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Food web structure in a double-basin ultra-oligotrophic lake in Northwest Patagonia, Argentina, using carbon and nitrogen stable isotopes

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ABSTRACT

Fish, benthic macroinvertebrates, macrophytes, biofilm, plankton, and terrestrial material were analyzed for stable nitrogen ($\delta^{15}\text{N}$) and carbon isotopes ($\delta^{13}\text{C}$) to study the food web structure of a double-basined, ultra-oligotrophic lake in northwest Patagonia. The $\delta^{15}\text{N}$ values of all biota were consistently higher in Moreno East basin, probably caused by a higher anthropogenic impact; however, the estimated trophic positions of fish showed no differences between basins, indicating that fish are feeding from food chains with different $\delta^{15}\text{N}$ baselines. Fish $\delta^{13}\text{C}$ values were higher in Moreno West basin but no pattern was observed for the rest of the organisms analyzed. The different hydrogeomorphic characteristics between the basins could lead to these differences in the C signatures of fish. SIAR (Stable Isotope Analysis in R) mixing model was used to study possible food sources for adult fish and the results were contrasted with published gut content analysis. According to the model, in both basins small size fish such as Small Puyen (*Galaxias maculatus*) and juvenile fish, had the highest relative isotopic contribution to introduced Rainbow Trout (*Oncorhynchus mykiss*) and Brook Trout (*Salvelinus fontinalis*), which had lower contributions from other sources such as insect larvae, decapods, and snails. On the contrary, decapods and insect larvae were isotopically more important for the native Creole Perch (*Percichthys trucha*) than small fish in both basins. An ontogenetic shift in the carbon source and trophic level was observed in Puyen Chico and Rainbow Trout from the West basin.

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Introduction

Lake Moreno is an ultra-oligotrophic lake in Nahuel Huapi National Park (NHNP) located in the northern Patagonia Andes along the Chile–Argentina border. Its most noteworthy characteristic is its hourglass shape with distinct double basins divided by a narrow connection into Lake Moreno East (ME) and Lake Moreno West (MW) (Fig. 1). Both basins in Lake Moreno contain similar fish (Barriga et al. 2012) and invertebrate species assemblages. However, ME has steeply sloping unproductive cobblestone beaches, surrounded by a dry ecotonal mixed forest of *Nothofagus dombeyi* and *Austrocedrus chilensis*. In contrast to ME, MW has gently sloping

sandy or gravelly shoreline lined with macrophytes and is surrounded by a more dense forest.

There is an extended periurban area in Lake Moreno's watershed. The North and East margins of ME exhibit medium to low density human settlements, moreover, Colonia Suiza, a small village located in the southern margin of ME is one of the oldest settlements in the lake's watershed and consequently has a higher anthropogenic impact in the area (Pereyra et al. 2005). Furthermore, there is a small rainbow trout farm (estimated annual production 50 tonnes year⁻¹) (Temporetti and Pedrozo 2000) in ME, and while well-regulated, may be exposing the lake to additional nitrogen-rich wastes. On the contrary, all the westernmost are of MW is a Protected Area with low anthropogenic impact, whereas the rest of the basin is less populated than ME, with isolated houses, hotels and a golf course located in the North margin of the lake.

Over the past two decades, stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope analyses (SIA) have been widely used to study carbon transfer dynamics, food web structure and to estimate trophic

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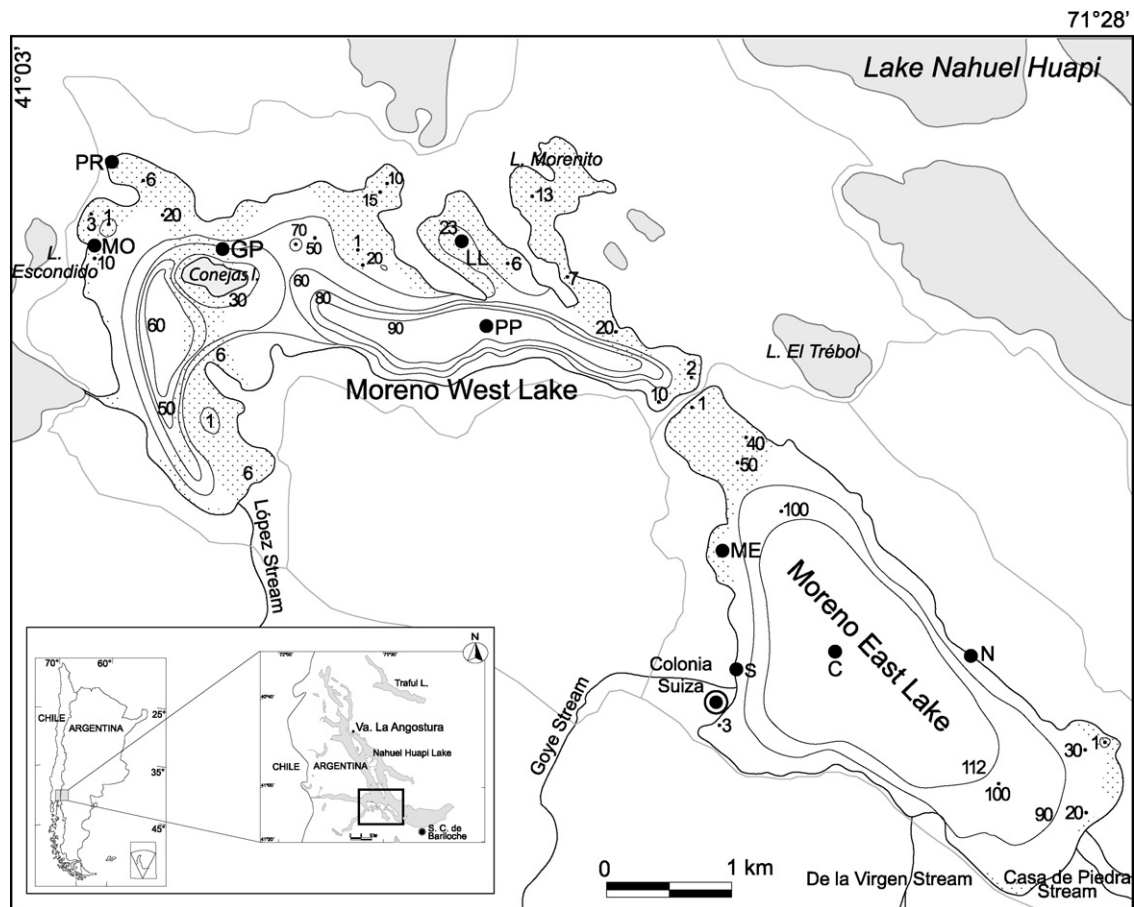


Fig. 1. Map of study area showing sampling points at Lake Moreno East and West, bathymetric data and aquatic vegetation distribution (shaded area). LL, PP, GP and C correspond to plankton sampling points, MO and ME are pelagic/demersal fish sampling points and N, S and PR are macrophyte, macroinvertebrate and littoral fish sampling points.

position, in freshwater lacustrine ecosystems around the world (e.g., Beaudoin et al. 2001; Campbell et al. 2003; Herwig et al. 2004). In addition, stable isotope analyses can be used to determine the impact of anthropogenic factors and energy flow within food webs by assessing the baseline isotopic values within food webs (e.g., Cabana and Rasmussen 1996; McClelland and Valiela 1998; Post 2002). Carbon and nitrogen stable isotope values are also useful in the study of ontogenetic shifts in the diet of fish as this technique provides a time-integrated measure of the trophic position of individual consumers (Vander Zanden et al. 2000) and the different energy pathways (benthic-littoral vs. pelagic) utilized (McIntyre et al. 2006). Understanding the ontogenetic shifts in fish diet and habitat is essential in ecological studies as these changes may affect species interactions in the food web (Grey 2001).

However, cross ecosystem comparison of food web structure using C and N stable isotopes are limited by the influence of a complex interacting series of biogeochemical factors, including variable species composition, anthropogenic factors affecting baseline stable isotope values and physicochemical influences on abiotic and biotic stable isotope values (e.g., Vander Zanden et al. 1997; Post 2002). Double-basined lakes containing similar species assemblages are ideal for such comparisons, as often many variables are sufficiently similar to assess the impact of one or two changes (e.g., Schindler 1974). Furthermore, oligotrophic lakes can be exceedingly sensitive even to low nutrient loadings and anthropogenic disturbances, so shifts in isotopic baselines would be immediately discernible.

