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Lake size, macrophytes, and omnivory contribute to food web linkage in temperate shallow eutrophic lakes

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Abstract Physical, chemical and biological processes facilitate cross-habitat connections in lakes, prompting food webs to be supported by different subsidies. We tested the hypothesis that the pelagic food web is subsidized by littoral resources and fish foraging behaviour plays a major role in carbon flux and on food web structure in shallow hypereutrophic lakes. We performed a fish diet and carbon and nitrogen isotope analyses to predict the linkage between littoral and pelagic habitats in three shallow hypereutrophic lakes. Lakes differed in morphology, fetch, macrophyte composition and width of the littoral zone. δ^{13}C signals of seston differed among lakes, but were similar to other producers. Macroinvertebrates and fish carbon signatures were more enriched in the lake co-dominated by emergent and submerged vegetation. Fish foraging behaviour indicates that more than the 80% of the carbon that sustain adult fish was channelled from the littoral. In conclusion, littoral carbon were relevant and sustain, in part, food web in these shallow lakes. Factors like the extension of the littoral zone, lake morphometry, and the dominance of multi-chain omnivorous fish facilitate the connection among lake compartments and the transference of littoral carbon to lake food web.

Keywords Carbon stable isotope · Nitrogen stable isotopes · Fish · Macroinvertebrates

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Introduction

Chemical, physical and biological processes contribute to habitat coupling among the distinct compartments of ecosystems and between the ecosystems and the landscape (Schindler & Scheuerell, 2002). Habitat coupling has an impact on different processes and attributes at the population, community and ecosystem level; affecting, for example, prey-predator interactions, nutrient recycling, food web structure and stability, and the flux of carbon and energy in lake ecosystems (Schindler & Scheuerell, 2002; Vander Zanden & Vadeboncoeur, 2002; Vanni et al., 2006; Vander Zanden et al., 2011; Vanni et al., 2013). The degree of habitat coupling in lakes, deep and shallow ones, depends from the interaction of lake characteristics (like morphometry, size, depth, productivity, width of the littoral zone) and several biological processes (like omnivory). Ecological interactions should be more important in lakes having a high perimeter:area relationship. In this sense, small and shallow lakes should have the strongest coupling as their perimeter:area ratio increases as lake area decrease (Schindler & Scheuerell, 2002). Further, fish can play an important role as habitat couplers or integers because they can lead to inter-habitat omnivory (depending on the plasticity of their foraging behaviour) and translocate nutrients from one compartment to another (as are mobile organisms) (Vander Zanden & Vadeboncoeur, 2002; Vadeboncoeur et al., 2005; Vanni et al., 2006). In turn, a high lake perimeter:area ratio and fish multichannel omnivory (Vadeboncoeur et al., 2005) should contribute to cross-habitat connections.

Pelagic food webs can be supported in a variable extent by different subsidies (Grey et al., 2001; Vander Zanden & Vadeboncoeur, 2002; Solomon et al., 2011; Vander Zanden et al., 2011; Wilkinson et al., 2013). The contribution of autochthonous or allochthonous subsidies seems to be related to lake productivity (Wilkinson et al., 2013). In oligotrophic systems, zooplankton could rely on terrestrial and pelagial carbon while zoobenthos and fish on terrestrial and benthic carbon (Solomon et al., 2011). In eutrophic and dystrophic lakes, autochthony is dominant and benthic and littoral resources can support in part zooplankton, zoobenthos and fish communities (Jones & Waldron, 2003; Mendonça et al., 2013; Wilkinson et al., 2013). Fish littoral-benthic reliance average

57% in a multi-lake study and was independent of the morphological and limnological characteristics of lakes, suggesting that benthic production pathways are disproportional important for sustaining fish species diversity in lakes (Vander Zanden et al., 2011).

Shallow lakes are complex ecosystems, and worldwide, many of these lakes are threatened by eutrophication shifting to a turbid state (Moss, 2007). It has been postulated that benthic pathways contribution is maximum in clear oligotrophic lakes while is close to zero in eutrophic lakes (in a range from 0 to 1) (Vadeboncoeur et al., 2008). Even in turbid lakes where benthic contribution is poor, pelagic and littoral food webs could be supported by littoral periphyton and also by detritus and dissolved organic carbon (DOC) derived from macrophytes and periphyton. Therefore, littoral areas can be a tremendous source of detritus and energy to lakes (Jones & Waldron, 2003). In turbid shallow lakes, two main factors can affect littoral contribution to lake food web; on one hand, the reduced water depth facilitates trophic interaction and on the other hand, the light water climate sets limits to littoral and benthic production, allowing the existence of distinct lake compartments despite the reduce depth. The relevance of littoral carbon for the pelagic food web in turbid systems would also be related to other physical and biological factors like lake size, water mixing by wind action, width of the littoral zone, contribution of detritus pathways, and multichain-omnivory by fish. All these factors can interact favouring or preventing not only the pelagic-littoral coupling, but also the stabilization of trophic interactions and food webs, and the trophic position of consumers (Vadeboncoeur et al., 2005; Attayde & Ripa, 2008; Stenroth et al., 2008).

In South America, a bimodal pattern for chlorophyll concentration and the existence of the clear and turbid phases in shallow lakes have been demonstrated (Kosten et al., 2012). In particular, the Pampa plain area is a region rich in shallow polymictic lakes which have been described in a clear or turbid regime, being turbidity the result of suspended organic and/or inorganic material (Quirós et al., 2002; Allende et al., 2009; Kosten et al., 2012). Low Secchi disc depth characterized most of these lakes, which have phytoplankton assemblages adapted to low light penetration and to water mixing to access light. As a consequence, in pampean shallow lakes light penetration limits the composition of the phytoplankton

community and the distribution of macrophytes (Diovisalvi et al., 2015; Izaguirre et al., 2015). Also in subtropical systems the degree of omnivory displayed by neotropical fish is far greater than in cold systems (Teixeira-De Mello et al., 2009; Colautti et al., 2015). All these characteristics make these eutrophic turbid shallow lakes an interesting model with the potential to clarify the importance of littoral carbon for pelagic food webs and the relevance of lake size, macrophyte type and littoral structure on food web subsidies and trophic position of consumers.

We combined carbon and nitrogen isotope with conventional fish gut analyses plus satellite image analyses to determine the relevance of the littoral area, lake size and fecht on the degree of linkage between the littoral and pelagic systems. We tested the hypothesis that the pelagic food web is subsidized by littoral resources and fish foraging behaviour play a major role in carbon flux and on food web structure in shallow eutrophic lakes. We aimed to establish: (1) the relevance of pelagic and littoral carbon sources to lake food web and to the dominant pelagic fish species, (2) the role of fish as integers of food webs, (3) fish trophic position, and (4) the factors that might affect carbon flux in shallow lakes.

Methods

Study area

We studied three shallow lakes located in the Pampa Plain area (Buenos-Aires Province, Argentina, South America): Lake Nahuel Rucá (37°37′S; 57°26′W), Los Carpinchos (37°03′S, 57°19′W) and Las Mostazas (37°10′S, 57°15′W). This area is scattered by shallow lakes and constitutes one of the largest area of wetlands of South America. Pampean lakes are shallow systems (1–3 m), in eutrophic state and most of them present a turbid regime. Eutrophic conditions are associated to land use, mainly to agriculture which has been intensified since nineteenth century (Quirós et al., 2002; Diovisalvi et al., 2015). These lakes behave as warm polymictic, having higher total phosphorous, nitrogen, and chlorophyll *a* concentration, and lower Secchi Disc transparency than lakes located in other temperate regions. Therefore, they stand at one extreme of the trophic state continuum (Diovisalvi et al., 2015).

