



Species- and habitat-specific bioaccumulation of total mercury and methylmercury in the food web of a deep oligotrophic lake



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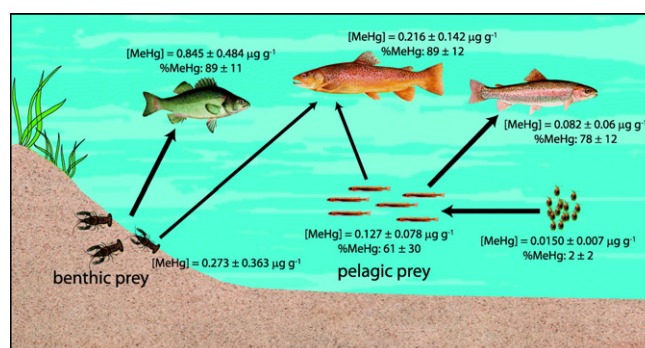
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HIGHLIGHTS

- MeHg biomagnified in the food web, however, THg was not related to trophic level.
- Total Hg and MeHg concentrations were higher in native than in introduced fish.
- Mercury bioaccumulation in top predator fish varied by foraging habitat.
- Carbon source was negatively related to THg and MeHg concentrations.

GRAPHICAL ABSTRACT



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ABSTRACT

Niche segregation between introduced and native fish in Lake Nahuel Huapi, a deep oligotrophic lake in North-west Patagonia (Argentina), occurs through the consumption of different prey. Therefore, in this work we analyzed total mercury [THg] and methylmercury [MeHg] concentrations in top predator fish and in their main prey to test whether their feeding habits influence [Hg].

Results indicate that [THg] and [MeHg] varied by foraging habitat and they increased with greater percentage of benthic diet and decreased with pelagic diet in Lake Nahuel Huapi. This is consistent with the fact that the native creole perch, a mostly benthivorous feeder, which shares the highest trophic level of the food web with introduced salmonids, had higher [THg] and [MeHg] than the more pelagic feeder rainbow trout and benthopelagic feeder brown trout.

This differential THg and MeHg bioaccumulation observed in native and introduced fish provides evidence to the hypothesis that there are two main Hg transfer pathways from the base of the food web to top predators: a pelagic pathway where Hg is transferred from water, through plankton (with Hg in inorganic species mostly), forage fish to salmonids, and a benthic pathway, as Hg is transferred from the sediments (where Hg methylation occurs mostly), through crayfish (with higher [MeHg] than plankton), to native fish, leading to one fold higher [Hg].

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1. Introduction

Mercury (Hg) is a naturally occurring element that is emitted to the atmosphere by natural processes and human activities, and that can be

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transported and deposited in remote aquatic and terrestrial ecosystems (Driscoll et al., 2013; Selin, 2009). Consequently, many aquatic systems that have never received direct discharges of mercury may register elevated levels of this metal (e.g. Chen et al., 2005; Hammerschmidt and Fitzgerald, 2006; Yang et al., 2002). Following deposition in aquatic ecosystems, Hg may be reemitted to the atmosphere, incorporated from the dissolved phase into pelagic basal organisms such as bacteria and phytoplankton or can be methylated by microorganisms such as sulfate-reducing bacteria in anoxic sediments to form methylmercury (MeHg) (Driscoll et al., 2007). Methylmercury is listed as one of the most dangerous chemicals in the world's environment (Gilbert and Grant-Webster, 1995), and it is a powerful neurotoxic compound even in low concentrations, that can also affect the genome, reproduction, and various body systems in humans (Zahir et al., 2005).

Once methylated, MeHg can be taken up by benthic and pelagic organisms from the base of the food web and be transferred to higher trophic level organisms such as fish (Lindqvist et al., 1991). Therefore, the effect of fish foraging in different habitats (benthic or pelagic), where Hg in prey can differ widely, must be quantified to understand and predict patterns of Hg accumulation in lake fish (Karimi et al., 2016). As food represents the main route of mercury exposure for fish (Hall et al., 1997) and due to its rapid accumulation and slow excretion, MeHg biomagnifies through food webs (Kidd et al., 2012; Trudel and Rasmussen, 1997), with an increase of the proportion of MeHg relative to total Hg (%MeHg) from about 10% in phytoplankton to >95% in top predator fish (Watras and Bloom, 1992). Studies of trophic transfer of Hg in lakes with similar environmental settings and Hg loading rates suggest that the extent of MeHg accumulation in top consumers, such as piscivorous fish, is driven by both community complexity and the trophic pathway (pelagic versus benthic) (Power et al., 2002).

There is some discrepancy among studies regarding the best predictor of Hg in top predator fish for both pelagic and benthic food webs as some researchers suggest that aqueous concentrations may be a more important driver of MeHg bioaccumulation than sediments (Chen et al., 2009), while others relate concentrations in biota to sediment concentrations (Tremblay et al., 1996, 1998a). There is also disagreement regarding to whether the pelagic-feeding pathway is more efficient in total Hg (THg) and MeHg transfer to higher trophic levels than benthic-feeding (Chételat et al., 2011; Ethier et al., 2008; Lavoie et al., 2010; Stewart et al., 2008) or vice versa (Eagles-Smith et al., 2008; Lescord et al., 2015). The variability in Hg bioaccumulation and biomagnification in freshwater fish can be explained in part by biological factors such as physiology, growth rate, length, age, weight, trophic position, and feeding habits (Cabana and Rasmussen, 1994; Gantner et al., 2010; Jenssen et al., 2010; Karimi et al., 2016; Sackett et al., 2013) as well as physical and chemical characteristics of the system. For instance, higher Hg concentrations in fish and other organisms have been described in large and deep lakes (Clayden et al., 2013), but others found the opposite trend (Kidd et al., 2012).

