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Aquatic Toxicology 84 (2007) 198–207

AQUATIC TOXICOLOGY

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Branchial cadmium and copper binding and intestinal cadmium uptake in wild yellow perch (*Perca flavescens*) from clean and metal-contaminated lakes

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Received 11 December 2006; received in revised form 22 March 2007; accepted 23 March 2007

Abstract

Branchial binding kinetics and gastro-intestinal uptake of copper and cadmium where examined in yellow perch (*Perca flavescens*) from a metal-contaminated lake (Hannah Lake, Sudbury, Ontario, Canada) and an uncontaminated lake (James Lake, North Bay, Ontario, Canada). An *in vivo* approach was taken for gill binding comparisons while an *in vitro* gut binding assay was employed for gastro-intestinal tract (GIT) uptake analysis. By investigating metal uptake at the gill and the gut we cover the two main routes of metal entry into fish. Comparisons of water and sediment chemistries, metal burdens in benthic invertebrate, and metal burdens in the livers of perch from the two study lakes clearly show that yellow perch from Hannah L. are chronically exposed to a highly metal-contaminated environment compared to a reference lake. We found that metal-contaminated yellow perch showed no significant difference in gill Cd binding compared to reference fish, but they did show significant decreases in new Cd binding and absorption in their GITs. The results show that gill Cd binding may involve low-capacity, high-affinity binding sites, while gastro-intestinal Cd uptake involves binding sites that are high-capacity, low-affinity. From this we infer that Cd may be more critically controlled at the gut rather than gills. Significant differences in branchial Cu binding (increased binding) were observed in metal-contaminated yellow perch. We suggest that chronic waterborne exposure to Cu (and/or other metals) may be the dominant influence in gill Cu binding rather than chronic exposure to high Cu diets. We give supporting evidence that Cd is taken up in the GIT, at least in part, by a similar pathway as Ca^{2+}, principally that elevated dietary Ca^{2+} reduces Cd binding and uptake. Overall our study reveals that metal pre-exposure via water and diet can alter uptake kinetics of Cu and Cd at the gill and/or the gut.
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Keywords: Yellow perch; Gill; Gastro-intestinal tract; Copper; Cadmium; Calcium

1. Introduction

Sudbury, Ontario, Canada is known for its abundant production of nickel and copper from mining operations; unfortunately this also makes it famous for its highly contaminated surrounding environment. An extensive study by Pyle et al. (2005) revealed that lakes in close proximity to the industrial operations compared to distant reference sites have very high concentrations of many metals (some of the highest in the world) in their water and sediment (Cu, Cd, Ni, Zn), as well as in the tissues of inhabitant fish.

Teleosts take up metals via two pathways, either by their gills and/or their gut. Mechanisms of branchial metal uptake have become increasingly well defined (Wood, 2001) while mechanisms of intestinal metal uptake have been less well characterized. This is despite the fact that nutritive metals, such as Cu, Fe, and Zn are taken up primarily by the gut and not the gill of fish under optimal growth conditions (Bury et al., 2003). The main route of entry of nonessential metals, such as Cd, may also be via the gut under certain circumstances (Wood et al., 2006).

Copper is thought to be taken up by fish gills via apical Na^+ channels (Grosell and Wood, 2002) while cadmium is believed to share a common transport pathway with Ca^{2+} (Verbost et al., 1987, 1989). The mechanisms of Cu uptake at the gastro-intestinal tract (GIT) of fish appear fundamentally different from those at the gills (Nadella et al., 2006, 2007). However, there is

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doi:10.1016/j.aquatox.2007.03.024

now some evidence that Cd, at least in part, is taken up by a similar route as Ca^{2+} in the GIT of rainbow trout, principally that elevated dietary Ca^{2+} reduces Cd uptake through the GIT both *in vivo* and *in vitro* (Franklin et al., 2005; Baldisserotto et al., 2006; Wood et al., 2006).

Fish feed on invertebrates, which are known to contribute a crucial link in the trophic transfer of metals through the food chain (Dallinger and Kautzky, 1985). It appears that resident invertebrates of Hannah Lake, a metal-contaminated lake near Sudbury have been severely impacted in that they are less abundant, smaller, less diverse (Iles and Rasmussen, 2005) and potentially poorer in their nutritive quality than those from nearby reference sites. The probable consequences for benthivorous perch would be retarded growth and high metal tissue burdens (Dallinger and Kautzky, 1985). However, to our knowledge invertebrates from Hannah L. have not been directly analysed for metal content or for their nutritive quality.

Pre-exposure to experimentally elevated dietary Cu concentrations causes trout to down-regulate their branchial Cu uptake and *vice versa* (Kamunde et al., 2001, 2002b). Therefore, Cu concentrations in the natural diets of fish in pristine and contaminated lakes may have an important controlling influence on the uptake of Cu at their gills. Moreover, fish consuming high Na^+ diets demonstrate reduced branchial Cu uptake and a subsequent reduction of Cu accumulation in tissues (Pyle et al., 2003; Kamunde et al., 2003). Similarly, fish consuming high Cd (Szebedinszky et al., 2001) or Ca^{2+} (Zohouri et al., 2001; Baldisserotto et al., 2004) diets have been demonstrated to down-regulate branchial Cd uptake. These results indicate that fish can regulate branchial metal uptake on the basis of dietary ionic composition, such that increased dietary Na^+ causes a reduction in branchial Cu uptake and increased dietary Ca^{2+} causes a reduction in branchial Cd uptake.

Yellow perch (*Perca flavescens*) were chosen as the test species for this study because of their predominance in the area of concern and their high metal tolerance (Pyle et al., 2005). Using these fish, rather than typical laboratory species such as rainbow trout, allows for more ecologically relevant testing in terms of environmental risk assessment. This paper focuses on Cu and Cd which allows for comparisons between binding and uptake rates of an essential and a nonessential 3 metal.

