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Stable isotope analysis of trophic structure, energy flow and spatial variability in a large ultraoligotrophic lake in Northwest Patagonia

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ABSTRACT

The food web structure of the largest lake in northwest Patagonia, Lake Nahuel Huapi (area 557 km²), was investigated. Fish, benthic macroinvertebrates, plankton and primary producers were analyzed for stable nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) isotopes in three sites with contrasting morphometry and environmental characteristics. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ revealed a similar food web structure between basins but with distinct isotopic baselines. The SIAR (Stable Isotope Analysis in R) mixing model was used to study potential food sources (benthic or pelagic) for adult fish. Results indicated that energy flow to fish from both habitats is different for each site and species, and does not depend on the basin morphometry. An ontogenetic shift in the carbon source and trophic level was observed in some of the fish species, revealing a coupling between the pelagic and benthic-littoral habitats of the lake. Our findings complement the existing literature on trophic interactions between introduced and native fish, and provide novel information on plankton composition, food web structure and energy flow in a large lake of North Patagonia that can be extrapolated to other understudied lakes in the area.

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Introduction

Lakes are complex ecosystems composed of several distinct subsystems or habitats. The pelagic habitat of lakes includes planktonic organisms, and fish and energy flow is largely through phytoplankton and bacterial pathways (Schindler and Scheuerell, 2002). In contrast, the benthic-littoral habitat is characterized by periphyton, macrophytes, macroinvertebrates, and fish, and the energy flow includes primary production from benthic algae and macrophytes, allochthonous inputs from the terrestrial system and sinking phytoplankton from the epilimnion (Fitzgerald and Gardner, 1993; Covich et al., 1999; Doi, 2009). Ecological interactions between the different habitats, the relative importance of the littoral habitat, and the potential for zoobenthos to contribute to whole-lake secondary production are determined by lake size and lake basin morphometry (Schindler and Scheuerell,

2002). Lake Nahuel Huapi is the largest (557 km²) and deepest (464 m) lentic water body of glacial origin in North Patagonia to the East of the Andes (Quirós and Drago, 1985; Iriondo, 1989). This lake has complex shoreline topography with seven branching arms, each with its own varying morphometric and environmental characteristics over a terrestrial gradient ranging from wet montane Andean forests in the north-west to dry temperate steppes in the east.

In nutrient-poor lakes, such as Lake Nahuel Huapi (Díaz et al., 1998), where low phytoplankton biomass and high transparency favor benthic primary production, littoral energy mobilization has been suggested to be of considerable importance for fish (Hecky and Hesslein, 1995; Vadeboncoeur et al., 2002). Large and deep lakes tend to have lower perimeter-lake area ratios, thereby reducing the potential contribution of benthic-littoral habitats in comparison with small and shallow lakes (Schindler and Scheuerell, 2002). However, for deep lakes with complex topography, such as Nahuel Huapi, Baikal (Central Asia), Malawi or Tanganyika (East Africa), the benthic-littoral habitats may become more important for certain bays with more shallowly tapering shorelines (e.g. Yuma et al., 2006). In this sense, Queimaliños et al. (2012) found that the neighboring connected lakes (Moreno West and Moreno East) have the same surface area but differential allochthonous indicators in the pelagic zone. This is related to the differences of

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perimeter-lake area ratio of both lakes, and the consequent dissimilarities of contact between the lake and the terrestrial environment.

Due to their high mobility, fish function as a strong link between benthic-littoral and pelagic habitats by feeding on prey from both habitats (Schindler and Scheuerell, 2002; Vander Zanden and Vadeboncoeur, 2002; Barriga et al., 2012; Reissig et al., 2015). In Patagonian lakes, exotic salmonids, particularly rainbow trout, play a major role in habitat coupling through predation in both benthic-littoral and pelagic habitats (Juncos et al., 2013). Recent investigations in Lake Nahuel Huapi have focused on the trophic interactions between top predator fish species and their prey through analyses of gut contents (Juncos et al., 2011, 2013; Juárez, 2012). However, there has been little research on whole food-web structure and the role of fish in connecting habitats within this oligotrophic lake. In particular, two native fish, the galaxiids *Galaxias platei* and *Galaxias maculatus*, are also an important link between benthic-littoral and pelagic habitats (Barriga et al., 2012; Reissig et al., 2015). This occurs in two ways: *Galaxias* spp. are an important link between their lower trophic invertebrate prey in both pelagic and benthic-littoral habitats and their piscivorous predators (Rechenq et al., 2011; Milano et al., 2013; Reissig et al., 2015), and *Galaxias* spp. will undergo ontogenetic migration from pelagic juvenile stages to benthic-littoral and deep-benthic adult stages, therefore connecting the two habitats through their lifecycle stages (Barriga et al., 2002).

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 position of the organisms in freshwater lacustrine ecosystems around the world (e.g., Post, 2002; Campbell et al., 2003; Solomon et al., 2011). The isotopic composition of the carbon and nitrogen in an animal reflects the nitrogen and carbon isotopic composition of its diet (DeNiro and Epstein, 1978, 1981). Consumers are typically enriched in ^{15}N relative to its diet; therefore, the $\delta^{15}\text{N}$ values can be used to estimate trophic position. In contrast, $\delta^{13}\text{C}$ values of consumers change little with trophic transfers (Post, 2002) consequently they can be used to determine original sources of dietary carbon (Layman et al., 2011). This approach has been used in North Patagonian lakes to analyze and compare the food web structure of the double-basin system of Lake Moreno (Arcagni et al., 2013), which is characterized by contrasting hydrogeomorphic characteristics in each basin (Queimaliños et al., 2012). Lake Moreno is an ultraoligotrophic lake that flows through a short river into Lake Nahuel Huapi and possesses a similar food web structure as the larger lake.

The similar species assemblages and the inter-basin differences between each branch of Lake Nahuel Huapi, as well as the use of stable isotope techniques to assess food web structure and energy flow, provided the opportunity to formulate the following questions: Are there differences in the food web structure between basins? Are there differences in the isotopic baselines between the basins? Is the contribution of pelagic and benthic-littoral energy sources to top predator fish related to the morphometry of the basins? In this context the objectives of this work are to characterize through stable isotope analysis the food web structure of Lake Nahuel Huapi in three basins with differential morphometric and environmental features, to compare the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the organisms between the basins, basins, and to determine the contribution of pelagic and benthic-littoral carbon sources to the top predator fish species in each branch.

Materials and methods

Study Site

Lake Nahuel Huapi (41°03'S, 71°25'W), located in Nahuel Huapi National Park (NHNP, Fig. 1), is an ultraoligotrophic system with a mean annual Secchi depth of 12 m, euphotic zone ($Z_{1\%}$) that extends to 48.8 m, total phosphorus of $5.1 \mu\text{g L}^{-1}$, and chlorophyll *a* of $0.6 \mu\text{g L}^{-1}$ (Alcalde et al., 1999; Caravati et al., 2010). Like all the lakes

in Andean Patagonia, Lake Nahuel Huapi is of glacial origin and has been classified as warm monomictic with summer stratification (Quirós and Drago, 1985; Diaz et al., 2007).

Because of the constant west winds and the Andes mountain range that play a crucial role in determining the precipitation regime of the area, there is a strong west–east climatic gradient. As a result, between the westernmost and easternmost margins of Lake Nahuel Huapi, precipitations shift from 3000 mm to less than 700 mm, influencing plant distribution. Hence, while mountain slopes on the west are covered by dense forest, on the east the vegetation is characterized by grass and shrub species typical of the Patagonian steppe.

Three sites were selected in Lake Nahuel Huapi for this study: Brazo Rincón (BR), Bahía López (BL), and Dina Huapi (DH) (Fig. 1). Brazo Rincón is situated on the northwestern part of the lake in the rainiest region of NHNP, with average precipitation of 2800 mm y^{-1} (data provided by the Autoridad Interjurisdiccional de Cuenca (AIC)). This branch is surrounded by the Andino-Patagonian forest characterized by *Nothofagus dombeyi* (Coihue), *Chusquea culeou* (Caña colihue) in the understory, and other native tree and shrub species. The branch is characterized by an extended littoral zone with sandy beaches, and the basin has a regular shape with a maximum depth of approximately 100 m and an area of around 7.7 km^2 .

Bahía López is a small, shallow, and closed bay (Fig. 1) with a maximum depth around 50 m and an area of approximately 1 km^2 . The average precipitation rates in BL are of 1200 mm y^{-1} (AIC), and the area is surrounded by a mixed forest of *N. dombeyi* and *Austrocedrus chilensis* (Cordilleran cypress). There are a few rocky beaches around the bay and Lake Moreno's connection with Lake Nahuel Huapi is on the south-east margin of the basin.

The third site, DH, is located in the main and largest branch of Lake Nahuel Huapi, near River Limay, the outflow of the lake, and downstream of the city of San Carlos de Bariloche (population 130,000) (Fig. 1). This sampling point is located in the driest region of the lake (500 mm y^{-1} , AIC) with a maximum depth of 236 m. Besides a few tree species that grow near the shoreline of DH, such as *Nothofagus antarctica* (Ñire), *Maytenus boaria* (Mayten), and exotic *Salix* sp. (Willow), the dominant vegetation is typical of the Patagonian steppe.

