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# Silver bioaccumulation in chironomid larvae as a potential source for upper trophic levels: a study case from northern Patagonia

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**Abstract** Silver (Ag) is a pollutant of high concern in aquatic ecosystems, considered among the most toxic metallic ions. In lacustrine environments, contaminated sediments are a source of Ag for the food web. Chironomidae (Insecta: Diptera) are the most abundant, diverse, and representative insect groups in aquatic ecosystems. Chironomid larvae are closely associated to benthic substrates and link primary producers and secondary consumers. Given their trophic position and their life habits, these larvae can be considered the entry point for the transference of Ag, from the benthic deposit to the higher trophic levels of the food web. Previous studies in lakes from Nahuel Huapi National Park (Northern Patagonia) showed Ag enrichment over background levels ( $0.04\text{--}0.1\ \mu\text{g g}^{-1}$  dry weight) both in biota (bivalves and fish liver) and sediments from sites near human settlements. The aim of this study was to analyze the role of chironomids in the transference of Ag from the benthic reservoir of Lake Moreno Oeste to the food web. The concentration of Ag in chironomid larvae tissue ranged from 0.1 to  $1.5\ \mu\text{g g}^{-1}$  dry

weight, reaching a bioaccumulation factor up to 17 over substrates and depending on the associated substrate type, feeding habitats, larval stage, and season. The main Ag transfer to higher trophic levels by chironomids occurs in the littoral zone, mostly from larvae inhabiting submerged vegetation (*Myriophyllum quitense*) and sediment from vegetated zones. This study presents novel evidence of the doorway role played by chironomid larvae in Ag pathways from the sediments into food webs of freshwater ecosystems.

**Keywords** Heavy metal · Bioaccumulation · Benthic organism · Lacustrine substrates · Lake Moreno Oeste · North Patagonia

## Introduction

Silver (Ag) is a natural occurring heavy metal, scarce in the Earth's crust ( $> 0.1\ \mu\text{g g}^{-1}$ ), which can be found at elevated concentrations in surface waters, associated with anthropogenic activities (Eisler 1996; Prucell and Peters 1998). This heavy metal is a pollutant of high concern for aquatic ecosystems, considered one of the most toxic metallic ions (Eisler 1996; Ratte 1999). In lakes from the Nahuel Huapi National Park (NHNP), northern Patagonia (Argentina), Ag contents were previously determined both in suspended load and biota (bivalves and fish liver) from sites near human settlements and the upper layers of sedimentary sequences. These studies revealed an enrichment of Ag over the background levels established for Patagonian environments ( $0.04\text{--}0.1\ \mu\text{g g}^{-1}$  dry weight). This increase is related to the large population expansion in the area. For example, brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) from Patagonian lakes have liver Ag concentrations ([Ag]) among the highest ever reported worldwide ( $10\text{--}29\ \mu\text{g g}^{-1}$  dry weight) (Ribeiro

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Guevara et al. 2005a; Revenga et al. 2011; Juncos et al. 2017). In Lake Nahuel Huapi (LNH), Ag enrichment was associated with the discharge of waste products from the photographic film processing industries, a well-known source of Ag in water bodies (Eisler 1996; Prucell and Peters 1998; Juncos et al. 2017).

Silver is an extremely particle-reactive metal, so it is easily scavenged from the water column and incorporated to the sediments, which are the main environmental reservoirs of this pollutant and a source of Ag for the aquatic food web (Rivera-Duarte and Flegal 1997; Prucell and Peters 1998). In Lake Nahuel Huapi, Juncos et al. (2017) observed that pelagic prey may not be important vectors of Ag for larger fish and piscivorous predators, suggesting a benthic uptake route for Ag. In Lake Moreno, biodilution processes of Ag were observed between primary producers and small forager fish when whole body [Ag] was considered (Revenga et al. 2011). Nevertheless, when considering whole food web biomagnification and hepatic [Ag] of top predator fish, a significant positive regression was found between [Ag] and trophic level (Revenga et al. 2011). Therefore, the understanding of Ag transference from the benthic reservoir to the aquatic biota is essential to understand Ag trophodynamics and the implications of Ag contamination for lacustrine ecosystems.

Chironomidae (Insecta: Diptera) are the most abundant, diverse, and representative group of aquatic insects (Walker 1995). Chironomid larvae are characterized by their wide spatial distribution, inhabiting different patches and substrates. Some species show a clear preference for a particular substrate, while others are versatile, utilizing different kinds of substrates (Pinder and Reiss 1983; Williams 2017). In Lake Moreno Oeste, chironomid larvae are present at the littoral, sublittoral, and deeper zones, inhabiting different types of substrates such as submerged decomposing leaves from the surrounding forest, stems of macrophytes, and bed sediments from vegetated and not vegetated zones at different depths (Williams 2017). These larvae are a frequent prey of both invertebrates and vertebrates (Tokeshi 1995). In Patagonian lakes, Chironomidae larvae are one of the main preys of small puyen (*Galaxias maculatus*) (Cervellini et al. 1993). In turn, this forager fish is the main food item for larger fish such as the introduced brown trout and rainbow trout and the native creole perch (*Percichthys trucha*) (Barriga et al. 2012; Juncos et al. 2013). It is then possible that chironomid larvae could be a link between the benthic reservoirs of Ag and upper trophic level organisms.

The aim of the present study was to determine the role of chironomid larvae in the uptake and the sourcing of Ag from the benthic reservoir to the food web in Lake Moreno Oeste (Northern Patagonia), assessing its bioaccumulation in relationship with their spatial and seasonal distribution and associated substrate type.

