Into the wild: escaped farmed rainbow trout show a dispersal-associated diet shift towards natural prey

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Abstract The feeding ecology of farmed fish escapees has seldom been assessed, although they are one of aquaculture's main environmental impacts. Here we tested if the diet of *Oncorhynchus mykiss* rainbow trout escapees was affected by their dispersal from farms in a reservoir in Argentine Patagonia by combining stomach content and stable isotope data, and compared their spatial patterns with those of caged and wild (previously naturalized) conspecifics. Our results reveal a shift in the stomach content and $\delta^{13}C$ values of escapees, reflecting a farm (pellets) to wild (mainly *Daphnia* sp.) diet transition associated to dispersal from farms. The $\delta^{13}C$ signal of escapees sampled within the farming area was close to that of caged fish, whereas the $\delta^{13}C$ of escapees captured far from it was indistinguishable from that of wild rainbow trout. Furthermore, escapee dispersal from farms was associated with a transition from indiscriminate surface feeding (on indigestible floating items) typical of caged fish to preying heavily on *Daphnia* sp. In contrast, wild fish diet was homogeneous across all sites. Farm escapees gradually acquiring the feeding behavior of their wild conspecifics as they disperse from the farms may promote competition for food and space, and increase their chances for survival in the wild.

Keywords Farm escapees · Feeding behavior · Freshwater aquaculture · *Oncorhynchus mykiss* · Stable isotopes · Stomach contents

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Introduction

Aquaculture is presently the top growing food production sector in the world (Troell et al., 2014; Edwards, 2015), but the potential for long-term environmental effects is compromising further growth in several countries (Bureau & Hua, 2010; Niklitschek et al., 2013; Osmundsen et al., 2017).

One of the main issues of aquaculture is the escapement of cultured organisms, particularly for

long-lived and highly dispersive fish species (Naylor et al., 2005; Ford & Myers, 2008), which may bring negative consequences on wild populations and ecosystems (McGinnity et al., 2003; Lacroix & Stokesbury, 2004). Fish escape from aquaculture facilities mostly due to human error, infrastructure malfunction, or predatory animals tearing holes in the nets (Jensen et al., 2010; Jackson et al., 2015), and a positive correlation has been shown between their incidence in waterbodies and the intensity of farming (Fiske et al., 2006).

Cultured fish escapees are known to thrive in many wild environments (Jacobsen & Hansen, 2001; Arechavala-Lopez et al., 2012), but the dynamics of their ecological roles across time or space have seldom been assessed, especially in freshwater environments. Diet, dispersal, and survival of farm escapees are key aspects to consider when quantifying their impact on receiving ecosystems (Olsen & Skilbrei, 2010; Sepúlveda et al., 2013). Long-term survival and dispersal is expected to result from the consumption of natural preys, but few studies have assessed their feeding habits, and contrasting results were found across environments, seasons, and species, particularly for salmonid escapees (e.g., Soto et al., 2001; Olsen & Skilbrei, 2010; Johnston & Wilson, 2015). Although a high proportion of escapees die shortly after escape (Patterson & Blanchfield, 2013; Hamoutene et al., 2018), their large absolute numbers ensures thriving populations of farmed fish in the wild (Thorstad et al., 2008; Dempster et al., 2016).

The dispersal and survival of farmed fish in the wild have shown a high variability across studies, with several factors influencing them, both in freshwater (e.g., Bridger et al., 2001; Blanchfield et al., 2009; Charles et al., 2017) and marine environments (e.g., Skilbrei, 2010; Skilbrei et al., 2015). Dispersal may depend on factors like fish size, season, and the relative availability of natural prey close to and far from the farms (Olsen & Skilbrei, 2010), as well as on the intensity of competition around the farms (Dempster et al., 2010; Arechavala-Lopez et al., 2013).

Understanding the long-term behavior of escapees could contribute to the mitigation of aquaculture negative impacts by providing useful information for recapture protocols (Hedger et al., 2017; Šegvić-Bubić et al., 2018), which rely on the knowledge about species- and environment-specific dispersal (Dempster et al., 2016; Izquierdo-Gomez et al., 2016). In this context, research focusing simultaneously on diet and dispersal could help to better understand the fate of the farm escapees. This approach has been applied in a wide range of wild animal taxa by the combination of spatial data with stable isotope analysis and stomach content data (e.g., Olson et al., 2010; Brush et al., 2012; Connan et al., 2014), thus integrating information from different time intervals. Escapees go through processes that vary greatly in spatio-temporal span. Generally, long rearing periods in farm cages are followed by very rapid escape events. With time, some individuals may learn to prey on wild organisms, disperse, and go through slower processes, like adopting energetically efficient spatial use and feeding behaviors, and even reproduction (Naylor et al., 2005). In the few studies where stable isotope analysis has been applied to farm escapees, no clear patterns were found in relation to dispersal distance, mainly because of low variability among recaptured escapees (probably caused by low survival; Abrantes et al., 2011; Bell et al., 2016) and high overlap in isotopic signals between farm pellets and natural preys (Johnston et al., 2010; Johnston & Wilson, 2015).

Argentinian Patagonia presents an excellent opportunity to study the environmental impacts of temperate freshwater aquaculture, where cage culture of rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), is an incipient but growing activity. Most of the production comes from the Alicurá reservoir, reaching 1800 ton year^{-1} in 2009 (Temporetti et al., 2001; Zeller et al., 2009). This reservoir is located in the upper reaches of the Limay river, where salmonids have successfully been introduced after sustained efforts during the twentieth century, constituting an internationally renowned fishery within Northern Patagonia (Pascual et al., 2007). Previously, we have shown that farm escapees are abundant in Alicurá reservoir (Cussac et al., 2014), and that they share resources and likely compete with wild fish (Nabaes Jodar et al., 2017), as reported in other environments (e.g., Jacobsen & Hansen, 2001; Johnston & Wilson, 2015). In this study, we assess the diet of rainbow trout escapees in relation to their dispersal from farms in the reservoir. First, we test the hypothesis that stomach contents and the isotopic signal of escaped rainbow trout are associated with dispersal. Second, we assess the extent to which the spatial trophic patterns of escapees correspond with those of their sympatric wild conspecifics.

Materials and methods

Sampling and stomach content analysis

The Alicurá reservoir is a large hydro-power reservoir (area: 67.5 km^2, mean depth: 48 m) located on the upper Limay River in southwestern Argentina, and has operated since 1985 (Fig. 1). It starts 58 km downstream of lake Nahuel Huapi, in the transition zone between the Andean forest and the Patagonian steppe, at the juncture of rivers Traful and Limay. Along with farm escapees and wild rainbow trout, six native fish species and three other salmonids have been reported in the reservoir (Cussac et al., 1998, 2014; Aigo et al., 2008; Nabaes Jodar et al., 2017). Only natives *Odontesthes hatcheri* (Eigenmann, 1909), *Percichthys trucha* (Valenciennes, 1833) and *Galaxias maculatus* (Jenyns, 1842), and exotic rainbow trout, *Oncorhynchus mykiss*, and brown trout, *Salmo trutta* Linnaeus, 1758, are frequently captured. During the time of sample collection at the reservoir, eight farms were active, totaling an annual production estimated between 700 and 1000 tons (pers. comm. from farm technicians). There are no reports of past nor present number of escapees.

