Aquatic Toxicology 120–121 (2012) 67–78

Contents lists available at SciVerse ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Subcellular metal partitioning in larvae of the insect *Chaoborus* collected along an environmental metal exposure gradient (Cd, Cu, Ni and Zn)

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ARTICLE INFO

Article history:
Received 19 February 2012
Received in revised form 27 April 2012
Accepted 1 May 2012

Keywords:
Subcellular partitioning
Chaoborus
Metals
Toxicity
Metallothioneins
Detoxification

ABSTRACT

Larvae of the phantom midge *Chaoborus* are common and widespread in lakes contaminated by metals derived from mining and smelting activities. To explore how this insect is able to cope with potentially toxic metals, we determined total metal concentrations and subcellular metal partitioning in final-instar *Chaoborus punctipennis* larvae collected from 12 lakes situated along gradients in aqueous Cd, Cu, Ni and Zn concentrations. Concentrations of the non-essential metals Cd and Ni were more responsive to aqueous metal gradients than were larval concentrations of the essential metals Cu and Zn; these latter metals were better regulated and exhibited only 2–3-fold increases between the least and the most contaminated lakes. Metal partitioning was determined by homogenization of larvae followed by differential centrifugation, NaOH digestion and heat denaturation steps so as to separate the metals into operationally defined metal-sensitive fractions (heat-denaturable proteins (HDP), mitochondria, and lysosomes/microsomes) and metal-detoxified fractions (heat stable proteins (HSP) and NaOH-resistant or granule-like fractions). Of these five fractions, the HSP fraction was the dominant metal-binding compartment for Cd, Ni and Cu. The proportions and concentrations of these three metals in this fraction increased along the metal bioaccumulation gradient, which suggests that metallothionein-like proteins play an important role in metal tolerance of *Chaoborus* living in metal-contaminated environments. Likewise, a substantial proportion of larval Zn was in the HSP fraction, but its contribution did not increase progressively along the metal gradient. Despite the increases in Cd, Ni and Cu in the HSP fraction along the metal bioaccumulation gradient, some accumulation of non-essential metals (Cd and Ni) was observed in putative metal-sensitive fractions (e.g., HDP, mitochondria), suggesting that metal detoxification was incomplete. In the case of Cd, there appears to be a threshold body concentration of about 50 nmol Cd g^{-1} dry weight, above which Cd detoxification becomes more effective and below which *Chaoborus* does not "turn on" its detoxification machinery to the fullest extent. We speculate that acclimation or adaptation of *Chaoborus* to these highly metal-contaminated environments may have resulted in a capacity to tolerate some metal spillover without comprising essential biological functions such as growth and reproduction.

1. Introduction

Essential trace metals are accumulated by living organisms and play key roles in numerous physiological and biochemical processes, acting as co-factors, as electron donors or acceptors in redox reactions, and as allosteric promoters (Finney and O'Halloran, 2003). However, these same metals can provoke toxicity when their concentrations exceed the metabolic requirements and storage capacity of living cells. Non-essential metals that enter the intracellular environment are subject to similar complexation reactions, but their involvement in these reactions is likely to lead to deleterious effects (Mason and Jenkins, 1995). Metal toxicity is assumed to occur when the intracellular concentration of a metal exceeds a threshold value, such that the metal binds to inappropriate, physiologically important sites such as biomolecules (small peptides, enzymes, DNA or RNA) or organelles (mitochondria, endoplasmic reticulum and nuclei), causing impairment of biological functions. Below such threshold values, the organism is assumed to be able to handle metals by controlling their rates of uptake or loss (Rainbow, 2002) or by sequestering them in intracellular compartments such as cytosolic metal-binding proteins, lysosomes and metal-rich granules (Marigómez et al., 2002; Mason and Jenkins, 1995; Wallace et al., 2003). In the cytosol, metallothioneins and metallothionein-like proteins are thought to be the main metal-binding molecules. These low molecular weight, heat-stable and cysteine-rich proteins bind essential and non-essential metals in most vertebrates and invertebrates (Amiard et al., 2006).

Metal resistance or tolerance has generally been attributed to the ability of an organism to preclude the binding of metals to inappropriate, physiologically important, metal-sensitive

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0166-445X/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aquatox.2012.05.001

sites (Campbell et al., 2008; Mason and Jenkins, 1995). In principle, subcellular metal partitioning procedures could be used to reveal internal metal compartmentalization strategies used by organisms to cope with potentially toxic metals. One can distinguish between "metal-sensitive" fractions and "metal-detoxified" fractions in cells. To investigate how organisms can tolerate metal-contaminated environments in the field, one would need a sentinel organism that was abundant, easy to collect and identify, widespread, of adequate size, and tolerant of high ambient metal concentrations. Larvae of the phantom midge *Chaoborus* exhibit all of these properties and they have been used as sentinels for estimating biologically relevant concentrations of Cd and Ni in lakes (Hare and Tessier, 1998; Ponton and Hare, 2009). The presence of metallothioneins or metallothionein-like proteins in *Chaoborus* larvae has been used to explain their persistence in metal-contaminated environments (Croteau et al., 2002), but little else is known about the subcellular compartmentalization of metals in the larvae.

