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Critical body residues, Michaelis–Menten analysis of bioaccumulation, lethality and behaviour as endpoints of waterborne Ni toxicity in two teleosts

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Abstract Traditionally, water quality guidelines/criteria are based on lethality tests where results are expressed as a function of waterborne concentrations (e.g. LC50). However, there is growing interest in the use of uptake and binding relationships, such as biotic ligand models (BLM), and in bioaccumulation parameters, such as critical body residue values (e.g. CBR50), to predict metal toxicity in aquatic organisms. Nevertheless, all these approaches only protect species against physiological death (e.g. mortality, failed recruitment), and do not consider ecological death which can occur at much lower concentrations when the animal cannot perform normal behaviours essential for survival. Therefore, we investigated acute (96 h) Ni toxicity in two freshwater fish species, the round goby (*Neogobius melanostomus*) and rainbow trout (*Oncorhynchus mykiss*) and compared LC, BLM, and CBR parameters for various organs, as well as behavioural responses (spontaneous activity). In general, round goby were more sensitive. Ni bioaccumulation displayed Michaelis–Menten kinetics in most tissues, and round goby gills had lower K_d (higher binding affinity) but similar B_{max} (binding site density) values relative to rainbow trout gills. Round goby also accumulated more Ni than did trout in most tissues at a given exposure concentration. Organ-specific 96 h acute CBR values tended to be higher in round goby but 96 h acute CBR50 and CBR10 values in the gills were very similar in the two species. In contrast, LC50 and LC10 values were significantly higher in rainbow trout. With respect to BLM parameters, gill log K_{NiBL} values for bioaccumulation were higher by 0.4–0.8 log units than the log K_{NiBL} values for toxicity in both species, and both values were higher in goby (more sensitive). Round goby were also more sensitive with respect to the behavioural response, exhibiting a significant decline of 63–75 % in movements per minute at Ni concentrations at and above only 8 % of the LC50 value; trout exhibited no clear behavioural response. Across species, diverse behavioral responses may be more closely related to tissue Ni burdens than to waterborne Ni concentrations. To our knowledge, this is the first study to link Ni bioaccumulation with behavioural endpoints. In future it would be beneficial to expand these analyses to a wider range of species to determine whether Ni bioaccumulation, specifically in the gills, gut and whole fish, may be a good predictor of behavioural changes from metal exposure; which in the wild can lead to ecological death.

Keywords Nickel · Bioaccumulation · Lethality · Behaviour · Teleosts

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Introduction

In most jurisdictions, current water quality guidelines/criteria for Ni rely on approaches such as hardness-correction

which were developed more than two decades ago (CCREM 1987; USEPA 1986, 1995), and do not reflect the effects of other water chemistry factors that are known to affect metal toxicity. Several promising newer approaches are now available and these take into account the receptor characteristics of target organisms and the bioavailability of the metal as a function of water chemistry. For example, the European Union (ECB 2008) has recently adopted Biotic Ligand Modeling (BLM) approaches (Di Toro et al. 2001; Paquin et al. 2000; Niyogi and Wood 2004) which utilize water chemistry parameters to predict the bioavailability of Ni in conjunction with the binding constants of the biotic ligand (log K values) to predict toxicity. Another developing approach, currently not being used to derive Ni water quality guidelines, is the tissue residue approach (TRA; Luoma et al. 2009; Adams et al. 2011; Schmidt et al. 2011) which utilizes Ni bioaccumulation within the whole organism or within an organ to predict toxicity. The TRA makes use of critical body residues (CBR values) calculated from the concentration of Ni bioaccumulated which correlates with mortality, and therefore, in this manner, bioaccumulation can be used to predict the toxicity within and across species. The BLM and TRA methods may be used independently or in combination as a tool for protection of aquatic organisms. Expressing toxicological effect as a function of the bioaccumulation of a metal such as Ni has many potential benefits: integration of all exposure routes (e.g. water column, food and sediments), independence from differences and/or changes in water chemistry that may affect uptake, and incorporation of toxicokinetics of different species (USEPA 2007). The available information to date for CBR for metals, specifically Ni, is not robust and therefore more data are required.

Other ways in which Ni bioaccumulation can be related to Ni toxicity are by characterization of binding constants from saturable metal binding tests using Michalis–Menten analysis, where binding affinity (K_d) and binding site density (B_{max}) can correlate with Ni toxicity. Previous analysis on invertebrates demonstrated that, in general, more sensitive organisms (lower LC50 values) exhibit a higher binding affinity (lower K_d) and lower binding site density B_{max} values for Ni than more tolerant organisms (Leonard and Wood 2013).

Both the traditional and more modern approaches (BLM, TRA) protect a species only against physiological death (e.g. mortality, failed recruitment), while the ecological and behavioural impacts can also greatly affect survival in nature (Scott and Sloman 2004). Such metal-induced "ecological death" can occur at much lower concentrations, as the organism itself may not be overtly harmed but also is unable to perform normal behaviours such as foraging, predator evasion, or searching for mates (Kania and O'Hara 1974; Little and Finger 1990; Barron 2002). To date, behavioural disturbances from exposure to metals have not been employed as an endpoint to derive water quality guidelines (Wood 2012). However, behaviour may be a very sensitive and valuable endpoint. For example, behavioural dysfunction has been shown extensively for the olfactory toxicity of Cu (Pyle and Mirza 2007; McIntyre et al. 2008; Green et al. 2010) and is of concern as well for Ni (Pyle and Couture 2012). Other behavioural endpoints include disruptions of predator avoidance, schooling behaviour, reproductive behavior, and formation and maintenance of social hierarchies (Atchison et al. 1987; Beitinger 1990; Scott and Sloman 2004; Sloman and Wilson 2006; Sloman 2007; Sopinka et al. 2012). The most frequently studied behavioural responses to contaminant-related toxicity in fish involve swimming activity (Little and Finger 1990). Swimming activity includes frequency and duration of movements, endpoints which are appropriate for a variety of species and relevant to survival (Rand 1984; Little and Finger 1990).

The information available on the impact of Ni on behaviour is scarce in comparison to other metals such as copper, cadmium and mercury (Scott and Sloman 2004). To address this deficit, we explored the effects of waterborne Ni exposure on two species of freshwater fish: the round goby and rainbow trout. The round goby (Gobiidae: *Neogobius melanostomus*) is an invasive species to the North American Great Lakes basin since the early 1990′s (Jude et al. 1995). It is known as a pollution-tolerant species (Pinchuk et al. 2003), and is benthic and philopatric, relying on shelters and burial into loose substrate to avoid predators (Belanger and Corkum 2003). The second species, rainbow trout (Salmonidae: *Oncorhynchus mykiss*) is a recreational sport fish that inhabits the pelagic zones of many lakes and rivers. Rainbow trout are one of the most sensitive fish species tested to date for Ni (Nebeker et al. 1985; USEPA 1995). These two species not only have different habitats and lifestyles but they may also be at different ends of the sensitivity spectrum for fish which makes them a good comparison to assess trends in bioaccumulation versus toxicity and behaviour.

Therefore, with this background in mind, our aims were: (1) to measure and compare organ and whole body Ni concentrations over a wide range of waterborne Ni concentrations in the two species as well as to calculate their K_d, B_{max}, 96 h acute CBR10 and CBR50 values; (2) to determine acute (96 h) LC10 and LC50 values for Ni in these two species and compare the resulting toxicity-derived log K values with those (log K_d) derived from the bioaccumulation tests; (3) to determine the impact of Ni on behavioural endpoints following an acute waterborne Ni exposure; (4) to assess the possible correlations between changes in behaviour (which may lead to ecological death) and either the Ni bioaccumulation or the Ni exposure concentration in these two species.

We hypothesized that round goby would be more resistant than rainbow trout, and that 96 h acute CBR50 values would vary less across species than LC50 values. As well, we expected that K_d and B_{max} values would be lower in the more sensitive species and that behaviour of round goby and rainbow trout would be affected by exposure to acute waterborne Ni at concentrations well below the LC50 value, and closer to LC10 values. Finally, we postulated that both Ni exposure concentration and Ni bioaccumulation would correlate to changes in behaviour (spontaneous movement).