Food web structure studies in Lake Moreno focused on the trophic relationship of the organisms of the plankton community

(e.g., Balseiro et al. 2001, 2004) or the predation of the introduced salmonids on the native fish species and other components of the food web (Macchi et al. 2007; Vigliano et al. 2009; Juncos et al. 2011), but there is a lack of studies that integrate all the components of the food web.

In South America, most stable isotope food web studies are from tropical regions (e.g., Da Silva et al. 2005; Garcia et al. 2006; Molina et al. 2011) but there is a significant gap for the temperate Southern Cone region. This study is the first one to use stable isotopes to focus on *whole food-web* trophic relationships in temperate mountain lakes in the Southern Cone region.

The main objective of this study was to analyze the food web structure of Lake Moreno using C and N stable isotopes comparing the East and West basins, which have different environmental, hydrogeomorphic characteristics, and anthropogenic effects.

Methods

Study site

Like all NHNP mountain lakes, Lake Moreno (Fig. 1) is a deep, warm monomictic lake from glacial origin, with high transparency (Secchi depth: 20 m), very low dissolved organic carbon (DOC), total phosphorous (TP), and nitrogen concentrations (TN) (Table 1), and with the vertical oxygen distribution following an orthograde curve year-around, typical of unproductive lakes during thermal stratification (Balseiro et al. 2001; Diaz et al. 2007). Lake Moreno system consists of two larger connected basins, Moreno East (ME), Moreno West (MW) and a smaller lake, Morenito (Fig. 1) connected

Table 1

Geographic and hydrogeomorphic characteristics (Queimaliños et al. 2012), and mean limnological and water chemistry parameters (Queimaliños and Diéguez personal communication) for Moreno East and West. Abbreviations are as follows: T – temperature, Cond – conductivity, Chl *a* – chlorophyll *a*, DOC – dissolved organic carbon, TN – total nitrogen, and TP – total phosphorus.

	Moreno East	Moreno West
<i>Geographic and hydrogeomorphic characteristics</i>		
Location	41°05'51"S 71°29'23"W	41°03'33"S 71°32'24"W
Area (km ²)	6.14	6.10
Max depth (m)	106	90
Mean depth (m)	67	33.5
Volume (km ³)	0.41	0.20
<i>Limnological and water chemistry characteristics</i>		
T (°C)	10.0	10.2
Cond (μS cm ⁻¹)	39.2	41.9
pH	7.16	7.39
Chl <i>a</i> (μg L ⁻¹)	1.45	1.12
DOC (mg L ⁻¹)	0.71	0.82
N-NH ₄ ⁺ (μg L ⁻¹)	2.72	1.88
TN (μg L ⁻¹)	132.0	144.1
TP (μg L ⁻¹)	4.02	3.89

to the larger lakes by a shallow channel open from mid-autumn to late-spring (Buria et al. 2007). Three streams (De la Virgen, Casa de Piedra, and Goye) flow into ME and López Stream into MW.

Fish, benthic macroinvertebrates, macrophytes, biofilm, plankton, tree leaves, and detritus were sampled four consecutive seasons (summer, fall, winter and spring) at 7 sites in ME and MW (Fig. 1). Fish were sampled in the years 2000–2001 and the rest of the samples during 2005–2006.

Fish sampling

Large fish such as the introduced salmonids *Oncorhynchus mykiss* (Rainbow Trout), *Salmo trutta* (Brown Trout), and *Salvelinus fontinalis* (Brook Trout) and the natives *Percichthys trucha* (Creole Perch), *Odontesthes hatcheri* (Patagonian Silverside), *Olivaichthys vielmensis* (Velvet Catfish), and the galaxiid *Galaxias platei* (Big Puyen) were captured using gill net gangs of six mesh sizes (15, 20, 30, 50, 60, and 70 mm bar) set perpendicularly or parallel to the coast line, from 2 m down to 50 m deep. Nets were set at dusk and raised early in the morning, approximately 12 h later.

Native galaxiid *Galaxias maculatus* (Small Puyen) and juvenile Patagonian Silverside, and salmonids (Brook Trout and Rainbow Trout) were collected using 40 cm × 8 cm, 2.5-mm double-funnel cylinder fish traps left at each site for 48 h or by using seine nets.

Fish were separated by species and their fork length and weight were recorded, and age was determined by scale reading. Muscle tissue was removed from the larger fish individuals (>90 mm) and homogenized individually or pooled with individuals of similar size.

Individual Small Puyen had insufficient mass to be analyzed individually, so up to 10 whole individuals (with heads and guts removed) were selected from drawing six size classes from <30 mm to >70 mm, and each size class sample set homogenized together.

Littoral sampling

The MW littoral site chosen for sampling is located at the westernmost side, which has a high abundance of submerged and emergent macrophytes and is surrounded by dense forest (PR in Fig. 1). The ME littoral sites were rocky beaches with sparse macrophytes and benthic algae in the south and north margins (S and N in Fig. 1). The traps on the north site (N) were damaged by unknown persons after a few sampling trips and after salvaging samples from

the N site, we had to abandon this site for the rest of the study. As a result, only the southern (S) in ME site was sampled regularly throughout this study, but both sets of samples were used in this study.

Benthic macroinvertebrates such as Trichoptera (caddis flies), Ephemeroptera (mayflies), Plecoptera (stoneflies), and Odonata larvae (dragonflies); decapods *Samastacus spinifrons* (crayfish) and *Aegla* sp. (crab), amphipods *Hyalella* sp., gastropods *Chilina* sp., bivalves *Diplodon chilensis*, and hirudeans (leeches) were hand-picked from submerged logs and stones and around macrophytes. Decapods and mollusks were removed from their carapaces/shells, the digestive tracts were removed and only muscle was analyzed. Whole bodies were analyzed of the remaining macroinvertebrates, including caddis flies which were removed from their cases. For all invertebrate taxa, individual sample masses were not sufficient for analyses so homogenate samples of pooled individuals, grouped by species and similar size, were prepared.

Macrophytes, both submerged (*Myriophyllum quitense* and *Nitella* sp.) and emergent (*Schoenoplectus californicus*, *Ranunculus* sp., *Hydrocotyle chamaemorus*, and *Galium* sp.) were collected by hand, and biofilm was obtained by scraping submerged stones. Those were washed in lake water to remove excess sediment and detritus, stored in clean plastic bags and processed in the laboratory. Macrophyte leaves and stems were cut into pieces and the biofilm obtained from the rocks was repeatedly washed with distilled water to remove debris by decantation and filtration.

Terrestrial sampling

To assess potential terrestrial inputs to the lake, leaves were collected from the most abundant plant species surrounding the shoreline sampling sites. Fresh leaves were obtained from live trees and shrubs, including *N. dombeyi* (Coihue) and *Luma apiculata* (Chilean myrtle) in both basins, *A. chilensis* (Cordilleran cypress), *Lomatia hirsuta* (Radal), and *Schinus patagonicus* (Laura) were sampled only in ME while *Crinodendron patagua* (Lily of the valley tree) and *Chusquea culeou* (Colihue) were sampled only in MW. As the leaves decompose under water and this process may change their stable isotope ratios (Fellerhoff et al. 2003), identifiable decomposing leaves were collected underwater from the littoral shoreline for comparison. This was only possible in MW, where the trees reach the shoreline and hence there was a deep layer of leaves and detritus inside the water; but in ME, as the forest grows farther from the coast, no leaves were found in the water.

Plankton sampling

One pelagic site (C in Fig. 1) was chosen for plankton sampling in ME due to its simple steep bathymetry, while 3 pelagic sites were chosen in MW for plankton sampling due to the complex bathymetry of the basin (LL, GP and PP in Fig. 1). Plankton samples were collected with vertical hauls with nets of three different mesh sizes (10, 53, and 200 μm), from 95 m deep in ME; 85 m, 40 m and, 15 m in PP, GP and LL, respectively, in MW (Fig. 1).

The plankton samples were concentrated as detailed by (Arribère et al. 2010a). In summary, Fraction 1 (F1) (10–53 μm) is dominated by large mixotrophic ciliates and by autotrophic dinoflagellates and in a lesser proportion by rotifers, immature stages of crustaceans (nauplii, copepodids, and cladoceran juveniles), and filamentous diatoms; Fraction 2 is composed of intermediate size classes (F2) (53–200 μm) including the same mixotrophic ciliates species as in F1, dinoflagellates, rotifers, and juveniles of cladocerans and F3 has the larger zooplankton sizes (F3) (>200 μm) dominated by larger copepods and cladocerans (Arribère et al. 2010a; Queimaliños et al. 2012).

