Stable isotope profiles of partially migratory salmonid populations in Atlantic rivers of Patagonia

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In the present study, profiles of stable isotope composition were characterized for two species with partially migratory populations in rivers along the latitudinal gradient of Patagonia, brown trout Salmo trutta and rainbow trout Oncorhynchus mykiss. The effects of factors (e.g. ontogeny of fishes, location, species and fasting) that may influence the stable isotope analysis (SIA) were evaluated, as was SIA evaluated as a tool to assign individual fish to their corresponding ecotype. Anadromous fishes exhibited enriched $\delta^{15}N$ (15.2 \pm 1.0%; mean \pm s.p.) and $\delta^{13}C$ (-19.2 \pm 1.3‰) relative to resident fishes' δ^{15} N (8.8 ± 1.1‰) and δ^{13} C (-23.2 ± 2.5‰). For both species, the difference in $\delta^{15}N$ was larger between resident (range 6.8–10.7‰) and anadromous (range 14.3–17.8‰) fishes than that in δ^{13} C. Values of δ^{13} C, while not as dramatically contrasting in rainbow trout, provided a powerful anadromy marker for brown trout in the region. Increases were found in both $\delta^{15}N$ and $\delta^{13}C$ during the spawning migration of anadromous rainbow trout, most likely due to fasting. Differences in stable isotopes between location, size and species were found, suggesting different stable isotopes base levels in freshwater environments and different trophic levels and feeding location of anadromous populations. The SIA was demonstrated as a powerful tool for ecotype discrimination in Patagonian Rivers, overriding any effect of sampling location, size or species. © 2008 The Authors

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Key words: anadromy; Patagonia; salmon; stable isotope analysis.

INTRODUCTION

The expression of anadromy in salmonids is driven by a trade-off between higher growth and fecundity, resulting from the exploitation of abundant marine resources, and lower survival associated with the physiological costs and predation risk of marine migrations (McDowall, 2001). In some species of salmonids, anadromy is facultative; populations can be monomorphic,

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composed exclusively of either anadromous or non-anadromous individuals, or polymorphic, also known as partially migratory, composed of both anadromous and non-anadromous individuals (Busby *et al.*, 1996).

Lakes and rivers of Patagonia have been stocked throughout the past 100 years with exotic salmonids, including species which are typically anadromous and others with facultative anadromy (Pascual et al., 2002; Pascual & Ciancio, 2007). This work is focused on two species that developed partially anadromous populations in Argentinean Patagonia: rainbow trout Oncorhynchus mykiss (Walbaum) of the Santa Cruz River (Pascual et al., 2001) and brown trout Salmo trutta (L.) of the Gallegos and Grande Rivers. There is growing evidence for partially anadromous species that intra-population variation for expressing anadromy may be closely related to habitat differences in fresh or salt water, rather than only due to genetic variation between alternative forms (Riva Rossi et al., 2007). The expression of partial anadromy in three different rivers of southern Patagonia provides a remarkable setting for contrasting life-history responses to environmental influences at the individual, population and interpopulation levels. Typical questions relate to identifying 'triggers' for the expression of anadromy in different rivers and for different species, and the specific ocean conditions met by species inhabiting particular rivers. From a more practical viewpoint, the need often arises to verify anadromy in recently discovered populations of unknown origin (Ciancio et al., 2005), requiring tools to discriminate anadromous from resident individuals (Doucett et al., 1999a, b; Charles et al., 2004).

Many techniques have been used to identify individual salmonids that migrate to and from the marine environment in the North Pacific and Atlantic Oceans (Doucett *et al.*, 1999*a*), including the use of scale and otolith growth pattern analysis, stable isotope analysis (SIA) and strontium to calcium ratios (Sr:Ca) in otoliths (Kalish, 1990; Kline et al., 1998; Doucett et al., 1999a; Charles et al., 2004; Curry, 2005). In Patagonia, scale patterns and otolith Sr:Ca ratios have been successfully used to determine and describe the life history of anadromous and resident individuals of Santa Cruz River rainbow trout (Pascual et al., 2001; Riva Rossi et al., 2007). The use SIA to detect anadromy, on the other hand, is an accurate and relatively inexpensive method, based on the existence of differences between stable isotope ratios of marine and freshwater biotas (McCarthy & Waldron, 2000; Rubenstein & Hobson, 2004). This technique has been used in South America to verify anadromy in Chinook salmon Oncorhynchus tshawytscha (Walbaum) spawning in the Santa Cruz River basin (Ciancio et al., 2005). In the present study, SIA was used to: (1) characterize the profiles of stable isotope composition of two species with sympatric anadromous-resident ecotypes in rivers along a latitudinal gradient in Patagonia, (2) evaluate the effect of factors that may influence SIA, *i.e.* species, river of origin, age, size and fasting and (3) assess the value of SIA as a tool to assign individual fish to their corresponding ecotype.