Lake Nahuel Huapi is the largest and deepest oligotrophic lake in Argentine North Patagonia (Quirós and Drago, 1985). Elevated concentrations of THg were recorded in plankton, up to 260 $\mu\text{g g}^{-1}$ dry weight (dw) in Brazo Rincón bay, mostly in the inorganic form as very low concentrations of MeHg were found in that compartment (Arcagni et al., 2017; Rizzo et al., 2014). Also THg concentrations in waters from Brazo Rincón catchment area and water column were high, ranging from 16.8 to 363 ng L^{-1} , being Hg^{+2} the predominant Hg species (% Hg^{+2} 96–99.8%), whereas MeHg was lower (%MeHg 0.02–2.10%) (Soto Cárdenas et al., 2017). In bed sediments from Brazo Rincón, Hg concentrations reached 1 $\mu\text{g g}^{-1}$ (Ribeiro Guevara et al., 2005). However, no biomagnification of THg was observed in its food web (Arcagni et al., 2017). When the main trophic transfer pathways to top predator fish were considered, i.e., benthic and pelagic pathways (Juncos et al., 2013, 2015), THg was found to biomagnify in the benthic food chains of native fish, while biodilution was observed in benthopelagic introduced salmonid food chains (Arcagni et al., 2017). In addition,

biomagnification of MeHg was recorded in a short food chain (i.e. from plankton to the benthivorous forage fish *Galaxias maculatus*) within individuals with a more benthic diet in contrast to individuals with a more pelagic (planktivorous) one (Rizzo et al., 2014), however, the trophic transfer of MeHg was not studied in the whole food web or in relation to the feeding habits of top predator fish. Therefore, the relative contribution of benthic and pelagic pathways in energy transfer in lake food webs has implications in Hg distribution in food webs and should be quantified to understand and predict the patterns of Hg accumulation in top predator fish in lakes.

In this study we compared the THg and MeHg concentrations in plankton, in macroinvertebrates, and in fish with different feeding habits in Lake Nahuel Huapi, a deep oligotrophic lake in Northwest Patagonia. We also analyzed the MeHg biomagnification in the food web using stable nitrogen isotopes and energy pathways using stable carbon isotopes. Since MeHg is mainly produced in the sediments while inorganic Hg predominates in the water column (Morel et al., 1998) and because MeHg is efficiently transferred to higher trophic level organisms through the diet (Hall et al., 1997; Kidd et al., 2012; Trudel and Rasmussen, 1997), we hypothesize that the foraging habitat and feeding habits (benthic versus pelagic) will influence the THg and MeHg concentrations in fish. Hence our general predictions are that 1) organisms relying on benthic energy will have higher THg and MeHg concentrations than organisms relying on pelagic energy and 2) we expect to find that native fish (creole perch, velvet catfish, and big puyen), which feeds mostly on benthic prey, have higher THg and MeHg concentrations than the introduced salmonids (rainbow trout and brown trout), which mostly relies on the benthopelagic small puyen.

2. Materials and methods

Lake Nahuel Huapi is the largest (557 km^2) and deepest (464 m) oligotrophic lake in Northwest Patagonia (Argentina) (Quirós and Drago, 1985; Fig. S1 in the Supplementary Information file). A more detailed description of this waterbody can be found in Arcagni et al., (2017, 2015). Samples were collected from three sites, Brazo Rincón, Bahía López, and Dina Huapi. The sampling sites and the lake's food web composition and structure were described by Arcagni et al. (2015).

Sampling of benthic invertebrates, plankton, and fish was performed in February and May 2011. Different techniques were used to collect and to process these organisms as described by Arcagni et al., (2015). Muscle tissue was removed from mollusks: mussels (*Diplodon chilensis*) and snails (*Chilina* sp.), large crustaceans: crayfish (*Samastacus spinifrons*) and crabs (*Aegla* sp.), and fish larger than 80 mm: rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), creole perch (*Percichthys trucha*), big puyen (*Galaxias platei*), and velvet catfish (*Oliveichthys viedmensis*). Head and guts were removed from fish smaller than 80 mm, small puyen (*Galaxias maculatus*) and from rainbow and brown trout juveniles, and the insect larvae were analyzed whole. Two plankton size fractions P1 class (53–200 μm) and P2 class (>200 μm) were obtained. P1 is mainly composed by mixotrophic ciliates (*Ophridium naumanii* and *Stentor araucanus*) and by rotifers, and P2 by copepods (*Bockella gracilipes* and cyclopoids) and cladocerans (*Ceriodaphnia dubia*, *Daphnia* sp., and *Bosmina longirostris*) (Arcagni et al., 2015). A more detailed composition and techniques used to collect and process this community can be found in Arcagni et al. (2015). All samples were homogenized with titanium and Teflon® devices and freeze-dried until constant weight, then grounded to fine powder using a Teflon® pestle and mortar for analysis.