Stable isotope analyses

All samples were taken immediately to the laboratory and processed as quickly as possible. Since the samples were also collected for trace metal analyses (Arribère et al. 2008, 2010a,b), all samples were rinsed with ultra-pure water, homogenized with titanium and Teflon® devices, frozen at -40°C and freeze-dried until constant weight, then ground to fine powder using a Teflon® pestle and mortar.

The isotopic composition of the carbon and nitrogen in an animal reflects the N and C isotopic composition of its diet (DeNiro and Epstein 1978, 1981). As consumers are enriched in ^{13}C by about 1‰ (avg. $0.8 \pm 1.1\text{‰}$, range -0.6 to 2.7‰) relative to its diet (DeNiro and Epstein 1978), $\delta^{13}\text{C}$ values can be used to evaluate the sources of carbon of a consumer when the isotopic signature of the sources are different (Post 2002). Benthic producers, such as periphyton, are enriched in ^{13}C compared to planktonic algae measured as particulate organic matter, and eventually these different $\delta^{13}\text{C}$ signatures will be reflected in pelagic and littoral consumers (France 1995). On the contrary, consumers are typically enriched in ^{15}N by an average of $3.4 \pm 1.1\text{‰}$ (between 1.3 and 5.3‰) relative to its diet (Minagawa and Wada 1984), so the $\delta^{15}\text{N}$ values can be used to estimate trophic level.

Stable isotope values (δ) were expressed in parts per thousand (‰) relative to the C and N reference materials (Pee Dee belemnite limestone and atmospheric nitrogen standard, respectively), as follows:

$$\delta(\text{‰}) = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000 \quad (1)$$

Stable carbon and nitrogen isotopes were measured via DELTAplusXP continuous flow stable isotope ratio mass spectrometer at the Queen's Facility for Isotope Research (QFIR), Queen's University, Kingston, ON, Canada. Duplicates and standard reference materials (Certified Reference Material [CRM] 8548, IAEA, Carbon Rod, Sulfanilamide) and in-house standards (lipid-extracted Atlantic Salmon, Red Tilapia and chicken blood) were analyzed to check the quality of analysis.

Data analyses

Stable isotope biplots for each basin were used to visually compare food web relationships among the species. Differences in stable isotope values between taxa and between basins were assessed using Student's t -tests and ANOVA followed by post hoc Tukey test.

Correlations between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ with length were assessed using Pearson's r to analyze possible ontogenetic dietary niche shifts in Small Puyen, Rainbow Trout, Brook Trout, and Creole Perch.

All statistical tests were performed using StatSoft Statistica v8.0 software.

To evaluate the relative contributions of the different food sources to the three fish species with enough data for analysis, Creole Perch, Rainbow Trout, and Brook Trout we fitted the Bayesian stable isotope mixing model SIAR v4.0 (Stable Isotope Analysis in R) (Parnell et al. 2010) using R v2.13.1 for Windows (R Development Core Team 2011) for each consumer separately. This model allows the inclusion of isotopic signatures and fractionation together with the uncertainty of these values within the model (Parnell et al. 2010). The number of iterations was 500,000, whereas 50,000 initial iterations were discarded. The information supplied to the model included all isotopic data of consumers, mean, and standard deviation (SD) of each source contributing to the consumers, Trophic Enrichment Factors (TEF, $\Delta^{15}\text{N}$, $\Delta^{13}\text{C}$), and elemental concentration values of each source (%C and %N).

Table 2

Fractionation factors ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$) calculated from Eqs. (2) and (3), respectively, for Rainbow Trout, Brook Trout, and Creole Perch from Moreno East and Moreno West.

Consumer	Moreno East		Moreno West	
	$\Delta^{15}\text{N}$ (‰)	$\Delta^{13}\text{C}$ (‰)	$\Delta^{15}\text{N}$ (‰)	$\Delta^{13}\text{C}$ (‰)
<i>S. fontinalis</i>	2.5 ± 0.2	3.2 ± 0.5	2.8 ± 0.3	2.4 ± 0.4
<i>O. mykiss</i>	2.5 ± 0.1	2.5 ± 0.3	2.9 ± 0.1	2.1 ± 0.3
<i>P. trucha</i>	2.7 ± 0.3	2.7 ± 0.6	3.2 ± 0.2	1.7 ± 0.3

TEFs or fractionation factors for each consumer (Table 2) were calculated using the following equations proposed by Caut et al. (2009) for fish muscle:

$$\Delta^{15}\text{N} = -0.281(\delta^{15}\text{N}) + 5.879 \quad (2)$$

$$\Delta^{13}\text{C} = -0.248(\delta^{13}\text{C}) - 3.4770 \quad (3)$$

TEFs were calculated for each consumer (Creole Perch, Rainbow Trout, and Brook Trout) sample using their $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values and average and SD TEFs were calculated to use in the SIAR model. Dietary sources for each consumer were chosen based on published gut content analyses (Bubach 2010; Juncos et al. 2011; Table 3). Following these authors, we chose Small Puyen, *S. spinifrons*, *Aegla* sp., and all the insect larvae together (they were not discriminated by taxa in the reference papers) as common food sources for Creole Perch, Rainbow Trout, and Brook Trout; juvenile fish (juvenile salmonids and Patagonian silversides) were included in the Brook Trout model, *Chilina* sp. in the Rainbow Trout model, and *Hyaella* sp. in the Creole Perch model.

In order to compare the food web structure in each basin, we normalized the stable isotope data to a common baseline by estimating the trophic position following Post et al. (2000) and Post (2002):

$$\text{TP} = \lambda \left(\delta^{15}\text{N}_{\text{fish}} - \frac{\delta^{15}\text{N}_{\text{mussel}} \cdot \alpha + \delta^{15}\text{N}_{\text{snail}} \cdot (1 - \alpha)}{\Delta_n} \right) \quad (4)$$

where λ is the trophic position ($\lambda = 2$) of the primary consumers chosen as baselines (the mussel *D. chilensis* and the snail *Chilina* sp.), Δ_n is the enrichment in $\delta^{15}\text{N}$ per trophic level calculated for each fish species using Eq. (2), and α is the proportion of carbon in the consumer ultimately derived from the base of the pelagic food web and can be estimated as:

$$\alpha = \frac{\delta^{13}\text{C}_{\text{fish}} - \delta^{13}\text{C}_{\text{snail}}}{\delta^{13}\text{C}_{\text{mussel}} - \delta^{13}\text{C}_{\text{snail}}} \quad (5)$$

Post (2002) suggested that long-lived primary consumers, such as snails and mussels, can be used as baseline organisms when estimating trophic position as they integrate the temporal variation and reflect the spatial variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ at the base of the food web. Following Post (2002) we chose *Chilina* sp. and *D. chilensis* as baseline organisms as they are very abundant and are the most conspicuous mollusks of Lake Moreno.

Results

$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values obtained for all the taxa collected from ME and MW are summarized in Tables 4 and 5. Overall, visually comparing the stable isotope diagrams for each basin (Fig. 2), it is clear that there are some marked differences, with all species from ME tending to have higher $\delta^{15}\text{N}$ than their counterparts in MW, but the $\delta^{13}\text{C}$ values do not follow a clear pattern between the basins when all the organisms are compared. Plankton had significant lower $\delta^{13}\text{C}$ values compared to benthic invertebrates and fish in both basins, and fish showed higher $\delta^{13}\text{C}$ values than macroinvertebrates in MW (post hoc Tukey test $p < 0.05$) but no

Table 3

Approximate food item percentages for Rainbow Trout, Brook Trout, and Creole Perch obtained from gut content analysis in Moreno Lake from Bubach (2010) and Juncos et al. (2011), and mean food item percentages obtained through SIAR mixing model in Moreno East and West for the same consumers.

Consumers	Food items	Percentage (%)		
		Bubach (2010)	Juncos et al. (2011)	This work
Rainbow Trout	Small Puyen	68	34	56 (ME), 69 (MW)
	Insect, adults	9	16	–
	Insect, larvae and pupae	5	24	19 (ME), 11 (MW)
	Plant material	5	–	–
	Molluscs	3	13	3 (ME, MW)
	<i>Aegla</i> sp.	4	6	11 (ME), 10 (MW)
	<i>S. spinifrons</i>	4	6	11 (ME), 7 (MW)
	Other fish	2	1	–
	Brook Trout	Other fish	44	13
Small Puyen		22	38	27 (ME), 25 (MW)
<i>Aegla</i> sp.		11	17	15 (ME), 14 (MW)
<i>S. spinifrons</i>		13	9	16 (ME), 11 (MW)
Insect, larvae and pupae		6	16	17 (ME), 20 (MW)
Plant material		3	–	–
Insect, adults		–	7	–
Amphipods		1	–	–
Creole Perch		<i>S. spinifrons</i>	47	19
	Insect, larvae and pupae	34	55	34 (ME), 5 (MW)
	Amphipods	8	11	20 (ME), 13 (MW)
	Small Puyen	4	12	27 (ME), 33 (MW)
	<i>Aegla</i> sp.	3	3	10 (ME), 18 (MW)
	Molluscs	3	–	–
	Other fish	1	–	–

differences were found in ME. In general, in both basins the fish community presented the highest $\delta^{15}\text{N}$, plankton and macroinvertebrates had intermediate values and primary producers showed the lowest values of the food web (Fig. 2).

