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Differential mercury transfer in the aquatic food web of a double basined lake associated with selenium and habitat

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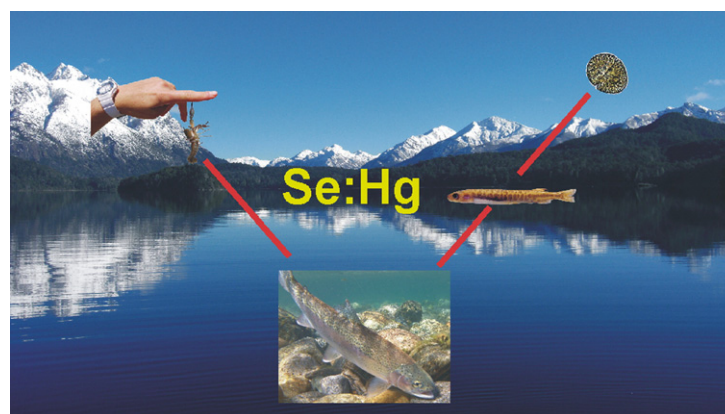
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HIGHLIGHTS

- Mercury was studied in the food web of Lake Moreno, Nahuel Huapi National Park.
- Mercury trophic transfer was assessed by nitrogen stable isotope ($\delta^{15}\text{N}$) analysis.
- Selenium was determined showing consistent source in pelagic and littoral organisms.
- High mercury concentrations, mostly inorganic, were determined in plankton.
- No mercury biomagnification was observed in Lake Moreno food web.

GRAPHICAL ABSTRACT



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ABSTRACT

Food web trophodynamics of total mercury (THg) and selenium (Se) were assessed for the double-basined ultraoligotrophic system of Lake Moreno, Patagonia. Each basin has differing proportions of littoral and pelagic habitats, thereby providing an opportunity to assess the importance of habitat (e.g. food web structure or benthic MeHg production) in the transfer of Hg and Se to top trophic fish species. Pelagic plankton, analyzed in three size classes (10–53, 53–200, and >200 μm), had very high [THg], exceeding 200 $\mu\text{g g}^{-1}$ dry weight (DW) in the smallest, and a low ratio of MeHg to THg (0.1 to 3%). In contrast, [THg] in littoral macroinvertebrates showed lower values (0.3 to 1.8 $\mu\text{g g}^{-1}$ DW). Juvenile and small fish species feeding upon plankton had higher [THg] (0.2 to 8 $\mu\text{g g}^{-1}$ muscle DW) compared to large piscivore fish species (0.1 to 1.6 $\mu\text{g g}^{-1}$ muscle DW). Selenium concentrations exhibited a much narrower variation range than THg in the food web, varying from 0.5 to 2.7 $\mu\text{g g}^{-1}$ DW. Molar Se:Hg ratios exceeded 1 for the majority of organisms in both basins, with most ratios exceeding 10.

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Using stable nitrogen isotopes as indicator of trophic level, no significant correlations were found with [THg], [Se] or Se:Hg. The apparent lack of biomagnification trends was attributed to elevated [THg] in plankton in the inorganic form mostly, as well as the possibility of consistent Se supply reducing the biomagnification in the food web of the organic portion of THg.

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1. Introduction

Mercury (Hg) is a global pollutant that reaches even the most remote regions by atmospheric transport. Deposited inorganic Hg (Hg^{2+}) is transformed in freshwater systems to organic Hg, mostly monomethylmercury (CH_3Hg^+ ; MeHg). MeHg bioaccumulates much more efficiently than Hg^{2+} , and it is a powerful neurotoxin affecting fish, wild life as well as humans through consumption of fish (Chen et al., 2005; Meili, 2001; Ullrich et al., 2001). Selenium (Se) is an essential element in biological systems, which can be toxic at high concentrations (Eisler, 1999). Selenium has a high chemical affinity with Hg in biological systems and can form insoluble Hg–Se complexes, sequestering Hg from the biological processes and neutralizing its toxic effects (Belzile et al., 2009; Khan and Wang, 2009; Peterson et al., 2009a,b; Sørmo et al., 2011). Elevated dietary Se has been linked with increased elimination of MeHg in freshwater fish (Bjerregaard et al., 2011). Therefore, the dual study of Se and Hg transfer in aquatic food webs is important to ascertain the potential impact of Hg in the ecosystem and the processes of Hg food web dynamics in a given aquatic system. The availability of Se

for binding Hg is frequently indicated by the Se:Hg molar ratio in a tissue, although Se could be combined with other elements; ratios greater than 1 indicate Se molar excess in the tissue, implying potential Se protection against Hg toxicity, whereas ratios lower than 1 suggest limited Se protection (Sørmo et al., 2011).

Food web structure and the portion of pelagic versus benthic habitat play important roles in trophodynamics of Hg and Se. While Hg frequently biomagnifies to elevated concentrations in food chains and Se is known to biomagnify in some lakes, the dietary sources and the importance of food source also are significant variables (Jardine et al., 2006; Stewart et al., 2004, 2008). It is challenging to compare different dietary sources and how Se modulates Hg in food webs in natural systems, controlling for diverse variables. Lake Moreno, a double-basined piedmont lake in Patagonia Argentina provides this opportunity (Fig. 1). Each basin has a different morphometry with the steeper basin having strong pelagic component in contrast to the other gently sloping basin having a greater littoral component (Queimaliños et al., 2012), which conforms to the typical morphometric influences on pelagic–littoral coupling in literature (Dolson et al., 2009). Pelagic–littoral

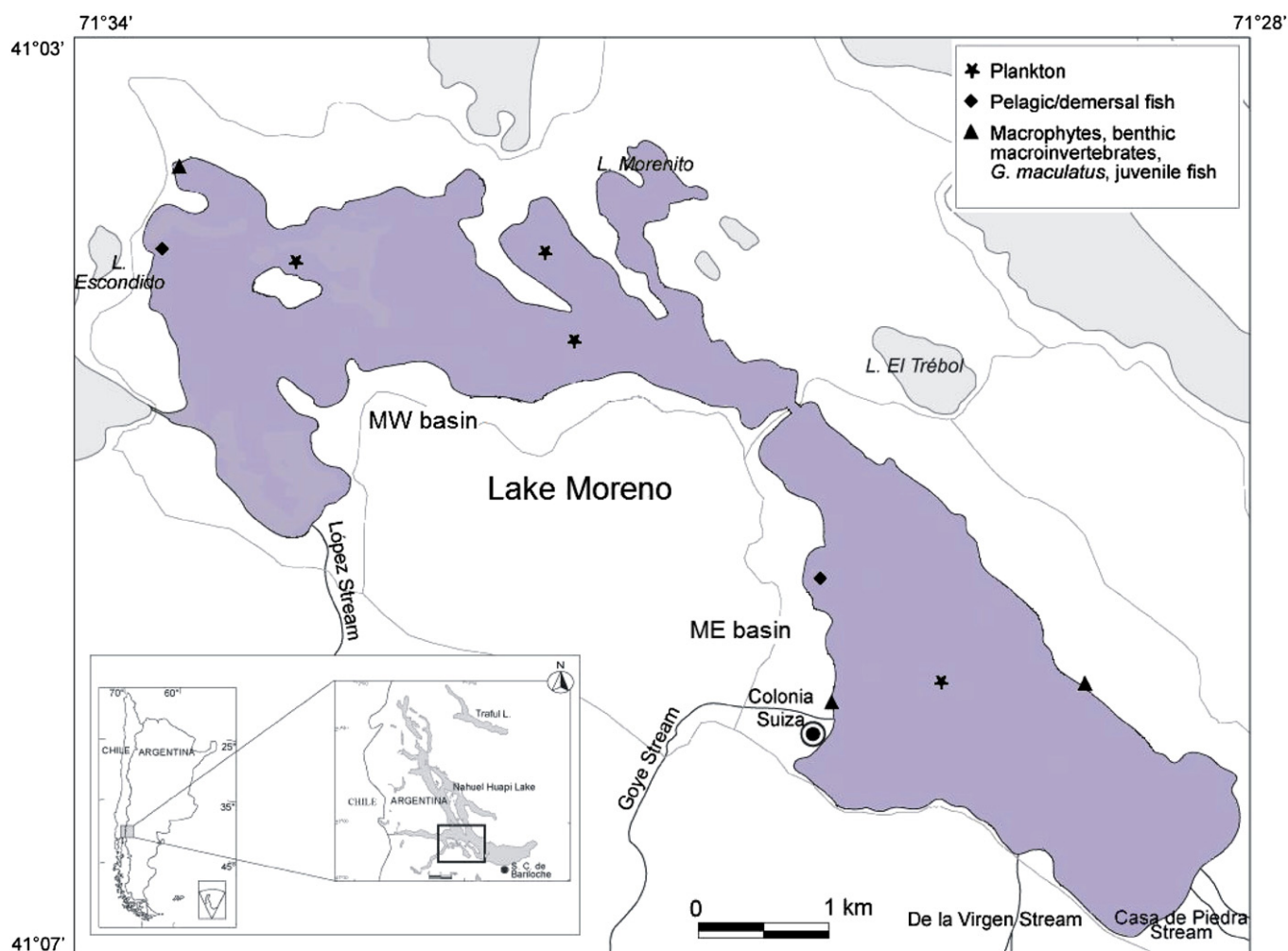


Fig. 1. Lake Moreno, Nahuel Huapi National Park, Northern Patagonia Andean Range. Biota sampling sites in both Lake Moreno basins, Moreno East (ME) and Moreno West (MW).

