The abundance of mixotrophic algae drives the carbon isotope composition of the copepod *Boeckella gracilipes* in shallow Patagonian lakes

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The importance of allochthonous versus authochthonous carbon in the diet of zooplankters has been discussed at length in the recent literature, the more so for lakes with dissolved organic matter (DOM) concentrations between 3.5 and 25 mg L^{-1}. Here, we investigated the sources of carbon for *Boeckella gracilipes* (Copepoda, Calanoida) in three Patagonian shallow lakes that have different but relatively moderate DOM concentrations (<5 mg L^{-1}). We linked their δ^{13}C values with parameters such as water color (absorbance 440 nm, $_{abs440}$), dissolved organic carbon (DOC) concentrations and the ratio Color$_{abs440}$:Chlorophyll *a* (Chl *a*). We also determined the isotopic composition of seston and phytoplankton composition. Our results showed a direct relationship between the δ^{13}C values of *B. gracilipes* and seston ($r^2 = 0.64$, $P<0.001$), and we demonstrated the importance of phytoplankton to *B. gracilipes* as its δ^{13}C signal was strongly related to the abundance of the mixotrophic nanoflagellate *Chrysochromulina parva* ($r^2 = 0.86$, $P < 0.001$). We further found an inverse relationship between DOM and the ratio Color$_{abs440}$:Chl *a* and *B. gracilipes* δ^{13}C values ($r^2 = 0.67$ and 0.56, respectively), which also supported the importance of authochthonous carbon for the copepod. We conclude that phytoplankton, particularly mixotrophic algae, rather than allochthonous carbon, supports the copepod *B. gracilipes* in shallow Patagonian lakes.

KEYWORDS: zooplankton; mixotrophs; carbon stable isotopes; dissolved organic carbon

INTRODUCTION

Freshwater zooplankton use food sources from aquatic primary production, but accumulating evidence suggests that cladocerans and also copepods are supported by organic matter produced outside of the aquatic environment, in the surrounding terrestrial landscape (e.g. Cole *et al.*, 2011; Rautio *et al.*, 2011; Karlsson *et al.*, 2012; Berggren *et al.*, 2014). Bacteria represent a key point of entry for terrestrial carbon through the uptake of dissolved organic matter (DOM) (Karlsson *et al.*, 2003; Jansson *et al.*, 2007); however, zooplankton take up bacterial carbon in different ways. While cladocerans directly ingest bacteria (Karlsson *et al.*, 2004; Taipale *et al.*, 2008), copepods tend not to feed directly on them; instead, their uptake of bacterial carbon is mostly channeled through consumption of bacterivorous protozoa and other microzooplankton (Jansson *et al.*, 1999; Berggren *et al.*, 2010). Also, some zooplankters are able to directly assimilate allochthonous particulate organic matter (POM) in the form of terrestrial detritus (e.g. leaf fragments or flocculated DOM) (Pace *et al.*, 2004; Cole *et al.*, 2006; Berggren *et al.*, 2014). The ability to determine carbon pathways is crucial for understanding pelagic ecosystem functioning; yet, existing evidence reveals contrasting results regarding both the amount and the type of allochthonous carbon used by the zooplankton in freshwater environments (Brett *et al.*, 2009; Cole *et al.*, 2011; Wilkinson *et al.*, 2013; Berggren *et al.*, 2014).

North Andean Patagonia (41°S 71°W) is an area characterized by aquatic environments with low nutrient concentrations and great influence of solar radiation (Morris *et al.*, 1995; Modenutti *et al.*, 2005). The shallow (<15 m) lakes in this area have distinct dissolved organic carbon (DOC) concentrations typically below 5 mg L^{-1} and particulate matter values ranging from 0.1 to 0.58 mg L^{-1} (Morris *et al.*, 1995; Bastidas Navarro *et al.*, 2009b; Bastidas Navarro and Modenutti, 2012). The surrounding landscapes and the precipitation patterns have been observed to affect the DOM content of these aquatic systems (Bastidas Navarro and Modenutti, 2012). Moreover, Bastidas Navarro *et al.* (Bastidas Navarro *et al.*, 2009b) found that the amount of available DOM is an important factor driving their balance between primary and bacterial production (Bastidas Navarro *et al.*, 2009b). In these systems, phytoplankton is dominated by mixotrophic organisms (Bastidas Navarro *et al.*, 2009b; Modenutti, 2014) that combine both autotrophic and heterotrophic nutrition. The crustacean zooplankton are represented by few species, including *Boeckella gracilipes*, an endemic calanoid copepod that dominates the pelagic communities (Balseiro and Modenutti, 1998). Balseiro

et al. (Balseiro *et al.*, 2001) showed that *B. gracilipes* consumes organisms <50 µm and that its diet comprises mainly moving organisms such as mixotrophic nanoflagellates and ciliates. Moreover, the laboratory experiments performed by Balseiro *et al.* (Balseiro *et al.*, 2001) and field data on a related species, *B. michaelseni* (Modenutti *et al.*, 2003), suggest that this copepod does not feed directly on bacteria or picocyanobacteria. Therefore, the route from terrestrial carbon to this copepod may pass through the consumption of bacteria by its mixotrophic prey.