The objectives of this study were fourfold: First to measure metal content and nutritive quality of natural food sources (benthic invertebrates) available to fish from Hannah L. and James L. Secondly, to compare gill Cu binding rates of fish from the two lakes. Penultimately, to characterize and compare the kinetics of both branchial and intestinal Cd uptake between the fish from the two lakes, and lastly to determine if Ca and Cd display competition for uptake sites in the GIT of the perch.

We hypothesized that invertebrates from Hannah L. would have elevated metal concentrations and poorer nutritive quality based on their chronic exposure. We further hypothesized that metal-contaminated fish would bind and take up less metal than reference fish because of either adaptive or regulatory changes. Based on experiments done on rainbow trout using Cd and Ca (Franklin et al., 2005; Baldisserotto et al., 2006; Wood et al., 2006) we also predicted that Ca would have a protective effect against Cd uptake in the gut of perch.

Fish and benthic invertebrates were collected from two lakes, Hannah L. (metal contaminated), and James L., located near North Bay, Ontario (approximately 150 km east of Hannah L.) which served as a reference location. The perch were evaluated for gill and/or GIT binding rates of Cd and Cu, each over a range of concentrations. An *in vivo* approach was taken for gill binding comparisons while an *in vitro* gut binding assay was employed for gastro-intestinal tract (GIT) uptake analysis. Benthic invertebrates were analysed for metals (Cd, Cu, Zn, Ni), major cations (Ca^{2+}, Mg^{2+}, Na^+), and nutrients (protein, carbohydrate and lipid concentrations).

2. Methods and materials

2.1. Experimental animals

Yellow perch (*P. flavescens*) (10–26 g) were collected from two lakes in north-eastern Ontario. James Lake (46°17′21″N, 78°59′26″W), located on the Nipissing University—Alcan Environmental Research Preserve near North Bay, served as an uncontaminated reference lake. Hannah L. (46°26′35″N, 81°02′24″W) represents a metal-contaminated lake located within the city limits of Sudbury. Yellow perch were sampled by angling from these lakes over a 2-day period in July 2006. At the time of capture the lake water temperature was approximately 16 °C. The caught fish were placed in 20 L tanks (Water-Pak® 8810-03) (20–25 fish in each) containing the fish's respective native lake water (16–20 °C). The perch were transported 5 h to McMaster University in Hamilton, Ontario immediately after final catch. Aeration was supplied using battery powered air pumps, and mortality during travel was minimal (∼5%). Upon arrival fish were placed into 200 L flow-through tanks containing a mixture of reverse osmosis water and dechlorinated Hamilton municipal tap water to produce relatively soft holding water (Ca = 125.7 μM, Na = 153.6 μM, Mg = 37.4 μM, hardness $\sim$16 mg L^{-1} as $CaCO_3$, dissolved organic carbon $\sim$ 1 mg L^{-1}, pH 6.8, temperature = 12 °C). Fish remained in these conditions for approximately 24 h before experimentation; no food was given to them during this time.

2.2. Gill ^{109}Cd kinetic binding assays

To determine the binding characteristics of cadmium to the gills of yellow perch, a radiolabeled ^{109}Cd *in vivo* binding assay was used (adapting the methods of Niyogi and Wood, 2004; Niyogi et al., 2004). Twenty-one fish from Hannah L. were randomly assigned to three 18 L Rubbermaid® containers for a total of seven fish per group. Each container represented a different Cd concentration, forming a range of exposures with nominal concentrations of 22, 44 and 90 nM. This was duplicated for James L. fish, for a total of six tanks. These containers were placed on a flow-through wet table which kept the temperature constant at 12 °C. Before the addition of fish, each container was filled with 6 L of water (same water as lab holding water) and subsequently spiked with Cd (as $Cd(NO_3)_2 \cdot 4H_2O$,

Fisher Scientific, Canada) and 3 μCi L^{-1} ^{109}Cd (as $CdCl_2$, specific activity = 3.65 μCi $μg^{-1}$, I.I.C.H., Kansas, USA) to achieve measured dissolved Cd concentrations of 31.4, 56.1, and 105.1 nM (for James L.) and 30.0, 54.2, and 100.0 nM (for Hannah L.).

Water samples (10 mL) were taken in duplicate at the beginning and end of the assay for each exposure container. Water samples were filtered through a 0.45 μm syringe tip filter (PALL Acrodisc™ 25 mm syringe filter) and acidified to 1% using concentrated trace-metal grade HNO_3 (Fisher Scientific, Canada). After a 3 h exposure period, the yellow perch were removed from their containers and euthanized by an overdose of MS-222 (600 mg L^{-1}). The entire GITs were removed by dissection for a subsequent ^{109}Cd *in vitro* gastro-intestinal binding assay (described below). Immediately after the removal of the GIT, the whole gill baskets were excised, rinsed with deionized water, blotted dry, and placed into individual 20 mL polyethylene scintillation vials for gamma counting.

2.3. Gill ^{64}Cu kinetic binding assays

To determine the binding characteristics of Cu to the gills of yellow perch, a radiolabeled ^{64}Cu *in vivo* binding assay was employed using the methods described above, with the addition of one more concentration in the James L. series (total of seven tanks). ^{64}Cu was prepared by irradiating dried $Cu(NO_3)_2$ (300 μg) at McMaster University Nuclear Reactor to produce a radioactivity level of 0.6 mCi (half life 12.9 h). Once irradiated, the dried $Cu(NO_3)_2$ was resuspended using 400 μL of 0.1 M HNO_3, 400 μL of 0.01 M $NaHCO_3$, and 1.7 mL deionized water. Assay water (6 L) was then spiked with the ^{64}Cu solution to produce measured total copper concentrations of 262.0, 352.4, 489.3, and 968.4 nM (for James L.) and 308.9, 597.8, and 1025.8 nM (for Hannah L.) in seven different tanks.