coupling in both basins of Lake Moreno is mediated mainly through ontogenetic development of key fish species, with larval and juvenile fish feeding upon plankton and shifting to benthic prey and fish as they grow (Macchi et al., 2007; Vigliano et al., 2009).

Stable carbon and nitrogen isotope analyses (SIA) have been widely used to study energy flow, food web structure and to estimate trophic position in aquatic ecosystems (Beaudoin et al., 2001; Campbell et al., 2003; Herwig et al., 2004). In addition, SIA can be used to study the sources and transfer of metals, elements and contaminants in food webs (Campbell et al., 2005a,b; Watanabe et al., 2008). Stable nitrogen isotope ratios ($\delta^{15}\text{N}$) tend to increase 2‰ to 4‰ with each trophic level, making it possible to identify Hg and Se biomagnification and trophodynamic processes by correlating $\delta^{15}\text{N}$ with the contaminant concentrations. Stable carbon isotope ratios ($\delta^{13}\text{C}$) show negligible fractionation (0.8 to 1‰) between trophic levels, but they can differ with carbon source (Campbell et al., 2000), and thus can be useful in evaluating dietary sources of Hg and Se sources in food webs.

In the present work we analyze Hg in the different compartments of the food web of the double-basined Lake Moreno, determined along with $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Based on previous studies in different biotic compartments of both Lake Moreno basins, we analyze the Hg trophodynamics by correlating with $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ to explain the apparent lack of Hg biomagnification (Rizzo et al., 2011), concurrent with very high Hg concentrations in planktonic organisms (Arribère et al., 2010b) and limited Hg contents in fish (Rizzo et al., 2011). Also, we study Se along with Hg to investigate the concentration patterns that could be associated with the incidence of Se in Hg transfer and bioaccumulation processes in the food web of Lake Moreno.

2. Experimental

Lake Moreno is a piedmont lake situated 768 m above sea level with a total combined surface of 12 km² (Fig. 1). It has two connected basins, Moreno East (ME) and Moreno West (MW), considered as independent lakes, with waters flowing from ME to MW (Queimaliños et al., 2012). Paleolimnological studies indicate Hg sources in Lake Moreno are likely from natural origins, with dated sediment [Hg] peaks corresponding with known dates of extended forest fires and volcanic activity (Ribeiro Guevara et al., 2010). ME is the deepest, with a maximum depth of 106 m, whereas MW maximum depth is 90 m. These lakes are warm monomictic and ultraoligotrophic, with low [N] and [P] (50 and below 4 $\mu\text{g L}^{-1}$ respectively) and low levels of dissolved organic carbon (0.4 to 0.7 mg L⁻¹), and an average pH of 6.8 (Díaz et al., 2007; Queimaliños, 2002; Queimaliños et al., 2012). Total Hg (THg) concentrations in bed sediments ranged from 0.3 to 0.6 $\mu\text{g g}^{-1}$ (Ribeiro Guevara et al., 2005a). The two basins differ in their morphometry and topography, with the shallower MW having a highly irregular and gently sloping shoreline, with peninsulas, bays and flooded areas surrounded by a *Nothofagus dombeyi*–*Austrocedrus chilensis* temperate rainforest. On the contrary, the shoreline of the deeper ME is more regular, with rocky beaches and less dense and drier rainforest–steppe ecotone vegetation. The coastline differences between the two basins result in different conditions for the proportion of littoral and pelagic habitats (and associated biotic communities), with MW having a more extensive littoral zone, and ME largely dominated by the pelagic zone (Queimaliños, 2002; Queimaliños et al., 2012).

The organisms collected for this study were grouped into pelagic organisms, littoral organisms, and fish. The pelagic organisms included plankton collected using vertical hauls of a 1 m diameter net. Plankton was separated into three size fractions by filtering through a succession of sieve mesh sizes: Fraction 1 (F1) included the 10 to 53 μm size class, consisting of haptophyte, cryptophytes, and dinoflagellates; Fraction 2 (F2) included the 53 to 200 μm size class consisting of larger phytoplankton (such as *Ceratium hirudinella*), rotifers, ciliates, and small crustaceans (such as immature stages of *Boeckella gracilipes* and *Bosmina longirostris*); and Fraction 3 (F3) composed by organisms

over 200 μm , which included larger copepods and cladocerans (Arribère et al., 2010b; Queimaliños et al., 2012).

Littoral organisms included primary producers and benthic macroinvertebrates. The benthic macroinvertebrates sampled were the decapods *Samastacus spinifrons* (crayfish) and *Aegla* sp. (crab); the mollusks *Diplodon chilensis* (mussel) and *Chilina* sp. (snail); insect larvae of Plecoptera (stonefly), Odonata (dragonfly), Ephemeroptera (mayfly), Trichoptera (caddis fly), and Diptera (Chironomidae larvae); Hirudinea (leeches), Oligochaeta (worms) and *Hyalella* sp. (amphipods). The benthic primary producers were biofilm, the submerged macrophytes *Myriophyllum quitense* (Andean watermilfoil) and *Nitella* sp. (stonewort), and the emergent *Schoenoplectus californicus* (California bulrush).

Tree and shrub leaves, another important carbon source for the lacustrine ecosystems, were collected in two ways: directly from the trees and shrubs in the lakeshore forest and the same leaf species that had fallen into the water. Leaves were collected for *N. dombeyi* (Coihue), *A. chilensis* (Cordilleran cypress), *Crinodendron patagium* (Lily of the valley tree), *Luma apiculata* (Chilean myrtle), *Lomatia hirsuta* (Radial), *Chusquea culeou* (Colihue), and *Schinus patagonicus* (Laura).

Fish were collected via various methods, including overnight gill nets (large fish), baited fish traps (juveniles and adult small fish) and plankton nets (small juveniles). The larger fish species were *Salmo trutta* (Brown Trout), generally considered the most piscivorous, *Oncorhynchus mykiss* (Rainbow Trout), and *Salvelinus fontinalis* (Brook Trout), and the native *Percichthys trucha* (Creole Perch) and *Odontesthes hatcheri* (Patagonian Silverside). Small fish species included the adults of *Olivaichthys viedmensis* (Velvet Catfish) and *Galaxias maculatus* (Puyen Chico), and juveniles of larger species (Brook and Rainbow Trout, and Patagonian Silverside).

2.1. Sample preparation

The samples were collected seasonally in both Lake Moreno basins (Fig. 1); sampling details and the clean trace metal protocols followed are described elsewhere (Arcagni et al., 2013; Arribère et al., 2008, 2010b; Rizzo et al., 2011). The cases, carapaces, shells and valves of Trichoptera larvae, *S. spinifrons*, *Aegla* sp., *D. chilensis*, and *Chilina* sp. are not completely digested by fish; therefore, hard parts were removed, and muscle and digestive (hepatopancreas) tissues were analyzed separately (Trichoptera larvae were removed from their cases and analyzed whole). The other macroinvertebrate samples were made-up of homogenized whole-body organisms since fish can fully digest them. No procedure to allow stomach content depuration of macroinvertebrates was carried out. Muscle and liver tissue was analyzed from large fish, individually or in pooled samples of similar size. *G. maculatus* and juvenile *O. hatcheri* were grouped by size in pooled samples of 10 to 30 individuals, analyzing whole body and whole body without head and guts samples. All samples analyzed (plankton, tree and shrub leaves, benthonic organisms, and fish) were freeze-dried to constant weight.