2.1. Mercury and methylmercury analysis

Instrument Neutron Activation Analysis (INAA) was used to determine THg concentrations, whereas THg in low concentrations was determined by a Direct Mercury Analyzer (DMA; Milestone DMA-80). Samples with intermediate THg concentrations were analysed via

DMA and INAA to confirm the equivalence of both techniques. INAA determination were performed by irradiating 1 to 200 mg of dried homogenized sample sealed in SUPRASIL-AN quartz ampoules at the RA-6 research nuclear reactor (Centro Atómico Bariloche, Argentina). Elemental concentrations were determined using the absolute parametric method. For DMA determinations, 20 to 40 mg of dried homogenized sample was thermally decomposed in a continuous oxygen flow. The elemental Hg released was trapped on a gold amalgamator and then desorbed to be measured by cold vapor atomic absorption spectrophotometry at 254 nm. INAA detection limits depend strongly on the sample composition and on the analytical conditions, particularly sample mass and irradiation, measurement, and decay times. INAA and detection limits ranged from 0.03 to 0.2 $\mu\text{g g}^{-1}$ and DMA detection limit was 0.005 ng of Hg. In each set of analysis Certified Reference Materials (CRMs) NRCC TORT-2 and DORM-2 for INAA determinations, and DORM-3 for DMA, were analyzed for analytical quality control; the results of the analysis matched with certified values (see Table S1 in the supplementary information file).

Methylmercury from freeze dried samples was isolated by distillation (Horvat et al., 1993) followed by an aqueous phase ethylation, purging, GC separation, pyrolysis and detection by cold vapour atomic fluorescence spectrophotometer – CV AFS (Horvat et al., 1993; Liang et al., 1994). The measurement step was implemented by an automated analyzer (Tekran Methyl Mercury Analyzer, model 2700). The detection limit for MeHg determinations was 0.1 ng of Hg. Distillation was performed in duplicate; MeHg was determined for each distilled sample also in duplicate. Analytical quality control was performed by analyzing CRM BCR 463 (tuna fish) (see Table S2 in the supplementary information file).

All total mercury [THg] and methylmercury [MeHg] concentrations are expressed in $\mu\text{g g}^{-1}$ dry weight (dw).

2.2. Stable isotope analysis

Stable carbon ($\delta^{13}\text{C}$) and nitrogen isotope ($\delta^{15}\text{N}$) analyses (SIA) have widely been used in ecotoxicological studies since $\delta^{15}\text{N}$ and biomagnifying element concentrations, such as Hg, tend to increase with increasing trophic level, therefore, a linear relationship between these elements and $\delta^{15}\text{N}$ in organisms within a food web can be used to evaluate biomagnification (Lavoie et al., 2013). In addition, as stable carbon isotope ratios ($\delta^{13}\text{C}$) can be used to estimate the C source of an organism (e.g. pelagic or littoral; France, 1995), they can be useful to evaluate the dietary sources of Hg in food webs (Lescord et al., 2015; McIntyre and Beauchamp, 2007; Wang et al., 2014). Trophic transference of an element can be calculated using the following equation:

$$\text{Log}_{10}[X] = b \times \delta^{15}\text{N} + a$$

where [X] is the concentration of the element, and *b*, the slope of the regression, is an indicator of the biomagnification rate when its value is positive (Kidd et al., 1995).

Stable carbon and nitrogen isotopes were measured via DELTA^{plus}XP continuous flow stable isotope ratio mass spectrometer at the Queen's Facility for Isotope Research at Queen's University, Kingston, ON, Canada. To check the quality of analysis for every 10 carbon/nitrogen samples we ran standard reference materials and in-house standards (see Table S3 in the supplementary information file). Duplicates were also run every 10 samples which produced a difference between samples of (\pm SD) ($\delta^{13}\text{C} = 0.13\%$, $\delta^{15}\text{N} = 0.27\%$, $n = 25$). Stable isotope values (δ) were expressed in parts per thousand (‰) relative to the C and N reference materials (Pee Dee Belemnite limestone and atmospheric nitrogen standard respectively).

2.3. Data analysis

The selection of the most important prey for each fish species was based on gut content analysis for these species in Lake Nahuel Huapi following Juncos et al. (2015). Prey with an Index of Relative importance (IRI) higher than 15% were chosen for each fish species according to the fish size range captured. Therefore, crayfish and insect larvae were chosen for creole perch, small puyen and crayfish for brown trout, insect larvae and small puyen for rainbow trout, and crayfish for big puyen and velvet catfish. The percentage of benthic (%benthic) versus pelagic (%pelagic) prey consumed by each fish species was calculated according to their size range. The %pelagic and %benthic were calculated by adding the %IRI corresponding to all pelagic (all life stages of *G. maculatus* and other fish) and benthic (crustaceans, mollusks, and insect larvae) prey, respectively consumed by one species of a determined size range (Juncos et al., 2015).

Interspecific differences in [THg] and [MeHg] were evaluated using ANOVAs followed by Tukey's post hoc test. All differences were regarded as statistically significant at a p-level of 0.05. The relationship between THg and MeHg and between THg and MeHg with %benthic and %pelagic were assessed by simple linear regressions and relationships between THg and MeHg and fish length by polynomial regressions (Tremblay et al., 1998b):

$$\text{Log}[X] = a + b \times \text{TL} + c \times \text{CTL}^2$$

where [X] is the concentration of the element, *a* the constant term, *b* the coefficient to total length (TL), and *c* the coefficient of the centered total length (CTL) (Simoneau et al., 2005).

All statistical tests were performed using XLSTAT v7.5 add-in for Microsoft Office Excel.

3. Results and discussion

Total mercury [THg] and methylmercury [MeHg] concentrations in individual samples spanned up to three orders of magnitude. The lowest [THg] corresponded to the introduced salmonids (0.027–0.039 $\mu\text{g g}^{-1}$) and the highest to plankton (15.8–19.1 $\mu\text{g g}^{-1}$). On the contrary, the lowest [MeHg] were recorded in plankton (0.003–0.006 $\mu\text{g g}^{-1}$) and the highest were from the native fish creole perch (1.27–1.82 $\mu\text{g g}^{-1}$) and velvet catfish (0.447–0.748 $\mu\text{g g}^{-1}$). Despite the high [THg] obtained in plankton, the MeHg to THg fraction (%MeHg) in that community was the lowest, $0.3 \pm 0.4\%$ in P1 and $1.9 \pm 2.3\%$ in P2, and the highest in fish (62–100%). Total Hg and MeHg concentrations in benthic macroinvertebrates were diverse: insect larvae and mussels had the lowest concentrations while decapods had the highest. The %MeHg in this group varied from 2% in molluscs to 100% in crayfish (Table 1).

