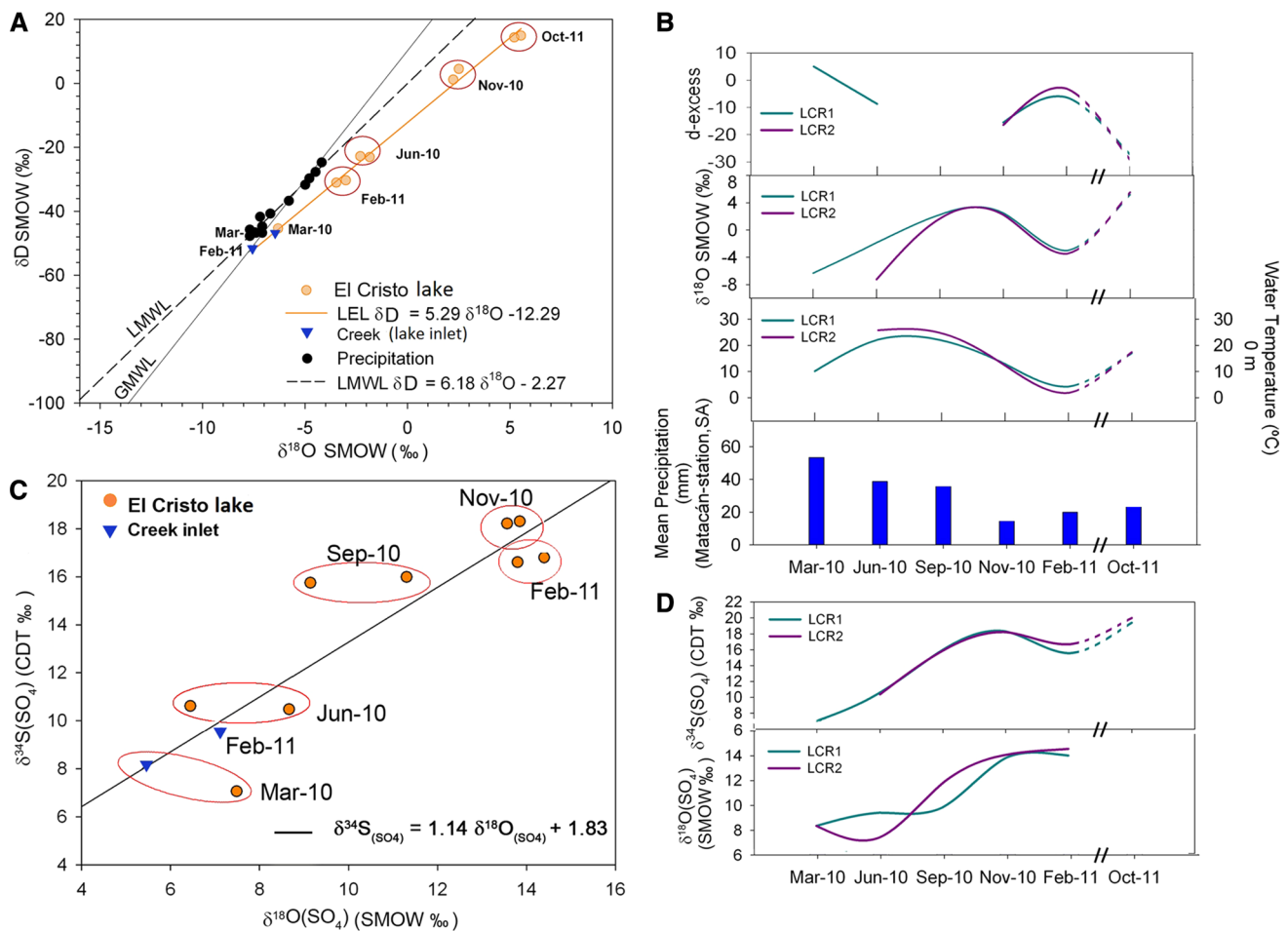


Fig. 4 a Plot of $\delta^{18}\text{O}$ versus δD values from lake and creek water. Global Meteoric Water Line (GMWL, Craig 1961) for reference. Sampled waters plot on a Local Evaporation Line (LEL). The Local Meteoric Water Line (LMWL) is determined from a linear regression of average precipitation values using the online isotopes in precipitation calculator (OIPC, <http://www.waterisotopes.org>). b Seasonal

