Food web reliance on allochthonous carbon in two high mountain lakes with contrasting catchments: a stable isotope approach

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Abstract: The carbon isotopic signature (δ^{13}C) of dissolved inorganic carbon and food web components was examined in two high mountain lakes. Río Seco Lake is partially surrounded by alpine meadows and has temporal inlets, whereas La Caldera Lake is located on rocky terrain and does not receive inputs from runoff. We assessed whether these contrasting catchments involve differences in the isotopic signature of the food web components and then in the reliance on terrestrial carbon. The δ^{13}C of dissolved inorganic carbon was not significantly different between lakes and reflected an atmospheric gas exchange origin. Unexpectedly, bulk particulate organic matter showed enriched δ^{13}C values in both lakes, suggesting a terrestrial vegetation influence. Bulk particulate organic matter was exploited mostly by the cladoceran *Daphnia pulicaria*, whereas the copepod *Mixodiaptomus laciniatus* was ^{13}C depleted relative to particulate organic matter, indicating a selective feeding on an isotopically lighter source, likely phytoplankton. The results obtained show that, despite contrasting catchments, the food web of both lakes might be partially supported by terrestrial carbon for which utilization is species specific.

Résumé : Nous avons déterminé les signatures isotopiques (δ^{13}C) du carbone inorganique dissous et des composantes du réseau alimentaire dans deux lacs de haute montagne. Le lac Rio Seco est partiellement entouré de prairies alpines et possède des tributaires temporaires, tandis que le lac La Caldera est situé sur un terrain rocheux et ne reçoit pas d'apport des eaux de ruissellement. Nous avons examiné si ces basins versants dissemblables entraînent des différences dans les signatures isotopiques des composantes du réseau alimentaire, ainsi que dans les dépendances du carbone d'origine terrestre. Le δ^{13}C du carbone inorganique dissous ne diffère pas significativement dans les deux lacs et reflète une origine par échanges gazeux atmosphériques. De façon inattendue, la matière organique particulaire grossière dans les deux lacs montre une enrichissement des valeurs de δ^{13}C, ce qui laisse croire à une influence de la végétation terrestre. La matière organique particulaire grossière est surtout exploitée par le cladocère *Daphnia pulicaria*, alors que le copépode *Mixodiaptomus laciniatus* est appauvri en ^{13}C par rapport à la matière organique particulaire, ce qui indique qu'il se nourrit de façon sélective d'une source moins riche en isotopes, vraisemblablement le phytoplancton. Les résultats obtenus montrent que, malgré la différence des bassins versants, les réseaux trophiques des deux lacs peuvent être alimentés en partie par du carbone d'origine terrestre, dont l'utilisation varie avec les espèces.

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Introduction

The use of carbon stable isotopes has increased significantly during the last two decades as a robust tool for tracing the flux of organic matter through food webs (Fry and Sherr 1984; Peterson and Fry 1987). Since isotopic fractionation of ^{13}C during trophic transfer is small, the isotopic signature of a consumer reflects closely that of its diet, showing a slight enrichment of 0.5‰–1‰ (DeNiro and Epstein 1978; Michener and Schell 1994). Therefore, carbon isotopes are mainly used to identify the different sources of organic matter to the food webs (Meili et al. 1996; Jones et al. 1998). Similarly, the isotopic signature of dissolved inorganic carbon (DIC) can provide useful information on the sources of inorganic carbon to lakes and the mechanisms that control the variations in the DIC pool (Leggett et al. 1999; Jones et al. 2001).

There is growing evidence that allochthonous sources of carbon can partially support plankton lake metabolism (Jones et al. 1998; Karlsson et al. 2003; Pace et al. 2004).

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Table 1. Chemical and biological characteristics of the study lakes.

	pH*	ANC (μequiv.·L^{-1})*	Free CO_2 (μmol·L^{-1})[†]	DIC (μmol·L^{-1})[†]	Chlorophyll a (μg·L^{-1})[‡]	BA (cells·mL^{-1})*
La Caldera Lake						
2001	7.0	254	57.7	311.4	0.8	1.0×10^6
2002	7.6	332	18.9	349.8	0.8	0.8×10^6
Río Seco Lake						
2001	6.7	90	40.8	130.7	1.0	1.9×10^6
2002	7.1	129	23.2	152.0	1.8	1.8×10^6

Note: Values represent the average for the sampling periods of 2001 and 2002. ANC, acid-neutralizing capacity; BA, bacterial abundance.

*Data from Pulido-Villena (2004).

[†]Estimated from pH and ANC (American Public Health Association 1992).

[‡]Data from Morales-Baquero et al. (2005).

The dependence of food webs upon organic matter derived from lake catchment might be expected to be more important in systems receiving significant amounts of external material. By contrast, lakes with negligible inputs of organic matter from their catchments might be expected to depend almost exclusively on organic carbon derived from algae production. Within lakes, the relative contribution of allochthonous sources of organic matter to the food web can change over time, depending on seasonal productivity (Grey et al. 2001).