Of all the fish captured in Lake Nahuel Huapi, 4 specimens (20%) of creole perch and 1 specimen (17%) of big puyen exceeded the U. S. Environmental Protection Agency [MeHg] threshold of 1.2 $\mu\text{g g}^{-1}$ dw for fish consumption. The %MeHg obtained in Lake Nahuel Huapi for plankton are in the same range than the values measured previously in Lake Moreno (0.2 to 3%; Arcagni et al., 2013), and are among the lowest found in the literature for both pristine and contaminated aquatic systems (e.g. from 10.9 to 90% Becker and Bigham, 1995; Chen et al., 2012; Gorski et al., 2003; Parkman and Meili, 1993; Watras et al., 1998; Watras and Bloom, 1992). On the contrary, %MeHg fraction in macroinvertebrates from this study are in the range of values from other lakes: between 11 and 100% (e.g. Becker and Bigham, 1995; Chumchal et al., 2011; Cremona et al., 2009; Surma-Aho et al., 1986; Tremblay et al., 1996). Methylmercury ratios in fish muscle from this study were between 60 and 100%, similar to other published studies with the same species or species with similar feeding type (e.g. Cappon, 1984; Jackson, 1991; Kim, 1995; Kwon et al., 2015; Wyn et al., 2009).

Table 1
Range, mean and standard deviation (SD) of total mercury (THg) and methylmercury (MeHg) concentrations, and %MeHg in biota collected in Lake Nahuel Huapi. Range of total length and percentage of benthic diet (%benthic) in fish are also recorded. N is the number of samples analyzed; the number in parentheses represents the total number of organisms collected. Total Hg and MeHg concentrations are presented in a dry weight basis.

Organism	Total length (mm)	N (n)	THg ($\mu\text{g g}^{-1}$)	MeHg ($\mu\text{g g}^{-1}$)	MeHg (%)	% benthic
Plankton						
Plankton 1 (53–200 μm)	–	5	0.340–19.1 7.59 \pm 7.36	0.0030–0.0060 0.0045 \pm 0.0013	0.1–0.9 0.3 \pm 0.4	–
Plankton 2 (>200 μm)	–	8	0.131–43.1 8.70 \pm 14.89	0.00065–0.251 0.0150 \pm 0.0079	0.06–7 2 \pm 2	–
Macroinvertebrates						
<i>Chilina</i> sp. (snail)	–	2 (20)	0.261–1.04 0.651 \pm 0.551	0.016–0.019 0.018 \pm 0.003	2–7 4 \pm 4	–
<i>Diplodon chilensis</i> (mussel)	–	5 (28)	0.081–0.560 0.223 \pm 0.198	0.013–0.031 0.018 \pm 0.007	2–25 13 \pm 8	–
<i>Aegla</i> sp. (crab)	–	5 (26)	0.142–2.72 0.749 \pm 1.109	0.086–0.249 0.172 \pm 0.072	7–765 53 \pm 27	–
<i>Samastacus spinifrons</i> (crayfish)	–	15 (29)	0.052–1.10 0.302 \pm 0.285	0.029–1.43 0.273 \pm 0.363	33–100 74 \pm 21	–
Insect larvae	–	5 (110)	0.147–0.272 0.198 \pm 0.066	0.031–0.039 0.034 \pm 0.004	12–23 19 \pm 6	–
Fish						
<i>Oncorhynchus mykiss</i> (rainbow trout)	240–627	16	0.0268–0.264 0.102 \pm 0.063	0.0189–0.257 0.0818 \pm 0.0598	62–100 78 \pm 12	54.6
<i>O. mykiss</i> (rainbow trout juvenile)	75–167	8	0.0476–0.100 0.0731 \pm 0.0158	0.0216–0.109 0.0662 \pm 0.0236	45–100 85 \pm 20	67
<i>Salmo trutta</i> (brown trout)	468–713	15	0.053–0.467 0.232 \pm 0.134	0.054–0.536 0.216 \pm 0.142	64–100 89 \pm 12	4.6–64.2
<i>S. trutta</i> (brown trout juvenile)	52–84	3	0.242–0.335 0.286 \pm 0.047	0.220–0.280 0.243 \pm 0.033	81–91 85 \pm 5	4.6
<i>Oliveichthys viedmensis</i> (velvet catfish)	266–395	2	0.499–0.719 0.609 \pm 0.156	0.447–0.748 0.597 \pm 0.213	90–100 95 \pm 7	96.9
<i>Galaxias maculatus</i> (small puyen)	35–58	18 (87)	0.080–1.00 0.241 \pm 0.199	0.024–0.272 0.127 \pm 0.078	5–100 61 \pm 30	–
<i>Galaxias platei</i> (big puyen)	190–232	6	0.407–1.72 0.777 \pm 0.486	0.354–1.58 0.745 \pm 0.444	83–100 93 \pm 7	87.1
<i>Percichthys trucha</i> (creole perch)	180–511	21	0.0904–2.33 0.917 \pm 0.576	0.0631–1.82 0.845 \pm 0.484	70–100 89 \pm 11	93.7

