

Discrimination between farmed and free-living invasive salmonids in Chilean Patagonia using stable isotope analysis

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Abstract In Chilean Patagonia relatively pristine aquatic environments are being modified by the introduction of exotic salmonids, initially through their deliberate release for sport fishing since the early twentieth century, and more recently via the accidental escape from fish farms. There is therefore a need to reliably distinguish between naturally reproducing and fugitive salmonids associated with the Chilean salmonid farming industry, the second largest in the world. We tested the ability of stable isotope analysis (SIA) and analysis of scale growth profiles to discriminate between farmed and free-living salmonids sampled around the Island of Chiloé. Juvenile Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) from aquaculture facilities were significantly more enriched in $\delta^{15}\text{N}$ and lipid-corrected $\delta^{13}\text{C}$ than river-caught individuals. Scale growth slopes during the first year in freshwater were significantly higher in farmed than in wild-caught rainbow trout, indicating faster somatic growth under hatchery conditions. Stable isotopes analysis classified 94% of juvenile Atlantic salmon and rainbow trout to their correct farm or free-living groups. Our results, therefore, can help to elucidate the origin and spread of exotic invasive

salmonids in Chile, and address one of the biggest threats to native freshwater fishes in Patagonia and other temperate zones of the Southern Hemisphere.

Keywords Stable isotope analysis · Invasive species · Salmonids · Aquaculture

Introduction

Salmonids are not native to the Southern Hemisphere, yet they are now abundant in many places in Chile and represent the most readily identified fish by local people (Soto et al. 2006). Concentrated around the Island of Chiloé (Los Lagos Region), the Chilean salmon industry has developed since the early 1980s into the second largest in the world, surpassed only by Norway (Bjørndal 2001; Soto et al. 2006). Low production costs in a ‘developing’ country, coupled with relatively pristine aquatic environments, provided perfect conditions for Chilean salmon farming (Bjørndal 2001). The majority of farmed salmonids in Chile are Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*Oncorhynchus kysutch*; Soto et al. 2001). However, unlike Atlantic and coho salmon, rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) were first introduced to Chile for recreational purposes in the early twentieth century (Basulto

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2003) and have since spread rapidly. Local streams provide ideal habitats for invasive trouts (Penaluna et al. 2009), which have successfully colonised similar streams in Argentina, the Falklands Islands, and New Zealand (McDowall et al. 2001; McDowall 2003; Pascual and Ciancio 2007).

Early introductions were justified by the State as it was argued that salmonids would simply occupy empty niches, not taken up by any native species (Soto et al. 2006). Yet, the introduction of invasive species—of which rainbow trout is rated as one of the ‘100 World’s Worst Invasive Alien Species’ (ISSG 2008)—contributes to the reduction of native species, and in some cases to their extinction (Barel et al. 1985). In New Zealand, the Falklands Islands and Argentina exotic salmonids have been shown to disrupt natural food-webs and impact upon native biodiversity, particularly on native galaxiid fishes (reviewed by McDowall 2006). Galaxiids and other native freshwater fishes have also declined in Chile, for which salmonid invaders may have also been responsible (Correa and Gross 2008; Lattuca et al. 2008; Arismendi et al. 2009; Penaluna et al. 2009). Galaxiids tend to be least abundant in places where salmonids are most numerous (Soto et al. 2001), suggesting that the presence of salmonids impacts upon native fish fauna. Salmonids may impact upon native fishes by aggressively feeding on shared resources (Glova et al. 1992; Young et al. 2009), but also by preying upon them (Arismendi et al. 2009).

Because farmed salmonids are selected for high growth and reared at very high densities, the potential for salmonid escapees to impact upon native fishes may differ greatly from the effects of wild, already ‘naturalised’ fish (Thorstad et al. 2008). For the successful conservation of native fish fauna, knowledge on the origin and spread of invasive salmonids through different routes is therefore essential. Differences in carotenoid pigments (natural astaxanthin vs. synthetic cantaxanthin) have often been used to distinguish wild from farmed salmonids (Sægrov et al. 1997; Youngson et al. 1997; Poole et al. 2000). However, with the advent of synthetic astaxanthin differences between farmed and wild salmonid may not be detectable by pigment analysis anymore.

