Branchial versus intestinal zinc uptake in wild yellow perch (*Perca flavescens*) from reference and metal-contaminated aquatic ecosystems

Soumya Niyogi, Gregory G. Pyle, and Chris M. Wood

Abstract: Zinc is an essential micronutrient for freshwater fish but can be toxic to them at elevated concentrations. Therefore, the regulation of zinc uptake is important in maintaining homeostasis when fish are chronically exposed to elevated zinc in nature. This study examined the kinetics of in vivo branchial and in vitro intestinal zinc uptake in wild yellow perch (*Perca flavescens*) from metal-contaminated and reference lakes in northern Ontario. The results showed that the branchial zinc uptake involves high-affinity transport sites, whereas the intestinal zinc uptake involves low-affinity transport sites. Interestingly, significant alterations in the branchial zinc uptake (reduced affinity, increased maximum transport rate) but no apparent changes in the intestinal zinc uptake characteristics were observed in the metal-impacted yellow perch population relative to the reference population. Subsequently, no differences in zinc concentrations of gill, liver, and whole body were recorded between reference and metal-impacted yellow perch populations. Overall, our study indicated that the gill, not the gut, likely plays a critical role in maintaining the zinc homeostasis in wild fish under chronic exposure.

Résumé : Le zinc est un micro-nutriment essentiel aux poissons d'eau douce, mais il peut devenir toxique aux concentrations élevées. C'est pourquoi la régulation de l'absorption du zinc est importante pour le maintien de l'homéostasie chez les poissons qui sont exposés de manière chronique à de fortes concentrations de zinc en nature. Notre étude examine la cinétique de l'absorption du zinc in vivo dans les branchies et in vitro dans l'intestin chez des perchaudes (*Perca flavescens*) sauvages du nord de l'Ontario provenant de lacs contaminés par le zinc et de lacs témoins. Nos résultats montrent que l'absorption du zinc dans les branchies se fait par des sites de transport de haute affinité, alors que l'absorption intestinale du zinc se fait par des sites de transport de faible affinité. Curieusement, nous observons chez la population de perchaudes affectées par le zinc, mais non chez la population témoin, des modifications importantes de l'absorption du zinc par les branchies (affinité réduite, accroissement du taux maximum de transport), mais aucun changement apparent dans les caractéristiques d'absorption intestinale du zinc. Par la suite, il n'y a pas de différences dans les concentrations de zinc des branchies, du foie et du corps entier chez la population témoin et la population de perchaudes affectées par le zinc. Dans son ensemble, notre étude montre que ce sont les branchies, et non le tube digestif, qui jouent un rôle critique dans le maintien de l'homéostasie du zinc chez les poissons sauvages dans des conditions d'exposition chronique.

[Traduit par la Rédaction]

Introduction

Zinc concentrations in freshwater fish, at both organismal and cellular levels, are very well regulated since its abundance can be toxic to fish. Excess zinc is excreted via the bile, gut sloughing (Handy 1996), or the gills (Hardy et al. 1987). Although it has been reported that excretion is the main route by which freshwater fish maintain zinc homeostasis (Shears and Fletcher 1983; Hardy et al. 1987), they are also known to regulate zinc accumulation.

Freshwater fish take up zinc from both water and food. It is widely believed that the gut is usually the primary route of absorption because most zinc is sequestered within sediments, while zinc in the water column is primarily particulate, adsorbed to dissolved organic and inorganic compounds (Florence et al. 1992; Rozan et al. 2000). However, it has been found that under laboratory-induced conditions of low dietary and (or) high waterborne zinc, the gill becomes increasingly important (Spry et al. 1988). The waterborne and dietary zinc uptake mechanisms in freshwater fish have been investigated in several laboratory-based studies using rainbow trout (*Oncorhynchus mykiss*) as a model species. Zinc uptake from water occurs primarily through the Ca^{2+} uptake pathway in gills (Hogstrand et al. 1994, 1996, 1998). In con-

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trast, the pathway of intestinal zinc uptake is yet to be fully characterized. Glover and Hogstrand (2002*a*) have demonstrated that intestinal zinc uptake in freshwater rainbow trout is saturable, indicating a carrier-mediated uptake pathway. The kinetic properties of the intestinal zinc uptake are quite different from those of the zinc uptake pathway in the gill. The gill appears to have a high affinity for zinc uptake, whereas the gut has a low affinity for the same (Glover and Hogstrand 2002*a*). Interestingly, it has been suggested that the mechanisms of intestinal zinc uptake are pharmacologically very different from that of gills in freshwater fish, the former being linked to potassium efflux (Glover et al. 2004). Moreover, Glover and Hogstrand (2002*b*) and Glover et al. (2003) have suggested that zinc could also be co-transported with histidine across the apical membrane of the rainbow trout intestine.

Previous studies on teleost fish kept under controlled laboratory conditions have demonstrated that both gills and gut play important roles in regulating zinc absorption. Hogstrand et al. (1994, 1995) have shown that rainbow trout, when chronically exposed to sublethal, waterborne zinc concentrations, down-regulate waterborne zinc uptake by significantly reducing the affinity of the common branchial Ca^{2+} and Zn^{2+} uptake pathway. Similarly, it has been reported that the proportion of zinc absorption in the gut decreases as the zinc load in the diet increases (Shears and Fletcher 1983; Hardy et al. 1987; Glover and Hogstrand 2002*a*), indicating the possibility of a mechanism for regulation of dietary zinc uptake in fish. However, whether this down-regulation of dietary zinc uptake is achieved by modulating the kinetic properties of the intestinal zinc uptake pathway has not been investigated to date. Furthermore, these findings have never been validated in feral fish naturally exposed to elevated zinc concentration in both the diet and water.

