Aquatic Toxicology 154 (2014) 141–153

Contents lists available at ScienceDirect

Aquatic Toxicology

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

Chronic nickel bioaccumulation and sub-cellular fractionation in two freshwater teleosts, the round goby and the rainbow trout, exposed simultaneously to waterborne and dietborne nickel

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ARTICLE INFO

Article history:
Received 22 January 2014
Received in revised form 16 April 2014
Accepted 27 April 2014
Available online 6 May 2014

ABSTRACT

Rainbow trout and round goby were exposed for 30 days to waterborne and dietary Ni in combination at two waterborne concentration ranges (6.2–12 μmol/L, 68–86 μmol/L), the lower of which is typical of contaminated environments. The prey (black worms; *Lumbriculus variegatus*) were exposed for 48 h in the effluent of the fish exposure tanks before being fed to the fish (ration = 2% body weight/day). Ni in gills, gut, and prey was fractionated into biologically inactive metal [BIM = metal-rich granules (MRG) and metallothionein-like proteins (MT)] and biologically active metal [BAM = organelles (ORG) and heat-denaturable proteins (HDP)]. Gobies were more sensitive than trout to chronic Ni exposure. Possibly, this greater sensitivity may have been due to the goby's pre-exposure to pollutants at their collection site, as evidenced by ~2-fold greater initial Ni concentrations in both gills and gut relative to trout. However, this was followed by ~2–16× larger bioaccumulation in both the gills and the gut during the experimental exposure. On a subcellular level, ~3–40× more Ni was associated with the BAM fraction of goby in comparison to trout. Comparison of the fractional distribution of Ni in the prey versus the gut tissue of the predators suggested that round goby were more efficient than rainbow trout in detoxifying Ni taken up from the diet. Assessing sub-cellular distribution of Ni in the gills and gut of two fish of different habitat and lifestyles revealed two different strategies of Ni bioaccumulation and sub-cellular distribution. On the one hand, trout exhibited an ability to regulate gill Ni bioaccumulation and maintain the majority of the Ni in the MT fraction of the BIM. In contrast goby exhibited large Ni spillovers to both the HDP and ORG fractions of the BAM in the gill. However, the same trend was not observed in the gut, where the potential acclimation of goby to pollutants from their collection site may have aided their ability to regulate Ni spillover to the BAM more so than in trout. Overall, chronic mortality observed in goby may be associated more with Ni bioaccumulation in gills than in gut; the former at either 4-d or 30-d was predictive of chronic Ni toxicity. BIM and BAM fractions of the goby gills were equally predictive of chronic (30-d) mortality. However, critical body residue (CBR50) values of the BIM fraction were ~2–4× greater than CBR50 values of the BAM fraction, suggesting that goby are more sensitive to Ni bioaccumulation in the BAM fraction. There was insufficient mortality in trout to assess whether Ni bioaccumulation was predictive of chronic mortality.

1. Introduction

Total and dissolved metal concentrations in the water alone can be poor predictors of environmental threats to aquatic organisms (Borgmann, 1983; Borgmann et al., 2004). Therefore, much emphasis has been placed on the role that chemical speciation of metals in the environment plays in metal toxicity (Pagenkopf, 1983; Morel, 1983; Campbell, 1995). However, in recent decades, the emphasis has shifted to the role of metal bioaccumulation in metal toxicity. For example, the Biotic Ligand Model (BLM) uses site-specific water chemistry parameters in conjunction with the binding constants of the biotic ligand to predict whether sufficient metal will bind to the organism to cause acute toxicity (Paquin et al., 2000; Di Toro et al., 2001; Niyogi and Wood, 2004). Short term metal bioaccumulation is used to predict longer term toxicity (e.g. Meyer et al., 1999). In addition, the Tissue Residue Approach (TRA) correlates tissue bioaccumulation with adverse biological effects (e.g. mortality) and in

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http://dx.doi.org/10.1016/j.aquatox.2014.04.028
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this manner bioaccumulation can be used to predict the toxicity within and across species (Connolly, 1985; McCarty and MacKay, 1993; Luoma et al., 2009; Borgmann et al., 2001; Adams et al., 2011; Schmidt et al., 2011). These methods may be used independently or together as a tool for assessing the toxicity to aquatic organisms.

Expressing toxicological effect as a function of the bioaccumulation of a metal such as Ni has many advantages: it integrates all exposure routes (e.g. water column and food), it incorporates changes in water chemistry over time, and it assimilates the toxicokinetics of different species (U.S. EPA, 2007). Indeed, bioaccumulation of metals has been found to be a better predictor of toxicity than exposure water concentrations (Borgmann et al., 1991, 2001; Borgmann and Norwood, 1997). However, metal bioaccumulation is not constant over time; there can be regulation of uptake and/or elimination, or sub-cellular compartmentalization, both of which can change rendering the metal more or less toxic (Adams et al., 2011).

In order to better understand the latter, Wallace et al. (2003) devised a protocol for separating intracellular fractions which can be operationally classified into biologically inactive metal (BIM; comprising metal-rich granules (MRG) and metallothionein protein or metallothionein-like proteins (MT)) and biologically active metal (BAM; comprising organelles (ORG) and heat-denaturable proteins (HDP); Rainbow, 2002). The fifth sub-cellular fraction is the cellular debris (CD) containing tissue fragments, cell membranes and other cellular components. The function of this fraction is currently unknown and is therefore not considered part of either the BIM or BAM fractions. This classification suggests that when metal levels exceed a threshold value in the BIM, there is metal spill-over into the BAM fractions which leads to deleterious biological effects. In this manner, the use of subcellular metal residues for the TRA may improve the effectiveness for toxicity assessment (Adams et al., 2011). Therefore assessing changes to bioaccumulation patterns both on a whole organ level and a sub-cellular level over a chronic time scale may further explain mechanisms of Ni toxicity.

Traditionally, the majority of information gained on metal toxicity has correlated gill metal bioaccumulation with mortality in models such as the BLM. Overall, gill metal (e.g. Ni) bioaccumulation from waterborne metal exposure is a good predictor of acute toxicity (Meyer et al., 1999); however, aquatic organisms such as fish use not only their gills, but also their gut to exchange the necessary nutrients and minerals, and to eliminate waste (Randall et al., 2002). In fact, in terms of metal exposure, many studies have shown that dietary exposure via prey can be as important as the waterborne route for metal bioaccumulation and associated toxicity (Spry et al., 1988; Munger and Hare, 1997; Zhang and Wang, 2005; Pyle et al., 2005; Farag et al., 2007). Therefore, in the present study, both the gills and the gut were assessed as potential biotic ligands for nickel following a joint waterborne and dietary exposure so as to best simulate their natural exposure conditions. The overall advantage of this approach is the integration of exposure routes when using the TRA as a method for predicting toxicity to aquatic organisms.

We chose to compare two fish species with different habitats and lifestyles: the round goby (*Neogobius melanostomus*) and the rainbow trout (*Oncorhynchus mykiss*). The round goby were collected from a "clean site" at LaSalle Park on Hamilton Harbour (Marentette et al., 2010), but the Harbour itself was designated by the International Joint Commission as a Canadian Area of Concern due to the many contaminants known to be problematic namely: polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and metals including cadmium, arsenic, lead, iron, mercury, zinc, and nickel (Hamilton Harbour Remedial Action Plan RAP, 1992, 2003). In contrast, the rainbow trout were purchased from a hatchery served with pristine water. The prey species, *Lumbriculus variegatus*, was chosen based on its ability to meet nutritional requirements, to be accepted by fish and its prevalence as a food source for these fish (Taraborelli et al., 2010; Mount et al., 2006). In our experimental design, the prey was pre-exposed to the same waterborne concentration of Ni before being fed to the two fish species, so as to simulate natural exposure conditions. Round goby are known to be a pollution-tolerant species (Pinchuk et al., 2003). However, a recent study conducted in our laboratory showed a greater sensitivity of this species to acute Ni toxicity (Leonard et al., 2014) in comparison to rainbow trout—one of the most sensitive teleosts to metals (Nebeker et al., 1985; U.S. EPA, 1995).

Therefore, our overall goal was to compare the Ni bioaccumulation patterns of these two species to joint waterborne and dietary exposures at an environmentally relevant waterborne Ni concentration (nominally 10 μmol Ni/L) and a more toxic concentration (nominally 60 μmol Ni/L). By way of reference, Ni levels as high as 17 μmol Ni/L have been reported in contaminated natural waters (Eisler, 1998), while levels of regulatory significance in Canada are 2.1 μmol Ni/L (a chronic value; CCREM, 1987) and in the United States are 10.6 μmol Ni/L (acute) and 1.2 μmol Ni/L (chronic), respectively (U.S. EPA, 1995). By assessing Ni bioaccumulation patterns on both a whole organ level as well as a sub-cellular level we aimed to determine if differences in toxic response were associated with different strategies of metal compartmentalization.

Therefore, with this background in mind, our specific objectives were: (1) to assess survival over chronic (30-d) exposures to combined waterborne and dietary Ni at two exposure concentrations, (2) to measure and compare whole-organ gill and gut Ni concentrations as well as Ni in their sub-cellular fractions at several time points over these chronic exposures; (3) to assess the changes in BIM and BAM fractions in the two organs; and (4) thereby to evaluate the use of acute subcellular bioaccumulation as the residue indicator of chronic Ni toxicity in rainbow trout and round goby.

2. Methods

2.1. Experimental organisms

Round goby, *N. melanostomus*, were collected during the weeks of Sept 26–Oct 7th, 2011 from Hamilton Harbour at LaSalle Park (43°18′1″ N, 79° 50′47″W), Lake Ontario, Canada. The background Ni concentration at this site averaged 0.31 ± 0.02 μmol/L. Commercial minnow traps baited with frozen corn and set at a depth of 1 m or less for 24 h were used to capture one hundred and eight round goby (mean body mass 11 ± 3 g). Fish were then transported back to the laboratory and acclimated for two weeks to laboratory conditions in 500-L containers served with flow-through, aerated, dechlorinated Hamilton (Ontario, Canada) tap water. PVC tubes were used for shelter and the round goby were fed ad libitum every second day with Big Al's Staple Flake Food (45% protein, 5% crude fat, 2% crude fiber and 8% moisture; Big Al's Aquarium Supercentres, Woodbridge, ON, Canada).

One hundred and eight rainbow trout, *O. mykiss*, 12 ± 1 g were purchased from Humber Springs Trout Hatchery, Orangeville, Ontario, Canada. They were initially contained in 500-L tanks receiving flow-through dechlorinated Hamilton tap water. Trout were fed 2% body weight, every second day, with Martin's commercial dried pellet feed (Martin Mills Inc., Ontario, Canada).

Black worms (*L. variegatus*) were purchased from Aquatic Foods (Fresno, CA, USA) and were kept in 80-L aquaria with a flow-through of continuously aerated dechlorinated Hamilton tap water at turnover rate of 20 L/day. *L. variegatus* were fed the same commercial ground flake food as the described above, once every two weeks.

All organisms were kept in Hamilton dechlorinated tap water with an ionic composition of (in mmol/L) Na (0.9), Cl (1.0), Ca

(1.0), K (0.04), Mg (0.4), and Ni ($<0.3 \times 10^{-5}$). Water hardness was ~140 mg/L as $CaCO_3$ equivalents; pH was 7.8, alkalinity 95 mg/L, water temperature was 12 ± 2 °C, dissolved organic carbon (DOC) was 2.2 mg/L and the photoperiod was 16:8 h light:dark.

