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Hg^{2+} incorporation in Andean Patagonian ultraoligotrophic lakes: insights into the role of pelagic protists

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ABSTRACT. In ultraoligotrophic lakes of Andean Patagonia, microbial assemblages at the base of pelagic food webs bear high THg concentrations compared to planktonic consumers. In this study, we evaluate experimentally the passive and active (trophic) uptake of Hg^{2+} using $^{197}Hg^{2+}$ to trace Hg incorporation in picoplankton (autotrophic and heterotrophic bacteria), in the photoautotrophic phytoflagellate, *Gymnodinium paradoxum*, and in the mixotrophic ciliates, *Stentor araucanus* and *Ophrydium naumanni*. The studied protists were found to incorporate substantial amounts of dissolved Hg^{2+}; however, their potential for Hg transference to higher trophic levels depends on their degree of Hg internalization (cytoplasmatic Hg), which varied widely among species.

Keywords: Andean Patagonian lakes, microbial assemblages, Hg^{2+} incorporation

1. Introduction

Andean North Patagonia has numerous deep clear ultraoligotrophic lakes (Queimaliños et al., 2012; 2019) with catchments impacted by active volcanoes of the Southern Volcanic Zone (Rizzo et al., 2014). Most of these lakes have very low levels of dissolved organic carbon (DOC < 0.7 mg L^{-1}) and, depending on the proximity to the volcanic source, show moderate to high levels of total mercury (THg), resulting in elevated THg:DOC ratios and thus, remarkable high availability for fractionation/bioaccumulation (Soto Cárdenas et al., 2018a). In the pelagic food webs of Andean Patagonian lakes, THg levels are consistently higher in the smaller planktonic fraction 10-53 µm comprising picoplanktonic and nanoplanktonic species, the main constituents of the microbial loop (Arribére et al., 2010; Rizzo et al., 2014; Soto Cárdenas et al., 2014). Larger planktonic fractions up to 200 µm, including protists and mixotrophic ciliates, rotifers and small crustaceans, show comparatively lower THg but higher levels than the fraction > 200 µm (Rizzo et al., 2014; Arcagni et al., 2018).

In this investigation, we experimentally evaluate the uptake of Hg^{2+} in key components of microbial assemblages of Andean lakes, including the picocyanobacteria *Synechococcus* sp., and three protists, the photoautotrophic phytoflagellate, *Gymnodinium paradoxum* (Chromista, Dinophyceae), and the mixotrophic ciliates, *Stentor araucanus* and *Ophrydium naumanni* (Protozoa, Ciliophora, Heterotrichida), both bearing the endosymbiotic green alga *Chlorella*. We hypothesize that the passive incorporation of Hg by the different species is determined by morphological traits [surface (S), volume (V), S:V], while the active Hg^{2+} incorporation depends on the trophic transfer through picoplankton consumption.

2. Materials and methods

2.1. Sample collection and processing

Water samples for experimental purposes were collected with a Kemmerer bottle at 20 m depth in Lake Moreno West (Nahuel Huapi National Park, Patagonia, Argentina). Organisms were collected sweeping with a 10 µm plankton net from 40 m depth to surface. Water samples were sterilized by filtration (0.22 µm, PVDF membranes, Millipore) and used as the experimental culture medium. Individuals of *Gymnodinium*, *Ophrydium* and *Stentor* were separated manually with a micropipette and put in filtered water. At least 30 individuals of each species were measured under the microscope. Natural picoplankton was obtained by sequential filtration of lake water through 20 µm and 2.7 m membranes, concentrated on 0.22 µm filters and resuspended in sterile lake water. The picocyanobacteria, *Synechococcus* sp., was obtained from laboratory cultures kept in the BG-11 culture medium.