The present study was designed to investigate the branchial and intestinal zinc uptake processes in wild freshwater fish collected from different metal-contaminated and reference lakes in and around Sudbury region of northern Ontario, Canada. The teleost chosen for this study was yellow perch (*Perca flavescens*), the most abundant endemic fish species of these aquatic ecosystems (Pyle et al. 2005). This percid is known to be quite tolerant to metals (Taylor et al. 2003; Niyogi et al. 2004), and it thrives in these soft, acidic, and metal-contaminated lakes of northern Ontario, where more sensitive standard laboratory species, such as rainbow trout and fathead minnows (*Pimephales promelas*), rarely inhabit (Pyle et al. 2005). The two main objectives of this study were (*i*) to characterize the kinetic properties of branchial and intestinal zinc uptake pathways in wild, metal-contaminated and reference yellow perch populations and (*ii*) to understand whether the branchial and (or) the intestinal zinc uptake pathways carry out the important regulatory function in fish living in zinc-contaminated natural environment.

Materials and methods

Field sites and sampling

The present study was conducted in two separate stages. The first stage was conducted during the summer of 2003 and followed by the second during the summer of 2004. This stage involved the characterization of branchial zinc uptake in reference and metal-contaminated yellow perch populations. It included three different lakes in and around the Sudbury region of northern Ontario: Geneva (46°45′N, 81°33′W), Hannah (46°26′N, 81°02′W), and Whitson (46°28′N, 80°58′W). Geneva Lake is located at a distance of more than 100 km northwest of the Sudbury Township. This lake was chosen as a reference lake, since it has not been contaminated by mining and smelting operations in Sudbury (Niyogi et al. 2004). Hannah and Whitson lakes are situated within a range of 20 km of the Sudbury smelters and are highly metal-contaminated (Pyle et al. 2005).

The second stage of this study involved the characterization of intestinal zinc uptake in reference and metal-contaminated yellow perch populations. It included two different lakes: Nosbonsing Lake (46°12′N, 79°13′W) as a reference site and Hannah Lake as a contaminated site. Nosbonsing Lake is located in the North Bay area of northern Ontario and is not contaminated with metals (Gauthier et al. 2006). We decided to work with a different reference lake for this part of the study because we could not go back to Geneva Lake owing to unavoidable logistical problems. Furthermore, despite repeated attempts, we were unable to catch any fish in Whitson Lake this time around.

Wild yellow perch (5–15 g, approximate age: 1–3 years) were captured live from all the lakes either by using a seine net and (or) by angling. Approximately 40 fish were collected per lake on each sampling day and returned to the laboratory live at McMaster University, Hamilton, in ambient water in a 40 L Rubbermaid™ container with a portable aerator unit. On arrival, fish were allowed to settle down at a stable temperature of 14–15 °C overnight in their respective lake waters before being used for experiments.

Surface water samples were collected with acid-washed 250 mL polyethylene bottles without any headspace from each study lake and transported to the laboratory at Nipissing University, North Bay. The pH of the water was measured by a standard pH meter (Fisher Scientific, Canada) immediately after arriving at the laboratory. Subsequently, the water samples were filtered through 0.45 µm nylon filters (Whatman Inc., USA), acidified to pH 2 with trace-metal-grade HNO_3, and stored at 4 °C until further analysis.

Characterization of branchial zinc uptake and new zinc accumulation in the gills

The whole-body zinc uptake rates via gills and newly accumulated zinc concentrations in the gills of reference and metal-contaminated fish were characterized using a 3 h in vivo assay as described by Alsop and Wood (2000). Twenty-eight fish collected from each lake were transferred into four polyethylene tanks (seven per tank) containing 15 L of reconstituted water ((0.25 mmol·L^{-1} $CaCl_2·2H_2O$, 0.25 mmol·L^{-1} $MgCl_2·6H_2O$, and 0.07 mmol·L^{-1} NaCl, dissolved in deionized (Nanopure, Barnstead) water, pH adjusted to 7.05 by adding from a stock solution of 1 mol·L^{-1} $KHCO_3$). The composition of the reconstituted water was formulated to approximately reflect the water chemistry conditions of the chosen lakes; water hardness and pH among these lakes range between 15 and 40 mg·L^{-1} as $CaCO_3$ and between 6.8 and 7.3, respectively (Gauthier et al. 2006). Each experimental tank was spiked with $ZnSO_4·7H_2O$ and

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then with 3 μCi·L^{-1} (1 Ci = 37 Gbq) of ^{65}Zn (as $ZnCl_2$, Oak Ridge National Laboratory, USA; specific activity = 1.97 mCi·mg^{-1}) to achieve approximate total waterborne zinc concentrations of 2, 4, 8 and 16 μmol·L^{-1}. These concentrations were chosen based on pilot experiments and previous trout studies (Hogstrand et al. 1994, 1996, 1998) so as to cover the saturable kinetic range of branchial zinc uptake. All tanks were placed in a water bath to maintain a constant temperature (14 ± 1 °C).