Scale growth profiles obtained from measurements of inter-circuli spacing have been found previously to provide a good index of relative growth in salmonids

(Fukuwaka and Kaeriyama 1997; Fisher and Pearcy 2005, Hubley et al. 2008), and could therefore also be used to discriminate between fast-growing hatchery fish and slower growing wild fish (Stokesbury and Lacroix 1997; Stokesbury et al. 2001). On its native range, Atlantic salmon escaping from marine net-pens have been identified from wild adults on the basis of differential scale growth patterns (Lund and Hansen 1991; Hansen et al. 1999), but the validity of scale discrimination to identify salmonid escapees in the Southern Hemisphere has rarely been tested. Variation in scale growth patterns was used to discriminate hatchery-reared from free-living Chinook salmon in New Zealand (Unwin and Lucas 1993), but discrimination at an early age can be more problematic and will probably depend on time at liberty (Fisher and Pearcy 1990; Bernard and Myers 1994).

Stable Isotope Analysis (SIA) has been widely employed in trophic ecology (Vander Zanden et al. 1999; McCarthy and Waldron 2000), as the ratios of carbon and nitrogen stable isotopes ($^{15}\text{N}:$ ^{14}N and $^{12}\text{C}:$ ^{13}C) are affected by diet and may be expected to vary with trophic status and feeding conditions (Satterfield and Finney 2002). Differences in stable isotope signatures (SIS) have previously been used to distinguish between farmed and wild salmonids, as the two groups have different diets (Aursand et al. 2000; Dempson and Power 2004), but the application of SIA to salmonid invasion biology is only recently beginning to be explored (McHugh et al. 2006, Ciancio et al. 2008a).

With Chile’s growing salmon aquaculture industry, Atlantic salmon escaping from fish farms are increasingly contributing to the already established trout invasions (Soto et al. 2006; Arismendi et al. 2009). For example, a recent earthquake and two severe storms resulted in the escape of several million Atlantic salmon and rainbow trout from open cages in the Aysen and Chiloé areas during 2007–2008 (Fish Farmer International 2007; The Patagonia Times 2009). In addition, there is a regular leakage of farmed salmonids at all life stages during culture (Gross 1998; Skilbrei and Wennevik 2006; Buschmann et al. 2009). Yet, fish farmers continue to argue that most invasive salmonids in Chile originate from historical introductions to boost sport fishing and not from fish farming, and that the majority of salmonid escapees are caught by artisanal fishermen and fail to

become established (Soto et al. 2001). Our aim therefore was to test the ability of stable isotopes and scale growth analysis to discriminate between juvenile Atlantic salmon and rainbow trout of farm or wild origin, in an effort to distinguish between the roles of commercial fish farming and natural colonization in the spread of invasive salmonids.

Materials and methods

Origin of samples

Free-living juvenile Atlantic salmon ($n = 25$) and rainbow trout ($n = 28$) were obtained by electro-fishing at seven different streams in the Los Lagos Region (southern Chile, Fig. 1) from November 2007 to September 2008. These were referred to as 'wild' fish. Farmed juvenile rainbow trout ($n = 25$) and Atlantic salmon ($n = 24$) were collected at two nearby fish farms on the Island of Chiloé (Fig. 1). Four free-living individuals (one salmon and three trout) had phenotypic traits normally associated with farmed fish, such as short opercula and eroded fins (McDonald et al. 1998; Erikson et al. 2006). These