## Study site

Lake Moreno Oeste (41° 03' 33" S; 71° 32' 24" W), as is the case with most lakes in the NHNP, is an ultraoligotrophic lake of glacial origin. It is located 758 m above sea level and has an area of 5.22 km<sup>2</sup> and a maximum depth of 90 m (Buria et al. 2007). It is a warm monomictic lake that stratifies from late spring to early autumn, has an extended euphotic zone (Secchi disk ~ 20 m), and dissolved organic carbon concentrations of approximately 0.8 mg L<sup>-1</sup>, chlorophyll *a* of 1 µg L<sup>-1</sup>, total phosphorus of 4 µg L<sup>-1</sup>, and total nitrogen of 140 µg L<sup>-1</sup>. This lake has a highly irregular coastline, with peninsulas, bays, and flooded areas occupied by native forests, dominated by *Nothofagus dombeyi* (coihue) and lesser amounts of *Austrocedrus chilensis* (cordilleran cypress). In the littoral zone, the macrophytes *Myriophyllum quitense*, *Nitella* sp. and *Schoenoplectus californicus* are among the most abundant aquatic vegetation (Arcagni et al. 2013 and references therein). Lake Moreno Oeste is connected to Lake Moreno Este (area of 5.42 km<sup>2</sup> and maximum depth of 106 m) by a narrow channel (Buria et al. 2007) (Fig. 1).

The food web structure of Lake Moreno system was described by Arcagni et al. (2013). The benthic community is dominated by insect larvae (Diptera, Trichoptera, Odonata, Ephemeroptera, and Plecoptera), mollusks (*Chilina* sp. and *Diplodon chilensis*), annelids (Oligochaeta), *Hyalella* sp. amphipods, the crab *Aegla* spp., and the crayfish *Samastacus spinifrons* (Philippi). The fish community is composed by exotic species such as the rainbow trout, brown trout, and brook trout (*Salvelinus fontinalis*) and the native species, like the creole perch (*Percichthys trucha*), big puyen (*Galaxias platei*), and small puyen (*Galaxias maculatus*) (Barriga et al. 2012).

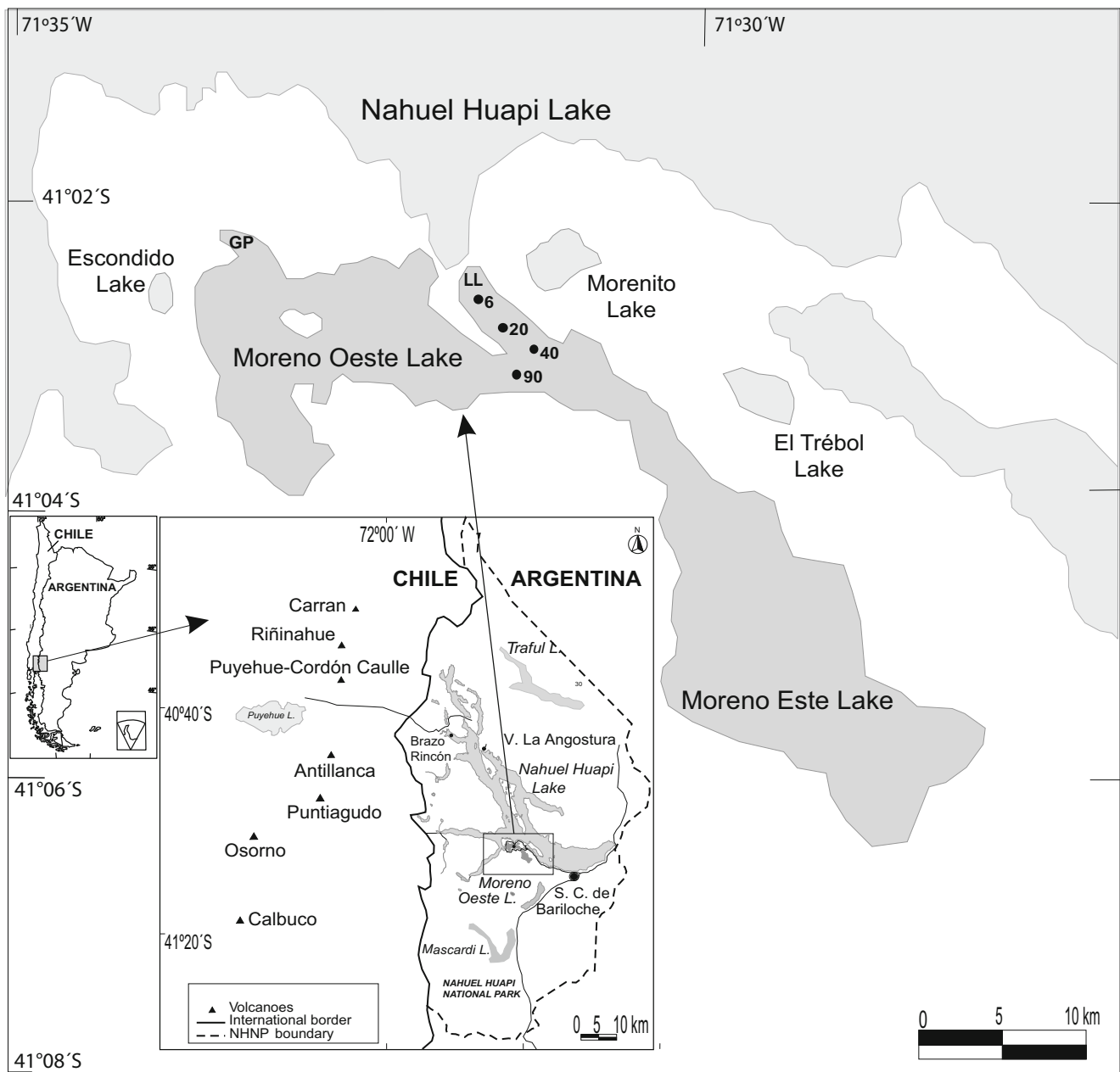
## Material and methods

### Sample collection

Substrate samples were collected seasonally, from April 2014 (austral autumn) to February 2015 (austral summer) from two bays in Lake Moreno Oeste: Llao Llao (LL) and Guardaparques (GP) (Fig. 1). Bed sediment samples were collected with an Eckman dredge (225 cm<sup>3</sup>) at 6, 20, 40, and 90 m deep (Fig. 1). In the littoral zone (from the shoreline to 6 m deep), sediment samples from vegetated areas (*M. quitense*, *Nitella* sp., and *S. californicus*), *M. quitense* leaves, and submerged riparian leaves were collected.