Duplicate water samples (10 mL) were taken 10 min after the spiking of ^{65}Zn into the water (time 0) and also at the end of 3 h exposure. At the latter, fish were anesthetized by transferring them to a bath containing 250 mg·L^{-1} buffered tricaine methanesulfonate (MS-222). Fish were then rinsed in a solution of ethylenediaminetetraacetic acid (EDTA; 1 mmol·L^{-1}, pH 7.1) for 1 min to displace loosely bound ^{65}Zn and blotted dry. Gills were excised and rinsed in EDTA solution again, and both gills and the remaining carcass were weighed separately. Gills, carcass, and one set of water samples were measured for ^{65}Zn activity in a gamma counter (MINAXI Gamma 5000 Series, Canberra Packard, USA). The other set of water samples were acidified to 1% HNO_3 and analyzed for total zinc concentrations using a certified standard for zinc (Fisher Scientific, Canada) on a flame atomic absorption spectrophotometer (220 FS, Varian, Australia). The analytical accuracy of the zinc analysis was verified by analyzing method blanks (acidified, reconstituted assay water without any spiked zinc), and quality control samples of known zinc concentration were obtained from the National Laboratory for Environmental Testing (NLET, Environment Canada, Burlington, Ontario). The blanks were below the detection limit for zinc at all times, and the measured zinc concentrations of the quality control samples were in good agreement with the known values. The rates of whole body zinc influx (J_{in}) across branchial epithelium (nmol·(kg wet weight)$^{-1}$·h^{-1}) at different waterborne zinc concentrations were determined by the following equation:

$$J_{in} = a[(bc^{-1})t]^{-1}$$

where a is the ^{65}Zn counts in fish (total counts·min^{-1}·(kg wet tissue)$^{-1}$, gill and carcass combined), b is ^{65}Zn counts in water (counts·min^{-1}·L^{-1}), c is the total zinc concentration in the water (μmol·L^{-1}), and t is duration of the exposure (h).

Subsequently, gill ^{65}Zn concentrations were converted to newly accumulated zinc concentrations using the following equation:

$$M_{new} = d(bc^{-1})^{-1}$$

where M_{new} is the newly accumulated zinc in the gills (nmol·(g wet weight)$^{-1}$), d is the ^{65}Zn counts·min^{-1}·(g wet gill tissue)$^{-1}$, b is ^{65}Zn counts in water (counts·min^{-1}·L^{-1}), and c is the total zinc concentration in the water (μmol·L^{-1}).

Characterization of intestinal zinc uptake

Intestinal zinc uptake rates in reference and metal-contaminated fish were characterized using a 2 h in vitro gut sac technique, as described by Nadella et al. (2006). It is important to note here that in vivo measurement of intestinal zinc uptake using a cannulation technique in live fish (e.g., Clearwater et al. 2000; Glover and Hogstrand, 2002*a*, 2002*b*) was not possible in this case because the sizes of the captured fish were quite small (5–15 g). Fish were killed by an overdose of MS-222 (250 mg·L^{-1}), and the entire gastrointestinal tract were dissected out and flushed with saline to remove food and faeces. Subsequently, intestinal sacs were prepared using the entire intestinal tract. The intestine was separated from the stomach just prior to the pyloric sphincter and anterior to the rectum. Each separated gut was fitted with a short length of heat-flared PE-tubing (PE-50) tied in place at the anterior end with double silk ligatures and was sealed at the posterior end with double silk ligatures. The catheter was used to fill and drain the gut sac.

The sacs were filled with 1–2.5 mL (depending on the size of the sac) of appropriate mucosal saline (modified Cortland saline (all in mmol·L^{-1}): NaCl 133, KCl 5, $CaCl_2 \cdot 2H_2O$ 1, $MgSO_4 \cdot 7H_2O$ 1.9, $NaHCO_3$ 1.9, $NaH_2PO_4 \cdot H_2O$ 2.9, glucose 5.5; pH 7.4; Wolf 1963) spiked with a mixture of cold zinc (as $ZnSO_4 \cdot 7H_2O$) and 0.05 μCi·mL^{-1} radioactive zinc (as $ZnCl_2$, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA; specific activity = 1.97 mCi·mg^{-1}) to achieve total zinc concentrations of 50, 100, 200 and 400 μmol·L^{-1}. This concentration range was chosen based on pilot experiments and previous studies (Glover and Hogstrand 2002*a*) so as to cover the saturable kinetic range of intestinal zinc uptake. Following filling, the PE tubing was sealed, the sac was blotted dry, and the preparation was weighed to the nearest 0.01 mg. Each sac preparation was placed in a fixed volume (12 mL) of the modified Cortland saline serving as the serosal bath and constantly aerated with a 99.7% O_2 and 0.3% CO_2 (i.e., P_{CO_2} = 2.25 torr; 1 torr = 133.3224 Pa) gas mixture. Temperature was maintained at 15 ± 2 °C. Seven gut sacs were exposed for 2 h at each zinc concentration mentioned before. The 2 h duration was selected based pilot experiments and observations from similar experiments done with copper in rainbow trout, which indicated that the metal and fluid uptake rates remain constant for up to 4 h in piscine gut sac preparations (Nadella et al. 2006).

Samples of serosal and mucosal saline were collected at the start and end of the flux period and counted for ^{65}Zn activity as described before. At the end of the flux period, each sac preparation was removed from the flux chamber, blotted dry, reweighed, and drained completely; all of these steps were completed within approximately 2–3 min. Subsequently, each sac was cut open by a longitudinal incision and washed in saline and EDTA (1 mmol·L^{-1}, pH 7.9). The washing was done to remove any loosely bound ^{65}Zn. The sac preparation was then blotted dry and gently scraped to remove mucus and epithelial cells using a glass slide. The gut tissue was spread out on a graph paper, and its gross surface area was determined by tracing its outline (Grosell and Jensen 1999). The tissue, serosal samples, wash solutions, and epithelial scrapings were counted separately for ^{65}Zn activity as noted earlier. The wash solutions represented loosely bound ^{65}Zn, while epithelial scrapings represented a combination of mucus and surface cells; these two sections accounted for only about 27% of net combined ^{65}Zn activity counted in all samples (data not shown). The gut tissue layer (approximately 51%) and the serosal sample (approximately 22%) collected at the end of flux period accounted for the bulk of ^{65}Zn activity and represented a conservative estimate of true intestinal zinc uptake. The rate of intestinal uptake

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(J_{in}) of zinc (nmol·(cm tissue)$^{-2}$·h^{-1}) was calculated by the following equation:

$$J_{in} = \mathrm{CPM}(\mathrm{SA}\cdot\mathrm{ISA}\cdot t)^{-1}$$

where CPM represents the total ^{65}Zn activity of the tissue plus serosal compartment, SA is the initial measured specific activity of zinc in the mucosal saline (^{65}Zn counts per litre divided by total zinc (µmol·L^{-1})), ISA is the intestinal surface area (cm^2), and t is time (h).