2.4. Gastro-intestinal sac preparation

An *in vitro* gastro-intestinal binding assay was used to determine GIT cadmium uptake rates. The method used was very similar to that employed by Nadella et al. (2006, 2007) in rainbow trout. As mentioned above, after yellow perch from Hannah L. and James L. were exposed for 3 h to radiolabeled Cd in the water for gill Cd binding measurements, they were euthanized with an overdose of MS-222 and used for a subsequent gut binding assay.

A pilot study validated the use of the same fish for both gill and gut fluxes. The study was carried out in same conditions described above for the gill ^{109}Cd kinetic binding assay, but rainbow trout (*Oncorhynchus mykiss*) were used in place of perch. After a 3 h exposure period, ^{109}Cd radioactivity was measured in different tissues of the trout. Results revealed that Cd in the fish originating from the exposure water was mostly localized in the gill baskets and that less than 1% of the Cd from the water had been transferred to the GIT.

The entire perch GIT was removed by dissection, then squeezed carefully and flushed with saline to remove any solid food, chyme, or faecal matter. Gastro-intestinal tracts were temporarily held in ice-cold saline while the visceral fat surrounding the GIT was gently pulled away. Unlike Nadella et al. (2006, 2007) who carried out sectional analysis of the GIT in large trout, we used whole GITs of the small perch to form single sacs. The posterior end of the intestine was tied closed using surgical silk while the other end was fitted with a short flared piece of polyethylene catheter tubing (Clay-Adams PE-50) and secured in placed with a silk ligature. The mucosal saline was infused and later drained through this catheter.

In a Cd GIT kinetic curve assay, intestinal sacs were filled with 0.5–2.0 mL (depending on size) modified Cortland saline (in mM: NaCl 124.0, KCl 5.0, $CaCl_2{\cdot}2H_2O$ 1.0, $MgSO_4{\cdot}7H_2O$ 1.9, $NaHCO_3$ 1.9, $NaH_2PO_4{\cdot}H_2O$ 2.9, glucose 5.5; Wolf, 1963). Cadmium was added in varying concentrations (as $Cd(NO_3)_2{\cdot}4H_2O$ Fisher Scientific, Canada) along with 0.5 μCi mL^{-1} radioactive ^{109}Cd (as $CdCl_2$, specific activity = 3.65 μCi $μg^{-1}$ (I.I.C.H., Kansas, USA)) giving total measured Cd concentrations of 1.1, 9.8, 47.3, 95.1 μM. A different modified saline (in mM: NaCl 133, KCl 5, $CaNO_3$ (1.6, 6.4, 11.9, or 61.1 measured), $MgSO_4{\cdot}7H_2O$ 1.9, glucose 5.5) was used for the Ca competition experiments to prevent Ca precipitating as carbonate or phosphate salts. For these latter tests both "cold" and radiolabeled Cd were added to the mucosal saline to achieve a measured total Cd concentration of 53.8 μM. All saline solutions were adjusted with NaOH to a pH of 7.4. After the gut bags were filled with the appropriate saline, they were sealed, blotted dry and weighed. The sacs were individually placed in different containers of 11 mL of "serosal" saline with measured osmolality (adjusted using mannitol) equal to that of the mucosal saline. Initial samples of the stock mucosal and serosal salines were taken at the beginning of each experiment. The serosal baths were aerated with a mixture of 99.7% O_2 and 0.3% CO_2 ($P_{CO_2} = 2.25$ Torr, similar to natural blood levels, Chowdhury et al., 2004). Temperature remained at approximately 15 °C throughout all gut sac experiments. After a 2 h exposure period, the GIT sacs were removed from the serosal saline, blotted dry, weighed, and then drained of remaining mucosal saline. A 5 mL sample of the serosal saline was taken and its ^{109}Cd activity was measured. The sacs were cut open by a longitudinal incision and were processed as described by Nadella et al. (2006).

2.5. Benthic invertebrate metal and nutritive analysis, and perch liver metal analysis

Invertebrates were collected from both lakes by D nets (with 1 mm mesh) using a "kick and sweep method". All invertebrates collected were hand-picked from the nets, pooled, and frozen at −20 °C. Frozen invertebrate samples from both lakes were placed in liquid nitrogen and ground to a fine powder using a mortar and pestle, and then acid digested (16 M trace metal grade concentrated HNO_3) at 50 °C for 12 h. They were subsequently diluted appropriately with 1% nitric acid and analysed for calcium, magnesium, sodium and zinc by flame atomic absorption spectroscopy (FAAS; Varian AA240FS, Mulgrave, Australia). A riverine water reference material for trace metals (SLRS-4) from the National Research Council of Canada was used as a

certified reference material to ensure accuracy of metal measurement. Cadmium, copper and nickel were analysed using a Varian GTA 120 graphite tube atomizer coupled to a Varian AA240FS spectrometer (Mulgrave, Australia). A certified reference material (TE88-4) originating from the National Research Council of Canada was measured to ensure accuracy.

Proteins were quantitatively analysed according to the instructions for the Coomassie Plus Bradford Assay from Pierce (Fisher Scientific, Canada) against a BSA standard curve. Total soluble carbohydrates (TSC) and glycogen were analysed as glucose equivalents using a protocol adapted from Kepler and Decker (1974) and Vadnais (2001). Prepared samples were compared to a glucose (Sigma, Canada) standard curve. The amount of glycogen was determined by subtracting the TSC glucose equivalents of non-digested samples from amyloglucosidase digested samples.

The livers of all the perch used in the GIT assays were removed during dissection and were immediately frozen. Soon thereafter, the livers were digested using 1N HNO_3 at 50 °C for 48 h in large bullet tubes. They were subsequently centrifuged for 2 min at 14 000 × *g* and their supernatants were analysed for Cd, Cu and Ni concentrations using a Varian GTA 120 graphite tube atomizer. A certified reference material (TM-15) from the National Research Council of Canada was used to ensure accuracy.