2.2. Analytical procedures

Total Hg and total Se concentrations were determined by Instrumental Neutron Activation Analysis (INAA). The specific methodology is described elsewhere (Arribère et al., 2008; Rizzo et al., 2011). The Certified Reference Materials (CRM) NRCC TORT-2, DORM-2, and DOLT-2 were analyzed for analytical quality control, and showed good agreement with the certified values. Methyl Hg concentrations were measured in lyophilized plankton via GC separation on an automated MeHg analyzer (model MERX; Brooks Rand Labs, Seattle, WA), after extraction in a potassium hydroxide solution in methanol (FDEP, 2011). The detection limit was 2–20 ng g⁻¹ dry weight (DW) depending on the initial sample mass. The CRM IAEA-405 and TORT-2 were analyzed for analytical quality control, showing good

agreement with the certified values. We use [THg], [MeHg] and [Se] to indicate concentrations, and %MeHg to indicate the fraction of MeHg to THg expressed in percentage.

Stable carbon and nitrogen isotopes were measured via DELTAplusXP continuous flow stable isotope ratio mass spectrometer. Stable isotope values (δ) were expressed in parts per thousand (‰) relative to reference materials (Pee Dee belemnite limestone for carbon and atmospheric nitrogen standard for N) (Arcagni et al., 2013). Duplicates and CRM IAEA-8548 (Carbon Rod, Sulfanilamide), and in-house standards (lipid-extracted Atlantic salmon muscle, red tilapia muscle, and chicken blood) were analyzed to confirm high quality of analysis, and are described elsewhere (Revenga et al., 2011).

Detailed INAA and stable isotopes QC results, as well as complete analytical results, are reported in the Supplementary data.

2.3. Statistics and data analyses

This study focused on the overall food web transfer patterns of Hg and Se in Lake Moreno, and thus average or grouped values were analyzed regardless of season, lake depth and specific sampling site, or biota developmental stage. Seasonal and location-specific analyses for individual elements were reported previously, and the consistency of the data supports the grouping of species-specific data points across seasons and sites within Lake Moreno (Arcagni et al., 2013; Arribère et al., 2008, 2010a,b; Rizzo et al., 2011). The only significant differences we had observed were on how carbon was transferred through the food web and differences in baseline $\delta^{15}\text{N}$ values, which were attributed to differences in pelagic vs. littoral habitat availability, and differences in anthropogenic inputs between the basins, respectively (Arcagni et al., 2013; Queimaliños et al., 2012). Average values are reported with standard deviations in parenthesis, whereas the analytical errors are reported after '±'. Differences between taxa within each basin were assessed with ANOVA followed by Tukey post-hoc test, whereas differences between taxa among lake basins were assessed using Student's t-test. Correlations between variables were determined by Pearson correlation test. Differences or correlations were considered significant at $p < 0.05$ for Type-II error.

3. Results and discussion

3.1. Pelagic organisms

Due to sampling methodology, plankton samples may include allochthonous materials, namely inorganic particles or organic detritus. The concentration of a geochemical tracer in biological tissues is very low or negligible; therefore, it can be associated with the geological material collected along with the planktonic organisms. Lanthanum, a rare earth element and geochemical tracer (Taylor and McLennan, 1988), was determined by INAA together with Hg and Se to roughly evaluate the contribution of inorganic particles in plankton samples. Lanthanum concentrations ([La]) in plankton samples are reported in Tables S3 and S4 in the Supplementary data. A typical [La] in Lake Moreno sediments was obtained from previous analyses of sedimentary sequences (Ribeiro Guevara et al., 2005a), considering the [La] of the upper layer corrected for the organic matter contents of those sediments. Concentrations ranged from 2 to 4 $\mu\text{g g}^{-1}$ DW (average per size class and lake basin) in the two smaller plankton size classes, decreasing in the $>200 \mu\text{m}$ class (0.5 and 0.7 $\mu\text{g g}^{-1}$ DW; average per lake basin), whereas [La] in bed sediments was 22 $\mu\text{g g}^{-1}$ (Ribeiro Guevara et al., 2005a). The contribution of inorganic particulate to each plankton sample was estimated comparing [La] in plankton samples to that in bed sediments. This contribution to F1 and F2 samples was 10–20 wt.%, and negligible to F3 samples (2–3 wt.%).

Total Hg concentrations in bed sediments ranged from 0.3 to 0.6 $\mu\text{g g}^{-1}$ (Ribeiro Guevara et al., 2005a), lower than those determined in F1 and F2 plankton samples (Table 1). Therefore, the inorganic particulate tends to dilute plankton [THg], and the actual [THg] would be

even higher than the reported values. It is not reasonable to include a correction in [THg] for F1 and F2 plankton samples for the dilution effect of the contribution of inorganic particulate since it is roughly estimated with [La], and it is not possible to accurately determine the [THg] of the inorganic fraction for each case. But [THg] increases in F1 and F2 plankton samples by the dilution effect is limited to the inorganic fractions determined, and tends to emphasize the observations that will be developed in further discussion.

The carbon to nitrogen concentration ratio in plankton samples (C:N, Tables S3 and S4 in the Supplementary data) reflects the allochthonous contributions of carbon, such as inorganic particles and organic detritus, with C:N values over 6.6 (Redfield proportion) revealing these allochthonous contributions (Stewart et al., 2008; Wetzel, 2001). Consistently with [La] determinations, the C:N ratios were similar in F1 and F2 samples (8.3–8.4; average per size class and lake basin), remaining near-Redfield proportion (6.6), associated hence with limited allochthonous contributions of carbon (Stewart et al., 2008). Furthermore, C:N ratios in F3 samples (4.9 and 5.1; average per lake basin) were lower than the Redfield proportion, associated with no relevant allochthonous contributions of carbon (Stewart et al., 2008).

Plankton [THg] was the highest measured for any organism in Lake Moreno, with values exceeding 200 $\mu\text{g g}^{-1}$ DW in F1 (Arribère et al., 2010b; Rizzo et al., 2011). Average [THg] decreased significantly with increasing plankton size in both basins ($p < 0.03$; Table 1). Despite the elevated plankton [THg], the proportions of MeHg to THg determined in the selected samples for both basins were exceedingly low, $<1\%$ MeHg in most cases, increasing to an average of 2.8% MeHg in F3 in ME (Table 1) ([MeHg] ranged from 6 to 90 ng g^{-1}). These MeHg fractions are particularly low compared to other plankton data around the world. Typical %MeHg ranges are between 11 and 83% in both pristine and contaminated aquatic systems (Belzile et al., 2006; Gorski et al., 2003; Kehrig et al., 2009; Watras et al., 1998) meaning that our plankton %MeHg results are one to two orders of magnitude lower, the lowest found so far in the literature (even considering the dilution effect in the [THg] of the inorganic material included in the samples mentioned before). This is despite the fact that [THg] in Lake Moreno plankton was considerably elevated, leading to [MeHg] similar to the reports cited.

The highest %MeHg in the $>200 \mu\text{m}$ plankton size class in ME was associated with an increase in $\delta^{15}\text{N}$ (Table 1). The lack of significant MeHg increase with increasing plankton size in MW could be due to differences in the community structure of the largest plankton. Cladocerans, with a wider omnivorous feeding spectrum, were more abundant in ME, while the predatory copepods were more abundant in MW (Arribère et al., 2010b). The smallest size classes of plankton (F1 and F2) did not have significantly different $\delta^{15}\text{N}$ values, but the largest size class had significantly higher $\delta^{15}\text{N}$ values ($p < 0.0001$), confirming a higher trophic level for the cladocerans and copepods. However, $\delta^{13}\text{C}$ was significantly lower in F3 compared with the smaller size classes ($p < 0.01$), which may indicate a mix of primary producers, heterotrophic organisms and consumers, and the presence of terrestrial and littoral detritus in F1 and F2 (Grey and Jones, 1999), in coincidence with C:N ratios discussed previously.