For the past decades, natural ratios of stable carbon isotopes (^{13}C:^{12}C, δ^{13}C) have been applied in studies of the sources and fluxes of organic matter in lake pelagic food webs (e.g. Grey *et al.*, 2001; Pace *et al.*, 2004; Cole *et al.*, 2011; Francis *et al.*, 2011; Berggren *et al.*, 2014). Despite its limitations (see Brett, 2014; Karlsson *et al.*, 2014), the natural isotope ratios combined with measurements of DOC and the optical properties of DOM can be used to trace the terrestrial contributions to zooplankton (Pace *et al.*, 2004; Carpenter *et al.*, 2005; Cole *et al.*, 2006; Berggren *et al.*, 2010). Particularly, the terrestrial carbon isotope signal is around 28‰ (Peterson and Fry, 1987), and specifically the forests around the Patagonian lakes have a signal of 28.4 ± 0.7‰ (Arcagni *et al.*, 2013), and the incorporation of allochthonous carbon by lake zooplankton can be positively related to the water color (colored DOC) (see Berggren *et al.*, 2014) and, especially, to the ratio between color and chlorophyll *a* (Chl *a*) (Carpenter *et al.*, 2005; Batt *et al.*, 2012).

Here, we followed the isotopic signal of the copepod *B. gracilipes* (δ^{13}C$_{Bg}$) in three shallow lakes during three different periods of the year with the aim of establishing their carbon sources. We indirectly estimated the allochthony status (incorporation of terrestrial carbon) of *B. gracilipes* by linking their δ^{13}C values with parameters such as DOC concentrations, water color (absorbance 440 nm, $_{abs440}$), and the ratio Color$_{abs440}$:Chl *a*. We further explored the importance of autochthonous carbon to *B. gracilipes* by relating the copepod δ^{13}C values with the abundance of its putative prey, for which we determined the phytoplankton composition on each occasion. We hypothesized that the values of carbon stable isotopes for *B. gracilipes* and seston would converge, approaching a terrestrial δ^{13}C signal (around 28‰), as DOC (and lake water color) increase and the copepod consumer becomes more dependent on allochthonous organic carbon (bacterial carbon incorporated by their mixotrophic prey). This cross-ecosystem comparison of the same zooplankter and its diet using stable isotopes may reveal the potential transfer pathways of organic matter to this consumer in systems with different, but rather moderate, DOM concentrations.

METHOD

Study sites

We studied three small (area $< 1 \text{ km}^2$) and shallow ($z_{max} < 12$ m) lakes, Morenito, Escondido and El Trébol, situated within the Nahuel Huapi National Park, Patagonia, Argentina ($41°$S $71°$W). This region of Patagonia is continental and humid cold-temperate, with a mean annual precipitation of 1500 mm that is mostly concentrated ($> 70\%$) in the autumn−winter period in the form of rain and snow (Paruelo *et al.*, 1998). The lakes are oligotrophic, with Chl *a* concentrations $< 5 \text{ μg L}^{-1}$, total phosphorus concentrations between 0.22 and 0.39 μM and dissolved inorganic nitrogen concentrations between 1.48 and 3.95 μM (Bastidas Navarro *et al.*, 2009b). The littoral zones of the lakes contain extensive areas of emergent and submersed aquatic vegetation, while the surroundings of the lakes are mixed native forests, mainly constituted by the evergreen Nothofagaceae *Nothofagus dombeyi* (Mirb.) Blume and the conifer *Austrocedrus chilensis* (D. Don) Florin et Boutleje.

Sample and field data collection

We sampled the lakes in summer (January), early autumn (March) and early spring (September) of 2012. On each sampling occasion, we measured water temperature, pH, conductivity and oxygen concentrations using a multiprobe (YSI 85; Biospherical Instruments Inc.). We took four 4 L water samples from each lake at 0, 4 and 8 m depth using a column sampler and placed them in acid-washed (HCl) containers. We also collected three integrated samples (0−8 m) of zooplankton using a 55 μm closing plankton net for zooplankton counts and for C:N and stable isotope analysis. The samples were carried to the laboratory in thermally insulated containers, zooplankton were placed in filtered lake water for 12 h for gut evacuation and then stored in the freezer at $-20°$C.

Laboratory determinations

We used an aliquot of water (500 mL) from each water sample to analyze Chl *a* and nutrients. We estimated Chl *a* using hot ethanol extraction of filter residues (GF/F Whatman, Maidstone, UK) according to Nusch (Nusch, 1980) using a fluorometer (Turner Designs, 10-AU). Also, we measured DOC and dissolved inorganic carbon (DIC) concentrations and Color$_{abs440}$ as estimates of terrestrial-derived DOC in the study lakes using a Shimadzu Total Organic Carbon analyzer (model TOC-Vcsh) and a Shimadzu UV2450 double-beam spectrophotometer, respectively. To perform these measurements, we filtered the water through pre-combusted GF/F filters. We also calculated the ratio Color$_{abs440}$:Chl

a as a proxy of the origin of the carbon pool, this index considers all the carbon that does not contain Chl *a* as allochthonous carbon (Pace *et al.*, 2004; Taipale *et al.*, 2007).

On each sampling occasion, we also preserved 300 mL of an integrated water sample (composed of aliquots of each depths, 100 mL each) in acid Lugol's solution for phytoplankton identification and counting with an inverted microscope at $15-40\times$ using 50 mL settling chambers.

Stable isotope samples

To prepare the seston samples, we pre-filtered 2 L of each sample through an 80 μm net followed by filtering through pre-weighed and pre-combusted GF/F filters. We then dried the filters at $60°$C for 2 days and stored them in a desiccator until packing them into tin capsules.