variation in $\delta^{18}\text{O}$ and d-excess of lake water (LCR) and their comparison with water temperature (this study) and monthly total precipitation (reference precipitation data from the Matacán-Salamanca weather station, <http://www.aemet.es>). c The relationship between the $\delta^{18}\text{O}$ and $\delta^{34}\text{S}$ values of dissolved sulphate. d Seasonal variation in $\delta^{34}\text{S}_{\text{SO}_4=}$ and $\delta^{18}\text{O}_{\text{SO}_4=}$

Deuterium excess ($\text{d-excess} = \delta\text{D} - 8\delta^{18}\text{O}$, Dansgaard 1964) can provide information about seasonality or moisture source of precipitation by reference to the GMWL which has d-excess of +10‰ (Froehlich et al. 2002). At El Cristo lake area, winter d-excess ranges from 10 to 16 ($\delta^{18}\text{O}$ and δD data from <http://www.waterisotope.org>), which indicates moisture derived from air masses of Atlantic origin (Celle-jeanton et al. 2001). Lower d-excess values in summer precipitation (from 8 to 9) are expected according the model of Froehlich et al. (2002). Generally, precipitation has seasonal cyclicality, with d-excess values higher for winter than for summer precipitation (Froehlich et al. 2002). Indeed, d-excess is often considered as an indicator of evaporation conditions (Merlivat and Jouzel 1979). Deuterium excess and $\delta^{18}\text{O}$ values of lake and creek samples show a strong positive

correlation ($r^2 = 0.98$, $n = 12$; figure not shown). These samples have relatively higher d-excess in spring and winter (+5 to +9‰) suggesting the contribution of direct precipitation from Atlantic weather fronts (Celle-jeanton et al. 2001) that results in surface run-off and groundwater table rise (Fig. 4b). In summer and autumn, d-excess values are lower (−29 to −4‰) as a result of direct evaporation and by the contribution of falling raindrops evaporated in a warm atmosphere with low relative humidity (Araguás-Araguás et al. 1995) (Fig. 4b). However, in February 2011 (colder dry winter month), the d-excess was lower in the lake (average −5‰) than in the creek (d-excess = +9‰) (Table 1). The low d-excess value is likely attributable to the longer residence time of lake water, in contrast with the ephemeral nature of surface run-off.

Seasonal isotopic variations of water-dissolved sulphate ($\delta^{34}\text{S}_{\text{sulphate}}$ and $\delta^{18}\text{O}_{\text{sulphate}}$)