2.2. Flow through exposure system

Ni stock solutions, made with $NiCl_2 \cdot 6\ H_2O$ (Sigma Aldrich, St. Louis, Missouri, USA, CAS no. 7791-20-0), were held in Mariotte bottles above the exposure tanks. Dechlorinated Hamilton tap water (750 mL/min) was mixed with 0.5 mL/min of Ni stock solutions from the Mariotte bottles in a mixing bucket before being administered to the exposure tanks containing the fish.

Mariotte bottle drip rates and flow rates of dechlorinated water were monitored daily. Water samples were taken every 24 h from each exposure to determine total and dissolved (0.45 μm filtration, see below) concentrations of Ni. Tanks were checked daily for impacted fish (defined as fish which had lost equilibrium and had turned upside down) which were removed immediately.

Thirty-six round goby and rainbow trout were transferred to each exposure tank (500-L) for 48 h prior to Ni exposure to allow time for acclimation to the new environment and were fed 2% body weight of black worms every second day throughout the 30-d trial. Nominal water exposure concentrations were 0, 10, 60 μmol Ni/L. Black worms were held in the out-flow from the fish exposure tanks for 48-h prior to being fed to fish. This allowed for the concentration of Ni in the prey to be constant throughout the experiment and attempted to better simulate the natural environment where both predators and prey are exposed to the same water chemistry. All black worms were either consumed or removed from the tank within 15 min.

2.3. Water chemistry

Mean water chemistry parameters for all experiments are shown in Supplementary Table 1. Measured total and dissolved Ni concentrations along with specific water chemistries were used to estimate the free ionic nickel (Ni^{2+}) concentrations and Ni^{2+} activity using Visual MINTEQ software (ver. 3.0, beta, KTH, Department of Land and Water, Resources Engineering, Stockholm, Sweden). The active fraction is a measure of the effective activity of Ni in these water chemistries, which is determined by concentration and by interactions (i.e. attraction or repulsion) of other molecules in solution. The NICA-Donnan model was used in the calculations to estimate the effect of DOC on Ni speciation. Average Ni water concentrations of each fraction were calculated: nominal, total, dissolved, ionic and active fractions of the metal, taking into account the measured water chemistry from Supplementary Table 1, and are reported in the Supplementary information section Table 2. All Ni water concentrations presented in this study are reported as the dissolved fractions of the metal, which were 92% of the total values (Supplementary Table 2).

2.4. Sub-cellular fractionation

The gills and gut of rainbow trout and round goby and the whole body of the prey, *L. variegatus*, underwent tissue homogenization followed by a differential centrifugation procedure to separate the tissues into five operationally defined fractions: metal rich granules (MRG), organelles (ORG), heat-denaturable proteins (HDP), metallothionein proteins (MT) and cellular debris (CD). The sub-cellular fractionation protocol generally followed the procedure of Wallace et al. (2003), Lapointe et al. (2009), and Ng et al. (2011). Tissues were stored at −80 °C and thawed on ice prior to subcellular fractionation. Tissues (required to be >0.2 g from 6 fish per treatment, or ~20 black worms) were weighed, then homogenized in 3–5 volumes of homogenization buffer which includes Tris–base 20 mM, pH 7.6, with 2 mM 2-mercaptoethanol and 0.2 mM phenylmethanesulfonylfluoride (PMSF). One third of the sample was used for measuring metal bioaccumulation for metal recovery. The remainder was then centrifuged at $1450 \times g$ at 4 °C for 15 min. The pellet was washed with buffer and re-centrifuged at the same speed to minimize the presence of cellular debris (unbroken cells, cell fragments and cell membranes). The pellet was washed with 2 mL of 1 N NaOH and heated in the water bath at 80 °C for 15–30 min. The mixture was then spun at $5000 \times g$ for 10 min to collect the cellular debris (CD; supernatant) and the metal rich granules (MRG; pellet). The supernatant collected after the $1450 \times g$ spin was centrifuged at $100{,}000 \times g$, 4 °C, for 1 h for separation of the organelles (ORG; pellet) and cytosol (supernatant). The cytosol fraction was then heated at 80 °C for 15 min and recentrifuged to separate the HDP (pellet) from the metallothionein (MT; supernatant) by centrifugation at $50{,}000 \times g$ for 10 min. Overall, recovery of Ni was $109\% \pm 9$ (sum of Ni in each fraction × 100%/Ni in homogenate).

In figures, the BAM fractions (comprising HDP and ORG) are shown above the zero line, whereas the BIM fractions (comprising MRG and MT) are represented below the zero line. The CD fraction is not presented in the Figures as it represents the broken cellular fragments during fractionation and it is not classified as either BIM or BAM. In the context of this study, the BIM fraction contains metal in its detoxified form whereas the fate of the metal in the CD fraction is unknown. On average, the CD fraction accounted for 35% of the total organ Ni, which is consistent with previously published values for Ni sub-cellular distribution in fish (Lapointe and Couture, 2009).

2.5. Tissue sampling

Six fish were sampled from each exposure concentration on days 0, 2, 4, 10, 20 and 30 of the exposure. Five sets of twenty black worms were sampled every other day for whole body Ni bioaccumulation (sub-cellular fractionation of prey was performed on the same days as the fish). Fish were euthanized with 0.80 mg/L of tricaine methanesulfonate (*MS-222*) (Syndel Laboratories Ltd. Nanaimo, BC, Canada; adjusted to pH 7.8 with NaOH) and rinsed briefly (1 min) in dechlorinated water. The body mass of each fish was measured and recorded. Gills and gut were surgically removed, rinsed with 0.9% NaCl solution, blotted dry and weighed and then stored in15-mL Falcon™ tubes at −80 °C for future analysis. Black worms were transferred to Hamilton dechlorinated water containing no added Ni for 5 min to remove adsorbed Ni, followed by a brief (5 s) rinse in nanopure water (18.2 MΩ cm, Millipore Corporation, Billerica, MA, USA). Organisms were then transferred to filter paper, patted dry, weighed and then stored in 2 mL bullet tubes at −80 °C for future analysis.

2.6. Analytical techniques

Fish sub-cellular fractions and homogenate for Ni recovery were digested with 2 N HNO_3 (trace metal grade, Fisher Scientific, Ottawa, ON, Canada) with a volume of 3–5 times the weight of the tissue in sealed vials. These were incubated in a Precision Oven (Jouan Inc., Virginia, USA) at 60 °C for 48 h, with vortexing at 24 h. Tissues are then stored at 4 °C for later analysis. Black worm sub-cellular fraction and homogenate were digested at room temperature with 65% HNO_3 (trace metal grade, Fisher Scientific, Ottawa, ON, Canada; 10 μL of HNO_3 per mg of tissue wet wt) for one week and then hydrogen peroxide (4 μL of H_2O_2 per mg of tissue wet wt) was added for 24 h to complete the digestion process (Croteau et al., 2002).

Ni concentrations in water samples and tissue fraction samples were measured using graphite furnace atomic absorption

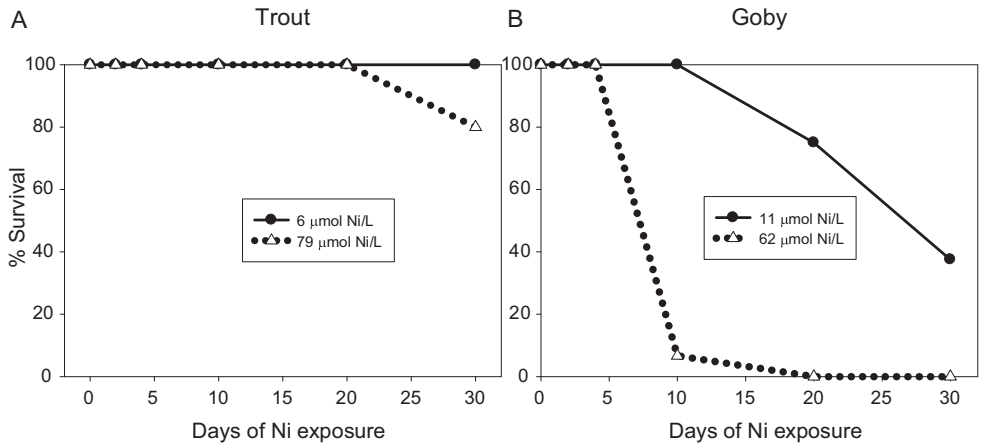

Fig. 1. Percent survival at each sampling time in both the lower and higher Ni exposure concentrations in rainbow trout (A) and round goby (B). Overall percent survival for each exposure concentration was calculated as the product of percent survival at the time of sampling multiplied by the percent survival from previous sampling time. $n = 1$ per treatment at each sampling time.

spectroscopy (GFAAS; Varian SpectrAA—220 with graphite tube atomizer (GTA—110), Mulgrave, Australia) against certified atomic absorption standards (Aldrich Chemical Company, Oakville, ON, Canada). Ni recovery in water samples was 93 ± 2.3% as determined by Environment Canada certified reference materials, TM-24.3 (lot no. 0310) and TM-25.3 (lot no. 0809). Quality control blanks were run every 20th sample to correct for background contamination. Background correction was not used and Ni concentrations were not corrected for recovery. Measurements were conducted at a wavelength and slit width of 232.0 nm and 0.2 nm, respectively, to obtain a lower working limit of 0.003 μmol/L.

Flame Atomic Absorption Spectroscopy (FAAS; Varian SpectrAA—FS-220, Mulgrave, Australia) was used to measure the concentrations of major cations (Na, Mg, Ca and K) in water samples. All water samples were diluted using 1% HNO_3 for Na analysis, 1% HNO_3 with 1% $LaCl_3$ for Ca and Mg analysis and 1% HNO_3 with 0.01% $CsCl_3$ for K analysis. Reference standard solutions for all ions were used to obtain standard curves (Fisher Scientific, Ottawa, ON, Canada). Water pH and DOC were measured using an Accumet® Basic AB15 pH meter (Fisher Scientific, Ottawa, ON, Canada) and a total organic carbon analyzer (Mandel Scientific Company Inc.; TOC–V_{CPN} series; Shimadzu, Kyoto, Japan), respectively.

As fish were sampled from the exposure tank at various timepoints, overall percent survival for each exposure concentration was calculated as the product of percent survival at the time of sampling multiplied by the percent survival from previous sampling time as is stated in the ASTM—E1241-05 Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fishes (ASTM, 2012).

2.7. Statistical analyses

Critical Body Residue 50 (CBR50) values with 95% confidence intervals (C.I.) were calculated using ToxCalc—Toxicity Data Analysis Software ver.5.0.32 (Tidepool Scientific Software, McKinleyville, CA, U.S.A.). The CBR50 was the Ni bioaccumulation in an organ on a specific day of exposure that corresponded to 50% chronic mortality at 30 d of exposure. Raw (no background correction) bioaccumulation data was used against chronic (30-d) mortality to calculate CBR50 values in ToxCalc.

Data have been presented as means ± SEM (n), where n is the sample size. All data reached normality, and conformed to homogeneity tests, or were transformed as necessary before statistical analyses were performed. Significant differences between two groups were evaluated by unpaired Student's t-tests (two-tailed). Comparisons among multiple treatment groups were assessed using a one-way analysis of variance (ANOVA) followed by Fisher LSD Method (Sigma Plot 10.0, Chicago, IL, USA). The Fisher LSD test was chosen as a powerful test protective against Type II error because our goal was to identify differences in subcellular distribution which remained consistent over different sampling times. For all tests, statistical significance was allotted to differences with $p < 0.05$.