Primary producers showed high intra- and inter-specific variability in their $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (e.g., $\Delta\delta^{15}\text{N}=19.4\text{‰}$ and $\Delta\delta^{13}\text{C}=9.4\text{‰}$ in *M. quitense* and biofilm, respectively, both from MW), although as a group tended to have the lowest $\delta^{15}\text{N}$ values in both basins (Fig. 2 and Table 5). Submerged macrophytes (*M. quitense* and *Nitella* sp.; -15.8‰ in MW and -13.2‰ in ME) showed statistically distinct $\delta^{13}\text{C}$ signals (post hoc Tukey test $p < 0.0001$) compared to biofilm (-24.4‰ in MW and -24.9‰ in ME), detritus

(-28.4‰ in MW), emergent aquatic plants (-27.8‰ in ME and -28.2‰ in MW), and tree leaves (-28.0‰ in ME and -29.6‰ in MW).

Within the plankton sample set, F1 and F2 did not show significant differences in the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values within each basin, but they showed significant lower $\delta^{15}\text{N}$ and higher $\delta^{13}\text{C}$ values than F3 (post hoc Tukey test $p < 0.05$). F1 and F2 did not show significant differences in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between basins, but F3 from ME had significantly higher $\delta^{15}\text{N}$ and lower $\delta^{13}\text{C}$ values than in MW (t -test $p < 0.05$).

The macroinvertebrate species were similar in both basins. In general, most of the invertebrate taxa sampled in both basins had

Table 4

Range, mean and standard deviation (SD) of the stable isotope ratios of nitrogen and carbon obtained in fish from Moreno East (ME) and Moreno West (MW). *N* is the number of samples analyzed; the numbers in parenthesis represents the total number of fish caught.

Species	Code	Lake	<i>N</i>	Length (cm)	Range (mean \pm SD)	
					$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
<i>Olivaichthys viedmensis</i> (Velvet catfish)	1	ME	7 (7)	17.9–26.0	8.0–9.4 (8.7 \pm 0.6)	–29.4 to –24.2 (–26.4 \pm 1.5)
<i>Galaxias maculatus</i> (Small puyen)	2	ME	15 (83)	3.2–7.9	7.8–10.4 (8.7 \pm 0.8)	–28.4 to –23.5 (–26.0 \pm 1.3)
		MW	12 (104)	3.2–6.0	7.5–10.0 (8.3 \pm 0.7)	–25.6 to –20.4 (–22.5 \pm 1.4)
<i>Galaxias platei</i> (Big puyen)	3	ME	1 (1)	34.7	8.9	–18.0
		MW	1 (1)	36.0	9.7	–19.4
<i>Odontesthes hatcheri</i> (Patagonian silverside)	4	ME	1 (4)	26.1	10.0	–31.0
<i>O. hatcheri</i> , juvenile	5	ME	2 (7)	3.7–5.9	7.6–8.9 (8.3 \pm 1.0)	–24.6 to –23.6 (–24.1 \pm 0.7)
		MW	2 (5)	3.5–6.8	6.4–9.4 (7.9 \pm 2.1)	–24.7 to –20.5 (–22.6 \pm 3.0)
<i>Oncorhynchus mykiss</i> (Rainbow trout)	6	ME	7 (49)	35.5–48.7	11.3–12.7 (12.0 \pm 0.5)	–25.7 to –22.0 (–24.3 \pm 1.2)
		MW	7 (18)	37.8–46.5	10.0–11.4 (10.6 \pm 0.5)	–24.1 to –20.5 (–22.7 \pm 1.3)
<i>O. mykiss</i> , juvenile	7	MW	9 (9)	8.9–18.2	6.8–9.7 (8.1 \pm 1.1)	–26.7 to –21.2 (–24.0 \pm 2.4)
<i>Percichthys trucha</i> (Creole perch)	8	ME	18 (53)	31.4–45.3	9.1–12.8 (11.2 \pm 1.0)	–28.1 to –17.4 (–24.9 \pm 2.6)
		MW	16 (63)	34.1–45.3	8.6–10.8 (9.5 \pm 0.6)	–23.4 to –18.0 (–20.9 \pm 1.3)
<i>Salmo trutta</i> (Brown trout)	9	MW	1 (1)	57.5	11.5	–23.5
<i>Salvelinus fontinalis</i> (Brook trout)	10	ME	7 (14)	26.3–44.5	11.0–12.8 (11.9 \pm 0.7)	–29.4 to –23.9 (–26.7 \pm 2.2)
		MW	6 (7)	24.0–54.0	9.9–12.5 (11.0 \pm 1.2)	–26.0 to –21.9 (–23.8 \pm 1.6)
<i>S. fontinalis</i> , juvenile	11	ME	2 (3)	11.0–11.5	11.2–11.3 (11.3 \pm 0.1)	–30.8 to –23.5 (–27.2 \pm 5.1)
		MW	3 (3)	13.5–22.5	9.5–10.5 (9.9 \pm 0.5)	–24.2 to –21.8 (–23.3 \pm 1.3)

Table 5
Range, mean and standard deviation (SD) of the stable isotope ratios of nitrogen and carbon for Moreno East (ME) and Moreno West (MW) in macroinvertebrates, plankton, primary producers, tree leaves, and detritus.