Sample collection and determination of limnological parameters

Samples were collected during fall, spring and summer of 2009 in Nahuel Rucá, 2009/2010 in Los Carpinchos and 2010/2011 in Las Mostazas.

Periphyton and macrophytes were collected at four different sites of the littoral zone in each survey.

Bulk water samples for limnological and isotope analyses were collected at 6 random sampling points in open waters using a van Dorn bottle, integrating 3 hauls from a depth of 1 m to the surface, and combined in two composite water samples for analyses. Chlorophyll *a* (Chl*a*) and total suspended solids (TSS) were determined after filtering 500 ml (or less, depending the lake) through Sartorius® MGC glass-microfibre filters. Chl *a* was extracted in 90% acetone and concentration was calculated according to the monochromatic method (Lorenzen, 1967). The precombusted Sartorius® MGC glass-microfibre filters were oven dried at 105°C for 1 h and, after that, ashed at 500°C during 1 h for the determination of TSS and its organic fraction, respectively (Wetzel & Likens, 1991; Apha, 1992). Total phosphorus (TP) and total nitrogen (TN) concentration were determined by standard methods after digestion with potassium persulphate at 125°C and 1.5 atm for 1 h (Valderrama, 1981; Apha, 1992). Bicarbonate and carbonate concentration was estimated in the field by titration with HCl (0.1 mol l^{-1}). pH and conductivity were recorded also in the field using a Hanna™ pHmeter and conductivimeter, respectively.

Zooplankton was obtained from vertical hauls from the bottom to the surface using a plankton net (mesh size: 50 μm). Macroinvertebrates and small littoral fish species were sampled using a dip net, sweeping littoral plants and lake bottom. Open water and benthic fish were captured by means of a rectangular funnel fish trap (1 m height, with lateral wings) placed in open waters during 12 h (Colautti, 1998).

The composition of the littoral macrophyte assemblage and the PVI (percent of volume occupied) of submerged macrophytes in the littoral zone was determined during invertebrate sampling in summer by means of 1–3 shore to shore transect samplings, depending on lake area and depth. Depth was also recorded. Lake surface and macrophyte cover was estimated from Landsat satellite imagery (http://earthexplorer.usgs.gov). Landsat 5 images were used

from dates close to spring or summer field work (Lake Carpinchos: LT52240862010015COA03, Lake Las Mostazas: LT52240862010255CUB01, and Lake Nahuel Rucá: LT52240862009268COA02). Lake boundary, macrophyte and open water covers were visually interpreted from the images based on field transect data and digitized on-screen in order to determine littoral area, maximum length, fetch (considering the predominant wind: South-Southeast) and total area for each lake.

Isotope sample preparation and analyses

For dissolved inorganic carbon (DIC) isotope analyses 10 ml of pre-filtered water was injected in an evacuated flask with silicone cap, prefilled with 2 ml of 85% H_3PO_4. The resulting CO_2 was cryogenically purified in laboratory.

Seston, periphyton and macrophyte samples were processed immediately and frozen. Seston was sampled by filtering lake water onto precombusted Sartorius MGC glass-microfibre filters. Periphyton was collected by shaking submerged macrophytes or the submerged portion of emergent plants into algae free water; afterward, the suspension was passed though precombusted Sartorius MGC filters. Macrophytes and emergent plants were rinsed with deionized water. The periphyton composition of each lake is described in Table 1.

Individuals of the dominant taxa from zooplankton and macroinvertebrates were maintained alive in filtered lake water for 4 and 24 h, respectively, to evacuate gut content. Subsequently, invertebrates were rinsed with deionized water. Two to three composite samples were prepared containing 200–700 individuals of zooplankton and 5–40 macroinvertebrate individuals. Cladocerans dominated the zooplankton assemblage, which composition is described in Table 1.

Small littoral fish species were processed as whole individuals. For large fish species, a small portion of muscle tissue was taken from either side of the dorsal fin above the lateral line.

All samples were oven dried at 60°C between 24 (periphyton, seston) to 48 h (all the animals). Dried samples of amphipods, *Palaemonetes argentinus* (Nobili, 1901) (grass shrimp) and gasteropods were further acidified with 1 M HCl, to remove inorganic

carbonates and oven dried again. All the samples were pulverized and stored frozen until isotope analyses.

For isotope and elemental composition analyses, samples were weighted, loaded in tin capsules and combusted on a Carlo Erba® Elemental Analyser, EA (CHONS) (Costech Technologies, Quebec, Canada) coupled to a Finnigan MAT Delta V® (Thermo Scientific, Bremen, Germany) continuous-flow isotope ratio mass spectrometer (CF-IRMS) through a Thermo ConFlo IV® (Thermo Scientific, Bremen, Germany) interface using internal standards. Helium was used as carrier gas. A working standard, pure CO_2 was measured prior to each sample. Three internal calibrated reference standards covering the entire ^{13}C range of the samples were also measured. Final results are expressed as $\delta^{13}C$ and $\delta^{15}N$ defined as

$$^{13}C = \left[\left(^{13}C/^{12}C\right)_{sample} / \left(^{13}C/^{12}C\right)_{V-PDB} - 1 \right] \times 1000 \qquad (1)$$

$$^{15}N = \left[\left(^{15}N/^{14}N\right)_{sample} / \left(^{15}N/^{14}N\right)_{AIR} - 1 \right] \times 1000, \qquad (2)$$

where $\delta^{13}C$ and $\delta^{15}N$ are the isotopic deviations in ‰; V-PDB is the international standard for carbon (Gonfiantini, 1978) and AIR for nitrogen (Coplen et al., 1992). For $\delta^{13}C$ determination of DIC in lake water samples, the resulting purified CO_2 was measured against Carrara Marble CO_2 in a dual inlet McKinney type Finnigan-Delta-S IRMS (Finnigan, Bremen, Germany). The isotopic deviation was expressed as in Eq. 1, considering the corresponding standard. The standard uncertainty was ± 0.1‰ for both isotopes.

Fish diet analysis

To obtain the high level of resolution required to discern complex trophic interactions, stable isotopes must be used in conjunction with other information, such as direct diet analyses (Post, 2002; Vander Zanden & Vadeboncoeur, 2002). Fish diet was analysed to understand C transference between the littoral and pelagic habitat. As only the fish inhabiting the pelagic could act as potential integers, the following fish species were selected: *Oligosarcus jenynsii*

Table 1 Mean limnological parameters, composition of isotopic samples, C:N ratio of primary producers, and $\delta^{13}C$ signatures of DIC (dissolved inorganic carbon) from open waters of Lake Los Carpinchos (LC), Nahuel Rucá (NR) and Las Mostazas (LM) during the studied period

	Los Carpinchos	Nahuel Rucá	Las Mostazas	$F_{2,7}$; P	Lake comparison
Limnological parameters					
Surface (km^2)	0.60	3.59	1.75		
Fetch (km)	0.65	1.15	1.40		
Depth (m)	1.10	0.79	1.09	4; = 0.05	
Maximum length (km)	0.82	3.2	1.7		
Conductivity (mS cm^{-1})	1.54	0.78	3.33	16; < 0.01	NR < LC, LM
pH	8.24	8.05	9.71	20; < 0.01	LM > LC, NR(P < 0.05)
Carbonates (mg l^{-1})	0	0	174.00		
Bicarbonates (mg l^{-1})	722.67	849.00	664.27	1, > 0.05	
TP (µg l^{-1})	455	661	328	0.26; > 0.05	
TN(µg l^{-1})	8256	7397	7026	1.7; > 0.05	
TSS (mg l^{-1})	24.24	101.79	42.85	13; < 0.01	NR > LC, LM
Chlorophyll a (µg l^{-1})[a]	51.16	41.39	96.23	1; > 0.05	
Secchi disc depth (m)	0.44	0.16	0.28	4; > 0.05	
Emergent macrophyte cover (%) (S. californicus)	37	54	15		
Submerged macrophytes PVI (%)	NP	NP	60		
Isotopic signals					
$\delta^{13}C$ DIC fall (‰)	− 5.4	–	− 5.3		
$\delta^{13}C$ DIC spring (‰)	− 9.2	− 9.6	− 7.6		
Sample composition					
Periphyton	Diatoms	Diatoms & Chlorophytes	Chlorophytes		
Zooplankton	Cladocera: *Daphnia spinulata* (Birabén, 1917), *Ceriodaphnia* sp., *Bosmina huaronensis* Delachaux, 1918, *Diaphanosoma* sp. Copepoda: *Notodiaptomus incompositus* (Brian, 1925), *Acanthocyclops* sp.	Cladocera: *Moina micrura* Kurz, 1874, *Ceriodaphnia* sp., Copepoda: *N. incompositus*, *Acanthocyclops* sp.	Cladocera: *M. micrura*, *Ceriodaphnia* sp., *Diaphanosoma* sp. Copepoda: *N. incompositus*, *Acanthocyclops* sp.		