The fish community of Lake Nahuel Huapi is represented by five native species, namely *Percichthys trucha* (creole perch), *Galaxias maculatus* (small puyen), *Galaxias platei* (big puyen), *Olivaichthys viedmensis* (velvet catfish), and *Odontesthes hatcheri* (patagonian silver-side), and three introduced salmonid species: *Oncorhynchus mykiss* (rainbow trout), *Salmo trutta* (brown trout), and *Salvelinus fontinalis* (brook trout) (Juncos et al., 2013). Information on the composition of the macroinvertebrate community of Lake Nahuel Huapi is scarce and the best sources of Nahuel Huapi freshwater invertebrate species are from published fish gut content analysis. Crustacean decapods (*Aegla* sp. and *Samastacus spinifrons*), amphipods (*Hyalella* sp.), gastropods (*Chilina* sp.), bivalves (*Diplodon chilensis*), and insect larvae are all important dietary items for littoral-feeding fish (Juncos et al., 2011; 2013).

The phytoplanktonic community is dominated by the bacillariophyceans *Cyclotella steligera*, *Rhizosolenia eriensis*, and *Synedra ulna*, the dinophycean *Gymnodinium* sp., the prymnesiophycean *Chrysochromulina parva*, and the cyanophycean *Dactylococcopsis raphidioides* (Diaz et al., 1998). Balseiro et al., (2007) identified the cladocerans *Bosmina longirostris*, *Bosmina chilensis*, and *Ceriodaphnia dubia* and the copepod *Boeckella gracilipes* as part of the zooplanktonic community.

Sampling and sample preparation

Sampling campaigns were carried out in two seasons, February (summer) and May (fall) 2011. Benthic macroinvertebrates, riparian tree leaves, and biofilm were sampled in summer when organisms are most abundant, fish in fall after the sport fishing season was over, and

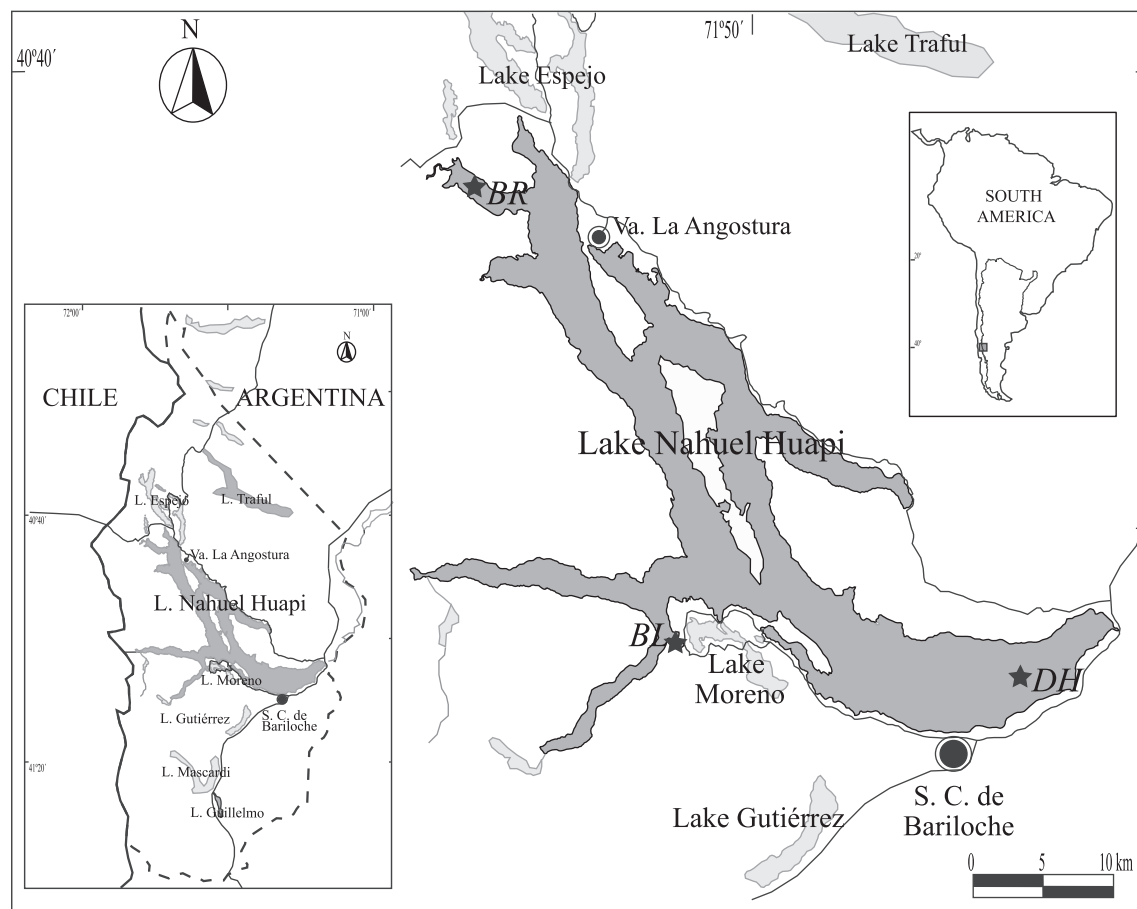


Fig. 1. Location of the study area in South America and in Nahuel Huapi National Park. Lake Nahuel Huapi area showing sampling points: BR—Brazo Rincón, BL—Bahía López, and DH—Dina Huapi.

plankton in both seasons. All samples collected at each site were taken to the laboratory and processed immediately within 24 h of sampling. Because the samples were also collected for trace metal analyses for separate studies (e.g. Rizzo et al., 2014), all samples were rinsed with ultra-pure water, homogenized with titanium and Teflon® devices, frozen at $-40\text{ }^{\circ}\text{C}$ and freeze-dried until constant weight, then ground to fine powder using a Teflon® pestle and mortar.

Large fish such as the introduced salmonids and the native creole perch, velvet catfish, and big puyen were captured using gill net gangs set at dusk perpendicular to the shore from 2 m down to 40 m deep, and raised early in the morning, averaging 12 h of soak time. Nets consisted of six 10-m-long panels of 15, 20, 30, 50, 60, and 70 mm bar mesh size. Native small puyen and juvenile salmonids were collected using double-funnel cylinder fish traps left at each site for 24 h or by using seine nets (12 m long and 1 mm bar mesh size). Fish were identified to species in the field, and their total length and mass recorded. Muscle tissue was removed in fish larger than 80 mm and homogenized individually. Muscle tissue could not be removed from fish smaller than 80 mm (e.g. small puyen) in sufficient quantity for analyses, so the head and the guts were removed from each individual and several individuals of similar size were pooled. Two of the five native (creole perch, small puyen) and two of the three introduced fish species (rainbow trout and brown trout) were captured in all three sites. Exceptions include big puyen which were captured in BR and DH only, and velvet catfish were only captured at BL and DH. The Patagonian silverside and brook trout were not captured in any of the sites despite extensive efforts to capture all species.

Benthic macroinvertebrates such as insect larvae, crustaceans, and snails were hand-picked from submerged logs and stones and the

mussel *D. chilensis* was collected by SCUBA divers between 2 and 15 m deep. Trichoptera (caddis flies) and Ephemeroptera (mayflies), the snail *Chilina* sp., the decapod *Aegla* sp., and the amphipod *Hyalella* sp. were collected at all the three sites. The crayfish *S. spinifrons* was collected at the three sites and the mussel *D. chilensis* and Chironomidae larvae (nonbiting midges), were collected only at BL and BR, respectively.

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 (*N. dombeyi*, *N. antarctica*, *Weinmannia trichosperma*, *Lomatia hirsuta*, *Luma apiculata*, *A. chilensis*, *M. boaria*, and *Amomyrtus luma*), shrubs (*Salix* sp., *Desfontainia spinosa*, *C. culeou*, *Escallonia rubra*, and *Gaultheria mucronata*), and herbaceous plants (*Hydrocotyle chamaemorus*). As many macroinvertebrates (e.g. Trichoptera larvae) were found living on submerged decomposing leaves (detritus), identifiable leaves were also collected underwater from the littoral shoreline.

Plankton samples were collected by vertical tows from 80 m to surface in BR and DH, and from the deepest point at 45 m to the lake surface in BL (Fig. 1). Nets of three different mesh sizes (10, 53, and 200 μm) were used in all cases. Samples were filtered in the field and in the laboratory as detailed by Arribé et al. (2010) to obtain three concentrated plankton fractions: P1 (≥ 10 and $< 53\ \mu\text{m}$ – phytoplankton and small mixotrophic ciliates), P2 (≥ 53 and $< 200\ \mu\text{m}$ – small zooplankton) and P3 ($> 200\ \mu\text{m}$ – large zooplankton). To evaluate species composition and biomass proportions in each fraction size, other water samples were obtained simultaneously with a 12-L Schindler-Patalas trap every 10 m between surface and bottom. A 250-mL sample of the lake water at each depth was preserved with acid Lugol's solution and immediately put in darkness to evaluate species composition of P1.

