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Nickel dynamics in the lakewater metal biomonitor *Chaoborus*

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ABSTRACT

Nickel (Ni) is a widespread contaminant present at toxic concentrations in aquatic systems in the vicinity of some mining and smelting operations. However, its accumulation by aquatic animals has been little studied and there are few biomonitors for this metal. Recently, larvae of the aquatic insect *Chaoborus* were shown to be effective as biomonitors for Ni concentrations in lakewater. Since animals are more effective as biomonitors when we understand how they take up their contaminants (from water or from food) and the rate at which they exchange contaminants with their surroundings, we set out to measure these parameters for *Chaoborus*. To achieve these goals, we exposed the components of a laboratory food chain (green alga, cladoceran, *Chaoborus*) to realistic Ni concentrations. We found that the majority (≈65%) of the Ni taken up by *Chaoborus flavicans* comes from lakewater, with the remainder coming from its planktonic prey (*Daphnia magna*). This result is consistent with the low mean efficiency (14%) with which *C. flavicans* assimilated Ni from its prey. To explain the low efficiency of Ni uptake from food we measured the subcellular distribution of Ni in prey, which predicted that the majority of the Ni in prey (≈55%) was available for assimilation by the predator. This potential Ni uptake efficiency was only reached in animals that ingested few prey, likely because their gut passage time was longer than those ingesting many prey. We also measured Ni uptake and loss by *C. flavicans* exposed to Ni in water then used these data to parameterize a mechanistic bioaccumulation model that allowed us to describe Ni exchange between this insect and water. Lastly, we used these model constants, along with field measurements of Ni in 10 Canadian lakes, to predict Ni concentrations in field populations of *Chaoborus*. Model predictions overestimated Ni concentrations in field populations by a factor of 4. We suggest that uncertainties in the rate constant for Ni uptake from water and a lack of measured Ni concentrations in the prey eaten by *Chaoborus* larvae in the field could explain this difference.

1. Introduction

Nickel (Ni) has many industrial and domestic applications, but its mining, processing and use can lead to the contamination of aquatic and terrestrial environments (Chau and Kulikovsky-Cordeiro, 1995; Mukherjee, 1998). Indeed, toxic effects on aquatic organisms living near one of the world's major metal smelting sites (Sudbury, Ontario; Gunn, 1995) are reported to be a consequence of elevated Ni concentrations in their environment (Borgmann et al., 2001). Thus evaluating Ni availability to such organisms is a priority. However, using metal measurements in sediments or water alone can be problematic because they do not consider the availability of these contaminants to organisms. In this regard, metal measurements in animals are often more useful because they integrate the chemical, biological and trophic aspects of a given system (Phillips and Rainbow, 1993; Hare et al., 2008).

A small number of invertebrates have been tested as biomonitors of Ni contamination in marine (Widdows, 1985; Gibb et al., 1996; Yeh et al., 2009) and freshwater (Tochimoto et al., 2003) systems. Recently, the phantom midge *Chaoborus* was added to this list (Ponton and Hare, 2009). Larvae of this insect are found in lakes worldwide, where they often bury themselves in sediment during the day, to avoid fish predation, and then migrate into the water column at night where they are predators of small planktonic invertebrates such as cladocerans, copepods and rotifers (Hare and Carter, 1987; Croteau et al., 2003a,b). Because *Chaoborus* incarnates many of the characteristics of a good biomonitor (widespread, abundant, easily collected and tolerant to the contaminants that it accumulates), it has been proposed as a biomonitor for estimating free Cd^{2+} and Ni^{2+} ion concentrations in lakewater (Hare et al., 2008; Ponton and Hare, 2009).

Biomonitors are used most effectively when the means and the rate at which they exchange contaminants are known. For example, if an animal accumulates a metal mainly from food then its metal concentrations will likely be influenced by the abundance and type of food available to it. Likewise, an animal that exchanges metals slowly with its surroundings would not likely

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be a good biomonitor of short-term changes in ambient metal concentrations.