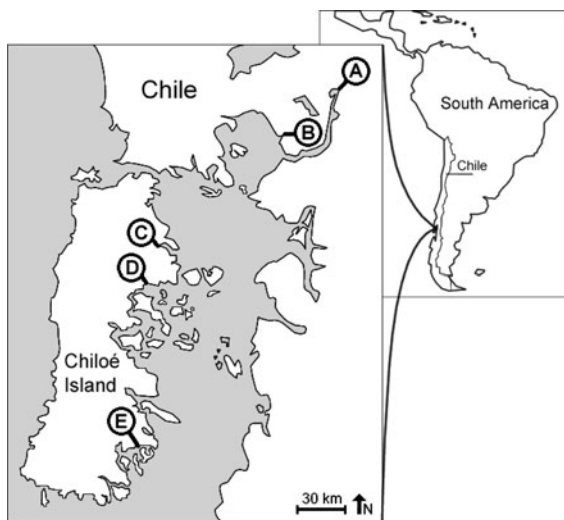


Fig. 1 Sampling locations of farmed and wild salmonids in the Los Lagos Region (S. Chile): (A) unnamed river U80A (5 wild *S. salar*), (B) River Chaica (1 wild *S. salar*), (C) River Cendoya (25 wild *O. mykiss*), Atlantic salmon farm (24 farmed *S. salar*), and unnamed rivers U32-U33 (19 wild *S. salar*), (D) unnamed rivers U69-U70 (3 wild *O. mykiss*), (E) rainbow trout farm (25 farmed *O. mykiss*)

were likely recent escapees but were included into the 'wild' group.

Total length, (TL, mm) was determined for most individuals, and scales were collected for age determination and growth profile analysis (see below). Approximately 50 mg of dorsal white muscle tissue was obtained from each fish and preserved in salt, which has been found to have little effect on isotopic signatures, and to be suitable for SIA (Arrington and Winmilller 2002).

Stable Isotope Analysis (SIA)

Samples of dorsal white muscle were oven dried at 50–60°C until constant weight for at least 24 h. Dried samples were then ground into a fine powder with a porcelain mortar and pestle. Tools and workspace were wiped thoroughly with ethanol to avoid cross-contamination between samples. One mg (± 0.005 mg) of ground muscle was combusted at 1,000°C in a PDZ Europa 20/20 Mass Spectrometer (Swansea University) for each fish. Oxides of nitrogen were reduced over hot copper and any traces of water removed. Carbon and nitrogen isotope ratios, which are expressed in delta notation (δ) as parts per thousand (‰), were calculated relative to the international standard for ^{13}C , the Vienna Pee Dee Belemnite (VPDB) standard (Craig 1957; Coplen 1995) after the following equation:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = \left(\left(\frac{R_{\text{Sample}}}{R_{\text{Standard}}} \right) - 1 \right) * 10^3;$$

$$R = {}^{13}\text{C}/{}^{12}\text{C}; \text{ or } R = {}^{15}\text{N}/{}^{14}\text{N}$$

$\delta^{13}\text{C}$ values were corrected for the effects of lipid concentration using a lipid normalisation equation as recommended by Post et al. (2007):

$$\delta^{13}\text{C}_{\text{corrected}} = \delta^{13}\text{C}_{\text{uncorrected}} - 3.32 + 0.99 * \text{C:N}$$

C:N ratios in the fish samples ranged from 3.13 to 4.22 for farmed fish, and from 3.13 to 4.23 for wild fish.

The C:N ratio references, atropine and acetanilide (OAS, Elemental Microanalysis, Okehampton, UK), were analysed between tissue samples to calibrate the machine and compensate for drift. Analytical precision, estimated as 1 standard deviation from mean C:N ratios of reference material, was 0.09 for acetanilide (mean = 6.85, $n = 34$) and 0.22 for atropine (mean = 14.43, $n = 10$).

Scale growth analysis

Scales from 104 of the fish used for SIA were also used for growth profile analysis, care being taken to choose only scales with intact, not regenerated nuclei (Lund and Hansen 1991). A digital microscope camera (Olympus DP10) was employed to take photographs at 6.3×10 times magnification, using Adobe Photoshop® 6.0 for adjustments of sharpness and contrast.