Large juveniles and adult fish were captured using gillnets composed of multiple bar mesh sizes (15 to 70 mm) working overnight in March and May 2014, September and November 2015 and January 2016, at a maximum of three out of four distinct locations each time, and in two to five depth strata (Table 1). Sampling locations included two sites between farms: Malalhuaca (MH) and farm site (FM), located 900 m and 100 m from the nearest farms, respectively, and two sites outside the farming area: Cola Limay (CL), 1500 m upstream (southward) of the southernmost farm, and Coloradas Fondo (CF), 22 km downstream (northward) from the northernmost farm (Fig. 1). In March 2014, sampling was performed in MH and CF sites, and in May 2014 the same two plus CL site were sampled (Table 1). In September and November 2015 and January 2016, only FM site was sampled (Table 1). Stomach content data were supplemented with rainbow trout samples collected in February and
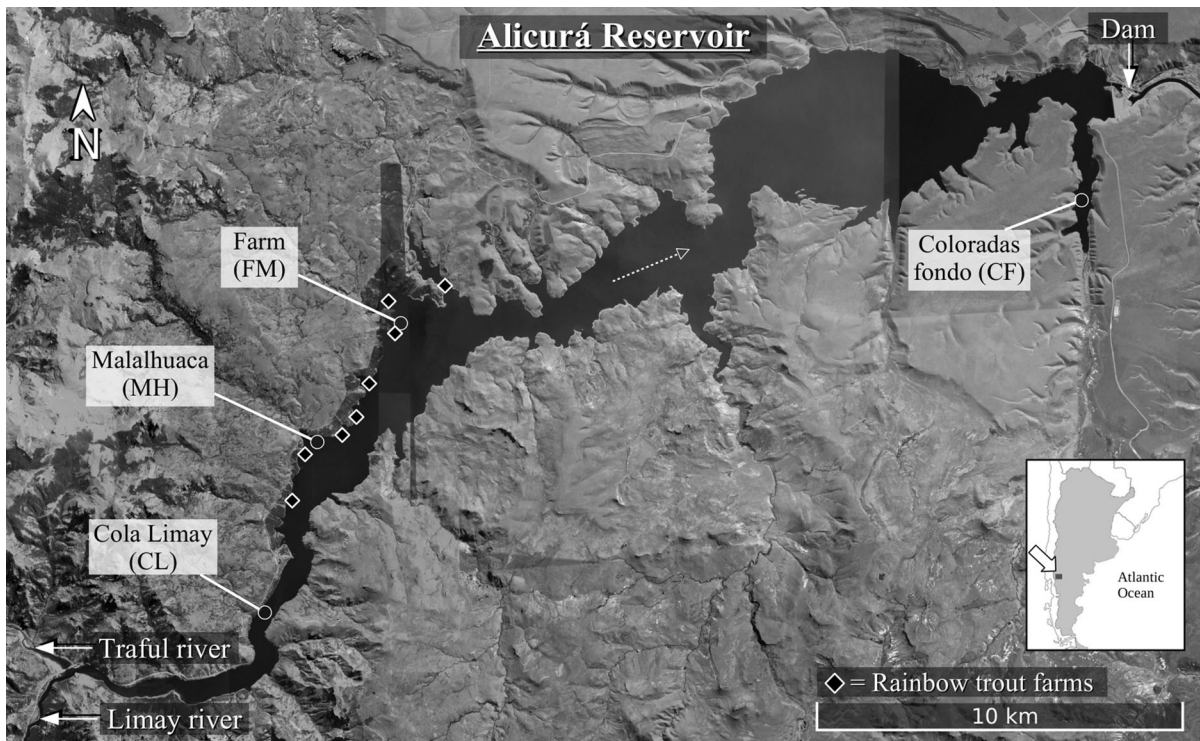
Fig. 1 Sampling sites Cola Limay (CL), Malalhuaca (MH), Farm (FM), and Coloradas fondo (CF) in Alicurá reservoir, and its location on a reference map of the region (inset). The dotted arrow indicates water flow direction. The main image was taken from Google Earth (Mountain View, California, USA)

Table 1 Escaped farm rainbow trout (ERT) and wild rainbow trout (WRT) samples utilized in the present study

Site	Date	No of captured individuals		No of stomachs analyzed with content (empty in brackets)		No of individuals analyzed for C–N isotopes	
		ERT	WRT	ERT	WRT	ERT	WRT
MH	2012 February	1	3	1 (0)	3 (0)	0	0
MH	2012 March	33	5	23 (4)	4 (1)	0	0
CL	2012 March	16	5	13 (3)	2 (2)	0	0
CF	2012 March	3	0	0 (0)	–	0	–
MH	2013 April	46	24	34 (3)	19 (5)	0	0
CL	2013 April	27	32	11 (1)	24 (0)	0	0
MH	2014 March	4	6	4 (0)	4 (2)	4	6
MH	2014 May	4	2	4 (0)	1 (1)	4	2
CL	2014 May	55	21	21 (1)	9 (0)	16	13
CF	2014 May	5	2	5 (0)	2 (0)	5	2
CF	2014 March	0	0	–	–	–	–
FM	2015 September and November	11	1	10 (1)	–	9	–
FM	2016 January	5	0	5 (0)	–	0	–

Samples are grouped according to sampling site and date. *FM* farm site, *MH* Malalhuaca, *CL* Cola, *CF* Coloradas fondo

March 2012, April 2013, and March 2014 in MH, CL, and CF sites to increase the power in assessing the influence of the distance from farms on the diet (Table 1).

Number of individuals, origin (escaped/wild), weight, and fork length were registered, and the stomachs dissected for diet analysis. Wild and farmed rainbow trout were identified according to their external characteristics, as farmed trout are characterized by shortened, rounded and/or wavy pectoral, dorsal, and tail fins (Fiske et al., 2005). Visual differentiation between escaped and wild salmonid conspecifics generally agrees with genetic identification (Johnston & Wilson, 2015). However, a study in Chilean Patagonia by Consuegra et al. (2011) reported that escaped rainbow trout (ERT) can sometimes exhibit no obvious phenotypic differences with wild rainbow trout (WRT). In consequence, fish identified in the present study as wild may include some relatively old farm escapees with regenerated fins, although the opposite is unlikely (Consuegra et al., 2011; Green et al., 2012).