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2.2. Experimental design

A first series of experiments was performed to study the passive Hg^{2+} incorporation (absorption and/or adsorption) in *Synechococcus*, *Stentor*, *Ophrydium*, and *Gymnodinium*. Organisms were placed in sterile lake water amended with Hg^{2+} (~8-16 ng L^{-1}) using the radioisotope $^{197}Hg^{2+}$. A second series of experiments was set up to evaluate the active uptake of Hg^{2+} in *Stentor*, *Ophrydium* and *Gymnodinium*, through similar incubations, except that we provided radiolabelled natural picoplankton (heterotrophic and autotrophic bacteria). After completing the incubations (14°C), organisms were recovered, and the activity of ^{197}Hg was evaluated according to Ribeiro Guevara et al. (2007). Three different Hg uptake factors were calculated, following Soto Cárdenas et al. (2014): a- the bioconcentration factor (BCF) based on individual Hg accumulation; b- the surface concentration factor (SCF) based on cell surface area; and, c- the volume concentration factor (VCF) indicative of the internalization of Hg^{2+}. The VCF to SCF ratio was calculated to characterize the degree of internalization.

3. Results

The S:V ratio indicated higher values in picoplankton (auto- and heterotrophic bacteria and *Synechococcus*; ca. 7.4 μm^{-1}), followed by *Gymnodinium* (ca. 0.2 μm^{-1}), *Ophrydium* (ca. 0.16 μm^{-1}) and *Stentor* (ca. 0.04 μm^{-1}).

In the passive Hg uptake experimental series, the BCF showed higher Hg^{2+} uptake in *Ophrydium*, followed by *Gymnodinium* and *Stentor*, whereas *Synechococcus* showed much lower uptake ($p < 0.001$; Fig. 1A). The SCF showed similar and much higher values in *Gymnodinium* and *Ophrydium* compared to *Stentor* and *Synechococcus* ($p < 0.001$; Fig. 1B). In terms of the VCF, *Synechococcus, Gymnodinium* and *Ophrydium* showed overall higher values than those measured in *Stentor* (Fig. 1C). The active uptake of Hg^{2+} recorded through the SCF and VCF resulted similarly higher in *Ophrydium* and *Gymnodinium* ($p > 0.05$) compared to *Stentor* ($p < 0.05$, respectively).

Stentor displayed higher active Hg^{2+} incorporation through the different bioconcentration factors compared to the passive ones ($p < 0.05$), indicating that consumption of Hg-bearing picoplankton enhanced its Hg uptake. In the case of *Gymnodinium*, the passive uptake of Hg was much higher than the active incorporation ($p<0.05$). In contrast, *Ophrydium* had similar passive and active Hg uptake, suggesting that it can incorporate Hg from the dissolved phase and also through ingestion.

4. Discussion and conclusions

Overall, the results obtained showed that the protists studied can incorporate substantial amounts of dissolved Hg^{2+}, although their passive and active incorporation differ among species. Different patterns of Hg^{2+} incorporation were detected in the studied protists.

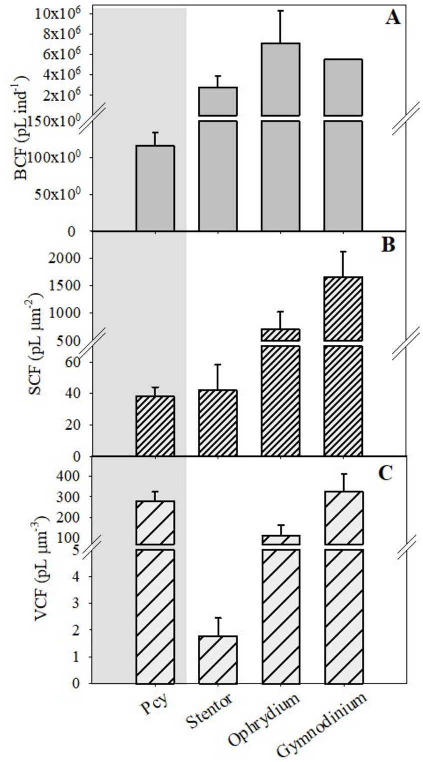

Fig.1. Hg^{2+} uptake ($^{197}Hg^{2+}$passive adsorption^{-1}) by different pelagic microbial species of Andean Patagonian lakes, measured in laboratory trials using natural lake water amended with $^{197}Hg^{2+}$ (THg:DOC = 21 ng mg^{-1}): **A**- Abundance-based Hg bioconcentration factor (BCF); **B**- Surface-based Hg concentration factor (SCF), and, **C**- Volume-based Hg concentration factor (VCF).