3. Results

3.1. Water chemistry

Ni water concentrations for Ni exposures to rainbow trout and round goby expressed as nominal, total, dissolved, ionic and active fractions of the metal, taking into account this measured water chemistry, are reported in the Supplementary Table 2, whereas corresponding water chemistry data are reported in Supplementary Table 1. While nominal concentrations for both species were 10 and 60 μmol/L, actual measured mean total concentrations were 12.0 and 68.3 μmol/L for goby and 6.2 and 85.6 μmol/L for trout; control concentrations averaged 0.05 μmol/L or less (Supplementary Table 2). All Ni water concentrations presented in this study are reported as the dissolved fraction of the metal, which averaged 92% of the total values in the low (11.2 and 5.8 μmol/L) and high exposures (62.0 and 79.0 μmol/L for goby and trout, respectively; Supplementary Table 2).

3.2. Survival over chronic exposure

Rainbow trout were more resistant to chronic waterborne and dietary nickel exposure than round goby. There was no mortality in the controls for either fish. There was no mortality at the lower exposure concentration and only 20% mortality in the higher exposure by day 30 for rainbow trout (Fig. 1A). In contrast, round goby mortality started on day 20 in the lower exposure with 38% survival by day 30. In the higher exposure, survival dropped steeply by day 10 to 7%, with no round goby surviving at the following two sampling times (Fig. 1B).

3.3. Comparison of whole-organ and sub-cellular fractional concentrations of Ni in gill and gut

Prior to Ni exposure in the laboratory, the gills and gut of round goby had ~2× higher Ni concentrations in comparison to rainbow trout and there was no significant difference in organ

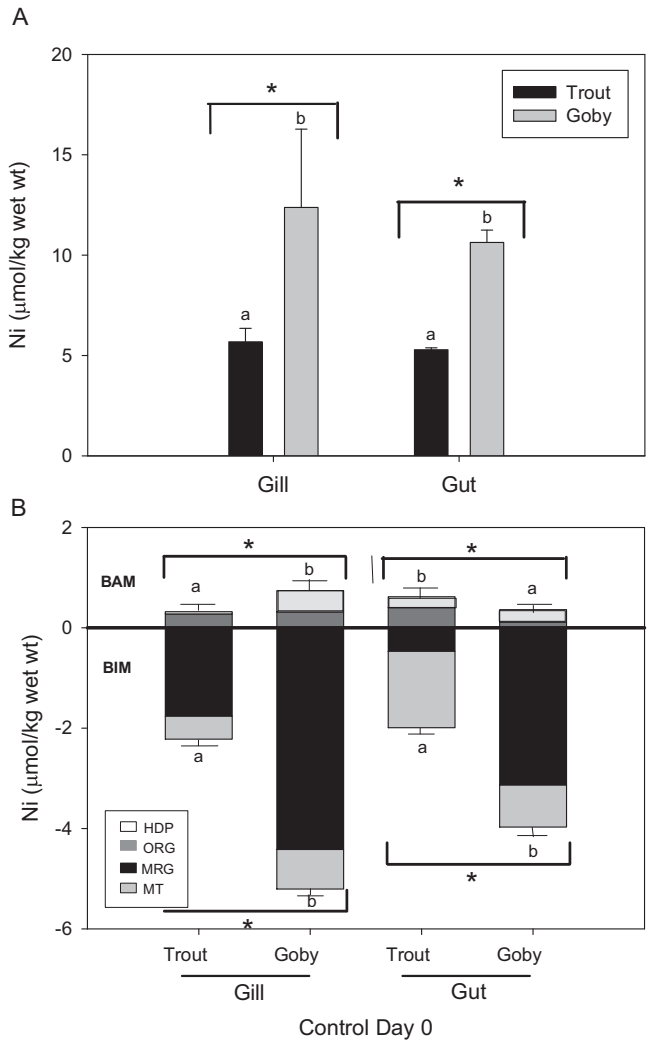

Fig. 2. Ni bioaccumulation in the gills and gut (A) and Ni bioaccumulation in the subcellular fractions (B) of rainbow trout and round goby at day 0 (i.e. prior to chronic Ni exposure in the laboratory) in control fish. HDP and ORG comprise the BAM fractions which are shown above the zero line, whereas, MRG and MT comprise the BIM fractions which are represented below the zero line. An * denotes a significant difference in the whole organ bioaccumulation between the two fish species (evaluated by unpaired Student's *t*-test), whereas different letters denote significant differences between organ bioaccumulation (A) or significant differences in either the BAM or BIM fractions of the organs (B); evaluated using a one-way analysis of variance (ANOVA) followed by Fisher LSD method; $p < 0.05$). Values are means ± S.E.M.; $n = 6$ per treatment.

Ni bioaccumulation within a species (Fig. 2A). The BIM fractions (MRG and MT) of the gills exhibited ~7× more Ni in comparison to the BAM fractions (ORG and HDP) in both rainbow trout and round goby (Fig. 2B). A similar trend was observed in the gut where ~3× and ~11× more Ni was in BIM fraction of rainbow trout and round goby, respectively (Fig. 2B). Within the gills, round goby had ~2.4× more Ni in both the BIM and BAM fractions in comparison to rainbow trout. The gut of round goby also had more Ni in the BIM (~2×) fractions in comparison to rainbow trout, whereas there was more Ni (~1.5×) in the BAM fractions of the rainbow trout (Fig. 2B).

3.4. Dietary component of Ni exposure from prey

The average Ni bioaccumulation both in the whole organism as well as the average sub-cellular distribution in the prey species, *L. variegatus*, is shown in Fig. 3. These worms were exposed for 48 h in the effluent of the fish exposure tanks before being fed to the fish. As the exposure concentration increased by either 13-fold or 6-fold in the rainbow trout and round goby exposure water, respectively, the Ni concentration within the *Lumbriculus* increased to a lesser extent with only 3.4 and 1.9-fold increases, respectively (Fig. 3A and B). The bioaccumulation of Ni in the BIM vs. BAM fractions was generally about equal. Within the BIM fraction, there was no significant difference between the percent Ni in the MRG and MT fractions, however, within the BAM fraction, there was on average 7× more Ni distributed to the ORG fraction in comparison to HDP fraction (Fig. 3C and D).

3.5. Whole organ Ni bioaccumulation at various time points

3.5.1. Gills

In the current study, gill Ni bioaccumulation in both species displayed a biphasic relationship of Ni loading into the gills in the initial days of exposure followed by a period of stabilization above control values (Fig. 4A and B). Rainbow trout gill Ni bioaccumulation steadily increased from day 0 to day 4 in the 6 μmol/L exposure and until day 10 in the highest exposure concentration, after which there were no significant changes (Fig. 4A). Across all sampling days, there was 2–3.5× more Ni in the gills of rainbow trout exposed to 79 μmol/L in comparison to 6 μmol/L, a 13-fold difference in exposure concentrations (Fig. 4A).

Gill Ni bioaccumulation in round goby was highest on day 2 followed by significant decreases of 70% and 55% by day 4 in the 11 μmol/L and 62 μmol/L exposure concentrations, respectively (Fig. 4B). In the 11 μmol/L exposure, gill Ni bioaccumulation remained constant until day 30 where it decreased by a further 60% (Fig. 4B). On days 2 and 4, there was 3–4× more Ni in the gills of round goby in the higher exposure in comparison to the lower exposure (Fig. 4B).

Gills of round goby bioaccumulated ~16× and ~5× more Ni by day 2 and day 4, respectively, in the higher exposure and an average of ~4 × more Ni in the lower exposure concentration in comparison to trout (Fig. 4A and B).

3.5.2. Gut

Acute (days 2 and 4) Ni bioaccumulation in the rainbow trout gut remained constant then increased by day 10 (in the 79 μmol/L exposure) or day 20 (at 6 μmol/L) and plateaued again until day 30 (Fig. 4C). Similar to the gills, the gut did not bioaccumulate Ni in proportion to the differences in the concentrations in the water or the prey. There was only ~2× more Ni in the gut of rainbow trout exposed to 79 μmol/L (83 μmol/kg wet wt in prey) in comparison to 6 μmol/L (24 μmol/kg wet wt in prey), a 13-fold difference in exposure water concentrations and a 3.4-fold difference in the prey concentration (Figs. 3A and 4C).

Gut Ni bioaccumulation of round goby was greatest on days 10 and 4 in the 11 μmol/L and 62 μmol/L exposure concentrations, respectively (Fig. 4D). There was a ~10-fold increase in the higher exposure, whereas, in the lower exposure concentrations, gut Ni bioaccumulation decreased steadily by 80% by day 30 (Fig. 4D).

In contrast to the gills, the gut Ni bioaccumulation did not differ as much between the two species with an average of ~2× more Ni in round goby gut in comparison to rainbow trout gut, with the exception of day 4 in the highest exposure where there was ~6× more in round goby (Fig. 4C and D).

3.6. Gill and gut subcellular Ni distribution

3.6.1. Gill

In the rainbow trout, there was 2–4× more Ni in the BIM fraction in comparison to the BAM fraction of the gills at the lower exposure (Fig. 5A) and approximately 2× more at the higher exposure

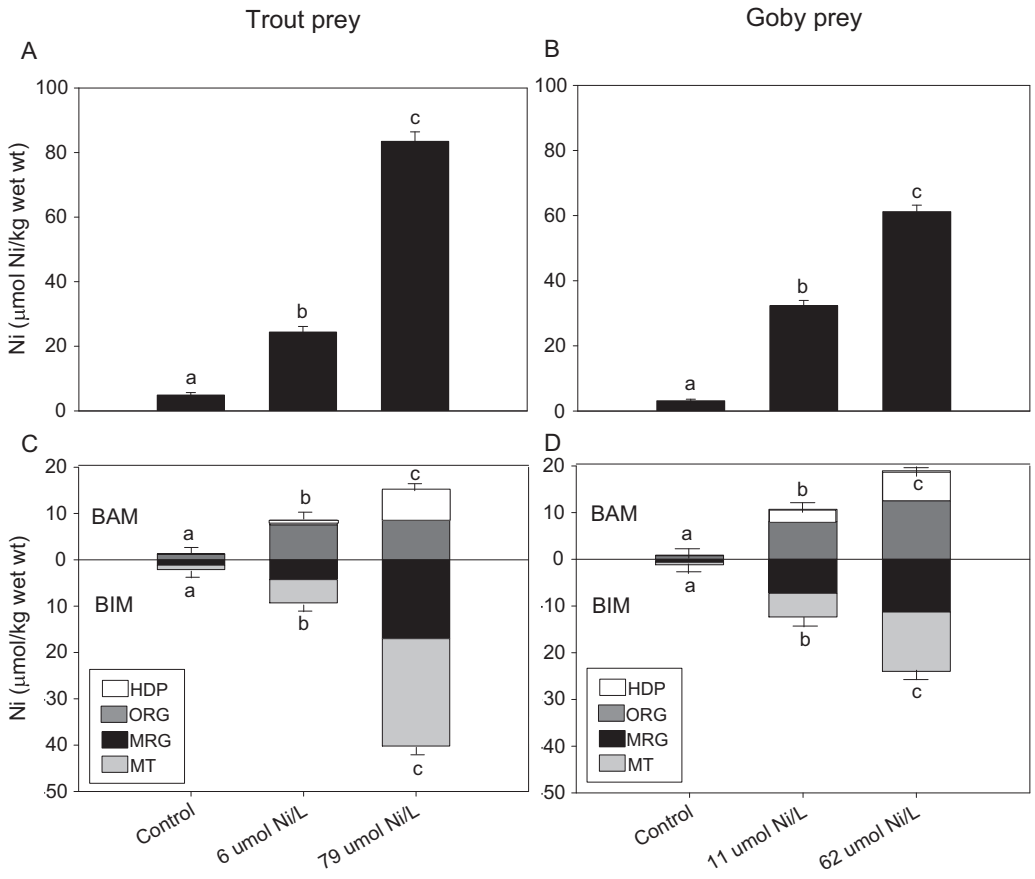

Fig. 3. Average whole body Ni bioaccumulation in prey Lumbriculus; ((A) and (B)) and distribution of Ni in sub-cellular fractions of prey ((C) and (D)) following 48-h exposure to Ni via effluent of rainbow trout ((A) and (B)) and round goby ((C) and (D)) exposure tanks. HDP and ORG comprise the BAM fractions which are shown above the zero line, whereas, MRG and MT comprise the BIM fractions which are represented below the zero line. Values are means ± S.E.M.; $n = 6$ per treatment. Different letters denote significant differences between organ bioaccumulation (panels (A) and (B)) or significant differences in either the BAM or BIM fractions at different exposure concentrations (panels (C) and (D)) and were evaluated using a one-way analysis of variance (ANOVA) followed by Fisher LSD method; $p < 0.05$.

concentration (Fig. 5C). Similarly, in the round goby, 2–4× more Ni bioaccumulated in the BIM vs. BAM fraction of the gills (Fig. 5B and D) in both exposure concentrations.