$\delta^{34}\text{S}_{\text{SO}_4=}$ and $\delta^{18}\text{O}_{\text{SO}_4=}$ values are positively correlated ($r^2 = 0.90$, $n = 11$) (Fig. 4c). Along time, $\delta^{34}\text{S}$ of water-soluble sulphates follows a sinusoidal trend, with lower values during the wet season (spring, + 7‰) and higher ones during the dry season (early autumn, +20‰) (Fig. 4d). The sulphate concentration in the lake water also shows seasonal variations, with lower values ($\approx 20\text{--}25 \text{ mg L}^{-1}$) in winter and autumn and higher ones ($\approx 40 \text{ mg L}^{-1}$) in spring and early summer. Potential sulphur sources are rainfall, dissolution of sulphate minerals, weathering of sulphides, mineralization of organic sulphur in soils, industrial and agricultural drainage, and so on (Krouse 1980; Krouse and Grinenko 1992). The lake sits on siliciclastic Miocene deposits devoid of sulphate minerals. The shale-rich rocks of the watershed (Lower Paleozoic metasedimentary rocks of Peña de Francia Range; Fig. 1c) include common sulphides averaging $\delta^{34}\text{S} = +9\text{‰}$ that overlap or are more depleted in ^{34}S than the dissolved sulphate in the lake. Oxidation of sulphides from Lower Paleozoic rocks has been proposed as the main source of dissolved sulphates (Jambrina et al. 2013), but sulphide oxidation should not result in significant isotopic fractionation. The observed seasonal isotopic variation, therefore, suggests that some additional mechanism is at work to change $\delta^{34}\text{S}_{\text{SO}_4=}$ over the year. Both $\delta^{34}\text{S}_{\text{SO}_4=}$ and $\delta^{18}\text{O}_{\text{SO}_4=}$ values are negatively correlated with sulphate concentrations ($r^2 = 0.72$, $n = 11$ and $r^2 = 0.84$, $n = 9$, respectively). Higher $\delta^{34}\text{S}_{\text{SO}_4=}$ values (ranging from + 18 to + 20‰) occur in autumn (November 2010 and October 2011) and are similar to the ones from seawater spray ($\delta^{34}\text{S} \sim +20\text{‰}$; Hoefs 2004), suggesting that marine sulphate may play a role as a potential sulphur source: the nearest coast is just over 200 km away from El Cristo lake in the prevailing wind direction (westerlies to northwesterlies). However, $\delta^{18}\text{O}$ values $\sim +14\text{‰}$ are higher than those of seawater spray ($\delta^{18}\text{O} \sim +9.4\text{‰}$, Hoefs 2004), suggesting that evaporation instead is the main mechanism for ^{18}O enrichment (Fig. 4c). On the other hand, an increase in $\delta^{34}\text{S}$ values could result from bacterial sulphate reduction processes, with production of HS^- plus isotopically heavy residual sulphate. Sulphides have been not detected in the lake floor sediments (which is not surprising, given the maximum 1.8 m depth of the lake: by product H_2S can easily escape to atmosphere; Hoefs 2004), but gypsum was identified as a trace mineral in the floor sediments (Jambrina et al. 2013). Presence of organic matter in the floor sediments allows bacterial sulphate reduction processes. In fact, in the driest years, a gypsum crust covers the exposed floor of the lake, as it occurred in October 2009 (Jambrina et al. 2013). Summer-dissolved sulphates (June and September 2010) are less enriched in ^{34}S ($\delta^{34}\text{S}$ value ranging from + 10 to + 16‰) and ^{18}O ($\delta^{18}\text{O}$

value ranging from + 6.4 to + 11.3‰), consistent with a combination of sulphate reduction and evaporative concentration mechanisms (Fig. 4d) that advances towards the end of the dry period. There is a close relationship between sulphate reduction and carbonate balance, as sulphate reduction results in the formation of CO_2 , which produces an increase in the concentration of HCO_3^- in the water (Table 1) and subsequent increase of alkalinity. The low $\delta^{34}\text{S}_{\text{SO}_4=}$ values in spring ($\delta^{34}\text{S} = + 7$ to + 8‰, March 2010) are due to the contribution of relatively lighter S from freshly weathered sulphides in the watershed rocks, as suggested by the similar $\delta^{34}\text{S}$ values of country rock sulphides and dissolved sulphate (Fig. 4d): sulphide oxidation to sulphate produces negligible fractionation (Krouse and Mayer 2000). It is worth noting in this regard that February 2011 $\delta^{34}\text{S}_{\text{SO}_4=}$ values in the lake were 7‰ higher than those of the creek. It has already been stated that winter 2011 was abnormally dry, so even when the tributary creek was flowing into the lake at the time of sampling, overall water balance was still dominated by resident, inherited lake water, enriched in the heavy isotopes because of the processed discussed above (Figs. 2; 4c), in contrast with creek water, carrying freshly produced sulphate by sulphide oxidation in the watershed. With regard to the variation of $\delta^{18}\text{O}_{\text{SO}_4=}$, the systematics of cause and effect are more complicated and not obvious in many cases (Clark and Fritz 1997; Kendall and Doctor 2003). During sulphide oxidation, sulphate-O derives from variable contributions of atmospheric oxygen and oxygen in water molecules (Clark and Fritz 1997 and references therein) and its $\delta^{18}\text{O}_{\text{SO}_4=}$ depends on the actual oxidation path. According to the cited authors, values of $\delta^{18}\text{O}_{\text{SO}_4=}$ show a strong contribution from atmospheric O_2 (after the equation of Van Everdingen and Krouse 1985, which propose that the proportion of the two oxygen sources varies according to the enzymes implicated in the biological reactions). If the extreme values of $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ for precipitation are taken into account ($- 3$ and $- 7\text{‰}$ for summer and winter average values), the proportion of atmospheric O_2 approximately ranges from 90% for summer to 55% for winter.