Plankton samples for P2 and P3 were obtained by sieving of 24-L water samples through a 53- μ m mesh and preserved with 4% formaldehyde. P1 and P2 were observed under direct microscope by scanning an entire 1 ml Sedgwick–Rafter chamber surface, and microcrustaceans (i.e. copepods and cladocerans) were identified under stereomicroscope in a 5-mL Bogorov chamber. Data are presented as mean proportions of zooplankton biomass in each fraction size. Littoral algal biofilm was obtained by scraping submerged stones and was repeatedly washed with distilled water to remove debris by decantation and filtration.

Decapods and mollusks were removed from their carapaces/shells and only muscle tissue was analyzed for stable isotopes. Whole bodies were analyzed for the remaining macroinvertebrates, including caddis fly larvae which were removed from their cases; but gut contents were not evacuated prior to analysis. For most invertebrate taxa, individual sample masses were not sufficient for analyses so homogeneous samples of pooled individuals, grouped by species and similar size, were prepared.

Stable isotope analyses

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

$$\delta(\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \cdot 1000.$$

Stable carbon and nitrogen isotopes were measured via DELTA^{PLUS}XP continuous flow stable isotope ratio mass spectrometer at the Queen's Facility for Isotope Research (QFIR), Queen's University, Kingston, ON, Canada. To check the quality of the analyses, for every 10 carbon/nitrogen samples, we ran one of the following standard reference materials and in-house standards (mean \pm SD): carbon standards "UC-1" ($\delta^{13}\text{C} = -25.6 \pm 0.2\text{‰}$, $n = 5$) and "Q-C" ($\delta^{13}\text{C} = -26.0 \pm 0.2\text{‰}$, $n = 8$), lipid-normalized Atlantic salmon (*Salmo salar*) ($\delta^{13}\text{C} = -18.3 \pm 0.1\text{‰}$, $\delta^{15}\text{N} = 8.5 \pm 0.1\text{‰}$, $n = 10$), red tilapia (*Oreochromis* sp.) ($\delta^{13}\text{C} = -20.7 \pm 0.1\text{‰}$, $\delta^{15}\text{N} = 5.8 \pm 0.1\text{‰}$, $n = 6$), and domestic chicken (*Gallus gallus*) blood ($\delta^{13}\text{C} = -19.9 \pm 0.1\text{‰}$, $\delta^{15}\text{N} = 4.0 \pm 0.5\text{‰}$, $n = 4$). Duplicates were also run every 10 samples and produced a difference between samples of (\pm SD $\delta^{13}\text{C} = 0.13\text{‰}$, $\delta^{15}\text{N} = 0.27\text{‰}$, $n = 25$).

Data analysis

Stable isotope biplots for each basin were used to visually compare food web relationships among the species. Multiple linear regressions were applied to the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of the most abundant ($n \geq 5$) fish species (rainbow trout, creole perch, and small puyen from the three sites; brown trout and big puyen from BR) against the fish total length and weight as indicators of fish growth, to assess if the observed variation in the isotopic values corresponded to ontogenetic dietary niche shifts that results in the consumption of prey with different isotopic values. Differences in stable isotope values of the organisms between the three sampling sites were assessed using non-parametric MANOVA (NPMANOVA). Multiple linear regressions were performed using XLSTAT v7.5 add-in for Microsoft Office Excel and NPMANOVA using Primer v7 with PERMANOVA + add on.

To evaluate the relative contribution of the pelagic vs the littoral food sources to salmonids and creole perch, we fitted the Bayesian stable isotope mixing model SIAR v4.2 (Stable Isotope Analysis in R) (Parnell et al., 2010) using R v3.11 for Windows (R Development Core Team, 2013) for each site and fish species separately. The number of iterations was 500,000 whereas 50,000 initial iterations were discarded. The information supplied to the model included the isotopic data of consumers (rainbow trout, brown trout, and creole perch), and those of their potential prey items representing two habitats, benthic-littoral prey was represented by the crayfish *Samastacus spinifrons*,

while pelagic prey was represented by zooplankton (P3). These items were chosen because they are directly or indirectly part of the consumer's diet and because they were sampled in the three sites. Salmonids and creole perch prey on crayfish (Juncos et al., 2013, 2015); and, although they do not feed directly on zooplankton, they prey over the main planktivorous fish in the lake, the small puyen (Cervellini et al., 1993; Reissig et al., 2003; Barriga et al., 2012; Juncos et al., 2015). The SIAR model also requires incorporation of a Trophic Enrichment Factor (TEF) for the food web as well as C and N elemental concentrations for dietary sources (obtained along with the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from the laboratory). As there is no literature on food web Trophic Enrichment Factors (TEFs) for Patagonian aquatic food webs, we chose to use the TEF values derived from McCutchan et al. (2003) meta-analysis for fish muscle ($\Delta^{15}\text{N} = 2.9 \pm 0.32$ and $\Delta^{13}\text{C} = 1.3 \pm 0.3$). These TEFs have been used in many mixing models with several fish species as consumers (e.g. Harrod et al., 2010; Hayden et al., 2013; Bergamino and Richoux, 2015).

Results

Dinophyceae was the dominant taxon in terms of biomass in P1 in BL in both seasons, in DH in summer, and in BR in fall, in contrast, small mixotrophic ciliates, which were less abundant, were the dominant group with more than 50% of the biomass in BR during summer (Fig. 2). In P2, larger mixotrophic ciliates (*Ophridium naumanni* and *Stentor araucanus*) were dominant in all sites and seasons except BL in summer where rotifers were the most abundant taxa (Fig. 2). The larger fraction, P3, was composed mainly of copepods (*Boeckella gracilipes* and cyclopoids) and cladocerans (*Ceriodaphnia dubia*, *Daphnia* sp., and *Bosmina longirostris*). In this fraction *B. gracilipes* was the most abundant species in summer at the three sites and in BR in fall. The second most abundant species in summer in BL and DH and in fall in BR, *B. longirostris*, was the dominant taxa in BL in fall (Fig. 2). The cladocerans *C. dubia* and *Daphnia* sp. were important in terms of biomass in BR during summer (Fig. 2).

In general, the $\delta^{15}\text{N}$ vs $\delta^{13}\text{C}$ biplots showed a similar food web structure among the three sites with creole perch, brown trout and rainbow trout in the highest trophic level as they are enriched in ^{15}N relative to other fish species and organisms sampled (Fig. 3). The $\delta^{15}\text{N}$ of small puyen and the largest plankton size fraction which includes zooplankton (P3) were similar to each other and higher than those of other fish such as juveniles of salmonids and velvet catfish. Macroinvertebrates were depleted in ^{15}N compared to fish and had similar $\delta^{15}\text{N}$ as the smaller plankton fractions (P1 and P2). Biofilm, leaves, and detritus were the most ^{15}N depleted items sampled. Within the macroinvertebrate community the decapods *Aegla* sp. and *S. spinifrons* presented the highest trophic level relative to other invertebrates, including insect larvae such as Ephemeroptera and Trichoptera, which were depleted in ^{15}N and ^{13}C (Tables 1 and 2, Fig. 3). In all the sites, the largest plankton size fraction (P3) were enriched in ^{15}N compared to the smaller plankton fractions and depleted in ^{13}C relative to P1, P2, fish, and macroinvertebrates in all the sites (Tables 1 and 2, Fig. 3).

In general, macroinvertebrates and small fish had mean $\delta^{13}\text{C}$ that fell in the range of the large fish $\delta^{13}\text{C}$ values, except for the snail *Chilina* sp. which was enriched in ^{13}C compared to the rest of macroinvertebrates and fish, being also enriched in ^{13}C (and ^{15}N) than their food source, biofilm (Fig. 3). Biofilm, leaves, detritus, and phytoplankton of P1, which are potential carbon sources for consumers, exhibited different carbon isotope ratios among them, with biofilm samples being relatively enriched in ^{13}C compared to phytoplankton and terrestrial material (Table 2, Fig. 3).