La Caldera and Río Seco lakes are two high mountain lakes located in the Sierra Nevada Mountains (Spain) at 3050 m above sea level. These lakes have contrasting catchment characteristics associated with the presence of terrestrial vegetation and inlets (Morales-Baquero et al. 1999). La Caldera Lake is located on rocky terrain with no terrestrial vegetation covering its catchment. In addition, it has no inlets and hence does not receive any inputs from stream runoff. Therefore, a high proportion of the organic matter in this lake is expected to have an algal origin. In contrast, Río Seco Lake is located in a catchment covered by alpine meadows and has temporal inlets that drain water from the catchment. Consequently, it is expected that organic matter in this lake will be derived from both autochthonous and terrestrial vegetation sources.

In this study, we determined the carbon isotopic signature (δ^{13}C) of DIC and food web components in these two lakes and assessed if their contrasting catchments (rocky terrain versus alpine meadows) involve differences in the reliance of food webs on allochthonous sources of carbon.

Material and methods

Study site

La Caldera Lake and Río Seco Lake show the typical characteristics of high mountain lakes (Table 1). They are ice covered for 8–9 months every year, they do not stratify, and Secchi depth equals maximum depth. Macrophytes are absent and both lakes have very simple food webs in which zooplankton represent the highest trophic level. Two crustaceans, the calanoid copepod *Mixodiaptomus laciniatus* and the cladoceran *Daphnia pulicaria*, represent almost the total zooplankton biomass in both lakes (Reche et al. 1997; Barea-Arco et al. 2001).

Sampling

The study lakes were sampled weekly during the ice-free periods of 2001 and 2002 for analyzing the δ^{13}C of DIC, particulate organic matter (POM), epilithon, and zooplankton. Samples for DIC and POM from La Caldera Lake (maximum depth $\approx$ 10 m) were collected by pumping water from depths of 9, 7, 5, 3, and 1 m and mixing it in equal parts to produce a single integrated sample. Since Río Seco Lake (maximum depth $\approx$ 3 m) is shallower than La Caldera Lake, samples for DIC and POM from this lake were collected using a column sampler (10 cm in diameter and 1 m in length) at depths of 0 to 1 m. Composite samples of zooplankton from both study lakes were collected with a 40-μm-mesh net carrying out horizontal and vertical transects. Epilithon was scraped from shallow rocks in at least four random sites of the littoral zone. Occasionally, composite samples of the terrestrial vegetation in Río Seco Lake catchment were also collected.

Sample treatment

Samples for δ^{13}C of DIC were obtained by adding in the field a 2-mL subsample of lake water to a sealed glass vial containing ultrapure phosphoric acid. DIC reacts with phosphoric acid to release CO_2, which is then analyzed for isotopic composition (Brand 1996). Samples of POM were obtained by filtering a 1500- to 2000-mL subsample of lake water through precombusted Whatman GF/C glass fiber filters excluding most bacteria. Prior to filtration, water samples were prefiltered through a 40-μm-mesh net to remove zooplankton and were acidified with concentrated hydrochloric acid to pH <2 to remove all inorganic carbon. Composite samples of zooplankton were taken to the laboratory immediately after sampling and stored in distilled water to allow gut evacuation of zooplankters. Crustacean zooplankton were later picked by hand using a fine pipette. Adult individuals of the most abundant species, the cladoceran *D. pulicaria* and the calanoid copepod *M. laciniatus*, were separated. In 2001, *D. pulicaria* in Río Seco Lake was infected by the epibiont chlorophyte *Korshikoviella gracilipes*. Since it was not possible to separate them, the cladoceran and its epibiont were considered as a single sample. However, previous measurements have revealed not significantly different δ^{13}C values between infected (–26.2 ± 0.5, $n = 2$) and uninfected (–24.5 ± 1.6, $n = 2$) samples. The single-species zooplankton samples were disposed of in precombusted porcelain bowls and dried at 60 °C. Epilithon was fil-