Seasonal variations in the stable carbon isotope composition of DIC

The isotopic signature of DIC is controlled by several biogeochemical processes, including dissolution of carbonates, photosynthesis and respiration of phytoplankton, OM oxidation, and exchange with atmospheric CO_2 (Hoefs 2004). During the warm season (summer and mid-autumn), the photosynthetic activity of aquatic plants, the evaporation of water, and more CO_2 degassing, particularly at early autumn when temperatures are high and the water level is lower, leads to more positive $\delta^{13}\text{C}_{\text{DIC}}$ values ($- 20$ to $- 14\text{‰}$) and higher pH (pH = 9), as compared

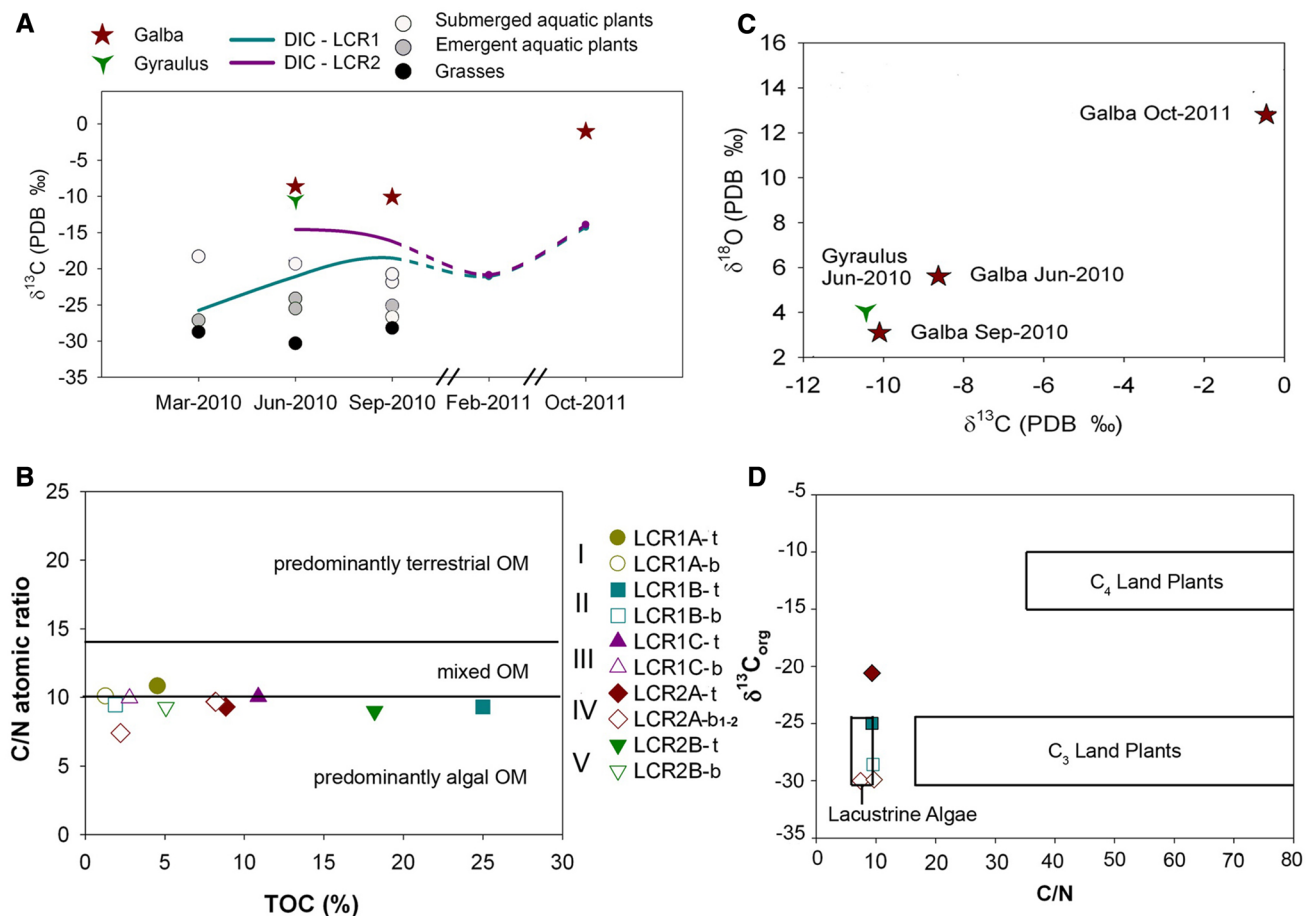


Fig. 5 **a** Seasonal variation of $\delta^{13}\text{C}_{\text{DIC}}$ and $\delta^{13}\text{C}$ values for the aquatic and land plants and modern gastropods (*Gyraulus* and *Galba*). **b** TOC versus C/N ratios for sedimentary organic matter. **c** $\delta^{18}\text{O}$ – $\delta^{13}\text{C}$ cross-

plot for *Gyraulus* and *Galba* data. **d** A cross-plot of C/N ratios versus $\delta^{13}\text{C}_{\text{org}}$ values indicates that the organic matter in the sediment is predominantly of lacustrine algal origin

with the wet season ($\delta^{13}\text{C}_{\text{DIC}} = -26$ to -21‰ ; $\text{pH} \sim 6\text{--}7$) (Fig. 5a). Most submerged freshwater macrophytes and algae obtain their carbon from the DIC species ($\text{CO}_{2(\text{aq})}$, HCO_3^- , depending on pH). At neutral pH and high $\text{CO}_{2(\text{aq})}$ levels, plants mostly use CO_2 for photosynthesis, whereas at pH above 8, relatively common in shallow productive lakes, HCO_3^- , whose $\delta^{13}\text{C}$ will be 7–12‰ higher than that of dissolved CO_2 , can be an additional carbon source for photosynthesis (Mook et al. 1974; Romanek et al. 1992). Moreover, the