# Acute cadmium biotic ligand model characteristics of laboratory-reared and wild yellow perch (*Perca flavescens*) relative to rainbow trout (*Oncorhynchus mykiss*)

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**Abstract:** This study evaluated the >400-fold tolerance to acute waterborne Cd of a metal-tolerant fish, yellow perch (YP, *Perca flavescens*), relative to a sensitive model fish, rainbow trout (RBT, *Oncorhynchus mykiss*), from the perspective of the acute Cd biotic ligand model (BLM). Three-hour gill binding characteristics for Cd and its competitor, Ca, in both species exhibited only small quantitative differences, but gill Cd accumulations at 3 h and 24 h, which were associated with 50% lethality at 96 h (3- and 24-h LA50s), were 52- to 60-fold higher in YP relative to RBT. However, the acute Cd BLM cannot be extended from RBT to YP by simple adjustments of LA50 values because unlike RBT, in YP, LA50s (3 and 24 h) were 26- to 47-fold greater than the capacity of the characterized set of Cd-binding sites. Moreover, 3-h gill Ca and Cd binding characteristics in wild YP, collected from one clean (Geneva) and two metal-contaminated softwater lakes (Hannah and Whitson) around Sudbury region, northern Ontario, revealed that chronic waterborne factors like hardness and Cd preexposure can influence both Cd and Ca binding in fish gills and could have major implications for the future refinement of the acute Cd BLM approach.

**Résumé :** Notre étude examine la tolérance à une exposition aiguë au cadmium (Cd) dans l'eau chez la perchaude (YP, *Perca flavescens*), un poisson tolérant aux métaux, tolérance plus de 400 fois plus élevée que celle de la truite arc-en-ciel (RBT, *Oncorhynchus mykiss*), un poisson modèle sensible, d'après le modèle du ligand biotique (BLM) de la toxicité aiguë du Cd. Les caractéristiques de liaison du cadmium et de son compétiteur, le calcium, au niveau de la branchie après 3 h n'affichent que de faibles différences quantitatives chez les deux espèces, mais les accumulations de cadmium après 3 h et 24 h, qui sont associées à une mortalité de 50 % après 96 h (LA50, 3 h et 24 h), sont de 52–60 fois plus élevées chez la perchaude que chez la truite. Cependant, le modèle BLM pour une exposition aiguë au cadmium ne peut être adapté de la truite à la perchaude par simple ajustement des valeurs de LA50 parce que, contrairement à la truite, la perchaude affiche des valeurs de LA50 (3 h et 24 h) 26–47 fois plus élevées que la capacité de la série caractérisée de sites de liaison du cadmium. De plus, les caractéristiques de liaison du calcium et du cadmium après 3 h dans la branchie de perchaudes sauvages, provenant d'un lac propre (Geneva) et de deux lacs contaminés (Hannah et Whitson), tous trois de faible dureté, de la région de Sudbury dans le nord de l'Ontario, démontrent que des facteurs aqueux chroniques, comme la dureté et l'exposition préalable au cadmium, peuvent affecter la liaison tant du cadmium que du calcium dans les branchies de poissons et peuvent avoir des conséquences majeures pour l'amélioration de la méthode BLM pour l'étude de l'exposition aiguë au Cd.

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## Introduction

The family Percidae is believed to be highly tolerant to many metals compared with the family Salmonidae, which is quite sensitive. For example, Percidae are reported to be 2–4 orders of magnitude more tolerant than Salmonidae to waterborne Cu (Taylor et al. 2003). However, virtually no information is available regarding differences in tolerance to waterborne Cd, a nonessential metal. Toxicity of Cd is generally attributed to the free divalent cation $Cd^{2+}$ (Pagenkopf 1983). Waterborne Cd appears to enter mainly through chloride cells in the gills (Wicklund-Glynn et al. 1994) and accu-

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mulates primarily in kidney, liver, and gills of fish (Hollis et al. 1999, 2000*a*, 2000*b*). At acute waterborne concentrations, Cd severely disrupts Ca homeostasis, which ultimately leads to death (Verbost et al. 1987; Reid and McDonald 1988). At the gill surface, Cd competes with Ca for high-affinity Ca-binding sites (Playle et al. 1993*a*, 1993*b*; Playle 1998) and once it enters inside the chloride cell blocks Ca uptake at the basolateral membrane through the inhibition of $Ca^{2+}$-ATPase (Verbost et al. 1987, 1989). The cumulative effect of these two processes causes acute hypocalcaemia in fish (Roch and Maly 1979; Verbost et al. 1987, 1989).