To obtain *B. gracilipes* samples, we separated 200 individuals (adults or late copepodite stages) from each sample under a stereomicroscope at $12.5\times$ and placed them into pre-combusted GF/F filters, which were then dried and packed into tin capsules as above. We prepared three samples on each sampling occasion except in autumn for Lake El Trébol when *B. gracilipes* individuals were not abundant enough to obtain the δ^{13}C values.

Elemental C and N and stable isotope ratios of C and N were analyzed at the Stable Isotope Facility, University of Davis, California, USA, using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa $20-20$ isotope ratio mass spectrometer (Sercon Ltd, Cheshire, UK). We expressed stable isotope data in part per mil (‰) deviations from international standards using the following equation: $\delta^{13}\text{C} = (R_{sample}/R_{standard} - 1) \times 1000$, where R is the ratio of heavy/light isotope content ($^{13}\text{C}/^{12}\text{C}$) and the working standards were glutamic acid and peach leaves. Internal precision was usually < 0.2‰. We used a mathematical normalization for lipid content based on C:N ratios (Post *et al.*, 2007).

Statistical analyses

We described the relationship between DOC and Color$_{abs440}$ with a linear function, although other more complex models fitted these data equally well. We ran linear regressions or Spearman correlations between $\delta^{13}\text{C}_{SES}$ and DOC, and between $\delta^{13}\text{C}_{Bg}$ and DOC, Color$_{abs440}$ and the index Color$_{abs440}$:Chl *a*. We averaged the values obtained across all depths for the Color$_{abs440}$, Chl *a* concentration, Color$_{abs440}$:Chl *a* ratio and DOC at each sampling event to present an integrated lake pattern. We also fitted a linear regression between $\delta^{13}\text{C}_{SES}$ and $\delta^{13}\text{C}_{Bg}$ and applied a stepwise multiple linear regression using the abundances of key

phytoplankton species to explain the $\delta^{13}C_{Bg}$. Finally, we ran linear regressions between the main putative prey of *B. gracilipes* as selected by the model and $\delta^{13}C_{SES}$ and DOC, respectively. For the regressions involving *B. gracilipes*, we averaged all values obtained for $\delta^{13}C_{SES}$ in each lake on each sampling occasion ($n = 3$), assuming that *B. gracilipes* consumes food from the entire water column. We tested the independence of the data by analyzing an autocorrelation plot of the residuals [the residuals of the variable of interest (e.g. $\delta^{13}C$) during a time (t) versus its residuals in a prior time (t^{-1})]. We also tested the homoscedasticity and normal distribution of the errors. Statistical analyses were performed using the software PASW Statistics version 18 and Table Curve 2D v5.01.

RESULTS

The lakes

The three lakes had similar surface temperatures on each sampling date, averaging 21°C in summer, 17°C in autumn and 9.5°C in spring (variations among lakes were lower than 1°C for all sampling events). However, during the summer sampling, Lakes Escondido and Morenito exhibited a thermal gradient with bottom temperatures reaching 14 and 17°C, respectively. We found aerobic conditions through the water column with concentrations at 100% saturation and comparable conductivities, around 60 μS cm^{-1}, on all sampling occasions within and among the lakes. pH ranged between 7 and 7.5.

We observed a trend in the values obtained for $Color_{abs440}$ (used to reflect the concentration of DOM) in the three lakes, the highest values were obtained in Lake Escondido, intermediate values in Lake Morenito and the lowest values in Lake El Trébol (Table I). However, $Color_{abs440}$ only differed between Lakes Escondido and El Trébol (ANOVA $P < 0.05$). DOC concentrations ranged between 2.5 and 4.3 mg L^{-1} and showed a similar trend as that of $Color_{abs440}$ (Table I). In particular, DOC increased as a linear function of $Color_{abs440}$ as follows: DOC $= 1.23 + (1.84 \times Color_{abs440})$ ($r^2 = 0.77$, $P = 0.002$). For DIC concentrations, we observed an opposite pattern with the highest values occurring in Lake El Trébol and the lowest in Lake Escondido (Table I). The optical index used to estimate the autochthonous versus allochthonous origin of carbon in the lakes, $Color_{abs440}$:Chl a, ranged from 0.27 to 1.0 and showed seasonal shifts (Table I), with the lowest variability being observed in Lake El Trébol and the highest in Lake Escondido. The highest value was measured in Lake Escondido in spring, indicating a greater influence of allochthonous carbon.

Table I: Values (mean $\pm$ 1 SE) of water color, $Color_{abs440}$, DOC concentrations, DIC concentrations and the ratio $Color_{abs440}$: Chl a for the three lakes

Lake	Season	$Color_{abs440}$ (m^{-1})	DOC (mg L^{-1})	DIC (mg L^{-1})	$Color_{abs440}$: Chl a (m^{-1} μg L^{-1})$^{-1}$
Escondido	Summer	1.30	4.18	8.06	0.61
	Autumn	1.57	4.35	8.05	0.50
	Spring	1.72	4.34	7.95	1.01
Morenito	Summer	1.01	3.01	8.75	0.40
	Autumn	1.31	3.17	8.58	0.58
	Spring	0.93	2.91	8.65	0.27
El Trébol	Summer	0.94	2.80	9.24	0.49
	Autumn	1.10	2.96	9.07	0.47
	Spring	0.71	2.87	9.24	0.40