A positive linear relationship between $\delta^{15}\text{N}$ and fish mass was observed in rainbow trout from BL and DH (BL - $F_{2,9} = 50.3$, $R^2 = 0.92$, $p \leq 0.0001$; DH - $F_{2,10} = 12.6$, $R^2 = 0.72$, $p = 0.002$) and brown trout in BR ($F_{2,25} = 599.5$, $R^2 = 0.98$, $p < 0.0001$), and a negative relationship was observed for small puyen in BR ($F_{2,8} = 12.7$, $R^2 = 0.76$, $p = 0.003$)

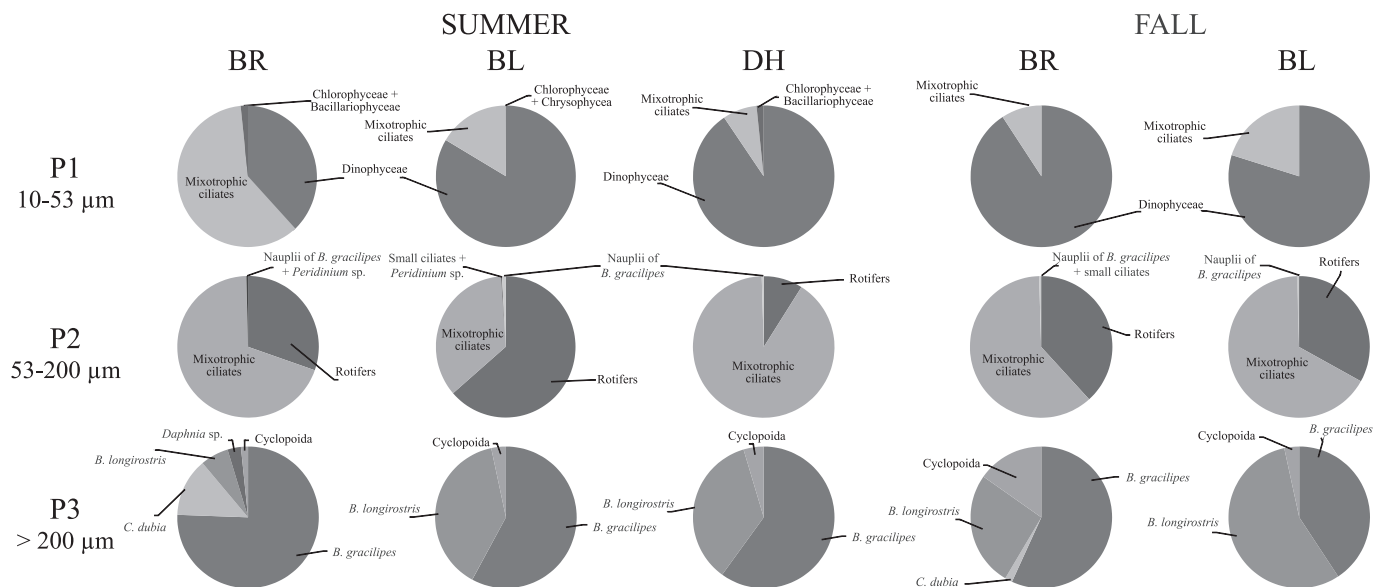


Fig. 2. Biomass proportion of plankton taxa present in Fraction 1 (P1 10–53 μm), Fraction 2 (P2 53–200 μm), and Fraction 3 (P3 > 200 μm), in Brazo Rincón (BR), Bahía López (BL), and Dina Huapi (DH) in summer and fall.

for the same variables. The $\delta^{13}\text{C}$ values showed a positive linear relationship with fish mass in brown trout from BR ($F_{2,25} = 77.2$, $R^2 = 0.86$, $p < 0.0001$) and in small puyen from DH ($F_{2,11} = 7.3$, $R^2 = 0.57$, $p = 0.01$); however, no relationships were observed for the rest of the fish species analyzed in the different sites.

When comparing the $\delta^{15}\text{N}$ – $\delta^{13}\text{C}$ values of the most common and abundant ($n \geq 5$) fish (creole perch, rainbow trout, and small puyen) and macroinvertebrates (crayfish) collected in the three sites, the analysis showed significant differences in the $\delta^{15}\text{N}$ – $\delta^{13}\text{C}$ values among the three sites (2-factor PERMANOVA $Pseudo-F_{11-91} = 5.28$, $p = 0.001$). Pairwise tests showed that the $\delta^{15}\text{N}$ – $\delta^{13}\text{C}$ values of creole perch (BL-BR $t = 2.06$, $p = 0.01$; BL-DH $t = 5.20$, $p = 0.001$, BR-DH $t = 8.12$, $p = 0.002$) and crayfish (BL-BR $t = 2.14$, $p = 0.04$; BL-DH $t = 3.66$, $p = 0.003$, BR-DH $t = 4.85$, $p = 0.001$) differed significantly among the sites; however, there were no significant differences in the $\delta^{15}\text{N}$ – $\delta^{13}\text{C}$ values of rainbow trout ($t = 0.539$, $p = 0.78$) and small puyen ($t = 1.02$, $p = 0.37$) between BL and BR.

The SIAR mixing model indicated that the use of pelagic and benthic-littoral habitats for salmonids and creole perch was different for each species depending on the site (Fig. 4). The pelagic contribution to brown trout from BR (mode: 99, 95% credibility limits: 81–100) and creole perch from BR (mode 99, 95% credibility limits: 92–100) and BL (mode 96, 95% credibility limits: 63–100) was higher than the benthic contribution. A similar contribution from the pelagic and benthic habitats was observed in rainbow trout from BR (pelagic: mode: 55%, credibility limits: 38–98; benthic: mode: 45%, credibility limits: 2–63) and BL (pelagic: mode: 51%, credibility limits: 29–77; benthic: mode: 49%, credibility limits: 23–71) (Fig. 4). In DH the relative contribution from benthic-littoral sources is significantly higher for both rainbow trout (mode: 88, 95%, credibility limits: 61–100) and creole perch (mode: 87%, credibility limits: 62–100) (Fig. 4).

Discussion

Macroinvertebrate consumers from the littoral zones of lakes have access to several different food sources such as epiphytes attached to macrophytes, vascular plants, phytoplankton, and decaying macrophytes (Cremona et al., 2010) that may differ in their isotopic value. In Lake Nahuel Huapi all primary producer and detritus sources exhibited distinctive $\delta^{13}\text{C}$ values, with the phytoplankton of P1, terrestrial vegetation, and detritus more depleted in ^{13}C compared to biofilm. In all three

sites, biofilm, phytoplankton from P1, and plant detritus are the most likely carbon sources for the primary consumers. Furthermore, in BL and BR, terrestrial vegetation is abundant and is close to the shoreline, and therefore provides a significant source of carbon to macroinvertebrates. The $\delta^{13}\text{C}$ of nearly all the macroinvertebrates collected spread in the range of the values of the primary producer C sources suggesting a mixed diet incorporating diverse sources of carbon. However, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the biofilm grazer snail *Chilina* sp. was consistent with specialized feeding on biofilm samples, which had also been observed for this species in the neighboring Lake Moreno (Arcagni et al., 2013).

In all the sites, the large plankton size fraction (P3) represented by copepods and cladocerans, was depleted in ^{13}C and enriched in ^{15}N compared to the smaller size fractions, P1 and P2. The higher $\delta^{15}\text{N}$ values observed in P3 are consistent with the feeding habits of the adult copepods (*B. gracilipes*) and cladocerans (*B. longirostris* and *C. dubia*). The copepod has an omnivorous diet composed mainly of ciliates and nanoflagellates of 4 to 33 μm (Balseiro et al., 2001) that correspond to components of P1 (10–53 μm) of this study and smaller organisms, and the cladocerans feed on phytoplankton and bacteria (Modenutti et al., 2010). P3 was depleted in ^{13}C relative to P1 and P2, contrary to what is expected, as consumers are usually slightly enriched in ^{13}C compared to their prey (Post, 2002). This is not limited to Lake Nahuel Huapi – a similar pattern was observed for size-fractionated plankton in neighboring Lake Moreno (Arcagni et al., 2013) and is actually a widespread phenomenon found in a wide range of lakes (del Giorgio and France, 1996), that may be caused by high lipid content in zooplankton that may result in a depletion of ^{13}C or by zooplankton relying on energy mobilized by phyto- and bacterioplankton not captured in our three size fractions (del Giorgio and France, 1996).

As expected, large piscivorous fish species such as salmonids and creole perch presented the highest $\delta^{15}\text{N}$ values, positioning them in the highest trophic level of Lake Nahuel Huapi. In general, in Lake Nahuel Huapi, creole perch has a more specialized diet preying mainly on the crayfish *S. spinifrons* while salmonids are more generalists preying mostly on all life stages of small puyen and also on macroinvertebrates, such as insect larvae and crayfish (Juncos et al., 2011; 2013; 2015). In addition, their diets are supplemented by other prey, such as adult insects, crabs, and mollusks, depending on the species, season and fish size (Juncos et al., 2013).

The galaxiids, small and big puyen, and salmonid juveniles were depleted in ^{15}N relative to those of the larger piscivorous fish species.