differential assimilation of one or the other C form is also influenced by species-specific variations in the efficiency of HCO_3^- transport into the leaf (Herzschuh et al. 2010). Measured $\delta^{13}\text{C}$ values (Table 1) suggest that the major external source of carbon contributing to DIC is CO_2 derived from the decay of organic matter, either transported from the watershed or autochthonous to the lake. The lower $\delta^{13}\text{C}_{\text{DIC}}$ in spring and winter is explained by the contribution of isotopically light C during the oxidation of organic matter and more allochthonous soil-derived CO_2 (Fig. 5a).

Stable carbon isotope in modern plants and bulk sedimentary organic matter

Terrestrial plants are usually classified into two main types (C3 and C4) according to their photosynthetic pathways. C3 plants have $\delta^{13}\text{C}$ values ranging from -34.0 to -22.0‰ , while the $\delta^{13}\text{C}$ values of C4 plants range from -14.0 to -10.0‰ (Deines 1980; O’Leary 1988). The $\delta^{13}\text{C}_{\text{plant}}$ values of terrestrial vegetation (grasses) in El Cristo lake area confirmed the C3 photosynthetic pathway (Table 3). Within the lake itself, emergent macrophytes largely utilize $\text{CO}_{2(\text{atm})}$ with $\delta^{13}\text{C} \approx -7.4\text{‰}$, that is significantly fractionated during photosynthetic uptake, leading to low $\delta^{13}\text{C}_{\text{plant}}$ values between -24 and -27‰ . In contrast, measured values for submerged aquatic plants show higher $\delta^{13}\text{C}_{\text{plant}}$ (-18 to -22‰ ; except *Potamogeton*: -27‰) (Fig. 5a). The availability of dissolved CO_2 controls the carbon isotopic composition of algal organic matter because isotopic discrimination against ^{13}C increases when pCO_2 is high and decreases when it is low (Fogel and Cifuentes 1993).