Fig. 1. Changes in the $\delta^{13}C$ of DIC in (*a*) La Caldera Lake and (*b*) Río Seco Lake during the ice-free periods of 2001 (■) and 2002 (□). The broken line represents the $\delta^{13}C$ of atmospheric CO_2.
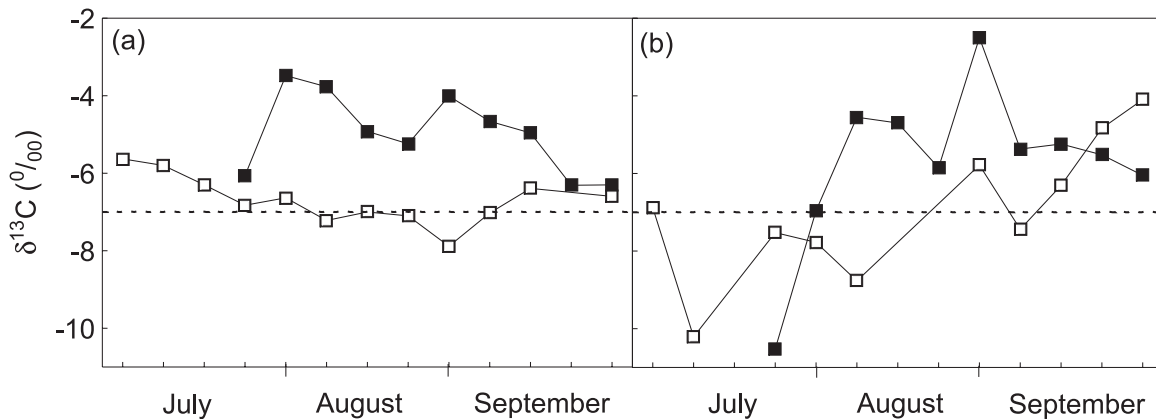
tered through a 40-μm-mesh net to remove aquatic insects and (or) benthic crustaceans. The filtrate was then collected on precombusted Whatman GF/C glass fiber filters after acidifying it to pH <2 with concentrated hydrochloric acid and dried at 60 °C. The samples of terrestrial vegetation from Río Seco Lake catchment were collected in precombusted porcelain bowls after grinding and acidifying them to pH <2.

Carbon isotopic composition analysis

The analysis of carbon stable isotope ratios was conducted by the Stable Isotope Laboratory at the Estación Experimental del Zaidín (CSIC, Granada, Spain). Carbon in biological samples (epilithon, POM, zooplankton, and terrestrial vegetation) was converted to CO_2 using an elemental analyzer based on the Micro-Dumas method (Fison® NA1500 NC). After water removal and chromatographic separation of CO_2, isotopic composition analysis was carried out in a Finnigan® MAT 251 triple-collector gas source mass spectrometer, where $\delta^{13}C$ of CO_2 from DIC samples was also analyzed. In 2001, carbon isotopic composition of replicate samples of zooplankton was also analyzed by the Natural Environmental Research Council (NERC) Stable Isotope Facility (Centre for Ecology and Hydrology, Merlewood, United Kingdom). Samples were combusted using an automated Carlo Erba NA1500 elemental analyzer coupled to a Dennis Leigh Technology® isotope ratio mass spectrometer. NERC data plotted against CSIC data showed a linear relationship (r^2 = 0.86, $p < 0.01$) for which the slope was not significantly different from 1 ($F_{[1,48]} = 0.25$, $p = 0.617$). Zooplankton isotopic composition during 2001 is given as the mean value of both measurements.

The reference materials used were secondary standards of known relation to the international standard for carbon, Pee Dee belemnite, and results are expressed as parts per thousand (‰) deviation from standards (δ notation).

Estimation of $\delta^{13}C$ of phytoplankton

Owing to the difficulty in separating phytoplankton from the other POM, we estimated the $\delta^{13}C$ of phytoplankton from the $\delta^{13}C$ of CO_2 and an assumed fractionation factor (ε_p) of 20‰, which is consistent with that observed in cultures and marine systems (Laws et al. 1995; Bidigare et al.

1997) and reported for other unproductive systems (Rau 1978; Meili et al. 1996). The $\delta^{13}C$ of CO_2 was calculated according to the method of Mook et al. (1974) from the measured $\delta^{13}C$ value of DIC and the concentration of CO_2 calculated from pH and acid-neutralizing capacity (American Public Health Association 1992).

Results

Carbon isotopic signature of DIC

In 2001, the $\delta^{13}C$ of DIC in La Caldera Lake showed the lowest values at the beginning and at the end of the ice-free period and two maxima were observed on 31 July and 28 August. In 2002, the $\delta^{13}C$ of DIC decreased by about 2‰ from the beginning of the ice-free period and, after reaching a minimum value of –7.9‰, increased slightly at the end of the ice-free period (Fig. 1*a*). In Río Seco Lake, in 2001, the minimum value of $\delta^{13}C$ of DIC was observed at the beginning of the ice-free period and, after a sharp increase, remained rather stable at around –5‰. In 2002, the seasonal pattern was characterized by a progressive ^{13}C enrichment of DIC throughout the ice-free period (Fig. 1*b*).

The $\delta^{13}C$ values of DIC in La Caldera Lake were, on average, significantly lower in 2002 than in 2001 ($t = 4.9$, df = 20, $p < 0.001$) (Table 2). The $\delta^{13}C$ values of DIC in Río Seco Lake appeared to be slightly lower in 2002 than in 2001, although the variation was high and there was no significant difference between years ($t = 1.2$, df = 18, $p = 0.231$) (Table 2). The average $\delta^{13}C$ values of DIC were not significantly different between the study lakes in either 2001 ($t = 1.6$, df = 18, $p = 0.139$) or 2002 ($t = 0.5$, df = 20, $p = 0.642$) (Table 2).