Total Hg and MeHg concentrations varied by foraging habitat and increased with greater percentage of benthic diet and decreased with pelagic diet (Fig. 1). This is consistent with the fact that native creole perch, a mostly benthivorous feeder, which shares the highest trophic level of the food web with the introduced salmonids (Arcagni et al., 2015 and Fig. S2 of the Supplementary Information file), had higher [THg] and [MeHg] than the more pelagic feeder rainbow trout and benthopelagic feeder brown trout (Tukey's test $p < 0.05$, Fig. 1). Other

native fish such as velvet catfish and big puyen, both in lower trophic level than creole perch and salmonids, but with a predominantly benthic foraging habitat (Arcagni et al., 2015, Fig. S2), also had higher [THg] and [MeHg] than the introduced fish (Tukey's test $p < 0.05$, Fig. 1). Moreover, no differences were observed in [THg] and [MeHg] between introduced rainbow and brown trout (Tukey's test $p > 0.05$), and between native creole perch and big puyen (Tukey's test $p > 0.05$; Table 1, Fig. 1). Additionally, [THg] and [MeHg] in creole perch and in

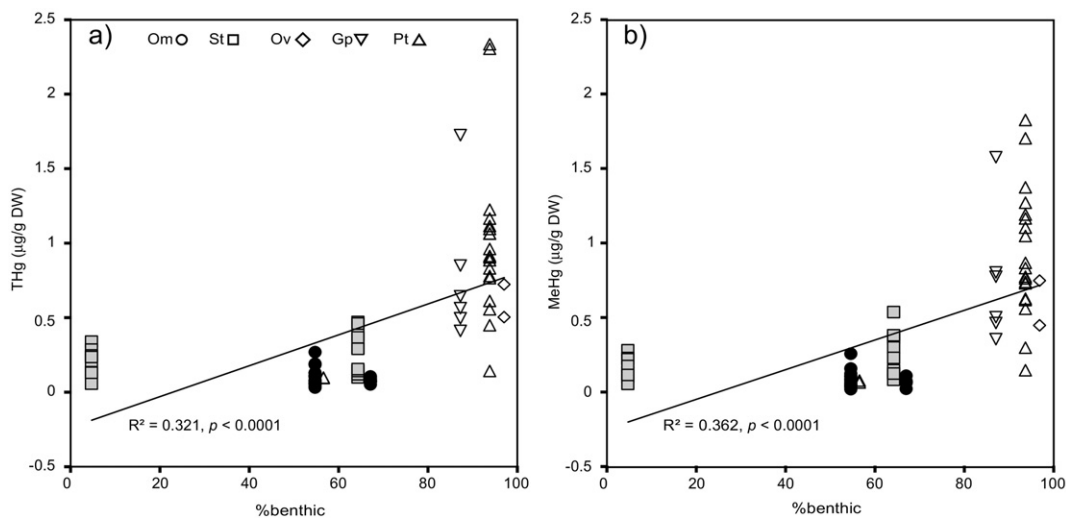


Fig. 1. Relationship between a) total mercury (THg) and b) methylmercury (MeHg) versus %benthic diet in fish from Lake Nahuel Huapi. Symbol colors represents foraging habitat: (white) benthic, (black) pelagic, and (grey) benthopelagic. Abbreviations are as follows: Om-*Oncorhynchus mykiss* (rainbow trout), St-*Salmo trutta* (brown trout), Ov-*Oliveichthys viedmensis* (velvet catfish), Gp-*Galaxias platei* (big puyen), and Pt-*Percichthys trucha* (creole perch).

big puyen were higher than in their preferred prey, crayfish and insect larvae, and crayfish and small puyen, respectively (Tukey's test $p < 0.05$). On the contrary, [THg] and [MeHg] were always lower or similar in rainbow trout and brown trout than in their prey (Tukey's test $p > 0.05$; Table 1).

The relationship between Log[THg] and Log[MeHg] with fish length was estimated using a polynomial regression, and significant relationships were obtained for creole perch and rainbow and brown trout ($p < 0.05$, Fig. 2). According to the regressions, Hg bioaccumulation with length seems to be different between creole perch and salmonids. Mercury seems to decrease with size approximately until 300 mm total length and then increases as the fish grew larger in salmonids, on the contrary, in creole perch, Hg seems to increase steadily with length in the range of sizes captured (Fig. 2), although, these differences may be due to the dissimilar size range of fish captured for each species. Many works have reported that since Hg bioaccumulates in fish, it generally increases with fish size (e.g. Bowles et al., 2001; Doyon et al., 1998; Driscoll et al., 1994; Evans et al., 2005), although not necessarily in a linear manner (Simoneau et al., 2005).

This differential Hg bioaccumulation with fish size observed in native and introduced fish from Lake Nahuel Huapi, provides more evidence to the results from Arcagni et al. (2017), where it was observed that there seems to be two main Hg transfer pathways from the base of the food web to top predators: a benthic and a pelagic pathway. In the benthic pathway, Hg is transferred from the sediments, through crayfish and other benthic macroinvertebrates, to native fish; and in the pelagic pathway, from water, through plankton, forage fish (small puyen), to introduced salmonids.