Submerged species (*Myriophyllum* and *Ranunculus section batrachium*) were enriched in ^{13}C relative to *Potamogeton* (Table 3). It is known that submerged *Myriophyllum* almost exclusively uses HCO_3^- (Herzschuh et al. 2010, and references therein). The contrasting ^{13}C -depleted carbon signature of *Potamogeton* is not unusual when HCO_3^- supply is plenty (see Herzschuh et al. 2010). Also, both physiological adaptations (*Potamogeton* acidifies the periplasmic space of the lower leaf side, converting HCO_3^- into CO_2 , that then diffuses into the chloroplast; Staal et al. 1989) and the uptake of airborne CO_2 when its leaves broke the water temporarily (Keough et al. 1998) result in low $\delta^{13}\text{C}_{\text{plant}}$ values. Submerged macrophytes collected in spring and early summer are higher ($\delta^{13}\text{C}_{\text{plant}} = -18\text{‰}$) than in late summer ($\delta^{13}\text{C}_{\text{plants}} = -22\text{‰}$), with an opposite $\delta^{13}\text{C}_{\text{DIC}}$ trend (Fig. 5a). The organic carbon isotopic composition of submerged macrophytes was not only controlled by the carbon isotopes of DIC, but also by other parameters such as temperature, humidity, precipitation and the physiology of the plant, that can affect $\delta^{13}\text{C}$ (Farquhar et al. 1989). Furthermore, the proportion of CO_2 and HCO_3^- consumed by the plant depends on physiological differences in the mechanism of carbon assimilation between species (Keely and Sandquist 1992), as well as on aquatic plant productivity (Herzschuh et al. 2010).

Total organic matter of lacustrine (floor) deposits consists of a mixture of materials from autochthonous and allochthonous sources that have distinct C/N ratios. Whereas algal sources have low C/N ratios, ranging from 4 to 10, C/N ratios in allochthonous, land plants organic matter are higher, and values of 20 or greater are common (Meyers 1994). C/N ratios measured in the lake floor were relatively low, ranging from 7.4 to 10.0, with a high positive correlation between TOC and TN ($r = 0.99$; figure not shown). This suggests a greater proportion of autochthonous material (aquatic vegetation) (Fig. 5b), confirmed by a cross-plot of C/N ratios against $\delta^{13}\text{C}_{\text{org}}$ values (Fig. 5c). $\delta^{13}\text{C}_{\text{org}}$ values (-25.0 to -21‰) occurring at the top of the sediment cores are higher than expected for lacustrine algae (Meyers and Lallier-Vergès 1999) and correlate with intervals characterized by higher TOC in Fig. 5b. Reduced availability of dissolved atmospheric CO_2 during periods of higher productivity (that in a shallow lake like El Cristo coincide with higher water temperature) and in waters where the ratio of HCO_3^- to CO_2 is kept elevated by an alkaline pH (Hassan et al. 1997) drives algae towards incorporation of isotopically heavier carbon and becomes buried in its sediment (Meyers 2003). In contrast, lower $\delta^{13}\text{C}_{\text{org}}$ values (-30 to -29‰) occur further down the cores, with lower TOC content, which suggests decreased lacustrine algal productivity. However, these reductions in TOC content and $\delta^{13}\text{C}_{\text{org}}$ values coincide with the lowest C/N atomic ratios and are not matched by a significant increase in terrestrial plant debris

from the catchment (Fig. 5b). The delivery of large amounts of isotopically light soil DIC ($\delta^{13}\text{C}_{\text{org}} \sim -12\text{‰}$) to a lake has been proposed to explain isotopically light algal organic matter ($\delta^{13}\text{C}_{\text{org}} \sim -32\text{‰}$) (Meyers 2003).

Carbon and oxygen isotope composition of modern gastropod shells

Although there is an obvious positive relationship between $\delta^{13}\text{C}_{\text{shell}}$ and $\delta^{13}\text{C}_{\text{DIC}}$ ($r = 0.89$, figure not shown), it is difficult to argue its statistical significance, since correlation is obviously influenced by one single outlier. In any case, measured $\delta^{13}\text{C}_{\text{shell}}$ is always markedly higher than $\delta^{13}\text{C}_{\text{DIC}}$ at the time of sampling, therefore ruling out equilibrium relationships.