In addition, net fluid transport rate (FTR) was determined from the change in total mass of the sac preparation over the flux period, which provided a gravimetric measure of changes in fluid content. This was normalized by taking into account the gross surface area of the exposed epithelium and time elapsed. FTR (µL·(cm tissue)$^{-2}$·h^{-1})) from mucosal to serosal compartments was determined as follows:

$$\mathrm{FTR} = [(\mathrm{IW} - \mathrm{TW})\mathrm{ISA}^{-1}]t^{-1}$$

where IW and TW are the initial and terminal weights (mg) of the sac preparation, respectively.

Zinc analysis in fish tissue and lake water

A subset of captured fish (seven per lake) was sacrificed as described before, and the gills, liver, and the remaining carcass were dissected out. All tissues were digested separately in five volumes of 1 mol·L^{-1} HNO_3 for 48 h at 60 °C. Total zinc concentrations were measured in digested samples after appropriate dilution with 1% HNO_3 (diluted in deionized (Nanopure, Barnstead) water) by flame atomic absorption spectroscopy as described earlier using a certified zinc standard (Fisher Scientific, Canada). The efficiency of the digestion method for zinc recovery was monitored using a National Research Council certified reference material (TORT-1) and method blanks of only 1% HNO_3. An average recovery rate of 96% ± 5% was recorded for zinc, and zinc was undetectable in blanks. Whole-body total zinc concentrations were calculated by dividing the sum of zinc contents (concentrations multiplied by weight) of all the tissues by the sum of weights of all the tissues.

Similarly, the filtered water samples collected during sampling in each lake were analyzed for dissolved zinc concentrations using the certified standard. Again, the quality assurance was maintained by analyzing the certified reference materials from NLET and method blanks as described previously. The free ionic concentration of zinc (Zn^{2+}) in the surface water of each lake was estimated using the geochemical speciation modeling program, MINEQL+ (version 4.5 for Windows, Environmental Research Software, Hallowell, Maine). For modeling input, the measured, dissolved zinc concentration and pH of the surface water were used, and the data of other required parameters (e.g., calcium, magnesium, alkalinity, and dissolved organic carbon (DOC)) for each selected lake were taken from Gauthier et al. (2006) and Niyogi et al. (2004). A conditional stability constant (log K) of 7.9 and a binding site density of 0.5 µmol·(mg DOC)$^{-1}$ were incorporated into the MINEQL+ files for modeling zinc–DOC complexation (Playle 2004).

Kinetic and statistical analyses

The kinetic properties of branchial and intestinal zinc uptake (maximum uptake rate (J_{max}) and substrate concentration at which uptake rate is half of J_{max} (K_m)) as well as the affinity (log K) and capacity (B_{max}) of zinc-binding in gills were calculated via nonlinear regression analyses with a hyperbolic curve fit (single rectangular two parameters: $y = ax(x + b)^{-1}$; Sigma Plot Version 8.0, Systat Software Inc., Point Richmond, California, USA) to fit the parameters to the Michaelis–Menten equation. The assumptions in the regression analyses (i.e., normality of residuals and homogeneity of variances) were tested using Shapiro–Wilk's test for normality and Levene's test for homogeneity of variance (both at $\alpha = 0.05$), respectively. All of the data met these assumptions. Significance of regressions and parameter estimates (J_{max} and K_m; log K and B_{max}) between reference and metal-contaminated populations were evaluated using a standard Student's t test ($p < 0.05$). Intestinal FTRs at different zinc concentrations within each yellow perch population as well as gill, liver, and whole-body zinc concentrations among different yellow perch populations were analyzed for statistical significance by employing a one-way analysis of variance followed by Bonferroni multiple comparison tests ($p < 0.05$). Again, the data were checked for normality and homogeneity of variances using Shapiro–Wilk's test and Levene's test, respectively (both at $\alpha = 0.05$). All the data met the assumptions.

Results

In vivo branchial zinc uptake and new zinc accumulation in the gills

The whole-body zinc uptake across gills exhibited saturation at a waterborne zinc concentration range of 0–20 µmol·L^{-1} in both reference (Geneva) and metal-contaminated (Hannah and Whitson) yellow perch populations. However, the rate of uptake was distinctly higher in fish from metal-contaminated lakes relative to those from reference lake, particularly at higher waterborne zinc concentrations, with Whitson fish exhibiting the maximum (Fig. 1*a*). The kinetic characterization of branchial zinc uptake showed significant increases of both J_{max} and K_m (the inverse of affinity) in metal-contaminated populations relative to the reference population (Table 1*a*). The J_{max} increased by about 57% in Hannah Lake and 88% in Whitson Lake relative to Geneva Lake perch. In contrast, approximately 48% and 36% increases of K_m (i.e., decreases in affinity) were observed in Hannah and Whitson lakes, respectively, relative to the Geneva Lake population.

The pattern of newly accumulated zinc concentrations in the gills of different perch populations was quite similar to that of whole-body zinc uptake from water — considerably higher in metal-contaminated populations relative reference population (Fig. 1*b*). The kinetic analyses of the data provided affinity (log K) values of 5.5, 5.1, and 5.2 and capacity (B_{max}) values of 11.2, 18.2, and 22.4 nmol·(g wet tissue)$^{-1}$ for gill–zinc binding in Geneva, Hannah, and Whitson perch populations, respectively. The log K and B_{max} values of gill–zinc binding in both contaminated populations (Hannah and Whitson lakes) were significantly different from the reference population (Geneva Lake).