The relative abundances of phytoplankton groups in Lakes Escondido and Morenito were dominated by nanoflagellates belonging to Haptophyceae, Cryptophyceae, Dinophyceae and Chrysophyceae classes (Fig. 1a and b). The main species representing each group were mixotrophic nanoflagellates such as *Chrysochromulina parva*, *Plagioselmis lacustris*, *Gymnodinium varians* and *Dynobrion divergens*. Phytoplankton in Lake El Trébol was characterized by the presence of diatoms (Bacillariophyceae) (Fig. 1c), mainly *Cyclotella meneghiniana*, but *C. parva* was the most characteristic mixotrophic nanoflagellate present in this lake, while other species (e.g. *P. lacustris*) were found in low abundances (Fig. 1c). Ciliates were scarce (Fig. 1a–c) and did not show any particular trend in the study lakes. Following Balseiro *et al.* (Balseiro *et al.*, 2001), we selected the phytoplankton species from the three lakes that could be considered potential food items for *B. gracilipes* (Table II). These included motile, non-colonial organisms with a size range below 30 μm as mixotrophic nanoflagellates and several ciliates (Table II).

The stable isotope signals

The $\delta^{13}C_{SES}$ values from the three lakes ranged from -27.3 to -33.9‰ showing pronounced differences among lakes and also seasonal variation (Table III). The $\delta^{13}C$ values of seston samples were very similar for all depths sampled in each season in each lake ($P > 0.05$). Lake Escondido showed the lowest $\delta^{13}C_{SES}$ values followed by Lake Morenito, while lake El Trébol had the highest values (Table III). The signals of $\delta^{13}C_{SES}$ were higher in El Trébol than in Morenito and Escondido in summer and spring, while Escondido had lower values than the other two lakes in autumn.

The $\delta^{13}C_{Bg}$ values also showed large fluctuations, both among lakes and seasons (Table III). The $\delta^{13}C_{Bg}$ in
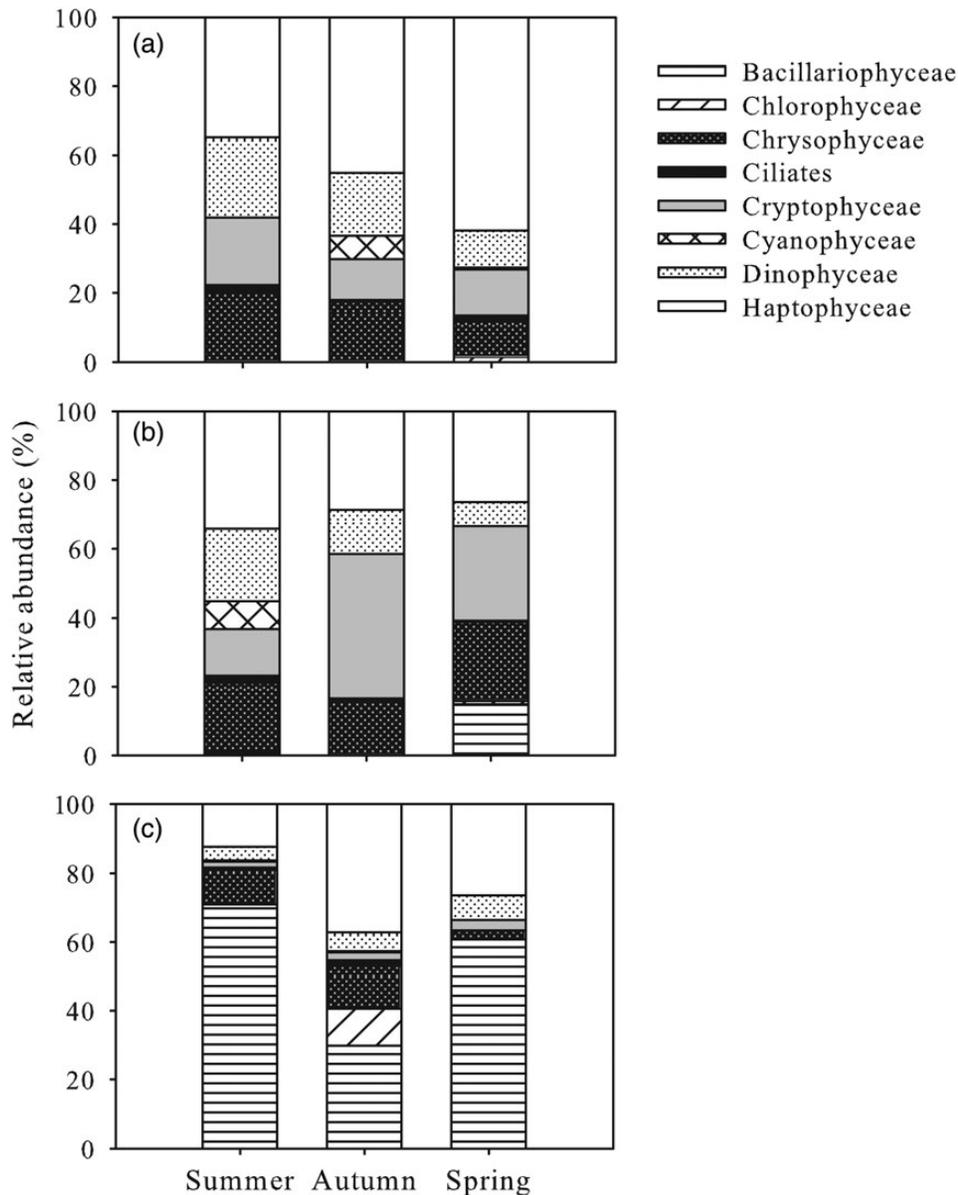
Fig. 1. The composition of phytoplankton groups in Lakes Escondido (**a**), Morenito (**b**) and El Trébol (**c**) during the three seasons.

the three lakes mirrored the trend obtained for the $\delta^{13}C_{SES}$, with the highest values in El Trébol, intermediate values in Morenito and the lowest values in Lake Escondido ($-31,0$, -33.9 and -37.4‰, respectively). *Boeckella gracilipes* had more ^{13}C-depleted values than $\delta^{13}C_{SES}$ (Table III).