Sediment samples from the zone dominated by *M. quitense* and *Nitella* sp. were collected with an Eckman dredge. The sediment samples in the area occupied by *S. californicus* were collected in two ways: the upper 3 cm corresponding to the water-sediment interface were extracted with a plastic syringe, whereas the deeper sediment (approx. 10 cm) was collected





**Fig. 1** Study area. LL (Llao Llao) and GP (Guardaparques) indicate chironomids sampling bays. Black dots indicate sites sampled in sublittoral and deeper zones (6, 20, 40, and 90 m). Modified from Arribére et al. (2010) and Daga et al. (2010)

with a short plastic corer. Samples of *M. quitense* leaves were collected manually. For the collection of submerged decomposing leaves, a 531-cm<sup>2</sup> area was delimited in the riparian zone, and submerged leaves in the area were collected manually. All samples were stored in plastic bags. Six replicates of each substrate type were collected at each sampling season.

At the laboratory, chironomid larvae were separated from each substrate sample and identified under a binocular glass until a subfamily or tribe level was reached (Prat et al. 2011). Samples for metal analysis were done on the main chironomid taxa if the weight of the sample was above 0.5 mg. When the sample weight was not enough, a sample composed of pooled

individuals was used (Table 1). Replicate samples were prepared in some cases to evaluate variability (Table 1). Biological (*M. quitense* and riparian leaves) and sedimentary samples were kept for analysis after extracting chironomid larvae (“Analytical procedures”).

### Chironomid’s purge

The gut content of aquatic insects can represent a significant fraction of the contaminant body burden, particularly in those species that feed on sediment or detritus. Therefore, this fraction can introduce a substantial bias when analyzing whole

**Table 1** Chironomid samples studied for Ag contents in Lake Moreno Oeste

Sampling season	Substrate	Chironomidae sample composition	Chironomidae samples analyzed <sup>c</sup>
Austral autumn 2014	Littoral riparian leaves	–	–
	<i>M. quitense</i>	T. Tanytarsini, T. Chironomini, Tanypodinae, Orthoclaadiinae	Orthoclaadiinae and T. Chironomini
	Sed. <i>M. quitense</i>	T. Chironomini, Tanypodinae, Orthoclaadiinae	Tanypodinae, T. Chironomini, Orthoclaadiinae
	Sed. <i>S. californicus</i> sp. <sup>a</sup>	T. Tanytarsini, T. Chironomini, Tanypodinae, T. Pseudochironomini	Tanypodinae and T. Chironomini
	Sed. <i>S. californicus</i> sp. <sup>b</sup>	–	–
	Sed. <i>Nitella</i> sp.	T. Chironomini, Tanypodinae	Tanypodinae and T. Chironomini <sup>d</sup>
	Bed sed. 6 m depth	Tanypodinae, T. Pseudochironomini, T. Chironomini	T. Pseudochironomini (3 <sup>c</sup> ) and T. Chironomini
Austral winter 2014	Bed sed. 20 m depth	Tanypodinae, T. Chironomini	T. Chironomini
	Littoral riparian leaves	–	–
	<i>M. quitense</i>	T. Chironomini, T. Pseudochironomini, Orthoclaadiinae	T. Chironomini and Orthoclaadiinae <sup>d</sup>
	Sed. <i>M. quitense</i>	Tanypodinae, Orthoclaadiinae, T. Chironomini	T. Chironomini and Tanypodinae <sup>d</sup>
	Sed. <i>S. californicus</i> sp. <sup>a</sup>	–	–
	Sed. <i>S. californicus</i> sp. <sup>b</sup>	–	–
	Sed. <i>Nitella</i> sp.	Tanypodinae, Orthoclaadiinae, T. Pseudochironomini, T. Chironomini	Tanypodinae
Austral spring 2014	Bed sed. 6 m depth	Tanypodinae, T. Pseudochironomini, T. Chironomini	T. Pseudochironomini (5 <sup>c</sup> )
	Bed sed. 20 m depth	Tanypodinae, T. Pseudochironomini, T. Chironomini	T. Chironomini
	Littoral riparian leaves	–	–
	<i>M. quitense</i>	T. Tanytarsini, T. Chironomini, Orthoclaadiinae	Tanypodinae
	Sed. <i>M. quitense</i>	T. Chironomini, Tanypodinae, T. Pseudochironomini	Tanypodinae and T. Chironomini
	Sed. <i>S. californicus</i> sp. <sup>a</sup>	–	–
	Sed. <i>S. californicus</i> sp. <sup>b</sup>	–	–
Austral summer 2015	Sed. <i>Nitella</i> sp.	T. Chironomini, Prodiamesinae, Tanypodinae, T. Tanytarsini	Tanypodinae
	Bed sed. 6 m depth	T. Tanytarsini, T. Chironomini, Tanypodinae, T. Pseudochironomini	T. Chironomini and T. Pseudochironomini <sup>d</sup> (2 <sup>c</sup> )
	Bed sed. 20 m depth	T. Chironomini, Tanypodinae	T. Chironomini
	Littoral riparian leaves	Tanypodinae	Tanypodinae
	<i>M. quitense</i>	T. Chironomini, T. Tanytarsini, T. Pseudochironomini, Orthoclaadiinae	1. T. Pseudochironomini and T. Chironomini
	Sed. <i>M. quitense</i>	T. Chironomini, T. Tanytarsini, T. Pseudochironomini, Tanypodinae, Orthoclaadiinae	T. Chironomini and T. Pseudochironomini <sup>d</sup> (3 <sup>c</sup> )
	Sed. <i>S. californicus</i> sp. <sup>a</sup>	Tanypodinae, T. Tanytarsini, T. Chironomini, T. Pseudochironomini	Tanypodinae and T. Pseudochironomini <sup>d</sup>
Austral summer 2015	Sed. <i>S. californicus</i> sp. <sup>b</sup>	T. Pseudochironomini, T. Tanytarsini, Tanypodinae	T. Tanytarsini and Tanypodinae <sup>d</sup>
	Sed. <i>Nitella</i> sp.	Tanypodinae, T. Chironomini	Tanypodinae
	Bed sed. 6 m depth	Tanypodinae, T. Chironomini	T. Chironomini
	Bed sed. 20 m depth	Tanypodinae, T. Chironomini	T. Chironomini and Tanypodinae <sup>d</sup>