MATERIALS AND METHODS

SAMPLE AREA AND COLLECTION

During 2001–2006, fishes were collected from four different rivers along the Atlantic coast of Patagonia, covering a latitude range from 41 to 54° S (Fig. 1): non-anadromous

rainbow trout from the Negro River (average annual discharge 845 m³ s⁻¹; 41° S), partially migratory rainbow trout from the Santa Cruz River (700 m³ s⁻¹; 50° S), partially migratory brown trout from the Gallegos River (38 m³ s⁻¹; 52° S) and partially migratory brown trout from the Grande River (40 m³ s⁻¹; 54° S). Fishes were captured using a combination of rod and reel, gillnets and electrofishing. Fork length (L_F) and mass of adult fishes were recorded except in the Grande River where L_F and mass were backcalculated from scale measurements.

Approximately 50 g of dorsal muscle (or muscle from the head in the case of the Grande River) was extracted from each individual and preserved frozen during transport to the laboratory. For ecotype identification and age determination, scales were removed from both sides of the fish from an area located below the anterior margin of the dorsal fin, approximately five scale rows above the lateral line. Three scales were cleaned from each of the fish collected; impressions were made on acetate sheets and inspected on a microfiche reader. Fishes were initially assigned to resident and anadromous ecotypes by inspection of growth patterns on the scales (Davis & Light, 1985; Richard & Bagliniere, 1990). All resident fishes from the Grande River were juveniles (<100 mm L_F) and were thus unambiguously assigned to the resident ecotype. Validation of ecotype assignments was provided by two independent analyses: profiles of Sr:Ca in otoliths of Santa Cruz rainbow trout (n = 5; Riva Rossi *et al.*, 2007) which allowed for unambiguous classification of anadromy, and growth patterns in the scales from five anadromous rainbow trout captured at sea.

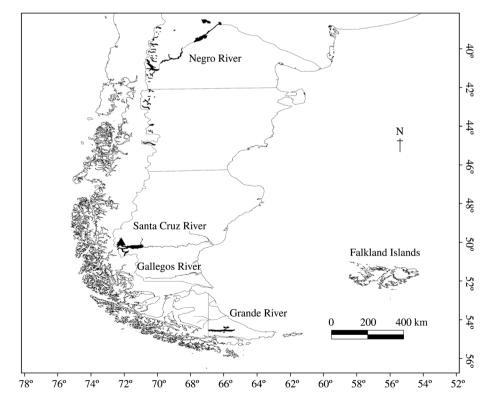


FIG. 1. Location of rivers sampled in Patagonia (Argentina).

STABLE ISOTOPE ANALYSIS

Carbon and Nitrogen SIA were conducted on dorsal muscle from 119 fishes from the main Atlantic-draining rivers of Patagonia. Samples were collected during the years 2001–2006 and included 90 rainbow trout (10 from the Negro River and 80 from the Santa Cruz River) and 29 brown trout (16 from the Gallegos River and 13 from the Grande River; Fig. 1). Samples were dried at 60° C for 48 h and ground to a fine powder. All samples were analysed for C and N content and stable isotopic signatures at the Stable Isotope Facility, University of California, Davis, U.S.A. The stable isotope ratios are expressed as δ values as ∞ : $\delta X = 10^3 [(R_{sample} R_{sjandard}^{-1})-1]$, where X is ¹³C or ¹⁵N and R is the corresponding ratio ¹³C:¹²C or ¹⁵N:¹⁴N. Standards used were Vienna Peedee belemnite for C and N₂ for N.

DATA ANALYSIS

All statistical analyses were carried out using the R software package 6.0 (http://www. r-project.org/). Variation in isotope ratios in consumers may result from the trophic level at which they feed, as well as from spatial and temporal changes in the isotopic composition at the base of the food web (Michener & Schell, 1994). Therefore, isotopic signatures are expected to vary not only between ecotypes but also between species and between rivers for a given ecotype. To evaluate the importance of such effects in Patagonian fishes, isotope signatures were compared for all combinations of species, rivers and ecotypes available, and for which appropriate data were available using a two-way ANOVA. Prior to analysis, the assumptions of normality and homoscedasticity of residuals were tested using Kolmogorov–Smirnov (KS) and Levene tests, respectively. Because δ^{13} C data did not meet these assumptions, these data were transformed using the reciprocal transformation. Residuals of δ^{15} N were normal and homocedastic, so data were not transformed.

