Interspecific differences in the bioaccumulation of arsenic of three Patagonian top predator fish: Organ distribution and arsenic speciation

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ABSTRACT

Interspecific differences in arsenic bioaccumulation and organ distribution (muscle, liver, kidney and gills) in three predator fish (creole perch, rainbow trout and brown trout) from a Patagonian lake impacted by volcanic eruptions were studied. Arsenic in fish organs were compared analyzing: 1) temporal (before and after volcanic eruption) and spatial (near and far from the volcano) influence of Puyehue-Cordón Caulle volcanic complex activity on arsenic concentrations; 2) the influence of growth (as total length), organ type and their interactions over arsenic accumulation; and 3) arsenic speciation and total arsenic relationship with carbon to nitrogen ratios (C:N), as a proxy of lipid presence, in fish muscle. In general, total arsenic concentrations in creole perch organs were 2–7 times higher than those recorded in the corresponding organs of salmonids. Arsenic was preferentially accumulated in liver and kidney in the three fish species. The influence of the volcanic activity over arsenic concentrations was more evident in creole perch: organs from creole perch captured closest to the volcano exhibited higher arsenic concentrations. Temporal variations were not so consistent. No clear relationship between arsenic and fish length was observed. Positive and linear relationship between arsenic in all pair of organs was found in creole perch, while rainbow trout showed a quadratic relationship between muscle and the remaining organs, indicating different arsenic assimilation-elimination relationships between organs and fish. The arsenic liver:muscle ratio in the three fish species was greater than 1, suggesting some level of arsenic stress. Arsenobetaine (AB) and dimethylarsinic acid (DMA) were the dominant arsenic species in muscle of these fish, having creole perch 3–4 times higher AB than rainbow trout. A positive relationship between C:N ratio and total arsenic concentrations was found, with higher C:N in creole perchs near the volcano. In terms of food safety, no inorganic arsenic compound were detected, therefore arsenic levels in fish from Lake Nahuel Huapi does not represent any health risk to consumers.

1. Introduction

Arsenic is a ubiquitous and potentially toxic metalloid distributed in the earth crust, soil, sediments, air, natural waters and living organisms (Mandal and Suzuki, 2002; Smedley and Kinniburgh, 2002). Natural processes (e.g., weathering reactions, biological activity, volcanic emissions) as well as anthropogenic activities (e.g., mining activity, arsenical pesticides, combustion of fossil fuels) can mobilize this element (Smedley and Kinniburgh, 2002). However, most negative environmental impacts from arsenic are the result of arsenic mobilization and transformation under natural redox conditions (Mandal and Suzuki, 2002; Nikolaidis et al., 2004; Smedley and Kinniburg, 2002).

Arsenic is an element of concern in many volcanic regions of the world (Bundschuh et al., 2012; Nicolli et al., 2012; Smedley and Kinniburgh, 2002) as it is a recurrent constituent of volcanic gases (Symonds et al., 1992) and tephras (Ruggieri et al., 2012). In eruptive

gases, it can be found predominantly in the form of arsenous acid (As(OH)3) which is the most toxic form (Pokrovski et al., 2002) and it can also be adsorbed onto ash surfaces and transported long distances from the point source where it can reach aquatic systems (Allard et al., 2000; Witham et al., 2005). Acids, metal salts and adsorbed gases on tephra surface are highly soluble, dissolving rapidly on contact with water (Bia et al., 2015; Ruggieri et al., 2012). As a result, arsenic can be released from tephras by desorption or dissolution under favorable conditions (Smedley and Kinniburgh, 2002) or can remain associated with particles and deposited in sediments (Ferguson and Gavis, 1972). Then arsenic enters the aquatic food chain through direct consumption of water or prey, and through the associated sediment particles in the case of benthic prey, and through non-dietary routes such as uptake through absorbing epithelia (Rahman et al., 2012).

In aquatic ecosystems, arsenic can occur in several oxidation states and chemical forms (Bissen and Frimmel, 2003; Phillips, 1990). These chemical forms of arsenic, namely species of arsenic, influence the ultimate biological availability to organisms. Arsenic speciation is important in terms of how arsenic undergoes biogeochemical cycling and metabolism, ultimately determining the toxicity (Bissen and Frimmel, 2003; Le et al., 2004). Arsenic species include both inorganic (e.g., arsenite and arsenate) and organic species (e.g., methylated species, arsenolipids and arsenosugars) the former being more toxic than the latter (Bissen and Frimmel, 2003). Inorganic forms of arsenic are the predominant species in abiotic compartments such as sediment and water, whereas organoarsenic compounds generally predominate in organisms (Rahman et al., 2012).

Arsenic speciation in freshwater organisms has been less frequently reported than in marine organism and there is little consensus on the major arsenic species present (Ciardullo et al., 2010; Schaeffer et al., 2006; Šlejkovec et al., 2004; Soeroes et al., 2005). Several authors have identified arsenobetaine (AB) as the predominant arsenic species in freshwater fish, although its occurrence is variable and appears to be mainly linked to the uptake through the diet (Ciardullo et al., 2010; McIntyre and Linton, 2012; Šlejkovec et al., 2004). However, the more toxic inorganic species may be dominant in fish tissues at locations with high aqueous contamination (Jankong et al., 2007). Therefore, since some arsenic species are more toxic than others, arsenic speciation analyses in edible parts of fish (i.e., muscle) are needed to fully understand the risk posed by their consumption, especially in environments subjected to arsenic inputs.

Lake Nahuel Huapi (41° 03´S, 71° 25´ W), a deep oligotrophic lake in Nahuel Huapi National Park (NHNP), is located near the Southern Volcanic Zone of the Andean Range, an active volcanic region with high historic eruptive frequency and high impact all over the Argentinean Patagonia (Collini et al., 2013; Martin et al., 2009; Naranjo and Stern, 2004; Stern, 2004). This lake has been historically affected by volcanic eruptions with significant amount of tephras deposited in sediments and in the surrounding water catchment (Daga et al., 2010). Approximately 50 km from the western limit of the lake is the Puyehue-Cordón Caulle volcanic complex (PCCVC; 40° 32´ S, 72° 02´ W), which last eruption in June of 2011, covered the entire lake with different grain size volcanic materials (Masciocchi et al., 2013).

Arsenic air pollution associated with the PCCVC eruptions was revealed by increases in arsenic concentrations in epiphytic lichens collected from the NHNP after the eruptive event (Bubach et al., 2012). Particularly in Lake Nahuel Huapi, changes in arsenic concentrations were found for a number of aquatic organisms collected before and after the volcanic eruption, with the highest concentrations and relative changes demonstrated for phytoplankton (Juncos et al., 2016). Regarding fishes, arsenic concentrations measured in muscle and liver of different species from this lake were in the range of concentrations recorded in fish tissues from low to moderate arsenic-polluted systems (Juncos et al., 2016). Since there are no records of relevant As-generating activities (e.g., mining, metallurgical industry) in the region it was hypothesized the PCCVC eruptions as a potential significant source of arsenic to Lake Nahuel Huapi and ultimately to aquatic organisms. Nonetheless, before PCVCC eruption creole perch (*Percichthys trucha*) had higher arsenic levels in muscle and liver than the salmonids rainbow trout (*Oncorhynchus mykiss*) and brow trout (*Salmo trutta*), which was attributed to differences in feeding habits (benthivorous and piscivorous, respectively) (Juncos et al., 2015, 2016).

This means that more than one factor is involved in the arsenic concentrations differences observed between fish species, and not all of them were explored in the previous study. Food web structure, fish species, transport and distribution between tissues, physiological factors such as ingestion rates, growth rates, lipid levels, dilution and excretion, can also be important (Rahman et al., 2012). Even more significant, from the perspective of food safety, is the form in which arsenic is being accumulated, particularly whether the more toxic inorganic arsenic forms are present.