Table 1 continued

	Los Carpinchos	Nahuel Rucá	Las Mostazas	$F_{2,7}$; P	Lake comparison
C:N ratio					
Schoenoplectus	137 ± 32	67 ± 1	109.6 ± 32		
Ceratophyllum	–	–	16.9 ± 1.6		
Periphyton	8.7 ± 0.2	12.4 ± 0.6	12.7 ± 2.6		
Seston	8.7 ± 2.7	9.5 ± 1.5	9.3 ± 1.3		

Significance (P level) and F values with the associated degrees of freedom (except for Chlorophyll) corresponding to One Way-ANOVA and pos hoc comparisons among lake parameters are showed

TP total phosphorous concentration, *TN* total nitrogen concentration, *TSS* total suspended solids, *NP* not present, – not determined

[a] Degrees of freedom for chlorophyll = 2, 11

(Günther, 1864), *Odontesthes bonariensis* (Cuvier & Valenciennes, 1835), *Cyphocharax voga* (Hensel, 1869) and *Rhamdia quelen* (Quoy & Gaimard, 1824). A total of 370 gut contents (stomach plus intestine) were examined under a stereoscopic microscope and the abundance of the organisms consumed was determined. The contribution of sediment and detritus to the total mass ingested was estimated by a modification of the weight difference determination method (Bowen, 1979; Ahlgren & Bowen, 1992). A subset of individuals (at least 30–50) of each prey taxon was measured under a microscope (total length or head capsule width) to estimate dry mass from published length-mass regressions (Dumont et al., 1975; Bottrell et al., 1976; Herman & Heip, 1982; Benke et al., 1999).

Data analysis

In order to establish the contribution of littoral and pelagic potential C-sources from primary consumer to lake food web, the Bayesian stable isotope mixing model SIAR v4 (R package Stable Isotope Analysis) (Parnell et al., 2010) was run. The upper and lower limits of the contribution of carbon sources were estimated for each invertebrate species in each lake. The mean trophic fractionation and standard deviation used was 0.4‰ (1 SD = 1.3‰) for δ^{13}C and 3.4‰ (1 SD = 1‰) for δ^{15}N (Post, 2002). The number of iterations for the model to run was 500,000 and the initial iterations to discard were 50,000. The performance of the model, indicated by a low correlation between sources, was checked by a diagnostic matrix plot showing the correlation coefficients and how pairs of posterior distributions were correlated. A high

negative correlation indicates that the model is struggling to differentiate between sources having similar and close signals.

In addition, to establish the relevance of littoral and pelagic carbon to the dominant fish species, fish diet data (see section above) and the elemental composition of each prey were used to estimate the contribution on C of each prey type to the total biomass ingested by fish. As animal are homeostatic respect to their elemental composition (Vanni et al., 2002), carbon body content (% of dry mass) was obtained from the literature (Andersen & Hessen, 1991; Vanni et al., 2002; Frost et al., 2003; Mcintyre et al., 2008; Small et al., 2010; Córdoba, 2012). Once calculated the total C contribution of each prey, the source of C was classified into littoral, benthic or pelagic carbon depending on prey habitat use (González Sagrario & Balseiro, 2010) (Table 2). Finally, fish foraging habitat revealed by fish diet allowed to determine the role of the dominant fish species as integers of lake food webs.

In order to determine fish trophic position and compare food web structure in each lake, the trophic position of each fish species was estimated normalizing the stable isotope data to a common littoral and pelagic baseline according to Post (2002):

$$\text{TP} = \lambda + (\delta^{15}\text{N}_{\text{fish}} - [\delta^{15}\text{N}_{\text{littoral}} \times \alpha + \delta^{15}\text{N}_{\text{pelagic}} \times (1-\alpha)])/\Delta_n, \quad (3)$$

where TP is the trophic position; λ is the trophic position of the organism used to estimated δ^{15}N$_{\text{littoral \& pelagic}}$ (in this case 2); δ^{15}N$_{\text{littoral}}$ is the baseline signal of the most abundant gastropod; δ^{15}N$_{\text{pelagic}}$ is

the baseline signal of zooplankton, and Δ_n is the enrichment in $\delta^{15}N$ per trophic level (3.4‰). α is the proportion of nitrogen in the consumer derived from the littoral food web and was calculated as

$$\alpha = (\delta^{13}C_{fish} - \delta^{13}C_{pelagic})/(\delta^{13}C_{littoral} - \delta^{13}C_{pelagic}). \quad (4)$$

To understand which factors might affect carbon flux in lakes, One-Way ANOVA and post hoc comparisons, performed by Tukey HSD test, (Crawley, 2007) were used to determine differences/similarities in chemical parameters between lakes using R (R Development Core Team https://cran.r-project.org/).

Finally, the $\delta^{13}C$ signature of phytoplankton was estimated in order to discriminate if the $\delta^{13}C$ of seston corresponded to a pelagial community or represented the signal of phytoplankton plus detritus/algae from other compartments. This information contributes to understand the carbon flux and mixing process in lakes. The $\delta^{13}C$ signature of phytoplankton was calculated considering the $\delta^{13}C$ of dissolved inorganic carbon (DIC) minus the fractionation (Δ) that occurred during photosynthesis ($\delta^{13}C_{phytoplankton} = \delta^{13}C_{DIC} - \Delta_{photosynthesis}$). The range of Δ considered was 20 and 29‰ (Goericke et al., 1994).

Results

Limnological characteristics

Table 1 summarizes the limnological characteristics of the studied lakes. The three lakes share several characteristics like their shallowness ($\sim$ 1 m), a hypereutrophic status according to chlorophyll a, total phosphorous (TP), and nitrogen (TN) concentration. All the lakes had high bicarbonate concentration, alkaline waters (pH varied between 8.0 and 9.7), low conductivity, and low Secchi disc depth. Lakes differed in surface, fetch, total suspended solids concentration (TSS), the width of the littoral zone and macrophyte composition. The highest pH values were recorded in Las Mostazas and the lowest conductivity in Nahuel Rucá. Lake Nahuel Rucá had the highest surface while Las Mostazas doubled the fetch of Los Carpinchos. Turbidity also differed among lakes, being TSS the highest in Nahuel Rucá. The distribution of emergent macrophytes in a ring fashion along the shore allowed the distinction between a well-defined littoral area from the pelagic zone. The littoral zone reached different extents, occupying the largest area in Nahuel Rucá. This area was dominated by the giant bulrush (*Schoenoplectus californicus* (C. A. Méy.) Soják) in all the lakes. Submerged macrophytes (*Ceratophyllum demersum* L. and *Myriophyllym* sp.) only occurred within the littoral area of Las Mostazas.