McConnaughey et al. (1997) stated that fully aquatic, gill-breathing snails produced shells with $\delta^{13}\text{C}$ values similar to DIC, whereas lung-breathing aquatic snails and land snails produced shells containing significant amounts of respired carbon, thus implying a possible physiologic control on the incorporation of metabolic carbon. Both *Galba* and *Gyraulus* are pulmonated snails, which can explain the variable relationship with DIC. The $\delta^{13}\text{C}$ contents of shells are probably influenced by a change in the balance between intake of the organic carbon in the diet and DIC consumption (Leng et al. 1999). The influence of vital effects, as have been documented in some recent lakes (Anadón et al. 2010; Shanahan et al. 2005), cannot be ruled out.

Similarly, it cannot be sustained that shells precipitated their oxygen in equilibrium with ambient water. The 8–10‰ difference in $\delta^{18}\text{O}$ values of *Galba* between samples collected in autumn 2011 and in summer 2010 cannot be accounted for by differences in $\delta^{18}\text{O}_{\text{water}}$, water temperature, or both. Taking June 2010 as a reference ($\delta^{18}\text{O}_{\text{water}} \sim -2\text{‰}$), water was $\sim 5\text{‰}$ higher in September and $\sim 7\text{‰}$ in October. Water temperature was 5 °C lower in autumn 2011 than in summer 2010. These differences cannot explain the observed changes in $\delta^{18}\text{O}_{\text{shell}}$: a decrease in temperature of about 10 °C can result in $\delta^{18}\text{O}$ differences of only 2 to 3‰ (Friedman and O'Neill 1977), although it is true that, even if not truly statistically significant, $\delta^{18}\text{O}_{\text{shell}}$ augments as $\delta^{18}\text{O}_{\text{water}}$ does.

The results indicate differences in $\delta^{13}\text{C}_{\text{shells}}$ and $\delta^{18}\text{O}_{\text{shells}}$ values between *Gyraulus* and *Galba*. In June, when the water temperatures are higher ($\sim 23\text{ °C}$), $\delta^{13}\text{C}_{\text{DIC}}$ ranges between -15 and -21‰ , and $\delta^{18}\text{O}_{\text{water}}$ values are around -2‰ , and the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of *Galba* were 1.9 and 1.6‰ higher than in *Gyraulus*, respectively, (Table 4). *Galba* specimens ($\delta^{13}\text{C}_{\text{shell}} = -8.6\text{‰}$; $\delta^{18}\text{O}_{\text{shell}} = +5.6\text{‰}$) were collected from the littoral area associated with emergent macrophytes (*Baldellia ranunculoides*; $\delta^{13}\text{C} = -24.1\text{‰}$), whereas *Gyraulus* ($\delta^{13}\text{C}_{\text{shell}} = -10.5\text{‰}$; $\delta^{18}\text{O}_{\text{shell}} = +4.0\text{‰}$) was picked from slightly deeper water dwelling on submerged

macrophytes (*Myriophyllum* $\delta^{13}\text{C} = -18.7\text{‰}$). Higher $\delta^{18}\text{O}$ values occurring on *Galba* are likely to be related to higher temperature and evaporation effect in the shallow littoral area and higher $\delta^{13}\text{C}$ with little input of recycled organic carbon into the water. Different isotopic ratios for Holocene *Gyraulus* and *Valvata* have been explained by differences in $\delta^{18}\text{O}_{\text{water}}$ and $\delta^{13}\text{C}_{\text{DIC}}$ between microhabitats within a lake (Jones et al. 2002). Moreover, substantial intraspecies and within-shell variation in the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values of shell aragonite have been described, even within the same environment (Shanaham et al. 2005).

At El Cristo lake, however, data rather hint at a relationship between isotopic values on the gastropod and those of its diet, with higher $\delta^{13}\text{C}_{\text{shell}}$ values for gastropods collected on plants with higher $\delta^{13}\text{C}_{\text{plant}}$.

Conclusions

This study underlines the application of stable isotope measurements to identify the hydrogeochemical changes and biogeochemical dynamics at El Cristo lake, a very sensitive system to seasonal fluctuations within the Mediterranean conditions of the NW Iberian Peninsula.