In this context, the objective of present work was to analyze the interspecific differences in arsenic bioaccumulation and organ distribution in three predator fish from a Patagonian lake. To this end, total arsenic concentration in four organs (muscle, liver, kidney and gills) of creole perch, rainbow trout and brown trout from Lake Nahuel Huapi were compared, analyzing: (1) the influence of an arsenic source such as a volcanic eruption in organ arsenic concentrations at two scales: spatial (one site near the PCVCC Complex and one far away) and temporal (with sampling carried out before and after a major volcanic eruption), (2) the variations of arsenic accumulation according to growth (as total length), organ type and the relationship between organs, (3) the different chemical species accumulated in fish muscle, identifying toxic inorganic and non-toxic organic species to evaluate the potential risk to human and wildlife fish consumers, and (4) the relationship of muscle total arsenic with C:N ratios, a proxy of lipid concentrations, to infer the potential incidence of lipid contents on the different arsenic accumulation patterns of fish.

2. Materials and methods

2.1. Study area

Lake Nahuel Huapi (40°55′ S, 71°30′ W; Fig. S1 Supplementary material) is a warm monomictic oligotrophic glacial lake located within NHNP, with a surface area of 557 km² and a maximum depth of 464 m (Díaz et al., 2007). There is a steady flow through the lake from the Andes mountain range stream runoff entering the lake in the northwest towards the River Limay in the southeast. The climate in the Andes mountains is cold temperate and due to the constant west winds, there is a strong west-east climatic gradient across the lake. The total annual precipitation varies from 3000 mm in the westernmost side of NHNP to less than 700 mm on the eastern side. This precipitation gradient shapes plant coverage and distribution, with the western side of the park covered by dense Andean-Patagonian *Nothofagus* forest, while the eastern borders are at the onset of the dry Patagonian steppe characterized by shrub lands (Juncos et al., 2017).

The sampling sites were selected following the West-East predominant wind direction and according to the distance to the PCCVC (Fig. S1). Brazo Rincón (BR) site is the northwestern branch of the lake, closest to the volcano and with a max depth of 100 m, and was the most affected site after the last eruption of the PCCVC, receiving approximately 10–30 cm of coarse ash fall. With a max depth of 236 m, Dina Huapi (DH) site is in the driest region of the lake, farthest away from the PCCVC and near the city of San Carlos de Bariloche. This was less affected site receiving 1.5–5 cm of accumulated ash directly from the volcano (Masciocchi et al., 2013; Fig. S1). However, this site received larger amounts of floating ash carried by the water from the rest of the lake draining through the River Limay.

2.2. Sampling and sample preparation

Creole perch, rainbow trout and brown trout were sampled on May 2011 (autumn before PCCVC eruption) and three years after the PCCVC eruption, on May 2014 at two sites, Brazo Rincón (BR) nearest the PCCVC, and Dina Huapi (DH) furthest from the PCCVC (Fig. S1). Gill nets made of six 10-m long panels of different mesh size, were set at dusk perpendicular to the shore from 2 m down to 40 m deep, and raised early in the morning (Juncos et al., 2016). Total length and weight were measured for all fish, then the liver, kidney, gill filaments and a portion of dorsal muscle were dissected from each fish using titanium and Teflon® devices. All tissues were repeatedly washed with deionized water repeatedly and were stored in acid-washed polyethylene containers at −40 °C, lyophilized and homogenized.

2.3. Analytical procedures

2.3.1. Total arsenic determination

Total arsenic concentrations were determined by Instrumental Neutron Activation Analysis (INAA) following published protocols outlined by Arribére et al. (2010). Aliquots ranging from 50 to 200 mg of lyophilized homogenized sample were sealed in SUPRASIL quartz ampoules and irradiated for 20 h in the RA-6 nuclear research reactor (Centro Atómico Bariloche, Argentina). Gamma-ray spectra were collected using a High Purity Germanium (HPGe) detector, 30% relative efficiency and a 4096-channel analyzer. The concentrations are reported in dry weight (DW) basis. The specific methodology is described in Campoy-Diaz et al. (2018). Arsenic concentrations were determined using the absolute parametric method. Analytical errors were computed as the propagation of the uncertainties associated with the nuclear parameters, the efficiency of the gamma-ray detection system, the neutron flux determinations, and the area of the specific emission considered (sample mass uncertainties were not relevant). The arsenic detection limit (determined from the minimum detectable area for the gamma emission considered), as well as the quantification limit, in INAA strongly depends on the analytical conditions of the analyzed sample, particularly the gamma-ray spectral background generated by other elements contained in the sample. Irradiation and measurement conditions were carefully selected to obtain significant values in all cases. Certified reference materials (CRMs) from the National Research Council of Canada (NRCC) TORT-2 and DORM-2 were analyzed together with tissues samples for analytical quality control; the results of the CRMs analysis match with certified values, considering uncertainties (Table S1).

2.3.2. Arsenic speciation analysis

Arsenic speciation analyses were performed for a subset of the May 2014 fish muscle samples, with 5 randomly selected adult creole perch and rainbow trout samples from each site (total of 20 samples). High performance liquid chromatography coupled to an inductively coupled plasma mass spectrometer (HPLC-ICP-MS); ICP-MS Xseries II, Thermo Scientific, Bremen, Germany and HPLC Spectra System MCS 1000, Thermo Scientific, Bremen, Germany) following the Thermo Scientific application note n° 40741 (Nash and McSheehy, 2005). Freeze-dried fish samples were weighed into disposable polypropylene tubes (about 0.30 g) and 10 mL of a solution at 1% v/v nitric acid and 1% v/v hydrogen peroxide were added to each sample. Vessels were capped, briefly vortexed and left over-night at room temperature. Samples were vortexed briefly before extraction in ultrasonic bath and every 15 min during extraction to ensure the dissolution of the sample. Extraction was performed in an ultrasonic bath at 55 °C for 2 h. Then the samples were centrifuged for 20 min at 4500 rpm and supernatants were filtered through a 0.20 μm mesh filter and diluted 1:1 with ultrapure water before subjected to HPLC-ICP-MS analysis. Chromatographic separation was performed on an ion-exchange column Hamilton PRP-X100, 5 mm, 100 A°, 4.6 × 150 mm, using carbonate and phosphate ammonium salts as mobile phases. The following arsenic species were investigated: DMA (Dimethylarsinic acid), MMA (Monomethylarsonic acid), AB (Arsenobetaine), iAs (inorganic arsenic, sum of arsenite and arsenate), and % of total arsenic was calculated using the INAA values for arsenic.

The limit of quantitation of this method (LOQ) was $0.020 \, \mu g \, g^{-1}$ DW. The extraction efficiency of arsenical was in the range of 68 – 72%.

2.3.3. C:N ratio analysis

In addition to the water-soluble arsenicals, other species such as lipid-soluble arsenic compounds might be present although non-extractable with the used methodology (Arroyo-Abad et al., 2016). In absence of direct lipid measurements, carbon to nitrogen ratios (C:N) were used as predictor of lipid content, because lipids is mostly carbon and very low nitrogen (Barnes et al., 2007). Therefore an increase in total lipid concentration in muscle correlates with increases in C:N ratios (Fagan et al., 2011). In summary, bulk %C and %N values were determined for the muscles of fish sampled in 2011, via DELTAplusXP continuous flow stable isotope ratio mass spectrometer at the Queen's Facility for Isotope Research (QFIR), Queen's University, Kingston, ON, Canada (see Arcagni et al., 2015 for detailed methodology and quality control).

2.4. Statistical analysis

Non-metric multidimensional scaling (NMDS) based on Bray–Curtis similarity matrix was performed on the arsenic concentrations by organ type, fish species, sampling site and year. Differences among groups were tested using a one-way analysis of similarity (ANOSIM), and when Global-R was significant with $\alpha < 0.05$, post-hoc pairwise comparisons were computed. NMDS results were considered to be sufficiently described in two dimensions when the stress value, a measure of the goodness of fit, was < 0.2 (Clarke and Warwick, 2001). Multivariate analysis was performed using PRIMER-E 6 statistical software (Clarke and Warwick, 2001).

To assess differences in total arsenic concentrations between the muscle, liver, kidney and gills within each fish species (tissue-specific differences) and between the concentration of total arsenic in each organ between the three fish species (species-specific differences), one-way analysis of variance (ANOVA) was performed, followed by a Tukey's multiple comparison test. Student's t-tests were used to assess significance in total arsenic concentrations between sampling years and between sampling sites, for each fish species and organ separately. When parametric assumptions were not fulfilled, Kruskal-Wallis one-way ANOVA on ranks (for multiple comparisons) followed by Dunn's t-test, or a Rank Sum test, were performed.