– no larvae were observed

<sup>a</sup> Sediment below the water-sediment interface

<sup>b</sup> Sediment from the water-sediment interface

<sup>c</sup> When replicates were analyzed, they are in parenthesis

<sup>d</sup> Pooled sample with similar amount of each group

organisms to assess the assimilation of metals into tissues (Cain et al. 1995). Trophic transfer of heavy metals occurs when they are incorporated in the tissues of a lower trophic level organism, which is in turn consumed by another organism from a higher trophic level. However, heavy metals present in

the gut, which are not assimilated, will not be incorporated and transferred to upper trophic levels (Suchanek et al. 2008). As the main objective of this work was to evaluate chironomids as a doorway for the incorporation of Ag from the benthic reservoir to the food web, each chironomid sample was purged

before analysis. This procedure consisted in keeping the chironomid larvae in beakers containing ASTM (American Society for Testing and Materials) type 1 water for 48–72 h until their guts were emptied (Brooke et al. 1996; Suchanek et al. 2008). Once the chironomids were purged, they were recovered and prepared for metal analysis. Feces (named here as purged material) were also recovered and prepared for analysis when the mass was significant (> 0.5 mg).

### Analytical procedures

Samples of chironomids, purged material, and biological substrates (*M. quitense* and riparian leaves) were placed in SUPRASIL quartz ampoules for analysis and freeze-dried until constant weight. The ampoules were sealed afterwards.

For sediment substrates, the fraction analyzed was < 63  $\mu\text{m}$  since chironomids ingest fine sediment particles. Thereby, sediment samples were also freeze-dried and sieved with a 63- $\mu\text{m}$  mesh size sieve. This sediment fraction was separated and placed in plastic vials for analysis.

All samples were irradiated in the RA-6 research nuclear reactor (Centro Atómico Bariloche, Argentina). Elemental concentrations were determined by Instrument Neutron Activation Analysis (INAA). The ampoules were irradiated for 20 h whereas sediments were irradiated for 6 h; two gamma-ray spectra were collected after irradiation at different decay times using an intrinsic High Purity Germanium (HPGe) detector and a 4096-channel analyzer. Elemental concentrations were determined using the absolute parametric method. The concentrations were reported on dry weight (DW) basis. Analytical uncertainty is reported after “ $\pm$ .”

### Geological material contamination

Inorganic particulates of geological origin may contaminate biota samples, biasing Ag determinations. Although in this work chironomid samples were carefully cleaned and purged, there is the possibility that they included geological particles. In order to obtain elemental concentrations representative of the biological tissues under study, it was necessary to correct the elemental concentration of biota samples by subtraction of geological particulate contributions. Geological particulate remains were detected in chironomid samples by the determination of lithophile elements, such as the rare earth element samarium (Sm). INAA is a multielemental technique which allows the simultaneous determination of up to 35 elements, including the geochemical tracer Sm. Samarium showed the highest sensitivity in the biological samples analyzed among the geochemical tracers that can be determined by INAA. It was therefore used to evaluate the contribution of geological particles in samples and to perform concentration corrections (Juárez et al. 2016; Juncos et al. 2017). The remaining material after the subtraction of geological contamination

corresponded to the biological fraction of the sample, calculated as follows:

$$F_b = 1 - \frac{C_{L,V}}{C_{L,G}}$$

- $F_b$  Biological fraction of the sample, determined by subtraction of the geological fraction evaluated by the determination of a lithophile element (Sm)
- $C_{L,V}$  Concentration of the lithophile element (Sm) measured in the biota sample
- $C_{L,G}$  Concentration of the lithophile element (Sm) measured in the geological material contaminating the biota sample

The correction can be computed only when a lithophile element is measured in the biota sample. Detection limits in INAA determinations depend on the irradiation conditions and on the composition of the sample, which can vary significantly among samples. In this case, detection limits for Sm were low enough to assure that any relevant geological particulate interference was below analytical uncertainties.

Another step to implement this correction is to determine elemental concentrations in the geological material contaminating biota samples. The results of the analysis of sediment samples were used to determine the biological fraction and to implement the correction of the [Ag] by geological material contamination in each chironomid sample. Concentration of Ag in chironomid samples discussed here includes this correction.

The correction of [Ag] in chironomid samples due to geological particulate contamination is not reliable for samples with low biological fractions, given that geological material is dominant. In chironomid samples with biological fractions below 50%, [Ag] were not considered for correction by geological particulate contamination. Samples with analytical corrections larger than 50% were also disregarded.

### Ag bioaccumulation factor

Determination of bioaccumulation factors (BAFs) is a method to evaluate the long-term average bioaccumulation of metals in aquatic organisms (Banerjee et al. 2015). Silver BAFs were calculated here for the chironomid larvae according to their respective substrates, in order to evaluate the seasonal incorporation of Ag in these organisms. This procedure permits the comparison of the bioaccumulation level between samples collected seasonally from different substrates. The formula used to calculate Ag BAFs is the ratio between the heavy metal concentrations in the organism according to their corresponding substrate, as follows:

$$BAF = \frac{[Ag]_{ch}}{[Ag]_s}$$

$[Ag]_{ch}$  [Ag] in chironomid larvae  
 $[Ag]_s$  [Ag] in chironomid substrate

## Results

### Silver in substrates

Biological substrates (*M. quitense* and riparian leaves) exhibited the lowest [Ag], with values ranging between 0.01 and 0.1  $\mu\text{g g}^{-1}$  DW (Fig. 2; Table I, supporting information). In these substrates, [Ag] were similar among seasons, being the mean value in *M. quitense* 0.072 (0.022)  $\mu\text{g g}^{-1}$  DW (standard deviation between brackets), similar to littoral riparian leaves 0.062 (0.013)  $\mu\text{g g}^{-1}$  DW. Sediment samples presented higher [Ag] than biological substrates, ranging from 0.037 to 6  $\mu\text{g g}^{-1}$  DW (Fig. 2; Table I, supporting information). Among sediment samples, those from vegetated zones ranged in lower levels, averaging 0.171 (0.107)  $\mu\text{g g}^{-1}$  DW, as well as sediment at 20 m deep, averaging 0.176 (0.048)  $\mu\text{g g}^{-1}$  DW. The highest [Ag] were measured in sediment from the sublittoral zone (at 6 m deep) averaging 2.36 (2.55)  $\mu\text{g g}^{-1}$  DW, reaching up to 6  $\mu\text{g g}^{-1}$  DW in spring (Fig. 2; Table I, supporting information). These values were tenfold higher than those found for the other sediment types.