The image analysis software ImageJ® 1.41 was employed to digitize the position of growth rings (circuli) and to measure the distances between consecutive circuli (growth increments) in order to derive measures of scale growth (Fukuwaka and Kaeriyama 1997; Fisher and Pearcy 2005, Hubley et al. 2008). Scale measurements and circuli counts were made from the centre of the scale nucleus (focus) to the edge of the scale along the longest scale axis (Hiilivirta et al. 1998). Measurements of inter-circuli spacing were used to age the fish, and to identify the position of the first winter (annulus). Growth profiles were obtained by plotting circuli number against back-calculated total lengths at each circulus, which assumes a simple proportional model of fish growth in relation to scale growth (Francis 1990). This is the most common method of back-calculation salmonid scales (Friedland et al. 1996), and produces accurate, reproducible results for freshwater growth in Atlantic salmon (Kuparinen et al. 2009). OLS regression was then used to determine growth slopes during the first year (0 + growth), measured between the scale focus and the scale edge in the case of under-yearlings, or to the beginning of the first annulus in the case of older fish.

Statistical analysis

Statistical analysis was carried out with SPSS v. 16.0 and SYSTAT v. 10. Prior to analysis, the assumptions of normality and homogeneity of variances were tested by the one-sample Kolmogorov–Smirnov and Levene's tests, respectively. When these assumptions were not met, log-transformed values or non-parametric methods were used for analysis.

It has been proposed that body size can have an influence on reaction time of isotopic incorporation (Wolf et al. 2009). Therefore, to account for the possible effects of body size on SIS, the correlation

coefficient was first inspected and, if significant, size-adjusted comparisons were carried out using TL as a covariate.

In rainbow trout, differences in SIS between hatchery and wild fish were assessed by MANOVA while statistically controlling for variations in body size. Differences in SIS between farmed and wild-caught Atlantic salmon were assessed by the Mann–Whitney test, as parametric assumptions could not be met. The 'leave-one-out' discriminant function analysis (DFA) was employed to investigate the ability of SIS and scale growth slopes to correctly classify salmonids as farmed or wild.

Results

Growth profiles of wild and farmed salmonids

Scale analysis showed that 20 out of 25 farmed trout (80%) and 17 out of 29 wild trout (59%) in our juvenile samples were younger than one winter (0 + age class). All farmed Atlantic salmon and 20 out of 24 of wild individuals (83%) were also 0 + . Four free-living fish caught by electro-fishing (one salmon and three rainbow trout) were suspected to be recent escapees, all of which were older than 1 year (1 + and 2 + age classes).

In Atlantic salmon, the first year growth slopes of farmed and wild fish were not statistically different (Fig. 2a; $t_{42} = -1.128$, $P = 0.266$). In contrast, the growth slopes of farmed rainbow trout were significantly higher than those of wild fish (Fig. 2b; $t_{54} = -6.158$, $P < 0.001$), indicative of faster growth rates under hatchery conditions.

SIS variation in wild and farmed salmonids

Bivariate $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$ SIS scatter plots for farmed and wild salmonids showed two distinct groups (Fig. 3a, b), and indicated that farmed fish tended to be more enriched in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ than fish caught in the wild (Table 1).

Total length (log-transformed) was positively correlated with both $\delta^{13}\text{C}$ ($r = 0.811$, $P < 0.001$) and $\delta^{15}\text{N}$ ($r = 0.853$, $P < 0.001$) in wild-caught trout, but body size had no significant effect on SIS of farmed trout or juvenile salmon. Farmed rainbow

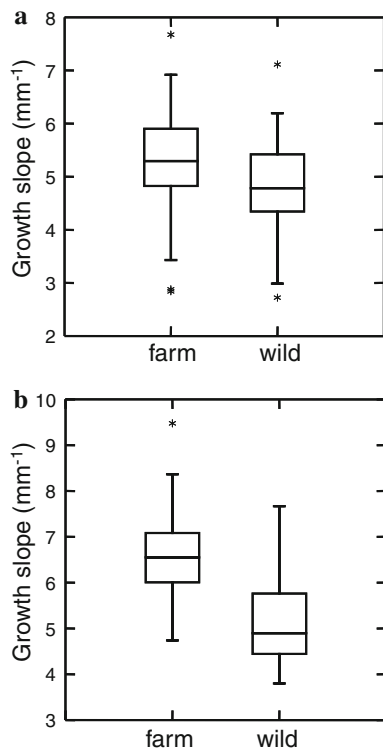


Fig. 2 Regression slopes of scale 0 + growth to the first annulus of (a) juvenile Atlantic salmon and (b) rainbow trout of farm and wild origins