The [THg] in the smallest size class was very elevated, decreasing in the larger classes. We hypothesized that this is due to passive uptake or adsorption of Hg^{2+} , mechanism that leads to greater Hg^{2+} concentrations in higher surface:volume ratio plankton, which correspond to the smaller species. Laboratory radiolabeled Hg^{2+} amendment experiments showed strong passive Hg^{2+} uptake in native algae *Cryptomonas erosa* incubated in Lake Moreno waters after 24 h (Diéguez et al., in press); radiolabeled Hg^{2+} experiments with natural plankton from Lake Moreno showed similar results (Diéguez et al., 2012). If this is the predominant uptake mechanism in natural conditions in Lake Moreno, then the Hg concentrations at the base of the food web depend strongly on the dissolved Hg^{2+} and on the dynamic of the plankton populations. The passive uptake mechanism, enhancing Hg^{2+} incorporation on the smaller plankton with limited transference to higher

Table 1
Plankton from Lake Moreno. Average values of the Hg, MeHg, Se, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ determinations, and Se:Hg molar ratio.^a

| Lake Moreno basin | Organism | Hg ($\mu\text{g g}^{-1}$) ^{b,c} | MeHg (%) ^d | Se ($\mu\text{g g}^{-1}$) ^{b,c} | Se:Hg ^{e,c} | $\delta^{15}\text{N}$ (‰) ^c | $\delta^{13}\text{C}$ (‰) ^c |
|-------------------|-------------------------------|--|-----------------------|--|----------------------|--|--|
| East | Plankton 10–53 μm | 64 (78) | 0.63 (0.76) | 1.34 (0.29) | 0.19 (0.29) | 5.5 (1.4) | −26.2 (2.0) |
| | Plankton 53–200 μm | 15 (24) | 0.192 (0.042) | 1.53 (0.46) | 2.2 (5.7) | 5.0 (1.7) | −26.1 (2.1) |
| | Plankton > 200 μm | 5.7 (7.7) | 2.8 (3.0) | 2.37 (0.56) | 5.9 (6.6) | 8.9 (1.9) | −29.4 (1.6) |
| West | Plankton 10–53 μm | 54 (85) | 0.72 (0.17) | 1.53 (0.26) | 0.34 (0.45) | 4.8 (1.5) | −26.3 (2.4) |
| | Plankton 53–200 μm | 13 (15) | 0.34 (0.15) | 1.63 (0.33) | 2.6 (4.2) | 4.5 (2.0) | −25.5 (2.1) |
| | Plankton > 200 μm | 5.4 (8.9) | 0.449 (0.089) | 2.30 (0.26) | 6.8 (7.8) | 7.77 (0.95) | −28.5 (1.5) |

^a Organisms collected in the pelagic environment. The analysis of each sample is reported in Supplementary data.

^b Dry weight basis.

^c Standard deviation of the average in parenthesis.

^d Fraction of MeHg to THg.

^e Molar ratio.

trophic levels, and the low %MeHg determined in Lake Moreno filtered waters (0.4 to 2.4%MeHg; 1 to 5 ng L^{−1} [THg]), which limit the direct incorporation of MeHg from the water column, would explain the decrease of the [THg] with larger plankton size classes.

In contrast with [THg], [Se] showed low variability within each plankton size class (standard deviation of the mean ranging from 11 to 30%; Table 1), and increasing concentration with plankton size (Table 1). Measured [Se] is somewhat higher than average literature values reported for plankton in other systems globally, with freshwater plankton from pristine lakes typically below 1 $\mu\text{g g}^{-1}$ DW, and up to 20 $\mu\text{g g}^{-1}$ DW in contaminated sites (Belzile et al., 2006; Muscatello and Janza, 2009; Saiki et al., 2012). In marine systems, average [Se] in plankton is lower, ranging from 0.02 to 3 $\mu\text{g g}^{-1}$ DW with reports of strong seasonal variability (e.g. from 0.11 to 5.3 $\mu\text{g g}^{-1}$ DW in Northwestern Mediterranean Sea) (Boisson and Romeo, 1996; Kehrig et al., 2009). There was a positive correlation between [Se] and $\delta^{15}\text{N}$ in plankton (Fig. 2), which provides evidence of Se biomagnification within the plankton community of Lake Moreno; in contrast, [Se] was negatively correlated with plankton $\delta^{13}\text{C}$ (Fig. 2). Se:Hg molar ratios consistently increased with plankton size in both basins, despite the high variability in [THg] (Table 1).

The relationship between [Se], $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and size, suggests a consistently pelagic Se source within the food web of Lake Moreno. Although the Lake Moreno plankton [THg] is extremely elevated, the consistent patterns of plankton [Se] and the Se:Hg molar ratios >1 (even showing high variability associated with [THg] variations) for the larger plankton size classes most frequently consumed by fish (Table 1), increases the potential for Se neutralization of the Hg toxic effects in upper trophic levels. This is in addition to the very low %MeHg at the base of the food web which reduces MeHg uptake and subsequently transfer rates within the food web.

3.2. Littoral organisms

The macroinvertebrate taxa showed lower $\delta^{15}\text{N}$ values than most Lake Moreno fish species, occupying hence lower trophic levels (Fig. 3). There was one exception only: Hirudinea, a leech not identified but probably belonging to the *Helobdella* genus, a predator of aquatic invertebrates (Siddall and Borda, 2003). Macroinvertebrate taxa with the lowest $\delta^{15}\text{N}$ values in MW, such as Oligochaeta, Chironomidae larvae and Plecoptera larvae, showed the highest average [THg] and the lowest Se:Hg molar ratios (Table 2). A similar situation was observed in ME, where *Chilina* sp. and Plecoptera larvae with relatively low $\delta^{15}\text{N}$ values (5.3 and 7.1‰, respectively; Table 2), had the highest [THg] (1.8 and 1.98 $\mu\text{g g}^{-1}$ DW, respectively; Table 2). On the contrary, macroinvertebrate species with higher $\delta^{15}\text{N}$ values such as *S. spinifrons* and *Aegla* sp. showed comparatively low THg values, and the Se:Hg molar ratios in *Aegla* sp. among the highest. The Se:Hg molar ratios for most macroinvertebrate species were >1, ranging from 1 to 30 (Table 2). The only correlations observed within the macroinvertebrate community were a negative one between $\delta^{13}\text{C}$ and

[THg] ($p < 0.001$) and a positive one between $\delta^{13}\text{C}$ and the Se:Hg molar ratio ($p < 0.05$; Table 2), both in MW.

The submerged decomposing tree leaves had [THg] from 0.20 $\mu\text{g g}^{-1}$ DW for *C. patagua* to 1.65 $\mu\text{g g}^{-1}$ DW for *C. culeou*, with $\delta^{13}\text{C}$ values (−26.3 to −29.8‰) similar to some insect larvae taxa (Table 2), supporting the hypothesis that these macroinvertebrates may be feeding upon terrestrial leaf detritus from the surrounding forest. Another possible source of THg and Se to primary consumers such as grazers (e.g. *Chilina* sp.) and scrappers (e.g. Plecoptera larvae) is biofilm, which had high [THg] (2.7 and 2.8 $\mu\text{g g}^{-1}$ DW in ME and MW, respectively) and the highest [Se] among primary producers (0.4–0.9 $\mu\text{g g}^{-1}$ DW), and also $\delta^{13}\text{C}$ values (−24.9 and −24.4‰ in ME and MW, respectively) similar to these macroinvertebrates. However, the maximum average [THg] and [Se] in those primary producer species (2.8 and 0.6 $\mu\text{g g}^{-1}$ DW, respectively) are still lower than those for plankton (Table 1). The aquatic macrophytes *M. quitense* and *Nitella* sp. had average [THg] of 0.9 and 1.4 $\mu\text{g g}^{-1}$ DW respectively, but they are an unlikely dietary source to the macroinvertebrate and fish community in Lake Moreno, which is consistent with the higher $\delta^{13}\text{C}$ values observed (Arcagni et al., 2013).

In littoral food webs, Se uptake from the detrital food chain may be considered one of the most important pathways for Se recycling (Orr et al., 2006). Se in the top layer of the sediment is generally the result of deposition of dead organic material from the water column. Macrophytes, phytoplankton, periphyton and terrestrial plant litter release Se to the ambient water through excretion, cell lysis or when grazed upon by herbivores (Stewart et al., 2010). The microorganisms at the base of the food web, such as bacteria and fungi, bioconcentrate Se up to 10⁶ fold relative to the water column (Wiramanaden et al., 2010), while Se is taken up primarily through diet in higher trophic levels (Hillwalker et al., 2006; Stewart et al., 2010). The high [Se] found in the detritivorous organisms, Chironomidae larvae and Oligochaeta (Paggi, 2009), may be attributed to the [Se] in detritus material. Aquatic insect larvae, Chironomidae specifically, are also an important link between Se biogeochemical sources (e.g. surface water and sediment) and higher trophic level organisms, such as fish and other macroinvertebrates (Hillwalker et al., 2006).