Niche segregation among native and introduced fish has been reported in Lake Nahuel Huapi food web (Juncos et al., 2015). Native

fish, especially creole perch, have a more benthivorous diet, feeding mostly on the omnivorous, closely associated to sediments, crayfish, while salmonids are more piscivorous preying heavily on the planktivorous small puyen (Juncos et al., 2013, 2015). Sediments are major repositories of various contaminants, including Hg (e.g. Barak and Mason, 1989; Timmermans et al., 1989; Ullrich et al., 2001), and are the principal location for Hg methylation (Morel et al., 1998). Once methylated in the sediment, MeHg can be taken up by benthic organisms by direct absorption of dissolved Hg from the sediment porewater and the overlying water by the gills during respiration or indirectly through the ingestion of food containing Hg (detritus), which is then transferred to higher trophic levels in the aquatic food web (Reinfelder et al., 1998). Crayfish ([MeHg] between 0.029 and 1.43 $\mu\text{g g}^{-1}$), which are polytrophic, may acquire Hg remobilized from the benthic habitat by feeding on small macroinvertebrates that dwell in the sediments (e.g. insect larvae, [MeHg] between 0.031 and 0.039 $\mu\text{g g}^{-1}$) or on detritus of plant or animal origin (Rudolph, 2002; Rudolph et al., 2010), and then transfer the accumulated Hg to the native benthivorous fish, creole perch, big puyen, or velvet catfish, resulting in the higher THg and MeHg concentrations within the fish community and %MeHg over 65% with a mean value of $90 \pm 12\%$. On the contrary, rainbow trout, which feed mostly on the planktivorous forage fish small puyen, had lower THg and MeHg concentrations than the native fish, but a %MeHg over 65% with a mean value of $81 \pm 15\%$ (Table 1, Fig. 1). Brown trout, with intermediate Hg concentrations between native fish and rainbow trout, relies on small puyen at smaller sizes (up to 540 mm standard size) and shift to a diet composed mainly by crayfish at larger sizes (Juncos et al., 2015).

There was no relationship between log-transformed [THg] and trophic level ($\delta^{15}\text{N}$), however, log-transformed MeHg concentrations increased

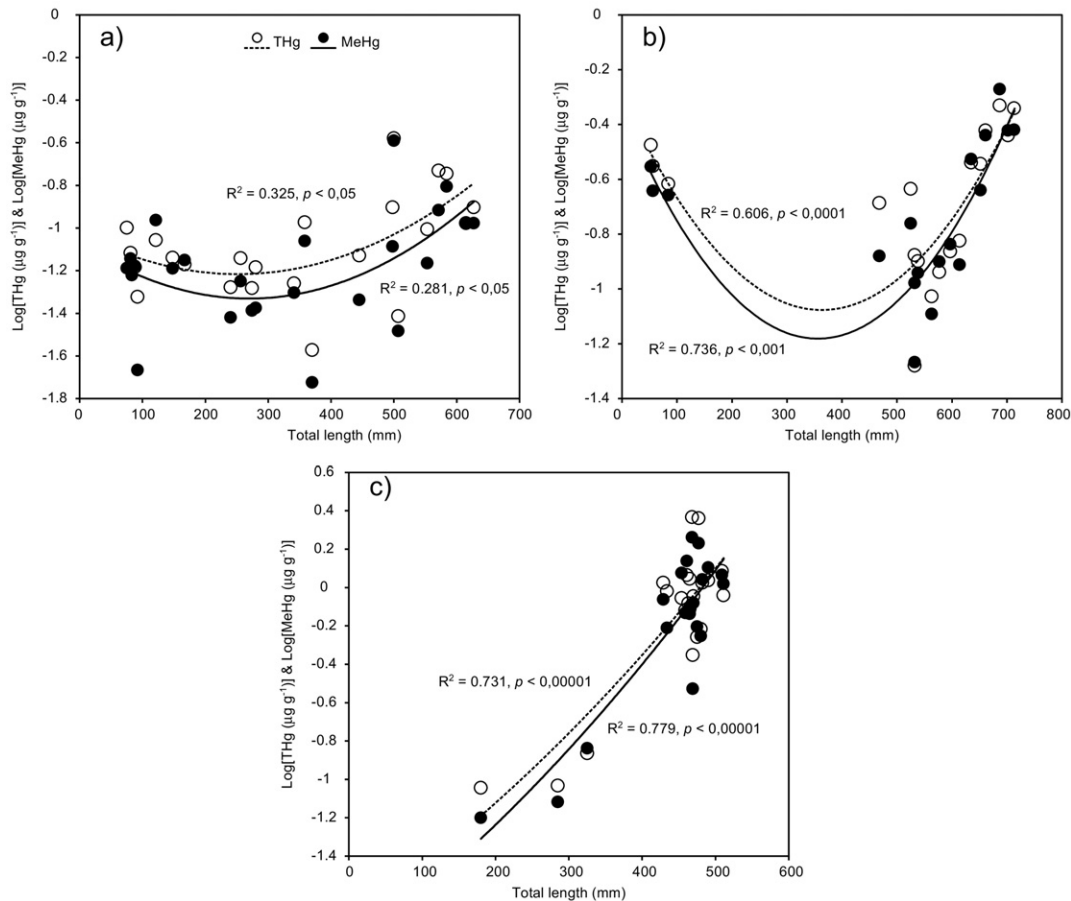


Fig. 2. Polynomial regression between log[THg] (open circles and dashed lines) and log[MeHg] (black circles and full lines) vs total fish length in a) rainbow trout, b) brown trout, and c) creole perch from Lake Nahuel Huapi.