Methods

Experimental organisms

Round goby, *N. melanostomus*, were collected from Hamilton Harbour at LaSalle Park (43°18′1″N, 79° 50′47″W; weeks of June 29–July 10th, 2009, background Ni concentration = 0.31 ± 0.03 μmol/L). Forty-eight round goby (mean body mass 10 ± 3 g), were caught in commercial minnow traps baited with frozen corn, set at a depth of 1 m or less, for 24 h. Fish were then transported back to the laboratory and acclimated to laboratory conditions in 5-L containers, with a flow-through of aerated, dechlorinated Hamilton (Ontario, Canada) tap water. PVC tubes, approximately 5–10 cm in length, were used for shelter and the round goby were fed ad libitum every other day with Big Al's Staple Flake Food (45 % protein, 5 % crude fat, 2 % crude fibre and 8 % moisture; Big Al's Aquarium Supercentres, Woodbridge, ON, Canada).

All round goby were treated with oxytetracycline (2.5 g/L for 2 h), a formalin dip (1:6,000 for 1 h) and chloramine T (2 mg/L for 4 h) to prevent and minimize infections. All treatments were completed 2 weeks prior to nickel exposure.

Forty-two rainbow trout, *O. mykiss*, 13 ± 1 g were purchased from Humber Springs Trout Hatchery, Orangeville, Ontario, Canada. They were initially held in 500 L tanks receiving flow-through dechlorinated Hamilton tap water. Trout were fed 2 % body weight, every other day, with Martin's commercial dried pellet feed (Martin Mills Inc., Ontario, Canada).

All fish were kept in Hamilton dechlorinated tap water with an ionic composition of (in mmol L^{-1}) Na (0.9), Cl (1.0), Ca (1.0), K (0.4), Mg (0.4), and Ni ($<4 \times 10^{-5}$). Water hardness was ~140 mg/L as $CaCO_3$ equivalents; pH was 7.8, alkalinity 96 mg/L, water temperature was 13 ± 2 °C, dissolved organic carbon (DOC) was 2.3 mg/L and the photoperiod was 16:8 h light:dark. Fish were fasted for 48 h prior to exposure to allow sufficient time for gut clearance and to standardize metabolic rate.

Table 1 Water chemistry for Ni exposures

	Round goby	Rainbow trout
Na	881 ± 4	856 ± 2
K	42 ± 3	40 ± 2
Cl	971 ± 6	980 ± 6
Ca	1,078 ± 3	1,069 ± 5
Mg	401 ± 2	370 ± 6
Hardness	148 ± 2	144 ± 5
DOC	2.4 ± 0.2	2.1 ± 0.4
Alkalinity	95 ± 5	97 ± 3
pH	7.8 ± 0.1	7.8 ± 0.1

All ion concentrations are represented in μmol/L with the exception of DOC (mg/L), hardness and alkalinity (mg/L as $CaCO_3$) and pH

Values are mean ± SEM, n = 20–30 per value

Flow-through exposure system

Ni stock solutions, made with $NiCl_2 6\ H_2O$ (Sigma Aldrich, St. Louis, Missouri, USA), were held in Mariotte bottles above the exposure tanks. A flow of 0.5 ml/min of Ni stock solutions from the Mariotte bottles was mixed with a flow of 750 ml/min dechlorinated Hamilton tap water 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.

Acute (96 h) LC50 tests

Mean water chemistry parameters for all experiments are shown in Table 1. Measured total and dissolved Ni concentration were generally close to the nominal values and dissolved values were used to determine LC50 values. Measured Ni concentrations in the exposure waters are shown in Supplementary Table 1. Eight round goby and six rainbow trout were transferred to each exposure tank (20 L) for 24 h prior to Ni exposure to allow time for acclimation to the new environment. Each concentration was tested in duplicate to assess acute toxicity. Impacted fish (defined as fish which had lost equilibrium and had turned upside down) were removed from the exposure tanks and euthanized.

Behavioural assay

Round goby

At the end of the 96 h tests, all surviving round goby in each exposure were isolated in a clear plastic bin

(75 × 30 cm) filled with 11 L of control water at 13 ± 2 °C. Fish were allowed to acclimate for 30 min before spontaneous activity was filmed from above with a video camera for 5 min. Each fish in each group was later scored from the video tapes, for the mean number of movements made per minute. Round goby are discrete movers, making single distinct hops or swims interspersed with long periods of inactivity that are easily observed and scored during video analysis (Marentette et al. 2011, 2012; Marentette and Balshine 2012).

Rainbow trout

Similarly at the end of the 96 h tests, all surviving rainbow trout in each exposure group were placed in the same clear plastic bin (75 × 30 cm) filled with 11 L of control water at 13 ± 2 °C. Fish were allowed to acclimate for 30 min before spontaneous activity was filmed from above for 5 min. A different video scoring system was used here because rainbow trout tend to move more smoothly and continuously than the round goby. Videotapes were converted to jpg images at 2 frames per second, and velocity in cm/sec determined from 1 min sequences using ImageJ's Manual Tracker plug-in (NIH). Each fish was assigned a mean velocity in cm/sec.

Tissue sampling

Following behavioural measurements, both rainbow trout and round goby were rinsed briefly (5 s) in nanopure water (18.2 MΩ cm, Millipore Corporation, Billerica, MA, USA) and then euthanized with 0.80 mg/L of tricaine methanesulfonate (*MS-222*) (Syndel Laboratories Ltd. Vancouver, BC, Canada; adjusted to pH 7.8 with NaOH). The body mass of each fish was measured and recorded. Gills, gut, kidney, and liver were excised, weighed and then preserved for further analysis. Gills and gut were rinsed with 0.9 % NaCl solution and blotted dry for 5 min before storing them in 15 ml Falcon™ tubes. The kidney, liver and brain were stored in 2 ml bullet tubes and the remaining carcass (consisting of the remainder of the fish which was largely muscle, bones, skin and scales) was stored in aluminum foil. All organs were kept at −4 °C for later tissue analysis.

Analytical techniques

The various organs and the carcass of each fish were digested in sealed vials 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. These were incubated in a Precision Oven (Jouan Inc., Virginia, USA) at 60 °C for 48 h, with vortexing at 24 h. Tissue digests were then stored at 4 °C for later analysis. Ni concentrations in water samples and tissue samples were measured using graphite furnace atomic absorption spectroscopy (GFAAS; Varian SpectrAA–220 with graphite tube atomizer (GTA–110), Mulgrave, Australia) against certified standards (Aldrich Chemical Company, Oakville, ON, Canada). Measurements were conducted at a wavelength and slit width of 232.0 and 0.2 nm, respectively, to obtain a lower working limit of 0.2 µg/L or 0.003 µmol/L. Ni recovery was 91 ± 2.3 % as determined by Environment Canada certified reference materials, TM-24.3 (lot # 0310) and TM-25.3 (lot # 0809). Ni concentrations were not corrected for recovery.

Concentrations of major cations (Na, Mg, Ca and K) in water samples were analyzed by flame atomic absorption spectroscopy (FAAS; Varian SpectrAA–FS-220, Mulgrave, Australia). Samples were diluted using 1 % HNO_3 for Na analysis and 1 % HNO_3 with 1 % $LaCl_3$ for Ca and Mg analysis. Na, K, Mg and Ca reference standard solutions (Fisher Scientific, Ottawa, ON, Canada) were used to obtain standard curves. 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.

Mean water chemistry parameters for all acute Ni exposures are shown in Table 1. Ni water concentrations in each exposure level tested were expressed as five fractions: nominal, total, dissolved, ionic and active fractions of the metal, calculated as in "Analytical techniques" section taking into account the measured water chemistry from Table 1, and are reported in the Supplementary Information Section (Supplementary Table 1). Note that the active fraction represents the concentration of the ion in its fully dissociated, freely diffusible form, and is less than the total ionic concentration because of the tendency of ions to interact with other atoms and molecules in solution (Hill et al. 2012). All water Ni concentrations presented in this study are reported as the dissolved fractions of the metal, which averaged 95 % of nominal values and 94 % of the total values (Supplementary Table 1).

Statistical analyses

Data have been presented as mean ± SEM (n), where n is the sample size. 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 (attraction or repulsion) of other molecules in solution. The NICA-Donnan model (Benedetti et al. 1995) was used in the calculations to estimate the effect of DOC

on Ni speciation. Acute LC50 and LC10 values with 95 % confidence intervals (CI) were calculated using ToxCalc–Toxicity Data Analysis Software ver.5.0.32 (Tidepool Scientific Software, McKinleyville, CA, USA). The 96 h acute critical whole-body residue (CBR50) was the Ni bioaccumulation in an organ or whole fish (combined organs) that corresponded to 50 % mortality. We used two different methods for determining the 96 h acute CBR50 values with 95 % CI (1) ToxCalc software (as above, substituting Ni bioaccumulation for waterborne Ni concentration) and (2) linear regressions of logit mortality versus log Ni bioaccumulation. For the latter method, non-linear regressions of log bioaccumulation versus logit mortality were used to determine the 96 h acute CBR10 and CBR50 values, and 95 % CI were derived for these CBR values in Sigma Plot for Windows version 10.0 (Systat Inc., Chicago, IL, USA). When the regression was significant at $p < 0.05$ or the coefficient of determination (r^2) was greater than 0.6, a goodness-of-fit curve also was fitted to the original data (see Leonard and Wood 2013). Ni bioaccumulation and survival were corrected for control levels prior to analysis.