### Silver in chironomids with respect to substrates (BAF)

In substrates from the littoral zone (sediment from vegetated zones, *M. quitense*, and submerged riparian leaves) and bed sediments at 20 m deep, larvae exhibited high [Ag] over their respective substrate, showing a range of BAF values between 0.86 and 17.4 (Table 2). In the sediments at 40 and 90 m deep, chironomid larvae were absent.

In *M. quitense* leaves, chironomid larvae recorded the highest [Ag] compared to the [Ag] measured in this macrophyte (Fig. 2), so that BAF values in this substrate were the highest, with a maximum of 17.4 in winter (Table 2). Although *M. quitense* showed low [Ag] in different sampling seasons, the larvae associated with it averaged 0.84 (0.53)  $\mu\text{g g}^{-1}$  DW. It should be noted that during spring, the BAF was four- to sixfold lower than those observed in other seasons, despite the fact that larvae from *M. quitense* recorded [Ag] higher than those from this substrate (Table 2). Larvae inhabiting this macrophyte belonged to Orthocladiinae and Chironominae (tribe Chironomini)

subfamilies, except during spring; those larvae were from the Tanypodinae subfamily (Table 1).

In submerged riparian leaves, chironomid larvae were observed only during summer, where the [Ag] was elevated compared with the substrate, recording a BAF value of 14.4. All these larvae belonged to the Tanypodinae subfamily (Tables 1 and 2).

The [Ag] in chironomid larvae of the sediments from the vegetated areas dominated by *M. quitense*, *Nitella* sp., and *S. californicus*, ranged from 0.1 to 2.5  $\mu\text{g g}^{-1}$  DW, and the BAF values fluctuated between 0.86 and 10.5. All these individuals belonged to the Tanypodinae, Orthocladiinae, and Chironominae subfamilies (tribe Chironomini) (Table 1).

Finally, larvae from sediments at 20 m deep registered [Ag] between 0.216 and 0.64  $\mu\text{g g}^{-1}$  DW, recording BAF values between 1.2 and 5.45 (Table 2). These larvae belonged to the Chironominae subfamily (Chironomini tribe), except in summer, where members of Tanypodinae were also observed (Table 1).

In contrast, chironomids from the sublittoral zone showed low [Ag] compared with those from the sediments, which represents the lowest BAFs in winter and spring (< 0.05) (Table 2). All these larvae belonged to the Chironominae subfamily (Pseudochironomini and Chironomini tribes) (Table 1).

### Silver in purged material

Nine samples of purged material were recovered for metal analysis. In general, purged material recovered from larvae inhabiting sediment substrates showed higher [Ag] (between 0.125 and 0.78  $\mu\text{g g}^{-1}$  DW) (Table II, supporting information) than those observed in both the corresponding larvae and sediments (Fig. 2; Table 2; Table I, supporting information). At the sublittoral zone, the purged material recorded elevated [Ag] in autumn, winter, and summer (0.83, 0.67, and 0.56  $\mu\text{g g}^{-1}$ , respectively) (Fig. 2; Table II, supporting information). However, these values were lower than the [Ag] observed in sediments.

In biological substrates, the [Ag] on purged material from larvae living on *M. quitense* and riparian leaves (0.6 and 0.83  $\mu\text{g g}^{-1}$  DW, respectively) is elevated compared with the [Ag] measured in these substrates (0.058 and 0.0596  $\mu\text{g g}^{-1}$  DW, respectively).