trout were significantly more enriched in SIS than wild-caught trout (MANOVA $F_{1,50} = 383.857$, $P < 0.001$) in both $\delta^{15}\text{N}$ (farm = 15.53, wild = 10.15) and $\delta^{13}\text{C}$ (farm = -26.16 , wild = -33.82) while statistically controlling for variation in body size. Similarly, farmed Atlantic salmon were significantly more enriched in $\delta^{15}\text{N}$ (Mann–Whitney $P < 0.001$) and $\delta^{13}\text{C}$ (Mann–Whitney $P < 0.001$) than juvenile salmon living in the wild (Table 1).

Prior to analysis, only one juvenile Atlantic salmon was suspected to be a recent escapee on the basis of its external appearance. This individual did not stand out of the wild group. In contrast, the three rainbow trout which were suspected to be recent escapees showed up as outliers and had $\delta^{15}\text{N}$ SIS which were more similar to those of farmed fish (Fig. 3b). With the exception of such escapees, maximum nitrogen SIS values for wild rainbow trout were in all cases smaller than the minimum nitrogen SIS values found amongst farmed individuals, and therefore completely separated the two groups on the basis of $\delta^{15}\text{N}$ alone. Wild Atlantic salmon were

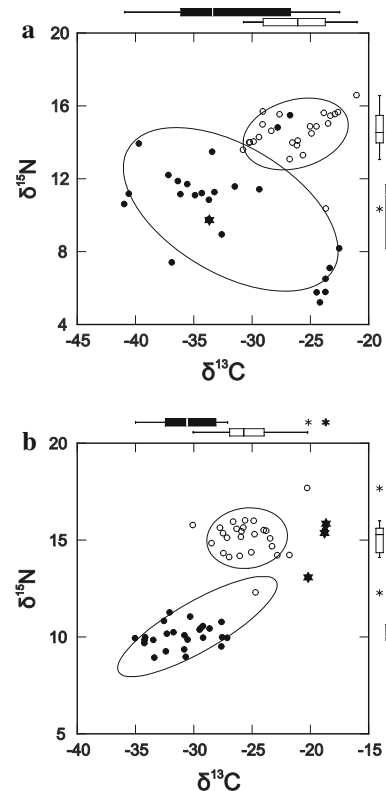


Fig. 3 Variation in lipid corrected carbon and nitrogen SIS of (a) juvenile Atlantic salmon and (b) juvenile rainbow trout of farm (open circle) and wild (filled circle) origins. Ellipses shown are centred on the SIS sample means, and have major axes determined by unbiased sample standard deviations and orientation determined by the SIS covariance. Four suspected recent escapees (one salmon, three trout) are identified by stars

significantly more variable in nitrogen SIS than wild rainbow trout (Levene's test $df = 1,51$, $P = 0.003$).

Discrimination of wild and farmed salmonids

Discriminant function analysis (DFA) assigned 95.2% of cross-validated Atlantic salmon to the correct wild or farmed group ($P < 0.001$) using three variables, carbon and nitrogen SIS and growth slope. With only nitrogen and carbon SIS, correct discrimination of wild and farmed salmon was still high (93.9%, $P < 0.001$). In contrast, discrimination of juvenile salmon on the basis of growth slopes alone was poor (57.1%) and not different from what would be expected by chance alone.

DFA assigned 94.3% of rainbow trout to their correct group using carbon and nitrogen SIS ($P < 0.001$). Inclusion of growth slope did not

Table 1 Variation in body size and SIS for juvenile Atlantic salmon and rainbow trout of wild and farm origin