Carbon isotopic signature of food web components

Changes in $\delta^{13}C$ of food web components in La Caldera Lake during the ice-free periods of 2001 and 2002 are shown in Fig. 2. During 2001, the $\delta^{13}C$ of the epilithon remained rather constant with values between –12.0‰ and –16.0‰. The $\delta^{13}C$ of POM was around –28.0‰ until the last weeks of the ice-free period when it showed a ^{13}C enrichment of about 5‰. The $\delta^{13}C$ estimates of phytoplankton in La Caldera Lake yielded an average value for 2001 of –32.5‰. The calanoid copepod *M. laciniatus* was more de-

Table 2. $\delta^{13}C$ (‰) of DIC, epilithon, POM, and zooplankton (*Daphnia pulicaria* and *Mixodiaptomus laciniatus*) in La Caldera Lake and Río Seco Lake.

	DIC	Epilithon	POM	*D. pulicaria*	*M. laciniatus*
La Caldera Lake					
2001	−5.0±1.0	−13.9±1.9	−26.1±2.7	−25.1±0.9	−31.3±1.8
2002	−6.7±0.6	−13.1±0.9	−23.0±1.1	−22.2±0.7	−30.3±1.6
Río Seco Lake					
2001	−6.0±1.8	−17.9±2.4	−27.0±3.2	−28.8±1.0	−31.9±1.5
2002	−7.0±1.8	−16.6±2.1	−26.3±0.8	−33.4±1.3	−32.4±1.1

Note: Values represent mean ± SD for the ice-free periods of 2001 and 2002.

Fig. 2. Changes in the $\delta^{13}C$ of POM (●), epilithon (■), and zooplankton (the calanoid copepod *Mixodiaptomus laciniatus* (□) and the cladoceran *Daphnia pulicaria* (○)) in La Caldera Lake during the ice-free periods of (*a*) 2001 and (*b*) 2002. The broken line represents the mean value of estimated $\delta^{13}C$ of phytoplankton.
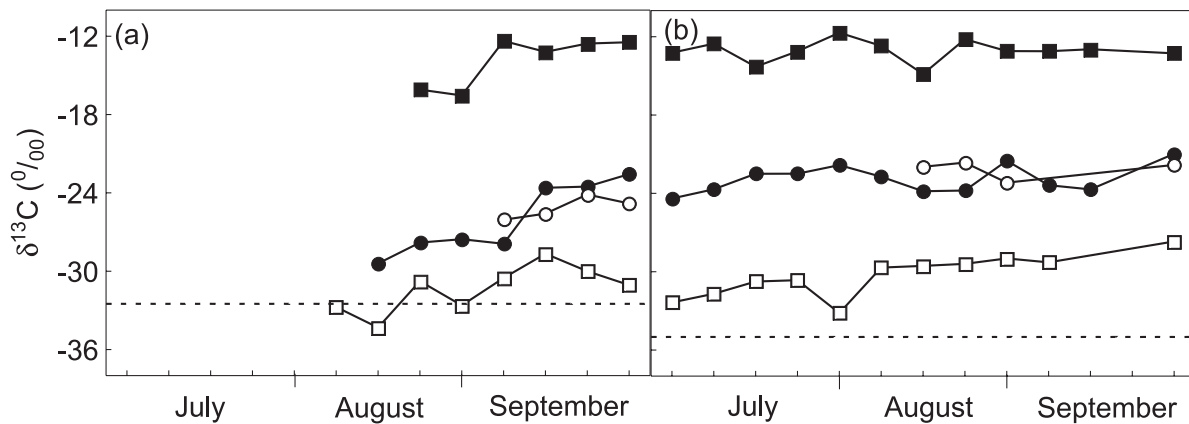

pleted in ^{13}C than POM, with $\delta^{13}C$ values ranging from −34.3‰ to −28.7‰. The $\delta^{13}C$ values of the cladoceran *D. pulicaria* are available only for September owing to its low abundance during July and August. During that month, the $\delta^{13}C$ was similar to that of POM (from −26.1‰ to −24.1‰). During 2002, the $\delta^{13}C$ of the epilithon ranged from −14.9‰ to −11.7‰ with no clear trend through the ice-free period. POM was, on average, 3‰ more enriched than in 2001 ($t = 3.6$, df = 17, $p < 0.01$) (Table 2) and it remained rather stable during the ice-free period. The $\delta^{13}C$ estimates of phytoplankton yielded an average value for 2002 of −35.0‰. The $\delta^{13}C$ of *M. laciniatus* was similar to that of the previous year (Table 2) and showed a progressive increase over the ice-free period. The $\delta^{13}C$ values of *D. pulicaria* were again similar to those of POM (from −23.2‰ to −21.6‰) and higher than in 2001 ($t = 5.4$, df = 6, $p < 0.01$) (Table 2).