Stomachs were extracted in the laboratory and individually preserved in 96% ethanol at $-20°C$. Content analysis was performed using a stereo microscope. Prey items were grouped based on their taxonomic linkage or spatial habits: (1) terrestrial insects, (2) aquatic nymph or larval insects, (3) Gastropoda, (4) *Daphnia* sp. (Cladocera), (5) fish, (6) rodents, (7) farm pellets, and (8) indigestible items (vegetable debris, synthetic fragments, pumice stones). Prey volume was determined by water displacement. The volume percentage that each prey category contributed to a stomach was selected as the measure of diet (Wallace, 1981). This method allows an equal representation of predators of different sizes (Ahlbeck et al., 2012), and works as a proxy of nutritional importance of each prey category independently of prey size (Hyslop, 1980; Wallace, 1981).

Stable isotope samples and analysis

Stable isotope analysis of carbon and nitrogen of muscle tissue was performed on a subset ($n = 61$) of rainbow trout individuals captured in March and May 2014, and September and November 2015. In addition, muscle tissue samples ($n = 15$) were obtained from five individuals sampled at each of three different farms in the reservoir, named A, B, and C (for anonymity concerns) throughout this work. The preys previously reported to be dominant for rainbow trout of Alicurá reservoir, i.e., *Daphnia* sp. and terrestrial insects (Nabaes Jodar et al., 2017) were also sampled. *Daphnia* sp. was collected in November 2015 at FM

site using a conical zooplankton net with 200 μm mesh size, 50 cm of mouth diameter, and 2 m long. Terrestrial insects were collected in November 2015 using yellow pan traps consisting of a plate filled with soapy water (Leather, 2005; Caut et al., 2014) set in the floor of a forested field, 20 m from the reservoir coastline at MH site. Each sample of plankton and terrestrial insects was thoroughly rinsed with distilled water. Four pools were prepared including several individuals of *Daphnia* sp. larger than 1 mm., and three specimens of each dipterans and wasps. Samples for stable isotope analysis were preserved frozen (− 20°C) until processed, except for farm trout samples, which were preserved in ethanol at 4°C. Since ethanol preservation may dilute lipids (which are depleted in ^{13}C) and, therefore, increase the relative abundance of ^{13}C in the sample, the potential effect of ethanol was estimated using muscle tissue from four escaped rainbow trout captured in MH site. Two tissue samples were obtained from each specimen, one for ethanol preservation and one for cold preservation (− 20°C).

Between 0.8 and 1.2 mg of oven-dried (60°C for 48 h) homogenized and pulverized samples were analyzed for C and N stable isotope composition at the Stable Isotope Facility of the University of California-Davis. An Elemental Analyzer-Isotope Ratio Mass Spectrometer composed of a PDZ Europa ANCA-GSL elemental analyzer interfaced to PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd, Cheshire, UK) was utilized. Isotope ratios are expressed as delta values (δ^{13}C and δ^{15}N) relative to international standards for carbon (Vienna PeeDee Belemnite) and atmospheric nitrogen. Secondary isotopic reference materials used were Bovine Liver ($SD_{\delta^{13}C} = 0.09$, $SD_{\delta^{15}N} = 0.10$), Nylon 5 ($SD_{\delta^{13}C} = 0.09$, $SD_{\delta^{15}N} = 0.15$), Glutamic Acid ($SD_{\delta^{13}C} = 0.07$, $SD_{\delta^{15}N} = 0.13$), and enriched Alanine ($SD_{\delta^{13}C} = 0.1$, $SD_{\delta^{15}N} = 0.09$), interspersed between samples (one every three samples).

Statistical analysis

Given that the effect of ethanol preservation in δ^{13}C (mean = + 0.23%) was minimal relative to the sample variability (as reported in Correa, 2012) and not significantly different from zero (Paired t test, $t = − 1.45$, P value = 0.24), data obtained from samples preserved in ethanol were not modified. As

the C:N ratios of all taxa were frequently above 3.5 (max. = 5.6), δ^{13}C values of all samples were normalized using the formula suggested by Post et al. (2007) for aquatic organisms.

Differences in δ^{13}C and δ^{15}N between the distinct origins of trout (caged, escaped, and wild) were assessed using a generalized least squares (gls) model with the variance function varIdent, which enables accounting for heteroscedasticity among factor levels, providing estimates of the standard deviations for each origin (Zuur et al., 2009).

To evaluate the effect of dispersal distance on escapee isotopic composition, a gls model was also applied, including site as a predictor (factor with four levels) and allowing variances to vary per site.

The combined effect of wild/escaped origin and site was only tested in two sites, i.e., MH and CL, given that no wild trout were captured in FM, and only two individuals were captured in CF. The same statistical procedure described above was employed to account for the different variances observed between origins. Significant terms of each model were assessed via likelihood ratio tests. Post hoc pairwise comparisons were made using modified Tukey contrasts according to Herberich et al. (2010) method for heteroscedastic data, using R packages 'multcomp' (Hothorn et al., 2008) and 'sandwich' (Zeileis, 2004).

Given the paucity of items that comprised most of the diet of rainbow trout in Alicurá reservoir (Nabaes Jodar et al., 2017; present results, Online Appendix 4), the three most consumed items (percentage of total volume), i.e., *Daphnia* sp., indigestible items and terrestrial insects, were selected to compare their incidence across sites and along stable isotope values. Also, these three items represent different feeding habits, i.e., at surface (indigestible items and terrestrial insects) versus in the water column (*Daphnia* sp.), as well as contrasting nutritional values, i.e., null (indigestible items) versus nutritious (insects and *Daphnia* sp.). Surface feeding is also associated to farm-feeding on pellets and, therefore, is expected to remain as a predominant behavior in recent escapees, as previously reported (Skilbrei et al., 2009).

The relation between δ^{13}C (as dependent variable) and the incidence of the three main diet items (% volume; fixed predictors) was evaluated, using a subset of individuals for which both stomach content and isotopic data were obtained. Given the nature of the data (relative abundances), negative correlation is

expected between the incidence of at least two of the three predictors. Therefore, in order to evaluate if the variation in the dominance of each item was associated with changes in $\delta^{13}C$, separate models were run, each with only one of the predictors. The model with % indigestible items as predictor required accounting for heterogeneous variance, which was done using the gls model function with varExp function (recommended when the variance covariate includes zeros, Zuur et al., 2009). The models with % *Daphnia* or % terrestrial insects as predictors did not show patterns in their residuals allowing a general linear model to be fitted for each predictor. For wild trout individuals with isotopic data ($n = 12$), the low variability and the extreme values in the volume percentage of each diet item, precluded statistical analysis of their relation to $\delta^{13}C$ (raw data and marginal box-plots of both types of data are presented in Online Appendix 3).