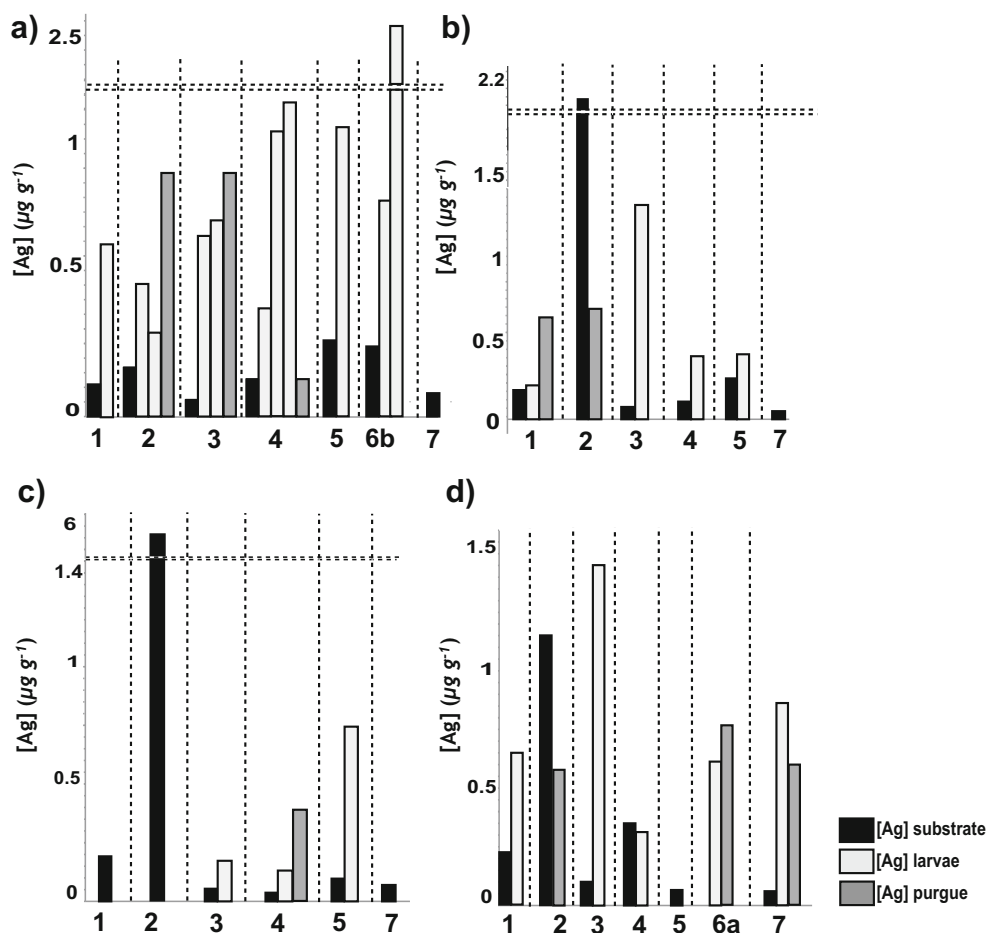
## Discussion

### Silver in substrates

In Lake Moreno Oeste, [Ag] measured in substrates from the littoral zone and sediments at 20 m deep exhibited comparable values with the background levels known for Patagonian environments (0.04–0.1  $\mu\text{g g}^{-1}$  DW) (Ribeiro Guevara et al.



**Fig. 2** Silver concentration ([Ag]) in substrates, chironomid larvae, and purged material from Lake Moreno Oeste. **a** Autumn. **b** Winter. **c** Spring. **d** Summer. 1. Sediment 20 m deep. 2. Sediment 6 m deep. 3. *M. quitense*. 4. Sediment *M. quitense*. 5. Sediment *Nitella* sp. 6a. Sediment from the water-sediment interface from *S. californicus*. 6b. Sediment below the water-sediment interface from *S. californicus*. 7. Riparian leaves



2005b; Juárez et al. 2016). In contrast, [Ag] recorded in the sublittoral zone is higher than the background levels, being comparable with previous reports associated with anthropic contamination from Lake Nahuel Huapi (1.68–4.58  $\mu\text{g g}^{-1}$  DW) (Ribeiro Guevara et al. 2002; Juncos et al. 2017). As in Lake Nahuel Huapi, these high values suggest contamination of anthropogenic origin in Lake Moreno Oeste (Ribeiro Guevara et al. 2002; 2005a, b; Juncos et al. 2017). Additionally, these results are comparable with those of anthropogenic pollution in European aquatic systems: 0.4–0.9  $\mu\text{g g}^{-1}$  DW (Grahn et al. 2006) and 0.4–6.7  $\mu\text{g g}^{-1}$  DW (Lanceleur et al. 2011).

#### Silver in chironomids with respect to substrates (BAF)

Different responses when analyzing BAF values of Ag were observed. For substrates from littoral zones and sediments at 20 m deep, BAFs presented high values, with the highest for chironomids from biological substrates (Table 2). In contrast, lower BAFs were observed in organisms from the sublittoral zone, particularly during winter and spring (Table 2). These BAF variations confirmed the importance of spatial dependent

variables, particularly substrate type, in studies about Ag uptake by chironomid larvae.

Highest BAFs were found in chironomid larvae inhabiting *M. quitense* leaves. These larvae belonged mainly to the Chironominae (Chironomini and Pseudochironomini tribes) and Orthoclaadiinae subfamilies; this latter is represented by *Parapsectrocladius escondido* Cranston & Añón-Suárez, 2000 (Williams 2017). Orthoclaadiinae larvae are mainly herbivorous (Coffman and Ferrington 1996; Armitage et al. 1995), with shredding or scraping habits. *Parapsectrocladius escondido* probably feeds on periphyton, phytoplankton, or detrital debris surrounding macrophyte structures. The feeding habits of these larvae are relevant, given that Revenga et al. (2011) recorded elevated [Ag] in phytoplankton ( $1.82 \pm 3.00 \mu\text{g g}^{-1}$  DW) for Lake Moreno Oeste. Previous works have established that because of their physiology and density, submerged plants contribute directly to the particulate organic pool used by invertebrate populations as a food source or indirectly through leaching of dissolved organic matter (Beaty et al. 2006; Christensen et al. 2009). Therefore, chironomid feeding habits in zones dominated by macrophytes seem to play a key role in enhancing Ag bioaccumulation.