The full segregation between rainbow and brown trout in the rivers analysed did not allow the separation of the effects of river and of species. Mean δ^{13} C and δ^{15} N values were therefore analysed as a function of ecotype (anadromous or resident) in three species–river complexes: Santa Cruz River rainbow trout (OmSC), Gallegos River brown trout (StGa) and Grande River brown trout (StGr). A priori planned comparison contrasts were used to test differences between anadromous and resident river complexes and between ecotypes from different species. Afterwards, *t*-tests were used to compare isotope signatures of resident rainbow trout in the Negro River (only resident fish inhabit this river) with those of resident rainbow trout of the Santa Cruz River.

Fishes may change their diet throughout their ontogeny and growth (Keeley & Grant, 2001), and this could be reflected in their stable isotopic signature (Menard *et al.*, 2007). To evaluate changes during ontogeny, regressions between stable isotopic signatures and L_F were developed for each ecotype within each species–river complex and for anadromous brown trout (Gallegos and Grande Rivers). $\delta^{15}N$ in anadromous rainbow and $\delta^{13}C$ in anadromous brown trout showed a significant relationship with L_F . For these two cases, the joint effect of L_F and categorical variables (age or river) was analysed with ANCOVA. First, ANCOVA was used to account for the effect of age (categorical variable) in analysing the relationship between $\delta^{15}N$ (dependant variable) and L_F (covariate) in Santa Cruz River anadromous rainbow trout. Second, an ANCOVA was used to account for the effect of river of origin (categorical variable) in analysing the relationship between $\delta^{13}C$ (dependant variable) in analysing the relationship and L_F (covariate) in analysing the Grande and Gallegos Rivers. As normality and homoscedasticity assumptions were met, data were tested untransformed.

Fasting of anadromous fishes during the reproductive migration may also affect stable isotopic signatures (Doucett *et al.*, 1999*c*). To test for such an effect in Patagonian salmonids, the relationship between stable-isotope ratio and month of in-river catch was tested using linear regressions. This analysis was performed for anadromous rainbow trout only, which has a well-defined run timing and long overwintering (Riva Rossi *et al.*, 2003) as brown trout exhibit multiple runs and the time each fish had spent in fresh water is unknown.

RESULTS

The $L_{\rm F}$ of fishes analysed ranged from 140 to 410 mm for resident rainbow trout, from 422 to 830 mm for anadromous rainbow trout, from 40 to 430 mm for resident brown trout and from 415 to 860 mm for anadromous brown trout. Overall, fishes were clearly segregated into two distinct groups in terms of their combined δ^{13} C– δ^{15} N signatures (Fig. 2). The first group, which included fishes with resident scale patterns and brown trout juveniles, exhibited isotopic signatures typical of freshwater environments (δ^{15} N 8·8 ± 1·1‰ and δ^{13} C –23·2 ± 2·5‰ mean ± s.D.; Fry & Sherr 1984; Michener & Schell, 1994), and was significantly δ^{13} C and δ^{15} N depleted compared to the second group (ANOVA, $F_{1,99}$ δ^{15} N P < 0.001 and δ^{13} C P < 0.001). The second group was significantly enriched, exhibited marine δ^{13} C and δ^{15} N signatures (δ^{15} N 15·2 ± 1.0‰, δ^{13} C –19·2 ± 1·3‰) and included anadromous fishes, fishes captured at sea (δ^{15} N 14·9 ± 1.9‰, δ^{13} C –18·7 ± 0.8‰), and fishes known to be anadromous by Sr:Ca ratios (δ^{15} N 15·4 ± 0·2‰, δ^{13} C –19·2 ± 0·8‰). Four brown trout, originally classified as resident, had an unusual combination of marine δ^{15} N (14·2 ± 0·6‰) and freshwater δ^{13} C (–30·4 ± 0·2‰) values (Fig. 2).