The model described above ($\delta^{13}C$ and volume % relations) was replicated for two subsets of the data containing only escapees captured (a) within the farming area (sites MH and FM), and (b) outside the farming area (sites CL and CF). In addition, the effect of areas with or without farming activity on the diet of escapees was assessed. For this, a Wilcoxon rank sum test (W; a non-parametric version of the two-sample t test) was used to compare volume % of *Daphnia* sp., indigestible items and terrestrial insects *between* individuals captured within and outside the farming area. This analysis included extra stomach content data from years 2012–2014 as described above. The same procedure was applied to wild trout stomach content data. Furthermore, fish length effects on the differences between sites in isotopic and stomach content data of wild and escaped fish were assessed. To do this, linear regressions were applied to test if fish length (dependent variable) varied between sites (predictor) including only individuals with diet data, and if fish length (as predictor) affected the muscle $\delta^{13}C$ (as dependent variable). Also, Spearman rank correlation tests were performed between fish length and stomach contents (i.e., *Daphnia* sp., indigestible items, and terrestrial insects).

All model assumptions were checked before and after model selection procedures using plots of fitted and predicted values versus residuals, as well as normal probability plots (Zuur et al., 2009; Warton & Hui, 2011).

Isotopic mixing models

A Bayesian mixing model was implemented with the package MixSIAR (Stock et al., 2018), for which information on sources of C and N was obtained by performing stable isotope analysis of the two main (digestible) diet items reported for rainbow trout in Alicurá reservoir, i.e., *Daphnia* sp. and terrestrial insects (Nabaes Jodar et al., 2017 and present study). Although rainbow trout of Alicurá reservoir has not been reported to contain farm pellets in their stomachs (Nabaes Jodar et al., 2017), and only three (all escapees captured at site FM) did in the present study, farm pellets were considered an important source to be included in the mixing models, since the isotopic turnover time of fish muscle is in the order of months (Dempson & Power, 2004; Buchheister & Latour, 2010). To do this, instead of using the isotopic signal of the pellets, whose compositions in local markets have significant temporal variability (pers. comm. from farm technicians), associated to a positive diet-tissue discrimination factor (DTDF), we used the isotopic data of caged farmed rainbow trout individuals fed solely with pellets from three farms in the Alicurá reservoir, associated to a DTDF of zero. This allows the software to interpret it as "pre-corrected" source values, and represents a potential source with a discrimination factor specific for both consumer and diet, as it has been done in aquatic herbivore fatty acid studies (Galloway et al., 2014a, b). In our particular case, there could be a fractionation offset because of differences in metabolism between caged and free-ranging rainbow trout, but it can be considered negligible compared to the uncertainty typically assumed when using trophic discrimination factors taken from the bibliography, which are generally not specific for the food source nature and/or its composition (Caut et al., 2009). Two of the three farm sources were combined since they did not show significantly different isotopic values (farms B and C), rendering only two potential sources of farm pellets (farm A and farm B–C).

The DTDFs for rainbow trout muscle were obtained by averaging all estimates reported in the literature for this species, fed with farm pellets in all cases (Rounick & Hicks, 1985; Pinnegar & Polunin, 1999; McCutchan et al., 2003; Abrantes et al., 2011). The DTDF for $\delta^{13}C$ was 1.5, and for $\delta^{15}N$ was 3.0, which are within the recommended values for fish muscle (Sweeting et al.,

2007a, b). Large standard deviations were assigned to all three DTDFs, i.e., $SD_{\delta^{13}C} = 1.02$ and $SD_{\delta^{15}N} = 0.4$, which are the largest reported for rainbow trout (Rounick & Hicks, 1985; Pinnegar & Polunin, 1999; McCutchan et al., 2003; Abrantes et al., 2011), to ensure the outputs of the model are conservative and robust to the diet-unspecific DTDFs (Galván et al., 2012). The model included a process × residual error structure (Stock & Semmens, 2016a), and Markov Chain Monte Carlo settings consisted in three chains of 100,000 chain length, 50,000 burn-in, and 50 thin. Finally, diagnostics were based on Gelman–Rubin and Geweke tests, which reflected sufficient convergence to accept the results (Stock & Semmens, 2016b).

Results

$\delta^{13}C$ and $\delta^{15}N$

Values of $\delta^{13}C$ were significantly different between farm (highest), escaped, and wild rainbow trout (lowest; Likelihood ratio test (LRT): $X^2_{(4)} = 101$, $n = 76$, $P < 0.0001$; Fig. 2A). Tukey contrasts revealed $\delta^{13}C$ of trout harvested from farm A was higher than from the other two farms, and the three of them were higher than those of escaped and wild rainbow trout ($P < 0.0001$; Fig. 2A). Escapees presented the largest standard deviation (1.9), followed by wild trout (1.1), while farm trout had the smallest (0.20–0.31).

Regarding the $\delta^{15}N$ model (LRT: $X^2_{(4)} = 47$, $n = 76$, $P < 0.0001$; Fig. 2B), Tukey contrasts revealed escapees did not differ from wild trout ($t = 0.5$, $P = 0.98$), but did so from farm trout ($P < 0.04$). Wild trout $\delta^{15}N$ differed from farms A and C ($P < 0.001$), but not so from farm B ($t = -2.5$, $P = 0.086$). Farm A $\delta^{15}N$ values were smaller than farms B and C ($P < 0.001$), and also smaller than escaped and wild trout ($P < 0.001$). These results showed a large separation of farm A $\delta^{15}N$ from all the other groups ($\sim 3\%$; Fig. 2B).

Escapees $\delta^{13}C$ varied across sites (LRT: $X^2_{(3)} = 20.4$, $n = 38$, $P = 0.0001$), separating sites within (MH and FM) from those outside the farming area (CL and CF) (Fig. 2C). Tukey contrasts revealed that escapees had higher $\delta^{13}C$ (more similar to farmed trout) in sites MH and FM than in sites CL ($t = -4.6$, $P < 0.001$ and

$t = -2.8$, $P = 0.034$, respectively) and CF ($t = -3.3$, $P = 0.012$ and $t = -2.4$, $P = 0.085$, respectively; the marginal significance of MH to CF comparison suffered the low number of samples in CF, $n = 5$, but escapees from CF showed all $\delta^{13}C$ values within the range of CL escapees; Fig. 2C).

Further analysis revealed that $\delta^{13}C$ differences between escapees and wild trout are site-dependent (LRT $X^2_{(3)} = 4.9$, $n = 38$, $P = 0.026$), being similar at CL (average difference = 0.7%, $t = -1.8$, $P = 0.22$) but different at MH site (average difference 2.59%, $t = -2.6$, $P = 0.038$) (Fig. 2D). Also, wild trout had relatively low and homogeneous $\delta^{13}C$ values at MH and CL sites (average difference = 0.75%, $t = -1.4$, $P = 0.44$) (Fig. 2D).