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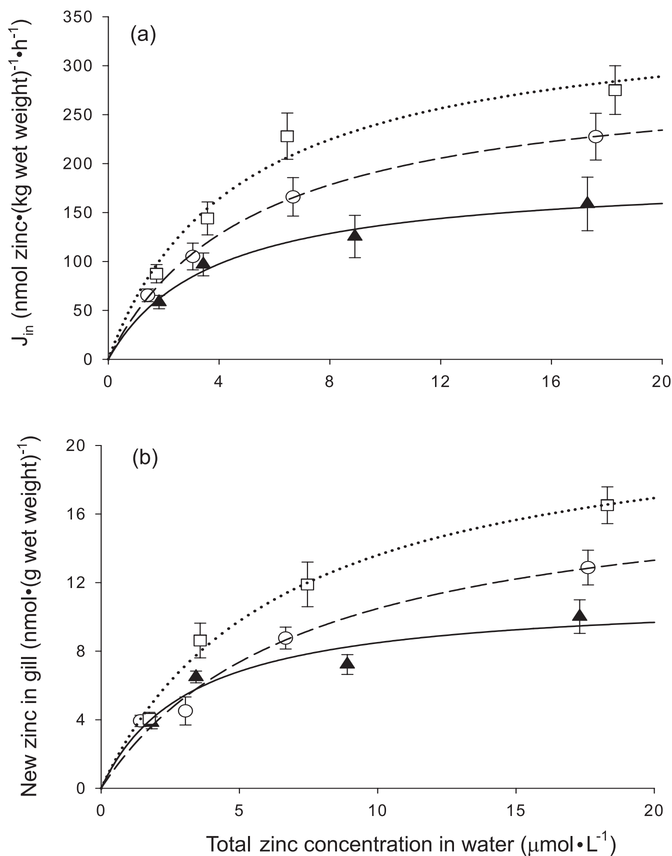
Fig. 1. In vivo (*a*) branchial zinc uptake rate and (*b*) new zinc accumulation in the gills at various waterborne zinc concentrations in different wild yellow perch (*Perca flavescens*) populations. Data are expressed as mean ± standard error of the mean (n = 7). The relationship of each regression line may be defined by the Michaelis–Menten equation: $f = ax(x + b)^{-1}$, where f is zinc transport rate (1*a*) or new gill zinc (1*b*); $a = J_{max}$ (1*a*) or B_{max} (1*b*); $b = K_m$; and x is total zinc concentration in water. The regressions of both Hannah and Whitson (contaminated) lake yellow perch populations were significantly different from that of the Geneva (reference) Lake population ($p < 0.05$). Geneva Lake, closed triangles; Hannah Lake, open circles; Whitson Lake, open squares.



In vitro intestinal zinc uptake

FTR remained constant during in vitro intestinal zinc uptake experiments in reference (Nosbonsing Lake), as well as metal-contaminated (Hannah Lake) yellow perch populations, as no significant differences were observed at any tested zinc concentrations in either population (Figs. 2*a* and 2*b*). As noticed in the gills, zinc uptake was saturable in the intestine in both reference and metal-contaminated populations (Fig. 3), although saturation occurred at a much higher range of substrate (luminal zinc) concentrations (up to 400 μmol·L^{-1}). However, no differences in the rates of intestinal zinc uptake were noted between the reference and metal-contaminated populations. Moreover, the kinetic characterization of intestinal zinc uptake revealed no significant difference in either J_{max} or K_m between the two populations (Table 1*b*).

Water quality and zinc accumulation in fish

The pH of surface waters from sampled lakes varied within the range of 6.8 to 8.1. Geneva and Whitson lakes were slightly acidic, whereas Nosbonsing and Hannah lakes were slightly alkaline (Table 2). The dissolved zinc concentrations in water were two- to three-fold higher in metal-contaminated lakes, with Whitson having the highest, followed by Hannah, relative to both reference lakes (Geneva and Nosbonsing) (Table 2). The estimation of free zinc (Zn^{2+}) concentrations in surface waters of sampled lakes by MINEQL+ revealed a similar profile to dissolved zinc concentrations; however, the difference increased by more than 10-fold when metal-contaminated lakes were compared with Nosbonsing Lake (reference) (Table 2). Interestingly, no significant differences in zinc concentrations were recorded in either gills, liver, or whole body among any reference and metal-contaminated yellow perch populations (Table 2).

Discussion

Contamination by various essential and nonessential metals (e.g., copper, cadmium, nickel, and zinc) in several lakes in the Sudbury region of northern Ontario is quite well documented (Rajotte and Couture 2002; Pyle et al. 2005; Gauthier et al. 2006). The two selected metal-contaminated lakes for this study (Hannah and Whitson) have two- to three-fold greater dissolved zinc and many-fold greater free zinc (Zn^{2+}) concentrations in their surface water relative to the reference lakes (Geneva and Nosbonsing), indicating the chronic history of zinc exposure to the resident fish populations.

To the best of our knowledge, the present study is the first to characterize the kinetics of short-term (2–3 h) branchial and intestinal zinc uptake in yellow perch. We have shown that in vivo branchial zinc uptake in wild yellow perch is saturable, indicating a carrier-mediated uptake as demonstrated previously in laboratory-reared rainbow trout by several other workers (Hogstrand et al. 1994, 1996, 1998). This is consistent with evidence indicating that the uptake of zinc from water occurs primarily through the apical Ca^{2+} channels in chloride cells of gills (Hogstrand et al. 1995, 1996, 1998). Hogstrand et al. (1995) reported J_{max} values of 240–410 nmol·kg^{-1}·h^{-1} and K_m values of 3.6–7.8 μmol·L^{-1} for branchial zinc uptake in control and zinc-exposed (2.3 μmol·L^{-1} zinc in moderately hard water — 120 mg·L^{-1} as $CaCO_3$) juvenile rainbow trout under laboratory conditions. The J_{max} values for branchial zinc uptake in our study varied between 189 and 355 nmol·kg^{-1}·h^{-1} among reference and metal-contaminated wild yellow perch populations, which is quite comparable to the findings of Hogstrand et al. (1995). Similarly, we recorded a variation of K_m values between 3.6 and 5.3 μmol·L^{-1} among different wild yellow perch populations, thereby suggesting that the affinities (inverse of K_m) of the branchial zinc uptake pathways are quite similar between these two fish species.