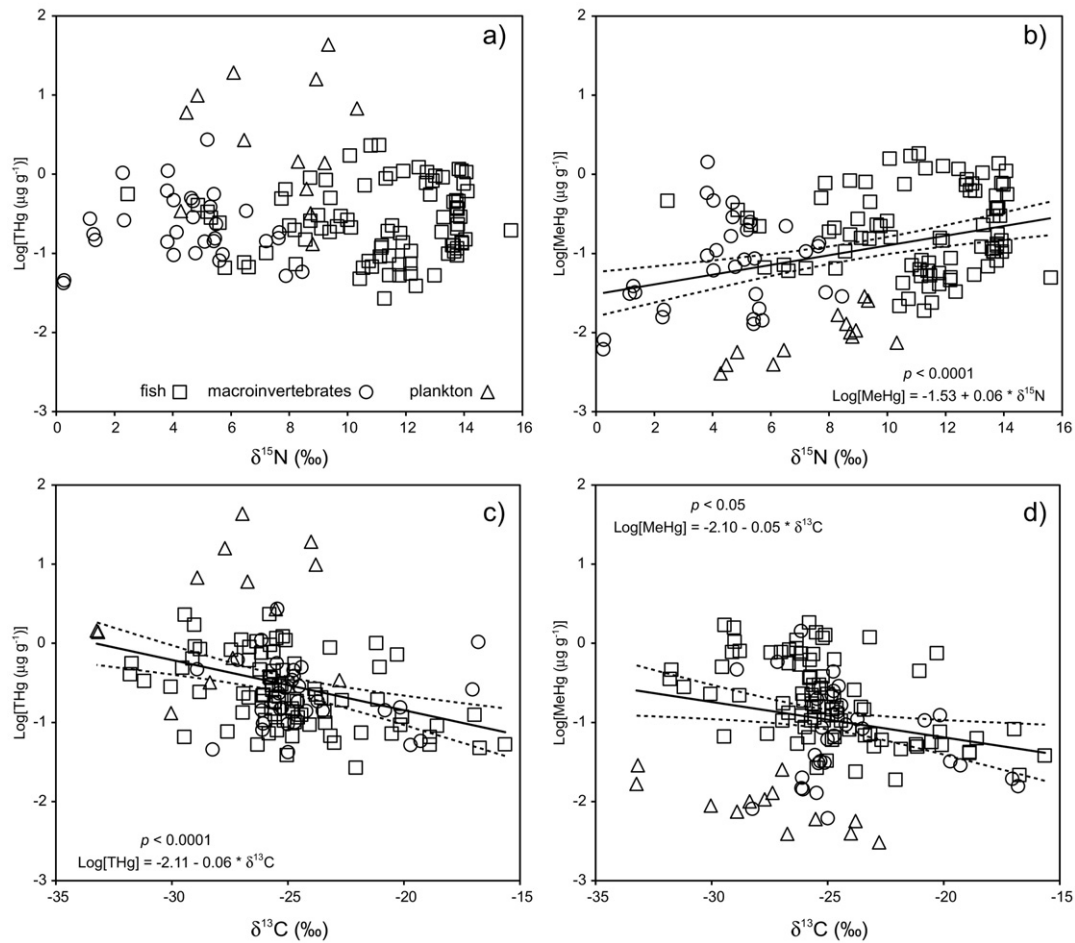


Fig. 3. Linear regression between a, c) $\log[\text{THg}]$ and b, d) $\log[\text{MeHg}]$ vs $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in the whole food web of Lake Nahuel Huapi. Only significant regression lines are shown.

with increasing trophic level, indicating that MeHg biomagnifies in the food web of Lake Nahuel Huapi with a biomagnification factor of 0.06 (Fig. 3a, b). The average biomagnification factor for freshwater ecosystems worldwide is 0.24 ± 0.08 (Lavoie et al., 2013), higher than the obtained Lake Nahuel Huapi. Many factors can affect biomagnification rates in food webs: latitude, food web composition, water chemistry, etc. (Lavoie et al., 2013), being higher in cold and low productivity systems, such as Lake Nahuel Huapi, but, the biomagnification rate was lower than expected.

Dietary carbon sources ($\delta^{13}\text{C}$) were negatively related to log-transformed [THg] and [MeHg] (Fig. 3c, d), implicating that taxa with lower $\delta^{13}\text{C}$ values (indicative of pelagic energy reliance, France, 1995), such as creole perch, big puyen, and zooplankton, have higher [THg] and [MeHg] than taxa enriched in ^{13}C (rainbow trout and snails, Fig. S2).

At a first glance, the pelagic habitat appears to be an important source of Hg to the food web as the smaller plankton fraction, between 10 and 53 μm which is composed by phytoplankton and small mixotrophic ciliates (Arcagni et al., 2015), presented very high [THg], reaching $260 \mu\text{g g}^{-1}$ dry weight (Arcagni et al., 2017). These elevated THg concentrations are not unusual in the area, as similar concentrations (up to $258 \mu\text{g g}^{-1}$ dw) were also observed in the same plankton fraction in Lake Moreno, which is a Lake Nahuel Huapi tributary (Arcagni et al., 2013). Despite the high [THg] measured, [MeHg] in plankton are the lowest in the food web, with %MeHg from 0.0059 to 7% in zooplankton (Table 1). Total Hg concentrations in waters from Brazo Rincón bay catchment area and water column ranged from moderate to high ($16.8\text{--}363 \text{ ng L}^{-1}$). The predominant Hg species in streams and in the lake was Hg^{+2} (% Hg^{+2} 96–99.8%), whereas MeHg was overall low (%MeHg 0.02–2.10%) indicating a reduced methylation potential

of the catchment area and waters in spite of high THg values (Soto Cárdenas et al. 2017), consistent with plankton results. The low %MeHg from Lake Nahuel Huapi plankton indicates that most of the [THg] in plankton is in the inorganic form and its transfer to upper trophic levels is inefficient, as it decreases from lower (phytoplankton) to higher trophic level fractions (zooplankton), and to small puyen (Arcagni et al., 2017; Rizzo et al., 2014). On the contrary, MeHg increases with increasing trophic level within the planktonic community and to forage fish (Table 1). As inorganic Hg binds to the cellular membrane and MeHg is sequestered in the cytoplasm of algal cells, inorganic Hg is poorly assimilated by zooplankters which digest the cytoplasmatic contents and defecate the membrane material (Mason et al., 1995, 1996). In this way, MeHg is more efficiently transferred than inorganic Hg from phytoplankton to zooplankton during grazing (Mason et al., 1996).