	<i>S. salar</i>		<i>O. mykiss</i>	
	Wild <i>n</i> = 25	Farm <i>n</i> = 24	Wild <i>n</i> = 28	Farm <i>n</i> = 25
TL (mm)				
Mean	107.4	81.5	167.8	105.7
SD	28.96	23.51	101.35	10.19
Minimum	51	46	89	88
Maximum	170	126	560	129
$\delta^{13}\text{C}$				
Mean	-31.93	-26.40	-32.65	-27.74
SD	5.83	2.90	5.14	2.6
Minimum	-41.13	-30.98	-39.05	-33.16
Maximum	-22.36	-21.47	-19.57	-21.47
$\delta^{15}\text{N}$				
Mean	10.32	14.46	10.54	15.10
SD	2.83	1.24	1.64	0.99
Minimum	5.19	10.34	8.91	12.28
Maximum	15.46	16.56	15.73	17.67
C:N ratio				
Mean	3.46	3.50	3.38	3.59
SD	0.26	0.16	0.20	0.21
Minimum	3.17	3.13	3.13	3.34
Maximum	4.23	3.82	3.91	4.22

improve discrimination of rainbow trout, which remained the same (94.3%). Using growth slope data as the only classification variable for DFA resulted in 75.0% correct classification of rainbow trout to farm or wild group ($P < 0.001$).

Discussion

This study shows that farmed and free-living invasive salmonids in Chilean Patagonia can be distinguished by their carbon and nitrogen stable isotopic signatures, most likely as a result of their different diets. This opens the possibility of identifying fish escaping from aquaculture facilities, one of the main causes for the spread of invasive salmonids outside their native range (Arismendi et al. 2009).

Several factors could affect the isotopic signatures of invasive salmonids, and therefore our ability to correctly identify aquaculture escapees from naturalised, free-living fish. For example, depletion

of $\delta^{13}\text{C}$ and enrichment of $\delta^{15}\text{N}$ have been observed in brown trout coinciding with a dietary shift towards terrestrial invertebrates (Cucherousset et al. 2007). Carbon isotopic signatures can also vary in juvenile salmonids along the river length, reflecting varying terrestrial contribution in riverine food webs (Finlay et al. 2002). A progressive downstream enrichment of $\delta^{13}\text{C}$ can be expected in riverine food webs because headwater fishes tend to rely more on allochthonous carbon sources than individuals living downstream (Doucett et al. 1996; Cunjak et al. 2005) due to differences in energy flow between stream algae, invertebrates and fish (Perry et al. 2003). Yet, farmed Atlantic salmon and rainbow trout in our study were substantially more enriched, particularly in relation to $\delta^{15}\text{N}$, than resident salmonids found over a wide area of Pacific and Atlantic Patagonia (Table 2), allowing for discrimination of the two groups and supporting the generality of our conclusions.

It seems likely that the factors most likely to affect the identification of aquaculture escapees will be anadromy, piscivory, anthropogenic inputs, and fasting because all these will tend to enrich the SIS of salmonids, and therefore make it more difficult to discriminate between the enriched isotopic signatures of farmed fish and the comparatively more depleted isotopic signatures of wild fish. Anthropogenic nitrogen inputs can result in artificially elevated $\delta^{15}\text{N}$ SIS of free-living fish (Lake et al. 2001; Anderson & Cabana 2005), but this is unlikely to be a significant problem in Patagonian watersheds, which are sparsely populated and relatively free from agriculture impacts. Data from nearby rivers Pescado, Blanco and Tepu indicate that $\text{NO}_3\text{-N}$ typically ranges from 2 to 65 $\mu\text{g/L}$ (Arismendi 2009). In general, Patagonian rivers and lakes inhabited by salmonids tend to be oligotrophic and relatively unproductive (Ciancio et al. 2005; Arismendi 2009), and consequently nitrogen enrichment due to anthropogenic inputs is unlikely to elevate the nitrogen SIS of free-living salmonids to values observed amongst farmed fish.

Another potential source of nitrogen enrichment may result from the diet switch that occurs when salmonids migrate to sea (anadromy) or become piscivorous. Anadromy and piscivory can result in $\delta^{15}\text{N}$ isotopic enrichment (Finlay et al. 2002; Jardine et al. 2008), and a stepwise 2–5‰ increase in $\delta^{15}\text{N}$ can be expected per trophic level (Cabana and Rasmussen 1994; Jennings et al. 2002), though the

Table 2 Variation in SIS of free-living resident salmonids in Patagonian streams (Means (min, max) are shown)