A comparison between the Ni bioaccumulated in the BIM and BAM fractions of the two species, shows approximately 3× more BIM Ni and 7× more BAM Ni in the round goby gills vs. the rainbow trout, respectively, at most times, regardless of exposure concentrations. However, day 2 was an exception where there was 15× and 40× more Ni bioaccumulated in the BIM and BAM fractions of round goby gills, respectively (Fig. 5).

3.6.2. Gut

The gut of rainbow trout exposed to either Ni exposure concentration, bioaccumulated ~3× more Ni in the BIM vs. BAM fraction (Fig. 6A and C). In contrast, the BIM fractions of the round goby gut bioaccumulated ~10× more Ni in comparison to the BAM fraction (Fig. 6B and D).

In general, in the lower exposure concentration, similar amounts of Ni bioaccumulated in the BAM fractions of the rainbow trout and round goby (Fig. 6A and B). Similarly, there was about the same amount of Ni in the BIM fractions up until day 20 where there was ~4× more Ni in the rainbow trout gut in comparison to round goby. In the higher exposure concentration, more Ni bioaccumulated in the BIM and BAM fractions of rainbow trout on day 2, whereas by day 4, ~5× and ~2× more Ni was in the BIM and BAM fractions of round goby in comparison to rainbow trout, respectively (Fig. 6C and D).

3.7. How Ni bioaccumulation and sub-cellular fractions correlate to mortality

We examined relationships between Ni bioaccumulation at various exposure times versus chronic (30-d) mortality to look for predictive indicators. For simplicity, only day 4 and day 30 data are shown in Fig. 7, but data on intermediate days exhibited similar relationships. Acute gill Ni bioaccumulation at day 4 was just as predictive of chronic (30-d) mortality as bioaccumulation at day 30 in the round goby (Fig. 7A). As 4-d and 30-d Ni bioaccumulation increased, there was increasing chronic mortality. Ni bioaccumulation in both the BIM and BAM fractions were also predictive of chronic (30-d) mortality (Fig. 7C and E). In addition, there was no significant difference (95% confidence intervals overlapped) between CBR50 values calculated either from 4-d or 30-d bioaccumulation for any of the fractions: whole gill or BIM or BAM fractions of the gills of round goby (Fig. 7A, C and E). Notably, in the gut, in contrast to the gills, there was no correlation between gut Ni bioaccumulation and chronic mortality with respect to the whole organ or BIM and BAM fractions (Fig. 7B, D and F).

The high survival of rainbow trout in both exposure concentrations did not permit a correlation to be made between early bioaccumulation and chronic mortality.

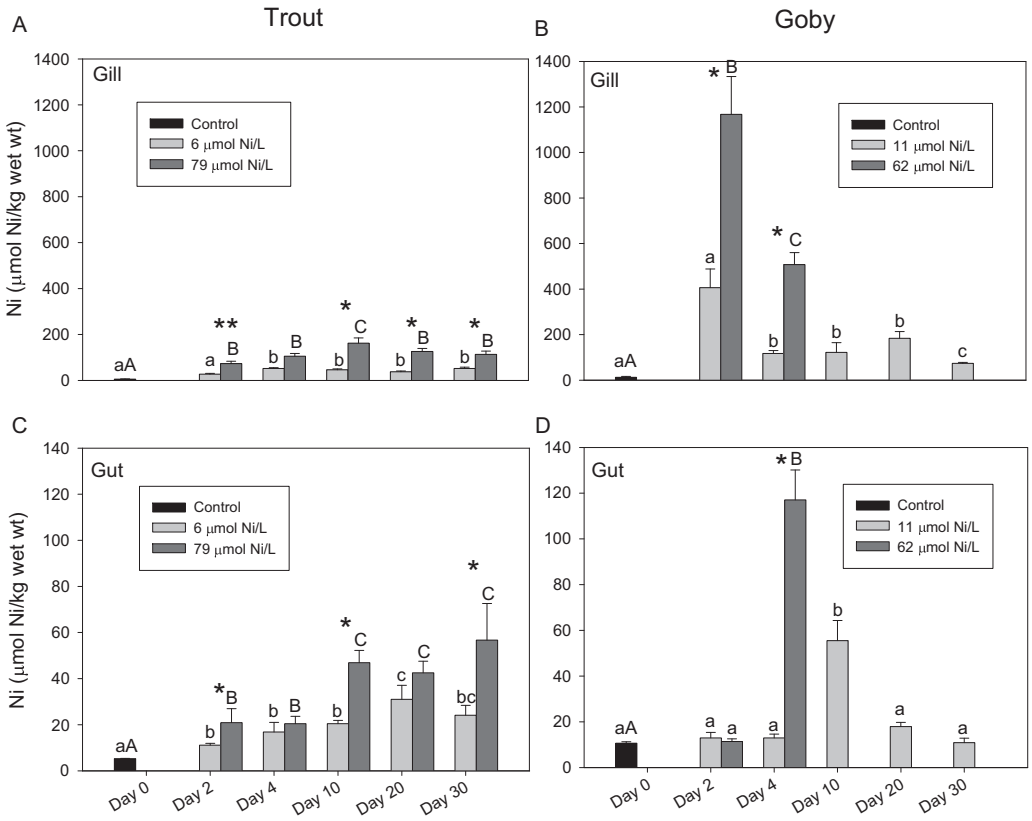

Fig. 4. Total Ni bioaccumulation in the gills and gut of rainbow trout ((A) and (C)) and round goby ((B) and (D)), respectively. Different letters denote differences within a concentration over time which were evaluated using a one-way analysis of variance (ANOVA) followed by Fisher LSD method. An * denotes a concentration-dependent difference at a time point ($p < 0.05$; evaluated by unpaired Student's *t*-test). Values are means ± S.E.M.; $n = 6$ per treatment.

4. Discussion

4.1. Overview

There are many benefits of acute mechanistic data on metal toxicity in terms of modeling approaches for protection of aquatic species; however, quite often the concentrations used in acute toxicity tests are not environmentally relevant and do not take into consideration dietborne metal exposure. In the current longer term study we have employed a joint waterborne and dietary Ni exposure at two concentrations, the lower of which is typical of a Ni-contaminated environment (<12 μmol Ni/L; Chau and Kulikovsky-Cordeiro, 1995; Eisler, 1998) and a higher level Ni exposure concentration which is not environmentally relevant but may give information on mechanisms of chronic Ni toxicity. To the best of our knowledge, only four other studies have assessed the subcellular distribution of Ni in fish tissues: the whole body of the fathead minnow, *Pimephales promelas* (Lapointe and Couture, 2009), olfactory epithelium and nerve of the northern pike, *Esox lucius* (Tallkvist et al., 1998) and the liver of wild yellow perch, *Perca flavescens* (Giguère et al., 2006; Campbell et al., 2008). However none of these assessed the sub-cellular distribution of Ni in the gills or the gut—the two organs responsible for the exchange of nutrients and minerals, and eliminating waste as well as the organs considered to be biotic ligands of metal binding.

We have shown that round goby are more sensitive than rainbow trout to chronic Ni exposure. Earlier, we reported that round goby were also more sensitive to acute Ni exposure (Leonard et al., 2014). Possibly, this greater sensitivity may be due to the round goby's pre-exposure to pollutants at their collection site, as evidenced by ~2-fold greater initial Ni bioaccumulation in both their gills and gut relative to trout. This is contrary to other studies, where fish acclimate to metal exposures causing a decrease in sensitivity (Zhang and Wang, 2005; Campbell et al., 2008). However, it may also be possible that this is simply a difference between farm reared and wild fish. Nonetheless, this higher initial Ni bioaccumulation was followed by ~2–16× larger bioaccumulation in both the gills and the gut during the experimental exposure. On a subcellular level, ~3–40× more Ni is associated with the BAM fraction of round goby in comparison to rainbow trout.

Assessing sub-cellular distribution of Ni in both the gills and gut of two fish of different habitat and lifestyles revealed two different strategies of Ni bioaccumulation and sub-cellular distribution. On the one hand, the rainbow trout exhibited an ability to regulate gill Ni bioaccumulation and maintain the majority of the Ni in the MT fraction as part of the BIM fraction. In contrast, the round goby exhibited large Ni spillovers to the BAM fraction of the gill, both to the HDP and ORG compartments. However, the same trend was not observed in the gut, where the potential acclimation of round goby to pollutants from their collection site may have aided their ability to regulate Ni spillover to the BAM fraction more so than the rainbow trout.

Gill Ni bioaccumulation at either 4-d or 30-d in the whole organ, or BIM or BAM fractions of the metal were predictive of chronic Ni toxicity in the round goby. There was not sufficient mortality in the rainbow trout to allow assessment of the Ni bioaccumulation which would be predictive of chronic mortality.