2.6. Analytical techniques and calculations

Samples from the gill assays (gill assay water and gill tissues), and from gastro-intestinal assays (rinse solutions, blotting paper, epithelial scrapings, serosal salines, and gut tissues) were counted individually for radioactivity using a 1480 Wallac Wizard 3 in. Automatic Gamma counter (Perkin-Elmer, Canada). Water samples were subsequently analysed by graphite furnace atomic absorption spectroscopy (GFAAS; Varian SpectrAA-220FS, Mulgrave, Australia) to determine dissolved cadmium concentrations (*c* in equation below). Analytical standards (TM-15) certified by the National Research Council of Canada were used to verify measured values.

Newly bound cadmium and copper in gill tissue was determined using the measured specific activity of the water: $a(bc^{-1})^{-1}$ where *a* is the ^{109}Cd or ^{64}Cu counts in gills (cpm g^{-1}), *b* the counts in the exposure water (cpm L^{-1}), and *c* is the total metal concentration the in water (μg L^{-1}). Log *K* values were calculated by: $\log(d^{-1})$ where *d* is the concentration of the substrate that provides a binding equivalent to half of the B_{max} in molar units.

Uptake rates (J_{in}) of Cd (pmol cm^{-1} h^{-1}) in the GIT were determined by the equation: $J_{in} = CPM(R\,SA\,t)^{-1}$ where CPM represents the sample ^{109}Cd activity, *R* the specific activity of the initial mucosal saline used (cpm $pmol^{-1}$), SA the tissue surface area, and *t* is the flux time (h). For the specific activity component of the above equation, the total Cd concentration of the initial mucosal saline was measured for each experiment using flame atomic absorption spectroscopy (FAAS; Varian SpectrAA-220FS, Mulgrave, Australia). Calcium concentrations in the various mucosal salines were also determined using FAAS, against a standard curve made from a reference standard from Fisher Scientific, USA.

2.7. Statistical analysis

Data have been presented as means ± standard errors, and significant differences are denoted by "*". A fiducial limit of $P < 0.05$ was used throughout. Cadmium and copper uptake kinetics were analysed using either least-squares linear regression or non-linear regressions employing a hyperbolic curve fit (single rectangular two parameters $y = ax(x+b)^{-1}$; SigmaPlot Windows version 8.0) as appropriate. Statistical analyses were performed using SPSS12 for Windows. Independent Student's *t*-tests (two-tailed) were used to compare mean GIT uptake rates of the fish from the different lakes exposed to the same Cd or Ca concentration. Two-way analysis of variance (ANOVA) or ANCOVA were used to determine if there were overall differences in uptake rates or binding based on metal concentration or lake of fish origin or in the calcium competition experiments, based on calcium concentration or the lake of origin. Data presented in Figs. 1A, 2A and B, and 3A and B underwent log transformation to meet the requirements of normality.

3. Results

3.1. Chemistry of water, sediment, and benthic invertebrates from James L. and Hannah L.

Both James L. and Hannah L. are softwater lakes low in Ca, but James L. has a slightly higher hardness value mainly due to its higher Mg levels. James L. also exhibits a higher concentration of DOC and a greater buffering capacity (alkalinity) than Hannah L. (Table 1). The two study lakes vary greatly with respect to the amount of metals present in the water, sediment (Table 1) and in resident invertebrate fauna (Table 2). In Hannah L., copper concentrations in benthic invertebrates, water and sediment were about 5, 17 and 63 times higher than in James L., respectively. Cadmium levels were about 3, 5 and 1.5 times higher in the invertebrates, water and sediment, respectively. Nickel levels were also notably higher in Hannah L. in each of the above-mentioned components, while differences in Zn concentrations were modest. These values indicate that fish living in the contaminated lake have a chronic history of Cu, Cd and Ni exposure both internally, by ingested food, and externally, from ambient water and contact with sediment. Not surprisingly, high Cu, Cd, and Ni burdens were found in the livers of perch from Hannah L. (Table 1).

The invertebrates collected from the two lakes were slightly different in terms of species composition (James L.: damselfly larvae, dragonfly nymph, snails, caddisfly larvae, dytiscid beetles, and dytiscid larvae were collected; Hannah L.: damselfly larvae, adult notonectids, dragonflies, and dragonfly nymphs). Hannah L. invertebrates had higher lipid concentration than James L. invertebrates (∼1.5 times), but there was little difference with respect to protein and carbohydrate content (Table 2). James L. benthic invertebrates had dramatically higher concentrations of Ca (approximately 20-fold) compared to Hannah L.

Table 1
Water quality parameters and concentrations of cations, including metals of interest, in water and sediment samples collected from Hannah and James Lakes

	Metals															Water quality parameters			
	Cd (MDL = 0.1)	Cu (MDL = 1)	Ni (MDL = 1)	Al (MDL = 1)	As (MDL = 1)	Ba (MDL = NA)	Ca (MDL = 100)	Fe (MDL = 50)	Mg (MDL = NA)	Mn	Pb	Rb	Se	Sr	Zn	Alkalinity	DOC	Hardness	pH
Total concentration in water																			
Hannah L.																			
Mean	0.5[a]	25	181	ND	2	24	11603	71	4137	251	NA	3	2	65	10	16	5[a]	46	7.6
S.E.M.	–	2	45	–	0	2.4	1150	4	377	19	–	0	0.3	6	3	0	–	4	0
James L.																			
Mean	0.1	1.5	1	19	0.3	19	16143	505	15537	186	0.3	1	NA	21	16	91	11	62	6.8
S.E.M.	0	0.1	0.2	5	0	2	1837	115	2957	88	0.1	0.04	–	1	13	7	1	4	0.1
Total concentration in sediment																			
Hannah L.																			
Mean	3	1450	1461	NA	87.7	NA	2906	34151	NA	264	67	NA	32	NA	148	–	–	–	–
S.E.M.	1	284	233	–	7.6	–	781	1913	–	10	6	–	3	–	31	–	–	–	–
James L.																			
Mean	2	23	15	NA	9	69	9796	12163	4639	247	45	2	32	NA	221	–	–	–	–
S.E.M.	0.3	4	3	–	2	15	1266	2228	631	57	12	0.5	2	–	35	–	–	–	–
Total concentration in liver																			
Hannah L.																			
Mean	5.36	102.18	5.94	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
S.E.M.	0.37	22.1	0.45	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
James L.																			
Mean	0.24	5.26	0.51	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
S.E.M.	0.02	0.46	0.04	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Note: Water and sediment chemistry from Hannah L. (Pyle et al., 2005); MDL = minimum detection limit; NA = not available; ND = not detected; S.E.M. = standard error of the mean; total metal concentrations in water and sediment are in $\mu g\ L^{-1}$ and $\mu g\ g^{-1}$ respectively; alkalinity, hardness and dissolved (DOC) are in $mg\ L^{-1}$; water and sediment samples ($n = 3$); total metal concentrations in liver tissue are in $\mu g\ g^{-1}$ wet weight; liver samples ($n = 20$).