To model significant relationships in arsenic concentrations between organ type for each fish species, simple linear regression analysis using $\log_{10}$-transformed values or polynomial regressions were used, with both sampling sites and years grouped to improve the statistical power. For modelling total arsenic in muscle for each grouped fish species and C:N ratios, simple regression analyses were also used.

To assess significance for muscle DMA and AB concentrations for creole perch and rainbow trout, two-way ANOVA followed by a Tukey's multiple comparison test were used. When arsenic species concentrations in samples were below the detection limit, for the calculations of mean concentrations and associated statistical analyses, a value of one-half the detection limit was used.

Data were analyzed using SPSS software version 15.0, with a 95% level of confidence ($\alpha = 0.05$).

3. Results

3.1. Total arsenic in fish tissues

3.1.1. General patterns

The mean concentration of total arsenic for all fish species ranged

Table 1
Mean ± standard deviation (range) total arsenic concentration in the muscle, liver, kidney and gills of three fish species caught in Lake Nahuel Huapi in May 2011 and May 2014, at two sites: Brazo Rincón (BR) and Dina Huapi (DH). The sample size (N) and total length and weights of fish are also indicated.

Species	Year	Site	N	Total length (mm)	Weight (g)	As muscle ($\mu g\,g^{-1}$)	As liver ($\mu g\,g^{-1}$)	As kidney ($\mu g\,g^{-1}$)	As gills ($\mu g\,g^{-1}$)
Creole perch (*Percichthys trucha*)	2011	BR	11	476 ± 24 (429–511)	1553 ± 208 (1158–1900)	1.16 ± 0.33 (0.72–1.79)	3.56 ± 0.88 (2.59–5.88)	3.24 ± 0.59 (2.28–4.11)	1.75 ± 0.45 (0.88–2.68)
		DH	5	357 ± 120 (180–450)	761 ± 560 (69–1402)	0.43 ± 0.23 (0.25–0.84)	2.31 ± 1.42 (0.83–4.10)	1.92 ± 0.69 (1.44–3.07)	1.26 ± 0.40 (0.93–1.73)
	2014	BR	5	438 ± 24 (410–470)	1230 ± 310 (805–1555)	0.81 ± 0.24 (0.44–1.07)	3.43 ± 1.45 (1.13–4.92)	4.31 ± 2.07 (1.98–7.42)	1.43 ± 0.50 (0.70–2.02)
		DH	6	422 ± 43 (360–470)	1049 ± 272 (701–1265)	0.43 ± 0.18 (0.13–0.65)	1.75 ± 1.00 (0.31–3.29)	1.88 ± 0.88 (0.67–3.00)	1.27 ± 0.37 (0.92–1.95)
Rainbow trout (*Oncorhynchus mykiss*)	2011	BR	5	577 ± 50 (500–620)	2376 ± 475 (1701–2780)	0.52 ± 0.27 (0.18–0.91)	0.52 ± 0.10 (0.38–0.62)	0.45 ± 0.21 (0.21–0.66)	0.28 ± 0.10 (0.15–0.40)
		DH	6	402 ± 121 (256–553)	892 ± 817 (161–2180)	0.21 ± 0.15 (0.1–0.41)	0.41 ± 0.13 (0.3–0.6)	0.66 ± 0.13 (0.44–0.79)	0.29 ± 0.18 (0.12–0.60)
	2014	BR	6	326 ± 92 (260–510)	515 ± 526 (233–1585)	0.38 ± 0.10 (0.22–0.5)	0.82 ± 0.38 (0.50–1.51)	1.07 ± 0.34 (0.73–1.67)	0.52 ± 0.20 (0.30–0.75)
		DH	5	306 ± 61 (240–395)	406 ± 315 (136–935)	0.39 ± 0.16 (0.26–0.61)	0.74 ± 0.35 (0.36–1.13)	0.76 ± 0.14 (0.59–0.98)	0.60 ± 0.11 (0.40–0.68)
Brown trout (*Salmo trutta*)	2011	BR	11	600 ± 75 (468–713)	2980 ± 1010 (1544–4600)	0.71 ± 0.25 (0.3–1.3)	0.34 ± 0.21 (0.14–0.9)	0.58 ± 0.30 (0.13–1.23)	0.41 ± 0.21 (0.03–0.78)
		DH	1	548	2180	0.78	0.5	1.08	0.24
	2014	BR	3	523 ± 38 (480–550)	1950 ± 490 (1495–2470)	0.30 ± 0.15 (0.13–0.42)	0.36 ± 0.01 (0.28–0.44)	0.56 ± 0.22 (0.34–0.79)	0.73 ± 0.55 (0.19–1.29)
		DH	2	(270–410)	(275–864)	(0.46–0.63)	(0.5–0.89)	(1.12–1.57)	(0.47–0.68)

from 0.21 to 1.16 $\mu g\,g^{-1}$ in muscle, 0.34–3.56 $\mu g\,g^{-1}$ in liver, 0.45–4.31 $\mu g\,g^{-1}$ in kidney, and 0.28–1.75 $\mu g\,g^{-1}$ in gills (Table 1). The NMDS analysis using all data allowed to visualize general patterns of arsenic distribution among fish according to organ accumulation, sampling sites and year (Fig. 1). Creole perch could be separated from both salmonid species (i.e., rainbow trout and brown trout) (ANOSIM; Global-R = 0.77, $p < 0.01$; Fig. 1), because of the elevated arsenic concentrations in all organs compared to salmonid group (Fig. 1). Mean arsenic concentrations for the native creole perch muscles ranged from 0.4 to 1.16 $\mu g\,g^{-1}$ and were significantly higher than arsenic concentrations in muscle of salmonids (mean concentrations ranged from 0.5 to 0.7 $\mu g\,g^{-1}$). Concentrations as high as 3.56 and 4.31 $\mu g\,g^{-1}$ in liver and kidney, respectively, were recorded in creole perch, while the highest concentrations recorded for these organs in salmonids were 0.82 and 1.07 $\mu g\,g^{-1}$, respectively. Each group could be further subdivided into two sub-groups, resulting in four significant groups (ANOSIM; Global-R = 0.64, $p < 0.001$). The group of creole perch was segregated according to sampling site in the horizontal dimension, into BR creole perch (90% of similarity) and DH creole perch (82% of similarity) sub-groups, with the DH creole perch having lower concentrations of arsenic in all organs than the BR creole perch. As for the salmonids group, the 2011 brown trout from both sites formed a separate sub-group (78% of similarity) due to the higher arsenic concentrations in muscle. The second salmonid sub-group included all rainbow trout from both sites and years and brown trout sampled in 2014 (78% of similarity; Fig. 1). The NMDS multivariate approach allowed to identify inter-specific differences in arsenic bioaccumulation and temporal and spatial variations in that bioaccumulation, which will be outlined below.

3.1.2. Temporal and spatial variations

The influence of the PCCVC was evaluated at two scales: temporal (between years) and spatial (between sites). Arsenic concentrations between sampling years (2011 vs 2014, three years after the volcanic eruption) was first investigated in the muscle, because muscle has less metabolic activity than the other organs in this study, and therefore could better reflect long-term effects, if any. At first glance, there seems to be a decrease in arsenic concentrations in muscle of the three fish species, three years after the volcanic eruption (i.e., 2014), in the site closer to the volcano, BR, but no in DH (Table 1; Fig. 2). However, the decrease was only significant for BR brown trout ($p = 0.02$). With regard to the other organs, significant temporal differences were observed only for rainbow trout kidney and gills from BR and gills from DH, whose arsenic concentrations were higher in 2014 than in 2011 ($p < 0.05$).

The effect of site in total arsenic concentrations was especially evident in creole perch organs at both sampling years. Muscle and kidney from 2011 creole perch, and muscle, liver and kidney from 2014 creole perch all showed higher arsenic concentrations in BR (closer to the volcano) than those from DH ($p < 0.001$). Only kidney from 2011 rainbow trout plus gills tissues from 2014 rainbow trout differed significantly between sampling sites, with arsenic concentrations being lower in BR than in DH ($p = 0.007$ and 0.01, respectively). The low numbers of brown trout caught in DH at both sampling dates ($N_{2011} = 1$; $N_{2014} = 2$) did not allow the inter-site comparison between organs for this species (Fig. 2).