3.3. Pelagic and juvenile fish species

G. maculatus is a native small fish that represents an important link between plankton and piscivorous fish (Juncos et al., 2011; Macchi et al., 2007; Vigliano et al., 2009). Pelagic *G. maculatus* larvae and juveniles are known to feed on plankton; *Boeckella gracilipes* and ciliates such as *Ophrydium* sp. and *Stentor* sp. (size class 53–200 μm) (Barriga et al., 2012; Kamjunke et al., 2009). The size class corresponding to these species had elevated [THg] and significant [Se] (Table 1), thereby serving as a potentially important pelagic conduit of THg and Se to the fish community. Correspondingly, *G. maculatus* small juveniles collected in the pelagic zone had the highest [THg] (max. 10.4 \pm 1.3 $\mu\text{g g}^{-1}$ DW, averaging 8.0 (4.3) and 1.7 (1.1) $\mu\text{g g}^{-1}$ DW in ME

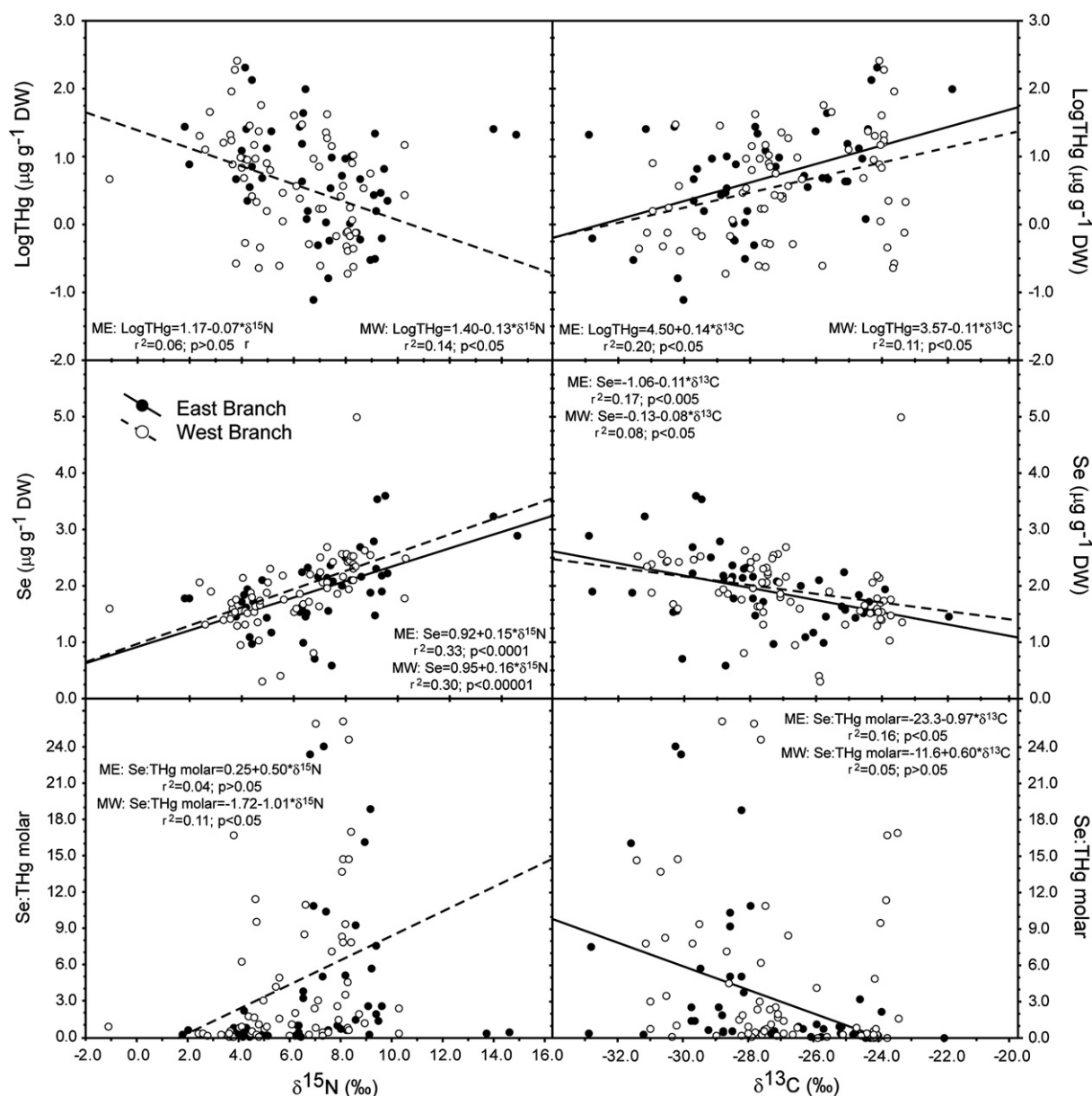


Fig. 2. Mercury and selenium concentrations, and Se:Hg molar ratios, in plankton from Lake Moreno East and West; plots vs. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Correlation parameters included.

and MW respectively; Table 3) of all fish species sampled, particularly compared to the largest piscivorous fish species. Pelagic *G. maculatus* [THg] are higher than those for littoral adults and juveniles (Table 3). The [Se] in pelagic *G. maculatus* is similar to plankton, with average Se:Hg molar ratios over 1 (Table 3). $\delta^{15}\text{N}$ values are higher in pelagic *G. maculatus* than in the smaller plankton size classes F1 and F2, and slightly higher than F3, in both basins (Tables 1 and 3). Taken together, [THg], [Se] and SIA suggest that the smaller plankton size classes are likely being a relevant food source for pelagic *G. maculatus*, which is in line with published data (Barriga et al., 2012; Kamjunke et al., 2009). It is also noteworthy to point out the high [THg] (up to $6 \mu\text{g g}^{-1}$ DW) in some small littoral specimens, which possibly migrated recently from the pelagic zone. Furthermore, littoral *G. maculatus* feed on plankton in addition to Chironomidae larvae and other benthic prey. While pelagic plankton had elevated [THg], benthic Chironomidae larvae, an important littoral food item for *G. maculatus*, also had high [THg] ($1.2 \mu\text{g g}^{-1}$ DW; Table 2). This indicates a dietary transition from high-[THg] small plankton fractions to moderate-[THg] Chironomidae larvae then lower-[THg] littoral prey through its ontogenetic development.

The juveniles of *O. mykiss* and *O. hatcheri* had similar [THg] trends as *G. maculatus*. *O. hatcheri* juveniles exhibit similar ontogenetic dietary shifts as *G. maculatus* (Cervellini et al., 1993), whereas *O. mykiss* juveniles may also feed on zooplankters (Kamjunke et al., 2009), and on smaller stages of *G. maculatus* (Juncos et al., 2011). Both trophic connections are supported by the trends in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values. Moreover, *O. mykiss* juveniles in MW had lower $\delta^{15}\text{N}$ values (8‰; Table 3) compared to the other large fish, but yet had about 5-fold higher [THg] than *O. mykiss* adults, and lower muscle Se:Hg molar ratio (Table 3). Furthermore, because *O. mykiss* juveniles also feed on *G. maculatus* larvae and juveniles they have an additional source of elevated THg and Se. These results underscore the importance of ontogenetic changes in fish diet and its relationship with Hg and Se trophodynamics in food webs. It is often assumed that the bigger more piscivorous stages of many fish species would exhibit higher concentrations than their juvenile counterparts. However, this pattern can be altered by particularly elevated concentrations in one habitat favored by juvenile individuals (e.g. pelagic vs. littoral in this case), and by weak pelagic–littoral coupling due to the morphometry of the lake. The same situation has been observed