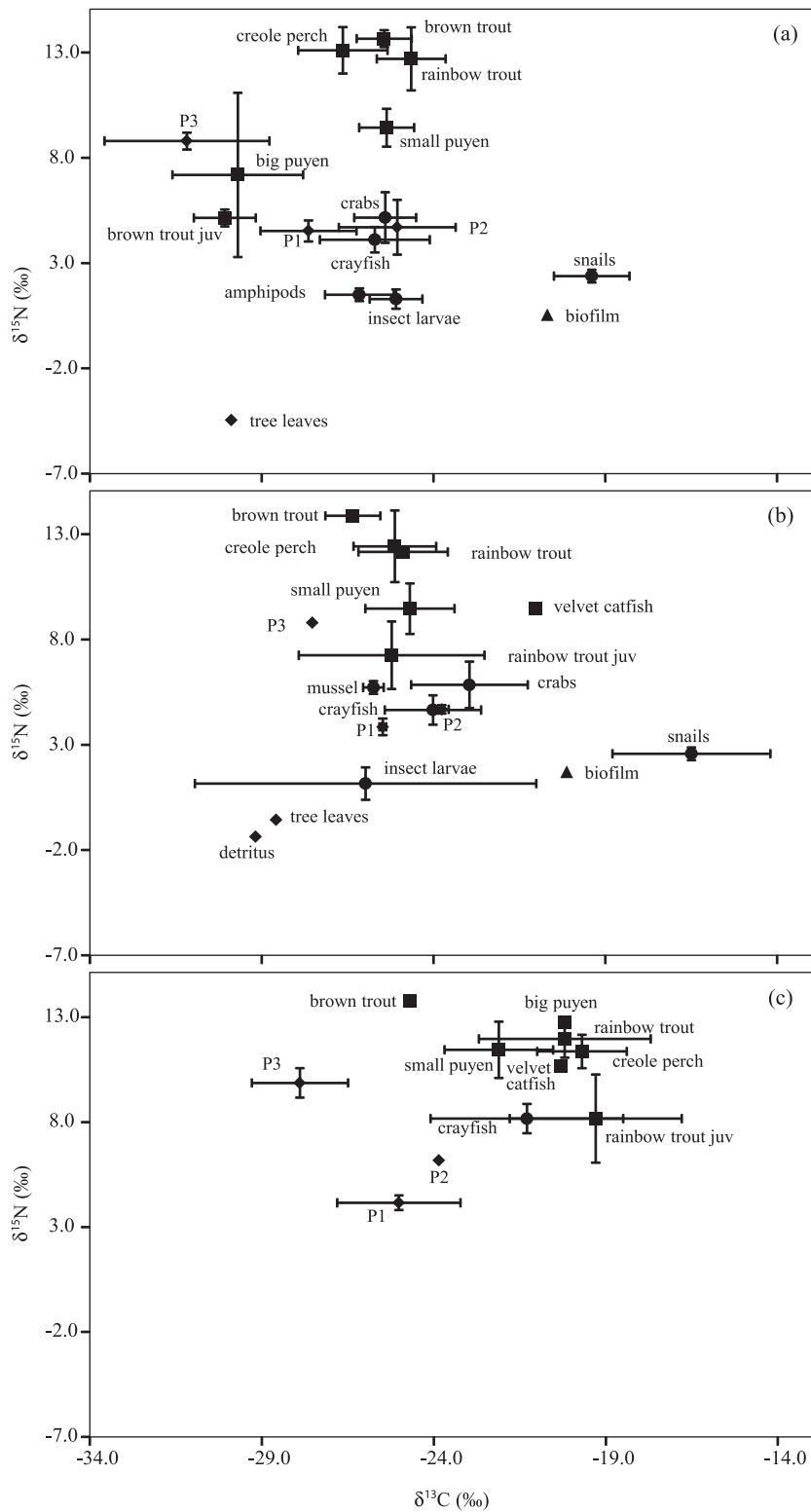


Fig. 3. Mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values and their standard deviations for fish (square symbols), macroinvertebrates (circles), and plankton (diamonds), in Brazo Rincón (a), Bahía López (b), and Dina Huapi (c).

Small puyen is the main planktivorous species of the lake, with the juveniles consuming mainly zooplankton and adults combining pelagic and littoral prey such as chironomid larvae (Cervellini et al., 1993; Reissig et al., 2003; Barriga et al., 2012; Milano et al., 2013). These differences in diet are reflected in the enriched ^{15}N observed in the juveniles

of small puyen. Meanwhile, adults also reflect a high consumption of pelagic prey enriched in ^{15}N relative to insect larvae and closer to P3. In this sense, Reissig et al. (2015) observed a high selection of small puyen towards pelagic prey for all fish sizes during night in the neighboring Lake Moreno. These authors demonstrated that juveniles and

Table 1

Range, mean and standard deviation (SD) of the stable isotope ratios of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$), and total length for fish collected in Bahía López (BL), Brazo Rincón (BR), and Dina Huapi (DH) from Lake Nahuel Huapi. *N* is the number of samples analyzed; the numbers in parentheses represent the total number of organisms collected.

Organisms	Sampling site	<i>N</i>	Mean \pm SD		
			Total length (mm)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
<i>Galaxias maculatus</i> (small puyen)	BL	8 (22)	48.6 \pm 5.5 42–61	9.2 \pm 1.3 7.7–11.4	–24.8 \pm 1.4 –26.2 to –22.2
	BR	8 (41)	50 \pm 4 43–58	8.9 \pm 0.5 8.2–9.8	–25.2 \pm 0.8 –26.1 to –23.6
	DH	9 (25)	45.5 \pm 3.5 41–56	11.6 \pm 1.6 9.9–15.6	–21.5 \pm 1.5 –23.8 to –19.1
<i>G. maculatus</i> juveniles	BL	2 (6)	35.3 \pm 0.8 35–37	10.3 \pm 0.0 10.2–10.3	–24.2 \pm 0.8 –24.7 to –23.6
	BR	3 (17)	35.8 \pm 1.6 33–39	10.6 \pm 0.6 10.0–11.1	–25.8 \pm 0.6 –26.5 to –25.4
	DH	5 (13)	34.2 \pm 1.6 30–37	10.9 \pm 0.2 10.7–11.3	–23.3 \pm 0.8 –24.0 to –21.9
<i>Galaxias platei</i> (big puyen)	BR	8 (8)	211 \pm 15 190–232	7.2 \pm 3.9 1.8–13.2	–29.7 \pm 1.9 –31.8 to –26.3
	DH	1 (1)	230	12.7	–20.2
<i>Oliveichthys viedmensis</i> (velvet catfish)	BL	1 (1)	266	9.4	–21.0
	DH	1 (1)	395	10.6	–20.3
<i>Oncorhynchus mykiss</i> (rainbow trout)	BL	4 (4)	448 \pm 118 341 – 584	12.1 \pm 0.2 11.8–12.3	–24.9 \pm 1.3 –26.0 a –23.0
	BR	5 (5)	577 \pm 50 500–627	12.7 \pm 1.5 10.0–13.7	–24.7 \pm 1.0 –26.1 to –23.8
	DH	10 (10)	408 \pm 144) 240–652	11.9 \pm 0.9 10.5–13.5	–20.2 \pm 2.5 –23.4 to –15.6
<i>O. mykiss</i> juveniles	BL	8 (8)	103 \pm 36 72–167	7.2 \pm 1.6 5.8–10.8	–25.2 \pm 2.7 –29.5 to –20.6
	DH	3 (3)	71 \pm 20 53–92	8.1 \pm 2.1 6.3–10.4	–19.3 \pm 2.5 –21.8 to –16.7
<i>Percichthys trucha</i> (creole perch)	BL	9 (9)	445 \pm 49 325–490	12.4 \pm 1.7 8.7–14.2	–25.1 \pm 1.2 –26.7 to –23.2
	BR	13 (13)	474 \pm 22 429–511	13.1 \pm 1.1 10.8–14.1	–26.7 \pm 1.3 –29.4 to –25.2
	DH	5 (5)	357 \pm 120 180–450	11.3 \pm 0.8 10.1–12.3	–19.7 \pm 1.3 –21.4 to –18.1
<i>Salmo trutta</i> (brown trout)	BL	2 (2)	597 \pm 91 532–661	13.8 \pm 0.1 13.7–13.9	–26.4 \pm 0.8 –27.0 to –25.8
	BR	23 (23)	608 \pm 62 468–713	13.6 \pm 0.4 12.5–14.5	–25.5 \pm 0.8 –26.9 to –24.1
	DH	1 (1)	548	13.7	–24.7
<i>S. trutta</i> juveniles	BR	5 (13)	55 \pm 17 40–84	5.1 \pm 0.4 4.5–5.6	–30.1 \pm 0.9 –31.2 to –28.8

adults of small puyen use low light “windows” as refuge from large visual predators as *P. trucha* and introduced salmonids to exploit high quality pelagic resources.

Juvenile salmonids, which tend to have a littoral diet consisting of insects, amphipods and immature stages of small puyen (Juncos et al., 2011; 2013), presented the lowest $\delta^{15}\text{N}$ of the fish community. The $\delta^{13}\text{C}$ values of the different prey items of salmonids and creole perch (e.g. small puyen, decapods, insect larvae) are in the range of the values observed for the fish, consistently with the stomach content analysis from Juncos et al. (2011; 2013).