3.1.3. Organ variations within- and between fish species

Regarding inter-organ differences within each fish species, arsenic was preferentially accumulated in liver and kidney, with concentrations decreasing in the following order: kidney ≥ liver > gill > muscle. In rainbow trout sampled in 2011 in DH and in BR in 2014, total arsenic concentrations in kidney were significantly higher than in muscle and gills ($0.001 < p < 0.006$), and liver arsenic concentrations of rainbow trout from 2014-BR were higher than in muscle ($p = 0.033$). Creole perch from both sites and sampling years, had arsenic concentrations
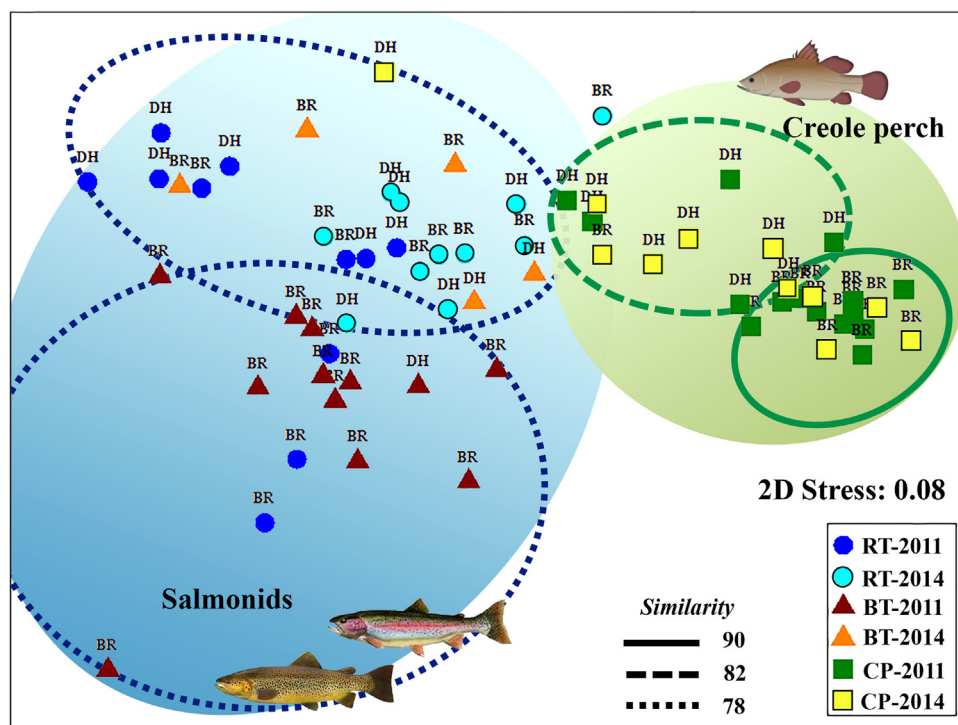
Fig. 1. Non-metric multidimensional scaling (NMDS) of the Bray–Curtis distances computed on arsenic concentrations determined for muscle, liver, kidney and gills of rainbow trout (RT), brown trout (BT) and creole perch (CP) sampled in Brazo Rincón (BR) and Dina Huapi (DH) from Lake Nahuel Huapi in 2011 and 2014. Shadowed areas are shown: the smaller green circle on the right and the larger blue circle on the left respectively enclose creole perch (native fish) and salmonids (introduced fish). The percent of within group similarity is indicated by solid (90% similarity) to dashed (78% similarity) circles.
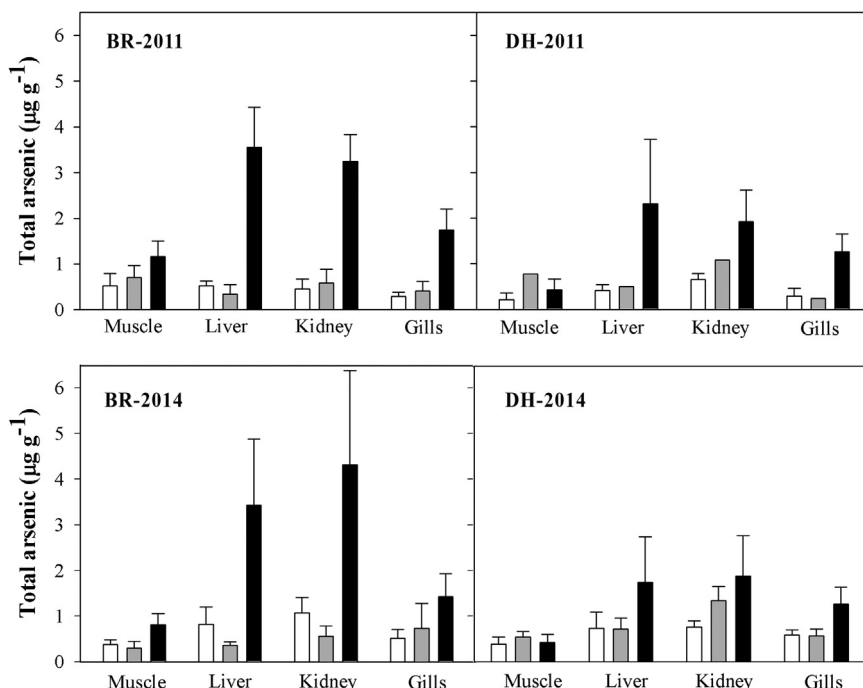

Fig. 2. Total arsenic concentrations (mean and standard deviation) in tissues of rainbow trout (white bars), brown trout (grey bars) and creole perch (black bars) sampled in 2011 and 2014 in Brazo Rincón (BR) and Dina Huapi (DH).

significantly higher in kidney and liver than in gills and muscle ($p < 0.01$) (Fig. 2). The exception was the 2011 BR brown trout (N = 1) with total arsenic concentrations being significantly higher in muscle than in liver and gills ($p = 0.003$).

Total arsenic concentrations accumulated differently between fish species (Fig. 2). In all organs of creole perch from BR from both years, concentrations were significantly higher than in the organs of rainbow and brown trout ($p < 0.005$). In DH 2011, where total arsenic concentrations in muscle of creole perch were significantly lower than in BR, no differences with muscles of salmonids were found ($p = 0.17$).

However, liver, kidney and gill had significantly higher total arsenic concentrations than the same salmonid organs ($p < 0.005$). In 2014-DH samples, only arsenic in gills were significantly higher in perch than in rainbow trout ($p = 0.004$). Arsenic in liver and kidney was higher in creole perch than in rainbow trout, although not statistically significant with p-values close to the limit of significance (0.06 and 0.05, respectively). No differences in arsenic concentrations were found between rainbow and brown trout organs in BR neither in 2011 nor in 2014 samples.