Table 2 Prey considered as a source of littoral, benthic or pelagic carbon preyed by fish inhabiting the pelagic in lakes Los Carpinchos, Nahuel Rucá and Las Mostazas

Pelagic	Source of carbon	
	Littoral	Benthic
Cladocera: (*Ceriodaphnia dubia* Richard, 1894, *Moina micrura*, *Bosmina huaronensis*, *Diaphanosoma* sp.)	Crustacea: Ostracoda, Chidorid Cladoceran, *Hyalella*, adults/juveniles of *Palaemonetes argentinus*	Crustacea: Harpacticoid Copepods, Ostracoda
Calanoid and Cyclopoid Copepods	Gasteropods: *Physa acuta*, *Biomphalaria peregrina*, *Uncancylus*, *Pomacea canaliculata*	Gasteropods: *Heleobia parchapii*
Zoea of *Palaemonetes argentinus*	Insecta: Corixidae, *Bellostoma*, *Cyanallagma bonariense*,	Insecta: Chironomidae
Fish: *Odontesthes bonariensis*, *Oligosarcus jenynsii*, *Cyphocharax voga*	*Aeshna*, Curculionidade, *Berosus*, *Megadytes*,	Fish: *Corydoras paleatus*, *Pimelodella laticeps*
	Ceratopogonidae, Trichoptera	
	Fish: *Cnesterodon desemmaculatus*, *Jenynsia multidentata*, *Astyanax* spp.	

Isotopic analysis

Carbon isotopic signatures of DIC varied seasonally ranging from -9.6 to $-5.3‰$, being ^{13}C-enriched during fall and more ^{13}C-depleted in spring (Table 1).

The δ^{13}C signals of seston and the different primary producers differed among lakes and producer type (Fig. 1). For example, the δ^{13}C signal of emergent macrophyte *S. californicus* was similar among lakes, having depleted values that ranged from -26.4 to $-25.45‰$, whereas the δ^{13}C signal of the submerged macrophyte *C. demersum* was enriched ($-16.35‰$) (Fig. 1, Las Mostazas). The δ^{13}C signals of seston showed different values in the three lakes, being more depleted in Los Carpinchos ($-29.15‰$), close to the emergent macrophyte signal in Nahuel Rucá and highly enriched in Las Mostazas ($-20.93‰$). In addition, only the δ^{13}C-seston of Los Carpinchos represents a planktonic signal as fall in the possible estimated range of δ^{13}C-seston signals of a pelagial community (-36 to $-27‰$), considering the reported mean values of δ^{13}C-DIC and photosynthesis fractionation. δ^{13}C-seston of Las Mostazas and Nahuel Rucá fall out of this range. Besides, seston in Las Mostazas had the highest proportion of organic matter (86%) (One-Way Anova: $F_{2,7} = 24$, $P < 0.001$; Tukey: $P < 0.01$ respect to the other two lakes) (Fig. 1). The carbon-to-nitrogen ratio (C:N) was similar and low (8–12) for periphyton and seston, and higher and variable for the emergent macrophyte *S. californicus* in all the lakes (Table 1).

Periphyton composition (Table 1) and its δ^{13}C and δ^{15}N signals differed among lakes (Fig. 1), ^{13}C-enriched values ($\sim -20‰$) occurred in Los Carpinchos and Las Mostazas while ^{13}C-depleted values in Nahuel Rucá. Periphyton δ^{15}N signatures exhibited great variation, being more enriched in Los Carpinchos than in Nahuel Rucá, and in Las Mostazas.

The δ^{13}C signals of zooplankton exhibited values close to the seston and littoral producers, and also differed among lakes, being more depleted in Los Carpinchos and Nahuel Rucá and highly enriched in Las Mostazas (Fig. 1).

The δ^{13}C signatures of macroinvertebrate species exhibited values in the range of seston and littoral primary producers (*Schenoplectus*, *Ceratophyllum*, periphyton) in all the lakes. However, δ^{13}C signatures of macroinvertebrates differed among lakes, being enriched in the lake with emergent and submerged vegetation and depleted in lakes dominated by emergent vegetation (Fig. 1). This pattern could not be related to the trophic functional group to which the each species belongs as all the groups showed the same pattern of δ^{13}C signature change among lakes. For example, the amphipod *Hyalella* sp. (omnivore) exhibited depleted carbon signatures ($\sim -28‰$) in Los Carpinchos (with only emergent vegetation) and enriched signatures and related to the one of the periphyton in Las Mostazas ($\sim -19‰$). This pattern was found in all trophic functional groups: omnivores (Corixidae), omnivore predators (*P. argentinus*), raptorial predators (the odonates *Aeshna* sp. and *Cyanallagma bonariense* (Ris, 1913)), fluid-feeder predators (the heteropteran *Bellostoma* sp.), and the grazer/detritivore gasteropod *Heleobia parchapii* (d' Orbigny, 1835). Gasteropod species exhibited the depleted δ^{15}N signatures of all macroinvertebrates, ranging from 3.3 to 4.85‰ and could be considered at the base of the food web (Fig. 1).

Stable isotope mixing model (SIAR) shows that the distributions of the contribution of the different C-sources to the diet of zooplankton and littoral macroinvertebrates were similar (Fig. 2). Indeed, the model detects high negative correlation between the posterior distribution of pair of sources in all the ecosystems, indicating that the primary producer's signatures could not be differentiated. For example, the contribution of periphyton was the lowest to the diet of macroinvertebrates and zooplankton from Los Carpinchos (Fig. 2). However, the correlation between periphyton-*Schenoplectus* and seston-*Schenoplectus* was high in all the cases for all consumers (range from -0.7 to -0.4), indicating that macroinvertebrates are incorporating these sources in similar proportions or/and that seston is also rich in detritus derived from *Schenoplectus*. Therefore, isotopic signals are similar and could not be discriminated. Similarly, submerged macrophytes contribution was similar to the other C-sources in Las Mostazas and also highly and negative correlated with seston, periphyton and *Schenoplectus* (range from -0.75 to -0.34) (Fig. 2).