Among various water quality parameters (hardness, pH, alkalinity, and dissolved organic matter (DOM))that could potentially influence the Cd uptake, hardness is often reported to be the major factor influencing Cd toxicity (Calamari et al. 1980; Hollis et al. 1997, 2000*b*). As water hardness (defined as the sum of Ca and Mg concentrations, expressed as $CaCO_3$ equivalents) increases, acute Cd toxicity decreases (Carrol et al. 1979; Pascoe et al. 1986). This is thought to be due to the fact that the major hardness cation, Ca, outcompetes Cd for binding sites on the gills and thereby reduces toxicity (Spry and Wiener 1991). Moreover, the bioavailability and toxicity of Cd are less affected by DOM than are the bioavailability and toxicity of Cu (Giesy et al. 1977; Winner 1984; Block and Pärt 1986) because Cd binds about 10-fold less well to DOM than does Cu (Alberts and Giesy 1983; Morel and Herring 1993), but Cd binds about 16-fold more strongly at fish gills than Cu (Playle et al. 1993*b*). Calcium is, therefore, the most important modifier of Cd toxicity in fish rather than water pH or DOM, both of which play important roles in modifying the toxicity of other metals like Cu (Playle et al. 1993*a*; Erickson et al. 1996; MacRae et al. 1999).

The development of the "biotic ligand model" (BLM) in recent years has attempted to better account for the bioavailability and toxicity of metals to aquatic life (DiToro et al. 2001; De Schamphelaere and Janssen 2002; Paquin et al. 2002). The BLM, which quantifies the affinity and capacity of the gills (biotic ligand) of fish to bind metals and relates this binding to acute toxicity, has been proposed as a method for modeling metal toxicity in the aquatic environment based on site-specific water quality parameters. The acute Cu BLM (Santore et al. 2001) has already been approved by the US Environmental Protection Agency in 2000, provisional acute BLMs for Ag (Paquin et al. 1999; McGeer et al. 2000) and Zn (Santore et al. 2002) have been published recently, and an acute Cd BLM is now under active development through industrial – US Environmental Protection Agency consortia. However, only a few fish species (mainly rainbow trout (RBT) (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*)) have been given emphasis so far for the development of BLM. There is a need to incorporate more species from different fish families, especially those present in metal-impacted environments, to widen the potential for its application.

Our study is mainly focussed on elucidating mechanistic differences in tolerance between yellow perch (YP) (*Perca flavescens*) (a resistant percid endemic to metal-impacted lakes of northern Ontario and Quebec) and a sensitive model species, RBT (a salmonid) from the perspective of the BLM. We compared the following aspects between the two species: (*i*) acute Cd toxicity (96-h LC50s (the concentrations of Cd lethal to 50% of the fish at 96 h)) in moderately hard water (120 mg $CaCO_3 \cdot L^{-1}$) to elucidate Cd sensitivity, (*ii*) the critical gill Cd burden at 3 and 24 h, which is associated with 50% lethality at 96 h (3- and 24-h LA50s), and (*iii*) short-term (3-h) gill Cd and gill Ca binding characteristics (binding affinity (log $K$) and binding capacity ($B_{max}$)). YP were selected for this study because of their abundance and endemic nature to metal-contaminated areas like Sudbury, northern Ontario, and Rouyn-Noranda, northern Quebec (two major mining and smelting locations in Canada) (Sherwood et al. 2000; Couture and Kumar 2003). In addition, we also evaluated the gill Cd and gill Ca binding characteristics in wild YP populations collected from one clean (Geneva) and two metal-contaminated lakes (Hannah and Whitson) around the Sudbury region to test in the natural environments the applicability of the gill binding constants (log $K$ and $B_{max}$) developed in YP populations reared in moderately hard water at our laboratory.