A positive relationship was found between escapees $\delta^{13}C$ and % indigestible items (LRT: $X^2_{(1)} = 4.9$, $n = 28$, $P = 0.02$, Fig. 3C), while a negative one was found between $\delta^{13}C$ and % *Daphnia* sp. (F test: $F_{(1)} = 19.9$, $n = 28$, $P = 0.00012$, Fig. 3C). No relationship was found among escapees $\delta^{13}C$ and % terrestrial insects ($F_{(1)} = 0.9$, $n = 28$, $P = 0.33$, Fig. 3C).

Wild rainbow trout showed low variability in both isotopic signal and stomach content (Online Appendix 3). There were only 12 individuals with isotopic data and non-empty stomachs, from which only two consumed indigestible items (5% and 20% in volume) and only three consumed terrestrial insects (15%, 50%, and 100% in volume). Eleven consumed *Daphnia* sp. abundantly, with nine of them having more than 85% of their stomach volume occupied with this prey (Online Appendix 3).

Escapees outside the farming area showed a negative relationship between percentage *Daphnia* sp. consumption and $\delta^{13}C$ (LRT: $X^2_{(1)} = 7.2$, $n = 14$, $P = 0.007$), and a positive one between indigestible items and $\delta^{13}C$ (LRT: $X^2_{(1)} = 7.0$, $n = 14$, $P = 0.008$), reflecting the same pattern as when escapees were analyzed all together. Only one individual with stable isotope data (captured outside the farming area) had terrestrial insects in its stomach, and this prey represented only 6% of the its stomach content volume. For escapees within the farming area, no associations were found between $\delta^{13}C$ and % *Daphnia* sp. (LRT: $X^2_{(1)} = 2.5$, $n = 16$, $P = 0.11$), indigestible items (LRT: $X^2_{(1)} = 0.05$, $n = 16$, $P = 0.82$), or terrestrial insects (LRT: $X^2_{(1)} = 0.17$, $n = 16$, $P = 0.7$).
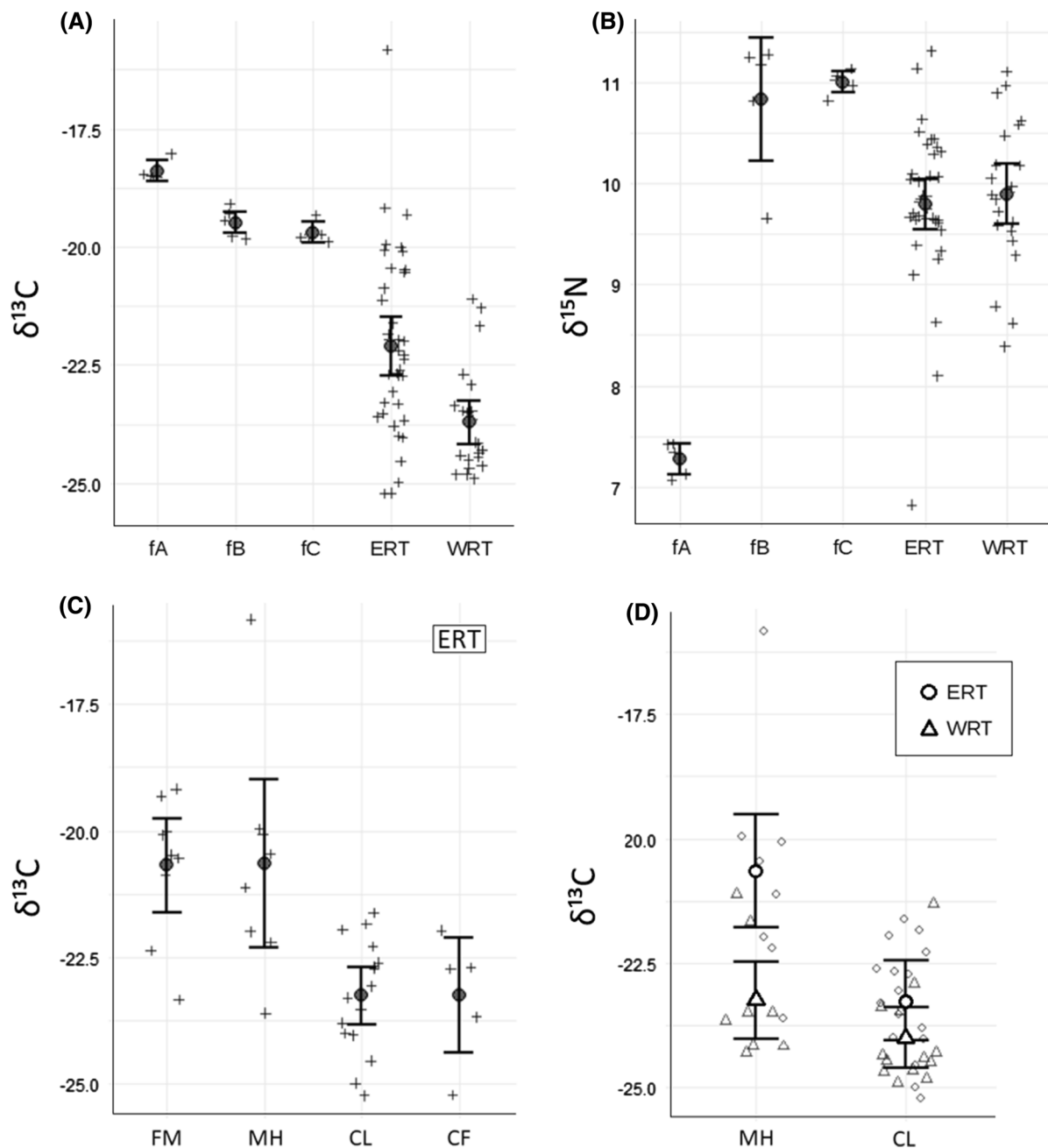
Fig. 2 Means, 95% confidence intervals and raw data from four GLS (Generalized Least Squares) models for **A** δ^{13}C and **B** δ^{15}N of rainbow trout muscle by fish origin, **C** δ^{13}C of escaped farmed rainbow trout (ERT) by site, and **D** δ^{13}C of ERT and wild rainbow trout (WRT) by site from Alicurá reservoir. fA, fB, and fC represent three distinct farms at the reservoir. *MH* Malalhuaca, *FM* farm site, *CL* Cola Limay, *CF* Coloradas fondo

Stomach content–site associations

Indigestible items were more consumed by escapees within the farming area ($n = 79$) than outside of it

($n = 51$; Wilcoxon rank sum test: W = 2514, $P = 0.011$), whereas the opposite was observed for *Daphnia* sp. (W = 1374.5, $P = 0.001$) (Fig. 3A). Terrestrial insect incidence in escapee diet showed

no differences between areas (W = 2236, P = 0.25). Wild trout diet was homogeneous between sites within (n = 31) and outside the farming area (n = 40; W = 536 to 590, P = 0.28 to 0.65, Fig. 3B).