Species	TL range (mm)	River	Latitude	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Reference
Rainbow trout						
	140–410	Sta. Cruz (Argentina)	50° S	−22.5 (−19.3, −28.4)	8.4 (6.9, 10.5)	Ciancio et al. (2008b)
	291–406	Negro (Argentina)	41° S	−21.8 (−20.9, −23.0)	9.7 (8.5, 10.2)	Ciancio et al. (2008b)
	100–650	Bueno (Chile)	40° S	–	(6.3, 7.4)	Arismendi (2009)
	100–340	Llico (Chile)	41° S	–	(7.7, 8.8)	Arismendi (2009)
	100–616	Pescado (Chile)	41° S	–	(5.5, 8.8)	Arismendi (2009)
	89–560	Chiloé streams (Chile)	41–42° S	−32.7 (−19.6, −39.1)	10.5 (8.9, 15.7)	This study
Brown trout						
	276–430	Gallegos (Argentina)	52° S	−25.1 (−23.1, −27.9)	9.9 (8.9, 10.7)	Ciancio et al. (2008b)
	43–53	Grande (Argentina)	54° S	−26.7 (−24.9, −29.8)	7.6 (6.8, 8.6)	Ciancio et al. (2008b)
	100–650	Bueno (Chile)	40° S	–	(5.0, 14.9)	Arismendi (2009)
	100–340	Llico (Chile)	41° S	–	(7.5, 8.6)	Arismendi (2009)
	100–616	Pescado (Chile)	41° S	–	(5.0, 11.6)	Arismendi (2009)
Atlantic salmon						
	51–170	Chiloé streams (Chile)	41–42° S	−31.9 (−22.4, −41.1)	10.3 (5.2, 15.5)	This study

precise extent of SIS enrichment due to piscivory may depend closely on metabolic turnover and prey composition (MacAvoy et al. 2001). The isotopic signatures of small juveniles will not generally be affected by anadromy or piscivory, but large anadromous salmonids returning from the sea to spawn in Atlantic Patagonia (Ciancio et al. 2008a, b) have shown enriched $\delta^{15}\text{N}$ SIS similar to values found amongst farmed fish in our study. In this case, scale analysis or Sr:Ca profiles (Ciancio et al. 2008b) may be required to identify anadromous individuals, which may not be readily separated from aquaculture escapees by SIA alone.

Cessation of feeding can also result in $\delta^{15}\text{N}$ enrichment due to mobilisation, reorganisation and catabolism of tissue (Doucett et al. 1999), but fasting is only likely to affect SIS of salmonids during spawning (Ciancio et al. 2008b), and perhaps also during the first few days following a escape event, when escapees may have empty stomachs while they learn to forage on natural prey (Munakata et al. 2000). The latter will tend to delay the time of isotopic incorporation, and therefore extend the length of time aquaculture escapees can be detected.

Our study indicates that farmed salmonids can be recognised by SIA, but we do not know for how long the enriched signature of farmed fish will persist after fish escape from fish farms. Fish white muscle can retain specific SIS from a few weeks to several

months following a shift in diet (Hesslein et al. 1993; Kennedy et al. 2005; Perga and Gerdeaux 2005), but the dynamics of isotopic incorporation can be complex (Dalerum and Angerbjörn 2005; Newsome et al. 2007) making it difficult to predict the temporal retention of diet-related SIS. Body size can have a strong influence on reaction time of isotopic incorporation (Barnes et al. 2008; Wolf et al. 2009) and SIS has been found to vary with salmonid body size in previous studies (e.g. Doucett et al. 1999; Jardine et al. 2005). However, in our study neither carbon nor nitrogen SIS varied with fish length in Atlantic salmon. Only in wild rainbow trout was the SIS positively correlated with body size, possibly due to size-dependent changes in foraging behaviour (Cury et al. 2003), as the small size of most of our free-living trout precluded the possibility of anadromy and piscivory. Somatic growth is likely to be the factor that affects isotopic discrimination of aquaculture escapees the most, because growth affects carbon and nitrogen turnover rates, and therefore the production of new tissue (Herzka and Holt 2000; Bosley et al. 2002; Jardine et al. 2004). Data from North America indicates that brook trout alevins originating from anadromous parents lose their maternal marine signature when they reach ~30 mm in length, corresponding to a fivefold increase in weight (Curry 2005). In the Connecticut River, juvenile Atlantic salmon retained distinct hatchery-specific SIS for

5 weeks, and C signatures from the hatchery were still detectable in the scales of 3 month-old fish (Kennedy et al. 2005).