Previous laboratory studies demonstrated that freshwater fish acclimate to chronic waterborne zinc exposure mainly by co-regulating the kinetic properties of Ca^{2+}–Zn^{2+} transporters in the gills. The K_m of Ca^{2+} uptake decreases with very little change in J_{max} (Hogstrand et al. 1994, 1995, 1998), whereas both J_{max} and K_m of branchial zinc uptake increase significantly (Hogstrand et al. 1998; Alsop and

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Table 1. Maximum uptake rate (J_{max}) and substrate concentration at which uptake rate is half of J_{max} (K_m) of branchial (*a*) and intestinal (*b*) zinc uptake in different wild yellow perch (*Perca flavescens*) populations.

Lake	J_{max}	K_m ($\mu mol \cdot L^{-1}$)
(*a*) Branchial zinc uptake.		
Lake	J_{max} (nmol·(kg wet tissue)$^{-1}$·h^{-1})	K_m ($\mu mol \cdot L^{-1}$)
Geneva (reference)	189.1±14.2	3.6±0.3
Hannah (contaminated)	296.1±17.1*	5.3±0.3*
Whitson (contaminated)	355.5±39.6*	4.9±0.6*
(*b*) Intestinal zinc uptake.		
Lake	J_{max} (nmol·(cm tissue)$^{-2}$·h^{-1})	K_m ($\mu mol \cdot L^{-1}$)
Nosbonsing (reference)	0.9±0.2	264.5±95.2
Hannah (contaminated)	1.1±0.2	374.9±105.2

Note: Data are presented as parameter value ± standard error of the mean (n = 28). Significant differences ($p < 0.05$) in J_{max} and K_m between reference and contaminated populations are indicated by an asterisk.

Fig. 2. The fluid transport rate (FTR) from mucosal to serosal compartments during in vitro intestinal zinc uptake at various mucosal zinc concentrations in (*a*) Nosbonsing (reference) and (*b*) Hannah (contaminated) lake yellow perch (*Perca flavescens*) populations. Data are expressed as mean ± standard error of the mean (n = 7). No significant differences were observed among the treatments in both populations ($p < 0.05$).

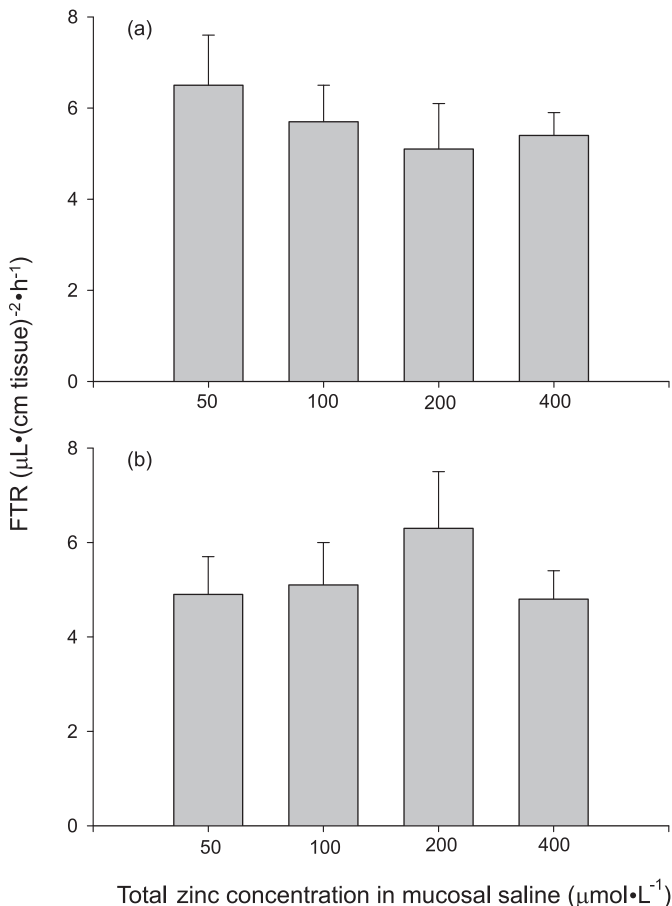
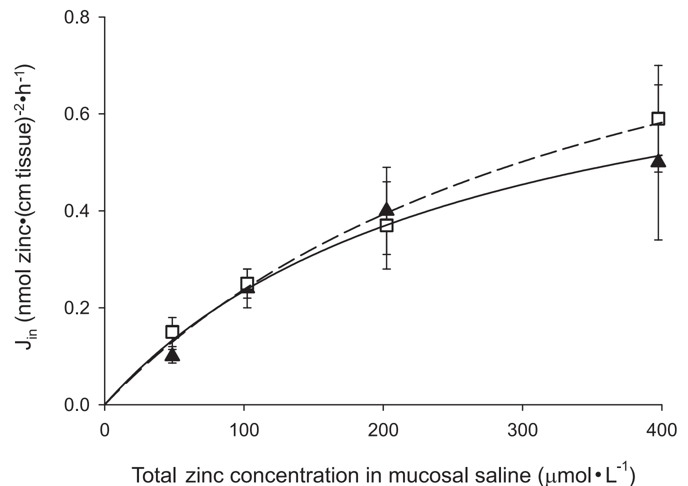


Fig. 3. In vitro intestinal zinc uptake rate at various mucosal zinc concentrations in different wild yellow perch (*Perca flavescens*) populations. Data are expressed as mean ± standard error of the mean (n = 7). The relationship of each regression line may be defined by the Michaelis–Menten equation: $f = ax(x + b)^{-1}$, where f is zinc transport rate; $a = J_{max}$; $b = K_m$; and x is total zinc concentration in mucosal saline. The regressions of Hannah (contaminated; open squares) and Nosbonsing (reference; closed triangles) lake yellow perch populations were not significantly different ($p < 0.05$).