## Materials and methods

### Studies with laboratory-reared fish

Juvenile RBT (8–12 g) and YP (8–12 g) were obtained from Humber Springs Fish Hatchery (Orangeville, Ont.) and Kinmount Fish Farm (Kinmount, Ont.), respectively. Fish were maintained at least for 1 month in 500-L polyethylene tanks supplied at 2 $L \cdot min^{-1}$ with dechlorinated Hamilton tap water of moderate hardness (12–14 °C, pH 8.0, 0.6 mmol $Na^+ \cdot L^{-1}$, 0.7 mmol $Cl^- \cdot L^{-1}$, 1.0 mmol $Ca^{2+} \cdot L^{-1}$, 0.15 mmol $Mg^{2+} \cdot L^{-1}$, 120 mg hardness$\cdot L^{-1}$ as $CaCO_3$, 95 mg alkalinity$\cdot L^{-1}$ as $CaCO_3$, 3 mg DOM$\cdot L^{-1}$) before the experiments. Fish were fed once a day at 2% of their body weight with commercial trout food (granulated hatchery feed; Corey Feed Mills Ltd., Fredericton, N.B.) and organic debris was siphoned out daily. No fish mortality was observed during this period.

#### *Acute toxicity and 24-h gill LA50 of Cd*

The 96-h LC50s of Cd together with 24-h gill Cd burdens were determined in moderately hard water for both species. Fish ($n$ = 10 per concentration) were exposed in duplicate (two sets per concentrations) in a flow-through system to five different Cd concentrations (YP: 16 548 ± 1067, 26 868 ± 801, 41 814 ± 887, 72 953 ± 2135, and 113 879 ± 3203 nmol$\cdot L^{-1}$, respectively; RBT: 51 ± 1, 136 ± 3, 237 ± 10, 394 ± 11, and 531 ± 7 nmol$\cdot L^{-1}$, respectively; $n$ = 9 for all values) in addition to control conditions (background Cd concentration 0.98 ± 0.2 nmol$\cdot L^{-1}$, $n$ = 9) at a temperature of 14 °C. Cadmium stocks ($Cd(NO_3)_2 \cdot 4H_2O$; Fisher Scientific, Canada) were supplied from Marriott bottles into diluent water in mixing head tanks. These tanks then fed 20-L polythene tanks at a rate of approximately 200 mL$\cdot min^{-1}$ each. The duplicate tanks (one for 96-h LC50 and the other for 24-h gill Cd burdens) for each concentration were interconnected so as to have the same water qualities. All tanks were allowed to equilibrate for 12 h before adding fish, individually covered, aerated, and checked for fish mortality every 6 h. No mortality was observed in the first 24 h for both fish species, and all 10 live fish were sampled from one of the tanks in each set of duplicate exposures.

The fish were killed individually by a blow to the head and entire branchial baskets were dissected out. Each branchial basket was rinsed separately for 20 s in "Nanopure" deionized water (Sybron Barnstead, Boston, Mass.) to remove excess blood and loosely adhered metal and blotted dry. Then, each basket was digested in five volumes of 1 mol·l$^{-1}$ $HNO_3$ (trace metal grade; Fisher Scientific, Canada) for 48 h at 60 °C and analyzed for Cd after appropriate dilution (e.g., 10×, 50×, 100×, etc.) with 1% $HNO_3$ using a graphite furnace atomic absorption spectrophotometer (GFAAS) (model GTA 110; Varian Australia) and a certified Cd standard for GFAAS (Fisher Scientific, Canada). The other set of exposures was continued for 96 h and dead fish, whenever found, were removed immediately and the total number of dead fish at the end of 96 h in each tank was recorded. Water samples (5 mL) were taken after every 12 h, acidified with 50 µL of concentrated trace metal grade $HNO_3$, and analyzed for total Cd by GFAAS as described above. The 96-h LC50 values (±95% confidence intervals) were determined by log probit analysis using mean measured waterborne Cd concentrations. Lethal gill Cd accumulation at 24 h associated with 50% mortality at 96 h (24-h LA50) was determined by a regression analysis of 96-h mortality logit versus the 24-h log gill Cd concentrations (MacRae et al. 1999) where logit = $\log[M(1 - M)^{-1}]$ and $M$ is mortality proportion.