4.2. Survival over chronic exposure

Round goby are expected to be a rather pollution tolerant species because of their prevalence in highly contaminated areas

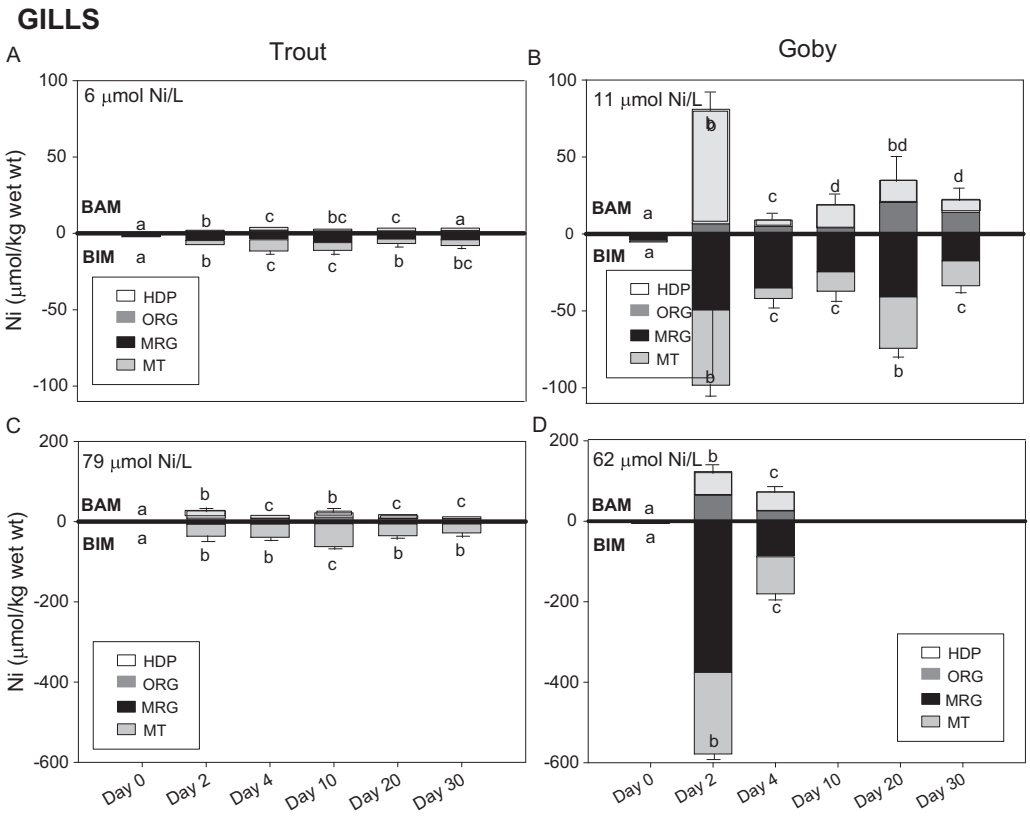

Fig. 5. Ni subcellular distribution in the gills of rainbow trout ((A) and (C)) and round goby ((B) and (D)). Dissolved Ni exposure concentrations are presented in the upper left quadrants. HDP and ORG comprise the BAM fractions which are shown above the zero line, whereas, MRG and MT comprise the BIM fractions which are represented below the zero line. Different letters denote significant differences, which were evaluated using a one-way analysis of variance (ANOVA) followed by Fisher LSD method, in either the BAM or BIM among the six sampling times ($p < 0.05$). Values are means ± S.E.M.; $n = 6$ per treatment.

(Pinchuk et al., 2003) including Hamilton Harbour (Marentette and Balshine, 2012). However, laboratory studies have demonstrated that this species is more sensitive to both acute Ni (Leonard et al., 2014) and chronic Ni (present study) toxicity in comparison to rainbow trout, a species which is known to be one of the most sensitive teleosts to metal toxicity (U.S. EPA, 1986; Brix et al., 2004). For example, the chronic no-observable effect concentration (NOEC) and lowest observable effect concentration (LOEC) for growth of trout are less than 0.6 μmol Ni/L (Nebeker et al., 1985). To the best of our knowledge, there are no previous studies which assess chronic Ni toxicity to round goby.

4.3. Comparison of whole-organ and sub-cellular fractional concentrations of Ni in the gill and gut

Prior to laboratory testing, the round goby had ~2× more Ni in the gills and the gut than the rainbow trout purchased from a hatchery. As well, on a sub-cellular level, the round goby had ~3× more Ni in the BIM and BAM fractions of the gills in comparison to rainbow trout. There was also ~7× more Ni in the BIM fraction of the gut of round goby in comparison to rainbow trout. This suggests that the round goby were closer to reaching or had reached their threshold concentration in the BIM fraction leading to spill over into the BAM fraction (Adams et al., 2011), which potentially caused adverse physiological effects. In fact, this may help explain the higher sensitivity of the round goby. Although the collection site (LaSalle Park on Hamilton Harbour) is considered to be a "clean site" (Marentette et al., 2010), the Harbour itself is in close proximity to industrial activities such as steel mills and many contaminants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and metals including cadmium, arsenic, lead, iron, mercury, zinc, and nickel are known to be of concern in some areas of the Hamilton Harbour (Hamilton Harbour Remedial Action Plan RAP, 1992, 2003; International Joint Commission, 1999). In fact, sediment Ni concentrations at LaSalle Park were 900 μmol/kg, a value which is above the lowest effect level according to the provincial sediment quality guidelines (Zeman, 2009) and liver concentrations of collected round goby were ~3 μmol/kg (Marentette et al., 2010), below levels of the gill and gut of the current study, however this may be due to low Ni bioaccumulation in the livers of teleosts in general (Leonard et al., 2014). Therefore, the residual influence of contaminants from Hamilton Harbour both via the diet and/or the water in addition to the laboratory Ni exposure may have overwhelmed the detoxifying strategies of the round goby and therefore reduced their overall tolerance.

4.4. Dietary component of Ni exposure from prey

Recently, the dietary transfer of metal to fish has been recognized as a critical exposure route which needs to be addressed in further detail as current WQC are based on waterborne exposures (Meyer et al., 2005; Béchard et al., 2009; Klinck et al., 2009). It has been suggested that dietary metal uptake from natural food (more typical prey) vs. artificial food may provide predators with a form of the metal which is more trophically available (Meyer et al., 2005; Ng and Wood, 2008; Béchard et al., 2009; Klinck et al., 2009).

Black worms were held in the out-flow from the fish tanks for 48-h prior to being fed to the fish. A steady-state condition in the prey was therefore not achieved. Previous research with Zn and Cd has shown that steady-state conditions were not reached in the caddisfly, *Mystacicks* sp., over 30 days of exposure (Timmermans et al., 1992). Additionally, in same black worm species used in the

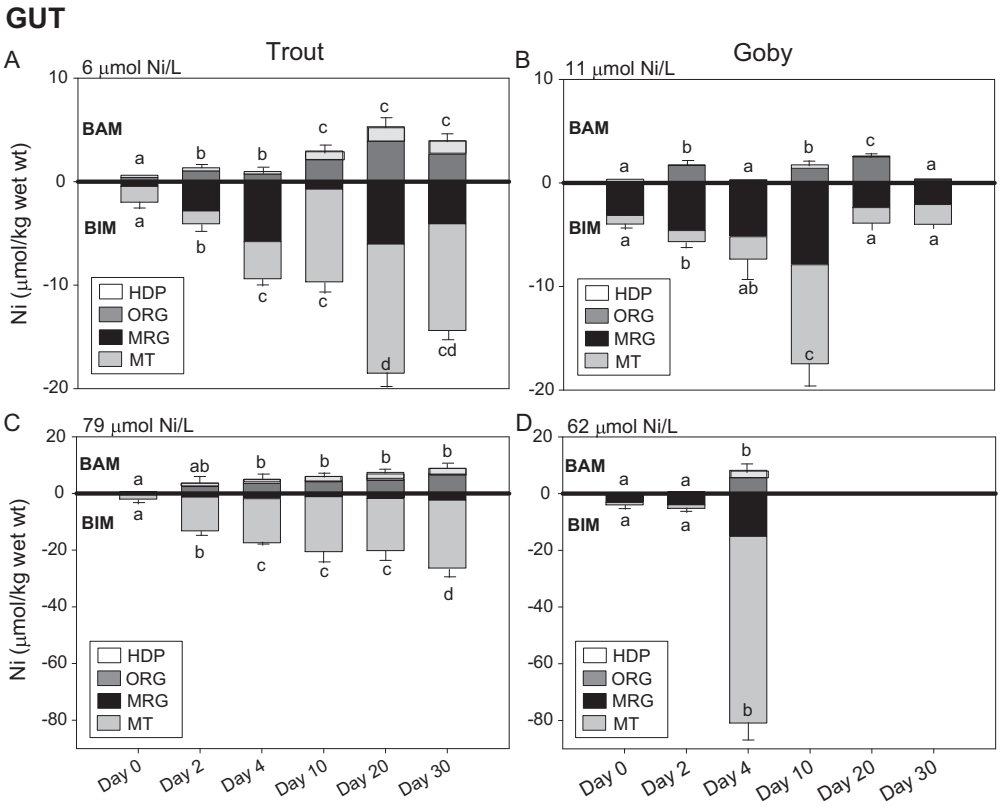

Fig. 6. Ni subcellular distribution in the gut of rainbow trout ((A) and (C)) and round goby ((B) and (D)). Dissolved Ni exposure concentrations are presented in the upper left quadrants. HDP and ORG comprise the BAM fractions which are shown above the zero line, whereas, MRG and MT comprise the BIM fractions which are represented below the zero line. Different letters denote significant differences, which were evaluated using a one-way analysis of variance (ANOVA) followed by Fisher LSD method, in either the BAM or BIM among the six sampling times ($p < 0.05$). Values are means ± S.E.M.; $n = 6$ per treatment.

present study, steady state conditions were not achieved over a 7 week exposure to waterborne Pb (Derek Alsop, personal communication). Therefore, we chose to expose the predators to a constant Ni concentration from the diet over the course of the 30-d exposure by exposing the black worms in an acute manner.

While it is possible that Ni accumulation in the gut tissue of the predators occurred due to input from waterborne sources (drinking, transport from the gills via the plasma), it seems more likely that the bulk of the bioaccumulation would have originated from the prey. In trout gut, Ni concentrations by day 30 were approximately equal to those in the prey, suggesting that equilibrium had been achieved. In round goby, at the lower Ni exposure concentration, Ni levels in the gut tissue were actually lower than in the prey, though the opposite occurred in the higher exposure concentration at day 4, the last sampling day before substantial mortality occurred. In the trout, at the lower Ni exposure concentration, there was significantly less of the total in the BAM fraction, and more in the BIM compartment than in the prey, suggesting that detoxification had occurred. However this was not true at the higher exposure concentration. In round goby, there was evidence of a greater detoxification capacity inasmuch as only about 10% of the accumulated Ni was partitioned into the BAM fraction, whereas 90% was stored in the BIM compartment at both exposure concentrations. These differences suggest that the round goby was more efficient at regulating dietary Ni uptake and detoxification in comparison to rainbow trout (see below Section 4.6). However, based on our experimental design, we cannot accurately evaluate the trophic transfer efficiency of Ni in these species nor distinguish the contribution of the two Ni sources (waterborne and dietary) to Ni bioaccumulation in the predators. Future studies should investigate the aspect of contaminant pre-exposure on the trophic transfer efficiency of metals.

4.5. Whole organ Ni bioaccumulation at various time points

Gaining an understanding of Ni toxicokinetics during chronic exposures may establish links between toxicity and exposure (McGeer et al., 2000; McCarty and MacKay, 1993). In the current study, gill Ni bioaccumulation in both species displays a biphasic relationship of Ni loading into the gills in the initial days of exposure followed by a period of stabilization above control values (Fig. 4A and B). This trend has been previously shown for Cu and Zn by Laurén and McDonald (1987), Grosell et al. (1997) and McGeer et al. (2000) and is suggestive of metabolic regulation characteristic of essential nutrients; it is not observed for Cd, a non-essential metal (McGeer et al., 2000). Ni essentiality has not been established in fish, however, homeostatic regulation of Ni has been shown in lake whitefish (Ptashynski and Klaverkamp, 2002), and rainbow trout (Chowdhury et al., 2008).

In general, the initial damage phase characteristic of chronic metal bioaccumulation occurs from bioaccumulation on or in the gills (McGeer et al., 2000; McDonald and Wood, 1993), which was observed in the present study where gill Ni bioaccumulation occurred at earlier time points than the gut Ni bioaccumulation (Fig. 4). In the gut, Ni bioaccumulation did not increase until day 4 or day 10 in either of the fish species, which correlates well to the commonly held view that diet-borne metals are unlikely to cause acute metal toxicity (Meyer et al., 2005) but play a more critical role in chronic metal toxicity.