Wood 2000). Interestingly, we also observed significantly higher J_{max} and K_m values in metal-contaminated wild yellow perch populations (Hannah and Whitson lakes) relative to a reference population (Geneva Lake). Therefore, overall our findings in relation to branchial zinc uptake in wild fish are remarkably similar to the findings of previous laboratory studies, even though the chronic zinc exposure levels (~0.1–0.2 $\mu mol \cdot L^{-1}$) were much lower than in the above-referenced laboratory studies (~2 $\mu mol \cdot L^{-1}$). However, it is also possible that these modulations in branchial zinc uptake in wild perch are induced, at least partially, by waterborne cadmium as well, since in addition to elevated zinc, both Hannah and Whitson lakes have approximately threefold higher cadmium levels in their surface waters relative to Geneva Lake (Niyogi et al. 2004). Cadmium, like zinc, shares the common Ca^{2+} uptake pathway in the fish gill (Verbost et al. 1987, 1989; Niyogi and Wood 2004*a*), and therefore fish can potentially modulate the Ca^{2+} transport pathway to down-regulate both waterborne Cd^{2+} and Zn^{2+} uptake.

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Table 2. Fish size, water quality, and gill, liver, and whole-body zinc concentrations in different wild yellow perch (*Perca flavescens*) populations.

Lake	Fish size (g) (n = 35)	Water pH (n = 1)	Measured dissolved zinc in water ($\mu mol \cdot L^{-1}$) (n = 1)	Modeled free zinc (Zn^{2+}) in water ($\mu mol \cdot L^{-1}$) (n = 1)	Gill zinc ($\mu mol \cdot (g\ wet\ weight)^{-1}$) ($n$ = 7)	Liver zinc ($\mu mol \cdot (g\ wet\ weight)^{-1}$) ($n$ = 7)	Whole-body zinc ($\mu mol \cdot (g\ wet\ weight)^{-1}$) ($n$ = 7)
Geneva	6.32±1.05	6.81	0.07	0.037	0.48±0.17	0.41±0.12	0.35±0.08
Nosbonsing	12.11±3.17	8.08	0.08	0.007	0.44±0.09	0.34±0.11	0.31±0.07
Hannah	6.22±0.52	7.35	0.18	0.096	0.59±0.19	0.53±0.17	0.39±0.11
Whitson	5.64±0.47	6.86	0.24	0.128	0.62±0.13	0.57±0.15	0.38±0.09

Note: Data for fish size, tissue, and whole-body zinc concentration are presented as mean ± standard error of the mean.

Consistent with the kinetic profile of whole-body zinc uptake via gills, the gill–zinc binding properties (affinity (log K) and capacity (B_{max})) also changed significantly in metal-impacted yellow perch populations (Hannah and Whitson lakes) relative to reference population (Geneva Lake). The affinity decreased by 2- to 2.5-fold in metal-contaminated fish, whereas the capacity increased by 1.5- to 2-fold. Alsop and Wood (2000) reported a log K value of 5.6 for gill–zinc binding in laboratory-reared rainbow trout, which is quite similar to the log K value (5.5) in our reference perch populations. Moreover, they also reported decreased affinity and increased capacity of gill–zinc binding in trout chronically exposed to waterborne zinc (3.8 $\mu mol \cdot L^{-1}$ zinc in moderately hard water — 120 $mg \cdot L^{-1}$ as $CaCO_3$) in a laboratory, as observed in our study. These findings have two important implications for the biotic ligand model (BLM) approach, which uses short-term metal–gill binding characteristics to predict lethal gill accumulation of metals (e.g., zinc) and thereby toxicity in fish (Niyogi and Wood 2004*b*). First, the current versions of fish zinc BLM (Santore et al. 2002; De Schamphelaere and Janssen 2004), developed essentially by using data from research with rainbow trout, may be extended to other fish species, since the affinity constant of gill–zinc binding appears to be quite similar between these two disparate species. Secondly, the zinc–gill binding properties are changeable and not fixed, contrary to the assumption of the current framework of BLM approach. Thus, it indicates that the chronic history of the resident fish populations needs to be incorporated into the current BLM framework to make more accurate predictions of acute toxicity.