[a] Iles and Rasmussen (2005).

Table 2
Concentrations of cations, including metals of interest, and nutritional parameters in benthic invertebrates collected from Hannah and James Lakes

	Metals and cations ($\mu g\,g^{-1}$)							Nutritional parameters ($\mu g\,mg^{-1}$)			
	Ca	Cd	Cu	Na	Ni	Mg	Zn	Protein	Mean total CHO	Mean soluble CHO	Lipid
Hannah L.											
Mean	477.13	0.92	38.14	934.80	47.55	247.67	45.20	17.70	0.046	0.041	150.75
S.E.M.	15.72	0.07	1.87	38.08	3.04	4.79	1.68	0.72	0.001	0.001	5.46
James L.											
Mean	9524.85	0.29	8.26	781.5	ND	263.34	17.30	19.40	0.042	0.043	102.39
S.E.M.	1716.61	0.09	1.00	9.36	ND	21.57	–	0.42	0.001	0.002	3.36

Interestingly, invertebrate Na and Mg concentrations were not different between the two study lakes (Table 2). All of the invertebrates listed above are known to be part of the natural diet of yellow perch (Jansen and Mackay, 1992; Thayer et al., 1997; Iles and Rasmussen, 2005).

3.2. Gill ^{109}Cd and ^{64}Cu kinetic binding assays

In yellow perch from both James L. (uncontaminated) and Hannah L. (metal-contaminated), the *in vivo* 3 h binding of Cd to the gills increased with increasing cadmium concentrations (Fig. 1A). The results were very similar for perch from the two sites. These increases in Cd binding to the gills were saturable within the concentration range of 30–105 nM. The log $K_{Cd\text{-}gill}$ and B_{max} for James L. were 7.5 and 0.527 nmol g^{-1}, respectively, whereas the log $K_{Cd\text{-}gill}$ and B_{max} for Hannah L. were 7.2 and 0.739 nmol g^{-1}, respectively—not significantly different in perch from the two lakes (determined by two-way ANOVA, $F_{3,37} = 1.667$, $p = 0.205$).

The 3 h *in vivo* binding of Cu to the gills of yellow perch from both James L. and Hannah L. increased with increasing copper concentration in a linear fashion (Fig. 1A). The lack of saturation prevented the determination of kinetic parameters (log $K_{Cu\text{-}gill}$ and B_{max}). However, further statistical analysis indicated a significant difference in gill Cu binding between the two lakes, with Hannah L. fish exhibiting a consistently higher binding than James L. fish (determined by ANCOVA, $F_{3,65} = 6.06$, $p = 0.016$).

3.3. Gastro-intestinal ^{109}Cd kinetic uptake rates

The amount of Cd measured in the gut muscle tissue combined with Cd found in the serosal fluid represents a conservative measure of metal transported into the blood compartment of the fish (absorbed Cd (i.e., as described by Nadella et al. (2006)). As Cd exposure concentrations increased in the *in vitro* assays, uptake rates increased non-linearly but showed little evidence of saturation (Fig. 2A), so B_{max} and log K values could not be calculated. The shapes of the curves suggest that Cd binding sites in the GIT are low-affinity, high-capacity sites. Overall, a two-way ANOVA indicated that fish from James L. (clean) absorbed significantly more Cd than yellow perch from Hannah L. (contaminated) ($F_{1,46} = 24.36$, $p < 0.001$); this difference was also significant at all Cd concentrations up to 47.3 μM (determined by Student's *t*-tests).

Radiolabeled cadmium measured in the rinse samples (i.e., saline and EDTA and blotting paper together (see Nadella et al., 2006 for details)) represents metal that was loosely bound to the mucus on the surface of the GIT (Fig. 2B). This Cd fraction also