Rainbow trout and brown trout exhibited size-related shifts in $\delta^{15}\text{N}$ values indicating ontogenetic shifts in diet composition. This variation is related to changes in the diet as the fish grows, from prey depleted in ^{15}N (insect larvae, amphipods and small puyen adults), to prey enriched in ^{15}N such as crayfish and juveniles of bigger fish (Juncos et al., 2015). The lack of variation between $\delta^{15}\text{N}$ and the fish length and weight for creole perch, rainbow trout from BR and big puyen in our sampling set may be caused by the narrow spectrum of sizes captured (between 300 and 500 mm for creole perch, between 500 and 600 mm for rainbow trout from BR, and around 200 mm for big puyen). Size-related diet shifts were observed in Lake Nahuel Huapi by analyzing stomach contents in creole perch (Juncos et al., 2015), but our sample set did not include the smaller sizes included in the diet analysis performed by those authors. The negative correlation between $\delta^{15}\text{N}$ and the fish

length and weight observed in small puyen in BR was also recorded for the same species in the neighboring Lake Moreno (Arcagni et al., 2013), and is associated to the more planktivorous diet in juveniles than adults. The trend suggests an ontogenetic dietary shift from plankton enriched in ^{15}N to a combined diet with some prey depleted in ^{15}N such as chironomid larvae. These results are supported by gut content analysis that showed that small puyen larvae consume plankton and as juveniles and adults combine larger littoral prey such as chironomid larvae with pelagic zooplankton (Cervellini et al., 1993; Barriga et al., 2012; Milano et al., 2013; Reissig et al., 2015). In general, plankton was enriched in ^{15}N in DH, particularly in P2 and P3. Karlsson et al. (2004) and Syväranta et al. (2008) observed that cladocerans were depleted in ^{15}N compared to copepods. However, the higher $\delta^{15}\text{N}$ observed in DH could not be due to a difference in species composition, as the proportion of copepods to cladocerans was higher only in BR. Crayfish and zooplanktivorous small puyen from DH were also enriched in ^{15}N relative to specimens from the other basins. Elevated $\delta^{15}\text{N}$ baseline values for the DH food web may help explain the differences between the nitrogen isotopic ratios of these taxa. The $\delta^{15}\text{N}$ values in phytoplankton usually reflect those of the inorganic nitrogen sources at the base of the food web, including, ammonium, nitrate or nitrite, and the fractionation associated with the assimilation processes (Syväranta et al., 2008; Mercado et al., 2010). The DH site is located at the east of the City of San Carlos de Bariloche, and it likely receives

Table 2
Range, mean and standard deviation (SD) of the stable isotope ratios of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) for macroinvertebrates, plankton and carbon sources collected in Bahía López (BL), Brazo Rincón (BR), and Dina Huapi (DH) from Lake Nahuel Huapi. *N* is the number of samples analyzed; the numbers in parenthesis represents the total number of organisms collected.

Organisms	Sampling site	<i>N</i>	Mean \pm SD Range	
			$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
Macroinvertebrates				
<i>Chilina</i> sp. (snail)	BL	8 (66)	2.5 \pm 0.3 2.0–2.9	–16.5 \pm 2.3 –19.6 to –13.1
	BR	8 (69)	2.4 \pm 0.3 2.0–3.0	–19.4 \pm 1.1 –22.0 to –18.3
<i>Diplodon chilensis</i> (mussel)	BL	15 (77)	5.7 \pm 0.3 5.3–6.5	–25.8 \pm 0.3 –26.2 to –25.2
<i>Aegla</i> sp. (crab)	BL	12 (72)	5.8 \pm 1.1 3.9–7.7	–23.0 \pm 1.7 –26.1 to –20.6
	BR	5 (26)	5.1 \pm 1.2 3.2–6.5	–25.4 \pm 0.9 –27.0 to –24.7
<i>Samastacus spinifrons</i> (crayfish)	BL	8 (18)	4.6 \pm 0.7 3.8–5.6	–24.0 \pm 1.4 –26.5 to –21.7
	BR	8 (14)	4.1 \pm 0.6 2.9–4.7	–25.7 \pm 1.6 –28.9 to –24.4
	DH	6 (8)	8.1 \pm 0.7 7.5–9.4	–21.3 \pm 2.8 –25.1 to –18.8
<i>Hyalella</i> sp. (amphipod)	BR	4 (123)	1.5 \pm 0.3 1.1–1.6	–26.2 \pm 1.0 –27.0 to –24.8
Ephemeroptera L. (mayfly)	BL	4 (92)	1.6 \pm 0.5 1.1–2.2	–25.7 \pm 6.2 –32.9 to –20.3
	BR	7 (201)	1.2 \pm 0.1 0.9–1.3	–24.9 \pm 0.8 –25.7 to –23.6
Trichoptera L. (caddis fly)	BL	2 (24)	0.3 \pm 0.02	–26.6 \pm 2.3 –28.2 to –25.0
	BR	1 (3)	0.4	–26.2 to –25.7 –24.9
Chironomidae L. (non-biting midge)	BR	2 (37)	2.0 \pm 0.02	–25.9 \pm 0.4
Plankton And Carbon Sources				
Plankton 1 (10–53 μm)	BL	2	3.8 \pm 0.4 3.5–4.1	–25.5 \pm 0.1 –25.6 to –25.4
	BR	4	4.5 \pm 0.5 3.9–5.0	–27.7 \pm 1.4 –29.1 to –25.8
	DH	2	4.1 \pm 0.4 3.8–4.3	–25.0 \pm 1.8 –26.3 to –23.8
Plankton 2 (53–200 μm)	BL	2	4.6 \pm 0.3 4.4–4.9	–23.8 \pm 0.03
	BR	4	3.5–6.5 4.7 \pm 1.3	–26.7 to –22.8 –25.1 \pm 1.7
	DH	2	6.1 \pm 0.01	–23.9 \pm 0.2 –24.0 to –23.7
Plankton 3 (>200 μm)	BL	2	8.8 \pm 0.2 8.6–8.9	–27.5 \pm 0.2 –27.7 to –27.4
	BR	4	8.8 \pm 0.4 8.3–9.2	–31.2 \pm 2.4 –33.2 to –28.4
	DH	2	9.8 \pm 0.7 9.3–10.3	–27.9 \pm 1.4 –28.9 to –27.0
Biofilm	BL	1	1.7	–20.1
	BR	1	0.5	–20.7
Detritus	BL	6	–1.4 \pm 3.3 –3.4 – 5.3	–29.2 \pm 1.5 –31.8 to –27.8
Leaves	BL	9	–0.6 \pm 2.0 –2.3 – 3.5	–28.6 \pm 1.1 –30.2 to –27.5
	BR	17	–4.5 \pm 2.2 –7.2 to –1.2	–29.9 \pm 1.0 –31.3 to –27.7

anthropogenic inputs of nitrogen from sewage effluents. Nitrogen compounds from sewage typically have very high $\delta^{15}\text{N}$ values compared with those from other watershed sources (Cabana and Rasmussen, 1996). This would consequently increase the $\delta^{15}\text{N}$ baseline for the affected food web and therefore the nitrogen isotopic values of all trophic levels, from phytoplankton to fish (Hansson et al., 1997).

Plankton, crayfish, and fish such as small puyen, creole perch and rainbow trout were depleted in ^{13}C in BR relative to the other sites. Differential biomass proportion of zooplankton species in P3 between sites may explain the depletion in ^{13}C in BR compared with

those from BL and DH. The proportion of copepods to cladocerans is higher in BR compared to BL and DH in summer and to BL in fall (Fig. 2). The differences in $\delta^{13}\text{C}$ of copepods and cladocerans may be explained by the selective feeding behavior of copepods for autochthonous resources depleted in ^{13}C (Pulido-Vilena et al., 2005) compared with cladocerans which directly graze on bacteria that rely on allochthonous organic matter which is usually enriched in ^{13}C (del Giorgio and France, 1996; Taipale et al., 2008; Queimaliños et al., 2012).