Species	Code	Lake	N	Range (mean \pm SD)	
				$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
<i>Molluscs</i>					
<i>Chilina</i> sp. (Snail) ^a	12	ME	2	3.8–6.7 (5.2 \pm 2.0)	–20.8 to –14.7 (–17.7 \pm 4.3)
		MW	7	0.8–3.3 (2.1 \pm 0.8)	–21.3 to –15.5 (–18.4 \pm 2.1)
<i>Diplodon chilensis</i> (Mussel) ^a	13	ME	1	5.1	–27.7
		MW	27	3.7–7.1 (5.4 \pm 0.8)	–26.4 to –24.6 (–25.5 \pm 0.4)
<i>Crustaceans</i>					
<i>Aegla</i> sp. (Crab) ^a	14	ME	5	6.1–10.2 (8.4 \pm 1.8)	–25.0 to –18.9 (–22.0 \pm 2.4)
		MW	2	4.4–7.4 (5.9 \pm 2.1)	–23.1 to –22.2 (–22.7 \pm 0.6)
<i>Hyalella</i> sp. (Amphipod) ^b	15	ME	5	7.2–8.4 (7.9 \pm 0.5)	–28.0 to –22.3 (–24.9 \pm 2.5)
		MW	4	2.1–4.3 (2.9 \pm 1.0)	–28.2 to –19.1 (–23.2 \pm 3.8)
<i>Samastacus spinifrons</i> (Crayfish) ^a	16	ME	4	7.9–9.3 (8.6 \pm 0.6)	–23.7 to –20.9 (–22.4 \pm 1.2)
		MW	15	4.0–6.6 (5.7 \pm 0.7)	–25.6 to –20.4 (–22.3 \pm 1.4)
<i>Annelids</i>					
<i>Hirudea</i> (Leech) ^b	17	ME	2	10.4–14.1 (12.3 \pm 2.6)	–27.6 to –23.7 (–25.6 \pm 2.8)
		MW	3	6.9–8.3 (7.6 \pm 0.7)	–27.6 to –23.5 (–25.1 \pm 2.2)
<i>Insect larvae</i>					
Ephemeroptera (Mayfly) ^b	18	ME	1	8.3	–23.5
		MW	4	4.4–6.5 (5.8 \pm 1.0)	–25.9 to –18.4 (–23.1 \pm 3.2)
Odonata (Dragonfly) ^b	19	ME	1	4.6	–20.0
		MW	5	3.1–6.2 (4.3 \pm 1.2)	–27.8 to –20.0 (–23.5 \pm 3.1)
Plecoptera (Stonefly) ^b	20	ME	4	6.0–9.4 (7.1 \pm 1.6)	–21.4 to –28.9 (–24.8 \pm 3.3)
		MW	2	3.7–3.9 (3.8 \pm 0.2)	–37.4 to –36.1 (–36.8 \pm 0.9)
Trichoptera (Caddis fly) ^b	21	ME	8	5.9–9.0 (7.6 \pm 1.0)	–30.7 to –22.4 (–27.9 \pm 2.5)
		MW	9	2.4–5.1 (3.7 \pm 0.9)	–33.5 to –24.5 (–27.4 \pm 3.0)
<i>Plankton</i>					
Fraction 1 (10–53 μm)	22	ME	7	4.2–7.9 (5.5 \pm 1.4)	–30.3 to –24.3 (–26.2 \pm 2.0)
		MW	12	3.6–8.4 (4.8 \pm 1.4)	–30.3 to –23.4 (–26.2 \pm 2.3)
Fraction 2 (53–200 μm)	23	ME	17	1.8–7.5 (5.0 \pm 1.6)	–30.1 to –22.0 (–26.0 \pm 2.1)
		MW	26	–1.1–7.0 (4.5 \pm 2.0)	–31.0 to –23.4 (–25.5 \pm 2.1)
Fraction 3 (> 200 μm)	24	ME	22	6.5–14.6 (8.9 \pm 1.9)	–32.9 to –27.2 (–29.4 \pm 1.5)
		MW	30	5.1–10.3 (7.8 \pm 0.9)	–31.4 to –26.8 (–28.5 \pm 1.5)
<i>Primary producers</i>					
Biofilm	25	ME	5	1.2–8.4 (5.2 \pm 2.6)	–27.7 to –23.2 (–24.9 \pm 1.8)
		MW	3	1.1–2.1 (1.5 \pm 0.5)	–27.9 to –18.5 (–24.4 \pm 5.1)
<i>Myriophyllum quitense</i> (Andean water milfoil)	26	ME	3	5.1–8.6 (6.7 \pm 1.8)	–15.9 to –10.4 (–13.2 \pm 2.7)
		MW	9	–12.2–7.2 (–3.5 \pm 6.8)	–20.2 to –10.2 (–14.7 \pm 3.6)
<i>Nitella</i> sp. (Stoneworth)	27	MW	4	–13.9 to –5.4 (–9.4 \pm 4.3)	–20.6 to –17.1 (–18.5 \pm 1.6)
<i>Schoenoplectus californicus</i> (California bulrush)	28	MW	1	2.3	–26.3
Tree leaves (<i>Austrocedrus chilensis</i> , <i>Chusquea culeou</i> , <i>Crinodendron patagua</i> , <i>Lomatia hirsuta</i> , <i>Luma apiculata</i> , <i>Nothofagus chilensis</i>)	29	ME	5	–0.7–4.6 (1.5 \pm 2.0)	–29.7 to –26.4 (–28.0 \pm 1.4)
		MW	4	–2.7–4.5 (0.4 \pm 3.1)	–30.9 to –28.2 (–29.6 \pm 1.1)
Detritus (<i>Chusquea culeou</i> , <i>Crinodendron patagua</i> , <i>Luma apiculata</i> , <i>Nothofagus chilensis</i> , <i>S. californicus</i>)	30	MW	5	–0.7–3.7 (0.6 \pm 1.8)	–29.8 to –26.3 (–28.4 \pm 0.7)
Emergent macrophytes (<i>Galium</i> sp., <i>Hydrocotyle chamaemorus</i> , <i>Ranunculus</i> sp.)	31	ME	8	3.4–10.2 (5.9 \pm 2.1)	–29.7 to –24.7 (–27.8 \pm 1.6)
		MW	8	–0.6–7.1 (3.4 \pm 2.2)	–32.3 to –26.1 (–28.2 \pm 2.1)

^a Muscle.^b Whole body.

consistently lower $\delta^{15}\text{N}$ values than fish and higher than those of primary producers (post hoc Tukey test $p < 0.05$). In both basins, the snails showed the lowest $\delta^{15}\text{N}$ values along with dragonfly larvae and mussels in ME, and amphipods and caddis fly larvae in MW. Hirudeans, crabs, and crayfish had the highest $\delta^{15}\text{N}$ values in both basins (Fig. 2 and Table 5). In fact, hirudeans from ME had similar $\delta^{15}\text{N}$ signatures than some salmonids (e.g., $12.3 \pm 2.6\%$ in hirudeans vs. $12.0 \pm 0.5\%$ in Rainbow Trout), possibly related to their scavenging role on dead organisms (Sarica et al. 2005). In both basins, snails showed the highest $\delta^{13}\text{C}$ values, and decapods and may fly larvae were also enriched in ^{13}C compared with the other taxa (Fig. 2 and Table 5). Caddis fly larvae and mussels had low $\delta^{13}\text{C}$ in both basins, and the rest of the taxa showed no clear trend (Fig. 2 and Table 5).

In both basins, adult salmonids presented the highest $\delta^{15}\text{N}$ values, followed by Creole Perch and juvenile salmonids with

significantly lower values and the native Small Puyen, Velvet Catfish, and juvenile Patagonian Silverside showed the lowest $\delta^{15}\text{N}$ signatures of all fish species (post hoc Tukey test $p < 0.05$). The most common sampled species in both basins, Rainbow Trout, Brook Trout, Creole Perch, and Small Puyen, presented significantly lower $\delta^{13}\text{C}$ values in ME compared to with MW (t -test $p < 0.05$; Fig. 3 and Table 4). On the contrary, the $\delta^{15}\text{N}$ values were significantly higher in ME than in MW in those fish species (t -test $p < 0.05$; Fig. 3 and Table 4). Although the absolute $\delta^{15}\text{N}$ values of most fish species are higher in ME than in MW, the estimated trophic position (see Eq. (3) in 'Methods' section) for each species is similar for both basins as there are no significant differences between ME and MW (t -test $p < 0.05$; Fig. 4).

Based on the results of the SIAR mixing model (Fig. 5a), juvenile fish and Small Puyen had highest relative contribution to the diet of Brook Trout in both basins, and the rest of the food items

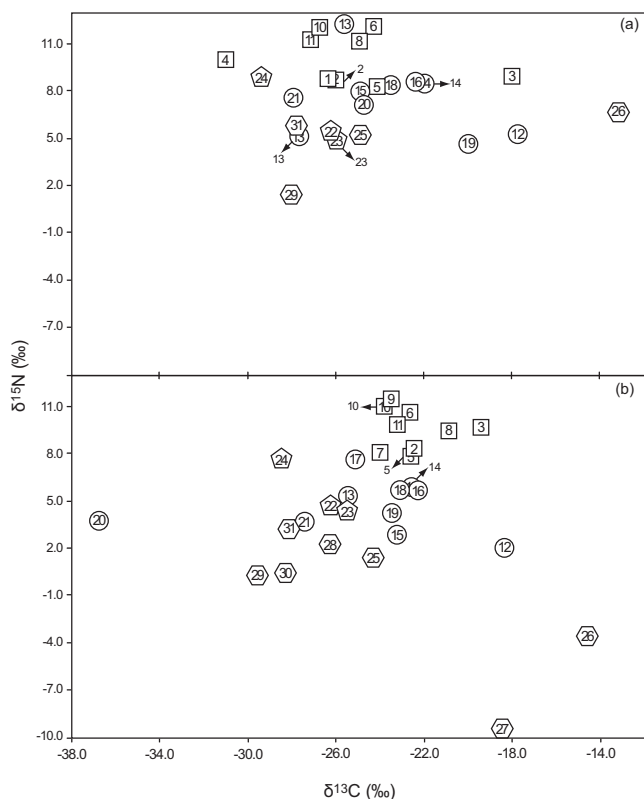


Fig. 2. Mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for fish (squares), macroinvertebrates (circles), plankton (pentagons) and primary producers (hexagons) in Moreno East (a) and West (b). Numbers correspond to 1 – Velvet Catfish, 2 – Small Puyen, 3 – Patagonian Silverside, 4 – Patagonian Silverside juvenile, 5 – Rainbow Trout, 6 – Rainbow Trout juvenile, 7 – Creole Perch, 8 – Brown Trout, 9 – Brook Trout, 10 – Brook Trout juvenile, 11 – *Chilina* sp., 12 – *Chilina* sp., 13 – *Diplodon chilensis*, 14 – *Aegla* sp., 15 – *Hyalella* sp., 16 – *Samastacus spinifrons*, 17 – Hirudea, 18 – Ephemeroptera larvae, 19 – Odonata larvae, 20 – Plecoptera larvae, 21 – Trichoptera larvae, 22 – plankton F1, 23 – plankton F2, 24 – plankton F3, 25 – biofilm, 26 – *Myriophyllum quitense*, 27 – *Nitella* sp. 28 – *Schoenoplectus californicus*, 29 – tree leaves, 30 – detritus, 31 – emergent macrophytes.