With these factors in mind, we set out to measure Ni uptake and loss by *Chaoborus* larvae since nothing is known about how Ni enters this predator (via water or its prey) or the rate at which this insect exchanges Ni with its surroundings. We measured Ni uptake from both water and food, the efficiency with which this predator assimilates Ni from its prey, and Ni efflux from this insect. We then used this information in a biodynamic model to predict Ni concentrations in larvae exposed to this metal in both the laboratory and the field. Since there is little information on Ni bioaccumulation in general, our results for this aquatic insect should aid in understanding how aquatic invertebrates interact with this environmentally important trace metal.

2. Methods

To describe and explain Ni accumulation by the biomonitor *Chaoborus*, we created a planktonic food chain consisting of the green alga *Pseudokirchneriella subcapitata*, the herbivore *Daphnia magna* (Crustacea, Cladocera) and the predator *Chaoborus flavicans* (Insecta, Chaoboridae). We used realistic exposure concentrations of 50 or 300 nmol L^{-1} free Ni ion ($[Ni^{2+}]$); these values are lower than those measured in many Ni-contaminated lakes (up to 2000 nmol L^{-1} total dissolved Ni; Ponton and Hare, 2009). Free Ni ion concentrations were estimated using the speciation program MINEQL+ (version 4.5, 1998, Environmental Research Software, ME, USA). All Ni-exposure media were prepared 1 day in advance to allow them time to reach equilibrium and pH was adjusted using NaOH (1 mol l^{-1}) and maintained constant by adding a pH buffer to the medium (10 mmol l^{-1} MOPS, 3-morpholinopropanesulfonic acid; pK_a 7.2). Daily pH measurements confirmed that pH remained constant, and *MOPS* buffer is reported to have a negligible influence on Ni speciation (Van Laer et al., 2006).

2.1. Nickel exposure: algae

Stock cultures of the green alga *P. subcapitata* were maintained in Bristol culture medium (Table 1) at 20 °C under "cool white" fluorescent lights (100 μE m^{-2} s^{-1}) on a 16/8 day/night cycle. Air was bubbled continuously into the algal cultures to maintain cells in suspension and to ensure an adequate supply of CO_2. Contaminated algae to be used as food for *D. magna* were cultured in Bristol medium (Table 1) having a $[Ni^{2+}]$ of 50 nmol L^{-1} that was held in polycarbonate Erlenmeyer flasks under the same light and temperature regimes used for the stock cultures. Six samples of algae were taken for Ni-analysis. Each 40-mL algal sample was centrifuged at 10,000 × *g* for 10 min, then the supernatant was removed and the pellet was centrifuged a second time with Ni-free 0.1 mM EDTA (to remove both the Ni-contaminated culture medium and Ni adsorbed on the algae), followed by a final centrifugation in Ni-free Bristol medium. Pellets were placed in acid-washed pre-weighed high-density polyethylene (HDPE) bottles and frozen at −80 °C until drying, digestion and Ni-analysis.

Table 1
Total macronutrient concentrations and trace metal (free ion) concentrations (calculated using MINEQL+) in the modified Bristol medium used in our experiments. The metal buffer EDTA and the pH buffer MOPS were also present in the medium.

Macronutrient	Concentration (total dissolved; mol L^{-1})	Trace metal	Concentration (free ion; mol L^{-1})
B	9.2×10^{-5}	Co^{2+}	1×10^{-12}
Ca	4.7×10^{-4}	Cu^{2+}	1×10^{-13}
Cl	2.5×10^{-3}	Fe^{3+}	1×10^{-19}
CO_3	2.0×10^{-4}	Mn^{2+}	1×10^{-09}
K	1.3×10^{-3}	MoO_4^{2-}	1×10^{-07}
Mg	2.1×10^{-4}	Zn^{2+}	1×10^{-10}
Na	1.6×10^{-3}		
NO_3	1.5×10^{-3}		
PO_4	7.0×10^{-9}		
SO_4	3.7×10^{-4}		