Non-linear regression analyses were performed to determine the concentration-dependent kinetics of Ni bioaccumulation, again as in Leonard and Wood (2013), with a hyperbolic curve fit (single rectangular two parameters $y = ax/(x + b)$; Sigma Plot for Windows version 10.0; Systat Inc., Chicago, IL, USA) in order to determine the parameters of the Michaelis–Menten equation:

$$\text{Specific binding} = (B_{max} \times [L])/([L] + K_d);$$

where [L] is the concentration of the ligand (Ni), B_{max} is the binding site density for the ligand (μmol kg^{-1} wet wt), and K_d is the binding affinity (expressed in μmol Ni/L).

Significant differences between two groups were evaluated using unpaired Student's t tests (two-tailed). Comparisons amongst multiple treatment groups were assessed using a one-way analysis of variance (ANOVA) followed by the Fisher LSD Method (Sigma Plot 10.0, Chicago, IL, USA) or by Dunnett's test for the behavioural analysis, in order to compare treatment groups to the control group. Spearman non-parametric rank correlations were performed to compare water Ni exposure concentration or Ni bioaccumulation in organs or whole fish against behavioural data. For all tests, statistical significance was allotted to differences with $p < 0.05$.

Results

Ni bioaccumulation and Michaelis–Menten parameters

Prior to Ni exposure in the laboratory, the gills, gut, carcass and whole fish of round goby had 1.2–2.6× more Ni in comparison to trout, however, the liver and brain of rainbow trout had 2.1 and 3.8× more Ni, respectively, than the round goby. There was no significant difference in the kidney Ni level. In general, as the Ni exposure concentration increased so did Ni bioaccumulation (this was true for all organs and for both species apart from trout carcass/muscle, Fig. 1). Ni bioaccumulation in organs and whole fish (all organs combined) were comparable between the two species on a per weight basis (Fig. 1). Primarily, Ni bioaccumulation occurred in the gills, kidney and gut, with less Ni in the guts of rainbow trout than round goby. Interestingly, little Ni was detected in the brain, suggesting that the blood–brain barrier is fairly efficient for this important behavioural control center. In round goby brains, elevated Ni were observed only at the highest exposure concentration tested (221.3 μmol Ni/L), while in rainbow trout brains, Ni bioaccumulation never exceeded the levels observed in the control fish.

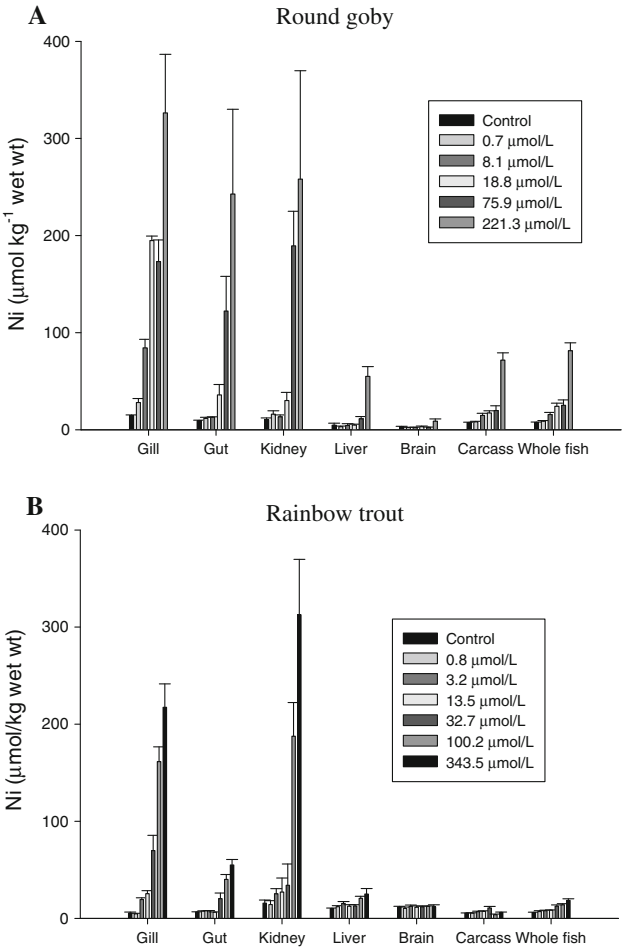

Fig. 1 Ni bioaccumulation, represented in μmol/kg wet wt, in organs of round goby and rainbow trout over a range of exposure concentrations following a 96 h waterborne exposure. Values are mean ± SEM. $n = 8$ per treatment for round goby and $n = 6$ per treatment for rainbow trout

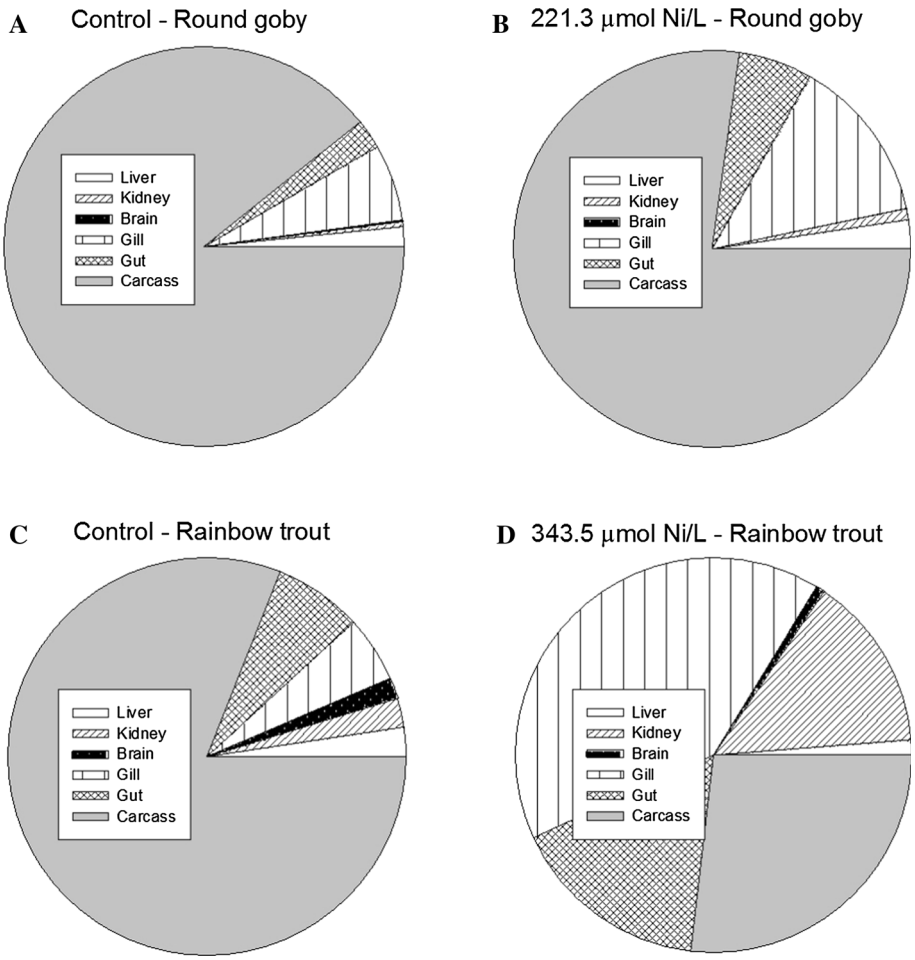

Fig. 2 Pie charts reflecting the average percentage Ni content (i.e. as a percentage of the whole body burden) in each organ in the (**a**, **c**) control fishes and (**b**, **d**) highest exposure fishes following an acute (96 h) waterborne exposure to Ni. $n = 8$ per treatment for round goby and $n = 6$ per treatment for rainbow trout

However, when accounting for the weight of the organs, the order of Ni bioaccumulation on a percent basis in organs from highest to lowest for both species was: carcass > gill > gut > kidney > liver > brain (Fig. 2). In comparison to control fish, the round goby from the highest exposure concentration had 2× more Ni bioaccumulated in their gills, gut and kidney. The pattern was even more pronounced in the trout with fish from the highest exposure concentration having 8, 2.5, and 6.4× more Ni in their gills, gut and kidney, respectively compared to control fish (Fig. 2).