Fish species showed ^{13}C-depleted signatures in Nahuel Rucá and Los Carpinchos, ranging from -28 to $-26‰$, and ^{13}C-enriched signatures in Las Mostazas (from -22 to $-20‰$) (Fig. 1). Fish that depend on basal resources, like zooplankton or periphyton, showed the lowest δ^{15}N signatures [juveniles of *Odontesthes bonariensis* (zooplankton), and
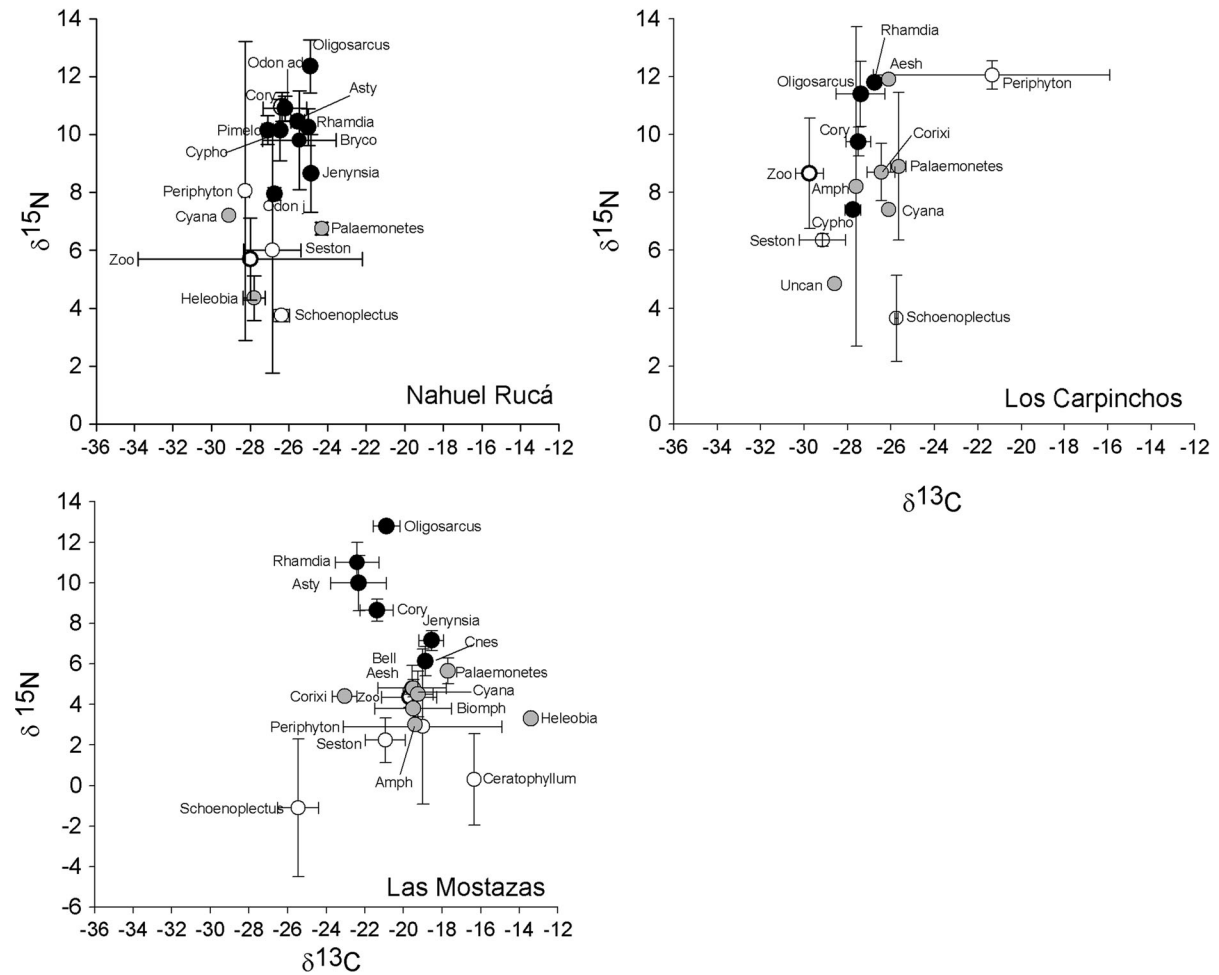
Fig. 1 Carbon and nitrogen isotope signals in food webs of Lake Nahuel Rucá, Los Carpinchos, and Las Mostazas. Circles of different colours represent: the primary producers and seston (white), the zooplankton (white and black), the macroinvertebrates (grey) and the fish (black). Acronyms for: primary producers: Schoenoplectus: *Schoenoplectus californicus*, Ceratophyllum: *Ceratophyllum demersum*; zooplankton: Zoo; macroinvertebrates: Palaemonetes: *Palaemonetes argentinus*, Amph: *Hyalella* sp.(Amphipoda), Uncan: *Uncancylus* sp. (Gasteropoda), Heleobia: *Heleobia parchappii* (Gasteropoda), Biomph: *Biomphalaria peregrina* (d´Orbigny, 1835)

(Gasteropoda),Corixi: Corixidae (Hemiptera), Bell: *Bellostoma* sp. (Hemiptera), Aesh: *Aeshna* sp. (Odonata), Cyana: *Cyanallagma bonariense* (Odonata); littoral small fish: Jenynsia: *Jenynsia multidentata*, Cnes: *Cnesterodon decemmaculatus*, Asty: *Astyanax* sp., Bryco: *Bryconamericus iheringii*; benthic fish: Pimelo: *Pimelodella laticeps*, Cory: *Corydoras paleatus*, Rhamdia: *Rhamdia quelen*; and pelagic fish: Cypho: *Cyphocharax voga*, Odon ad and j: adults and juveniles of *Odontesthes bonariensis*, Oligosarcus: *Oligosarcus jenynsii*. Please, note the different scales of the ordinate axes

Cnesterodon decemmaculatus [Jenyns, 1842] and *Jenynsiia multidentata* [Jenyns, 1842] (periphyton and invertebrates)] (Fig. 1, Las Mostazas and Nahuel Rucá). The δ^{15}N ranged from 8.64 to 11‰ for benthic (B), littoral (L) and pelagic (P) omnivorous fish species (B: *Corydoras paleatus* (Jenyns, 1842*), Pimelodella laticeps* (Eigenmann, 1917)*, R. quelen*; L: *Astyanax* sp., *Bryconamericus iheringii* (Boulemger, 1887), P: *O. bonariensis*). The piscivore

Oligosarcus jenynsii exhibited the highest nitrogen signature. Also, *O. bonariensis* changed its trophic position when adult, incrementing by 0.6‰ the δ^{13}C and by 2.9‰ the δ^{15}N signals (Fig. 1, Nahuel Rucá).

Fish diet and trophic position

Figure 3 presents the relative contribution in abundance of different prey types and basal resources. All
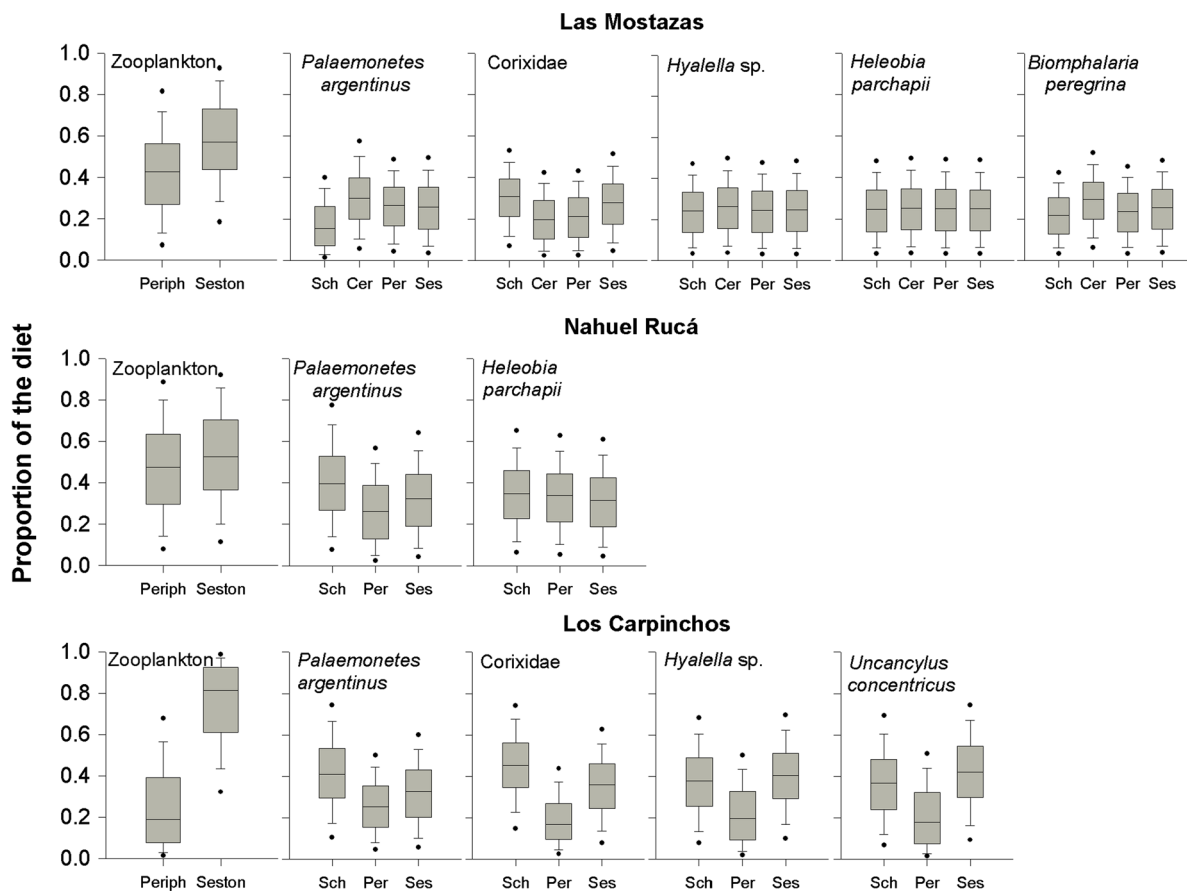
Fig. 2 Potential proportion of contribution of the different carbon sources for zooplankton and littoral macroinvertebrate diets in each lake. The contribution was calculated using the Mixing Isotope Model in R (SIAR v4, Parnell et al. 2010). The boxes showed the mean proportion of contribution to the diet and the 5, 25, 75 and 95% credibility intervals