Fish length effects

Fish fork length did not vary across sites for escaped (F test: $F_{(3)}$ = 0.52, n = 129, P = 0.67), nor wild rainbow trout (F test: $F_{(2)}$ = 0.38, n = 68, P = 0.69). Also, fish length did not affect δ^{13}C of escapees (F test: $F_{(1)}$ = 0.35, n = 30, P = 0.56), nor of wild rainbow trout (F test: $F_{(1)}$ = 0.30, n = 12, P = 0.60). Finally, Spearman rank correlations between fish length and volume % of *Daphnia* sp. (escaped: ρ = − 0.09, P = 0.3; wild: ρ = − 0.048, P = 0.7), indigestibles (escaped: ρ = 0.132, P = 0.13; wild: ρ = 0.13, P = 0.28), and terrestrial insects (escaped: ρ = 0.021, P = 0.81; wild: ρ = 0.04, P = 0.73) were non-significant for both escapees (n = 129) and wild individuals (n = 68).

Isotopic mixing models

The isotopic signal of escapees captured within the farming area was highly influenced by that of pellets from farms B and C, and to a smaller extent by *Daphnia* sp. The opposite resulted for escapees captured outside the farming area as well as wild individuals from both areas, with their isotopic signal highly influenced by *Daphnia* sp. (Fig. 4; Online Appendix 1). 95% credibility interval (95% CI) for the percentage contribution of pellets from farms B and C was 46% to 78% (median = 65%) for escapees within the farming area, whereas it was 23% to 47% for escapees outside, and 16% to 49% and 19% to 45% for wild individuals captured within and outside the farming area, respectively (Online Appendix 1). In the case of *Daphnia* sp. percentage contribution, the 95% CI for escapees within the farming area was 14% to 34% (median = 23%), while for escapees outside of it was 48% to 70%, and for wild individuals 43% to 75% and 52% to 77% within and outside the farming area, respectively. Estimates of the 95% CI for the contribution of farm A and terrestrial insects included 0% for every consumer group (Online Appendix 1).

Discussion

We found and characterized a significant shift in the diet and stable isotope signal of rainbow trout escapees, reflecting a farm- to wild-based diet transition associated with dispersal distance from farms. Escapees captured within the farming area had δ^{13}C close to that of caged fish and showed a predominance of indiscriminate surface-feeding behavior, whereas the great majority of escapees sampled outside the farming area were comparable to their wild conspecifics by showing lower δ^{13}C and preying predominantly on zooplankton.

In accordance with our results, escaped and wild rainbow trout captured far from farms in Lake Huron (Canada) showed equivalent isotopic signals, supporting escapee ability to feed on wild preys (Johnston & Wilson, 2015). In a Tasmanian aquaculture-impacted fjord, a study found that *c.* 25% of rainbow trout escapees fed on native fauna (Abrantes et al., 2011), once again showing their ability to adjust feeding behaviors to the receiving ecosystem. However, escapees in that study largely retained the farm pellet isotopic signal both close to and far (10 km) from the farms probably due to the very low survival of escapees in that environment, where turbidity is high and wild prey availability is low (Abrantes et al., 2011; Bell et al., 2016).