Ni bioaccumulation displayed a hyperbolic, saturable relationship with respect to exposure concentration in the gills (Fig. 3a), gut (Fig. 3b), kidney (Fig. 3c) and whole fish (organs combined; Fig. 3g). Table 2 shows the calculated Michaelis–Menten constants from these relationships. Note that in Fig. 3g, the B_{max} for whole fish (organs combined) in the round goby occurred at concentrations well beyond the highest Ni concentration tested, so this value should be interpreted with caution. Between species, the binding site density for Ni, or B_{max} value, of the gut was 7.5× higher in round goby in comparison to rainbow trout. In round goby, the various organs had similar B_{max} values, but in rainbow trout, B_{max} values (on a per weight basis) decreased with the increasing size of the organ (kidney > gill > gut > whole fish (all organs combined; Table 2)).

In both species, the binding affinity for Ni (K_d values) did not differ significantly across the various organs: gills, gut, kidney and whole fish. However, the K_d values (inverse relationship to binding affinity) were 4.9× lower in the gills and 2.0× higher in the guts of round goby in comparison to rainbow trout (Table 2).

In the liver of both species and in the carcass of round goby, there was a linear relationship between Ni bioaccumulation and exposure concentration and therefore no Michaelis–Menten parameters were calculated (Fig. 3d, f).

96 h acute critical body residues

Critical body residues (CBR values) are the concentrations of Ni in tissues which correlate to a percent mortality (in the case of the present study: 96 h acute CBR50 and CBR10; Fig. 4). However, methods for calculating these

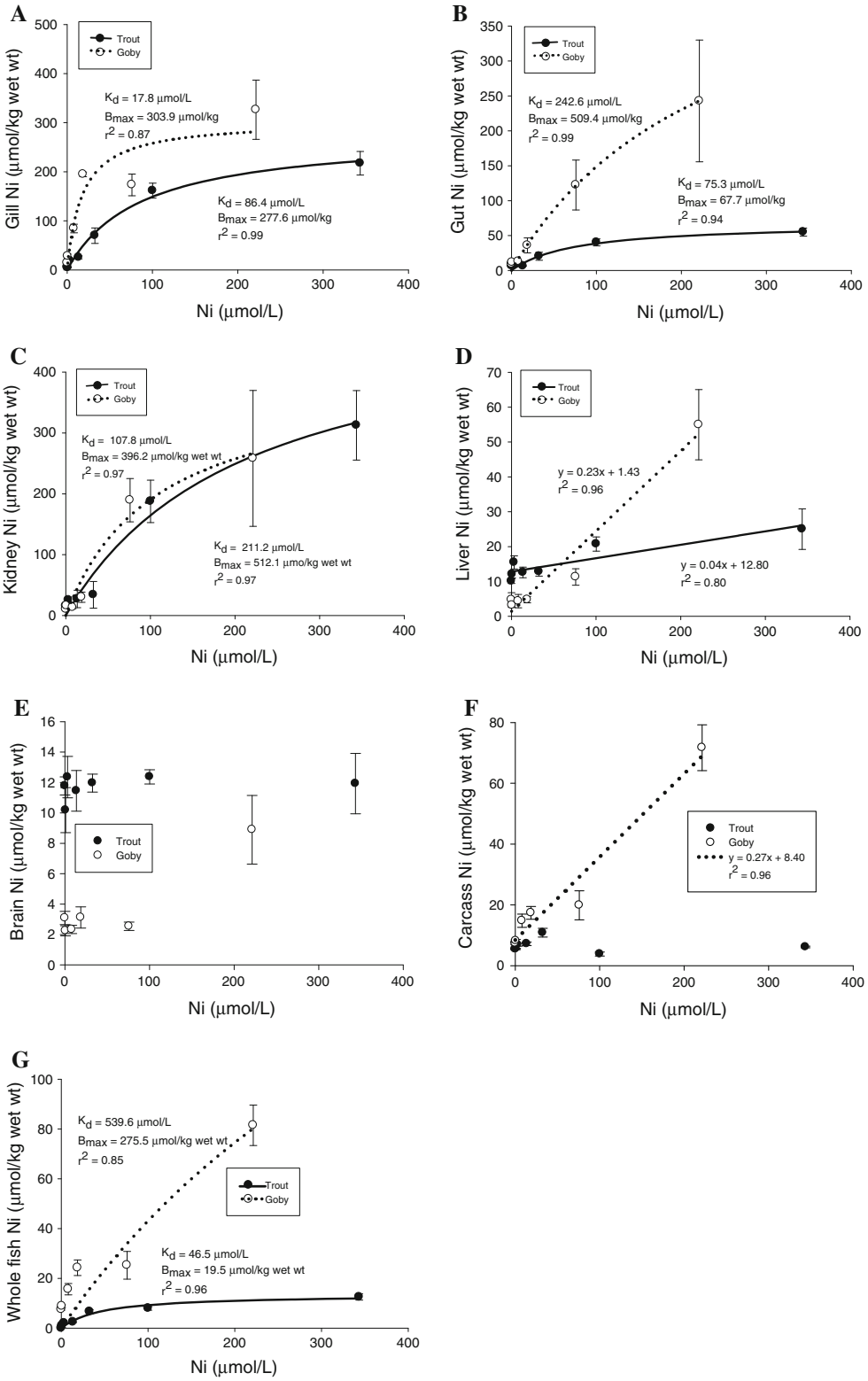

Fig. 3 Ni bioaccumulation in **a** gills, **b** gut, **c** kidney, **d** liver, **e** brain, **f** carcass and **g** whole fish (organs combined) over a range of exposure concentrations following a 96 h exposure of round goby and rainbow trout. Values are mean ± SEM.; $n = 8$ per treatment for round goby and $n = 6$ per treatment for rainbow trout. Non-linear regression analyses were performed to determine the concentration-dependent kinetics of Ni bioaccumulation with a hyperbolic curve fit. *Curves* were not fitted if the relationship between exposure Ni concentration and Ni bioaccumulation was not saturable

values are not standardized in the literature; therefore we have included a comparison of two commonly published methods: logit mortality versus log bioaccumulation (Ng et al. 2012; Leonard and Wood 2013) and Toxcalc for determining CBR values (Table 3). In general, both methods calculate similar CBR values, however, CBR values were more variable (95 % confidence intervals were larger) when calculated by the logit mortality versus log

Table 2 Michaelis-Menten kinetic constants (B_{max} and K_d) for saturable Ni bioaccumulation in the gill, gut, kidney and whole fish of round goby and rainbow trout

	B_{max} (μmol/kg wet wt)	K_d (μmol Ni/L)	r^2
Goby			
Gill	303.9 ± 54.1[a]	17.8 ± 11.7[a]	0.87
Gut	509.4 ± 71.8[a]	242.6 ± 58.1[a]	0.99
Kidney	396.2 ± 84.6[a]	107.8 ± 52.0[a]	0.97
Whole fish	275.5 ± 422.4[a]	539.6 ± 1,116.8[a]	0.85
Trout			
Gill	277.6 ± 20.1[a]	86.4 ± 16.8[a,c]	0.99
Gut	67.7 ± 9.6[b,c]	75.3 ± 29.5[a,c]	0.94
Kidney	512.1 ± 101.8[c]	211.2 ± 86.4[a]	0.97
Whole fish	19.5 ± 1.9[b]	46.5 ± 15.6[a]	0.91

The B_{max} and K_d values were not calculated for the liver in either species and the carcass for round goby as the relationship between Ni exposure concentration and organ Ni bioaccumulation was linear. As well, in the brain and carcass of trout, no relationship was found between Ni exposure and organ Ni concentration

Values are mean ± SEM

[a,b] Significant difference between B_{max} and K_d values within a species

[c] Significant difference between a B_{max} and K_d values of an organ or whole fish between the two species

bioaccumulation method. In addition, the Toxcalc method calculates CBR values which correspond slightly better to CBR values interpolated from the goodness-of-fit lines (Table 3; Fig. 4).