The sources of carbon for *B. gracilipes*

Our results showed that the values obtained for $\delta^{13}C_{SES}$ and $\delta^{13}C_{Bg}$ were related to DOC (Fig. 2a and b). The $\delta^{13}C_{SES}$ values significantly decreased with increasing

DOC ($r^2 = 0.45$, $P < 0.001$, Fig. 2a). Concurrently, the relationship between $\delta^{13}C_{Bg}$ and DOC was inverse and significant, hence increasing DOC concentrations correlated with lower ^{13}C signals in *B. gracilipes* (Fig. 2b, $r^2 = 0.67$, $P < 0.001$). We also found a statistically significant negative correlation between $Color_{abs440}$ and $\delta^{13}C_{Bg}$ ($r^2 = 0.65$, $P < 0.001$) (Fig. 3a) and also between $Color_{abs440}$:Chl *a* and $\delta^{13}C_{Bg}$ ($r^2 = 0.54$, $P < 0.001$) (Fig. 3b).

The $\delta^{13}C$ signals of the consumer and the seston fraction were closely associated (Fig. 3c), the $\delta^{13}C_{Bg}$ values became more negative mirroring the trend obtained for

Table II: Potential food items for B. gracilipes *selected following Balseiro* et al. *(Balseiro* et al.*, 2001) from the phytoplankton identified in the three lakes*

Potential prey	Group	ESD (μm)	Abundance range (%)
Chrysochromulina parva Lackey	Haptophyceae	3.74	12–62
Plagioselmis lacustris	Cryptophyceae	6.01	1–39
Gymnodinium varians Maskell	Dinophyceae	8.92	3–20
Cryptomonas spp.	Cryptophyceae	11.22	0.3–2.6
Balanion planctonicum (Foissner, Oleksiv and Müller) Foissner, Berger and Kohmann	Ciliate	12.73	0–0.75
Peridinium sp.	Dinophyceae	16.03	0–5
Rimostrombidium humile (Penard) Petz and Foissner	Ciliate	16.03	0–1.3
Rimostrombidium lacustris (Foissner, Skogstad and Pratt) Petz and Pratt	Ciliate	23.64	0–0.2
Urotricha sp.	Ciliate	25.03	0–0.4
Limnostrombidium viride (Stein) Krainer	Ciliate	30.96	0–1.2
Gymnodinium paradoxum Schill.	Dinophyceae	32.65	0–2.6
Pelagohalteria viridis (Fromentel) Foissner, Skogstad and Pratt	Ciliate	33.06	0–0.2

ESD, equivalent spherical diameter.

Table III: Values (mean $\pm$ 1 SE) of $\delta^{13}C$ of seston (SES) and B. gracilipes *(Bg) for the three lakes during the seasons*

Lake	Season	$\delta^{13}C_{SES}$ (‰)		$\delta^{13}C_{Bg}$ (‰)	
		Mean	SE	Mean	SE
Escondido	Summer	−31.3	0.7	−36.4	0.2
	Autumn	−33.1	0.5	−35.0	0.4
	Spring	−32.9	0.1	−39.9	0.1
Morenito	Summer	−30.4	0.2	−34.3	0.6
	Autumn	−28.8	0.5	−32.3	0.0
	Spring	−32.5	0.4	−34.0	0.1
El Trébol	Summer	−28.2	0.3	−30.2	0.2
	Autumn	−27.3	0.0	[a]	—
	Spring	−29.4	0.4	−31.8	0.1

[a]Not enough material to make a reliable measurement of $\delta^{13}C$.

$\delta^{13}C_{SES}$ in the lakes ($r^2 = 0.64$, $P < 0.001$). The $\delta^{13}C_{Bg}$ values were closely associated with the abundance of the nanoflagellate *C. parva* and the ciliates *Limnostrombidium viride* and *Balanion planctonicum* in the food pool ($r^2 = 0.95$, $P < 0.001$), increased abundance of these food items resulted in more negative values of $\delta^{13}C_{Bg}$. The function that described the relationship was: $\delta^{13}C_{Bg} = -28.1 - (0.15 \times C.\ parva) - (2.1 \times L.\ viride) - (2.9 \times B.\ planctonicum)$. Notably, the abundances of *C. parva* alone explained 86% of the total variance in $\delta^{13}C_{Bg}$ values (Fig. 3d), while *L. viride* added 7% and *B. planctonicum* only 2% to the model variance. The three prey items selected by the model comprise the full prey size spectra described for *B. gracilipes* by Balseiro *et al.* (Balseiro *et al.*, 2001) (Table II). Finally, we explored the relationship between *C. parva* and the $\delta^{13}C_{SES}$ values and between DOC concentrations and *C. parva* abundance and found that increasing *C. parva* abundance did, indeed, result in more negative values of $\delta^{13}C_{SES}$ (Fig. 4a, $r^2 = 0.45$, $P < 0.001$) and that increasing DOC concentrations resulted in increasing *C. parva* abundances (Fig. 4b, $r^2 = 0.60$, $P < 0.015$).