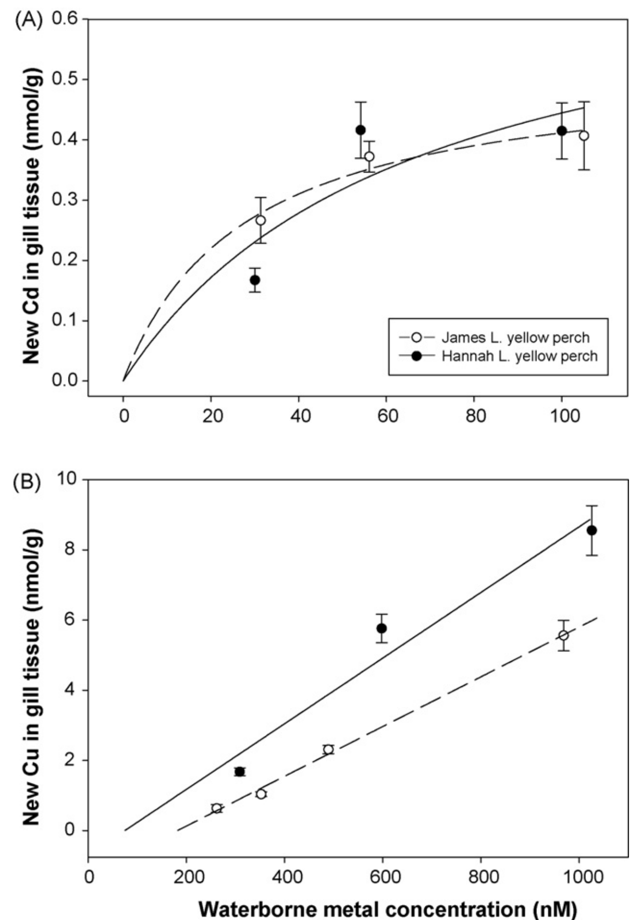

Fig. 1. (A) Newly accumulated ^{109}Cd in gill tissue of yellow perch at various waterborne ^{109}Cd concentrations in softwater after a 3 h exposure. Non-linear regressions were fitted to the data of James L. (clean) ($r^2 = 0.938$) ($B_{max} = 0.527$ nmol/g wet tissue; log $K_{Cd\text{-}gill} = 7.5$) and Hannah L. (metal-contaminated) ($r^2 = 0.710$) ($B_{max} = 0.739$ nmol/g wet tissue; log $K_{Cd\text{-}gill} = 7.2$). There were no significant differences between the uptake rates based on the lake of origin of the fish ($F_{3,37} = 1.667$, $p = 0.205$). (B) Newly accumulated ^{64}Cu in gill tissue of yellow perch at various waterborne ^{64}Cu concentrations in softwater after a 3 h exposure. SigmaPlot 8.0 was used to fit linear regressions for James L. (clean) ($r^2 = 0.996$) and Hannah L. (metal-contaminated) ($r^2 = 0.952$). Note lack of saturation. There was a significant difference between the two lakes as determined by a two-way ANCOVA ($F_{3,65} = 6.06$, $p = 0.016$).

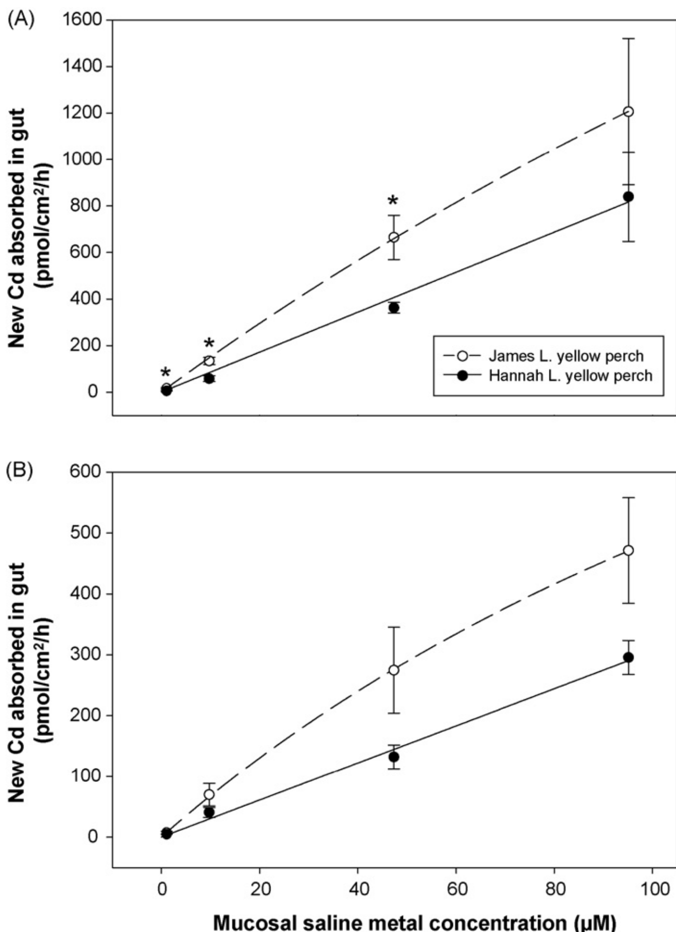

Fig. 2. (A) Cadmium uptake kinetics *in vitro* in gastro-intestinal tract of yellow perch exposed for 2 h to various mucosal Cd concentrations in modified Cortland saline. Means ± S.E.M. (n = 6). Non-linear regressions were fitted to the data of James L. (clean) ($r^2 = 0.68$) and Hannah L. (metal-contaminated) ($r^2 = 0.70$). Means with "*" indicate a significant difference ($p < 0.05$) between Hannah L. and James L. fish exposed to same [Cd], determined by unpaired Student's t-test. There was also an overall a significant difference ($F_{1,46} = 24.36$, $p < 0.001$) between the lakes as determined by two-way analysis of variance (uptake rates were log normalized before statistical test). (B) Cd loosely bound to epithelial cells in gastro-intestinal tract of yellow perch exposed *in vitro* for 2 h at various mucosal Cd concentrations in modified Cortland saline. Means ± S.E.M. (n = 6). Non-linear regressions for James L. (clean) ($r^2 = 0.70$) and Hannah L. (metal-contaminated) ($r^2 = 0.89$) were fit. A two-way analysis of variance determined that there was a significant lake effect ($F_{1,46} = 7.51$, $p = 0.009$).

exhibited close to linear uptake kinetics (Fig. 2A). Again, there was a significant lake effect by two-way ANOVA ($F_{1,46} = 7.51$, $p = 0.009$), but no individual differences; overall James L. fish had significantly higher amounts of loosely bound ^{109}Cd compared to Hannah L. fish. At comparable exposure concentrations, the amount of Cd bound to the surface mucus was about half of that of Cd absorbed by the GIT.