As in the gills, in vitro zinc uptake in the intestine of wild reference and metal-impacted yellow perch was saturable, again indicating a carrier-mediated uptake. Glover and Hogstrand (2002*a*) also observed saturable intestinal zinc uptake under a similar luminal zinc concentration range during in vivo experimentation with laboratory-reared rainbow trout. Whether this saturable process detected for intestinal zinc uptake is governed by a single transporter is a debatable issue, since zinc may be transported by more than one transport system in the piscine intestine (see Bury et al. 2003 for review). Nevertheless, Glover and Hogstrand (2002*a*) reported a K_m of 309 $\mu mol \cdot L^{-1}$ in rainbow trout intestine in vivo, which is quite consistent to our results (265–375 $\mu mol \cdot L^{-1}$) in wild yellow perch, again indicating the similarity in the affinity of intestinal zinc uptake pathway in two disparate fish species. Moreover, the K_m values of intestinal zinc uptake were about two orders of magnitude higher than the corresponding values of branchial zinc uptake in wild yellow perch populations, a phenomenon reported previously in laboratory-reared rainbow trout (Hogstrand et al. 1995; Glover and Hogstrand 2002*a*). We derived the J_{max} of intestinal zinc uptake per unit intestinal surface area (cm^2), whereas Glover and Hogstrand (2002*a*) evaluated it per unit body weight (kg). Interestingly, the conversion of our data, based on the average intestinal surface area (data not shown) to body weight ratio, yielded J_{max} values of approximately 1960 and 1660 $nmol \cdot kg^{-1} \cdot h^{-1}$ for whole-body zinc uptake via intestine in Nosbonsing and Hannah populations, respectively. Again, these values are quite comparable to the reported J_{max} of intestinal zinc uptake in laboratory-reared rainbow trout (933 $nmol \cdot kg^{-1} \cdot h^{-1}$ by Glover and Hogstrand

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(2002*a*)). Therefore, our results further corroborate the notion that intestinal zinc transport occurs through a high-capacity and low-affinity transport pathway in comparison with that in gills.

The high capacity and low affinity of intestinal zinc uptake process should not be surprising, since zinc is much more concentrated in the food relative to the water column, because most of the zinc in aquatic ecosystems is sequestered within sediments, natural organic matters, and (or) biota (Florence et al. 1992; Rozan et al. 2000). Interestingly though, in contrast with the gill, no difference in the kinetic properties of intestinal zinc transport was recorded between the reference and metal-contaminated yellow perch populations. This is also consistent with the findings of Spry et al. (1988), as they reported that zinc uptake from the water and the diet in freshwater fish are largely independent of each other even when the zinc concentration in the diet is much higher than the normal requirement.

One of the primary objectives of this study was to explore the probable mechanisms by which wild freshwater yellow perch regulate zinc uptake while living in a metal-contaminated environment. The fish from both contaminated lakes (Whitson and Hannah) did not show any increase of zinc concentrations in target organs or in whole body relative to reference lakes despite the fact that dissolved zinc levels in water were two- to three-fold higher in contaminated lakes. This suggests that zinc is very well regulated in these metal-contaminated populations. The present results provide evidence only for the gill, and not for the intestine, as a site of this regulation. Specifically, the gill contributes to the homeostatic zinc regulation by reducing the affinity of waterborne zinc transport pathway and thereby down-regulating waterborne zinc uptake in a zinc-contaminated ecosystem. However, it is instructive to look in detail at the consequences of the gill adjustment, because J_{max} values also increased. Using the Michaelis–Menten equation and applying the kinetic constants of Table 1*a* together with the measured, dissolved, waterborne zinc concentrations in the respective lakes, branchial zinc uptake rates of 3.6 (Geneva), 9.72 (Hannah), and 16.6 $nmol \cdot kg^{-1} \cdot h^{-1}$ (Whitson) are predicted, proportionately quite similar to the difference in respective waterborne zinc concentrations. Thus the gill adaptation does not provide protection at the background concentrations in the contaminated lakes, but it is likely that these uptake rates are still in the nutritive rather than the toxic range and are supplemented by intestinal uptake. However if waterborne zinc concentration were to surge to a much higher level (e.g., 2 $\mu mol \cdot L^{-1}$), then the gill adaptation would provide effective protection, yielding branchial zinc uptake rates of 81.1 (Hannah) and 103.0 $nmol \cdot kg^{-1} \cdot h^{-1}$ (Whitson) in comparison with a similar rate of 67.5 $nmol \cdot kg^{-1} \cdot h^{-1}$ in the perch of the reference lake (Geneva). Thus, the real importance of the gill adaptation may be to deal with the much higher and potentially toxic metal levels that accompany rainstorm and snowmelt events in contaminated watersheds (Spry et al. 1981; Rodushkin et al. 2004).

With respect to possible intestinal adaptation, Glover and Hogstrand (2002*a*) reported increased secretion and sloughing of mucus that led to sequestration and removal of excess zinc from the lumen at acutely elevated luminal zinc concentrations in rainbow trout during in vivo experimentation. They argued that this process is an important mechanism by which freshwater fish can down-regulate the intestinal transport of zinc at elevated dietary zinc levels. However, we could not verify the occurrence of such a mechanism in wild, metal-impacted yellow perch populations in our in vitro gut sac preparations. Clearly, in future studies it would be useful to know the metal levels in the diet and in the chyme of perch from reference and metal-contaminated lakes, so as to be able to predict intestinal zinc uptake rates in the various populations. However, it is generally recognized that freshwater fish are much less sensitive to zinc in the diet than in the water, since reports of toxicity arising from increased dietary zinc exposure are absent even at a concentration as high as 26 $\mu mol \cdot g^{-1}$ in the food (i.e., 26 000 $\mu mol \cdot L^{-1}$) in rainbow trout (Wekell et al. 1983). The main reason why fish are sensitive to waterborne zinc is that Zn^{2+} in water directly competes with Ca^{2+} for the high-affinity uptake sites in the gill. As a consequence, it causes a disruption of branchial Ca^{2+} uptake leading to hypocalcemia, a serious physiological condition that may have lethal implications (Hogstrand et al. 1996, 1998). Therefore, it is conceivable that fish living in the zinc-contaminated lakes are mainly susceptible to zinc in water rather than the diet and at critical times are required to regulate uptake from water so as to maintain ionic homeostasis and survival.

In summary, our study further strengthens the notion that zinc transport in intestine occurs through a low-affinity transport pathway relative to that in gills in freshwater fish. More importantly, it also suggests that the gill, not the intestine, plays a vital role in regulating zinc uptake and homeostasis in feral fish exposed to chronic elevations of zinc throughout their lives.

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