DISCUSSION

We studied three lakes with a gradient in DOC concentrations and Color$_{abs440}$, and also in the isotopic values of seston and the copepod *B. gracilipes* ($\delta^{13}C_{SES}$ and $\delta^{13}C_{Bg}$). Our results showed relationships between $\delta^{13}C$ and Color$_{abs440}$ and especially DOC concentrations. However, contrary to what we hypothesized, in the lakes with the highest DOC levels, the values of $\delta^{13}C_{SES}$ and $\delta^{13}C_{Bg}$ were the most depleted in ^{13}C relative to $\delta^{13}C$ of terrestrial carbon in the surrounding landscape (around 28‰). The explanation behind this result is that the factor modulating the changes in the carbon isotopes of *B. gracilipes* in North Patagonian lakes was the abundance of certain phytoplankton species, mainly mixotrophic algae.

Previous studies on the transfer of allochthonous carbon to zooplankton have shown that the allochthony of copepods was lower than the allochthony of cladocerans (Pace *et al.*, 2007; Solomon *et al.*, 2011; Wilkinson *et al.*, 2013; Berggren *et al.*, 2014). These results point to a significant effect of zooplankton feeding strategy on the amount of allochthony and have been supported by the observation that cladocerans directly graze on bacteria that allochthonous DOM (Karlsson *et al.*, 2004; Taipale *et al.*, 2008), while copepods are usually more selective for autochthonous resources (Pulido-Villena *et al.*, 2005). However, the incorporation of allochthonous DOM into copepods could be favored by the bacterivorous behavior of their nanoplanktonic prey (Karlsson *et al.*, 2003; Jansson *et al.*, 2007). Particularly, in the studied Patagonian lakes, bacteria have been shown to actively
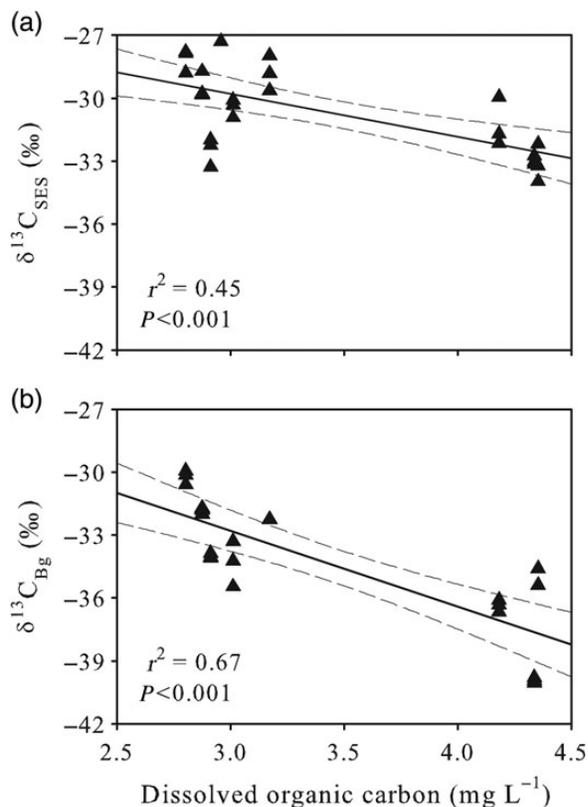
Fig. 2. Relationships between DOC concentrations and (**a**) $\delta^{13}C_{SES}$ and (**b**) $\delta^{13}C_{Bg}$. Dashed lines are predicted values and 95% confidence intervals from simple linear regression.

consume and rely on terrestrial DOC, probably more so at times where the exudates of phytoplankton may constitute a small portion of the DOC (Bastidas Navarro *et al.*, 2009a). Concurrently, the mixotrophic species in these lakes benefit from the higher DOC (i.e. more allochthonous) environment as they are more mobile than other phytoplankton species, rendering them better competitors for nutrients and light (Laybourn-Parry, 2002; Modenutti, 2014). Following this line of thought, we expected high allochthony by the calanoid copepod *B. gracilipes* in Patagonian lakes due to its high consumption of mixotrophic flagellates and ciliates (Balseiro *et al.*, 2001), which are key bacterial grazers (Balseiro *et al.*, 2004).

Our study showed that the allochthonous DOM present in the lakes does not necessarily reach *B. gracilipes*. We found a significant negative relationship between the $\delta^{13}C$ values of *B. gracilipes* and DOC and Color$_{abs440}$, suggesting that the allochthony of this pelagic consumer was inversely related to the amount of allochthonous DOM present in the lakes. This last statement was particularly supported by the negative relationship between $\delta^{13}C$ of *B. gracilipes* and the ratio Color$_{abs440}$:Chl *a*, which

is usually a good indicator of the relative use of allochthonous carbon by consumers (Carpenter *et al.*, 2005). Moreover, *B. gracilipes*, which tends to be a selective feeder (Balseiro *et al.*, 2001), had $\delta^{13}C$ values coupled with those of the seston pool (Fig. 4a). The consumption of ^{13}C-depleted phytoplankton by *B. gracilipes* during the periods of relatively higher DOC in the lakes could explain the observed trends in $\delta^{13}C_{Bg}$ values; moreover, this hypothesis is supported by our findings that the main factor driving the changes in $\delta^{13}C_{Bg}$ values was the abundance of mixotrophs as the nanoflagellate *C. parva* and the ciliate *L. viride* in the food pool. The strong inverse relationship between the $\delta^{13}C_{Bg}$ values and the abundances of these food items indicates that these mixotrophs can have a low $\delta^{13}C$ signal, considering that the trophic enrichment is $\sim 0.4 - 1.4‰$ (reviewed by Caut *et al.*, 2009).