**Table 2** Biological fraction and silver concentration [Ag] in chironomid samples

Sampling season	Substrate	Chironomidae samples analyzed <sup>a</sup>	Biological fraction (%)	Ag conc. ( $\mu\text{g g}^{-1}$ ) <sup>e</sup>	Ag conc. ( $\mu\text{g g}^{-1}$ ) <sup>f</sup>	Bioaccumulation factor (BAF)
Austral autumn 2014	Littoral riparian leaves	–	–	–	–	–
		<i>M. quitense</i>	Orthocladiinae	100	0.61 ± 0.15	0.61 ± 0.15
	Sed. <i>M. quitense</i>	T. Chironomini	98	0.66 ± 0.15	0.67 ± 0.16	11.6
		Tanypodinae	99	0.365 ± 0.083	0.368 ± 0.084	2.85
		T. Chironomini	83	0.91 ± 0.36	1.07 ± 0.43	8.29
	Sed. <i>S. californicus</i> sp. <sup>b</sup>	Orthocladiinae	93	1.10 ± 0.56	1.17 ± 0.61	9.07
		Tanypodinae	82	0.62 ± 0.29	0.74 ± 0.36	3.08
		T. Chironomini	56	1.45 ± 0.55	2.53 ± 0.95	10.5
	Sed. <i>S. californicus</i> sp. <sup>c</sup>	–	–	–	–	–
	Sed. <i>Nitella</i> sp.	Tanypodinae and T. Chironomini <sup>d</sup>	94	1.04 ± 0.49	1.09 ± 0.52	4.19
	Bed sed. 6 m deep	T. Pseudochironomini (3 <sup>a</sup> )	70	0.358 ± 0.072	0.460 ± 0.091	2.72
		T. Chironomini	100	0.289 ± 0.088	0.289 ± 0.088	1.71
	Bed sed. 20 m deep	T. Chironomini	49	0.35 ± 0.12	0.60 ± 0.25	5.45
	Austral winter 2014	Littoral riparian leaves	–	–	–	–
<i>M. quitense</i>		T. Chironomini and Orthocladiinae <sup>d</sup>	94	1.24 ± 0.53	1.32 ± 0.57	17.4
Sed. <i>M. quitense</i>		T. Chironomini and Tanypodinae <sup>d</sup>	83	0.33 ± 0.11	0.38 ± 0.14	3.49
Sed. <i>S. californicus</i> sp. <sup>b</sup>		–	–	–	–	–
Sed. <i>S. californicus</i> sp. <sup>c</sup>		–	–	–	–	–
Sed. <i>Nitella</i> sp.		Tanypodinae	74	0.321 ± 0.095	0.40 ± 0.14	1.59
Bed sed. 6 m deep (2) <sup>d</sup>		T. Pseudochironomini (5 <sup>a</sup> )	72	0.226 ± 0.057	< 0.1	< 0.05
		T. Chironomini	76	0.188 ± 0.068	0.216 ± 0.091	1.20
Austral spring 2014	Littoral riparian leaves	–	–	–	–	–
	<i>M. quitense</i>	Tanypodinae	99	0.162 ± 0.071	0.163 ± 0.072	3.02
	Sed. <i>M. quitense</i>	Tanypodinae	98	0.120 ± 0.060	0.121 ± 0.061	3.27
		T. Chironomini	22	< 0.9	–	–
	Sed. <i>S. californicus</i> sp. <sup>b</sup>	–	–	–	–	–
	Sed. <i>S. californicus</i> sp. <sup>c</sup>	–	–	–	–	–
	Sed. <i>Nitella</i> sp.	Tanypodinae	74	0.56 ± 0.22	0.73 ± 0.30	7.53
	Bed sed. 6 m deep	T. Chironomini and T. Pseudochironomini <sup>d</sup> (2 <sup>a</sup> )	51	0.35 ± 0.16	< 0.1	< 0.02
T. Chironomini		34	1.21 ± 0.20	–	–	
Austral summer 2015	Littoral riparian leaves	Tanypodinae	99	0.86 ± 0.25	0.86 ± 0.25	14.4
	<i>M. quitense</i>	T. Pseudochironomini	26	0.59 ± 0.18	–	–
		T. Chironomini	98	1.43 ± 0.22	1.45 ± 0.23	14.4
	Sed. <i>M. quitense</i>	–	48	0.33 ± 0.11	0.30 ± 0.20	0.86

**Table 2** (continued)

Sampling season	Substrate	Chironomidae samples analyzed <sup>a</sup>	Biological fraction (%)	Ag conc. ( $\mu\text{g g}^{-1}$ ) <sup>e</sup>	Ag conc. ( $\mu\text{g g}^{-1}$ ) <sup>f</sup>	Bioaccumulation factor (BAF)
		T. Chironomini and T. Pseudochironomini <sup>d</sup> (3 <sup>a</sup> )				
	Sed. <i>S. californicus</i> sp. <sup>b</sup>	Tanypodinae and T. Pseudochironomini <sup>d</sup>	< 10	0.79 ± 0.32	–	–
	Sed. <i>S. californicus</i> sp. <sup>c</sup>	T. Tanytarsini and Tanypodinae <sup>d</sup>	79	0.53 ± 0.17	0.61 ± 0.22	> 3
	Sed. <i>Nitella</i> sp.	Tanypodinae	26	0.69 ± 0.17	–	–
	Bed sed. 6 m deep	T. Chironomini	38	< 0.3	–	< 0.3
	Bed sed. 20 m deep	T. Chironomini and Tanypodinae <sup>d</sup>	100	0.64 ± 0.11	0.64 ± 0.11	2.87

<sup>a</sup> Replicates in parenthesis; values reported are replicates average

<sup>b</sup> Sediment below the water-sediment interface

<sup>c</sup> Sediment from the water-sediment interface

<sup>d</sup> Pooled sample with similar amount of each group

<sup>e</sup> Concentrations measured

<sup>f</sup> Concentrations corrected by geological particulate contamination

In spring, although larvae from *M. quitense* recorded [Ag] higher than the macrophyte, the BAF value was lower than those observed during other seasons (Table 2). Larvae from this substratum were mainly from the Tanypodinae subfamily (*Apsectrotanypus* sp.), and based on their larger size, these larvae corresponded to larval stages III and IV (Williams 2017), which are characterized by their predatory habits, unlike earlier stages (I and II) which are collectors (Coffman and Ferrington 1996; Ashe et al. 1987). Therefore, the different responses between specific chironomid groups could be related with dissimilar feeding habits and ecological preferences. The findings presented here could be indicative of biodilution processes between different trophic levels of the family Chironomidae. The results presented in this work are in line with previous studies conducted in Lake Moreno Oeste, where [Ag] decrease with increasing trophic level (biodilution), between primary producers and small forage fish (Arribère et al. 2010; Revenga et al. 2011). In this context, it is suggested that biodilution processes also occur between different functional groups of chironomids.

In submerged riparian leaves, the larvae belonged to early stages (I and II) of *Alotanypus vittigera* and *Ablabesmyia* sp. (Tanypodinae) based on their smaller sizes (Williams 2017). Due to the collector habits of larval stages I and II, they are at a lower trophic level than stages III and IV, therefore are more exposed to higher Ag bioaccumulation. Collector larvae in submerged riparian leaves exhibited high enrichment with respect to the substrate, confirming the high potential for Ag bioaccumulation of chironomid larvae at the base of the food web. This occurrence has been reported previously by

Krantzberg (1989), who found higher concentrations of different metals (Al, Cd, Ni) in smaller chironomids, compared to larger larvae. Given that no essential function has been attributed to Ag, it is possible that the changes in Ag bioaccumulation with time may represent changes in the organism's ability to eliminate Ag, or to the exposure level to bioavailable Ag. Life history traits, like metabolic and feeding rates and food preferences, differ greatly between different sized animals; hence, it would be also likely that exposure to and incorporation of metals can change throughout the life of an individual (Krantzberg 1989).

The elevated [Ag] recorded in chironomid larvae from sediments of vegetated zones are possibly related with the ability of plants to accumulate a high [Ag] in their root systems (Ratte 1999), enhancing metal accumulation in larvae inhabiting and feeding in the surrounding sediments.