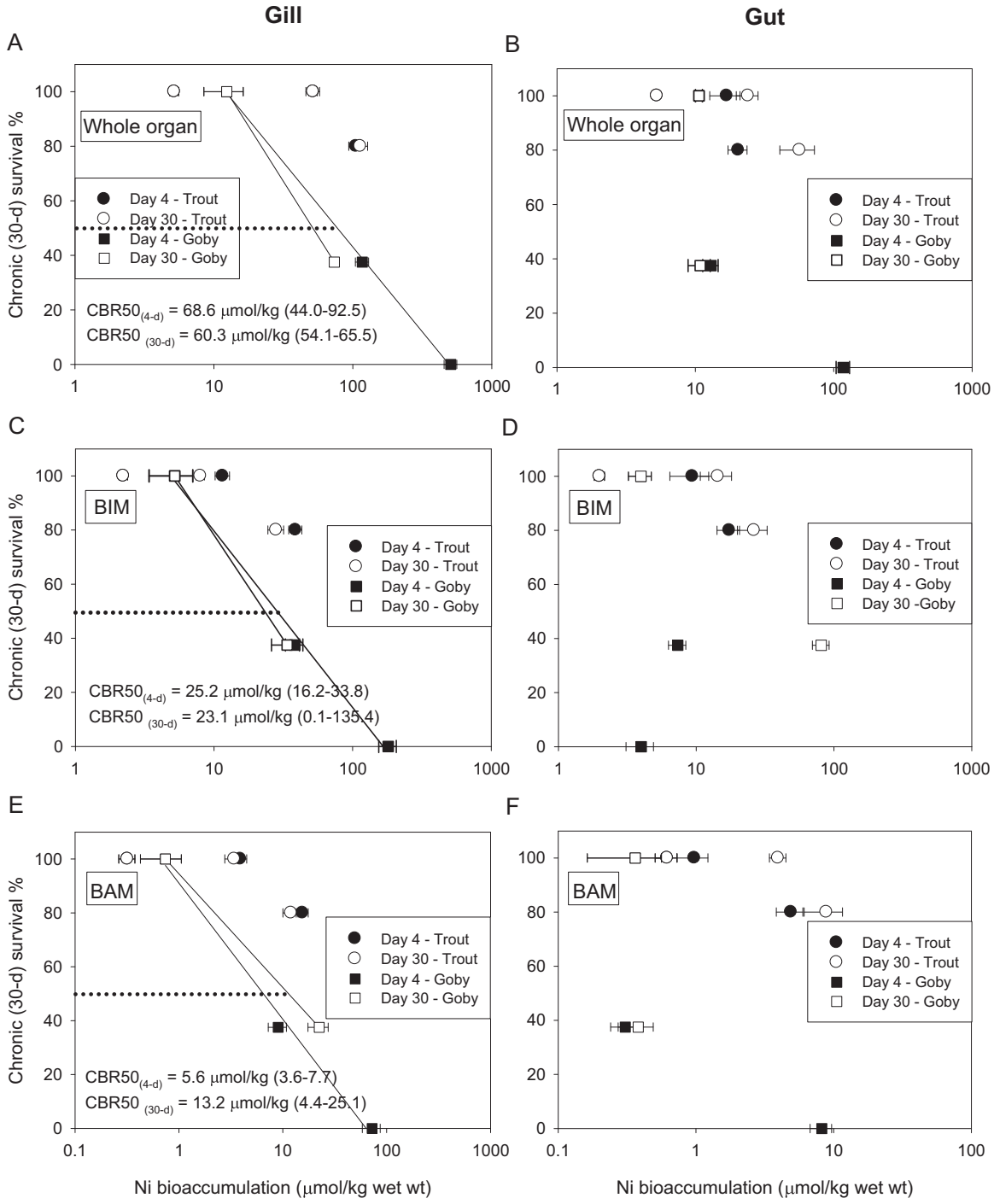

Fig. 7. Relationships between chronic (30-d) survival (%) and acute (4-d) and chronic (30-d) Ni bioaccumulation in the gills (A), BIM (C) and BAM (E) fractions of the gills, as well as in the gut (B), BIM (D) and BAM (F) fractions of rainbow trout and round goby. The lines at 50% survival intersect the bioaccumulation vs. mortality relationships at the CBR50 values, which are indicated on the figure panel for round goby gill tissue only and were calculated using Toxcalc software. Note the lack of relationship for the gut tissue of round gobies, and also the high survival of rainbow trout at tissue bioaccumulations associated with 50% chronic mortality in round gobies. Values are means ± S.E.M.; $n = 6$ per treatment.

4.6. Gill and gut subcellular Ni distribution

4.6.1. Gill

In general, similar trends were observed for both the lower, environmentally relevant Ni exposure and the higher, more toxic Ni concentrations; therefore, for simplicity; results will be discussed together for the two exposure concentrations.

In the gills of rainbow trout, the MT fraction was a major location of Ni bioaccumulation (Fig. 5A and C). This fraction is considered part of the BIM fraction and is therefore detoxified (Wallace et al., 2003). MTs are low molecular weight, cysteine-rich metal binding proteins which are induced from exposure to metals, namely Cu, Cd, and Zn (Roesijadi, 1992; Mason and Jenkins, 1995; Amiard et al., 2006). The information on MT induction by Ni is much less robust; however, Ptashynski et al. (2002) showed MT induction in the intestine of lake whitefish (*Coregonus clupeaformis*) following a dietary exposure to Ni and Giguère et al. (2006) demonstrated MT induction in the liver at low chronic Ni exposures in the yellow

perch, *Perca flavescens*. Mercury (Hg), Cd, Ag (silver) and Zn were also found to induce MT production in the gills of the carp, *Cyprinus carpio*, exposed for 7-d to each respective metal (Cosson, 1994), as well as Cd in the gills of rainbow trout following a 96-h waterborne exposure (Kamunde, 2009) and following a four week dietary Cd exposure from *L. variegatus* to rainbow trout (Ng and Wood, 2008). The binding of Ni by MTs appears be to a successful strategy for survival of this species (Figs. 1 and 5A and C).

In general, the gills of round goby appear to use MT and MRG as mechanisms of detoxifying Ni (Fig. 5B and D). In invertebrates, MRG formation involves the precipitation of a metal into insoluble concretions normally including Ca or Mg phosphate (Roesijadi, 1980; Brown, 1982; Vijver et al., 2004), rendering the metal detoxified. To the best of our knowledge, there is no direct evidence for the use of insoluble granules as a detoxification mechanism in fish; however, the presence of Ni in this fraction is not a novel finding. It has been previously shown in the whole body of fathead minnows and the relative proportion in this fraction increased with Ni exposure (Lapointe and Couture, 2009). Nonetheless, studies on invertebrates have suggested that MRG formation plays a role in chronic tolerance to metal exposure, while MT mainly acts to protect against acute metal exposure (Vijver et al., 2004). Within the 30 day Ni exposure, there was no defined pattern that either supports or rejects this theory. However, the use of both detoxifying strategies in round goby may suggest they were exhausting both detoxification strategies.

Nonetheless, Ni bioaccumulation in the gills of either species was not constrained to the BIM fraction. Even at sub-lethal levels of Ni exposure, both rainbow trout and round goby gills bioaccumulated more Ni than controls (Day 0) in the BAM fraction, suggesting that even at this low level of exposure metal detoxification was not entirely successful (Fig. 5). Previous research has shown complete metal detoxification by the BIM fraction (MTs and/or MRG) under conditions of low metal exposure, with partial detoxification (or spillover) into the BAM fraction under conditions of greater exposure (Wallace et al., 2003). However these studies have been primarily done on naïve organisms and the dietary component of metal exposure has not been taken into consideration. Kamunde (2009) also observed a similar trend to that of the present study for Cd in the gills and liver of rainbow trout where even background Cd bioaccumulation partitioned into all of the subcellular compartments analyzed. Simultaneous Ni bioaccumulation in both the BIM and BAM fractions suggests that detoxification strategies cannot entirely cope with the metal challenge.

A comparison between the two species demonstrates ~3–40× more Ni in the BAM fraction of the round goby gills in comparison to the rainbow trout. Early spillover into this fraction occurred in HDP, which decreased and plateaued by later time points. This is in contrast to the liver of wild yellow perch where steady-state Ni partitioning showed greatest Ni bioaccumulation in the HDP fraction in comparison to any other fraction (Giguère et al., 2006). In the current study, Ni spillover to the ORG fraction of the gills occurred later in the exposure (Days 20 and 30; Fig. 5). Interpretation of the ORG fraction should be done with caution as there may be some functional overlap between BIM and BAM fractions. The inclusion of lysosomes as part of the BAM fraction is not ideal as Ni bioaccumulation in this organ may be indicative of either Ni storage for eventual elimination and detoxification (i.e. BAM classification incorrect) or Ni storage in a biologically active form (i.e. BAM classification correct). The latter could occur if lysosomes become "leaky" following exposure to metals releasing hydrolytic enzymes into the cell (Viarengo et al., 1987). However, the other two components of the ORG fraction: mitochondria and microsomes are appropriately labeled as the BAM fraction; microsomes contain fragmented endoplasmic reticulum responsible for protein synthesis and transport (Fowler et al., 1989), and metal bound to the mitochondria has been shown to reduced metabolic capacities or increase oxidative stress (Silverberg, 1976; Lapointe and Couture, 2009). Therefore, round goby are not as adept at regulating gill Ni uptake and detoxification, leading to higher mortality in this species (Fig. 5).

4.6.2. Gut

Similar to the gills of rainbow trout, the majority of the Ni in the gut of trout was in the MT component of the BIM fraction; however, there was early spillover to the BAM fraction, mainly in the ORG in comparison to the HDP fraction (Fig. 6A and C). A somewhat different trend was observed in the gut of round goby, where the Ni was mainly found in the MRG fraction in comparison to the MT fraction (Fig. 6B and D). However, Ni in the BAM fraction was primarily associated with the ORG fraction. Therefore, it appears that spillover in the BAM fraction of the gut in both species occurs primarily in the ORG fraction, potentially leading to problems with protein synthesis and cellular respiration in the gut tissue.

The goby gut appears to be more efficient than the gills at regulating Ni bioaccumulation, whereas the opposite is true for the rainbow trout (Figs. 5 and 6). Round goby, which were collected from Hamilton Harbour, may have fed on pollution-tolerant benthic invertebrates such as dipterans and oligochaetes known to bioaccumulate toxicants (Seidman et al., 1986). It has been shown that wild yellow perch collected from metal-contaminated lakes had lower rates of Cd absorption in the gut compared to control fish (Klinck et al., 2007). These findings suggest that physiological changes may occur along the gut of fish to allow for better coping strategies in polluted aquatic environments (Klinck et al., 2007). In the gills, it is well established that when fish are chronically exposed to sublethal metal concentrations there is an increase in the low-affinity, high capacity binding sites (Niyogi and Wood, 2003). Acute studies with Ni have shown that the gut of round goby displays low affinity, high capacity transport systems, whereas, the gut of naïve rainbow trout exhibits high affinity, low capacity uptake parameters (Leonard et al., 2009, 2014). Therefore, the ability of the gut of round goby to better regulate gut Ni bioaccumulation and to detoxify the Ni to the BIM fraction may be due to the fish's previous exposure to contaminants via the diet. While this may have caused a change in the binding kinetics of the metal, however, it was not sufficient to protect this species against chronic mortality. Overall, chronic mortality observed in round goby (and not in rainbow trout) appears to be more closely related to the gill Ni bioaccumulation and not to the gut Ni bioaccumulation.