In Alicurá reservoir, changes in δ^{13}C signal of farm escapees were expected after shifting their diet from farm pellets to *Daphnia* sp., given the pronounced difference in δ^{13}C between these food sources. Large individuals of *Daphnia* sp. are the main food item across fish species (Nabaes Jodar et al., 2017), and may represent an easy prey to catch for rainbow trout escapees. On the contrary, the consumption of indigestible items was not expected to have a direct influence on the isotopic signal. However, the positive association found between the proportion of consumed indigestible items and δ^{13}C values of escapees could be indirect, being both variables affected by the shifting behavior from surface feeding to zooplanktivory at the water column. The relative incidence of indigestible items, wild preys, and fish δ^{13}C signal in relation to location further suggest that dispersal is associated with a transition in feeding behavior from an indiscriminate feeding typical of caged fish (Rikardsen & Sandring, 2006), to preying on *Daphnia* sp. A different situation was found for terrestrial
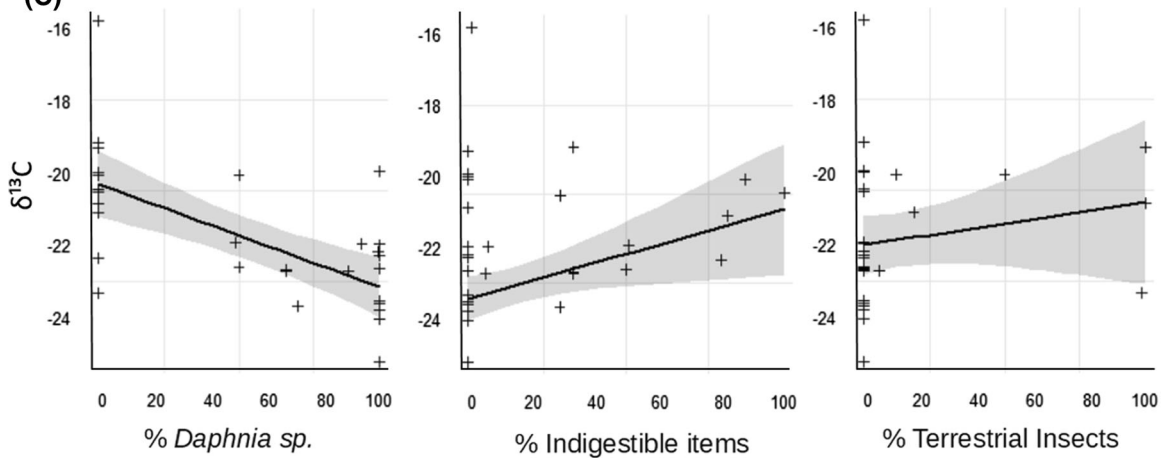
(A) Escaped rainbow trout:
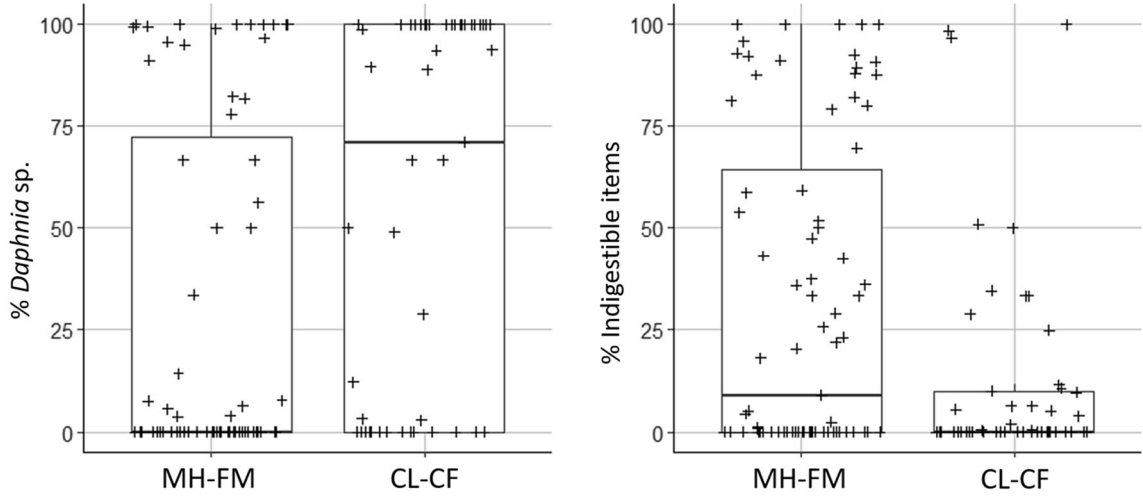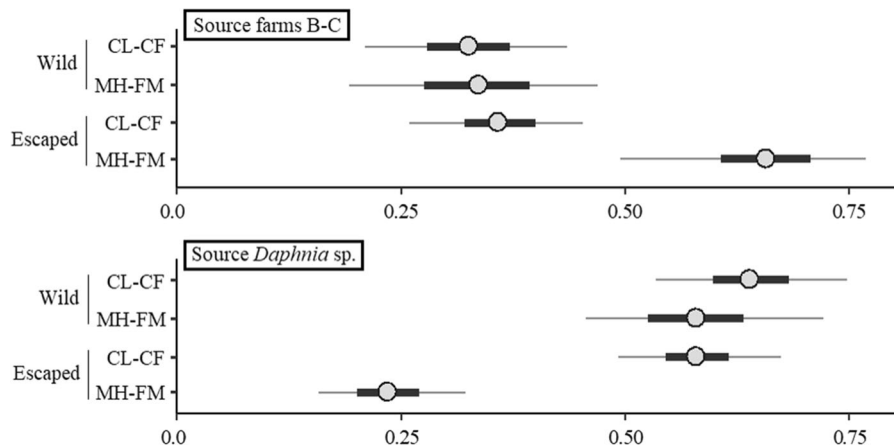
(B) Wild rainbow trout:
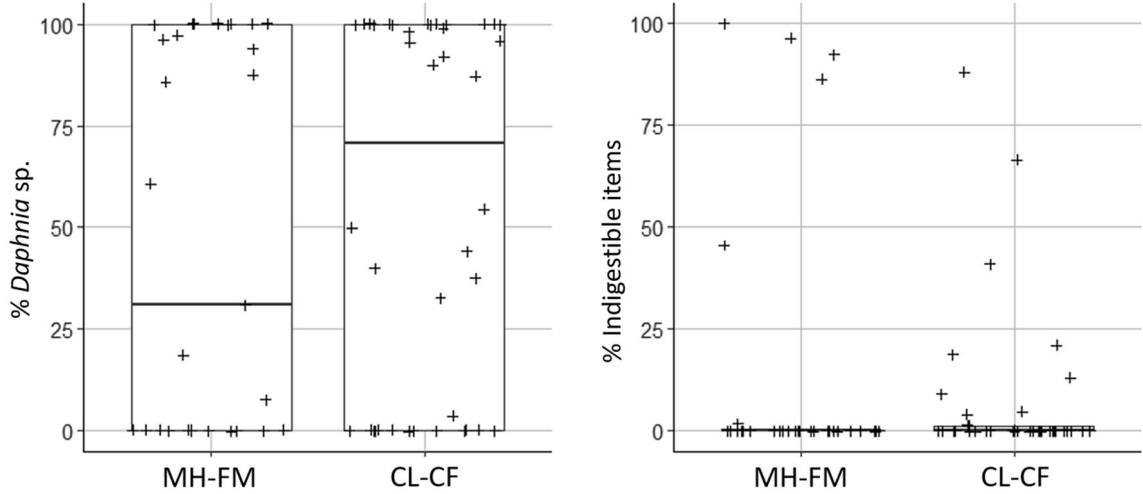
(C)

◄**Fig. 3 A**, **B** Box-plots and raw data (randomly scattered horizontally) comparing individual stomach contents (% volumetric incidence) of wild and escaped rainbow trout captured within (MH–FM) and outside (CL–CF) the farming area in Alicurá reservoir. The significance of each comparison (Wilcoxon rank sum tests) is showed in parentheses: "*": $P < 0.05$, "ns": $P > 0.05$. **C** Output from models (plus 95% confidence intervals) relating the % volumetric incidence of the three main prey items with muscle $\delta^{13}C$ for escaped rainbow trout

insects, whose incidence in stomach contents had no clear association with the $\delta^{13}C$ of escapees, nor with the distance to farms. Despite being distributed mainly in the water surface, thus favoring consumption by new escapees searching for farm pellets, the high nutritional value of terrestrial insects could also attract older, more experienced escapees that likely discern insects from indigestible items better than recent escapees, balancing their consumption across sites. However, the ratio of ingested to assimilated carbon and nitrogen from consumed insects could be lower than from soft-bodied *Daphnia* sp., causing the small representation of the former in the MixSIAR model estimates. This is supported by the tendency of rainbow trout to prey on large zooplankton whenever it is highly available, which increases their growth rate (Tabor et al., 1996; Ciancio et al., 2008; same pattern for other species: Mehner et al., 2005).

Our results suggest a high degree of tissue turnover in many escapees, meaning these fish escaped at least

several months before being captured (Dempson & Power, 2004; Buchheister & Latour, 2010). Also, numerous escapees showed a wide range of $\delta^{13}C$ values falling between the extremes presented by farmed and wild fish. This pattern could be the consequence of two non-exclusive processes, i.e., partial and ongoing tissue turnover towards the isotopic signal of wild rainbow trout (a process mostly dependent on time since escape), or differences in the feeding ecologies of escapees (a steady state of within-population variability). The low incidence of pellets in the stomachs of escapees (Nabaes Jodar et al., 2017 and present results) supports the former process. Thus, the large variability in $\delta^{13}C$ values indicates that escape events occurred somewhat continuously during the weeks and months before sampling, as escapees captured close to the farms are likely to have escaped more recently than those captured far from them, supporting a time-dependent dispersal distance.