Changes in $\delta^{13}C$ of the food web components in Río Seco Lake over the study ice-free periods are shown in Fig. 3. In 2001, the $\delta^{13}C$ of the epilithon was rather variable throughout the ice-free period with values ranging from −15.3‰ to −21.5‰. The $\delta^{13}C$ values of POM remained quite stable around −26‰ over the ice-free period, except for a sharp decrease to −34.0‰ in mid-September. The $\delta^{13}C$ estimates of phytoplankton in Río Seco Lake yielded an average value for 2001 of −32.2‰. As in La Caldera Lake, the calanoid copepod *M. laciniatus* had lower $\delta^{13}C$ values than *D. pulicaria*, ranging between −29.2‰ and −34.4‰. The cladoceran *D. pulicaria* was generally slightly ^{13}C depleted relative to POM and remained rather stable over the period

of study, with $\delta^{13}C$ values around −29‰. In 2002, $\delta^{13}C$ values of epilithon were similar to those measured in 2001 (Table 2) and showed a progressive increase over the ice-free period. The $\delta^{13}C$ values of POM were very stable throughout the period of study, with values similar to those in 2001 (Table 2). Composite samples of terrestrial vegetation from Río Seco Lake catchment collected during both study years yielded an average $\delta^{13}C$ value of −26.0‰ ± 0.4‰ ($n = 5$). The $\delta^{13}C$ estimates of phytoplankton yielded an average value for 2002 of −35.7‰. *Mixodiaptomus laciniatus* showed $\delta^{13}C$ values similar to those in 2001 (Table 2) and remained quite stable over the ice-free period. The $\delta^{13}C$ of *D. pulicaria* showed a significant decrease in 2002 relative to the previous year ($t = 8.3$, df = 17, $p < 0.001$) (Table 2) with values that remained around −33‰ over the whole ice-free period. This low $\delta^{13}C$ of *D. pulicaria* in 2002 coincided with a higher chlorophyll *a* content compared with the previous year (Table 1). To explore if this shift was due to a potential dietary change (from detritivorous to herbivorous), we performed a regression analysis between $\delta^{13}C$ of *D. pulicaria* and chlorophyll *a* concentration. The results obtained ($r^2 = 0.49$, $p = 0.002$, $n = 19$) revealed that the higher the chlorophyll *a* concentration, the lighter the $\delta^{13}C$ of *D. pulicaria* (Fig. 4).

In general, the food web components were more ^{13}C enriched in La Caldera Lake than in Río Seco Lake in both study years. These between-lake differences were greater in 2002. The $\delta^{13}C$ values of the epilithon were higher in La Caldera Lake than in Río Seco Lake in both 2001 ($t = 3.2$, df = 10, $p < 0.01$) and 2002 ($t = 5.6$, df = 23, $p < 0.001$) (Ta-