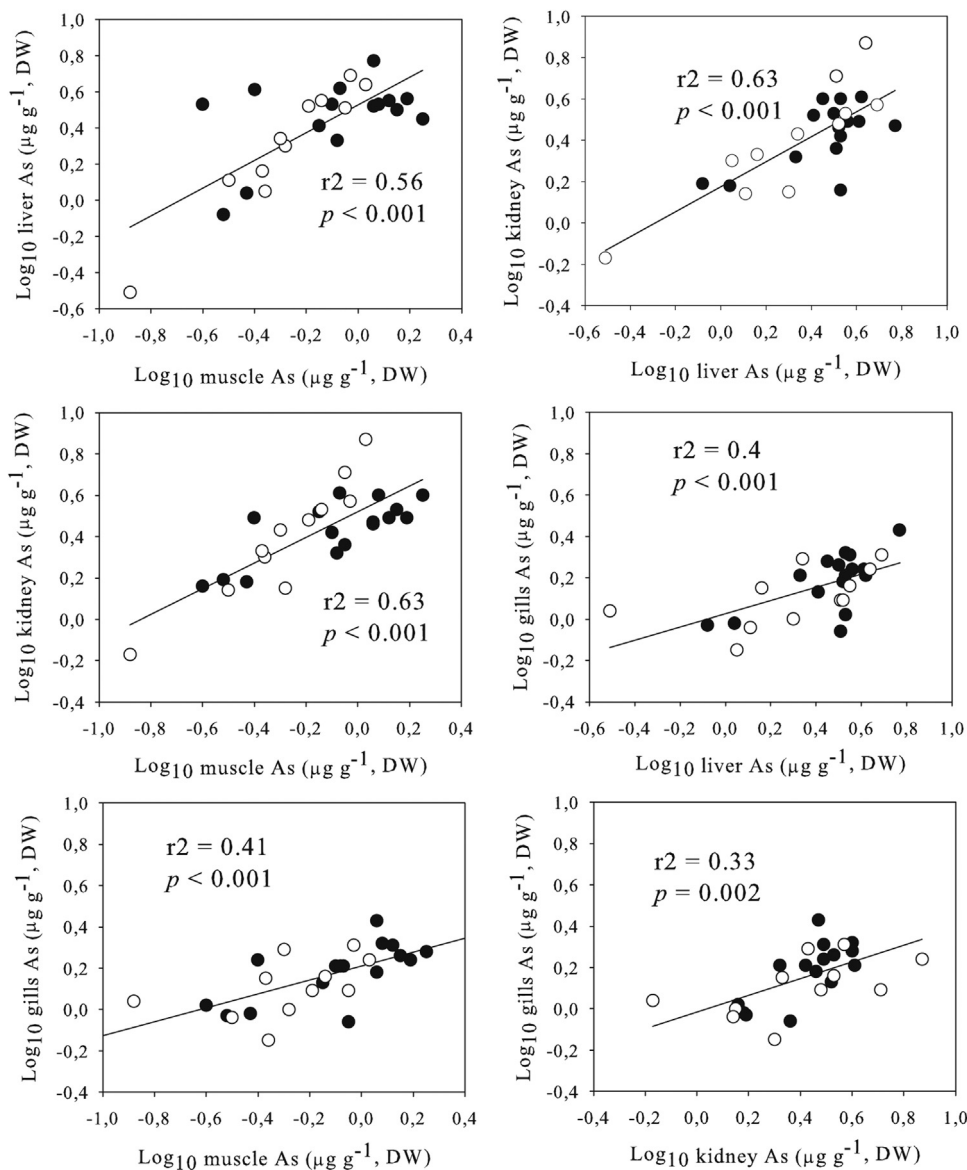The range of sizes obtained was limited and did not allow to

Fig. 3. Relationships between Log10-arsenic concentrations in various tissues for creole perch sampled from both sites in Lake Nahuel Huapi in 2011 (black circles) and 2014 (white circles). Regression lines and model parameters are shown were relationships were significant.

properly establish arsenic-length relationships in the analyzed organs at each sampling site and year. However, some trends became evident when the sampling sites were analyzed together by year. Positive significant relationships between arsenic organ concentrations and fish length were observed only for 2011 rainbow trout muscle ($R^2 = 0.82$; $p < 0.001$) and liver ($R^2 = 0.67$, $p = 0.002$), and 2011 creole perch muscle ($R^2 = 0.53$; $p < 0.001$). However, the case of creole perch might be an artifact caused by the inter-site differences previously indicated: DH individuals accumulated lower arsenic and have a wider size-range than perch from BR, which were represented by larger individuals with higher arsenic concentrations.

3.1.4. Inter-organ relationships

Inter-organ relationships for the concentrations of total arsenic showed different patterns between rainbow trout and creole perch (Figs. 3 and 4). Linear regression of log_{10}-transformed arsenic concentrations in all pair of organs combinations showed that there were positive significant relationships between all organs for creole perch (Fig. 3) indicating that muscle samples would be good indicator of body arsenic burden for this species. In rainbow trout, the relationship

between Log_{10}-arsenic concentrations of muscle-liver, muscle-kidney, and muscle-gills better adjusted to a quadratic regression, showing joint increases in the arsenic concentrations in the organs and muscle, until reaching a value of arsenic in muscle (around $0.3 \, \mu g \, g^{-1}$), from which, the concentrations in the other organs begin to decrease while they increase in the muscle (Fig. 4). No significant relationship between organs was found for brown trout, but low numbers of samples may be a challenge for this model approach.

The relative arsenic content of the liver, kidney and gills relative to muscle (organ:muscle ratio) is presented in Table S2. Ratios of arsenic concentrations in liver, kidney and gills to that in muscle were in most cases greater than 1, meaning that the muscle usually had a lower concentration of arsenic than the other organs. As was previously noted, the exception to this were the brown trout sampled in 2011, which had organ:muscle ratios less than 1, due to the greater accumulation of arsenic in the muscles regarding to other organs. Ratios of arsenic concentrations in liver, kidney and gills to that in muscle for creole perch were more than 1.5 times higher than that of salmonids (Table S2).
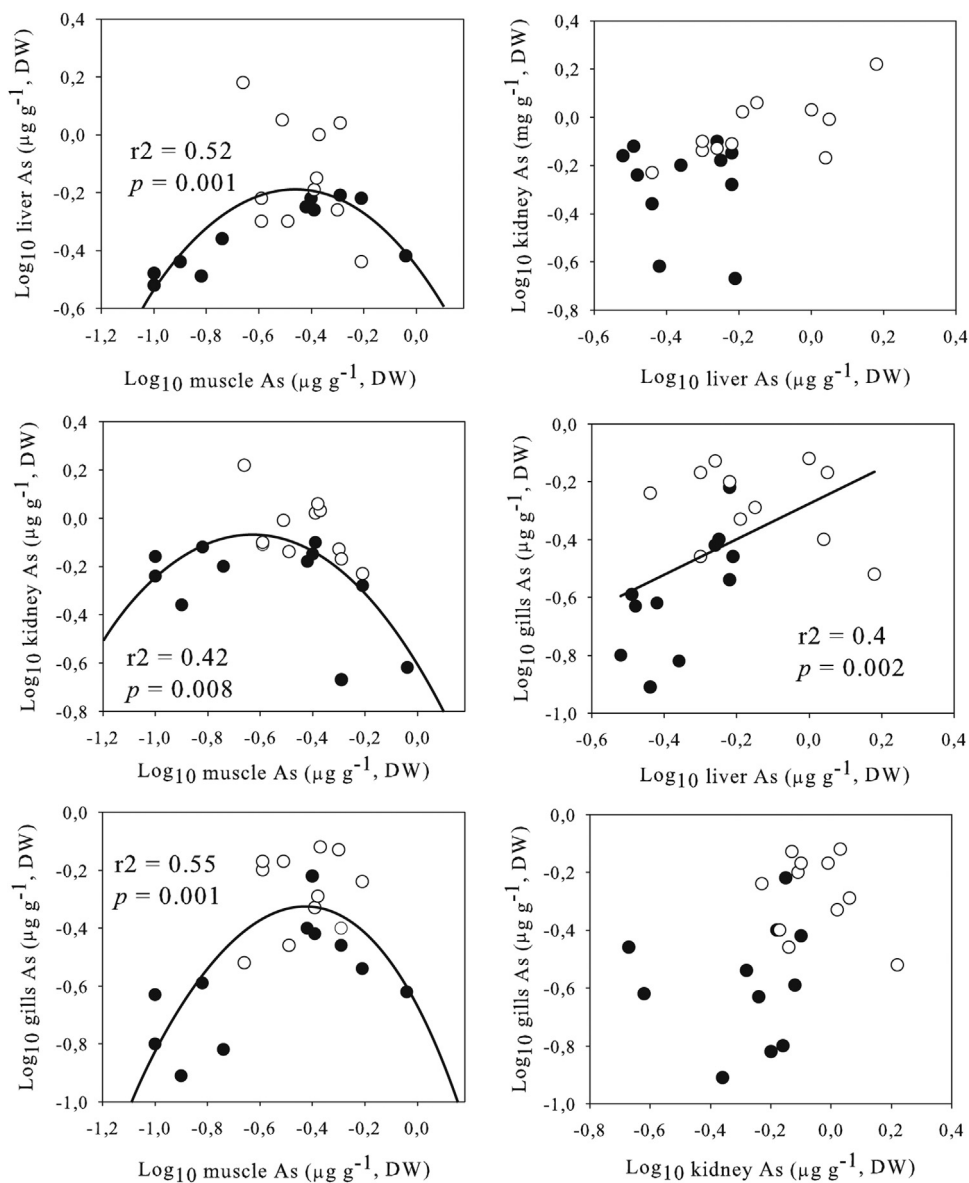
Fig. 4. Relationships between Log10-arsenic concentrations in various tissues for rainbow trout sampled from both sites in Lake Nahuel Huapi in 2011 (black circles) and 2014 (white circles. Regression lines and model parameters are shown were relationships were significant.