The differences observed in $\delta^{13}\text{C}$ of P1 and P2 among sites could not be explained by a different species composition. P2 from BR and DH in

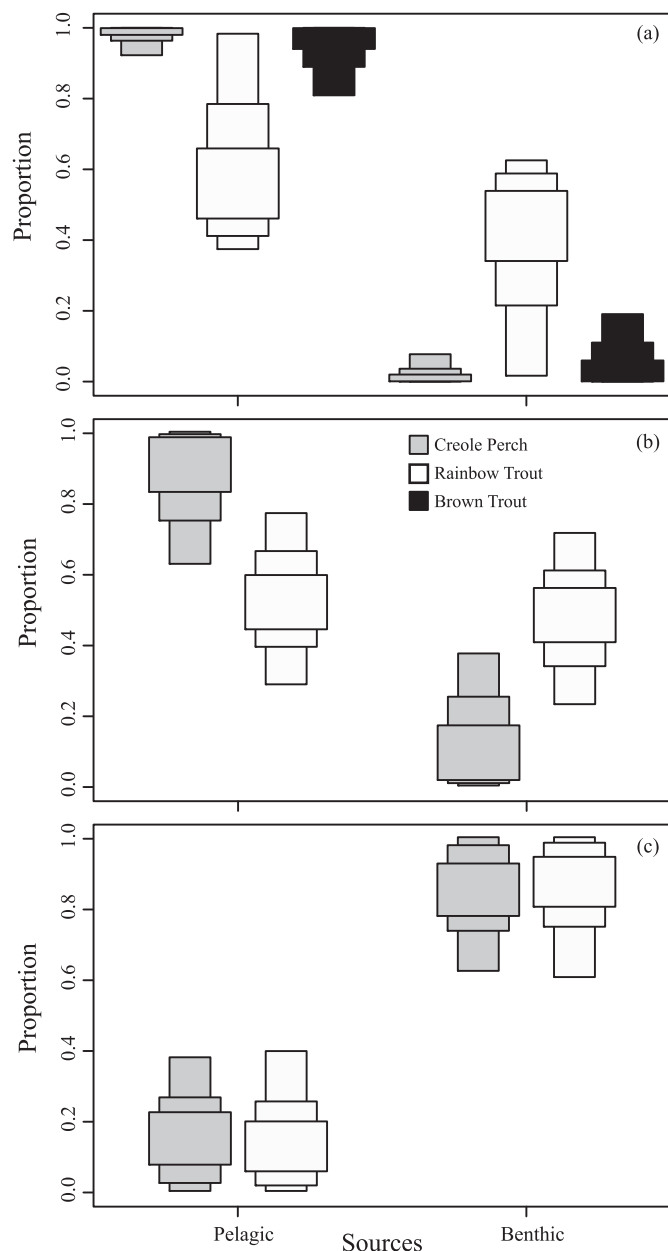


Fig. 4. Results of SIAR (95, 75 and 25% credibility intervals) showing estimated habitat contribution to creole perch (gray boxes), rainbow trout (white boxes) and brown trout (black boxes) in Brazo Rincón (a), Bahía Lopez (b), and Dina Huapi (c).

summer was composed mainly of mixotrophic ciliates, and in BL rotifers were the predominant taxa (Fig. 2). In fall, P2 from both BL and BR were taxonomically similar, with mixotrophic ciliates more abundant than rotifers. Despite these similarities between BR and DH in summer and BR and BL in fall, P2 was more depleted in ^{13}C in BR than the other sites in both seasons. There was a higher biomass proportion of mixotrophic ciliates in P1 from BR in summer than in BL and DH where the highest proportion was Dinophyceae (Fig. 2). In fall the proportions of taxa were similar in both sites sampled, with Dinophyceae being much more abundant than mixotrophic ciliates (Fig. 2). Despite the dissimilarities in taxonomic composition among sites in both seasons, P2 from BR was more depleted in ^{13}C than in the other two sites (Table 2). Consequently, the depletion of ^{13}C from BR may be due to other factors such as the degree of stable carbon isotope fractionation during the uptake of dissolved inorganic carbon and subsequent photosynthesis or the isotopic value of the inorganic carbon source in each

basin affected by the geochemistry of the ecosystem (Grey et al., 2000), that may result in different $\delta^{13}\text{C}$ baselines in each branch.

The relative importance of benthic-littoral habitats is minor in larger basins than in smaller ones (Schindler and Scheuerell, 2002). The Dina Huapi basin is deeper and larger than BL and BR, therefore, it has a lower perimeter-to-area ratio, consequently it was expected that in DH zoobenthos would contribute less to fish than planktonic production and that the opposite situation would occur in BL and BR. However the mixing model for creole perch and rainbow trout from DH, indicated that the isotopic contribution from the benthic habitat was greater than from the pelagic habitat. Also, there was a higher isotopic contribution from the pelagic habitat to the diet of brown trout in BR and creole perch in BL and BR, and a similar contribution from both habitats to rainbow trout in BR and BL. According to the SIAR model output based on two key prey items representing pelagic and littoral input, these fish species rely mostly on small puyen, which reflects the isotopic signal of the pelagic habitat as it preys on zooplankton in BR and BL, and in DH crayfish from the benthic-littoral habitat seems to isotopically contribute more to larger fish diets than zooplanktivorous small puyen. Salmonids and creole perch are generalist fish with a similar diet composition; however, Juncos et al. (2015) observed that crayfish dominate adult creole perch diet and brown trout larger than 500 mm, while small puyen is more important in the diet of rainbow trout. Furthermore, Rizzo et al. (2014), based on methylmercury-to-total mercury ratios in small puyen from the same sites in Lake Nahuel Huapi, concluded that mercury (which is transferred from prey to consumer) has its origins in the pelagic habitat in DH and in the benthic-littoral habitat in BR and BL. Nevertheless, the results indicate that the energy flow between habitats is different for each species depending on the site and is not related to the morphometry of the basin. Several factors, such as the trophic enrichment factors and dietary items chosen (Bond and Diamond, 2011) or the low number of samples for some fish consumers may have affected the results.

Habitat coupling by fish has important effects on nutrient cycles in lakes (Schindler and Scheuerell, 2002; Reissig et al., 2003). In Lake Nahuel Huapi, stable isotope values, complemented by published stomach content analysis, showed that native and introduced fish species play that role by moving between the pelagic and benthic-littoral habitats and feeding in both areas. This movement between habitats occurs ontogenetically, and the resulting diet shift is traced by a change in isotopic signals.

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References

- Alcalde, R., Labollita, H., Pedrozo, F., 1999. Lago Nahuel Huapi. Estudio de calidad de aguas. Autoridad Interjurisdiccional de Cuencas (AIC), Departamento de Aguas (DPA), Universidad del Comahue, Río Negro, Argentina.
- Arcagni, M., Campbell, L.M., Arribé, M., Kyser, K., Klassen, K., Casaux, R., Miserendino, M.L., Ribeiro Guevara, S., 2013. Food web structure of Lake Moreno (Northwest Patagonia, Argentina) using C and N stable isotopes. *Limnología* 43, 131–142.
- Arribé, M., Diéguez, M.C., Ribeiro Guevara, S., Queimaliños, C.P., Fajon, V., Reissig, M., Horvat, M., 2010. Mercury in an ultraoligotrophic North Patagonian Andean lake (Argentina): concentration patterns in different components of the water column. *J. Environ. Sci.* 22, 1171–1178.
- Balseiro, E.G., Modenutti, B.E., Queimaliños, C.P., 2001. Feeding of *Boeckella gracilipes* (Copepoda, Calanoida) on ciliates and phytoflagellates in an ultraoligotrophic Andean lake. *J. Plankton Res.* 23, 849–857.