A priori planned comparisons showed that for all rivers and species, the anadromous ecotype was enriched in both $\delta^{15}N$ and $\delta^{13}C$ as compared to their resident counterpart (Table I). There were also some differences in enrichment levels between species and river complexes within ecotypes. Within the resident ecotype, Santa Cruz River rainbow trout were significantly enriched in $\delta^{13}C$ compared with brown trout from both the Grande and Gallegos Rivers, but no significant differences were found between the latter two populations. Resident brown trout from the Gallegos River were significantly enriched in $\delta^{15}N$ relative to resident Santa Cruz River rainbow trout, and the latter were significantly enriched relative to resident brown trout from the Grande River.

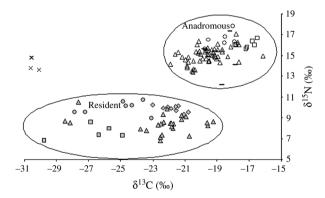


FIG. 2. Stable carbon and nitrogen isotopes plot. The first group (δ¹⁵N 8·8 ± 1·1‰ and δ¹³C −23·2 ± 2·5‰, mean ± s.p.) is significantly ¹³C and ¹⁵N depleted (enriched group, 15·2 ± 1·0‰, δ¹³C −19·2 ± 1·3‰). The first group included fishes of both species of freshwater origin, whereas fishes in the second group included marine origin fishes. Anadromous: rainbow trout *Oncorhynchus mykiss* Santa Cruz River (Δ), brown trout *Salmo trutta* Gallegos River (☉), brown trout Grande River (□) and rainbow trout marine capture (—). Residents: rainbow trout Negro River (♦), rainbow trout Santa Cruz (Δ), brown trout Gallegos River (☉) and brown trout Grande River (□). Gallegos River brown trout were plotted separately (★).

	Species and river complexes	Mean		Anadromous			Resident		
		$\delta^{13}C$	$\delta^{15}N$	StGa	StGr	OmSC	StGa	StGr	OmSC
Anadromous	StGa	-18.9	16.4	_	0.42	***	***		
	StGr	-17.9	16.1	0.03		***		***	
	OmSC	-19.6	15.0	0.14	***				***
Resident	StGa	-25.1	9.9	***				***	0.02
	StGr	-26.7	7.6		***		0.19		***
	OmSC	-22.5	8.4			***	***	***	

TABLE I. Results of planed comparisons of two-way ANOVA (d.f. = 1,19). Below the diagonal are results for $\delta^{13}C$ comparisons and above the diagonal are results for $\delta^{15}N$ comparisons

OmSC, rainbow trout Santa Cruz River; StGa, brown trout Gallegos River; StGr, brown trout Grande River.

***P < 0.001.

Resident rainbow trout from the Negro River were significantly enriched in δ^{15} N relative to the Santa Cruz River resident rainbow trout (*t*-test, P < 0.05) (Fig. 2). Within the anadromous ecotype, brown trout from the Grande River were enriched in δ^{13} C compared with both Gallegos River brown and Santa Cruz River rainbow trout, but no significant differences were found between the latter two populations. For δ^{15} N, anadromous brown trout from the Gallegos and Grande Rivers were significantly enriched relative to Santa Cruz River rainbow trout though no significant differences were found between the former two populations (Table I).

A positive correlation between stable isotopes signatures and $L_{\rm F}$ was found for both δ^{15} N ($r^2 = 0.6$, P < 0.05) and δ^{13} C ($r^2 = 0.62$, P < 0.05) in resident rainbow trout from the Negro River but not in resident rainbow trout from the Santa Cruz River. δ^{15} N increased significantly with $L_{\rm F}$ in Grande River anadromous brown trout ($r^2 = 0.68$, P < 0.05). δ^{15} N was also positively correlated to $L_{\rm F}$ of Santa Cruz River anadromous rainbow trout ($r^2 = 0.68$, P < 0.05), though there was no evidence for an additional effect on enrichment of age (ANCOVA, d.f. = 4,44, P > 0.05 differences in slopes and intercepts). As a group, anadromous brown trout were significantly enriched in δ^{13} C according to $L_{\rm F}$ ($r^2 = 0.03$, P < 0.05; Fig. 3). The relationship between δ^{13} C and $L_{\rm F}$ for this species was similar between the Grande and Gallegos River populations (ANCOVA, d.f. = 1,10, P > 0.05 for differences in slopes and intercepts), with no apparent river effect.

Significant increases in both δ^{15} N ($r^2 = 0.09$, P < 0.05) and δ^{13} C ($r^2 = 0.21$, P < 0.001) were found in Santa Cruz River rainbow trout caught in the course of the overwintering period between February and October (Fig. 4).