2.2. Nickel exposure: the herbivore Daphnia magna

D. magna was cultured at 20 °C in artificial lakewater containing M4 medium as a supplement (Elendt and Bias, 1990), at a hardness of 150 mg L^{-1} ($CaCO_3$ equivalence), and fed the green alga *P. subcapitata* (see Section 2.1). Juvenile *D. magna* to be used as Ni-contaminated prey for *Chaoborus* were removed from the rearing aquaria and held for 1 day in HDPE containers filled with Ni-contaminated ($[Ni^{2+}]$ = 50 nmol L^{-1}) water (Table 1) where they were fed Ni-rich algae in excess (see Section 2.1; the mean (±SD; n = 6) [Ni] in algae was 2140 ± 60 nmol g^{-1}). This exposure time allowed juvenile *D. magna* ample time to reach a steady state in their Ni concentrations (steady state is reached in ~6 h; D.E. Ponton, unpublished data). Nickel contaminated *D. magna* (2–5 days old) to be offered daily as prey to *Chaoborus* were first held in Ni-free Bristol medium (Table 1) and offered Ni-free *P. subcapitata* for 30 min to remove Ni-contaminated gut contents and Ni adsorbed on the body surface. To measure the Ni content of *D. magna*, 2–3 samples (each comprising 15–30 individuals) were placed on acid-washed, pre-weighed, Teflon sheeting in acid-washed microcentrifuge tubes and frozen at −80 °C for later Ni-analysis.

2.3. Ni distribution in the cells of Daphnia magna

To estimate the proportion of Ni in *D. magna* that is potentially available for transfer to *Chaoborus*, we held Ni-contaminated *D. magna* (see Section 2.2) for 3 h in Ni-free water (Table 1) and with Ni-free food (*P. subcapitata*) to remove both Ni-rich gut contents and Ni adsorbed on the body surface. These depurated prey (≈50 pooled individuals per sample; n = 10) were placed in acid-washed, pre-weighed 1.5 mL polypropylene microcentrifuge tubes, weighed, placed on ice and homogenized in physiological buffer (25 mmol l^{-1} TRIS; pH 7.4) (1:3 tissue to buffer, w/v ratio) using a Pellet Pestle (Kontes) for 2 s, at 30 s intervals, over a period of 5 min.

To measure metal concentrations in whole animals, aliquots of the homogenate were placed in pre-weighed 1.5 mL polypropylene microcentrifuge tubes and frozen at −80 °C. Remaining homogenates were centrifuged three times at 800 × *g*. Following each centrifugation, the supernatant was removed and the pellet was diluted again with physiological buffer at a tissue to buffer ratio of 1 to 2. The 3 supernatants were combined into pre-weighed 1.5 mL polypropylene microcentrifuge tubes (final tissue to buffer ratio of 1 to 7). These samples were then subjected to a centrifugation, digestion and heat-treatment protocol (Wallace et al., 2003 modified slightly according to Dumas and Hare, 2008) that gave the following operationally defined subcellular fractions: cellular debris (includes membranes and unbroken cells), granules (and other NaOH resistant particles), organelles (microsomes, lysosomes and mitochondria), heat-denatured proteins (HDP; includes enzymes) and heat-stable proteins (HSP; includes metallothionein, MT). The organelles fraction was further separated, by centrifugation, into a mitochondrial fraction and one containing other organelles (Giguère et al., 2006). By comparing metal burdens in aliquots of the homogenate to the sum of those measured in the various fractions, we determined that metal losses during the fractionation procedure were negligible (mean (±SD) recovery was 120 ± 28%). The 50,000 and 100,000 × *g* centrifugations were carried out using a Beckman TL-100 centrifuge with a TLA-100.3 rotor, whereas all other centrifugations involved the use of a