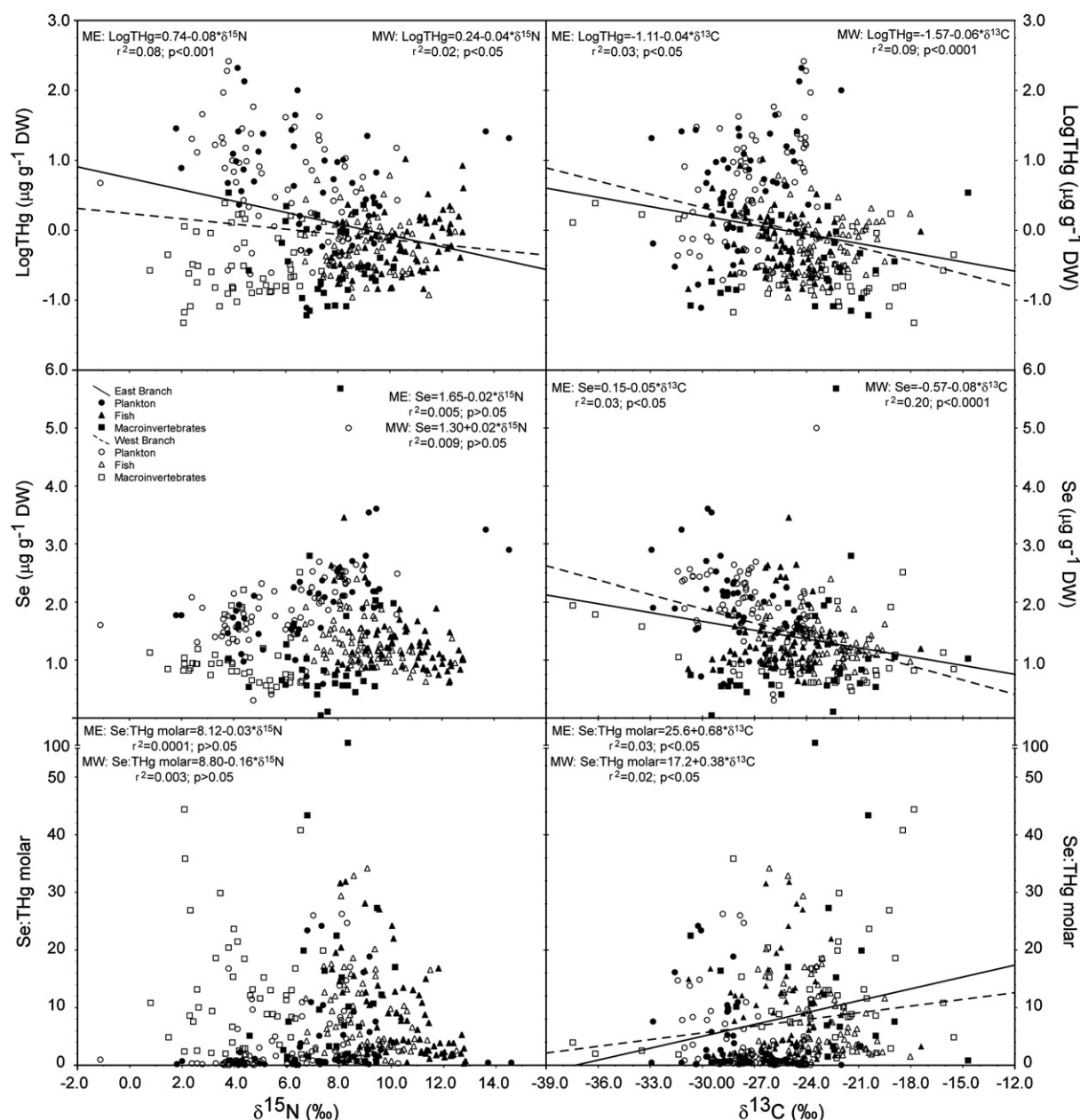


Fig. 3. Mercury and selenium concentrations, and Se:Hg molar ratios, in the food webs of Lake Moreno East and West; plots vs. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Correlation parameters included.

for organochlorine biomagnification in a Canadian Rocky Mountain lake, with copepods bioaccumulating organochlorines to higher concentrations (due to their high lipid content) than seen for the piscivore trout species, resulting in lack of biomagnification trends (Campbell et al., 2000).

To further emphasize the importance of pelagic sources of Hg and Se in Lake Moreno, it is useful to consider the juveniles of *S. fontinalis*, which include littoral benthic feeding. *S. fontinalis* juveniles exhibited a different trend as *O. mykiss* juveniles. *S. fontinalis* juveniles and adults had similar $\delta^{15}\text{N}$ in both Lake Moreno basins, but higher than those of *O. mykiss* juveniles (Table 3). Correspondingly, *S. fontinalis* juveniles had low average [THg] (0.225 and $0.530 \mu\text{g g}^{-1}$ DW in ME and MW respectively; Table 3) with adults having consistently higher [THg] (1.6 and $0.85 \mu\text{g g}^{-1}$ DW). *O. viedmensis*, another fish species with benthic-feeding habits, exhibited similar $\delta^{15}\text{N}$ as *G. maculatus* and *O. hatcheri* juveniles but higher [THg] and lower [Se] (Table 3), leading to an average Se:Hg molar ratio of 2.00, among the smaller values for littoral organisms, and more in line with Se:Hg ratios

measured in bigger fish (Table 3). This further emphasizes the combined importance of life-history traits and dietary habits on contaminant exposure.

3.4. Large fish

Adult fish had similar average $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in ME, consistently ranging between 11.2 and 12.0‰, and -24 and -27% respectively (Table 3). The exception to this similarity in stable isotope values is *O. hatcheri* with 10.0 and -31% respectively. The lower stable isotope values of *O. hatcheri* can be explained by its predominant feeding on plankton (Wegrzyn and Ortubay, 2009). The $\delta^{15}\text{N}$ values for the juvenile stage of *O. hatcheri* is much lower than in the adult (8.2‰; Table 3), revealing a lower trophic level. Large fish species from MW tended to have lower $\delta^{15}\text{N}$ and higher $\delta^{13}\text{C}$ values than those in ME, although they still were consistent among all species (9.8 to 11.5, and -21 to -24% respectively; Table 3) (Arcagni et al., 2013).

Table 2Littoral organisms from Lake Moreno. Average values of the Hg, Se, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ determinations, and Se:Hg molar ratio.^a

| Organism | Lake basin | Hg ($\mu\text{g g}^{-1}$) ^b | Se ($\mu\text{g g}^{-1}$) ^b | Se:Hg ^{b,c} | $\delta^{15}\text{N}$ (‰) | $\delta^{13}\text{C}$ (‰) |
|-------------------------------------|------------|--|--|----------------------|---------------------------|---------------------------|
| Ephemeroptera larvae whole body | East | <0.08 | 1.76 \pm 0.38 | – | 8.3 | –23.5 |
| Odonata larvae whole body | | 0.266 \pm 0.054 | 0.533 \pm 0.063 | 5.1 | 4.6 | –20.0 |
| Plecoptera larvae whole body | | 1.98 (0.57) | 1.3 (1.1) | 1.04 (0.45) | 7.1 (1.6) | –24.8 (3.4) |
| Trichoptera larvae whole body | | 0.79 (0.85) | 0.55 (0.24) | 6.79 (8.5) | 7.6 (1.0) | –28.0 (2.5) |
| <i>Aegla</i> sp. muscle | | 0.30 (0.11) | 1.68 (0.55) | 17 (11) | 9.77 (0.38) | –23.6 (1.3) |
| <i>Diplodon chilensis</i> muscle | | 0.342 \pm 0.071 | 3.21 \pm 0.34 | 23.8 | 5.1 | –27.7 |
| <i>Chilina</i> sp. muscle | | 1.8 (2.4) | 0.93 (0.13) | 10 (14) | 5.3 (2.1) | –17.8 (4.3) |
| <i>Hyaletella</i> sp. whole body | | 0.75 (0.65) | 1.5 (2.4) | 6.3 (6.2) | 7.92 (0.51) | –24.9 (2.5) |
| Hirudinea whole body | | 0.595 \pm 0.058 | 2.3 (1.2) | 6.36 | 12.3 (2.6) | –25.7 (2.8) |
| <i>Samastacus spinifrons</i> muscle | | 0.29 (0.13) | 0.69 (0.15) | 6.8 (3.0) | 8.55 (0.63) | –22.4 (1.2) |
| Chironomidae larvae whole body | West | 1.2 (1.0) | 1.65 (0.84) | 3.8 (1.3) | 2.3 | –23.85 (0.50) |
| Ephemeroptera larvae whole body | | 0.52 (0.27) | 1.46 (0.76) | 14 (18) | 5.73 (0.95) | –23.1 (3.3) |
| Odonata larvae whole body | | 0.57 (0.67) | 0.801 (0.084) | 13 (12) | 4.3 (1.2) | –23.5 (3.1) |
| Plecoptera larvae whole body | | 1.84 (0.78) | 1.87 (0.11) | 2.9 (1.4) | 3.80 (0.14) | –36.75 (0.92) |
| Trichoptera larvae whole body | | 0.60 (0.59) | 1.04 (0.23) | 9.0 (6.7) | 3.73 (0.87) | –27.4 (3.1) |
| <i>Aegla</i> sp. muscle | | 0.270 (0.022) | 2.07 (0.09) | 19.47 (0.69) | 6.7 (1.5) | –22.43 (0.45) |
| <i>Diplodon chilensis</i> muscle | | 0.25 (0.17) | 1.91 (0.34) | 30 (20) | 5.20 (0.77) | –25.56 (0.41) |
| <i>Chilina</i> sp. muscle | | 0.34 (0.37) | 0.94 (14) | 17 (15) | 2.06 (0.77) | –18.4 (2.1) |
| <i>Hyaletella</i> sp. whole body | | 0.56 (0.76) | 1.24 (0.46) | 16 (15) | 2.90 (0.96) | –23.2 (3.9) |
| Hirudinea whole body | | 0.95 (1.01) | 1.72 (0.24) | 16 (14) | 7.63 (0.70) | –25.1 (2.2) |
| Oligochaeta whole body | | 1.7 (2.2) | 2.7 (2.8) | 6.9 (4.6) | 1.25 (0.07) | –23.65 (0.21) |
| <i>Samastacus spinifrons</i> muscle | | 0.26 (0.32) | 0.73 (0.30) | 10.9 (5.1) | 5.54 (0.74) | –22.2 (1.5) |