DISCUSSION

The overall stable isotope signatures reported in this study for rainbow and brown trout in Atlantic rivers of Patagonia are consistent with those of native

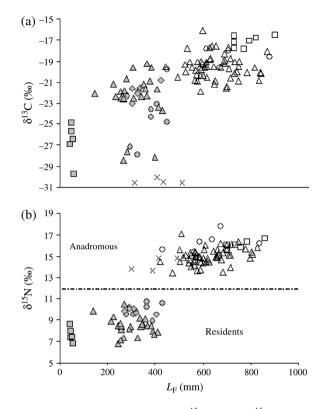


FIG. 3. Relationship between fork length (L_F) and (a) $\delta^{13}C$ and (b) $\delta^{15}N$ (see Fig. 2). Rainbow trout marine captures are not included. (b) Line separates anadromous from freshwater fishes in $\delta^{15}N$ plot.

populations from the northern hemisphere, with the expected enriched $\delta^{13}C$ and $\delta^{15}N$ in anadromous fishes as compared to resident fishes (Fig. 5). In general, anadromous fishes of all species (including previously reported exotic Chinook salmon; Ciancio et al., 2005) constitute a clearly distinct group with respect to both $\delta^{15}N$ and $\delta^{13}C$ levels, both exhibiting low variability within groups. The SIA has previously been successfully applied to assign these same anadromous species to distinct trophic levels (Ciancio et al., 2008). The SIA presented herein, however, clearly differentiates anadromous fishes from their resident counterparts. Resident groups, on the other hand, display larger variability in their isotopic signatures, in particular with respect to δ^{13} C levels. Differences between ecotypes reported in previous studies were particularly strong for δ^{13} C (Charles *et al.*, 2004, 2006; Curry, 2005). On the other hand, in the present study, a particularly strong contrast between ecotypes was found for $\delta^{15}N$ (Fig. 5), with differences between the most enriched resident individual and the most depleted anadromous individual exceeding 3‰. Anadromous fishes of Patagonia are consistently enriched in $\delta^{15}N$ compared with northern hemisphere populations (Fig. 5). In particular, anadromous rainbow trout are enriched with respect to North American steelhead (O. mykiss). Santa Cruz rainbow trout have a particular life cycle, with several short ocean migrations

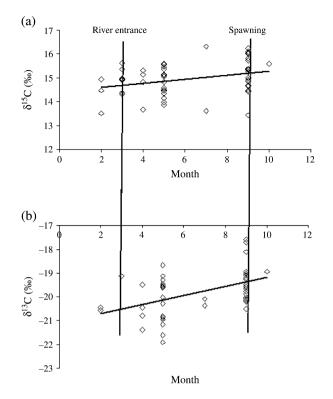


FIG. 4. Effect of fasting during reproductive migration of anadromous rainbow trout on (a) δ^{15} N and (b) δ^{13} C in dorsal muscle tissue. Significant increases in δ^{15} N ($r^2 = 0.09$, P < 0.05) and δ^{13} C ($r^2 = 0.21$, P < 0.001) were observed. The curves were fitted: (a) $y = 0.819x \pm 14.45$ and (b) y = 0.1869x - 21.064.

during their lifetime and a more coastal distribution (Pascual *et al.*, 2001; Riva Rossi *et al.*, 2007), very different from the oceanic distribution of North Pacific steelhead (Kaeriyama *et al.*, 2004). Enriched δ^{15} N levels are characteristic of the Patagonian Continental Shelf (Ciancio *et al.*, 2008).

Resident rainbow trout of both the Negro and the Santa Cruz Rivers are enriched in δ^{13} C with respect to resident brown trout of both the Gallegos and the Grande Rivers. This difference could be due in part to species-specific differences, but the rivers themselves are very different and may have characteristic isotope signatures. Whereas the former two are large rivers (700–845 m³ s⁻¹ discharge) where endogenous production may be significant, the two brown trout rivers are smaller (38–40 m³ s⁻¹ discharge) and may have a much larger input of headwater nutrients and a stronger stable isotope signature due to these terrestrial inputs (Doucett *et al.*, 1996). Resident rainbow trout from the Negro River are δ^{15} N enriched with respect to resident rainbow trout from the Santa Cruz River (Fig. 2). There are two possible explanations for the observed pattern: longer food chains, as there are 10 latitudinal degrees between rivers, or enriched δ^{15} N at the base of the food web due to natural conditions or enrichment due to human activities (Lake *et al.*, 2001).