4.7. How Ni bioaccumulation and sub-cellular fractions correlate to mortality

One of the fundamental concepts of the BLM is that early (acute) metal bioaccumulation is predictive of chronic toxicity. For example, Meyer et al. (1999) demonstrated that 24-h gill Ni bioaccumulation (LA50) in the fathead minnow (*P. promelas*) was a constant predictor of 96-h LC50 regardless of water chemistry parameters. In the current study, round goby gill CBR50 values calculated from either 4-d or 30-d gill Ni bioaccumulation against chronic (30-d) mortality were not significantly different which strongly suggests that either acute (4-d) or chronic (30-d) gill Ni bioaccumulation can be used for tissue residue-based risk assessment, even if equilibrium has not yet been reached. This is supported by a study on Cd in *Tubifex tubifex* where 4- to 17-d CBR50 values appeared to be independent of exposure time (Redeker and Blust, 2004). In addition, Ng et al. (2012) showed a similar trend where 7-d Cu bioaccumulation and chronic (28-d) survival were qualitatively similar to those when mortality and Cu were measured at the same chronic endpoint of 28-days.

CBR50 values of the BIM fraction were ~2–4× more than CBR50 values of the BAM fraction (Fig. 7). Therefore, the predicted 50%

mortality occurs at a lower Ni level in the BAM fraction than the BIM fraction, suggesting that round goby are more sensitive to Ni bioaccumulation in the BAM fraction. In addition, in the current study, BIM and BAM fractions of the goby gills are equally predictive of chronic (30-d) mortality. This is in contrast to Cu in the invertebrate, *L. variegatus*, where measurements in BIM, rather than BAM, gave a better indication of metal impact on a cellular level (Ng et al., 2012).

There were no relationships between gut Ni bioaccumulation, or BIM and BAM fractions, at any time point with chronic (30-d) mortality, suggesting that the gut is not the site of key toxic action, thereby supporting our acute study on these two fish species (Leonard et al., 2014) which emphasized the gills as the main site of toxic action and the best organ for prediction of both acute and chronic mortality.

These data also suggest that the concentration of the toxicant within the target tissue, the gills, that produces a certain effect is independent of time. Future studies should expand more upon this to determine whether CBR50 values are also independent of exposure conditions, such as water chemistry. In rainbow trout, which were more resistant to Ni exposure, there was insufficient mortality (Fig. 7) to calculate CBR50 values; however, from Fig. 7 we observe that similar gill Ni concentrations in rainbow trout and round goby are associated with different chronic percent survivals. This same trend is observed in the BIM and BAM fractions, suggesting that CBR50 values of the two species would not be similar which would have implications in terms of the predictive capacity of a chronic TRA model.

Earlier studies conducted in our laboratory demonstrated that CBR50 values were more consistent than exposure concentrations either between different water hardness values within a species or between different species (Leonard and Wood, 2013; Leonard et al., 2014). Data from the current study show promise that gill tissue Ni residues can be used to predict chronic metal toxicity. Together, these data support one of the main advantages of the TRA where tissue concentrations are generally less variable than exposure concentrations with respect to a toxicity response.

5. Conclusions

In this study, we have employed a joint waterborne and dietary Ni exposure at two concentrations over a chronic (30-d) time frame and assessed the sub-cellular distribution of Ni in the gills or the gut. Round goby were more sensitive than rainbow trout to chronic Ni exposure. This was possibly due to their pre-exposure to pollutants at their collection site, their higher bioaccumulation of Ni in both the gills and gut during the laboratory exposure, and/or the more Ni that associated with the BAM fraction of the gills in round goby in comparison to rainbow trout.

Gill Ni bioaccumulations at either 4-d or 30-d in the whole organ, or BIM or BAM fractions of the metal correlated to chronic Ni toxicity in the round goby. There was not sufficient mortality in the rainbow trout to assess the Ni bioaccumulation which would be predictive of chronic mortality.

Acknowledgments

We wish to thank Kris Knorr for his assistance in fish collection, and Warren Norwood, Patty Gillis, Jim McGeer, and two anonymous reviewers for constructive comments on the MS. Special thanks to Bill Adams of Rio Tinto for facilitating this research. This project was supported by a NSERC Strategic Grant, with support from Rio Tinto Alcan and Environment Canada, to CMW and Jim McGeer. CMW is supported by the Canada Research Chairs Program.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aquatox.2014.04.028.

References

Adams, W.J., Blust, R., Borgmann, U., Brix, K.V., DeForest, D.K., Green, A.S., Meyer, J., McGeer, J.C., Paquin, P., Rainbow, P.S., Wood, C.M., 2011. Utility of tissue residue approach for predicting effects of metals on aquatic organisms. Integr. Environ. Assess. Manage. 7, 75–98.

Amiard, J.-C., Amiard-Triquet, C., Barka, S., Pellerin, J., Rainbow, P.S., 2006. Metallothioneins in aquatic invertebrates: their role in metal detoxification and their use as biomarkers. Aquat. Toxicol. 76, 160–202.

ASTM, 2012. Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fishes: E1241–05. ASTM International, West Conshohocken, PA.

Béchard, K.M., Gillis, P.L., Wood, C.M., 2009. Trophic transfer of Cd from larval chironomids (*Chironomus riparius*) exposed via sediment or waterborne routes, to zebrafish (*Danio rerio*): tissue-specific and subcellular comparisons. Aquat. Toxicol. 90, 310–321.

Borgmann, U., 1983. Metal speciation and toxicity of free metal ions to aquatic biota. In: Nriagu, J.O. (Ed.), Aquatic Toxicology. Wiley Series in Advances in Environmental Science and Technology. John Wiley and Sons, New York, NY, p. 47.

Borgmann, U., Néron, R., Norwood, W.P., 2001. Quantification of bioavailable nickel in sediments and toxic thresholds to *Hyallela azteca*. Environ. Pollut. 111, 189–198.

Borgmann, U., Norwood, W.P., 1997. Toxicity and accumulation of zinc and copper in *Hyalella azteca* exposed to metal-spiked sediments. Can. J. Fish. Aquat. Sci. 54, 1046–1054.

Borgmann, U., Norwood, W.P., Babirad, I.M., 1991. Relationship between chronic toxicity and bioaccumulation of cadmium in *Hyalella Azteca*. Can. J. Fish. Aquat. Sci. 48, 1055–1060.

Borgmann, U., Norwood, W.P., Dixon, D.G., 2004. Re-evaluation of metal bioaccumulation and chronic toxicity in *Hyalella azteca* using saturation curves and the biotic ligand model. Environ. Pollut. 131, 469–484.

Brix, K.V., Keithly, J., Deforest, D.K., Laughlin, J., 2004. Acute and chronic toxicity of nickel to rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 23, 2221–2228.

Brown, B.E., 1982. The form and function of metal-containing 'granules' in invertebrate tissues. Biol. Rev. 57, 621–667.

Campbell, P.G.C., 1995. Interactions between trace metals and aquatic organisms: a critique of the free-ion activity model. In: Tessier, A., Turner, D.R. (Eds.), Metal Speciation and Bioavailability in Aquatic Systems. John Wiley and Sons, Chichester, pp. 45–102.

Campbell, P.G.D., Kraemer, L.D., Giguère, A., Hare, L., Hontella, A., 2008. Subcellular distribution of cadmium and nickel in chronically exposed wild fish: inferences regarding metal detoxification strategies and implications for setting water quality guidelines for dissolved metals. Hum. Ecol. Risk Assess. 14, 290–316.

CCREM (Canadian Council of Resource and Environment Ministers), 1987. Canadian Water Quality Guidelines. Task Force on Water Quality Guidelines.

Chau, Y.K., Kulikovsky-Cordeiro, O.T.R., 1995. Occurrence of nickel in the Canadian Environment. Environ. Rev. 3, 95–117.

Chowdhury, M.J., Bucking, C., Wood, C.M., 2008. Pre-exposure to waterborne nickel downregulates gastrointestinal nickel uptake in rainbow trout: indirect evidence for nickel essentiality. Environ. Sci. Technol. 42, 1359–1364.

Connolly, J.P., 1985. Predicting single-species toxicity in natural water systems. Environ. Toxicol. Chem. 4, 573–582.

Cosson, R.P., 1994. Heavy metal intracellular balance and relationship with metallothionein induction in the gills of carp. Biol. Trace Elem. Res. 46, 229–245.

Croteau, M.N., Hare, L., Campbell, P.G.C., Couillard, Y., 2002. Metallothionein-like protein in the biomonitor *Chaoborus*: occurrence and relationship to ambient metal concentrations in Lakes. Environ. Toxicol. Chem. 21, 737–741.

Di Toro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R., Santore, R.C., 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical basis. Environ. Toxicol. Chem. 20, 2383–2396.

Eisler, R., 1998. Nickel hazards to fish, wildlife, and invertebrates: a synoptic review. In: Biological Science Report, 1998–2001. US Geological Survey, Biological Resources Division.

Farag, A.M., Nimick, D.A., Kimball, B.A., Church, S.E., Harper, D.D., Brumbaugh, W.G., 2007. Concentrations of metals in water, sediment, biofilm, benthic macroinvertebrates, and fish in the Boulder River watershed, Montana, and the role of colloids in metal uptake. Arch. Environ. Contam. Toxicol. 52, 397–409.

Fowler, B.A., Lucier, G.W., Hayes, A.W., 1989. Organelles as tools in toxicology. In: Hayes, A.W. (Ed.), Principles and Methods in Toxicology. Raven Press Ltd., New York, NY, pp. 815–833.

Giguère, A., Campbell, P.G.C., Hare, L., Couture, P., 2006. Sub-cellular partitioning of cadmium, copper, nickel and zinc in indigenous yellow perch (*Perca flavescens*) sampled along a polymetallic gradient. Aquat. Toxicol. 77, 178–189.

Grosell, M.H., Hogstrand, C., Wood, C.M., 1997. Cu uptake and turnover in both Cu-acclimated and non-acclimated rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 38, 257–276.