Chrysochromulina parva belongs to a group of mixotrophs (Group C described by Jones, 1997) that includes mixotrophic protists whose primary mode of carbon acquisition is phototrophy; however, they ingest bacteria to supplement their nutrition proportionally to the light intensity (Balseiro *et al.*, 2004). Thus, consumers, as in our case *B. gracilipes*, may encounter the same food species at different times with differing biochemical compositions and therefore different isotopic signals. Particularly, Lake Escondido had the lowest $\delta^{13}C_{Bg}$ values and the highest abundances of *C. parva* (35−62%). Therefore, although terrestrial inputs form a major portion of DOM in Lake Escondido, *B. gracilipes* carbon is probably obtained from a light C source (linked to primary production) and/or the consumption of autotrophic picoplankton (e.g. picocyanobacteria, Balseiro *et al.*, 2004) by, for example, *C. parva* or *L. viride*. Matthews and Mazumder (Matthews and Mazumder, 2006) found vertical partition of the origin of the carbon in stratified lakes, with lower $\delta^{13}C$ values in the deep waters than in the surface waters; however, in our study lakes, this is not possible because they are continuously mixed. We also found that the increase in $\delta^{13}C$ values of *B. gracilipes* concurred with the presence of *C. meneghiniana* in Lake El Trébol. As a reference, diatoms $\delta^{13}C$ values ranged between −30.6 and −26.6‰ in meso-eutrophic Finland lakes (Vuorio *et al.*, 2006). The likely consumption of the diatom *C. meneghiniana* by *B. gracilipes* is surprising as this copepod preferentially preys on moving organisms (Balseiro *et al.*, 2001); yet, in Lake El Trébol, the abundances of motile prey were generally low. In this sense, Laundry (Landry, 1981) showed that zooplankton may switch to a more abundant prey at low concentrations of preferred phytoplankton species. Also, if little turnover of carbon took place during *B. gracilipes* development, then some of the carbon could have been incorporated in the early life stages
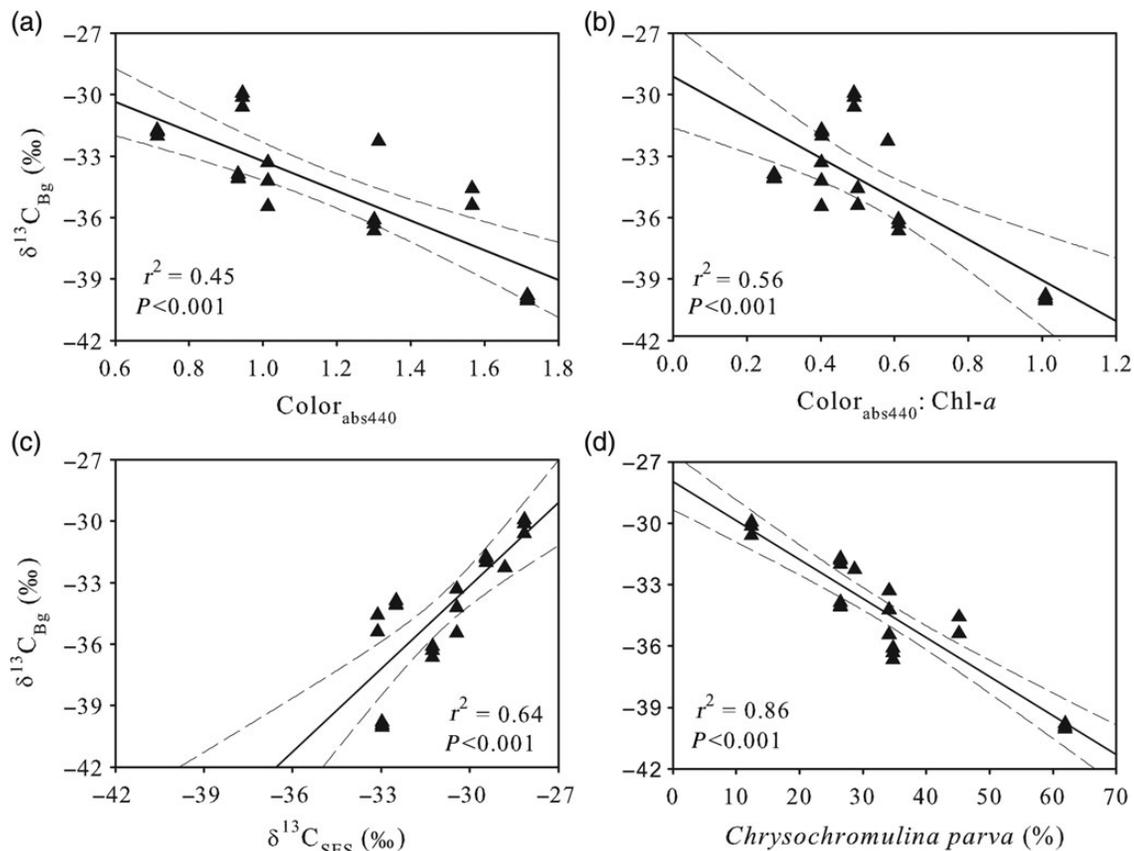
Fig. 3. Relationships between $\delta^{13}C_{Bg}$ and: (**a**) $Color_{abs440}$, (**b**) the ratio $Color_{abs440}$:Chl a, (**c**) $\delta^{13}C_{SES}$ and (**d**) *C. parva* abundances. Dashed lines are predicted values and 95% confidence intervals from simple linear regression.
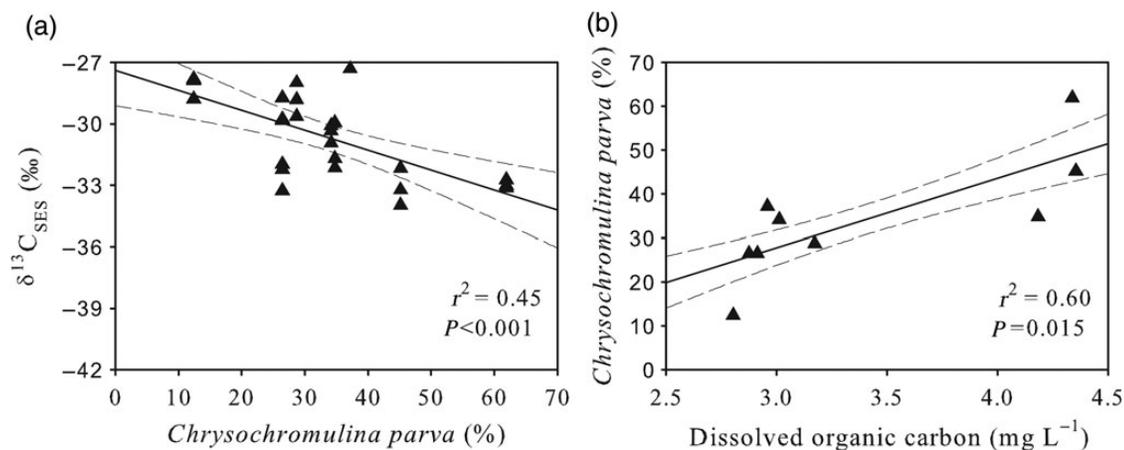