The trend of increasing MeHg and decreasing inorganic Hg with each successive trophic level in the pelagic habitat, ends in the small puyen-rainbow trout feeding link, as the salmonid had lower or similar Hg concentrations than its prey and similar %MeHg (Table 1). On the contrary, in the benthic habitat, both THg and MeHg increase with increasing trophic level from insect larvae and crayfish to creole perch and higher %MeHg in fish than in their prey (Table 1). Brown trout, with intermediate MeHg and THg concentrations between rainbow trout and creole perch, has lower Hg concentrations than their benthic diet, crayfish, and similar concentrations than their pelagic diet, small puyen. These results are supported by the regression between stable nitrogen isotope ratios ($\delta^{15}\text{N}$) and [THg], that showed a positive relationship between both variables for creole perch and big puyen and a negative relationship for salmonids in Lake Nahuel Huapi (Arcagni et al., 2017). These

differences in feeding habits among fish seem to be responsible for the observed differences in Hg bioaccumulation in Lake Nahuel Huapi, being higher in benthic feeders than in pelagic feeders.

Many studies have shown that in large, deep lakes with extensive pelagic zones, pelagic feeding fish have higher Hg concentrations than benthivorous fish (Gorski et al., 2003; Kahilainen et al., 2016; Karimi et al., 2016; Kidd et al., 2003; Power et al., 2002; Willacker et al., 2013). This differential bioaccumulation of Hg between pelagic and benthic habitats may be related with higher concentrations in zooplankton and other pelagic prey or by differences in bioenergetic processes at the base of the food web (Karimi et al., 2016; Kidd et al., 2003). In Lake Nahuel Huapi, benthic primary consumers had higher [MeHg] than pelagic primary consumers (plankton 1), however, differences in Hg concentrations between the main benthic and pelagic prey are not so evident, as no differences in [THg] and [MeHg] between benthic and pelagic prey (small puyen and crayfish) were found. This could mean that other factors besides Hg concentration in prey could be affecting metal bioaccumulation in fish.

Fish physiology can affect dietary uptake of Hg by influencing its assimilation and elimination as well as fish growth (Wang, 2012). For example, a high growth rate can result in lower Hg concentrations in fish by growth dilution (e.g. Simoneau et al., 2005; Ward et al., 2010), which could be the case of rainbow trout in Lake Nahuel Huapi. Growth rates data were not available for Lake Nahuel Huapi fish, however the growth efficiency (weight gain relative to consumption) was higher and the biomass of prey consumed was lower in creole perch and brown trout than in rainbow trout (Juncos et al., 2013), which might affect Hg bioaccumulation in these species leading to higher Hg concentrations in creole perch and brown trout than in rainbow trout. Temperature can influence growth rate, along with other factors such as water quality, season, habitat, food availability, sex, and age (Beitinger and Fitzpatrick, 1979). In most fish species growth increases with temperature (Beitinger and Fitzpatrick, 1979), therefore, in a cold and deep system like Lake Nahuel Huapi, it is expected that fish have a slower growth rate that could lead to higher Hg bioaccumulation (Harris and Bodaly, 1998).

The fish species analyzed in this study belong to three different orders, Salmoniformes, Osmeriformes, and Perciformes, each with a different physiology. For example, metabolic traits, such as MeHg elimination rates from tissues or energy allocation and growth rates can vary among fish species (Trudel and Rasmussen, 1997). This proves challenging when comparing Hg bioaccumulation among different species if their Hg uptake, assimilation, and excretion processes are poorly known. Therefore, the knowledge of these processes should be improved in order to discard effects that could influence Hg bioaccumulation in fish and make proper comparisons.

The differential THg and MeHg bioaccumulation observed in native and introduced fish provided evidence to the hypothesis that there are two main Hg transfer pathways in Lake Nahuel Huapi food web from the base of the food web to top predators: a benthic and a pelagic pathway. In the pelagic pathway, Hg is transferred from water, through plankton (being Hg mostly in the inorganic form), forage fish (small puyen), to introduced salmonids, where Hg is mostly in the organic form. In the benthic pathway, it is transferred from the sediments where Hg methylation occurs mostly, through crayfish and other benthic macroinvertebrates, to native fish, leading to one fold higher Hg levels. The higher Hg concentrations in native fish, some exceeding the consumption limits, may pose a health risk for vulnerable populations such as children and pregnant woman if consumed regularly. Therefore we suggest that the local regulatory authorities set guidelines for fish consumption of these species focusing on vulnerable populations.

4. Conclusions

In this work we showed that in Lake Nahuel Huapi, there was a clear pattern of differential THg and MeHg bioaccumulation between benthic and pelagic top predator fish which was strongly revealed by the

difference in Hg concentrations between introduced and native fish and by the different bioaccumulation pattern of Hg with fish size. Total Hg in muscle of benthic-littoral native fish, feeding mostly on benthic invertebrates, especially crayfish, increased with increasing trophic level. On the contrary, introduced salmonids to these environments, which mainly feed on planktonic forage fish, presented lower THg and MeHg concentrations than their diet and than native species (Table 1, Fig. 1).

Total mercury did not biomagnify in the food web of Lake Nahuel Huapi but MeHg did, with a biomagnification factor of 0.06, lower than that observed for similar systems.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2017.08.260>.

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