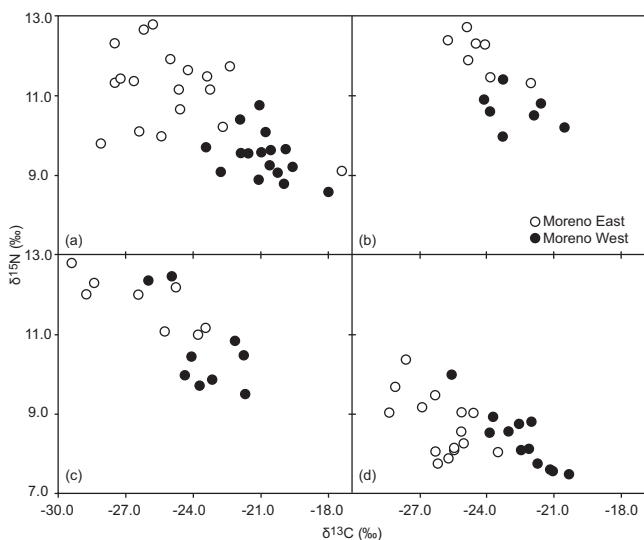


Fig. 3. Biplot of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for (a) Creole Perch, (b) Rainbow Trout, (c) Brook Trout, and (d) Small Puyen, comparing Moreno East (open circles) and West (full circles).

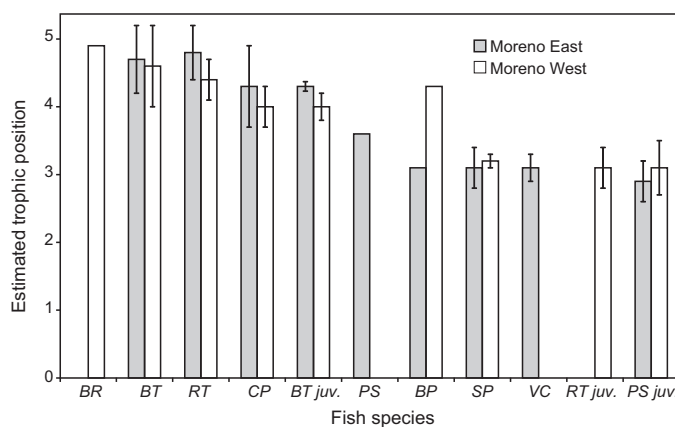


Fig. 4. Mean and standard deviation of estimated trophic position of fish from Moreno East (grey bars) and West (white bars). Abbreviations are as follows: BR – Brown Trout, BT – Brook Trout, RT – Rainbow Trout, CP – Creole Perch, SP – Small Puyen, BP – Big Puyen, VC – Velvet Catfish, and PS – Patagonian Silverside.

(crustaceans and insect larvae) had an equal and minor contribution (Table 3 and Fig. 5a). Small Puyen clearly had the highest relative contribution to Rainbow Trout in both basins (56% in ME and 69% in MW) than the other sources. Snails had the lowest relative contribution to Rainbow Trout with 3% mean of the total diet in both basins (Table 3 and Fig. 5b).

Insect larvae showed the highest mean relative contribution to the diet of Creole Perch in ME (34%), followed by Small Puyen (27%) and the amphipods (20%), while crustacean decapods had minor relative contributions. On the contrary, in MW, crayfish (34%) and Small Puyen (33%) had the highest relative contribution to the diet of Creole Perch while insect larvae (5%), had the lowest relative contribution to the total diet of this native fish in both basins (Table 3 and Fig. 5c).

To assess the ontogenetic shift in the diet of the fish species in Moreno Lake, fish size was plotted against $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for the most abundant fish species collected: Rainbow Trout, Brook Trout, Creole Perch, and Small Puyen. Body length was negatively correlated with $\delta^{15}\text{N}$ and positively correlated with $\delta^{13}\text{C}$ in both basins (Pearson's $r p < 0.01$) in Small Puyen and positively correlated with $\delta^{15}\text{N}$ in Rainbow Trout in MW (Pearson's $r p < 0.001$), but no correlation was observed between size and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in the other fish species (Fig. 6).

Discussion

A significant time period (about 3 years) occurred between fish sampling and the collection of the benthic and pelagic organisms analyzed that could mislead the comparison between both communities. A preliminary sampling of food items consumed by fish (insect larvae, whole crustaceans, and snails) was performed together with the fish sampling, showing similar stable isotope ratios when compared with the items collected in the second sampling campaign. Also, no significant event or modification of a relevant environmental condition was registered in Lake Moreno watershed in the period between the sampling campaigns. Both aspects support the implicit assumption that there was no relevant variation in the trophic connections between fish and their food items in the 3 years between the sampling campaigns. The fish food items of the preliminary sampling were ruled out from the analysis for consistency, considering the different turnover in pelagic and benthonic communities. However we are aware that the time lapse between sampling periods should be taken into account when drawing conclusions about Lake Moreno food web.

Possible carbon sources for consumers in the base of the food web in Lake Moreno include phytoplankton, biofilm, detritus from

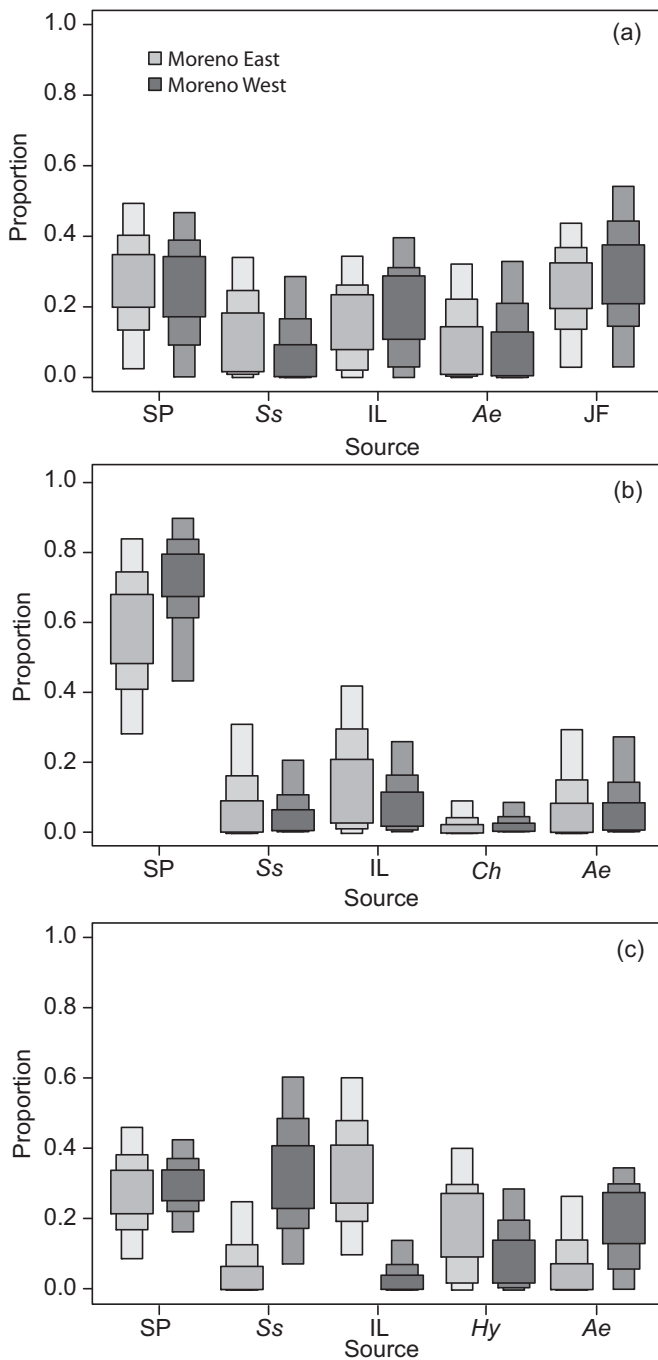


Fig. 5. Results of SIAR (95, 75, and 25% credibility intervals) showing estimated prey contribution to (a) Brook Trout, (b) Rainbow Trout, and (c) Creole Perch in Moreno East (light boxes) and Moreno West (dark boxes). Abbreviations of sources are as follows: SP – Small Puyen, Ss – *Samastacus spinifrons*, IL – insect larvae, Ae – *Aegla* sp., JF – Juvenile fish, Ch – *Chilina* sp., and Hy – *Hyaletella* sp.