Fig. 4. Relationships between *C. parva* and (**a**) $\delta^{13}C_{Bg}$ and (**b**) DOC concentrations. Dashed lines are predicted values and 95% confidence intervals from simple linear regression.

(Grey, 2000; Ventura and Catalan, 2008). Thus, in our case, the carbon signal of *B. gracilipes* adults may reflect a cumulative signal during *B. gracilipes* development.

In addition, inverse relationships between allochthonous carbon and zooplankton $\delta^{13}C$ have been reported by other studies, for instance, by France *et al*. (France *et al.*,

1997), Jones *et al.* (Jones *et al.*, 1999) and Lennon *et al.* (Lennon *et al.*, 2006). These studies suggest increased importance of an isotopically light carbon source with increasing concentrations of DOC. Lennon *et al.* (Lennon *et al.*, 2006) studied numerous lakes in north-eastern US belonging to a gradient of terrestrial-derived DOC and associated the variability in planktonic δ^{13}C with enhanced CO_2 recycling with increasing DOC concentrations. The rationale behind this suggests that with the increase in DOC, the heterotrophic respiration of terrestrial DOC contributed more to the DIC pool, which in turn led to higher growth of ^{13}C-depleted phytoplankton (France *et al.*, 1997; Lennon *et al.*, 2006). Our study lakes showed a direct relationship between DIC concentrations and $\delta^{13}C_{Bg}$ values (see Tables I and III).

Finally, methane production and subsequent oxidation by methanotrophs provide a highly depleted source of δ^{13}C in lake food webs (Jones *et al.*, 1999; Bastviken *et al.*, 2003; Kankaala *et al.*, 2006). The study lakes have well oxygenated water columns; hence, the potential for this source of carbon to be important in supporting zooplankton is likely limited. However, a recent study by Grossart *et al.* (Grossart *et al.*, 2011) provided direct evidence of the occurrence of microbial methane production under well-oxygenated conditions; in our case, the methanogenesis may be associated with, for instance, cleavage of methyl-esters (methylated substrates) by UV radiation, yet, such an assertion is purely speculative.

To summarize, the relative importance of terrestrial carbon for the support of aquatic food webs has been discussed at length in the recent literature. In this sense, there are a fair number of investigations highlighting the role of allochthonous carbon fuelling zooplankton communities, to different degrees, in temperate and boreal lakes with DOC concentrations between 3.5 and 25 mg L^{-1} (e.g. Jones *et al.*, 1999; Cole *et al.*, 2006; Berggren *et al.*, 2014). Here, we examined the carbon sources of *B. gracilipes* in rather moderate DOM lakes ($<$4.5 mg L^{-1}) and we demonstrated that the carbon isotope composition of the copepod was strongly related to the abundance of some of its putative prey. Particularly, the δ^{13}C values of both *B. gracilipes* and seston significantly decreased with increasing abundances of the mixotrophic nanoflagellate *C. parva* in the phytoplankton pool. We further found that the relative abundance of *C. parva* was directly linked with the amount of allochthonous carbon (DOC) in the lakes in the study period. We conclude that the carbon isotope composition of *B. gracilipes* indicates heavy reliance on autochthonous rather than allochthonous organic matter; essentially, the abundance of mixotrophic algae is the factor that drives the δ^{13}C values of this calanoid copepod in shallow North Patagonian lakes.

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