the fish species inhabiting the pelagic, behaved as omnivores preying on more than one trophic level. All fish species resulted eurytopic, exploiting pelagic, benthic and littoral resources, except *C. voga* which forages mainly on the benthic habitat. For example, *O. jenynsii* preyed upon littoral small fish and macroinvertebrates as adults and mostly upon zooplankton during the juvenile stage. *Odontesthes bonariensis* preyed mostly on zooplankton, but also consumed zoobenthos (mainly gasteropods) while *C. voga* consumed detritus and zoobenthos. The catfish *R. quelen* exploited all habitats and prey types, showing the broadest diet spectrum, and also consumed all the fish species available in the lakes. *Rhamdia*, *Odontesthes* and *Oligosarcus*, shared several resources like small littoral fish species (*Astyanax* sp.) and littoral and benthic macroinvertebrates, specially the grass shrimp

P. argentinus, the amphipod *Hyalella* sp. and chironomid larvae.

For all fish species, the mean abundance contribution of pelagic, littoral and benthic resources showed contrasting patterns when compared with mean carbon contribution (Fig. 4). Likely, the contribution of pelagic prey on abundance exceeds the 80% for *Oligosarcus* and *Odontesthes*, but the major origin of the carbon consumed was littoral (represented by littoral macroinvertebrates and small fish). In the case of *R. quelen*, the 60% of the carbon ingested is derived from the pelagic fish *C. voga* and *O. jenensii*, which in turn depended on benthic and littoral resources, respectively. Accordingly, fish in the pelagic habitat is sustained, directly or indirectly, by littoral or benthic resources. In particular, more than the 80% of the carbon that sustain adult *Oligosarcus*, *Odontesthes* and *Rhamdia* is channelled from littoral resources and
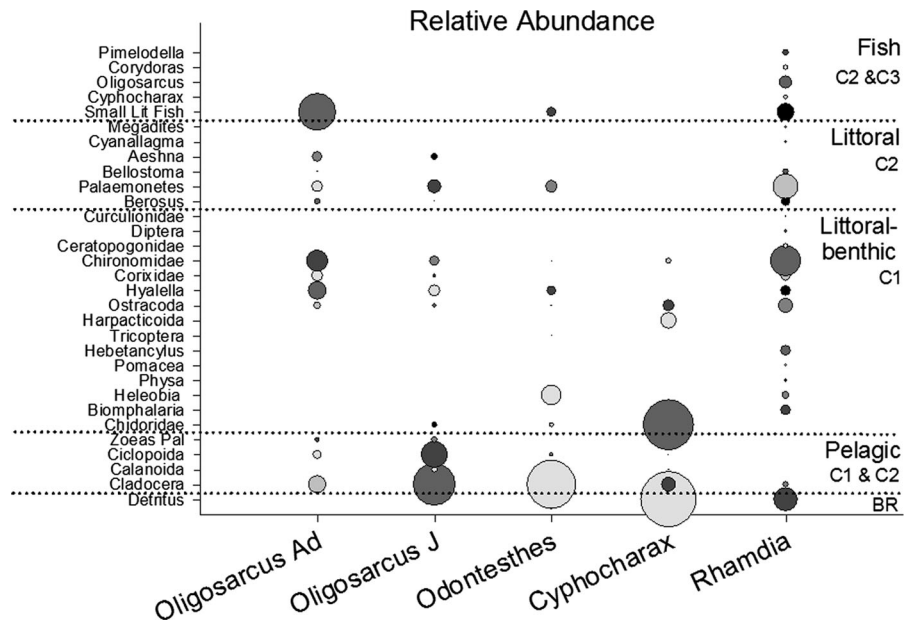
Fig. 3 Relative abundance of prey and basal resources, represented as bubble size, for the dominant fish species found in open waters in Lake Nahuel Rucá, Los Carpinchos and Las Mostazas. Size of bubbles is comparable among prey for each fish species. Dotted lines separate lake habitats, fish prey and type of resources consumed. Acronyms for resources: BR: basal resources, C1: primary consumer, C2: secondary consumer, C3: tertiary consumer, Pimelodella: *Pimelodella laticeps*, Corydoras: *Corydoras paleatus*, Oligosarcus: *Oligosarcus jenynsii*, Cyphocharax: *Cyphocharax voga*, Small Lit fish: *Jenynsia multidentata*, *Cnesterodon decemmaculatus* and *Astyanax* sp., Cyanallagma: *Cyanallagma bonariense*, Palaemonetes: *Palaemonetes argentinus*, Pomacea: *Pomocea canaliculata* (Lamarck, 1828), Physa: *Physa acuta* (Draparnaud, 1805), Heleobia: *Heleobia parchappii*, Biomphalaria: *Biomphalaria peregrina*. Acronyms for predators: Oligosarcus Ad: *O. jenynsii* adults, Oligosarcus J: *O. jenynsii* juveniles, Odontesthes: *O. bonariensis* adults, Cyphocharax: *C. voga* adults, Rhamdia: *R. quelen* adults

almost more than the 90% of the carbon consumed by *C. voga* belongs to the benthic habitat.

Estimated trophic position of fish species differed among lakes (Fig. 5), being higher in Nahuel Rucá than in the other lakes and lowest in Las Mostazas. The pelagic fish *O. jenynsii* had the highest trophic position in all lakes and the species that included detritus in its diet, like *Cyphocharax*, *Corydoras* or *Pimelodella*, had a lower trophic position than carnivorous species (*Astyanax*, *Jenynsia*, *Oligosarcus*). *Jenynsia multidentata*, a littoral fish, had a high trophic position similar to the one estimated for the pelagic carnivore *O. jenynsii*.

Discussion

The complemented isotope and fish diet analyses have revealed that littoral carbon are relevant and sustain in part the pelagic food webs in shallow temperate hypereutrophic lakes, supporting directly or indirectly fish biomass. Furthermore, our results stressed the role of omnivory and fish foraging plasticity in cross-habitat connections in shallow hypereutrophic lakes.