the surrounding forest and decaying macrophytes, and submerged and emergent macrophytes, which differ in their $\delta^{13}\text{C}$ values. The higher ^{13}C values observed in the emergent macrophytes *M. quitense* and *Nitella* sp. compared with the rest of the food web items indicates that they are not a carbon source for the primary consumers in both basins of Lake Moreno. On the contrary, some insect larvae such as caddis fly and stonefly larvae are slightly enriched in ^{13}C and ^{15}N compared to detritus and biofilm, indicating that they might be a C source for those consumers. *Chilina* sp. is known to graze on biofilm that grows on submerged rocks and logs (Díaz Villanueva et al. 2004) but surprisingly their $\delta^{13}\text{C}$ values were

highly enriched ($\Delta\delta^{13}\text{C} = 7.2\text{‰}$ in ME and 6.0‰ in MW) compared to their C source in more than the 1‰ usually expected between a consumer and its diet (DeNiro and Epstein 1978). Although the biofilm samples were carefully washed to remove detritus and sediments, it is possible that some remained causing a dilution in the carbon signal and thus lowering the $\delta^{13}\text{C}$ values.

In both basins, plankton F1 composed by autotrophic and heterotrophic organisms (Arribére et al. 2010a), had similar $\delta^{13}\text{C}$ values as F2 but both fractions were enriched (about 3‰) compared to F3, which is dominated by copepods and cladocerans (Queimaliños et al. 2012). del Giorgio and France (1996) proposed several mechanisms to explain this widespread phenomenon observed in freshwater plankton, as larger zooplankton are expected to be slightly enriched in ^{13}C compared to smaller plankton on which they feed (Fry and Sherr 1984). As expected, Patagonian Silverside juveniles and Small Puyen presented higher $\delta^{13}\text{C}$ values than the plankton fractions that are part of their diet. Phytoplankton can be considered as a carbon source of the pelagic food web as it is consumed by zooplankton, which is then preyed by low trophic level fish such as Small Puyen and immature stages of Big Puyen and Patagonian Silverside (Cervellini et al. 1993; Reissig 2005; Rechenq et al. 2010). Small fish are in turn, preyed by higher trophic level fish (Juncos et al. 2011), thus they play an important role in nutrient cycling as they are a major link between the pelagic and littoral habitat (Reissig 2005; Rechenq et al. 2010).

Salmonids and Creole Perch have generalistic opportunistic feeding habits across Lake Moreno (Macchi et al. 1999, 2007; Wegrzyn and Ortubay 2009) which support our interpretation of a wide dietary range based on their stable C and N isotope values (Fig. 2). As expected, adult salmonids have the highest $\delta^{15}\text{N}$ values of all biota sampled in both basins, and followed by Creole Perch. The 95% credibility intervals obtained for the main sources in the SIAR mixing model for Brook Trout are similar, ranging from 0% to about 30% in juvenile fish and from 0% to about 40% in Small Puyen (Table 3 and Fig. 5a). Published gut content data for Brook Trout in Lake Moreno (Table 3) indicate that fish (Small Puyen, juvenile Creole Perch, and others) were the most consumed items, followed by *Aegla* sp., *S. spinifrons* and insect larvae, and pupae (Bubach 2010; Juncos et al. 2011). These results indicate that we obtained similar dietary proportions for Brook Trout with the SIAR model as the ones obtained from gut content analysis.

Our SIAR mixing model output for Rainbow Trout indicate that Small Puyen had the highest relative isotopic contribution to the diet in both basins, with minor contributions being evenly distributed among insect larvae and decapods (Table 3 and Fig. 5b). This output mirrors the known real-world diets for those fish species (Table 3). Stomach content published by Bubach (2010) and Juncos et al. (2011) showed that Rainbow Trout from Lake Moreno had typically a high proportion of Small Puyen with other food items such as adult, larvae and pupae of insects plus a lower proportion of mollusks and crustaceans (*Aegla* sp. and *S. spinifrons*).

In the case of Creole Perch from ME, our SIAR model output suggests that insect larvae (34%) were the most important isotopic contribution (Table 3 and Fig. 5c). However, a different trend was found in MW, with crayfish (34%) and Small Puyen (30%) were found to be the most isotopically important and insect larvae (5%) being less isotopically important to Creole Perch. Published gut content analysis (Bubach 2010; Juncos et al. 2011; Table 3) also indicated the importance of insect larvae, as the most important items consumed by the native Creole Perch in addition to *S. spinifrons*. However, the SIAR mixing model output did not indicate *S. spinifrons* as an isotopically important contributor to Creole Perch in ME, only contributing 9% to the isotopic value (Table 3 and Fig. 5c). Published gut content data also identified a smaller proportion of Small Puyen and amphipod *Hyaletella* sp. in Creole Perch stomachs (Table 3). Note that zooplankton was not chosen as a food

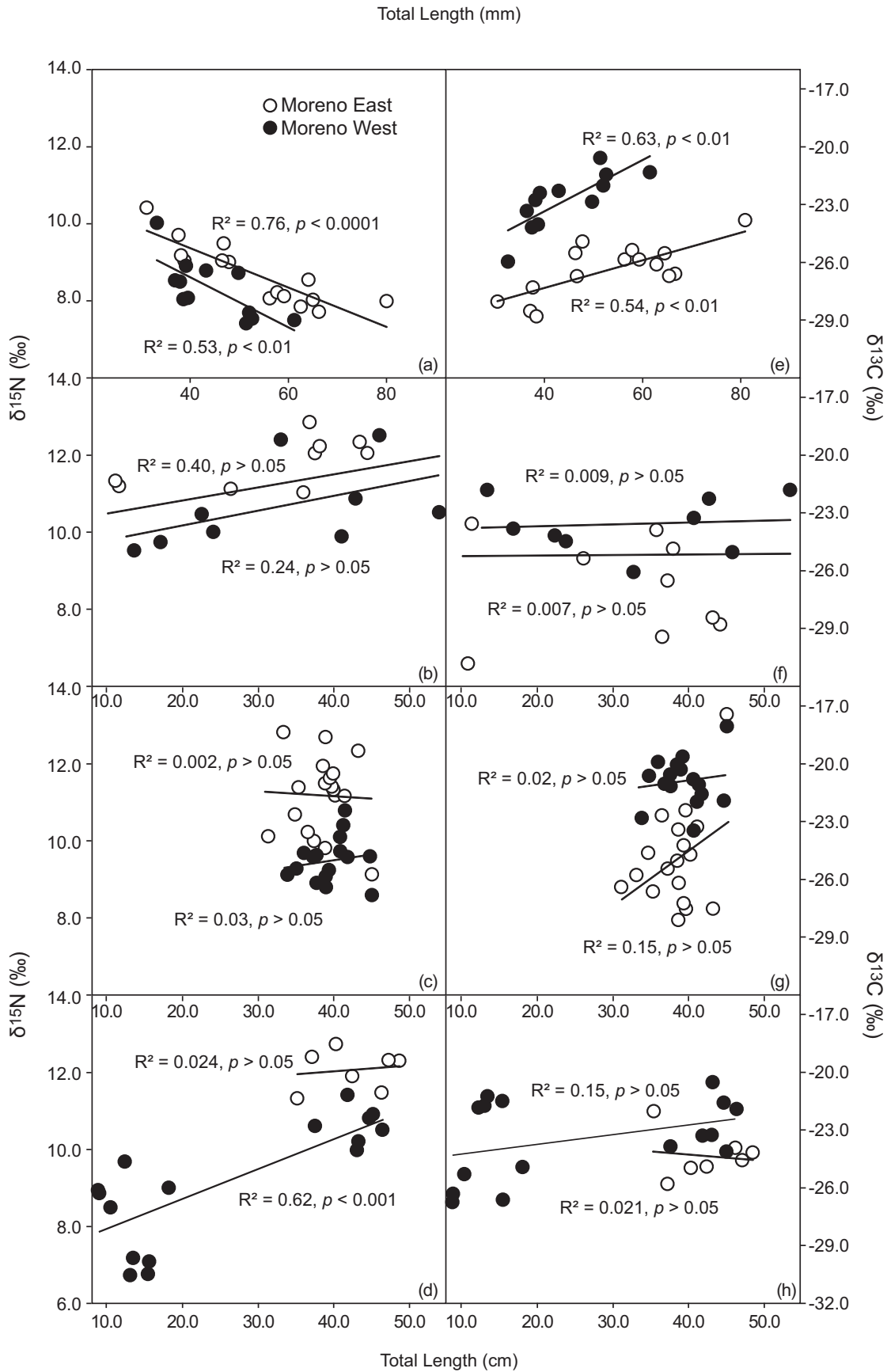


Fig. 6. Change in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values with increasing size for (a and e) Small Puyen, (b and f) Brook Trout, (c and g) Creole Perch, and (d and h) Rainbow Trout in Moreno East (open circles) and Moreno West (full circles).

source for any of the fish species analyzed with the mixing model, because it was reported only in the gut contents of Patagonian Silversides, Small Puyen and juvenile fish (Macchi et al. 1999; Reissig 2005; Wegrzyn and Ortubay 2009).