As fish growth slows down with age, older fish escaping from fish farms are likely to retain their enriched SIS for longer than fish escaping at a younger age. On the other hand, fish escaping at a young age will not be affected by anadromy, piscivory, or reproductive fasting—factors that may complicate the identification of older fish by SIA. Scale analysis may give some indication of time at liberty, and in this sense the combination of SIA and scale analysis may prove particularly useful for detecting the regular, low-intensity leakage of fish from freshwater hatcheries, an insidious source of invasive salmonids in Chile that is seldom reported and goes largely unnoticed (Sepúlveda et al. 2009). A small number of suspected escapees in our samples of free-living trout stood out of the rest, and were consistent with a farmed origin. Here, the independent assumption made prior to analysis about the origin of these individuals, that they were escapees, was supported by SIA.

Unlike for rainbow trout, scale analysis of Atlantic salmon did not detect differences in first year growth rates between hatchery and wild-caught fish, and performed poorly for group discrimination. In this context, SIA appeared to be a more powerful tool: where the analysis of salmonid scales could not detect differences between groups, SIA was able to discriminate between wild and farmed fish with 94% accuracy. Scale pattern analysis allowed for age estimates, which revealed a majority of 0 + amongst wild Atlantic salmon. The SIS of wild-caught juvenile salmon were very different from the SIS of fish of similar size and age sampled at a nearby hatchery. It is thus possible that the free-living juvenile salmon in our river samples were the offspring of naturally reproducing Atlantic salmon, though as this has not been reported in Chile yet (Arismendi et al. 2009) it would require further analysis. Alternatively, it is possible that all of our wild-caught Atlantic salmon were fish of hatchery origin that may have escaped at a very early age, and had been in the wild long enough for isotopic incorporation.

The muscle $\delta^{15}\text{N}$ SIS of hatchery-reared juvenile salmonids in Chile (mean = 14.5–15.1‰) is very similar to that reported by Kennedy et al. (2005) for hatchery juvenile salmon in the Connecticut River (14.5‰), but substantially more enriched than values

found for resident salmonids throughout Pacific and Atlantic Patagonia (mean = 7.6–10.5 ‰, Table 2). The diet of farmed rainbow trout studied here ('Golden Optima' 4 mm pellet) was not available for SIA, but aquaculture feeds, made up of fish meal and fish oil, are high in lipids and proteins and tend to be derived from organisms that are trophically higher than the natural prey items of juvenile salmonids (Molkentin et al. 2007). This would explain the nitrogen enrichment observed amongst farmed fish, as $\delta^{15}\text{N}$ is expected to increase at each trophic level (Vander Zanden and Rasmussen 1999). Carbon to nitrogen ratios can be used as proxy for lipid content (Jardine et al. 2008) and our study indicates that farmed salmonids in Chile tended to have higher C/N ratios than free-ranging fish (Table 1), reflecting the typically high fat content of cultured salmonids.

Wild salmonids in Chile showed more variation in carbon and nitrogen SIS than farmed individuals, and amongst wild fish, Atlantic salmon showed more variation in nitrogen SIS than wild rainbow trout. This probably reflects a more diverse, richer diet, but perhaps also the contribution of escapees having spent different times at liberty. Future SIA studies on farmed species could usefully incorporate experiments on isotopic incorporation, in which farmed salmonids could be shifted to a natural diet, as reported recently for sea bass (Barnes et al. 2008). It would be useful to determine experimentally for how long farmed individuals need to be at liberty before they reach equilibrium with the isotopic composition of their new diet.

In summary, the results of this study indicate that a combination of SIA and scale analysis can successfully discriminate between farmed and free-living salmonids, opening new possibilities for evaluating the extent and origin of salmonid encroachment, one of the biggest threats to native fish biodiversity in Chile (Soto et al. 2006; Young et al. 2010).

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