Using the Toxcalc method, 96 h acute CBR50 values in organs of round goby ranged from 23.5 μmol Ni/kg wet wt in the liver to 247.5 μmol Ni/kg wet wt in the gills, whereas, in rainbow trout, 96 h acute CBR50 values ranged from 16.9 μmol Ni/kg wet wt in the whole fish to 264.9 μmol Ni/kg wet wt in the kidney. In general 96 h acute CBR values were higher in round goby (i.e. a greater Ni bioaccumulation was associated with a given level of mortality), but only a few of the differences were statistically significant. An organ comparison between the two species showed similar 96 h acute CBR50 values for the gills, in fact there was no significant difference by the logit mortality versus log bioaccumulation method, and only a small difference by the Toxcalc method (Table 3). There was also little variation between the 96 h acute CBR50 values in the kidneys and livers of the two species. Although the 96 h acute CBR50 values of the gut and whole fish were ~2–3 fold different between the two species, these differences were not significant by the logit mortality versus log bioaccumulation method (Table 3).

The 96 h acute CBR10 values (similar to threshold values) for organs and whole body ranged from 7.5 to 193 μmol/kg wet wt in the two species (Table 3). An organ comparison between the two species revealed no significant difference between gill, gut or whole fish 96 h acute CBR10 values whereas there were 4.0 and 2.8× differences for the kidney and liver, respectively (Table 3).

Acute Ni LC10 and LC50 values for round goby and rainbow trout and comparison between log K values derived from toxicity versus bioaccumulation

The LC values in the two species were significantly different with the round goby being more sensitive (lower LC50 and LC10 values) than the rainbow trout (Table 4). The difference in LC50 values was 2.19 fold, and the difference in LC10 values was 3.80 fold.

Table 5 compares the log K_{NiBL} values derived from the ionic component of the LC50 value (toxicity) with those derived from the ionic component of the K_d (ionic Ni concentration causing half saturation of Ni bioaccumulation in the whole organism).

The log K_{NiBL} values for toxicity were 4.09 for round goby and 3.76 for rainbow trout. Round goby log K_{NiBL} values for bioaccumulation ranged from 3.38 to 4.86, whereas these values ranged from 3.78 to 4.44 in rainbow trout (Table 5). Notably, for both species, the gill log K_{NiBL} values for bioaccumulation are higher by 0.4–0.8 log units than the log K_{NiBL} values for toxicity.

Impact of Ni on behavioural endpoints following an acute waterborne Ni

The behavioral effect of acute Ni exposure on round goby was much more pronounced than in rainbow trout. In round goby, the selected behavioural endpoint of movements per minute significantly decreased by 63–75 % at all exposure concentrations of 8.1 μmol Ni/L or higher (up to 221.3 μmol Ni/L), indicating a clear locomotion threshold between 0.7 and 8.1 μmol Ni/L (Fig. 5a). The lowest observed effect concentration (LOEC) represents 8 % of the LC50 or 28 % of the LC10 (cf. Table 4). In contrast, in the rainbow trout, there was no significant difference in the mean velocity or swimming speed between the fish in control versus exposure treatments, although the mean velocity significantly declined in the 32.6 μmol Ni/L exposure concentration in comparison to the 0.8 μmol Ni/L (Fig. 5b).

Linking physiological endpoints (bioaccumulation) to ecological endpoints (behaviour)

To determine if the very different patterns in behavioral responses in the two species could be integrated by either the Ni exposure concentrations or the Ni bioaccumulation data, Spearman rank correlation analyses were conducted.

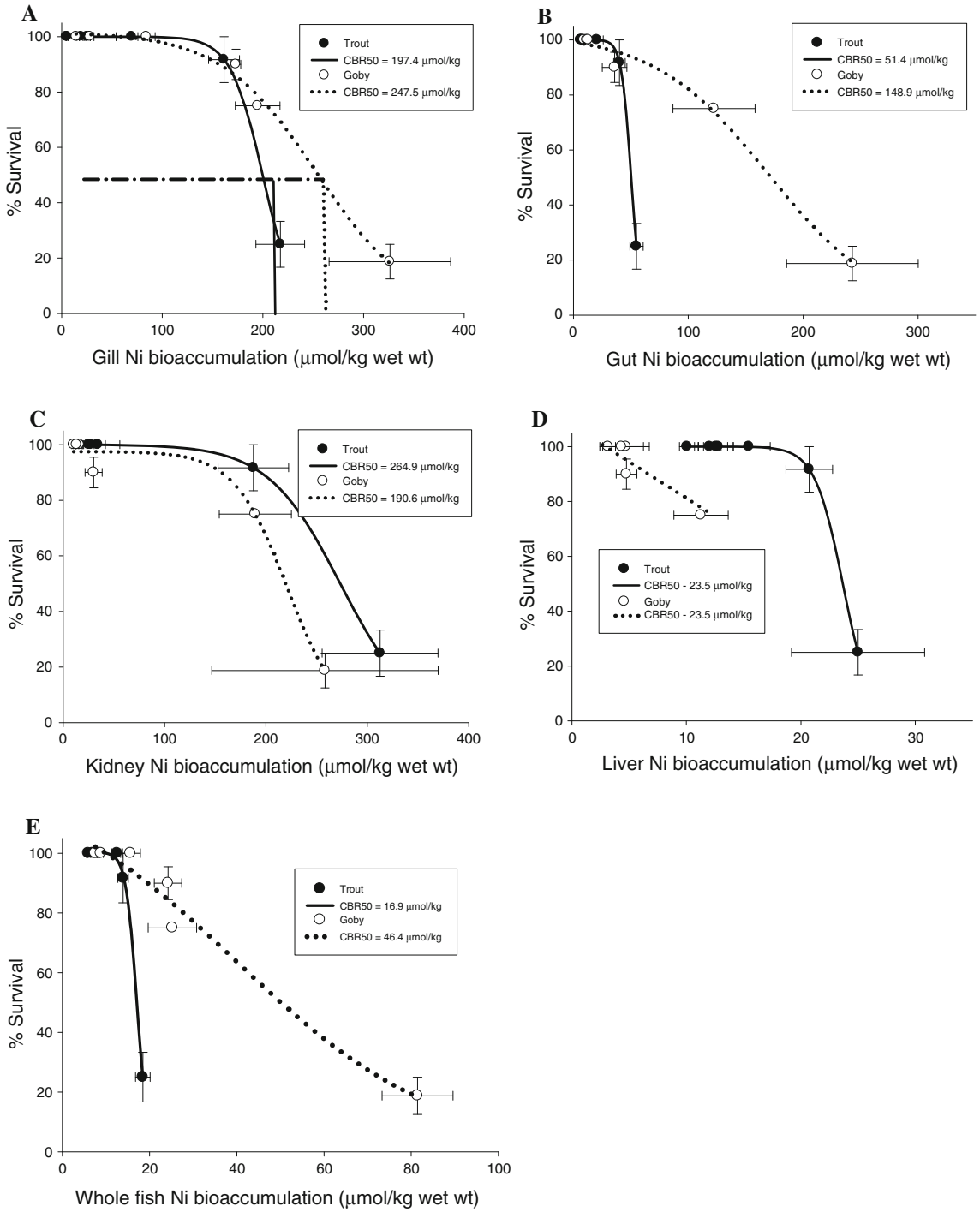

Fig. 4 Correlation between survival and **a** gill, **b** gut, **c** kidney, **d** liver Ni bioaccumulation in round goby and rainbow trout. Values are mean ± SEM; $n = 2$ replicate toxicity tests with 6 (rainbow trout) and 8 (round goby) per exposure concentration for % survival and $n = 6$–8 for Ni bioaccumulation. The lines at 50 % survival intersect the bioaccumulation versus mortality relationships at the 96 h acute CBR50 values, which are indicated on the Figure panel and were calculated using Toxcalc software

As Ni bioaccumulation increased in the gills and whole fish, behaviour/movement decreased with Spearman rank correlations of rho $= -0.6044$, $p = 0.03$. A slightly weaker correlation was observed with Ni bioaccumulation in the gut (rho $= -0.5604$, $p = 0.05$) (Table 6). There was also a trend suggesting that increased exposure Ni concentrations correlated with decreased movement (rho $= -0.544$, $p = 0.05$). There were no significant correlations between Ni bioaccumulation in the other organs (kidney, liver, brain or carcass) and changes in behaviour (Table 6).