In this study, the isotopic signatures of primary producers and seston could not be differentiated by the mixing isotope model, implying that these signals were similar. Indeed, in the lakes with highest surface, macrophyte cover and fetch, $\delta^{13}C$-seston signatures felt out of the possible range of $\delta^{13}C$ signature for phytoplankton, being more enriched than expected for a pelagial signal in relation to the measured $\delta^{13}C$-DIC (Nahuel Rucá and Las Mostazas). On the contrary, the most pelagial, and depleted $\delta^{13}C$ seston signature ($\sim -29‰$) occurred in Los Carpinchos, the smallest lake with almost half of the macrophyte cover and fetch than the other lakes (Table 1). Enriched $\delta^{13}C$ seston signatures could be the result of different mechanisms like climatic events (monsoons) that supplies allochthonous materials (Lee et al., 2013),
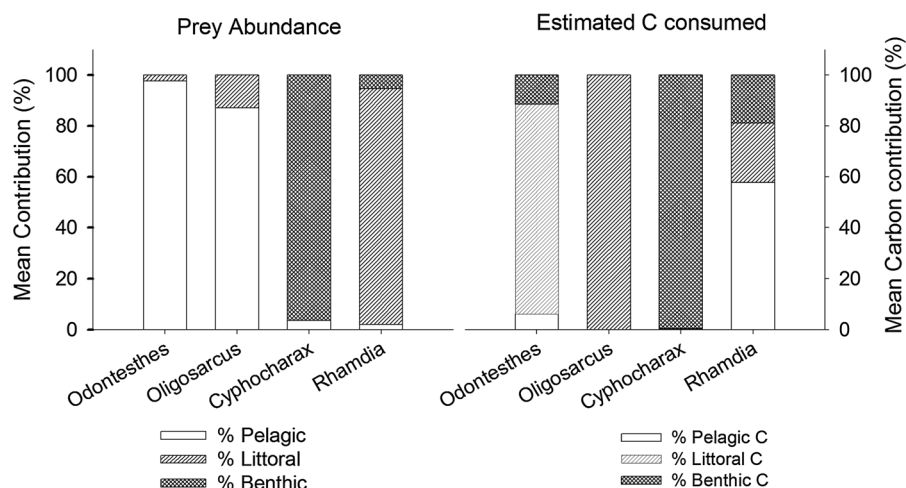
Fig. 4 Contribution of pelagic, littoral and benthic resources to total abundance of prey consumed (left panel) and contribution of estimated carbon consumed from the different lake zone (right panel) for the dominant fish species inhabiting the pelagic in Lake Nahuel Rucá, Los Carpinchos and Las Mostazas. Acronyms: Oligosarcus: *Oligosarcus jenynsii*, Odontesthes: *Odontesthes bonariensis*, Cyphocharax: *Cyphocharax voga*, Rhamdia: *Rhamdia quelen*
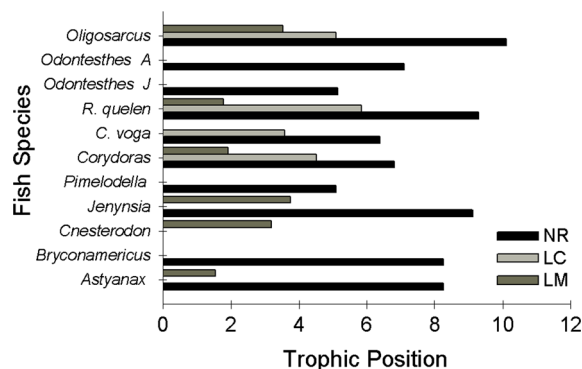
Fig. 5 Trophic position of fish species in Lake Nahuel Rucá (NR), Los Carpinchos (LC) and Las Mostazas (LM). Acronyms: Oligosarcus: *Oligosarcus jenynsii*, Odontesthes: adults (A) and juveniles (J) of *Odontesthes bonariensis*, R. quelen: *Rhamdia quelen*, C. voga: *Cyphocharax voga*, Corydoras: *Corydoras paleatus*, Pimelodella: *Pimelodella laticeps*, Jenynsia: *Jenynsia multidentata*, Cnesterodon: *Cnesterodon decemmaculatus*, Bryconamericus: *Bryconamericus iheringii*, and Astyanax: *Astyanax* sp

reduced fractionation at high algae cell number or gross rates or the switch to utilizing HCO_3^- as CO_2 concentration is depleted (France et al., 1997). $\delta^{13}C$ seston signatures did not show large variations for each lake; therefore, the enriched values observed could not be related to reduced fractionation or competition for CO_2 with emergent macrophytes (which dominated all lakes) as macrophytes can use atmospheric CO_2. For these hypereutrophic lakes, the most plausible explanation is that the phytoplankton signature must have been masked by the contribution of littoral detritus, as is usually enriched in ^{13}C relative to phytoplankton. The same mechanism has been suggested for other hypertrophic lakes with a ratio of littoral to pelagic high and a littoral zone dominated by emergent macrophytes (Grey et al., 2000). Our evidence, as other from South American shallow lakes, indicates that the discrimination of the different $\delta^{13}C$ signatures of primary producers is difficult due to wind mixing that renders a seston composed by phytoplankton plus littoral-derived detritus (Mendonça et al., 2013). Therefore, lake morphology, fetch and an extended macrophyte cover must have contributed to a seston subsidized by littoral inputs, and also suggests the relevance of the macrophyte-periphyton association as a source of littoral carbon to lake food web. In our study, water chemical composition, light climate and the $\delta^{13}C$-DIC in the water was similar among lakes as well as periphyton and seston C:N ratios; however, signatures of the whole food web differed among lakes, showing more depleted values when emergent macrophytes dominated the littoral area and enriched values when submerged macrophytes occurred (Fig. 1). Experimental studies have shown how the detritus originated from different macrophytes composed the seston without fractionation or change on the $\delta^{13}C$ respect the macrophyte from which was originated, but with a change on the

C:N ratio (Fellerhoff et al., 2003). For this reason, C:N ratio could not be a marker of detritus origin. In fact, macrophytes can contribute substantially to the total organic carbon content of lake sediments (Aichner et al., 2010), that once resuspended conform the particulate organic matter pool of lakes. As a consequence, we suggest that shallowness ($\sim$ 1 m), wind action (fetch), and the extension and composition of the littoral zone (that might favor periphyton development) could have an impact on the functioning of these shallow hypereutrophic lakes, facilitating part of the carbon flux between littoral and pelagic compartments throw the homogenization of pelagial and littoral carbon fractions.

Besides morphologic and geometric characteristics of lakes, habitat coupling it is also facilitated by mobile organisms as fish (Schindler & Scheuerell, 2002). It has been argued that pelagic food webs must have been subsidized by autochthonous and/or allochthonous resources (Polis et al., 1997). Several years after, it has been recognized that benthic, littoral or even terrestrial subsidies support lake ecosystems (Vanni et al., 2006; Solomon et al., 2011; Vander Zanden et al., 2011; Wilkinson et al., 2013). For example, terrestrial contribution is low in eutrophic and distrophic lakes, being autochthonous resources the main subsidies for pelagic consumers (Wilkinson et al., 2013). Carbon subsidies (periphyton, macrophytes, phytoplankton) might be different between lake types (shallow, deep, floodplain lakes). Indeed, fish relied in a 50% on benthic secondary production in deep lakes (Vander Zanden & Vadeboncoeur, 2002) while reliance on periphyton production ranged between 0 and 45% in shallow lakes (Jones & Waldron, 2003). In some cases, benthic algae plus terrestrial organic matter supported fish production in floodplain lakes, while on other cases macrophytes are involved in supplying carbon to aquatic consumers (Herwig et al., 2004; Zhang et al., 2013; Saigo et al. 2016). In our study, fish diet analyses revelled the relevance of the littoral zone as a foraging area and a source of carbon. Indeed, fish diet analyses showed that all fish species inhabiting the pelagic zone were eurytopic, exploiting pelagic, benthic and littoral resources, except *Cyphocharax voga* which forages mainly on the benthic habitat. All fish species preyed upon several trophic levels displaying a widespread omnivory. Even when pelagic resources seem to be the major contribution to the diet in terms of abundance,

the pattern based on biomass and carbon contribution was the opposite. The estimated contribution of littoral carbon accounted over the 80% of the biomass ingested by pelagic fish species through the consumption of littoral macroinvertebrates and/or small littoral fish species like *Cnesterodon*, *Jenynsia* or *Astyanax* (Figs. 3, 4). The only pelagic fish that did not rely on the littoral habitat was *C. voga*, which depended mostly on benthic resources: detritus, chydorids and chironomids, representing the 90% of the carbon consumed (González Sagrario & Ferrero, 2013). Macroinvertebrates and small littoral fish species showed a distribution completely associated to littoral macrophytes, without displaying any pattern of horizontal migration to open waters (González Sagrario & Balseiro, 2010), which indicates that the ultimate source of carbon for them is littoral primary producers or them derived detritus. In fact, all the fish species in these lakes (*Cyphocharax*, *Odontesthes*, *Oligosarcus* and *Rhamdia*) have been acting as integers of lake compartments and behaving as multi-chain omnivores, deriving energy directly from the benthic/littoral consumers. Meanwhile mixing isotope model could not identify the exact contribution of each primary producers, fish diet analyses revealed a fish reliance on littoral prey higher than the 80%, which supports evidence of a carbon flux from the littoral to the pelagic food web.