Fig. 3. Changes in the δ^{13}C of POM (●), epilithon (■), and zooplankton (the calanoid copepod *Mixodiaptomus laciniatus* (□) and the cladoceran *Daphnia pulicaria* (○)) in Río Seco Lake during the ice-free periods of (*a*) 2001 and (*b*) 2002. The broken line represents the mean value of estimated δ^{13}C of phytoplankton.
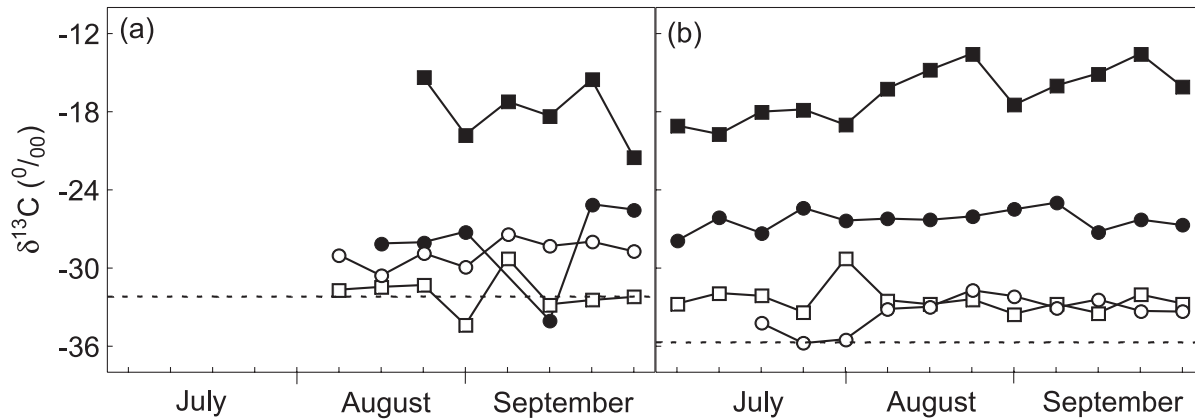
Fig. 4. Relationship between the δ^{13}C of *Daphnia pulicaria* and chlorophyll *a* concentration in Río Seco Lake for the ice-free periods of 2001 and 2002. Chlorophyll *a* concentration data are from Morales-Baquero et al. (2005).
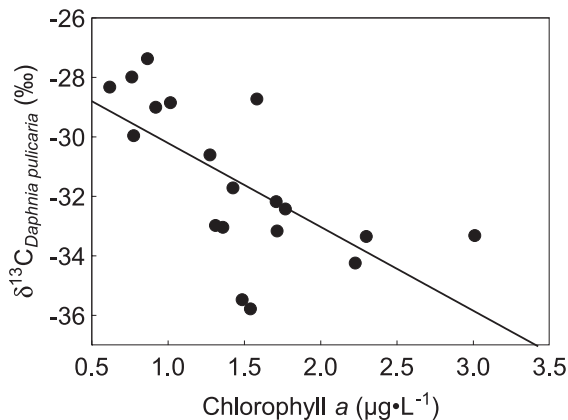
ble 2). There was no significant difference between the study lakes in δ^{13}C of POM in 2001 ($t = 1.2$, df = 13, $p = 0.262$). However, in 2002, the δ^{13}C of POM was higher in La Caldera Lake than in Río Seco Lake ($t = 9.1$, df = 23, $p < 0.001$) (Table 2). *Mixodiaptomus laciniatus* had similar δ^{13}C values in both study lakes in 2001 ($t = 0.7$, df = 16, $p = 0.480$). In 2002, the average δ^{13}C of this copepod was 2‰ higher in La Caldera Lake than in Río Seco Lake ($t = 3.9$, df = 22, $p < 0.001$) (Table 2). *Daphnia pulicaria* showed higher δ^{13}C values in La Caldera Lake than in Río Seco Lake in 2001 ($t = 6.1$, df = 11, $p < 0.001$). The difference between lakes in the δ^{13}C of this zooplankter was spectacular in 2002 ($t = 16.5$, df = 13, $p < 0.001$) with an average value in La Caldera Lake 11‰ higher than that in Río Seco Lake (Table 2).

Discussion

Carbon isotopic composition of DIC

The isotopic signature of DIC in lakes is influenced by the relative contribution of the different sources of inorganic carbon, which have distinct δ^{13}C values. Major allochthonous sources of DIC to lake systems are atmospheric gas exchange, mineralization of soil organic matter, and carbonate rock weathering (Amiotte-Suchet et al. 1999). Within lakes, respiration is the key process affecting the isotopic signature of DIC (Rau 1978; Leggett et al. 1999; Bade et al. 2004).

The study lakes are located on a catchment of mainly siliceous rocks. Accordingly, the input of DIC to the lakes from carbonate rock weathering is presumed negligible. On the other hand, the input of DIC from mineralization of soil organic matter is expected to be significant only in Río Seco Lake. Consequently, atmospheric gas exchange might be the most important allochthonous source of DIC to La Caldera Lake, whereas in Río Seco Lake, the main sources of DIC might be atmospheric gas exchange and mineralization of soil organic matter from its catchment. Thus, it might be expected that DIC is more ^{13}C depleted in Río Seco Lake than in La Caldera Lake. However, average δ^{13}C of DIC in Río Seco Lake was not significantly different from that in La Caldera Lake in any of the study ice-free periods. This suggests that, contrary to what it was expected, soil organic matter derived CO_2 is not a significant source of DIC to Río Seco Lake, and therefore, the contrasting catchments of the study lakes did not involve significant differences in the external sources of DIC. On the other hand, δ^{13}C of DIC in both lakes was similar to that of atmospheric CO_2, indicating that in these unproductive systems, respiration does not seem to be a major contributor to the DIC pool.

Carbon isotopic composition of food web components

One of the frameworks of isotopic analysis of food webs is that the δ^{13}C of an animal reflects closely that of its diet (DeNiro and Epstein 1978; Fry and Sherr 1984). The δ^{13}C of the cladoceran *D. pulicaria* always reflected the δ^{13}C of POM, except in Río Seco Lake in 2002. This strongly suggests a trophic reliance of this cladoceran on bulk POM in both study lakes. By contrast, the calanoid copepod *M. laciniatus* was, on average, 6‰ depleted in ^{13}C relative to POM in both study lakes. Such depletion in ^{13}C of zooplankton relative to POM has been previously reported (del Giorgio and France 1996; Grey and Jones 1999; Karlsson et al. 2003).