Table 3 96 h acute CBR values for Ni in μmol/kg wet wt. with lower and upper 95 % confidence intervals in brackets

	96 h CBR50 values calculated from logit vs. log (μmol/kg wet wt)	Fold difference	96 h CBR50 values calculated from Toxcalc (μmol/kg wet wt)	Fold difference	96 h CBR10 values calculated from Toxcal (μmol/kg wet wt)	Fold difference
Gills						
Goby	263.0 (210.2–305.3)	1.09	247.5 (234.0–262.5)	1.25	167.0 (152.9–178.5)	1.02
Trout	239.3 (190.7–267.7)		197.4[a] (191.4–203.8)		164.3 (155.0–171.3)	
Gut						
Goby	141.8 (63.0–254.7)	3.02	148.9 (91.4–297.6)	2.89	49.7 (10.9–82.9)	1.21
Trout	130.9 (8.57–512.9)		51.4[a] (49.5–53.5)		41.0 (38.2–43.2)	
Kidney						
Goby	173.4 (125.2–230.1)	1.73	190.6 (70.1–3,335.2)	1.38	48.6 (0.1–113.7)	3.98
Trout	299.8[a] (279.2–358.8)		264.9 (251.2–279.8)		193.3[a] (147.9–207.7)	
Liver						
Goby	9.0 (ND)	1.90	23.5 (14.9–49.8)	1	7.5 (3.8–11.7)	2.79
Trout	17.5 (8.4–26.7)		23.5 (23.1–24.0)		20.9[a] (20.2–21.5)	
Whole fish						
Goby	60.9 (16.1–357.3)	2.40	46.4 (36.3–66.4)	2.74	22.0 (14.9–28.1)	1.53
Trout	25.4 (8.1–37.2)		16.9[a] (16.5–17.4)		14.4[a] (13.8–14.8)	

The 96 h acute CBR50 values were not calculated for brain and carcass as the regression analysis between organ Ni bioaccumulation and survival was not significant at $p > 0.05$ or the coefficient of determination (r^2) was less than 0.6

96 h acute CBR50 values were calculated either by logit versus log method or by Toxcalc

[a] Significant difference in 96 h acute CBR50 or CBR10 values between the two species within an organ or whole fish

Table 4 96 h LC10 and LC50 values for Ni in μmol/L with lower and upper 95 % confidence intervals in brackets for round goby and rainbow trout

	LC10 (μmol/L)	Fold difference	LC50 (μmol/L)	Fold difference
Goby	29.0 (12.2–46.8)	3.80	104.1 (68.8–160.4)	2.19
Trout	110.3[a] (89.2–129.7)		228.1[a] (201.5–257.5)	

[a] Significant difference in toxicity values between the two species

Discussion

Overview

Contrary to our predictions, round goby were more sensitive to acute waterborne Ni exposure than rainbow trout; however, as expected the 96 h acute CBR50 values varied less between the two species than 96 h acute LC50 values. As well, in agreement with published data, the K_d values calculated from bioaccumulation and toxicity for the gills were lower (higher affinity) in the more sensitive round goby in comparison to rainbow trout. The Ni concentration that reached a behavioural threshold of reduced movement was well below the LC10, and the inhibition of movement was already maximal at this concentration, indicating that this is a very sensitive endpoint in the round goby. In marked contrast, no consistent behavioural effects were observed in rainbow trout.

Overall, one of the fundamental concepts of the BLM is that gill metal bioaccumulation is a good predictor of acute toxicity (Meyer et al. 1999; Pane et al. 2004). This study assessed whether this concept could be expanded to other organs for the purposes of the BLM and TRA and also if this framework could be extended to another endpoint such as behaviour. In general, gill, gut and whole fish Ni bioaccumulation were the most consistent predictors of toxicity and there was promising evidence that these organs may be used to predict behavioural endpoints. More work on a wider range of taxa is now needed.

Organ Ni bioaccumulation and Ni bioaccumulation parameters

On a per weight basis, Ni primarily bioaccumulated in the gills, kidney and gut (Fig. 1). A similar trend was previously observed in adult rainbow trout exposed for 117 h to 198 μmol/L of waterborne Ni (Pane et al. 2003). In previous studies, gill Ni bioaccumulation was found to be cellularly incorporated or loosely bound versus being blood-bound (Pane et al. 2004). Also a high Ni bioaccumulation in the gut contradicts the general belief that freshwater fish do not drink. Pane et al. (2003) suggested stress-induced drinking as a potential mechanism for

Table 5 Log K_{NiBL} values based on bioaccumulation (K_d values, various organs) and log K_{NiBL} values based on toxicity (LC50) values in round goby and rainbow trout

	Bioaccumulation			Toxicity		
	K_d value (μmol Ni/L)	Ionic Ni component of K_d value (μmol Ni/L)	$\log K_{NiBL}$ values	LC50 value (μmol Ni/L)	Ionic Ni component of LC50 value (μmol Ni/L)	$\log K_{NiBL}$ values
Goby						
Gill	17.8 ± 11.7	13.9 ± 9.1	4.86[a,e] (4.64–5.32)	104.1 (68.8–160.4)	80.2 (53.0–123.5)	4.09 (3.91–4.28)
Gut	242.6 ± 58.1	189.2 ± 45.3	3.72[b,e] (3.63–3.84)			
Kidney	107.8 ± 52.0	81.9 ± 39.5	4.09[c] (3.92–4.37)			
Whole fish	539.6 ± 1,131.8	415.5 ± 871.5	3.38[a,b,c] (ND)			
Trout						
Gill	86.4 ± 16.8[d]	67.4 ± 13.1	4.17[a,e] (4.09–4.26)	228.1 (201.6–257.5)	175.6 (155.2–198.3)	3.76 (3.70–3.81)
Gut	75.3 ± 29.5[d]	58.7 ± 23.0	4.23[a,e] (4.09–4.45)			
Kidney	211.2 ± 86.4	164.7 ± 67.4	3.78[b,e] (3.63–4.01)			
Whole fish	46.5 ± 15.6	36.0 ± 12.0	4.44[a,e] (4.32–4.62)			

There was no significant difference between K_d values for various organs within a species

[a,b,c] Significant difference between log K_{NiBL} values within a species

[d] Significant difference between K_d values of an organ or whole fish between the two species

[e] Significant difference between log K_{NiBL} values for bioaccumulation and toxicity

gastric Ni bioaccumulation, and ruled out hepatic clearance and subsequent biliary excretion as the cause due to the low level of bioaccumulation in the liver—as was also observed in the current study (Figs. 1, 3d). Both the brain and carcass (which is primarily comprised of muscle, bones, skin and scales) are not organs of large Ni bioaccumulation on a per weight basis, which is consistent with studies by Pane et al. (2003). Therefore, on a per weight basis, the gills, gut and kidney are likely the prime target organs.

However, when the relative weights of each of the organs are considered on a percent basis the carcass is the main sink of Ni bioaccumulation in the whole animal as would be expected from an "organ" comprising 90 % of the total weight of the fish (Fig. 2). Ni bioaccumulation in gills and gut correspond to ~8–12 % of the total Ni in the control fish and 20–60 % of the total Ni in the highest exposure. The contributions of the other organs to Ni bioaccumulation are relatively minor.

Saturable Ni binding in the gills, gut, kidney and whole fish (all organs combined) and subsequent calculation of Michaelis–Menten parameters allows for the characterization of Ni binding (Fig. 3; Table 2). To the best of our knowledge this is the first study to show a comparison of binding constants (affinity and capacity) in organs other than the gills and to compare between two fish species of different habitat and lifestyle, though we have recently made such measurements in invertebrates (Leonard and Wood 2013). Neither this study nor Leonard and Wood (2013) found a strong correlation between capacity values (B_{max}) and Ni toxicity in different species, but this was not the case with affinity values (K_d). The more sensitive species (lower LC50 value; round goby in this case) exhibited a higher affinity for Ni (lower K_d) in the gills (and although not significant, also in the kidney) than the more tolerant organism, rainbow trout (Table 2). This same trend of a lower K_d correlating with higher sensitivity was previously observed in a range of invertebrates acutely exposed to Ni (Leonard and Wood 2013).

The opposite was true for the gut, where the affinity of the rainbow trout gut for Ni was over 3× higher (i.e. lower K_d) than that of the round goby. The gut of round goby sampled from Hamilton Harbour contained mainly dipterans, oligochaetes, cladocerans, copepods, ostracods and dreissenids, some of which, namely dipterans and oligochaetes (Taraborelli et al. 2010) are predominant species in polluted aquatic environments (Winner et al. 1980), and are known to bioaccumulate toxins to a high degree (Seidman et al. 1986). Therefore, the gut of the round goby may have been previously exposed to contaminants via the diet. It is well established that when fish are chronically acclimated to sublethal metal concentrations there is an increase in the low-affinity, high capacity binding sites (Niyogi and Wood 2003). Therefore, this may be reflective of the "pre-exposure" in Hamilton Harbour to Ni and other contaminants (see below).