Multi-chain omnivory has stabilizing effects on food webs similar to those of single chain omnivory (Vadeboncoeur et al., 2005). Omnivorous organisms are favoured as food abundance is always higher at lower trophic levels and their dietary plasticity allow them to survive in changing environments and thus, under fluctuating resources. Pampean lakes are examples of fluctuating environments, subject to recurrent floods and drought periods, displaying considerable inter-annual differences in lake area and water column depth (Bohn et al., 2011; Diovisalvi et al., 2015). All these features make these lakes vulnerable to the colonization of omnivore species which dominate the composition of food webs. Although all species behave as multi-chain omnivores, different trophic positions have been recorded for each fish species across lakes, indicating changes on food web length. Food web can be lengthened in two different ways: (1) increasing diversity and thus leading to an increase number of links, or (2) omnivores can shift diet and increase its feeding to a higher trophic level. More

productive systems will sustain more basal resources; therefore, will be more energy available to add another trophic level at the top of the food chain [productivity hypothesis, (Oksanen et al., 1981)]. Several findings demonstrate that species can change from herbivorous to omnivorous and finally to a carnivorous type of feeding along a productivity gradient (Stenroth et al., 2008; France, 2012). In this study, the trophic position of shared fish species among these lakes follows the pattern NR > LC > LM (Fig. 5), even though lakes share the same trophic status and have similar nutrient levels. Therefore, the change in trophic position cannot be subscribe to an increase diversity due to a higher productivity, but must be related to a differential accessibility to food resources. Submerged macrophytes function as refuge areas for macroinvertebrates and small littoral fish species in these lakes (González Sagrario & Balseiro, 2010); thus, the access to more mobile organisms such as small fish prey become very difficult when the lake is colonized by submerged macrophytes. Consequently, the decrease in trophic position in Las Mostazas might be related with the structure provide by submerged macrophytes in the littoral zone that force omnivorous species to prey on more accessible prey belonging to lower trophic levels. Indeed, a diet shift rather than an increase number of trophic links might be responsible for the increase of fish trophic position in Nahuel Rucá and Los Carpinchos respect to Las Mostazas, as emergent macrophytes provide less refuge than submerged macrophytes (Dionne et al., 1990). Habitat quality is an influential factor for differences in diet and trophic position in omnivores (Stenroth et al., 2008). Inter-site differences in food web properties are probably best explained as resulting from environmental differences rather than mathematical/demographic epiphenomena (Winemiller, 1990). As an example, the degree of omnivory and the trophic position of lake trout in deep lakes changed depending on habitat accessibility on lakes with different morphometry (Dolson et al., 2009).

Lake size can influence physical response of lakes which also have an effect on different community attributes (Read & Rose, 2013). The estimated mixing depth for the studied lakes ($\sim$ 4 m) according with lake mixing model (Fee et al., 1996) exceeds the mean depth of them ($\sim$ 1 m), indicating that all these systems are highly mixed. According with lake fetch, the degree of mixing was higher in the larger lake without submerged vegetation (Nahuel Rucá) than in the small one (Los Carpinchos) or in the lake colonized also by submerged plants (Las Mostazas). In concordance with these facts, periphyton $\delta^{13}C$ signatures were depleted ($-$ 28‰) in the largest lake with the maximum fetch (Nahuel Rucá), while highly enriched ($\delta^{13}C \sim -$ 19‰) in the smallest lake or the one colonized by submerged macrophytes (Table 1, Fig. 1). Our results show that wind action in Nahuel Rucá assured the replenishment of ^{12}C in the boundary layer surrounding periphyton algae, rendering depleted signals in opposition to calm systems where signatures were enriched (Los Carpinchos, Las Mostazas). In concordance with our results, more negative $\delta^{13}C$ values were associated with plant growing in fast moving water (Osmond et al., 1981; Keeley & Sandquist, 1992). In some cases, different $\delta^{15}N$ signatures among lakes and large standard values were recorded for periphyton signals. These differences could be related to algae composition and its associated fractionation, the degree of mixing in the environment and/or reflect the signal of the $\delta^{15}NO^3$ in the water (De Brabandere et al., 2007). For example, diatoms have a higher fractionation factor for nitrate uptake (Δ: 9–12‰) than chlorophytes (Δ: 2–3‰) and enriched signatures in unstirred than in well-mixed cultures (Goericke et al., 1994), and periphyton had enriched signals under reduce flow in macrophyte beds (Goericke et al., 1994; De Brabandere et al., 2007). As the periphyton was exposed to different flow regimes and its composition varied during the sample period and among lakes (Table 1), the pattern found must be related to the associated fractionation of each group and water stability conditions.

Conclusions

Several physical, morphologic and biological factors interact and determine the relevance of the littoral subsidy in shallow lakes. In this case, we found that the combination of an extended littoral zone, macrophyte type, lake size, high fetch and the dominance of omnivorous fish render a high connection among lake compartments and a relevant transference of littoral carbon to pelagic food webs. In these systems, primary producer C-signature could not be differentiated, suggesting that lake morphology and the extended littoral areas are the principal factors that contribute to

a seston composed by a mix of phytoplankton and littoral detritus. Fish diet analyses demonstrated that all fish inhabiting the pelagic depended in a high extent on littoral or bentic carbon through the consumption of littoral/benthic macroinvertebrates and fish. Therefore, littoral carbon supports in part the pelagic fish production and this carbon flux is facilitated by the broad feeding plasticity of the assemblage of multi-chain omnivorous fish. The magnitude of this subsidy might explain the important top-down control over herbivorous zooplankton, which is characterized by small-sized species. Recent results considering pelagic and benthic food webs coupling showed that the strength of trophic cascades should increase with nutrient enrichment and that the relevance of the detritus pathways is one of the mechanisms affecting the resilience of alternative regime in shallow lakes (Attayde & Ripa, 2008). It has been demonstrated that pelagic and benthic food chains differed in their efficiency (carnivore production:herbivore production) (Rowland et al., 2015). The effectiveness of the conversion of littoral carbon into pelagic production (food chain efficiency) should be evaluated in the future including top fish species that integrates benthic and/or littoral and pelagic food chains. It seems that littoral and pelagic food chain are limited by *N* and *P* (Rowland et al., 2015), respectively; thus, pelagic predators can confront mismatches between their elemental requirement and food quality. Future studies should focus on how efficient littoral carbon is converted in pelagic production as well as how pelagic predator counterbalance (or not) the potential mismatches of different food quality and which mechanisms are involved. Research considering and integrating multiple pathways and the role of littoral carbon in food web are necessary as this carbon flux should not be minimized. Also, cross-habitat connections should be considered when management strategies and restoration programs would be performed.

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