Although the leeches collected were not identified to the species level, they probably belong to the *Helobdella* genus as this genus was identified and widely found in NHNP lakes and ponds (Añón Suárez 1991; Gullo 2006, 2009). Members of *Helobdella* are predators of aquatic invertebrates (Siddall and Borda 2003). The high $\delta^{15}\text{N}$ values of leeches, similar to those of salmonids, indicate that leeches may be scavenging on preys enriched in ^{15}N such as crustaceans and fish.

Cabana and Rasmussen (1996) accounted that 68% of the among-lake variation in the $\delta^{15}\text{N}$ values of primary consumers was due to anthropogenic impacts such as sewage and Hansson et al. (1997) found that discharges from a sewage treatment plant increased significantly the whole food web $\delta^{15}\text{N}$ values. Some nitrogen sources such as sewage and fertilizers are enriched in ^{15}N compared to other sources as the result of isotope fractionation during ammonification (Heaton 1986), thus the $\delta^{15}\text{N}$ values of primary producers increase (McClelland and Valiela 1998) and the isotopic signals of anthropogenic nitrogen are transferred to consumers through trophic relationships (Cabana and Rasmussen 1996). In our study the differences in the $\delta^{15}\text{N}$ values observed between the basins might be explained in terms of higher anthropogenic influence such as sewage or fertilizers, in one basin than in the other, as the population density around ME basin is higher than in MW. Also, the presence of a small fish farm in ME may influence the $\delta^{15}\text{N}$ levels of the food web. Elevated $\delta^{15}\text{N}$ related to fish farms have been found in marine and freshwater systems worldwide (e.g., Kullman et al. 2009). Farmed fish are feed with fish pellets and kept in floating cages in the lake that allow the water to flow through them. Nitrogen-rich fish faeces and the uneaten pellets (rich in N and P) will sink and accumulate in the sediments around the cages (Temporetti and Pedrozo, 2000). Although we had no means of measuring these anthropogenic inputs to the lake, the higher $\delta^{15}\text{N}$ values observed in the base of the littoral and pelagic food web, aquatic macrophytes, periphyton and plankton from ME, could be the result of the differential anthropogenic impact between the basins.

The lower $\delta^{13}\text{C}$ values observed in ME zooplankton and fish respect to MW are consistent with the distinct morphometry of the basins. Although the area of both basins is very similar (Table 1 and Fig. 1), the perimeter-to-lake area ratio is almost twice as great in MW as in ME (Queimaliños et al. 2012). Therefore, the interface between the lake and the terrestrial environment is larger in MW, which is compatible with a higher influence of allochthonous carbon to this basin (Queimaliños et al. 2012).

In oligotrophic lakes, subsidization from terrestrial habitats is important for the entire food web. For example, Doi (2009) and Cole et al. (2006) estimated that in lakes, carbon derived from terrestrial inputs will make up to 70%, 85%, and 50% of carbon in zooplankton, benthic invertebrates, and fish, respectively. del Giorgio and France (1996) found that detritus from terrestrial or littoral origin to be enriched in ^{13}C compared to phytoplankton. Queimaliños et al. (2012) found lower rates of allochthony in ME compared to MW, associated with the upper landscape position, larger volume and water residence time and lower perimeter:lake area ratio. The higher $\delta^{13}\text{C}$ values observed in zooplankton from MW than ME may be related to this different carbon sources to the lake, with more littoral/allochthonous sources derived from terrestrial inputs in MW and more autochthonous primary production in the deeper ME basin. Small Puyen and juvenile fish are enriched in ^{13}C compared to the zooplankton on which they feed, and also reflect the difference in $\delta^{13}\text{C}$ values between ME and MW. Large fish, especially salmonids, consume a high proportion of smaller size fish

(Bubach 2010; Juncos et al. 2011; SIAR results in this work) and also presented higher $\delta^{13}\text{C}$ values in MW than in ME. Benthic macroinvertebrates are also part of the diet of salmonids and Creole Perch (Bubach 2010; Juncos et al. 2011; SIAR results in this work) however no differences were found in the $\delta^{13}\text{C}$ values between basins. The significant differences in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values observed in salmonids and Creole Perch from each basin and between taxa groups also suggest that although these top predator species are able to move freely from one basin to the other, and feed in either of them, their movement might be restricted to the basin where they have been captured. Movement of Creole Perch in Lake Moreno was studied by Buria et al. (2007) during the pre-spawning season. They observed that fish released in MW after attaching a tracking device stayed in that basin or migrated to lake Morenito to spawn, demonstrating that Creole Perch is capable of moving throughout the interconnected basins, although they did not give insights about the feeding habits of this species when moving through the system.

Our study shows that $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values are different at the base of the food web in ME and MW. The absolute $\delta^{15}\text{N}$ values were consistently higher in most of the organisms sampled in ME (higher $\delta^{15}\text{N}$ baseline); however, the estimated trophic position for each of the fish species were similar in both basins, indicating a similar trophic role for fish among basins and that they are feeding from food chains with a higher $\delta^{15}\text{N}$ baseline in ME.

In Lake Moreno, Small Puyen exhibited a positive relationship between length and $\delta^{13}\text{C}$ from -27.6% to -23.5% in ME and from -25.6% to -21.1% in MW and a negative relationship between length and $\delta^{15}\text{N}$, which varied from 10.4% to 10.0% in the smaller individuals (about 30 mm) to 8.0% and 7.6% in the bigger ones (> 6 mm) in ME and MW, respectively. These results indicate an ontogenetic shift between the pelagic zone of the lake where they feed on preys with high trophic level (high $\delta^{15}\text{N}$ values), to the littoral area where their $\delta^{15}\text{N}$ values were lower. This is supported by gut contents as the larger small puyen consumes amphipods, zooplankton, and chironomid larvae, from the littoral zone (Reissig 2005) which have lower $\delta^{15}\text{N}$ and higher $\delta^{13}\text{C}$ values while the smaller Puyen larvae consume pelagic zooplankton (Cussac et al. 1992; Barriga et al. 2002) with higher $\delta^{15}\text{N}$ and lower $\delta^{13}\text{C}$ values.

The lack of correlation between the carbon and nitrogen isotopic ratios in the other fish species analyzed with the exception of Rainbow Trout in MW may be caused by the fact that only adults and a few juveniles were sampled and a wider size spectrum might be needed to observe a trend (Fig. 6). The length of Creole Perch sampled ranged, between 314 and 453 mm in both basins and, in MW where the correlation between $\delta^{15}\text{N}$ and size was positive for Rainbow Trout, the size range was wider (89–465 mm) because some juveniles were caught in this case. On the contrary, in ME only adults between 355 and 487 mm were captured with gillnets. Although a few juveniles of Brook Trout were obtained in both basins, it seems that the size range was not large enough to establish a correlation between the variables analyzed.

This is the first study using C and N stable isotope analyses to assess food web structure in a double-basined lake in northern Patagonia. Studies in double-basined lakes are rare (e.g., Schindler 1974; Schindler et al. 1992) so Lake Moreno presents a good opportunity to study two connected systems with similar species assemblage, different hydrogeomorphic characteristics, and anthropogenic impact. Different baseline $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values between the basins are likely associated with these differences (e.g., greater perimeter-to-lake area ratio in one basin the other) that led to ME food web having a higher $\delta^{15}\text{N}$ baseline and carbon values derived from more pelagic sources as indicated by lower $\delta^{13}\text{C}$ values. SIA also allowed us to assess the ontogenetic change in the diet of the native Small Puyen and by using a Bayesian mixing model

we were able to analyze the potential trophic relationships of the most abundant fish in Lake Moreno food web.

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