- Balseiro, E.G., Modenutti, B.E., Queimaliños, C.P., Reissig, M., 2007. *Daphnia* distribution in Andean Patagonian lakes: effect of low food quality and fish predation. *Aquat. Ecol.* 41, 599–609.
- Barriga, J.P., Battini, M.A., Macchi, P.J., Milano, D., Cussac, V.E., 2002. Spatial and temporal distribution of landlocked *Galaxias maculatus* and *Galaxias platei* (Pisces: Galaxiidae) in a lake in the South American Andes. *N. Z. J. Mar. Freshw. Res.* 36 pp. 345–359.
- Barriga, J.P., Battini, M.A., García-Asorey, M., Carrea, C., Macchi, P.J., Cussac, V.E., 2012. Intraspecific variation in diet, growth, and morphology of landlocked *Galaxias maculatus* during its larval period: the role of food availability and predation risk. *Hydrobiologia* 679, 27–41.
- Bergamino, L., Richoux, N.B., 2015. Spatial and temporal changes in estuarine food web structure: differential contributions of marsh grass detritus. *Estuar. Coasts* 38, 367–382.
- Bond, A.L., Diamond, A.W., 2011. Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. *Ecol. Appl.* 21, 1017–1023.
- Cabana, G., Rasmussen, J.B., 1996. Comparison of aquatic food chains using nitrogen isotopes. *Proc. Natl. Acad. Sci. U. S. A.* 93, 10844–10847.
- Campbell, L.M., Hecky, R.E., Wandera, S.B., 2003. Stable isotope analyses of food web structure and fish diet in Napoleon and Winam Gulfs, Lake Victoria, East Africa. *J. Great Lakes Res.* 29, 243–257.
- Caravati, E., Callieri, C., Modenutti, B.E., Corno, G., Balseiro, E.G., Bertoni, R., Michaud, L., 2010. Picocyanobacterial assemblages in ultraoligotrophic Andean lakes reveal high regional microdiversity. *J. Plankton Res.* 32, 357–366.
- Cervellini, P.M., Battini, M.A., Cussac, V.E., 1993. Ontogenetic shifts in the diet of *Galaxias maculatus* (Galaxiidae) and *Odontesthes microlepidotus* (Atherinidae). *Environ. Biol. Fishes* 36, 283–290.
- Covich, A.P., Palmer, M.A., Crowl, T.A., 1999. The role of benthic invertebrate species in freshwater ecosystems. *Bioscience* 49, 119–127.
- Cremona, F., Planas, D., Lucotte, M., 2010. Influence of functional feeding groups and spatiotemporal variables on the $\delta^{15}\text{N}$ signature of littoral macroinvertebrates. *Hydrobiologia* 647, 51–61.
- Del Giorgio, P.A., France, R.L., 1996. Ecosystem-specific patterns in the relationship between zooplankton and POM or microplankton $\delta^{13}\text{C}$. *Limnol. Oceanogr.* 41, 359–365.
- DeNiro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* 42, 495–506.
- DeNiro, M.J., Epstein, S., 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim. Cosmochim. Acta* 45, 341–351.
- Diaz, M.M., Pedrozo, F.L., Temporetti, P.F., 1998. Phytoplankton of two Araucanian lakes of differing trophic status (Argentina). *Hydrobiologia* 369 (370), 45–57.
- Diaz, M., Pedrozo, F., Reynolds, C., Temporetti, P., 2007. Chemical composition and the nitrogen-regulated trophic state of Patagonian Andes. *Limnologia* 37, 17–27.
- Doi, H., 2009. Spatial patterns of autochthonous and allochthonous resources in aquatic food webs. *Popul. Ecol.* 51, 57–64.
- Fitzgerald, S.A., Gardner, W.S., 1993. An algal carbon budget for pelagic–benthic coupling in Lake Michigan. *Limnol. Oceanogr.* 38, 547–560.
- Grey, J., Jones, R.L., Sleep, D., 2000. Stable isotope analysis of the origins of zooplankton carbon in lakes of differing trophic state. *Oecologia* 123, 232–240.
- Hansson, S., Hobbie, J.E., Elmgren, R., Larsson, U., Fry, B., Johansson, S., 1997. The stable nitrogen isotope ratio as a marker of food-web interactions and fish migration. *Ecology* 78, 2249–2257.
- Harrod, C., Mallela, J., Kahilainen, K.K., 2010. Phenotype–environment correlations in a putative whitefish adaptive radiation. *J. Anim. Ecol.* 79, 1057–1068.
- Hayden, B., Holopainen, T., Amundsen, P., Eloranta, A.P., Knudsen, R., Præl, K., Kahilainen, K.K., 2013. Interactions between invading benthivorous fish and native whitefish in subarctic lakes. *Freshw. Biol.* 58, 1234–1250.
- Hecky, R.E., Hesslein, R.H., 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. *J. N. Am. Benthol. Soc.* 14, 631–653.
- Iriondo, M., 1989. Quaternary lakes of Argentina. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 70, 81–88.
- Juárez, S.M., 2012. Estructura del ensamble de peces del litoral somero del lago Nahuel Huapi. Universidad Nacional del Comahue, San Carlos de Bariloche, Argentina (M.Sc. Thesis).
- Juncos, R., Milano, D., Macchi, P.J., Alonso, M.F., Vigliano, P.H., 2011. Response of rainbow trout to different food web structures in Northern Patagonia: implications for growth, bioenergetics, and invasiveness. *Trans. Am. Fish. Soc.* 40, 415–428.
- Juncos, R., Beauchamp, D.A., Vigliano, P.H., 2013. Modeling prey consumption by native and nonnative piscivorous fishes: implications for competition and impacts on shared prey in an ultraoligotrophic lake in Patagonia. *Trans. Am. Fish. Soc.* 142, 268–281.
- Juncos, R., Milano, D., Macchi, P.J., Vigliano, P.H., 2015. Niche segregation facilitates coexistence between native and introduced fishes in a deep Patagonian lake. *Hydrobiologia* 747, 53–67.
- Karlsson, J., Jonsson, A., Meili, M., Jansson, M., 2004. $\delta^{15}\text{N}$ of zooplankton species in Subarctic Lakes in Northern Sweden: effects of diet and trophic fractionation. *Freshw. Biol.* 49, 526–534.
- Layman, C.A., Araujo, M.S., Boucek, R., Hammerschlag-Peyer, C.M., Harrison, C., Jud, Z.R., Matich, P., Rosenblatt, A.E., Vaudo, J.J., Yeager, L.A., Post, D.M., Bearhop, S., 2011. Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biol. Rev.* 87, 454–562.
- McCutchan, J.H., Lewis, W.M.J., Kendall, C., McGrath, C.C., 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102, 378–390.
- Mercado, J.M., Ramirez, T., Cortésand, D., Liger, E., 2010. Isotopic composition of particulate organic nitrogen and its relationship to nitrate assimilation in the Mediterranean Sea. *Sci. Mar.* 74, 745–753.
- Milano, D., Aigo, J.C., Macchi, P.J., 2013. Diel patterns in space use, food and metabolic activity of *Galaxias maculatus* (Pisces: Galaxiidae) in the littoral zone of a shallow Patagonian lake. *Aquat. Ecol.* 47, 277–290.
- Modenutti, B.E., Albariño, R.J., Bastidas Navarro, M.A., Diaz Villanueva, V., Souza, M.S., Trochine, C., Laspoumaderes, C., Cuassolo, F., Mariluán, G., Buria, L., Balseiro, E.G., 2010. Structure and dynamic of food webs in Andean North Patagonian freshwater systems: organic matter, light and nutrient relationships. *Ecol. Austral* 20, 95–114.
- Parnell, A.C., Inger, R., Bearhop, S., Jackson, A.L., 2010. Source partitioning using stable isotopes: coping with too much variation. *PLoS ONE* 5 (e9672).
- Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83, 703–718.
- Pulido-Villena, E., Reche, I., Morales-Baquero, R., 2005. Food web reliance on allochthonous carbon in two high mountain lakes with contrasting catchments: a stable isotope approach. *Can. J. Fish. Aquat. Sci.* 62, 2640–2648.
- Queimaliños, C.P., Reissig, M., Diéguez, M.C., Arcagni, M., Ribeiro Guevara, S., Campbell, L.M., Soto Cárdenas, C., Rapacioli, R., Arribé, M.A., 2012. Influence of precipitation, landscape and hydrogeomorphic lake features on pelagic allochthonous indicators in two connected ultraoligotrophic lakes of North Patagonia. *Sci. Total Environ.* 427–428, 219–228.
- Quirós, R., Drago, E., 1985. Relaciones entre variables físicas, morfológicas y climáticas en lagos patagónicos. *Rev. Asoc. Ciencias Nat. Litoral* 16, 181–199.
- R Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria 3-900051-07-0. <http://www.R-project.org/>.
- Rechenq, M., Sosnovsky, A., Macchi, P.J., Alvear, P.A., Vigliano, P.H., 2011. Extensive diel fish migrations in a deep ultraoligotrophic lake of Patagonia Argentina. *Hydrobiologia* 658, 147–161.
- Reissig, M., Queimaliños, C.P., Balseiro, E.G., 2003. Effects of *Galaxias maculatus* on nutrient dynamics and phytoplankton biomass in a North Patagonian oligotrophic lake. *Environ. Biol. Fishes* 68, 15–24.
- Reissig, M., Queimaliños, C.P., Modenutti, B.E., Balseiro, E.G., 2015. Prey C:P ratio and phosphorus recycling by a planktivorous fish: advantages of fish selection towards pelagic cladocerans. *Ecol. Freshw. Fish* 24, 214–224.
- Rizzo, A.P., Arcagni, M., Campbell, L.M., Koron, N., Pavlin, M., Arribé, M.A., Horvat, M., Ribeiro Guevara, S., 2014. Source and trophic transfer of mercury in plankton from an ultraoligotrophic lacustrine system (Lake Nahuel Huapi, North Patagonia). *Ecotoxicology* 23, 1184–1194.
- Schindler, D.E., Scheuerell, M.D., 2002. Habitat coupling in lake ecosystems. *Oikos* 98, 177–189.
- Solomon, C.T., Carpenter, S.R., Clayton, M.K., Cole, J.J., Coloso, J.J., Pace, M.L., Vander Zanden, M.J., Weidel, B.C., 2011. Terrestrial, benthic, and pelagic resource use in lakes: results from a three-isotope Bayesian mixing model. *Ecology* 92, 1115–1125.
- Syväranta, J., Tirola, M., Jones, R.L., 2008. Seasonality in lake pelagic $\delta^{15}\text{N}$ values: patterns, possible explanations, and implications for food web baselines. *Fundam. Appl. Limnol. für Hydrobiol.* 172, 255–262.
- Taipale, S., Kankaala, P., Tirola, M., Jones, R.L., 2008. Whole-lake dissolved inorganic ^{13}C additions reveal seasonal shifts in zooplankton diet. *Ecology* 89, 463–474.
- Vadeboncoeur, Y., Zanden, M.J., Vander, Lodge, D.M., 2002. Putting the lake back together: reintegrating benthic pathways into lake food web models. *Bioscience* 52, 44–54.
- Vander Zanden, M.J., Vadeboncoeur, Y., 2002. Fishes as integrators of benthic and pelagic food webs in lakes. *Ecology* 83, 2152–2161.
- Yuma, M., Timoshkin, O.A., Melnik, N.G., Khanav, I.V., Ambali, A., 2006. Biodiversity and food chains on the littoral bottoms of Lakes Baikal, Biwa, Malawi and Tanganyika: working hypotheses. *Hydrobiologia* 568, 95–99.