3.2. Interspecific chemical differences in muscle arsenic accumulation

3.2.1. Relationship between muscle arsenic concentrations and C:N

C:N ratios can be useful indicators of lipid content (Fagan et al., 2011). When examining the relationship between total arsenic concentrations and C:N ratios in muscle of the three fish species, a significant and positive relationship was found ($R^2 = 0.46$; $p < 0.001$), having creole perch from BR the highest C:N ratios and total arsenic concentrations, rainbow trout from DH the lowest C:N and arsenic concentrations, and brown trout intermediate values (Fig. 5). Mean C:N ratios of creole perch from BR (5.2) were significantly higher than ratios of creole perch from DH (3.7) and rainbow trout from DH (3.5).

3.2.2. Arsenic speciation in fish muscle

The mean muscle concentrations of soluble arsenic species detected in rainbow trout and creole perch are presented in Table 2. Only organic arsenicals were above the detection limit. The major arsenic compounds present in muscle of the two fish species analyzed were arsenobetaine (AB), representing between 18% and 42% of the total arsenic in muscle, followed by dimethylarsinic acid (DMA),
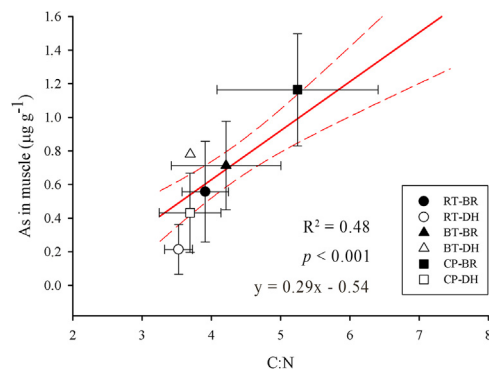

Fig. 5. Relationship between arsenic concentrations (mean ± standard deviation) and C:N ratios (mean ± standard deviation) for muscle tissue from all creole perch (CP), rainbow trout (RT) and brown trout (BT) sampled in 2011 in Brazo Rincón (BR) and Dina Huapi (DH) in Lake Nahuel Huapi (Fig. 1). Regression line with 95% C.I. and model parameters are included.

Table 2

Concentrations (mean ± standard deviation) of total arsenic, arsenobetaine (AB), dimethylarsenic acid (DMA), monomethylarsonic acid (MMA), and inorganic arsenicals (iAs) in the muscle of creole perch and rainbow trout caught in Brazo Rincón (BR) and Dina Huapi (DH) of Lake Nahuel Huapi in May 2014. Percent of total arsenic for the arsenic species recorded is also showed. Values below the limit of quantification (0.020 μg g^{-1}) are indicated as < LOQ.

Species	Site	N	Total arsenic (μg g^{-1})	Arsenic species (μg g^{-1})				Percent of total arsenic (%)	
				AB	DMA	MMA	iAs	AB	DMA
Creole perch (*Percichthys trucha*)	BR	5	0.81 ± 0.24	0.20 ± 0.18	0.05 ± 0.02	< LOQ	< LOQ	25	6
	DH	5	0.66 ± 0.35	0.28 ± 0.11	0.03 ± 0.02	< LOQ	< LOQ	42	4
Rainbow trout (*Oncorhynchus mykiss*)	BR	5	0.33 ± 0.09	0.06 ± 0.01	0.02 ± 0.01	< LOQ	< LOQ	18	6
	DH	5	0.43 ± 0.19	0.08 ± 0.03	0.04 ± 0.02	< LOQ	< LOQ	19	9

representing between 4% and 9% of the total arsenic measured. Monomethylarsonic acid (MMA) and inorganic arsenic were undetected (< LOQ). The concentrations of AB in the muscle of the native creole perch were highly variable, ranging from 0.06 to 0.3 μg g^{-1}, and were higher than in rainbow trout (Table 2). Significant differences ($p < 0.05$) in mean AB concentrations were found between creole perch and rainbow trout at both sampling sites. On the other hand, DMA concentrations were less variable and no statistically significant differences between samples were found. Despite the higher concentrations of both arsenic species in creole perch, no significant differences were found between fish in the proportions of individual arsenic species (AB, DMA) relative to total arsenic in muscles.

4. Discussion

4.1. Interspecific spatial and temporal variations in arsenic tissue concentrations

The accumulation of arsenic in the tissues of the fish from Lake Nahuel Huapi varied between fish species and between tissues within species. In turn, these variations in some cases were influenced by the location with respect to the volcano and to a lesser extent, by the year in which the fish were caught.

In general, the native benthic creole perch had higher total arsenic concentrations than salmonids in most analyzed organs at both sampling sites and years. In most cases, total arsenic concentrations in creole perch organs were 2–7 times higher than those recorded in the corresponding organs of salmonids. The range of arsenic concentrations recorded in rainbow trout and brown trout organs from Lake Nahuel Huapi were similar to that generally reported in the literature for salmonids and other fish from non-polluted to moderately- polluted freshwater bodies (Bordajandi et al., 2003; Burger et al., 2002; Culioli et al., 2009; Dsikowitzky et al., 2013; Has-Schön et al., 2008; Rosemond et al., 2008; Table S3). For example, in a low contaminated section of the Presa river (water arsenic concentration: 43 μg L^{-1}), arsenic concentrations in organs of *Salmo trutta* ranged from 0.13 to 0.39 μg g^{-1} DW, while in a moderately contaminated section (water arsenic concentration: 108 μg L^{-1}) arsenic ranged from 0.49 to 1.20 μg g^{-1} DW (Culioli et al., 2009; Table S3). These values are in line with the values recorded for brown trout in this study. Meanwhile, the highest arsenic concentrations recorded in organs of creole perch caught closest to the volcano (Brazo Rincón) were comparable to- or even higher than the levels found in other Perciformes species living in rivers and lakes strongly affected by anthropogenic contamination (e.g., abandoned mine, domestic and industrial discharges) (Dsikowitzky et al., 2013; Kirby and Maher, 2002; Rosemond et al., 2008; Zheng and Hintelman, 2004; Table S3). However, total arsenic concentrations in all organs from creole perch sampled at the furthest site of the volcano (DH) were between 20% and 60% lower than in perch from BR, with muscle having arsenic concentrations in the levels of salmonid muscle, and closer to levels of low-polluted environments. These might be indicating that the activity of the PCCVC impacts the arsenic concentrations in creole perch from this lake. In this context, it could be hypothesized

that BR creole perch is somehow more exposed to arsenic than are creole perch from DH since all analyzed organs accumulated higher arsenic in BR. Although DH perch organs concentrations were lower than in BR; liver and kidney had concentrations that are 3–5 times higher than in muscle, a difference that is similar to that between the same tissues in BR.

Bioaccumulation of metals and metalloids in organisms is complex since it is influenced by several factors, such as metal-specific chemistry and abundance (i.e., environmental concentration), geochemical influences on bioavailability, exposure route (e.g., diet and solution), and species-specific physiological attributes (Luoma and Rainbow, 2005). As a consequence, variable patterns of tissue distribution occur among species. Differences in total arsenic concentrations in salmonid muscles compared to that of creole perch have been previously reported in Lake Nahuel Huapi, and these differences were attributed to differences in feeding habits of these species (i.e., the exposure routes) and their location with respect to a volcanic source (i.e., environmental concentrations and geochemical influences) (Juncos et al., 2016).