^a Organisms collected in the littoral environment. The analysis of each sample is reported in Supplementary data.^b Dry weight basis; standard deviation of the average in parenthesis. In the case of pooled samples, the average is weighted by the number of individuals composing them.^c Molar ratio; standard deviation of the average in parenthesis.

Muscle [THg] was similar in *S. fontinalis* and *P. trucha* in both basins, but in was lower in adult *O. mykiss* muscle [THg] (Table 3). In ME, adult *O. hatcheri* had muscle [THg] similar to *S. fontinalis* and *P. trucha* (1.3 to 1.6 $\mu\text{g g}^{-1}$ DW; Table 3), despite its significant lower $\delta^{15}\text{N}$ values. In MW, a single *S. trutta* specimen was analyzed, which had the lowest muscle [THg] seen for all Lake Moreno fish species. To remark, the predominant Hg species in large fish muscle tissues is MeHg, ranging in 80–100%MeHg (Ullrich et al., 2001). It is noteworthy that *S. trutta* muscle tissue had the highest Se:Hg molar ratio (16) of all fish species (Table 3).

The Se:Hg molar ratio of *O. mykiss* muscle was higher than *P. trucha* in both basins, and than *S. fontinalis* in MW (Table 3). Furthermore, the [Se] in liver tissues of *O. mykiss* (average 23 and 26 $\mu\text{g g}^{-1}$ DW in ME and MW respectively) and *S. trutta* (10 $\mu\text{g g}^{-1}$

DW) was 2 to 6 times higher than those in the species with higher muscle [THg] *P. trucha* and *S. fontinalis* (average 4.1 to 4.4 $\mu\text{g g}^{-1}$ DW), associated probably with higher Se dietary uptake. These results could be consistent with the protective effect of Se to explain the low muscle [THg] in *O. mykiss* and *S. trutta* adults (Belzile et al., 2009; Peterson et al., 2009a,b; Sørmo et al., 2011), especially in light of recent evidence showing increased elimination of MeHg with increased Se intake in freshwater fish (Bjerregaard et al., 2011).

As already discussed above, Hg in plankton is largely in the inorganic form. Therefore, if the high [THg] determined in juveniles of *G. maculatus*, *O. hatcheri*, and *O. mykiss* is associated with plankton feeding, the %MeHg of THg in those fish species would be also low. This would obscure any Hg biomagnification trends in the whole food web, together with the Se protective effect.

Table 3Fish from Lake Moreno. Average values of the Hg, Se, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ determinations, and Se:Hg molar ratio.^a

| fish species | Lake basin | Hg ($\mu\text{g g}^{-1}$) ^b | Se ($\mu\text{g g}^{-1}$) ^b | Se:Hg ^{b,c} | $\delta^{15}\text{N}$ (‰) | $\delta^{13}\text{C}$ (‰) |
|--|------------|--|--|----------------------|---------------------------|---------------------------|
| <i>Galaxias maculatus</i> juveniles ^d | East | 8.0 (4.3) | 1.52 (0.32) | 1.8 (3.3) | 11.0 (1.0) | –27.08 (0.80) |
| <i>Galaxias maculatus</i> ^e | | 0.32 (0.12) | 1.87 (0.75) | 16.0 (7.4) | 8.8 (1.3) | –26.1 (3.2) |
| <i>Olivaichthys viedmensis</i> ^f | | 1.32 (0.54) | 0.898 (0.064) | 2.00 (0.87) | 8.74 (0.56) | –26.4 (1.6) |
| <i>Percichthys trucha</i> ^f | | 1.29 (0.43) | 1.19 (0.15) | 2.54 (0.68) | 11.44 (0.72) | –24.9 (1.7) |
| <i>Salvelinus fontinalis</i> juvenile ^f | | 0.225 (0.071) | 1.03 (0.16) | 12.1 (2.5) | 11.23 (0.06) | –25.9 (4.2) |
| <i>Salvelinus fontinalis</i> ^f | | 1.6 (1.2) | 1.04 (0.18) | 2.6 (1.6) | 11.99 (0.56) | –27.1 (2.0) |
| <i>Oncorhynchus mykiss</i> ^f | | 0.60 (0.29) | 0.74 (0.11) | 4.20 (2.5) | 11.97 (0.40) | –24.24 (0.94) |
| <i>Odontesthes hatcheri</i> juvenile ^f | | 0.161 (0.038) | 1.31 (0.24) | 21.0 (1.4) | 8.16 (0.70) | –24.17 (0.54) |
| <i>Odontesthes hatcheri</i> ^g | | 1.58 \pm 0.15 | 1.25 \pm 0.12 | 2.01 | 10.0 | –31.0 |
| <i>Galaxias maculatus</i> juveniles ^d | West | 1.7 (1.1) | 1.28 (0.17) | 2.4 (1.1) | 8.38 (0.95) | –24.15 (0.50) |
| <i>Galaxias maculatus</i> ^e | | 1.1 (1.3) | 1.26 (0.47) | 6.7 (5.2) | 8.22 (0.52) | –22.3 (1.1) |
| <i>Percichthys trucha</i> ^f | | 1.12 (0.25) | 1.154 (0.061) | 2.77 (0.67) | 9.79 (0.70) | –20.93 (0.88) |
| <i>Salvelinus fontinalis</i> juvenile ^f | | 0.530 (0.034) | 0.925 (0.074) | 4.43 (0.11) | 9.90 (0.53) | –23.3 (1.3) |
| <i>Salvelinus fontinalis</i> ^f | | 0.85 (0.57) | 1.07 (0.15) | 4.6 (3.7) | 11.0 (1.1) | –23.6 (1.6) |
| <i>Oncorhynchus mykiss</i> juvenile ^f | | 2.3 (1.8) | 1.36 (0.35) | 2.2 (1.4) | 8.1 (1.1) | –24.0 (2.4) |
| <i>Oncorhynchus mykiss</i> ^f | | 0.39 (0.19) | 0.806 (0.098) | 6.2 (2.3) | 10.65 (0.37) | –22.7 (1.5) |
| <i>Odontesthes hatcheri</i> juvenile ^f | | 0.80 (0.26) | 0.93 (0.15) | 3.22 (0.97) | 7.0 (1.4) | –21.3 (1.9) |
| <i>Salmo trutta</i> ^g | | 0.117 \pm 0.038 | 0.76 \pm 0.11 | 16.39 | 11.5 | –23.5 |

^a The analysis of each sample is reported in Supplementary Data.^b Dry weight basis; standard deviation of the average in parenthesis. In the case of pooled samples, the average is weighted by the number of individuals composing them.^c Molar ratio; standard deviation of the average in parenthesis.^d Small juveniles collected in the pelagic zone of the lake. Whole body pooled samples; the average is weighted by the number of individuals composing each pooled sample.^e Whole body without head and guts; the average is weighted by the number of individuals composing each pooled sample.^f Muscle samples.^g Single sample.