Finally, larvae from the sediments at 20 m deep exhibited a lower BAF during winter compared to autumn and summer (Table 2; Fig. 2). In Lake Moreno Oeste, water temperature in winter was 8 °C (10 °C lower than in summer). The decrease in the metabolic rate associated with low temperature and organic matter content could explain this response during the winter season, since a lower metabolic rate implies all biological processes, including Ag uptake, are slower (Nichols and Playle 2004; Kirschbaum 2006). Another explanation comes from the proportion of larvae types in each season: samples in summer recorded a higher proportion of early stages (I and II) of Tanypodinae (*Apsectrotanypus* sp.) compared to winter, which was composed only by members of Chironominae (Chironomini tribe) (Williams 2017). This is consistent with

the results described for the biological substrates, given that early stages of Tanypodinae have collector habits and higher Ag BAFs. Furthermore, the sediment at 20 m deep is characterized by a finer granulometry compared with littoral samples. Sediments composed by a small particle size are characterized by high metal concentration due to the higher surface/volume ratio (Moore et al. 1989); therefore, the metal accumulation could be higher in this zone.

In Lake Moreno Oeste, larvae from the sublittoral zone presented lower [Ag] than their sediments. This zone was dominated by the subfamily Chironominae (Pseudochironomini and Chironomini tribes), mainly *Riethia truncatocaudata*, *Cryptochironomus* sp., and *Dicrotendipes* sp. These taxa were observed in close association with the bed sediment, burrowing in the upper layers (Williams 2017). They are known to feed on detritus, which includes all non-living particulate organic matter and associated non-photosynthetic microorganisms (Coffman and Ferrington 1996; Ashe et al. 1987). A possible explanation for the lower [Ag] in these larvae compared to their substrate is the low assimilation of the ingested material (cellulose, lignin, and ash) by detritivorous invertebrates, which rapidly passes through the gut (Berg 1995), enhancing the rapid elimination of contaminants. In the case of other highly nutritious food sources, the explanation could be the increased metabolic rate in the presence of such food type, which would allow a greater detoxification rate (De Haas et al. 2006). In either case, contaminants would be eliminated rapidly; therefore, the toxic effects could have a lower impact in these organisms.

### Silver in purged material

Higher [Ag] in purged material compared to sediment samples could be related to the fact that heavy metals are adsorbed onto the surface of particles, so sediments composed by small particle size have higher metal concentration due to the higher surface/volume ratio (Moore et al. 1989). A possible explanation of the higher [Ag] recorded in purged material compared with the respective sediment could be that larvae ingest particles of the sediment of smaller fraction than those analyzed in this work ( $< 63 \mu\text{m}$ ). Concurrently, it has been reported that several collector species of Chironominae and Orthocladiinae ingest sediment particles between 0.5 and 75  $\mu\text{m}$ , a range denominated “very fine particulate organic material” (Henriques-Oliveira et al. 2003).

In biological substrates with a lower geological particulate uptake, elimination of heavy metals as a response to metabolic activity could explain the rather high [Ag] in purged material, which is a potential indication of a highly efficient detoxification mechanism (Fig. 2).

### Conclusion

In Lake Moreno Oeste, the [Ag] in chironomid larvae varied within a broad range, from 0.1 to 2.5  $\mu\text{g g}^{-1}$  dry weight, reaching a bioaccumulation factor up to 17-fold according to the type of substrate, feeding habits, larval stages, and seasonality. The main Ag transference from the benthic deposit to chironomid larvae takes place at the littoral zone, specifically from submerged vegetation (*Myriophyllum quitense*) and sediments from vegetated zones. Submerged riparian leaves and sediments at 20 m deep also provided Ag inputs, but only during summer.

In Patagonian lakes, chironomid larvae are an important prey for small puyen, and this small fish is the main item in the diet of exotic and native fishes (Barriga et al. 2012; Juncos et al. 2013). Considering the Ag enrichment in larvae from Lake Moreno Oeste compared to their environment, our results indicate that these larvae could act as a possible source of Ag to higher trophic levels of the food chains of Patagonian lacustrine environments.

In general, the [Ag] in substrates from the littoral and deeper zones was similar to the known background levels for Patagonian environments (0.04–0.1  $\mu\text{g g}^{-1}$  DW). The exceptions were the sediments from the sublittoral zone, which had the highest [Ag] (up to 6  $\mu\text{g g}^{-1}$  DW). These values are comparable to sites in Lake Nahuel Huapi (1.68–4.58  $\mu\text{g g}^{-1}$  DW) associated with urban development and anthropic contamination. However, the chironomid larvae inhabiting the sublittoral zone presented the lowest [Ag] ( $< 0.1 \mu\text{g g}^{-1}$  DW) and therefore the lowest BAFs ( $< 0.05$ ), suggesting a low rate of Ag assimilation or an efficient elimination of contaminants. This finding is consequential with the high [Ag] registered in the purged material, which was higher than those found for both the larvae and the substrates.

The present study suggests that the relationship between Ag bioaccumulation in larvae is dependent on the feeding habits of the different functional groups of Chironomidae. Larvae of collector, shredder, and scrapper feeding habits (Chironominae, Orthocladiinae, and larval stages I and II of Tanypodinae) recorded [Ag] higher than larvae of predator habits (larval stages III and IV of Tanypodinae), which are at upper trophic levels in the food web than the former.

The contrasting results obtained in this research confirm the importance of sampling several substrates and seasons, to better represent the underlying variability in order to understand metal contamination and accumulation in chironomid larvae. Given the variability among seasons, chironomid taxa, and substrates, future works on metal-contaminated systems should consider a broad range of taxa, larval stages, functional groups, substrate types, and sampling periods, to pinpoint the key factors influencing metal bioavailability in lentic benthic communities.



Therefore, we conclude that the bioavailability, and so the bioaccumulation, of Ag to higher trophic levels of the food web through chironomid larvae will depend on certain ecological aspects, such as the type of substrate, the feeding habitats, the larval stages, and the seasonal changes. The high [Ag] found in chironomids after purging and correction for geological particulate contamination and the elevated enrichment over substrates suggest that chironomid larvae could be acting as a potential source of Ag to the food web of Patagonian lacustrine environments.

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