3.4. Cd–Ca competition gastro-intestinal assay

Hannah L. perch absorbed significantly less Cd in their GITs with increased Ca concentrations. Although not statistically significant, a similar decreasing trend appeared in James L. fish (Fig. 3A and B). There was a 28% and 33% decrease in

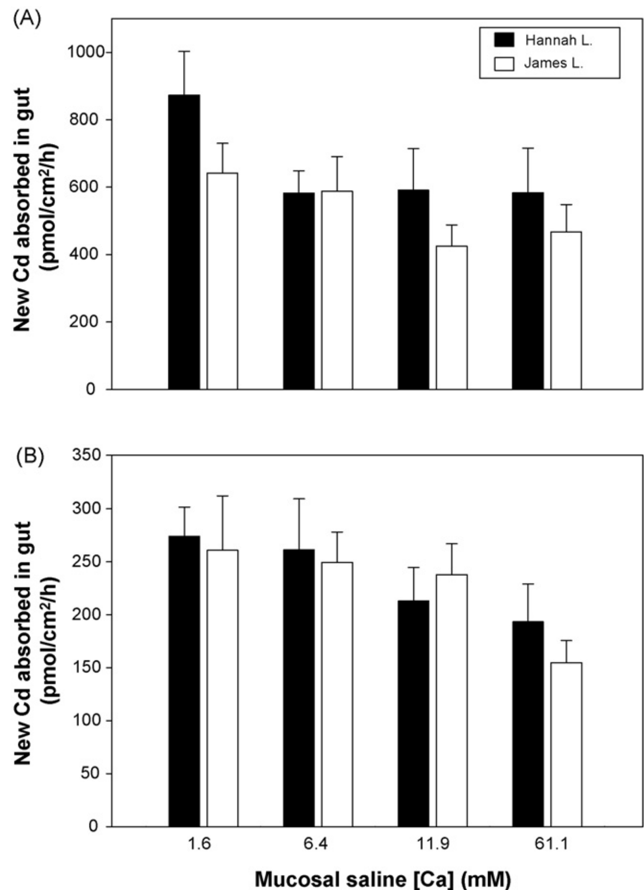

Fig. 3. (A) Uptake rates of absorbed Cd by the gastro-intestinal tract of yellow perch exposed *in vitro* for 2 h to 53.8 μM Cd with varying concentrations of Ca (1.6, 6.4, 11.9, or 61.1 mM). Means ± S.E.M. (n = 5 or 6 per treatment). There was no significant effect of Ca ($F_{3,46} = 2.55$, $p = 0.071$) or lake ($F_{1,46} = 1.81$, $p = 0.071$). (B) Cd loosely bound to epithelial cells of the gastro-intestinal tract of yellow perch exposed *in vitro* for 2 h to 53.8 μM Cd and various Ca concentrations. Means ± S.E.M. (n = 5 or 6 per treatment). There was no significant difference between lakes ($F_{1,46} = 0.14$, $p = 0.707$) or between Ca concentrations ($F_{3,46} = 2.54$, $p = 0.070$).

the mean absorbed Cd and a 41% and 29% reduction in the loosely bound Cd between the lowest (1.6 mM) and the highest (61.1 mM) Ca concentrations for James L. and Hannah L. fish, respectively. No significant lake effect was present (absorbed: $F_{1,46} = 1.81$, $p = 0.071$; rinse: $F_{1,46} = 0.14$, $p = 0.707$), and Cd uptake in Hannah L. fish was generally higher than in James L. fish.

4. Discussion

Yellow perch from Hannah L. clearly live in a highly metal-contaminated environment based on our reported water and sediment levels (Table 1), and we have shown that they are likely eating metal-contaminated invertebrates (Table 2). Liver tissue analysis of Hannah L. perch shows high concentrations of Cu, Cd, and Ni compared to perch from James L. (Table 1). We speculate that the differences in our results of branchial binding and intestinal uptake between the fish from the two different lakes, are due to these factors.

We have shown that perch from a metal-contaminated lake have significantly higher branchial Cu uptake rates. Kamunde et al. (2002a) pre-exposed rainbow trout to increased waterborne Cu levels (28 days to 22 $\mu g L^{-1}$) and found that Cu-acclimated fish exhibited a biphasic response. There was lower gill binding of new Cu when pre-exposed trout were acutely exposed to low waterborne copper concentrations (0 to ~93 nM, i.e., 0–6 $\mu g L^{-1}$ Cu) compared to control fish (acclimated to 2 $\mu g L^{-1}$ Cu). However, they found the opposite results when they acutely exposed the trout to higher concentrations of Cu (~300–1300 nM, i.e., ~19–83 $\mu g L^{-1}$ Cu), where pre-exposed fish actually bound more Cu at the gill than control fish. Notably, however, this greater gill binding was associated with reduced rather than elevated rates of Cu uptake from the water at the pre-exposure concentration. The yellow perch used in our experiment were chronically exposed to almost identical copper levels as in the experiment done by Kamunde et al. (2002a) described above (Hannah L. = 25 $\mu g L^{-1}$, James L. 1.5 $\mu g L^{-1}$, compared to 22 $\mu g L^{-1}$ and 2 $\mu g L^{-1}$ in their experiment). Our acute exposures were between 250 and 1000 nM (i.e., ~16–64 $\mu g L^{-1}$ Cu) which are comparable to the higher Cu range used by Kamunde et al. (2002a). Our findings of increased Cu gill binding in pre-exposed fish therefore fit very closely to the pattern they found. Kamunde et al. (2002a) attributed the phenomenon to an increase in low-affinity sites which are normally not involved in Cu transport into the internal compartment of the fish.

Elevated levels of Na in the diet will cause inhibition of Cu uptake at the gills (Pyle et al., 2003; Kamunde et al., 2005; Kjoss et al., 2005), but our invertebrate analysis revealed comparable Na concentrations (Table 2) and therefore Na is probably not an influential factor in this case. It is also known that chronic exposure to Cu via the diet can cause a reduction in rainbow trout branchial uptake of Cu (Kamunde et al., 2001). There is an elevated Cu concentration in the invertebrates of Hannah L. (38 μg Cu g^{-1}; Table 2), but this was far less than in the studies of Kamunde et al. (2001) and did not cause a reduction in Cu binding at the gill of yellow perch, contrary to the rainbow trout described above.