It does not appear that much can be drawn from the Michaelis–Menten parameters in the whole fish; combining the organs may have obscured the results, and this is

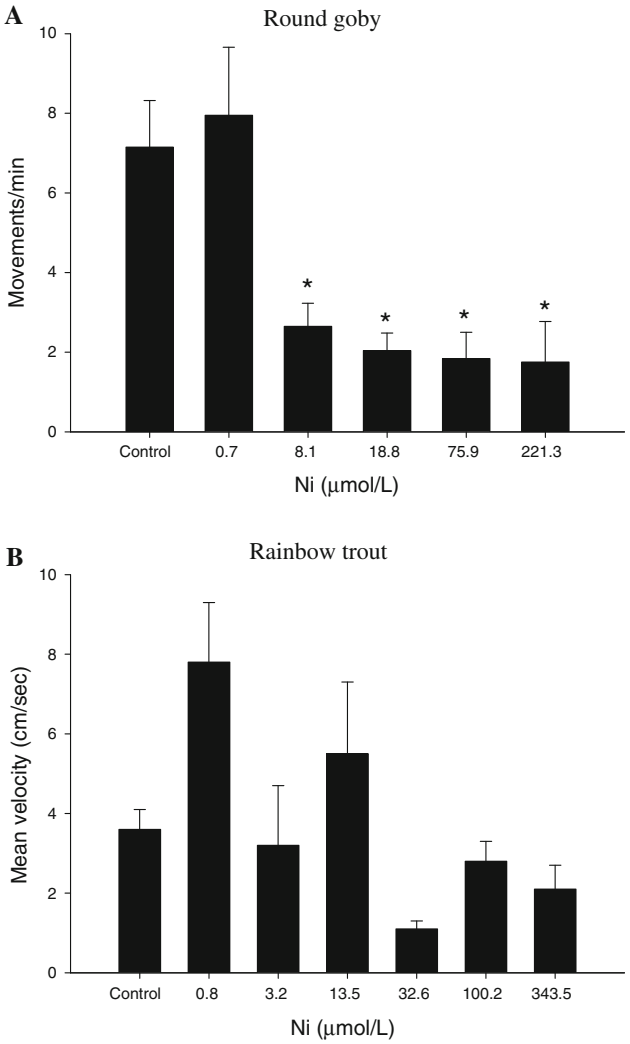

Fig. 5 Impact of Ni on behavioural endpoints in round goby (**a** movements/min) and rainbow trout (**b** mean velocity/min) exposed to 96 h of waterborne Ni. Values are mean ± SEM; $n = 8$ per treatment for round goby and $n = 6$ per treatment for rainbow trout. Statistical significance was determined using Dunnett's test to compare treatment groups to the control group and * denotes a significant difference from control value. There was no significant difference in mean velocity/min relative to the control value in rainbow trout at any exposure concentration

reflected in the high 95 % confidence intervals on K_d and B_{max} estimates (Table 2). Nevertheless, the much greater accumulation of Ni in the carcass and therefore in the whole fish in the round goby (Fig. 3g) may relate to its greater sensitivity to Ni.

If a sigmoidal relationship is observed between % survival and Ni bioaccumulation, CBR values can be calculated, specifically a 96 h acute CBR50 value which corresponds to the tissue concentration at 50 % mortality (Fig. 4) and a 96 h acute CBR10 value which represents a threshold value after which survival decreases as Ni bioaccumulation increases (Fig. 4). Both these parameters

Table 6 Spearman rank correlation between fish behavioral inhibition and Ni concentration in the exposure water or Ni bioaccumulation within an organ or whole fish

1st variable	2nd variable	Spearman rank correlation coefficient (rho value)	*P* value
Behaviour	Water exposure concentration	−0.544	0.05
	Gill	−0.6044	0.03
	Gut	−0.5604	0.05
	Kidney	−0.4835	0.09
	Liver	−0.0769	0.80
	Brain	−0.0055	0.98
	Carcass	−0.3846	0.19
	Whole fish	−0.6044	0.03

A negative Spearman correlation coefficient corresponds to a decrease in behaviour as the Ni exposure concentration or Ni bioaccumulation within an organ or whole fish increases

$P < 0.05$ is considered significant

facilitate a standardized comparison of different tissues and different organisms. This sigmoidal relationship has also been observed in two invertebrate species (*Daphnia pulex* and *Lumbriculus variegatus*) acutely exposed to Ni (Leonard and Wood 2013), as well as for Cu exposed *Lymnaea stagnalis* (Ng et al. 2012).

When comparing the two different methods for determining the 96 h acute CBR50 values with 95 % CI (ToxCalc software and linear regressions of logit mortality versus log Ni bioaccumulation), 96 h acute CBR50 values were less variable when calculated using the Toxcalc method in comparison to the logit mortality versus log bioaccumulation method. This is most likely because the Toxcalc method is less influenced by 0 and 100 % mortalities. However, both methods derive similar 96 h acute CBR50 values (Table 3). In addition, the Toxcalc software allows for calculation of more sensitive endpoints such as CBR10 values.

External effect concentrations (LC values) can vary due to water chemistry's influence on Ni bioavailability, however, internal effect concentrations (CBR50 values) should in theory vary much less. For Ni, Meyer et al. (1999) demonstrated that 24 h gill Ni bioaccumulation (LA50) in the fathead minnow (*Pimephales promelas*) was constant across wide range of water hardness even though the 96 h acute LC50s varied by tenfold. In the current study, we made a comparison between two different species rather than varying the external water chemistry. There was a 2.19 fold difference between 96 h acute LC50 values whereas there was only a 1.09–1.25 fold difference (depending on the method used) between 96 h acute CBR50 values for the gills between these two species (Table 3). This suggests that 96 h acute CBR50 values for the gills are less species-dependent than 96 h

acute LC50 values, which supports one of the main objectives of the TRA (and fundamental concept of the BLM) where tissue concentrations of the target organ (the gills) are generally less variable than exposure concentrations with respect to a toxicity response (Meyer et al. 1999; Luoma et al. 2009; Adams et al. 2011; Schmidt et al. 2011). Evidence similar to that of the current study which supports the TRA for metals is expanding (e.g. Redeker and Blust 2004; Leonard et al. 2011; Ng et al. 2012; Leonard and Wood 2013). This suggests that despite the large differences between these two fish (as well as the round goby's possible pre-exposure to Ni and other contaminants in Hamilton Harbour—see below), the threshold concentrations of Ni within a tissue which causes an effect are quite similar.

The 96 h acute CBR50 values are similar to the gill LA50 parameter (lethal accumulation which causes 50 % mortality) which is incorporated into the calculation of the BLM. In the present study, 96 h acute CBR50 values were 263 μmol/kg wet wt in goby and 239 μmol/kg wet wt in trout. Reported 96 h gill LA50 values were 2,079 μmol/kg wet wt (Brix et al. 2004) for rainbow trout and 250 μmol/kg wet wt for *P. promelas* (Meyer et al. 1999). The Brix et al. (2004) study used rainbow trout 18-d post-swim-up which averaged 1.6 g wet wt, 6–8× smaller than the fish in the current study, possibly explaining the much larger 96 h acute CBR50 value. The value for *P. promelas* correlates relatively well with the 96 h acute CBR50 values of the gill in the current study.

The fold differences for kidney (1.38–1.73), liver (1.00–1.90), and kidney (1.38–1.73) 96 h acute CBR50 values (Table 3) were also less variable than the differences in 96 h acute LC50 values. More information is required to determine whether these organs would be good predictors of Ni toxicity.

In the same manner, we can also derive 96 h acute CBR10 values using the Toxcalc method which are representative of a threshold value of the fish. There were no significant differences in 96 h acute CBR10 values of the gills and gut between the two species (Table 3), suggesting that in both species a similar bioaccumulation of Ni in these organs correlates to the onset of mortality. There was a significant difference between kidney, liver and whole fish 96 h acute CBR10 values, perhaps due to the larger concentration of Ni in the trout vs the goby prior to laboratory exposure to Ni.

It should be noted that using the logit mortality versus log bioaccumulation method, there is no significant difference between 96 h acute CBR50 values in the gills, gut, liver and whole fish however, this is due to the larger 95 % confidence intervals, as we see this trend does not exist when using the Toxcalc method which derives less variable 95 % confidence intervals (Table 3).