3.5. Food web trends

There were no correlations between $\delta^{15}\text{N}$ and [THg], [Se] or Se:Hg for the whole food web (Fig. 3). In particular, this was an unexpected finding for Hg, as Hg tends to biomagnify consistently in food webs world-wide (Campbell et al., 2005b; Chen et al., 2005). A typical slope value representing the biomagnification rate, would be roughly around 0.2 for log-10 transformed Hg vs $\delta^{15}\text{N}$ regression equations, regardless if the food web data was collected from tropical, temperate or polar regions, marine or freshwater (Campbell et al., 2005a). Even so, the lack of any significant relationships for Lake Moreno is not unique although is an uncommon situation: insignificant relationships also have been found for systems subject to differential distribution of bioavailable Hg at the base of the food web (Fowlie et al., 2008), unusual size structuring of the fish community (Kwon et al., 2012) and fish migrations and other food web shifts which result in less-than-clear stable isotope trends (Jardine et al., 2006). Unlike Hg, Se does not have a consistent food web biomagnification trend in literature: studies either find positive relationships with $\delta^{15}\text{N}$ (Orr et al., 2006; Stewart et al., 2004), or a lack of correlation (Campbell et al., 2005b; Gantner et al., 2009). This may be associated with the type of Se forms within the system and the mechanism of exposure from primary Se source within the waterbody (Orr et al., 2006). [Se] and [THg] both are negatively correlated with $\delta^{13}\text{C}$ (Fig. 3), indicating that biota with lighter $\delta^{13}\text{C}$ values, e.g. pelagic organisms, are key biota for the uptake of Hg and Se, but those elements are not being transferred through the food web.

Our list of hypotheses regarding the apparent lack of [THg] trophic biomagnification for the whole food web in both basins of Lake Moreno includes the following: (a) the top predators migrate frequently between the various connected lakes with different amounts of bioavailable Hg; (b) [THg] is biomagnifying up individual food chains leading to the top trophic fish species, but is being obscured by omnivory and variable concentrations in prey items; (c) some of the lower trophic species have particularly elevated [THg] that are not bioavailable obscuring the overall trend; or (d) [THg] biomagnification is suppressed throughout the food web, either via Se–Hg binding, growth rates or other factors.

In regards to Hypotheses (a) and (b), a previous SIA study showed evidence of relatively stationary habits within each Lake Moreno basin, with limited migration between interconnecting lakes, for the salmonid species and *P. trucha* (Arcagni et al., 2013). Furthermore, a closer examination of food chains leading to each top predator (e.g. large zooplankton – *G. maculatus*–*O. mykiss*; detritus–insect larvae – *O. viedmensis*) indicates that there was some increase in [THg] with trophic level, but it was not possible to elicit statistically significant correlations within those food chains. As already discussed above, elevated [THg] in plankton is largely in the inorganic form, which is not very bioavailable. This would further obscure any Hg biomagnification trends in the whole food web.

Therefore, we may consider Hypotheses (c) and (d). In both basins, the lack of correlations with $\delta^{15}\text{N}$ and the apparent relationship with $\delta^{13}\text{C}$, point to the importance of plankton for the uptake of [Se] and [Hg] through the whole food web.

Selenium is known to sequester Hg in stable compounds in tissues, and was reported associated with increased MeHg elimination due to high dietary Se in large fish species in fresh water systems (Bjerregaard et al., 2011; Sørmo et al., 2011), although the protective action of Se on Hg toxicity is well known for long time in marine systems (Ikemoto et al., 2004; Palmisano et al., 1995). This is due to the high affinity of Hg and Se in biological systems to form, with equimolar relation, mercury selenide (HgSe, also called tiemannite), a stable and insoluble compound (Cuvín-Aralar and Furness, 1991). The formation of inert Hg–Se compounds is the last step of Hg detoxification by Se, transforming Hg organic forms to these stable compounds in the hepatic tissue. Mercury demethylation and sequestration of inorganic

forms result in the accumulation of inert Hg–Se compounds in liver tissues (Palmisano et al., 1995). Selenium, unlike Hg, is an essential element for metabolic activity, and its concentration is physiologically regulated. Higher Se availability may increase the Se protective action on the toxicity of organic Hg; Se:Hg molar ratios over 1 imply Se molar excess in the tissue respect to HgSe, increasing the chance to participate in Hg detoxification. This is the case under study; Se:Hg molar ratios are consistently over 1 all along the food web, providing evidence, although not conclusive, that the high Se availability could be associated with the low THg transfer to the upper levels of the food web, and with the lack of Hg biomagnification.

To properly evaluate the incidence of Se in Hg demethylation and transformation to Hg–Se stable compounds, and hence Se incidence in Hg transfer along the food web given that mostly organic Hg bioaccumulates and biomagnifies, co-speciation of Hg and Se in the key tissues of the organisms is necessary. The target would be, basically, muscle and hepatic tissues. Actually, this is an analytical challenge. Some effort was carried on in Se speciation in muscle and liver tissue of *S. trutta*, *O. mykiss*, *S. fontinalis*, and *P. trucha* from North Patagonia lakes, including Lake Moreno (Kristan et al., 2013), but the results did not allow any conclusion related to Se protective effect on Hg toxicity. Moreover, researchers on heavy metals in marine organisms claimed that other elements, such as silver (Ag), may compete with Hg for binding sites on Se, thus limiting the Se:Hg molar ratio as a parameter to evaluate the potential protective effect of Se on Hg toxicity. Selenium could form a Ag stable compound, Ag₂Se, also detoxifying Ag (Agusa et al., 2008; Becker et al., 1995; Ikemoto et al., 2004). This could happen in Lake Moreno food web, given the elevated [Ag] determined in different organisms, particularly in fish liver (0.3 to 10 $\mu\text{g g}^{-1}$ DW) (Revenge et al., 2011; Ribeiro Guevara et al., 2005b), emphasizing the complexity in obtaining conclusive evidence of the Se protective effect when analyzing a entire food web.

4. Conclusions

The pelagic source of Hg to Lake Moreno food web was the first concern of the present work, regarding the elevated [THg] determined in plankton. Average [THg] in plankton ranged from 5 to 64 $\mu\text{g g}^{-1}$ DW, but the MeHg fraction was found to be very low (average 0.2 to 2.8%MeHg), implying limited pelagic Hg transfer to the entire food web. Passive uptake of Hg²⁺ was hypothesized as the main mechanism leading to so high [THg], enhancing incorporation on the smaller plankton but with limited transference to higher pelagic trophic levels. On the other side, the positive correlation of MeHg with $\delta^{15}\text{N}$ in the East basin of Lake Moreno provides evidence of MeHg biomagnification in the plankton community. The incidence of those high pelagic [THg] was observed in *G. maculatus*, a small planktivorous fish that combines pelagic and benthic feeding habits, with higher [THg] in pelagic specimens than littoral ones. Juveniles of *O. mykiss* and *O. hatcheri*, with similar ontogenetic dietary shifts as *G. maculatus*, showed also similar [THg] trends. These results underscore the importance of ontogenetic changes in fish diet in Hg trophodynamics in food webs. Particularly, the largely predominant inorganic form of pelagic Hg would obscure Hg biomagnification trends in the whole food web, linked by *G. maculatus* and juveniles of *O. mykiss* and *O. hatcheri*.

Littoral and pelagic organisms showed a consistent Se source to the entire food web in both Lake Moreno basins, with [Se] from 0.5 to 3 $\mu\text{g g}^{-1}$ DW (average values). Selenium biomagnification was observed in plankton. In fish muscle tissues [Se] ranged in similar values, increasing in liver tissues up to 100 $\mu\text{g g}^{-1}$ DW. The Se:Hg molar ratios were over 1 consistently along the food web, with values from 10 to 290 (average) in liver tissues of large fish, evidence of Se availability to participate in Hg detoxification. Higher [Se] in liver tissues were determined in fish species that exhibited lower [THg] in muscle tissues, result compatible with Se action on Hg detoxification. Although this is not conclusive evidence, the analysis of [Se] and Se:Hg molar ratios is

compatible with Se protection against Hg toxicity in Lake Moreno food web.

No positive correlation between $\delta^{15}\text{N}$ and [THg] was observed in both basins of Lake Moreno food web, and therefore, no THg biomagnification. These results, although are not unique, are unusual in food webs world-wide. Based on the data discussed, two concurrent factors could explain the apparent lack of Hg biomagnification in Lake Moreno food web; a large fraction of the elevated [THg] observed in the species at lower trophic levels is not bioavailable, and [THg] biomagnification suppression either via Se–Hg binding or other unidentified factors.

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Appendix A. Supplementary data

Tables S1 and S2 contain analytical QC results. Tables S3 to S20 report the analytical data ([THg], [Se], Se:Hg molar ratio, MeHg fraction, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) of the organisms studied in Lake Moreno food web. Supplementary data associated with this article can be found, in the online version, at doi: <http://dx.doi.org/10.1016/j.scitotenv.2013.03.008>.

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