Ontogenetic differences in isotope signatures were found for different species and ecotypes. Anadromous rainbow trout from the Santa Cruz River and

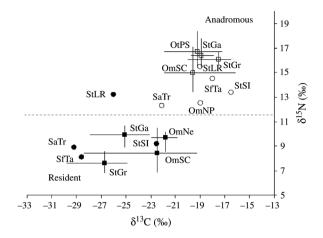


FIG. 5. δ¹⁵N and δ¹³C plot of Patagonian anadromous and resident populations (□, ■) showing ranges and populations of reference fishes (○, ●). Resident (■, ●) and anadromous (□, ○) fishes are shown. Species-river complexes are the same as those in Table I for Patagonian Rivers. Populations are: OtPS, *Oncorhynchus tshawytscha* from Patagonian Shelf (Ciancio *et al.*, 2005); StLR, *Salmo trutta* from La Roche Brook (Charles *et al.*, 2006); StSI, *S. trutta* from England and Ireland (McCarthy & Waldron, 2000); StTa, *Salvelinus fontinalis* from Tabusinac River (Doucett *et al.*, 1999a); SaTr, *Salvelinus* Riviere de la Trinite (Doucett *et al.*, 1999a); OmNP, *Oncorhynchus mykiss* North Pacific (Kaeriyama *et al.*, 2004).

resident trout rainbow from the Negro River showed enriched $\delta^{15}N$ with size, which is likely to reflect increasing trophic level occurring with growth. Anadromous brown trout showed an enrichment of $\delta^{13}C$ with size, which may reflect different feeding areas for older, larger fish. Resident fishes from the Gallegos River displayed enriched $\delta^{15}N$ with respect to resident fishes from the Grande River. This may reflect river-specific differences but may well be due to a lower trophic position of the latter, which were all juveniles. Four brown trout from the Gallegos River had an unusual combination of marine $\delta^{15}N$ and freshwater $\delta^{13}C$ isotope signatures. All these were mid-size fish ranging from 300 to 490 mm and weighing <1 kg. The exceptional stable isotopic signatures of these fish could be due to estuary feeding for a short period of time (Elliott, 1994). McCarthy & Waldron (2000) found similar stable isotope patterns in brown trout sampled in Loch Eck, in Scotland, U.K., concluding that they had been feeding in a brackish or marine environment.

The long river overwintering in Santa Cruz anadromous rainbow trout affects their isotopic signatures, as reflected by increases in both $\delta^{15}N$ and $\delta^{13}C$ between February and October. Isotopic enrichment can be explained by the fact that animals 'eat themselves' during fasting (Hobson *et al.*, 1993; Estrada *et al.*, 2005). The enrichment of $\delta^{13}C$ was stronger than that of $\delta^{15}N$. These results are similar to those found by Doucett *et al.* (1999*c*) for Atlantic salmon *Salmo salar* (L.). Atlantic salmon red muscle shows no changes in $\delta^{15}N$ but significant enrichment in $\delta^{13}C$ during upriver migration most likely due to continued reductions in lipids levels (Doucett *et al.*, 1999*c*). Lipids are $\delta^{13}C$ isotopically depleted relative to other tissue components (DeNiro & Epstein, 1977) and are consumed by anadromous salmonids as fuel during migration. Spawning fishes probably used much of their lipids reserves on spawning migration and gamete production.

While species, river of origin, overwintering and ontogen all appear to significantly affect isotopic signatures, the effect of ecotype is overriding for both species across samples. As populations expand in rivers of Patagonia and new populations are being discovered. SIA can become an effective tool for identifying anadromy and residency. Applications include verifying anadromy in newly discovered spawning populations and assessing the relative reproductive contribution of sympatric anadromous and freshwater-resident fishes to progeny through SIA of eggs or alevins (Curry, 2005). In particular, values of $\delta^{15}N$ were distinct and non-overlapping between ecotypes for all species and rivers (Fig. 5), providing a unique marker for anadromy in new target populations of brown and rainbow trout in Atlantic Rivers. Values of $\delta^{13}C$, while not as dramatically contrasting in rainbow trout, provide a powerful marker for brown trout in the same region. Meanwhile, the agreement of these data with those of other species and from other parts of the world points to the analysis of the combined value of δ^{13} C and δ^{15} N as a more universal tool to identify anadromy, with potential applications to rivers in a wider region around Tierra del Fuego and along the Pacific coast of southern Chile.

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