The patterns described above seem to be robust to potential seasonal effects, since $\delta^{13}C$ values of escapees captured at MH site during the end of the summer and autumn are very similar to those of escapees captured in spring (at FM site; Fig. 2C). On the contrary, escapees captured outside the farming area (CL and CF) during the end of the summer and autumn showed markedly different $\delta^{13}C$ values, having a large portion of the variability explained by the proximity to the farming area (Fig. 2C), with no

Fig. 4 Proportion estimates from the MixSIAR Bayesian mixing model of the contribution of farm pellets (estimated using caged farmed rainbow trout muscle isotopic data, associated to a DTDF of zero) and *Daphnia* sp. to the diet of rainbow trout grouped by origin (escaped and wild) and capture site (within the farming area: MH-FM; outside the farming area: CL–CF). Points: posterior medians; thick lines: 50% credible intervals; thin lines: 90% credible intervals. A DTDF specific to the consumer species was used, obtained by averaging estimates reported for rainbow trout fed with farm pellets

difference between summer–autumn and spring captures. Regarding the potential variation in prey availability, the abundance of *Daphnia* sp. seems to be high during all the seasons sampled as inferred from the stomach content data (Fig. 3A, B), and, specifically for the spring, from the effortless success at collecting this prey with a net (see "Materials and methods"; Nabaes Jodar D. N., unpublished density data). On the contrary, the falling of terrestrial insects onto the reservoir is expected to vary between seasons, with peaks in spring and summer, but no patterns were found for the consumption of this prey between locations for either fish origin.

Intense competition and predation around farm cages has been suggested to limit the access of new escapees to uneaten pellets exiting the cages (Skilbrei, 2012). Also, the probability that fish around farms have pellets in their stomachs has been positively associated with fish length (Carss, 1990). These observations may well be supported by the imbalance between the consistent inputs of escapees to the environment versus the limited amount and ephemeral nature of uneaten pellets. Depending on the number of fish escaping each farm, as well as on the density of cages, competition for wild preys at some distance from farms could also be high, promoting the dispersal of new escapees farther away from the farm-influenced area. In Alicurá reservoir, numerous large rainbow trout are typically seen around cages (pers. obs. Nabaes Jodar D. N.), though harvest consists of 300 g fish, and to a lesser extent 1.5 kg fish, suggesting that fish around farms are not recent escapees. It is highly likely that the smaller sizes of new escapees diminish their competitive ability, supporting their widespread dispersal interpreted from our stable isotope data. Rapid dispersal rates have also been reported for tagged rainbow trout escapees in Lake Huron, where trout farming has been operative for two decades (Patterson & Blanchfield, 2013). In contrast, high fidelity of escapees to a new and unique farm was reported in a small experimental lake (Blanchfield et al., 2009; Charles et al., 2017). This discrepancy could result from the significant size difference between these two lakes, but also due to the different periods of time each has supported cage culture activity, causing fish around the cages in Lake Huron (Johnston et al., 2010) to increase competition, and promote dispersal of new escapees.

Although aquatic ecosystems are being increasingly impacted by farmed fish escapees worldwide (Troell et al., 2014; Dempster et al., 2016; Lima et al., 2016), very low survival rates are commonly reported (Abrantes et al., 2011; Bell et al., 2016; Hedger et al., 2017). However, there are examples where escapees have had great success and readily switched to a wild prey diet, including Norwegian, North American, and Chilean fjords, Lake Huron in Canada, several freshwater environments of Brazil, and Alicurá reservoir studied here (Soto et al., 2001; Jensen et al., 2013; Johnston & Wilson, 2015; Lima et al., 2016; Nabaes Jodar et al., 2017). Besides the possible bias of methods for the estimation of survival, its magnitude has been associated with environmental features (Bell et al., 2016; Dempster et al., 2016), age at escape (Rikardsen & Sandring, 2006; Skilbrei, 2010), and season of escape events (Bridger et al., 2001; Olsen & Skilbrei, 2010; Skilbrei et al., 2015). It is important to consider that studies on escaped fish ecology generally gather information using one of two methods, i.e., by tagging, releasing and, shortly after, recapturing a limited number of fish (Hansen, 2006; Blanchfield et al., 2009; Olsen & Skilbrei, 2010), or by capturing escaped fish living in the wild since unknown periods of time (Soto et al., 2001; Arismendi et al., 2009; Jensen et al., 2013; Johnston & Wilson, 2015). It is not surprising that in the former case, poor adaptation to the wild is more frequently reported than in the latter, where the population sampled consists of abundant successfully adapted fish, resulting from large numbers of accidental escapes.

Both the homogeneity of wild rainbow trout isotopic composition captured in the Alicurá reservoir and the isotopic values of its main prey item, *Daphnia* sp. (sampled close to the farms), suggest that there is no significant carbon nor nitrogen flux from farm effluents to the lower trophic levels of the pelagic compartment. However, farm effluents still have the potential to impact the pelagic food web through other compounds, like the various forms of phosphorus they release that can heavily alter community dynamics and composition (Baffico & Pedrozo, 1996; Temporetti et al., 2001; Wellman et al., 2017). Aquaculture-origin phosphorus could be assimilated by primary producers, which are then preyed upon by herbivorous zooplankton, therefore constituting a flux independent from those of carbon and nitrogen. In the Canadian Lake 375, the increase in lake productivity following

aquaculture has been proposed to indirectly generate higher growth rates of the wild lake trout, *Salvelinus namaycush* (Walbaum, 1792), through the proliferation of a prey fish (Charles et al., 2017, and references therein). This indirect effect of farm effluents seems to exist also in Alicurá reservoir given that, as in Lake 375 (Wellman et al., 2017), wild salmonids do not prey on farm pellets (Nabaes Jodar et al., 2017; present results), but consume high quantities of *Daphnia* sp. The importance of this cladoceran among preys in Alicurá reservoir was very low at the onset of aquaculture activity *c.* 25 years ago (Macchi et al., 1999), thus posing farm effluents as the probable cause for the growth of *Daphnia* sp. population, since its abundance is highly dependent on low C:P ratios (Balseiro et al., 2007). Techniques for tracing aquaculture phosphorus in different compartments of the environment (Elsbury et al., 2009; Davies et al., 2014) could provide new insights on the impacts of this productive activity worldwide.

Considering that the origin of components in pellets is in many cases foreign to the site where farms are established (Waite et al., 2014), a wide geometry in the isotopic space of the food sources (i.e., wild vs. farm) could often be expected. The wide geometry of the isotopic space formed by wild, escaped, and farmed rainbow trout of Alicurá reservoir allowed us to perform a thorough examination of escapee behavior in the wild. This information, in combination with stomach contents and spatial data, represents an enriching approach for the study of the ecology of aquaculture escapees.

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