In autotrophic bacteria (picoplankton), Hg uptake was characterized by high internalization. The other protists showed particular Hg uptake characterized by: a- higher active than passive incorporation efficiency (*Stentor*), b- similar active and passive incorporation (*Ophrydium*) and c- higher passive incorporation (*Gymnodinium*), likely depending on their degree and mode of mixotrophy. Active Hg incorporation likely depends on bacteria consumption in *Stentor* and at a lesser extent in *Ophrydium.* In the case of *Gymnodinium*, the passive uptake clearly prevails, and the values of active incorporation recorded could be atributted to bacteria attachment to its surface rather than bacteria ingestion (Soto Cárdenas et al., 2018b).

The differences detected in the internalization of Hg^{2+} through the VCF in the different organisms tested would determine their potential for Hg transfer from the dissolved phase to the pelagic food web through consumption (Fig. 2). Higher internalization means a high potential for trophic transfer, as cytoplasmatic Hg can be more efficiently incorporated in consumers than Hg adsorbed to membranes (Wang, 2002; Twining and Fisher, 2004). These species may also transfer Hg to the benthic food web through their senescence and sinking and also regenerate Hg to the dissolved phase by excretion (Fig. 2) (Twiss and Campbell, 1995; Soto Cárdenas et al., 2014; 2018b; 2019). Thus, protists of pelagic microbial food webs of Andean lakes contribute to transfer Hg from the dissolved phase to higher trophic levels of pelagic and benthic lake food webs,

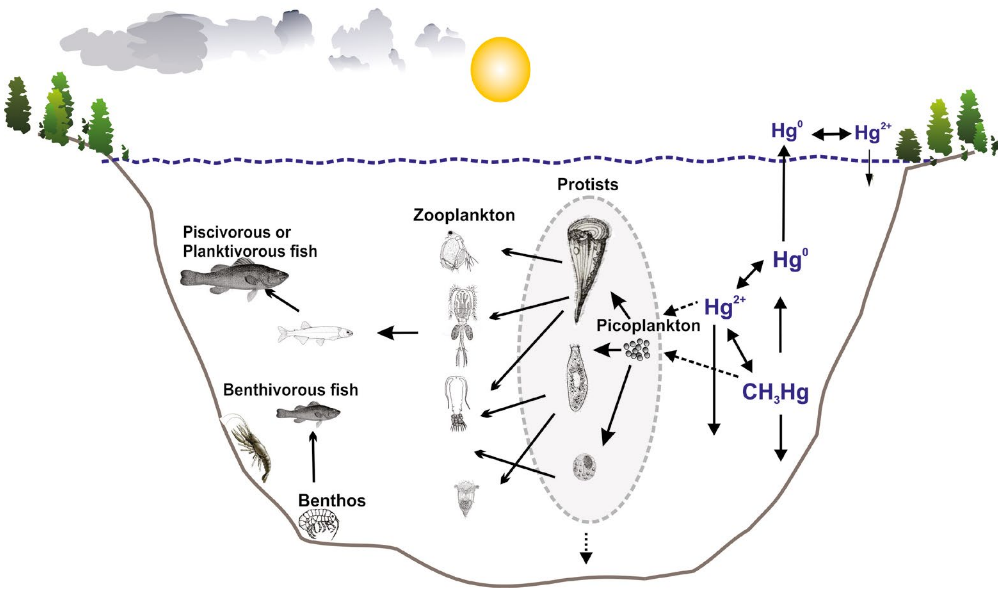

Fig.2. Schematic Hg^{2+} pathways involving pelagic microbial organisms of Andean Patagonian lakes.

linking the abiotic and biotic compartments in the Hg cycle.

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Conflict of interest

The authors declare no conflict of interest.

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