Several mechanisms have been proposed to explain this discrepancy between δ^{13}C values of zooplankton and POM.

First, lipids are ^{13}C depleted relative to whole organisms (McConnaughey and McRoy 1979); hence, part of this depletion can be due to lipid storage. Several studies have reported an average value of lipid-normalized $\delta^{13}C$ around 1–2‰ more positive than the uncorrected $\delta^{13}C$ (Kling et al. 1992; del Giorgio and France 1996). This difference might explain the slight depletion in ^{13}C of *D. pulicaria* relative to POM observed on several occasions. However, this mechanism by itself cannot explain the mean 6‰ difference between the $\delta^{13}C$ of *M. laciniatus* and the $\delta^{13}C$ of POM.

The most plausible explanation for this discrepancy is that POM in the study lakes was composed not only of phytoplankton but also of terrestrial organic matter, which is usually enriched in [13]C (del Giorgio and France 1996; Jones et al. 1998). This assertion is supported by the estimated $\delta^{13}C$ of phytoplankton. Despite the uncertainty of this estimation owing to the use of a unique assumed fractionation factor (ε_p) of 20‰, this value is likely suitable for the study lakes, since CO_2 concentration is higher than that typically reported as limiting for photosynthesis (Hein 1997; Burkhardt et al. 1999). Moreover, ε_p appears to be inversely dependent on the ratio of phytoplankton growth rate to CO_2 concentration (μ:CO_2) (Goericke et al. 1994; Laws et al. 1997). In La Caldera Lake, for instance, μ is about 0.75·day^{-1} (Reche et al. 1997), and hence, the application of the equation proposed by Laws et al. (1997) would lead to a fractionation of about 21‰, a value similar to that used in this study. On the other hand, the application of a lower ε_p (e.g., 11‰; Cole et al. 2002; Pace et al. 2004) would result in phytoplankton $\delta^{13}C$ values markedly higher than those measured for *M. laciniatus*. This inconsistency indicates either that there is another ^{13}C-depleted carbon source for zooplankton or that the $\delta^{13}C$ of phytoplankton is in the range estimated in this study. Hypolimnetic bacteria can constitute a light source of carbon for zooplankton (Jones et al. 1999; Bastviken et al. 2003). However, this alternative has low probability in the well-mixed study lakes where there is not an anoxic hypolimnium. Within epilimnetic layers, dissolved organic matter (DOM) usually shows an isotopic signature either similar to or heavier than POM (Benner et al. 1997; Jones et al. 1999; Ziegler et al. 2003). The enrichment in ^{13}C of DOM relative to POM can be associated with photoreactions (Osburn et al. 2001). In the study lakes, particularly in La Caldera Lake, DOM is highly photoreactive (Reche et al. 2001), and hence, the DOM isotopic signal should be particularly heavy. Since bacteria and DOM show similar isotopic signals (Ziegler et al. 2003), bacteria in the study lakes likely have an isotopic signature either similar to or heavier than POM owing to the assimilation of photochemically enriched DOM.

Other evidence supporting that, in the study lakes, phytoplankton is likely a light source of carbon is the observed change in the $\delta^{13}C$ of *D. pulicaria* related to chlorophyll *a* concentration in Río Seco Lake. Previous studies have reported that zooplankton might change from bulk POM to phytoplankton reliance during more productive periods (Grey et al. 2001). In particular, cladocerans have been observed to change feeding activity from detritivorous to herbivorous depending on phytoplankton concentration (Echevarría et al. 1994). It is possible that, in 2001, phytoplankton in Río Seco Lake was insufficient to support *D. pulicaria* re-

quirements, leading to the apparent reliance on bulk POM. By contrast, in 2002, the higher chlorophyll *a* concentration could have been enough to support a higher proportion of *D. pulicaria* requirements, explaining the observed ^{13}C depletion.

Finally, previous studies have observed a significant relationship between chlorophyll *a* concentration (surrogate of phytoplankton production) and the $\delta^{13}C$ of POM, indicating that POM is affected by autochthonous carbon input (Jonsson et al. 2001; Lehmann et al. 2004). By contrast, we did not find any significant relationship between chlorophyll *a* concentration and the $\delta^{13}C$ of POM in either La Caldera Lake ($r = 0.34$, $p = 0.159$, $n = 19$) or Río Seco Lake ($r = 0.30$, $p = 0.220$, $n = 19$). This fact strongly suggests that phytoplanktonic carbon is not the only contributor to the POM pool.

Overall, the analysis of the $\delta^{13}C$ of the zooplanktonic community in the study lakes revealed species-specific differences in their food sources. The calanoid copepod *M. laciniatus* selected a carbon source lighter than POM, likely phytoplankton. By contrast, the cladoceran *D. pulicaria* relied mainly on bulk POM, shifting to a more herbivorous behaviour in Río Seco Lake during the most productive ice-free period.