- (1) Lake water hydrochemistry is very sensitive to water level changes induced by direct precipitation and evaporation, but also to groundwater level oscillations within the surficial aquifer of the Tertiary detritic and Quaternary “raña” deposits. Lake water is of the bicarbonate type. However, sulphates are the major anion in spring due to solubilization of minerals from the substrate via groundwater flows and resolubilization of salts precipitated during the dry season.
- (2) Based on $\delta^{18}\text{O}$ and δD of lake water, and calculated d-excess values, we conclude that El Cristo lake is recharged by meteoric water, which is very quickly subjected to substantial evaporation. The correlation between $\delta^{18}\text{O}$ and δD in lake water defines an evaporation line (LEL: $\delta\text{D}=5.29\delta^{18}\text{O}-12.29$), which significantly deviates from the local meteoric water line in precipitation (LMWL: $\delta\text{D}=6.18\delta^{18}\text{O}-2.27$), confirming that evaporative enrichment had a significant impact on the isotopic signature of lake water.
- (3) Evaporation is also a factor in the enrichment of ^{18}O and ^{34}S in water-dissolved sulphate. $\delta^{34}\text{S}_{\text{SO}_4=}$ and $\delta^{18}\text{O}_{\text{SO}_4=}$ allow to assess the relative importance of different water mass mixing and mineralogical changes. Isotopically lighter dissolved sulphate derived from weathering (oxidation) of sulphides in the catchment area (Peña de Francia Range) is delivered to the lake via groundwater inflow; subsequent enrichment in ^{34}S and ^{18}O of this dissolved sulphate within the lake is mainly related with bacterial sulphate reduction just below the water–sediment interface, and evaporative mechanisms. At the onset of a new rainy season, resolubilization of salts precipitated during the former dry season could lead to transient higher $\delta^{34}\text{S}_{\text{SO}_4=}$ and $\delta^{18}\text{O}_{\text{SO}_4=}$ values.
- (4) Seasonal variations are also reflected by $\delta^{13}\text{C}_{\text{DIC}}$ values, which are higher in summer than in winter. The isotopic signature of DIC, which is the main form of inorganic C in the lake, is controlled by several biogeochemical processes, such as OM oxidation, photosynthesis, and respiration of aquatic vegetation and phytoplankton, and by temperature-dependent exchange with atmospheric CO_2 . The major source of carbon contributing to DIC is isotopically light CO_2 derived from the decay of organic matter in the soils of the catchment, delivered to the lake by surface run-off or via groundwater. Subsequent enrichment in $^{13}\text{C}_{\text{DIC}}$ is related with the preferential assimilation of ^{12}C during photosynthesis at periods of high primary productivity and relatively low water level.
- (5) The low $\delta^{13}\text{C}_{\text{plant}}$ values of modern terrestrial and emergent aquatic plants confirm the C3 photosynthetic pathway. Enrichment in ^{13}C in submerged aquatic *Myriophyllum* and *Ranunculus section batrachium* indicates that HCO_3^- is the main carbon source for photosynthesis. The depletion in ^{13}C in submerged *Potamogeton* is consistent with its physiology, and the opposite trend between $\delta^{13}\text{C}_{\text{DIC}}$ and $\delta^{13}\text{C}_{\text{plants}}$ suggests that the organic carbon isotopic composition of aquatic plants was not only controlled by the isotopic composition of DIC, but by plant physiology as well. TOC and $\delta^{13}\text{C}_{\text{org}}$ values and C/N ratios on sedimentary OM indicates a largely autochthonous contribution.
- (6) The carbon isotope composition of modern gastropods is decoupled from $\delta^{13}\text{C}_{\text{DIC}}$, but covary with $\delta^{13}\text{C}_{\text{Plant}}$ of the aquatic vegetation on which they were collected instead. The $\delta^{18}\text{O}$ values of shells are highly variable and can reach values as high as $+14\text{‰}$ due to evaporation. $\delta^{13}\text{C}_{\text{shell}}$ and $\delta^{18}\text{O}_{\text{shell}}$ variations between *Galba* and *Gyraulus* could be explained by differences between microhabitats.
- (7) Results reported provide a starting point for further isotopic research in Mediterranean shallow flow-through lakes nourished by siliciclastic aquifers, as well as a snapshot of modern lake isotope variability to be applied towards paleoenvironmental reconstructions.

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