Acute Ni toxicity values and comparison between log K values derived from toxicity versus bioaccumulation

To the best of our knowledge, there are no previous acute Ni toxicity studies on round goby. However, other studies on rainbow trout have reported acute 96 h LC50 values for Ni of 138 and 255 μmol Ni/L with water hardness of 22 and 120 mg/L as $CaCO_3$, respectively (Atchison et al. 1987; Pane et al. 2003) and are in good agreement with the current LC50 of 228 μmol Ni/L at a water hardness of 140 mg/L as $CaCO_3$.

Contrary to our predictions, round goby were 2.19 times more sensitive to waterborne Ni than rainbow trout with LC50 values of 104.1 and 228.1 μmol Ni/L, respectively. We had expected round goby to be more resistant to Ni due to the prevalence of this species in highly contaminated areas (Pinchuk et al. 2003). Potentially, while resistant to many toxicants, the round goby might be quite sensitive to Ni. However, a possible alternate explanation is that the goby used in this study had been collected from the wild. The collection site (LaSalle Park on Hamilton Harbour) is considered to be a "clean site" (Marentette et al. 2010), but Hamilton Harbour itself is a Canadian Area of Concern designated by the International Joint Commission (International Joint Commisson 1999). Many contaminants are known to be at problematic levels in other areas of Hamilton Harbour, namely: polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and metals including cadmium, arsenic, lead, iron, mercury, zinc, and nickel (Hamilton Harbour RAP 1992, 2003). Indeed, the goby had 2× more Ni in the gills and the gut than the rainbow trout purchased from a hatchery. It is possible that some residual influence of the Ni and other contaminants in either the diet or the water reduced the overall tolerance of the gobies leading to a higher sensitivity to Ni.

One of the main concepts of the BLM is that there is a strong overall correlation between log K values for gill binding and acute toxicity (Niyogi and Wood 2004) to the extent that measurement of binding affinity based on gill metal binding as an acceptable alternative to measurement of toxicity and vice versa (MacRae et al. 1999; Niyogi and Wood 2004). In the present study, we evaluated whether this concept could be extended beyond the biotic ligand of the gills (the known target side for acute toxicity of most metals in fish) to other organs. Specifically, in Table 5, we have compared the log K_{NiBL} values derived from the ionic component of the LC50 value (toxicity) with those derived from the ionic component of the K_d (ionic Ni concentration causing half saturation of Ni bioaccumulation in the whole organism). The log K_{NiBL} values calculated from the ionic component of the K_d value were considerably higher than log K_{NiBL} values for the ionic component of the LC50 value (toxicity) for the various organs or whole fish

(Table 5). Specifically for the gills (considered the toxic site of action), there is a 0.8 log unit difference between the log K_{NiBL} for bioaccumulation versus toxicity in round goby and 0.4 log unit difference in rainbow trout. This suggests that a BLM built on bioaccumulation would be more protective than one built on toxicity. A similar trend was observed for invertebrates (namely *Lymnaea stagnalis* and *Lumbriculus variegatus*) where the log K_{NiBL} for bioaccumulation was higher ($\sim$0.3–0.5 log units) than for toxicity (Leonard and Wood 2013). However, the kidney log K_{NiBL} values are the same for round goby and very similar for rainbow trout (Table 5), indicating the kidney as an organ of interest with regards to Ni as was previously shown by Pane et al. (2005, 2006). The correlation between log K_{NiBL} values for binding and acute toxicity does not appear to extend to other organs (gut or whole fish) which are not considered the toxic site of action.

Impact of Ni on fish behaviour

In the current study, we have shown that swimming activity, specifically movement, is a sensitive endpoint in comparison to lethality in round goby where the significant decline in movements per minute occurred at only 28 % of the LC10. This is consistent with other studies that showed that the average toxicant exposure concentration that caused significant alterations in swimming behaviour was less than 16 % of the concentration that caused 50 % mortality (Little and Finger 1990). To the best of our knowledge little is known regarding the effects of Ni on behavioural endpoints, however, acute sub-lethal concentrations of Ni (25–85 µmol/L) have been shown to impact respiratory and aggressive behaviour as well as cause stress-related discomfort movements in the tilapia, *Oreochromis niloticus* (Alkahem 1994).

The acute behavioural effect concentration in round goby was at or below 8.1 µmol Ni/L (Fig. 5a). Currently, the Canadian water quality guideline (WQG) for Ni at a water hardness of 140 mg/L as $CaCO_3$ is 2.1 µmol Ni/L (calculated using the water hardness based equation $e^{0.76*\ln[\text{hardness}]+1.06}$ (expressed in µg/L); CCREM 1987). In the United States, the Criterion Maximum Concentration (CMC-acute) for Ni is also based on water hardness ($\text{CMC} = e^{0.846*(\ln \text{ hardness}) + 2.255}$; USEPA 1995) and is 10.6 µmol Ni/L at 140 mg/L as $CaCO_3$. For the European Union, the Water Framework Directive, Environmental Quality Standard (EQS; based on a "user friendly" BLM which incorporates Ca, DOC and pH (ECB 2008)) is 0.043 µmol Ni/L (based on water chemistry from Table 1). It should be noted that the Canadian WQG and European Union's EQS values are chronic values. Therefore, round goby may be at risk of ecological death in the United States based on the current CMC for Ni; but, the criterion continuous concentration-chronic is 1.2 µmol Ni/L and therefore round goby are protected by the chronic criteria and are protected by Ni water quality guidelines in Canada and the European Union. The behavioural effects of chronic exposures to Ni in round goby, however, remain uncertain.

The behavioral effect of acute Ni exposure on rainbow trout was much less pronounced than in round goby (Fig. 5b). Metals often, but not always, affect swimming activity at levels lower than the LC50, in a range of fish taxa (Little and Finger 1990; Scott and Sloman 2004). The 96 h LC50 for rainbow trout exposed to copper was approximately 0.5 µmol/L (hardness as $CaCO_3$ of 30–102 mg/L, Howarth and Sprague 1978), while LOECs for homing behaviour after 37–40 weeks (Saucier et al. 1991, Saucier and Astic 1995) were 0.3 µmol/L, at 61 mg/L hardness. The 96 h LC50 for rainbow trout exposed to cadmium was 0.2 µmol/L at 140 mg/L hardness (Szebedinksy et al. 2001, Hollis et al. 1999), while LOECs for alarm substance responses after 7 days (Scott et al. 2003) and agonistic behaviours after 24 h (Sloman et al. 2003a, b) were 0.02 µmol/L at 120 mg/L hardness, respectively. It may be the case that Ni, like Cu, only affects trout behaviours at levels comparable to the 96 h LC50; however it is also possible that if we had measured activity levels after different exposure times to Ni, or different behaviours altogether (such as olfaction or aggression), different patterns would have been revealed.

Other metals have been shown to cause effects on behaviour either through the olfactory system, the brain (see Sloman 2007 for review), or through alterations to metabolic load (Allin and Wilson 1999). The results of the current study, suggest there is an efficient blood–brain barrier against Ni in both species. However, we cannot exclude indirect effects on the brain as a copper-exposed carp showed no accumulation of Cu in the brain, but exhibited an indirect effect of decreased 5-HT in the brain (De Boeck et al. 1995).

Link between changes in behaviour and Ni exposure concentration or Ni bioaccumulation

Traditionally, most toxicity endpoints are linked to the concentration of the metal, in this case Ni, in the environment (Meyer et al. 1999; Di Toro et al. 2001; Niyogi and Wood 2004); however, there is increasing evidence that Ni bioaccumulation may also be a good predictor of toxicity endpoints (see above; Luoma et al. 2009; Adams et al. 2011; Schmidt et al. 2011; Leonard and Wood 2013), and perhaps even behavioural endpoints. Here we have compared the correlation between changes in behaviour with both Ni exposure concentrations and Ni bioaccumulation in organs or whole fish (Table 6). We observe a

stronger correlation between Ni bioaccumulation in the gills or whole fish and behaviour than is found between Ni exposure concentration and behaviour. To our knowledge, this is the first study to link Ni bioaccumulation with behavioural endpoints such as swimming/spontaneous movement. Our integrative approach explored the response of two very different species, one of which showed a clear behavioural effect of exposure to Ni. In future it would be of interest and benefit to expand these analyses to a wider range of species to determine whether Ni bioaccumulation, specifically in the gills, gut and whole fish, may be a good predictor of behavioural changes from metal exposure; which in the wild can lead to ecological death.

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Conflict of interest The authors declare that they have no conflict of interest.

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