As discussed above, it is reasonable to assume that POM of both study lakes was composed not only of phytoplankton but also of more ^{13}C-enriched organic matter. Typically, terrestrially derived organic matter has higher $\delta^{13}C$ values than those derived from phytoplankton (Fry and Sherr 1984). Lakes with important inputs of organic matter from terrestrial vegetation of the catchment show $\delta^{13}C$ values of POM typical of those of terrestrial detritus, which can mask completely the isotopic signature of phytoplankton (Gu et al. 1994; Meili et al. 1996; Jones et al. 1998). Río Seco Lake is surrounded by terrestrial vegetation and it has temporal inlets that drain water from the catchment. In Río Seco Lake, mean $\delta^{13}C$ values of POM (–28‰ in 2001 and –26‰ in 2002) clearly reflected the isotopic signature of the organic matter derived from its catchment vegetation (mean value –26‰). This indicates that POM is composed not only of phytoplankton but also of terrestrial organic matter derived from its catchment. By contrast, in La Caldera Lake, inputs of terrestrial organic matter from the catchment are expected to be negligible owing to lacking terrestrial vegetation and stream inflows to the lake. Therefore, it would be expected that POM showed $\delta^{13}C$ values typical of phytoplankton-derived organic matter. However, the average $\delta^{13}C$ values of POM (–26‰ in 2001 and –23‰ in 2002) indicate either that there is a contribution of autochthonous ^{13}C-enriched organic matter (e.g., decomposed organic matter, epilithon) to the POM pool or that this lake receives inputs of terrestrial organic matter from sources other than the catchment. Previous studies have observed both a ^{13}C enrichment (Wu et al. 1999; Guo et al. 2004) and a ^{13}C depletion (Benner et al. 1997) of POM with depth associated with decomposition. In La Caldera Lake, previous measurements of $\delta^{13}C$ of POM collected from the surface yielded an average value (–22‰) similar to that obtained in this study for integrated samples. Therefore, it is not likely that the heavy signal of POM in La Caldera Lake reflects the presence of decomposed material. On the other hand, the epilithon in the study lakes showed a

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heavy isotopic signature, in agreement with previous studies that have shown a ^{13}C enrichment in benthic compared with pelagic algae, likely related to the greater diffusion resistance of CO_2 (France 1995*a*, 1995*b*). The incorporation of the epilithon to POM samples may also explain the enriched signature of POM. However, microscopic examination of Lugol-stained samples revealed the absence of epilithic algae in the water column (Pulido-Villena 2004).

It arises from the arguments presented above that La Caldera Lake is likely receiving ^{13}C-enriched organic carbon derived from allochthonous sources. There is growing evidence that atmospheric deposition constitutes a major input of nutrients to oligotrophic systems (Herut et al. 1999; Ridame and Guieu 2002; Markaki et al. 2003). It is also known that rainfall delivers significant amounts of soluble organic carbon to the ocean (Willey et al. 2000). The study lakes are located in the south of Spain, an area that receives significant amounts of Saharan dust mainly during spring and summer (Rodríguez et al. 2001). La Caldera Lake receives an annual cumulative flux of atmospheric soluble organic carbon of 9.2×10^5 mmol of carbon, mainly derived from Saharan dust (I. Reche, E. Pulido-Villena, and R. Morales-Baquero, unpublished). Considering that the volume of La Caldera Lake is about 46×10^6 L (Morales-Baquero et al. 1999), this allochthonous input of organic carbon would yield a DOC concentration of about 20 μmol·L^{-1}, which represents around 33% of the lake's DOC concentration (60 μmol·L^{-1}; Pulido-Villena 2004). These data provide evidence that atmospheric deposition can be a significant source of soluble organic carbon to La Caldera Lake, which, moreover, does not receive other different allochthonous inputs. Assuming the significant contribution of atmospheric inputs to the lake's DOC pool, the next step is to explore if the isotopic signature of organic carbon associated with atmospheric dust is heavy enough to explain the observed signal of POM. A recent study by Eglinton et al. (2002) reported that the δ^{13}C of total organic carbon in atmospheric dust derived from the Sahara Desert is around −18‰, suggesting the presence of biomass and burning residues derived from predominantly C4 vegetation accumulated in soils. Therefore, atmospheric inputs of organic carbon could explain the heavy signal of POM in La Caldera Lake.

In conclusion, carbon stable isotope analysis in the study lakes revealed that, despite contrasting catchments, both lake food webs might be partially supported by organic carbon of terrestrial origin, the utilization of which is species specific, dominated by *D. pulicaria*. These results underline that allochthonous sources different from the catchment (i.e., atmospheric inputs), as well as zooplankton community structure, should be considered in assessing the reliance of lake food webs on terrestrially derived organic matter.

Acknowledgements

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