Arsenic has a considerable tendency to accumulate in bottom sediments (Smedley and Kinniburgh, 2002), consequently, most of the arsenic in lakes without continuous inputs of soluble arsenic (as could be the case of mine tailings) is accumulated in bottom sediments while concentrations in waters are usually very low (Rahman and Hasegawa, 2012). For instance, in Lake Nahuel Huapi, deep bottom sediment concentrations varies regarding the distance to the PCCVC: closest to the volcano concentrations range from 11 to 73 μg g^{-1} (BR), and from 5 to 7 μg g^{-1} farthest the volcano (near DH) (Juncos et al., 2016). In water column, arsenic concentrations were only detected in BR, three month after the PCCVC eruption (below the LOQ 0.5 μg L^{-1}); and was undetected from six months onwards after the eruption (Pérez Catán et al., 2016). On the other hand, in the sediment-water interface arsenic concentrations increased from undetectable to detectable levels (3.0 ± 0.2 μg L^{-1}) six month after the PCCVC eruption in samples close to the volcano whereas the increase was smaller (1.2 ± 0.12 μg L^{-1}) at the furthest site (Pérez Catán et al., 2016). This is consistent with the increases in arsenic concentrations reported by Juncos et al. (2016) in zooplankton and the small puyen *Galaxias maculatus*, a few month after the PCCVC eruption, increases that were not reflected in predatory fish concentrations. In other words, the effect of the volcanic eruption on arsenic concentrations is very short in time and limited to water and plankton associated organisms. Three years after the eruptive event (i.e., 2014 samples), the effect is less visible, and no clear pattern was found in arsenic concentrations in organs of the analyzed fish, regarding 2011 eruption. However, volcanic influence on arsenic levels of fish from Lake Nahuel Huapi was evidenced at the spatial scale (regarding the distance to the source). It is assumed that the greater contribution of arsenic to the biota of this lake come mainly from the sediments (reservoirs of arsenic) through the organisms living in close association with them, which accumulate higher arsenic in their tissues than pelagic organisms (Juncos et al., 2016). Creole perch is a benthivorous fish in Patagoninan lakes, feeding mainly on the benthic crayfish *Samastacus* sp.; while the exotic salmonid species, rainbow and brown trout, are mainly piscivores, preying heavily

on the forage bentho-pelagic fish, small puyen (Juncos et al., 2013, 2015). Almost 50% more arsenic have been reported in crayfish muscle than in small puyen (Juncos et al., 2016). This partially explains the observed perch-salmonids differences in organ arsenic concentrations. Other studies have also shown differences in arsenic body burdens related to feeding habits. For example, arsenic concentrations in muscle of rainbow trout from a Patagonian fish farming feeding commercial food, were found higher ($1.5 - 3.2\,\mu g\,g^{-1}$ DW) than similar sized wild rainbow trout analyzed in this study, and that was consistent with the higher arsenic concentration present in the commercial food provided (Bubach et al., 2018). Moreover, in other freshwater systems higher body levels of total arsenic were found in bottom feeding fish compared to forage fish, while predatory fish presented the lowest body levels overall (Chen and Folt, 2000; Kirby and Maher, 2002; Suhendrayatna and Maeda, 2001).

Differences in bioaccumulation between introduced salmonids and the native creole perch in Lake Nahuel Huapi have also been observed for other metals measured in muscle and livers; such as Hg (Arcagni et al., 2017), Ag (Juncos et al., 2017), and Zn (Montañez et al., 2018). Although these differences have generally been attributed to differences in diet, the consistent patterns in differential trace element concentrations between salmonids and creole perch may also be indicating some taxonomy-associated pattern that could be the outcome of specie-specific metabolic processes. This has been observed for representatives of the salmonid family whose similarities in arsenic speciation clearly separated them from other fish families (Ciardullo et al., 2008, 2010; Shiomi et al., 1995; Šlejkovec et al., 2004).

4.2. Intraspecific organ distribution of arsenic and inter-organ relationship

Net bioaccumulation is the result of a balance among uptake rate from diet, uptake rate of dissolved forms, and loss rates (Luoma and Rainbow, 2005). However, whatever the route of exposure and income (dietary or waterborne exposure), ultimately the level of a given element in fish will be determined by the balance between uptake-elimination processes, and growth (Reinfelder et al., 1998). As a result of metal absorption, regulation, storage, biotransformation and excretion mechanisms, the distribution of each trace element inside the fish into different organs and tissues is different (Ciardullo et al., 2008). Therefore, arsenic distribution between the different tissues reflects not only the mode of exposure, i.e., dietary and/or aqueous exposure, but also the role of each tissue in metal processing.

In this study, arsenic was more evenly distributed between the organs of salmonids compared with creole perch, with small differences (when they existed) in arsenic concentrations between organs (e.g., slightly higher levels of arsenic were found in liver and kidney). In creole perch, arsenic levels were not evenly distributed between the different organs, showing clear and outstanding differences between the arsenic concentrations in muscle and the remaining organs, especially liver and kidney, being the pattern of arsenic accumulation liver = kidney > gills > muscle. This is consistent with most laboratory and field studies that indicated that arsenic tend to be accumulated into metabolic organs, such as liver and kidney (Jankong et al., 2007; Mason et al., 2000; Pedlar et al., 2002; Takatsu et al., 1999), contrary to muscle, that many studies showed is not a major repository for arsenic (Pedlar et al., 2002; Pedlar and Klaverkamp, 2002; Sorensen, 1991).

The liver is considered the most important organ responsible for detoxification and storage of toxic materials (Omar et al., 2013; Wood, 2012) and therefore is involved in the possible biotransformation of arsenic (e.g., transformation of inorganic arsenic into organoarsenicals prior to excretion; Sorensen et al., 1991); while kidney is considered to be important in elimination processes (Ciardullo et al., 2008; Wood, 2012). Both organs, play a major role in detoxification and excretion of metals through the induction of metal-binding proteins such as metallothioneins (McIntyre and Linton, 2012). On the other hand, gills are the dominant route of uptake for most waterborne metals and

metalloids, because of their large surface area, thin water-to-blood diffusion distance, and abundance of active transport pumps for nutrient ions from the external water (Wood, 2012). For this reason, gills are usually good indicators of the concentration of elements in the water where the fish lives. Nevertheless, arsenic uptake from water through the gills is usually negligible under low levels of contamination (McIntyre and Linton, 2012), as is the case of Lake Nahuel Huapi waters.

Gills from fish analyzed in Lake Nahuel Huapi were the organ that showed the least variation in arsenic levels between sampling sites and years within the same fish species. The low levels of arsenic that characterize the waters of this lake are probably not enough to induce modifications in arsenic concentrations in this organ. However, interspecific variations in gill arsenic concentrations were observed, creole perch having arsenic concentrations 4 times higher than salmonids, suggesting that gills in this species could be also a storage/elimination site for arsenic. Arsenic excretion through the gills has been confirmed in experimental studies for other fish (Oladimeji et al., 1984). This might explain the linear relationship between arsenic concentrations in all organs regarding gills in creole perch. The significant linear regressions of arsenic concentrations (as Log_{10} transformed values) between all pair of organs indicate that co-accumulation of arsenic is occurring in their organs, suggesting that arsenic is transported by blood to all organ's tissues in a form suitable for accumulation (Maher et al., 1999).

The relationship of arsenic accumulation between organs in rainbow trout was different. From a certain value of arsenic in the muscle (around $0.3\,\mu g\,g^{-1}$), the arsenic in the liver, kidney and gills, which was increasing together with the muscle, stabilized or even tends to decrease as arsenic muscle concentrations increase. Considering that arsenic accumulation in tissues is a function of the uptake and clearance rates of individual organs, and that not all tissues receive the same blood flow and in consequence, supply of arsenic to tissues will be different (Luoma and Rainbow, 2005; Reinfelder et al., 1998); therefore it is likely that after a certain level of accumulation in the liver, kidney and gills of rainbow trout, these organs begin to process and eliminate some form of arsenic at a higher rate than in creole perch. So, different arsenic regulation mechanisms are likely involved between both species.

The differences in the uptake and elimination rates also determine the different organ:muscle arsenic ratios found between species (here "organ" = all the organs examined except muscle). In this sense, it was observed that creole perch has higher organ:muscle ratios than salmonids, especially liver and kidney to muscle ratios. As was previously mentioned, higher concentrations of metals in liver and kidney compared to muscle have been attributed to the greater presence of metal-binding proteins (e.g., metallothioneins) in the tissues forming that organs (McIntyre and Linton, 2012). As was observed for other metals (e.g., copper and zinc; Miller et al., 1993), variations in the amount of metallothioneins could cause the observed differences between fish arsenic accumulation in this lake. Furthermore, inter-specific differences in arsenic bioaccumulation can be due to different arsenic absorption efficiencies of tissues. For example, Pedlar and Klaverkamp (2002) found that arsenic accumulation between two fish species differed after a 20-day experiment with dietary arsenic, due to differences in arsenic absorption through gastrointestinal tract, which is the tissue were arsenic is readily accumulated and from where it is distributed to organs and tissues via the circulatory system (Pedlar et al., 2002).