Hamilton Harbour Remedial Action Plan (RAP), 1992. Hamilton Harbour Stage 1 Report: Environmental Conditions and Problem Definition. Hamilton Harbour Remedial Action Plan (RAP), Burlington.
Hamilton Harbour Remedial Action Plan (RAP), 2003. Remedial Action Plan for Hamilton Harbour: Stage 2 Update 2002. Hamilton Harbour Remedial Action Plan (RAP), Burlington, ON.
International Joint Commission, 1999. Hamilton Harbour Area of Concern Status Assessment, Windsor.
Kamunde, C., 2009. Early subcellular partitioning of cadmium in gill and liver of rainbow trout (*Oncorhynchus mykiss*) following low-to-near-lethal waterborne cadmium exposure. Aquat. Toxicol. 91, 291–301.
Klinck, J.S., Green, W.W., Mirza, R.S., Nadella, S.R., Chowdhury, M.J., Wood, C.M., Pyle, G.G., 2007. Branchial cadmium and copper binding and intestinal cadmium uptake in wild yellow perch (*Perca flavescens*) from clean and metal-contaminated lakes. Aquat. Toxicol. 84, 198–207.
Klinck, J.S., Ng, T.Y.T., Wood, C.M., 2009. Cadmium accumulation and in vitro analysis of calcium and cadmium transport functions in the gastro-intestinal tract of trout following chronic dietary cadmium and calcium feeding. Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 150, 349–360.
Lapointe, D., Couture, P., 2009. Influence of the route of exposure on the accumulation and subcellular distribution of nickel and thallium in juvenile fathead minnows (*Pimephales promelas*). Arch. Environ. Contam. Toxicol. 57, 571–580.
Lapointe, D., Gentes, S., Ponton, D.E., Hare, L., Couture, P., 2009. Influence of prey type on nickel and thallium assimilation, subcellular distribution and effects in juvenile fathead minnows (*Pimephales promelas*). Environ. Sci. Technol. 43, 8665–8670.
Laurén, D.J., McDonald, D.G., 1987. Acclimation to copper by rainbow trout, *Salmo gairdneri*: physiology. Can. J. Fish. Aquat. Sci. 44, 99–104.
Leonard, E.M., Marentette, J.R., Balshine, S., Wood, C.M., 2014. Critical body residues, Michaelis–Menten analysis of bioaccumulation, lethality and behaviour as endpoints of waterborne Ni toxicity in two teleosts. Ecotoxicology 23, 147–162.
Leonard, E.M., Nadella, S.R., Bucking, C., Wood, C.M., 2009. Characterization of dietary Ni uptake in the rainbow trout, *Oncorhynchus mykiss*. Aquat. Toxicol. 93, 205–216.
Leonard, E.M., Wood, C.M., 2013. Acute toxicity, critical body residues, Michaelis–Menten analysis of bioaccumulation, and ionoregulatory disturbance in response to waterborne nickel in four invertebrates: *Chironomus riparius*, *Lymnaea stagnalis*, *Lumbriculus variegatus* and *Daphnia pulex*. Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 158, 10–21.
Luoma, S.N., Cain, D.J., Rainbow, P.S., 2009. Calibrating biomonitors to ecological disturbance: a new technique for explaining metal effects in natural waters. Integr. Environ. Assess. Manage. 6, 199–209.
Marentette, J.R., Balshine, S., 2012. Altered prey responses in round goby collected from contaminated sites. Ethology 118, 1–9.
Marentette, J.R., Gooderham, K.L., McMaster, M.E., Ng, T., Parrot, J.L., Wilson, J.Y., Wood, C.M., Balshine, S., 2010. Signatures of contamination in invasive round gobies (*Neogobius melanostomus*): a double strike for ecosystem health? Ecotoxicol. Environ. Saf. 13, 1755–1764.
Mason, A.Z., Jenkins, K.D., 1995. Metal detoxification in aquatic organisms. In: Tessier, A., Turner, D. (Eds.), Metal Speciation and Bioavailability in Aquatic Systems. John Wiley & Sons, Chichester, pp. 479–608.
McCarty, L.S., MacKay, D., 1993. Enhancing ecotoxicological modeling and assessment. Environ. Sci. Technol. 27, 1719–1728.
McDonald, D.G., Wood, C.M., 1993. Branchial mechanisms of acclimation to metals in freshwater fish. In: Rankin, J.C., Jensen, F.B. (Eds.), Fish Ecophysiology. Chapman and Hall, London, pp. 297–321.
McGeer, J.C., Szebedinszky, D., McDonald, D.G., Wood, C.M., 2000. Effects of chronic sublethal exposure to waterborne Cu, Cd or Zn in rainbow trout 2: tissue specific metal accumulation. Aquat. Toxicol. 50, 245–256.
Meyer, J.S., Adams, W.J., Brix, K.V., Luoma, S.N., Mount, D.R., Stubblefield, W.A., Wood, C.M. (Eds.), 2005. Toxicity of Dietborne Metals to Aquatic Organisms. SETAC Press, Pensacola.
Meyer, J.S., Santore, R.C., Bobitt, J.P., DeBrey, L.D., Boese, C.J., Paquin, P.R., Allen, H.E., Bergman, H.L., DiToro, D.M., 1999. Binding of nickel and copper to fish gills predicts toxicity when water hardness varies, not free-ion activity does not. Environ. Sci. Technol. 33, 913–916.
Morel, F., 1983. Principles of Aquatic Chemistry. John Wiley and Sons, New York, NY.
Mount, D.R., Highland, T.L., Mattson, V.R., Dawson, T.D., Lott, K.G., Ingersoll, C.G., 2006. Use of the oligochaete, *Lumbriculus variegatus*, as a prey organism for toxicant exposure of fish through the diet. Environ. Toxicol. Chem. 25, 2760–2767.
Munger, C., Hare, L., 1997. Relative importance of water and food as cadmium sources to an aquatic insect (*Chaoborus punctipennis*): implications for predicting Cd bioaccumulation in nature. Environ. Sci. Technol. 31, 891–895.
Nebeker, A.V., Savonen, C., Stevens, D.G., 1985. Sensitivity of rainbow trout early life stages to nickel chloride. Environ. Toxicol. Chem. 4, 233–239.
Ng, T.Y.T., Pais, N.M., Dhaliwal, T., Wood, C.M., 2012. Use of whole-body and subcellular residues of *Lumbriculus variegatus* to predict waterborne Cu toxicity in fresh water. Chemosphere 87, 1208–1214.
Ng, T.Y.T., Pais, N.M., Wood, C.M., 2011. Mechanisms of waterborne Cu toxicity to the pond snail *Lymnaea stagnalis*: physiology and Cu bioavailability. Ecotoxicol. Environ. Saf. 74, 1471–1479.
Ng, T.Y.T., Wood, C.M., 2008. Trophic transfer and dietary toxicity of Cd from the oligochaete to the rainbow trout. Aquat. Toxicol. 87, 47–59.
Niyogi, S., Wood, C.M., 2003. Effects of chronic waterborne and dietary metal exposures on gill metal-binding: implications for the biotic ligand model. Hum. Ecol. Risk Assess. 9, 813–846.
Niyogi, S., Wood, C.M., 2004. Biotic ligand model, a flexible tool for developing site-specific water quality guidelines for metals. Environ. Sci. Technol. 38, 6177–6192.
Pagenkopf, G.K., 1983. Gill surface interaction model for trace-metal toxicity to fishes: role of complexation, pH, and water hardness. Environ. Sci. Technol. 17, 342–347.
Paquin, P.R., Santore, R.C., Wu, K.B., Kavvadas, C.D., Di Toro, D.M., 2000. The biotic ligand model: a model of the acute toxicity of metals to aquatic life. Environ, Sci. Poll. Res. 3, 175–182.
Pinchuk, V.I., Vasil'eva, E.K., Vasil'ev, V.P., Miller, P.J., 2003. *Neogobius melanostomus* (Pallas 1814). In: Miller, P.J. (Ed.), The Freshwater Fishes of Europe. AULA-Verlag, Wiesbaden, pp. 293–345.
Ptashynski, M.D., Klaverkamp, J.F., 2002. Accumulation and distribution of dietary nickel in lake whitefish (*Coregonus clupeaformis*). Aquat. Toxicol. 58, 249–264.
Ptashynski, M.D., Pedlar, R.M., Evans, R.E., Baron, C.L., Klaverkamp, J.F., 2002. Toxicology of dietary nickel in lake whitefish. Aquat. Toxicol. 58, 229–247.
Pyle, G.G., Rajotte, J.W., Couture, P., 2005. Effects of industrial metals on wild fish populations along a metal contamination gradient. Ecotoxicol. Environ. Saf. 61, 287–312.
Rainbow, P.S., 2002. Trace metal concentrations in aquatic invertebrates: why and so what? Environ. Pollut. 120, 497–507.
Randall, D.J., Burggren, W., French, K., 2002. Eckert's Animal Physiology, Mechanisms and Adaptations. Freeman, New York, NY, pp. 752.
Redeker, E.S., Blust, R., 2004. Accumulation and toxicity of cadmium in the aquatic oligochaete *Tubifex tubifex*: a kinetic modeling approach. Environ. Sci. Technol. 38, 537–543.
Roesijadi, G., 1980. The significance of low molecular weight, metallothionein-like proteins in marine invertebrates: current status. Mar. Environ. Res. 4, 167–179.
Roesijadi, G., 1992. Metallothioneins in metal regulation and toxicity in aquatic animals. Aquat. Toxicol. 22, 81–114.
Schmidt, T.S., Clements, W.H., Zuellig, R.E., Mitchell, K.A., Church, S.E., Wanty, R.B., San Juan, C.A., Adams, M., Lamothe, P.J., 2011. Critical tissue residue approach linking accumulated metals in aquatic insects to population and community-level effects. Environ. Sci. Technol. 45, 7004–7010.
Seidman, L.A., Bergtrom, G., Gingrich, D.J., Reisen, C.C., 1986. Accumulation of cadmium by the fourth instar of the fly *Chironomus thummi*. Tissue Cell 18, 407–418.
Silverberg, B.A., 1976. Cadmium-induced ultrastructural changes in mitochondria of freshwater green algae. Phycologia 15, 155–159.
Spry, D.J., Hodson, P.V., Wood, C.M., 1988. Relative contributions of dietary and waterborne zinc in the rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci. 45, 32–41.
Tallkvist, J., Henriksson, J., D'Argy, R., Tjälve, H., 1998. Transport and subcellular distribution of nickel in the olfactory system of pikes and rats. Toxicol. Sci. 43, 196–203.
Taraborelli, A.C., Fox, M.G., Johnson, T.B., Schaner, T., 2010. Round goby (*Neogobius melanostomus*) population structure, biomass, prey consumption and mortality from predation in the Bay of Quinte, Lake Ontario. J. Great Lakes Res. 36, 625–632.
Timmermans, K.R., Spijkerman, E., Tonkes, M., Govers, H., 1992. Cadmium and zinc uptake by two species of aquatic invertebrate predators from dietary and aqueous sources. Can. J. Fish. Aquat. Sci. 49, 655–662.
U.S. Environmental Protection Agency (U.S. EPA), 1986. Quality criteria for water. In: EPA 440/5-86-001. U.S. EPA, Washington, DC.
U.S. Environmental Protection Agency (U.S. EPA), 1995. Updates: water quality criteria documents for the protection of aquatic life in ambient water. In: EPA-820-B-96-001. Office of Water, Washington, DC.
U.S. Environmental Protection Agency (U.S. EPA), 2007. Framework for metals risk assessment. In: EPA 120/R-07/001. U.S. Environmental Protection Agency Office of the Science Advisor, Washington, DC.
Viarengo, A., Moore, M.N., Mancinelli, G., Mazzucotelli, A., Pipe, R.K., Farrar, S.V., 1987. Metallothioneins and lysosomes in metal toxicity and accumulation in marine mussels: the effect of cadmium in the presence and absence of phenanthrene. Mar. Biol. 94, 251–257.
Vijver, M.G., Van Gestel, C.A.M., Lanno, R.P., Van Straalem, N.M., Peijnenburg, W.J.G.M., 2004. Internal metal sequestration and its ecotoxicological relevance: a review. Environ. Sci. Technol. 38, 4705–4712.
Wallace, W.G., Lee, B.G., Luoma, S.N., 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. I. Significance of metal-sensitive fractions (MSF) and biologically detoxified metal (BDM). Mar. Ecol. Prog. Ser. 249, 183–197.
Zeman, A.J., 2009. Contaminated sediments in Hamilton Harbour: compilation and evaluation of sediment databases, publications and reports, 1975–2008. In: Environment Canada Water Science and Technology Directorate WSTD Contribution No. 09-263.
Zhang, L., Wang, W.X., 2005. Effects of Zn pre-exposure on Cd and Zn bioaccumulation and metallothionein levels in two species of marine fish. Aquat. Toxicol. 73, 353–369.