The above findings suggest that chronic waterborne exposure to Cu (and/or other metals) may be the dominant influence in gill Cu binding rather than chronic exposure to high Cu diets. Indeed high concentrations of other metals in the resident water might out-compete Cu for common transporters. In turn, the gill may compensate by up-regulating low-affinity transporters to maintain essential Cu levels.

Although Hannah L. perch are chronically exposed to higher waterborne and dietary concentrations of Cd (Tables 1 and 2), they display similar patterns to James L. fish of saturable kinetics for gill Cd binding (Fig. 1A). A tendency for lower log $K_{\text{Cd-gill}}$ and higher B_{max} was not significant. In contrast, Niyogi et al. (2004) earlier reported a lack of saturability in the gill Cd binding kinetics of Hannah L. perch relative to perch from a different reference lake (Geneva L.), but worked over a fivefold higher concentration range than the present study, so the results are not directly comparable.

With rainbow trout, Hollis et al. (1999, 2000a,b) and Szebedinszky et al. (2001) found a lower log $K_{\text{Cd-gill}}$ and an increased B_{max} in fish chronically exposed to waterborne Cd, while Szebedinszky et al. (2001) found even greater effects (reduced log $K_{\text{Cd-gill}}$, increased B_{max}, a tendency towards lack of saturability) of chronic exposure to dietary Cd. However, the waterborne and dietary Cd concentrations used in these trout studies were considerably higher than the elevations in contaminated Hannah L. water and invertebrates to which the wild yellow perch of the current study were exposed (Tables 1 and 2). Furthermore, diets with low Ca (as in the Hannah L. invertebrates, Table 2) might be expected to have a counteracting effect, based on several studies showing that elevated Ca diets reduce gill Cd binding and uptake in trout (Zohouri et al., 2001; Baldisserotto et al., 2004, 2005; Wood et al., 2006). We suggest that these factors in combination may have resulted in the lack of significant difference of gill Cd binding kinetics of Hannah L. fish versus James L. fish.Hannah L. fish consistently exhibited lower rates of absorbed and loosely bound Cd in the *in vitro* gut sac assays over all concentrations compared to James L. fish (Fig. 2A and B). The response of the GIT was therefore different from that of the gills discussed above. It appears that fish from Hannah L. have adjusted to their metal-contaminated diets by down-regulating transporters involved in Cd uptake at the GIT (such as DMT1, zinc transporters, and Ca pathways, for example; Wood et al., 2006). *A priori*, the response would seem to have clear adaptive value against metal uptake and subsequent toxicity.

Our results contrast with those of Baldisserotto et al. (2006) who reported that rainbow trout chronically fed diets with much higher Cd concentrations (300 μg Cd g^{-1}) exhibited more loosely bound Cd in their mid- and posterior intestine after an acute *in vitro* Cd gut sac assay similar to ours. For absorbed Cd, they found that there was no difference between control and Cd exposed fish. Differences in results may have arisen due to the fact that Baldisserotto et al. (2006) used unrealistically high levels of dietary Cd (300 times greater than the level measured in invertebrates from Hannah L.), compartmentalized intestinal tracts rather than whole GITs, and laboratory-reared rainbow trout rather than wild yellow perch.

There is strong evidence for a shared pathway between Ca and Cd at the gill (Verbost et al., 1989) and there is increasing evidence for a similar mechanism in the gut (Franklin et al., 2005; Baldisserotto et al., 2006; Wood et al., 2006). Our results, although not statistically significant, suggest that Ca and Cd compete for binding sites, reducing loosely bound and absorbed cadmium (Fig. 3A and B). It is possible that fish may be able to modulate Ca transport to reduce Ca uptake, which simultaneously may reduce Cd uptake. Baldisserotto et al. (2006) gave evidence of this using rainbow trout chronically exposed to a Ca spiked diet (50 mg Ca g^{-1} for 30 days). This being said, it would probably be difficult for Hannah L. fish to choose diets of higher Ca content, as the available natural food is highly Ca-deficient compared to that found in James L. (Table 2). Indeed, based on Ca levels in the diet the fish feed on, opposite results would be expected.

Overall, our Cd results differ from a very recent and comparable study of Zn gill binding and GIT uptake in wild perch from Hannah L. versus different reference sites (Nosbonsing and Geneva L.; Niyogi et al., in press). Niyogi et al. (in press) reported that this essential metal showed significant differences (decreased log $K_{\text{Zn-gill}}$ and increased $B_{\max}$) in the binding of branchial Zn but no obvious changes in GIT Zn uptake. They concluded that the gill and not the gut played the dominant role in maintaining zinc homeostasis. Our Cu results, although obtained only for the gill, suggest that the same may be true for this second essential metal. However, our results for Cd suggest that the opposite may be true for this non-essential metal, which appears to be more critically controlled at the gut rather than the gills.

Acknowledgements

The authors thank the NSERC-MITHE (Metals in the Human Environment) research network for their support. CMW is supported by the Canada Research Chair Program. We thank Natalie Carreau, Josée Charland, Fathima Iftikar, Colleen Inglis, Karthik Nadella, Patrick Ryan, and Jeremy St. Onge for help with the collection of benthic invertebrates and yellow perch. We thank Linda Diao for her sampling and technical help and Dr. Dave Vadnais for his ion and nutrient analysis of the invertebrate samples. Furthermore, we would like to thank Dr. John Gunn (Laurentian University) for the use of his laboratory space while sampling invertebrates from Hannah L. We thank Dr. Peter Macdonald for his help with the statistical analysis of the data. We also thank Dr. Peter Chapman, Sara Klinck, and the reviewers of this manuscript who gave helpful and insightful comments.

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