Based on the known detoxification function of the liver, and given that the muscle can be considered an isolated compartment regarding arsenic uptake, the arsenic liver:muscle ratio has been used as an indicator of the level of arsenic stress under which an organism is subjected (Azcue and Dixon, 1994). Higher concentrations in the muscle relative to the liver, indicated by values less than 1, suggest low excretory activity, whereas higher liver concentrations, indicated by values greater than 1 suggest enhanced excretory activity (Azcue and

Dixon, 1994). Liver:muscle arsenic ratios in Lake Nahuel Huapi fish were mostly greater than 1, suggesting an enhanced excretory activity that might be indicative of some level of arsenic stress.

4.3. Arsenic speciation and C:N in muscle

Speciation is also important in determining arsenic bioaccumulation because it influences the uptake and elimination of a trace element (Bissen and Frimmel, 2003; Phillips, 1990). The major soluble arsenic species present in muscle of rainbow trout and creole perch from Lake Nahuel Huapi were AB followed in a lower proportion by DMA, while MMA and inorganic arsenic were below the limit of quantification (LOQ < 0.02 $\mu g\,g^{-1}$ DW). Arsenobetaine was 3–4 times higher in muscle of creole perch than in rainbow trout, while DMA presented similar values between both fish. Therefore the difference in total arsenic bioaccumulation in muscle between creole perch and salmonids might be determined by AB presence. Arsenobetaine is the predominant species of arsenic found in marine animals (Amlund and Berntseen, 2004; Schaeffer et al., 2006), but its dominance is much less consistent in freshwater fish, having been registered as the dominant arsenical in some freshwater fish species (Shiomi et al., 1995; Šlejkovec et al., 2004) and as very scarce in others (Shiomi et al., 1995; Soeroes et al., 2005). This is partially explained by the higher percentage of AB usually found in the food of marine fish compared to freshwater fish (McIntyre and Linton, 2012). For example, Kirby and Maher (2002) suggested that arsenic compounds presented in marine fish tissues may vary according to different diets. In freshwater fish, although the occurrence of AB is variable, it also appears to be primarily linked to its uptake via the diet (McIntyre and Linton, 2012). Absorption of AB from feed and efficient transfer and disposition of the unchanged compound from blood into muscle tissue is a common pattern in teleosts (Ciardullo et al., 2008). Moreover, AB is usually more easily assimilated than inorganic arsenic along the food chain (Zhang et al., 2016, 2017). Francesconi and Edmonds (1989) demonstrated that yelloweye mullet *Aldrichetta forsteri* fed with different arsenic compounds had low retention of arsenate in their muscle, while fish feed with AB had elevated levels of arsenic in their muscles. Similar results were obtained by Zhang et al. (2017) who showed that the goby fish *Mugilogobius chulae* feed on a diet richer in AB assimilated more AB than when feed on a diet with higher proportions of inorganic arsenic. Given the different feeding habits of creole perch and rainbow trout, a difference in arsenical compounds proportions in their main prey could offer a partial explanation to the higher AB concentration found in creole perch compared with rainbow trout. One hypothesis to be tested would be that crayfish have higher AB concentrations than the small puyen.

It is worth noting that the extraction yields of the water-soluble arsenicals from the muscle samples analyzed in this study were relatively low, between 27% and 30% in rainbow and 39–67% in perch, but comparable to the extraction efficiencies reported in other works (Jankong et al., 2007; Schaeffer et al., 2006; Soeroes et al., 2005). In freshwater fish recoveries reported for arsenic species are often low compared with high recoveries reported in marine organisms (usually greater than 70%) (Miyashita et al., 2009; Schaeffer et al., 2006; Shiomi et al., 1995). Arsenobetaine is a polar molecule readily soluble in both methanol and water, the most common solvents used for extraction in arsenic speciation analyses. For this reason, when AB is the dominant arsenical in the sample, as is the case in most marine animal samples, high extraction yields are obtained (Schaeffer et al., 2006; Soeroes et al., 2005). On the contrary, in freshwater organisms, AB concentrations are usually low or absent, and the presence of other non-extractable arsenicals became significant (Schaeffer et al., 2006). Therefore, non-extractable arsenic species could be present in Lake Nahuel Huapi fish tissues, and could be the reason of the low recoveries observed.

Higher lipid content in a tissue lowers the volume into which lipophobic compounds, such as AB, can be distributed, consequently

lowering the absorption efficiency of AB (Amlund et al., 2006). In absence of direct lipid measurements, C:N ratios are a useful proxy for lipid content and condition of individual fish, because lipids contain mostly C and little-to-no N, and therefore an increase in total lipid concentrations correlates with an increase in C:N ratios (Fagan et al., 2011). The highest C:N ratios and total arsenic concentrations found in muscle of creole perch from BR are coincident with a lower proportion of AB. On the contrary, in muscle of creole perch from DH lower arsenic levels and C:N ratios and higher proportion of AB were recorded. This result suggests that arsenic bioaccumulation could be favored by lipids, at least in creole perch, which in turn may be indicating the presence of arsenic bounded to lipid (arsenolipids). With lower C:N ratios, rainbow trout is presumed to have less fat; however AB proportion in its muscle was lower than in perch. Lower proportion of AB in rainbow trout compared to perch might be indicating the presence of other non-extractable arsenicals in salmonids. Although arsenolipids are usually reported in marine fish, a few arsenolipid species were detected and identified in four freshwater fishes from the River Elbe in Germany (Arroyo-Abad et al., 2016). However, more research is needed to explain the observed interspecific differences in arsenic speciation in top predator fish from this lake.

Finally, it is worth noting that, despite the high levels of arsenic found in the creole perch muscle compared to that of salmonids and to some other perciforms around the world, the presence of inorganic arsenic compounds was not registered in any of the fish analyzed. That is to say that in terms of food safety, arsenic in Lake Nahuel Huapi salmonids and creole perch does not represent any health risk to consumers.

5. Conclusions

This is the first study that analyzes the distribution of arsenic between different organs of fish from Patagonian lakes and that characterizes the chemical forms of arsenic present in edible tissues of Patagonian fish, thus contributing to the growing global knowledge regarding arsenic speciation in freshwater fish.

Arsenic in the three analyzed top predator fish from Lake Nahuel Huapi was higher than could be expected in unpolluted water bodies, suggesting that the volcanic activity has some influence on arsenic burdens in this lake. However, the levels and chemical forms of arsenic found in edible parts (i.e., muscle) of the analyzed fish are safe for consumption. Highest arsenic bioaccumulation recorded in organs of the benthivorous creole perch compared with salmonids, provides partial evidence of a diet-route arsenic incorporation. The influence of the volcanic activity on arsenic concentrations was more evident in creole perch, which showed higher arsenic levels closest to the volcano; while no inter-site differences were found in salmonid tissues, probably due to a more pelagic diet. Therefore, there is an indirect volcanic influence, through the prey and their association to the sediments.

Arsenic concentrations in fish can also be influenced by the metabolic role of organs and the different arsenic assimilation-elimination relationships between them. In fish from Lake Nahuel Huapi the highest arsenic concentrations were found in the organs with an active metabolism and detoxifying role, i.e., liver and kidney. The dominant arsenic species in the muscles of the analyzed fish were AB and DMA, the creole perch having 3–4 times higher AB concentrations in their muscle than rainbow trout. Given than the AB present in fish comes mainly from the food, this difference provides another evidence of the implication of the diet in the variations observed in arsenic concentrations among the fish in this lake. The low recovery of soluble arsenic species gave insight to the presence of other arsenic species that could not be extracted with the methodology used in this study. Finally, the positive relationship between C:N ratio (used as a proxy of lipid content) and total arsenic concentrations suggests that arsenic might be present bound to lipids in this fish. Therefore, the presence of arsenolipids could also be involved in the interspecific arsenic concentrations

differences found in Lake Nahuel Huapi fish, and should be investigated.

The results of this work highlight the diversity of factors involved in arsenic bioaccumulation in freshwater fish and the complexity of its study.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2018.10.077.

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