We aimed to determine the relative distribution of Cd, Cu, Ni and Zn among various subcellular fractions in *Chaoborus* larvae living in lakes along an environmental metal gradient. To achieve this goal, we collected final-instar larvae of *Chaoborus punctipennis* from a dozen lakes located in the vicinity of metal smelters located in Rouyn-Noranda (Quebec) and Sudbury (Ontario), Canada. A subcellular partitioning procedure, using differential centrifugation, NaOH digestion and heat-denaturation steps, was then applied to separate larvae into metal-sensitive fractions (cytosolic enzymes, organelles) and detoxified metal fractions (metallothionein-like proteins, metal-rich granules). To determine how metal subcellular partitioning responded as a function of the metal bioaccumulation gradient, trace metal concentrations were measured in each subcellular fraction and in the whole insect. In addition, dissolved metal concentrations in lake water were measured to assess metal concentrations in its surroundings. Our results highlight the importance of the cytosolic heat-stable protein (HSP) fraction in metal detoxification in the intracellular environment of this insect, presumably allowing the larvae to cope with elevated concentrations of bioavailable trace metals in the environment.

2. Materials and methods

2.1. Study areas and sampling protocol

The 12 Canadian Precambrian Shield lakes we chose for study were contaminated to various degrees by historical emissions from two metal smelters (Table 1), emissions that have been largely curtailed since the 1980s. Lakes near both smelters are contaminated with Cd, Cu and Zn (Banic et al., 2006; Perceval et al., 2006). Ni is also a metal of concern in the Sudbury area (Borgmann et al., 1998). From each of the 12 lakes we collected larvae of the insect *Chaoborus* (Diptera; Chaoboridae) in late May and early June of 2009 and 2010 (Table 1). Water samples were collected concurrently, with the exception of lakes Crowley and Silver for which we used aqueous metal concentrations measured in 2007 (Ponton and Hare, 2009). In each lake, larvae and water were collected at a single station with the exception of Lake Dasserat in which two widely separated sampling sites were treated as individual "lakes". One station (DSII) was situated in a bay (Baie Arnoux) receiving acid mine drainage (Baie Arnoux) and the other (DSI) was located at the opposite (northwest) end of the lake (Goulet and Couillard, 2009).

2.2. Collection of water samples

In each lake, triplicate water samples were collected in the epilimnion, where *Chaoborus* larvae feed nocturnally, using in situ

Table 1
Location, sampling date and chemical composition (pH, dissolved organic carbon (DOC), dissolved metal concentrations and free-ion metal concentrations) of water collected in the epilimnion of our study lakes.

Lake (code)	Location	Sampling date	pH	[DOC] mg L^{-1}	[Cd] (nM)	[Cu] (nM)	[Ni] (nM)	[Zn] (nM)	$[Cd^{2+}]$ (nM)	$[Cu^{2+}]$ (nM)	$[Ni^{2+}]$ (nM)	$[Zn^{2+}]$ (nM)
Rouyn-Noranda, Quebec												
Dasserat-I (DSI)	48°14′N, 79°23′W	2010	7.6	5.1	1.8	86	15	400	0.50	0.034	7.6	93
Dasserat-II (DSII)	48°16′N, 79°23′W	2010	7.8	5.9	0.92	97	15	500	0.35	0.022	7.7	110
Dufault (DU)	48°20′N, 79°07′W	2010	7.7	3.8	3.3	160	14	714	1.6	1.1	8.7	240
Marlon (MA)	48°15′N, 79°03′W	2010	7.7	7.8	1.4	180	13	53	0.28	0.053	5.5	9.1
Opasatica (OP)	48°03′N, 79°16′W	2010	6.7	6.7	0.16	45	19	7.9	0.02	0.0015	6.4	0.7
Osisko (OS)	48°14′N, 79°00′W	2010	8.5	2.3	0.48	53	33	6.5	0.17	0.0016	16	10
Sudbury, Ontario												
Crooked (CK)	46°25′N, 81°02′W	2010	6.7	4.2	1.1	350	1500	100	0.54	2.2	1000	46
Crowley (CW)	46°23′N, 80°59′W	2007	6.3	3.2	0.91	130	870	120	0.41	1.3	540	29
Hannah (HA)	46°26′N, 81°02′W	2010	7.9	3.5	0.47	200	1100	6.8	0.18	0.028	650	1.9
Lohi (LO)	46°23′N, 81°02′W	2009	6.7	3.6	0.77	110	780	110	0.4	1.7	540	38
Silver (SL)	46°22′N, 81°03′W	2007	5.9	2.7	2.2	160	1600	240	1.2	1.4	1100	130
Swan (SW)	46°21′N, 81°03′W	2009	5.9	2.1	1.9	90	1100	110	0.95	6.6	800	51
Ratio Max/Min			400[a]	3.7	21	7.8	123	110	80	4400	200	342

[a] Ratio of H^+-ion concentrations, not pH.