### *Three-hour lethal gill Cd accumulation (3-h LA50)*

Once the 96-h LC50 values were known for the two species, a simpler approach was used to determine the 3-h LA50 values. Ten individuals each of YP and RBT were exposed statically for 3 h in 20-L polyethylene tanks containing 10 L of aerated dechlorinated Hamilton tap water at 14 °C to 72 420 and 169 nmol waterborne Cd·L$^{-1}$ (as $Cd(NO_3)_2{\cdot}4H_2O$ (Fisher Scientific, Canada)), respectively (i.e., at the corresponding 96-h LC50 concentrations for each species), radiolabeled with 3 µCi $^{109}Cd{\cdot}L^{-1}$ (as $CdCl_2$, specific activity = 3.45 mCi·mg$^{-1}$, 1 Ci = 37 GBq) (Perkin-Elmer, USA)). After 3 h, fish were sacrificed with a blow to the head. Gill arches were dissected out, rinsed for 20 s in Nanopure deionized water, blotted dry, and placed in 20-mL polyethylene vials. Water samples (5 mL) were taken in duplicate at the beginning and end of the 3-h static exposure and acidified with 50 µL of concentrated trace metal grade $HNO_3$. Both water and gills were counted for radioactivity using a gamma counter (Packard Minaxi Auto-Gamma 5000 series). The appearance of radioactivity in the gills allowed for the calculation of "new Cd" in the gills (i.e., with no background). Water samples were analyzed for total Cd as described earlier. Gill $^{109}Cd$ concentrations were converted to absolute values (new Cd) using the measured specific activity ($bc^{-1}$) of the water:

$$a(bc^{-1})^{-1}$$

where $a$ is $^{109}Cd$ counts in the gills (counts per minute per gram wet tissue weight), $b$ is $^{109}Cd$ counts in water (counts per minute per litre), and $c$ is the total Cd concentration in the water (micrograms per litre).

### *Short-term (3-h) gill Cd binding characteristics*

To determine interspecific differences in the short-term nature of Cd binding in gills, we characterized the high-affinity gill Cd-binding sites using the 3-h radiolabeled $^{109}Cd$ technique (Hollis et al. 1999). Twenty-eight fish (of each species) were transferred into four clear plastic bags (seven per bag) containing 3 L of aerated synthetic water of moderate hardness (1 mmol $Ca^{2+}{\cdot}L^{-1}$ added as $Ca(NO_3)_2{\cdot}4H_2O$, 0.6 mmol $Na^{+}{\cdot}L^{-1}$, and 0.6 mmol $Cl^{-}{\cdot}L^{-1}$ added as NaCl plus 1.9 mmol $KHCO_3{\cdot}L^{-1}$ (all from Fisher Scientific, Canada) to achieve a pH of 8.0) and placed in a water bath to maintain stable temperature (14 °C). The concentrations of major ions ($Ca^{2+}$, $Na^{+}$, and $Cl^{-}$), alkalinity, and pH in the synthetic water were kept similar to dechlorinated Hamilton tap water in which fish were held before experiments. Each experimental polyethylene bag was spiked with $Cd(NO_3)_2{\cdot}4H_2O$ (Fisher Scientific, Canada) and then with 3 µC $^{109}Cd{\cdot}L^{-1}$ (as $CdCl_2$, specific activity = 3.45 mCi·mg$^{-1}$ (Perkin-Elmer, USA)) before fish were transferred to achieve approximate total Cd concentrations of 45, 89, 223, and 446 nmol·L$^{-1}$ respectively.

Water samples (5 mL) were taken in duplicate at the beginning and end of the 3-h static exposure and acidified with 50 µL of concentrated trace metal grade $HNO_3$. At the end of the exposure, fish were sampled and killed by a blow to the head. Gills were dissected out, rinsed in Nanopure water for 20 s, blotted dry, and placed in 20-mL polyethylene vials. Both water and gills were counted for radioactivity using a gamma counter (Packard Minaxi Auto-Gamma 5000 series). Water samples were further analyzed for total Cd by GFAAS as described before and for total Ca and Na by flame atomic absorption spectrophotometer (FAAS, Varian Australia, Model 220FS). Gill $^{109}Cd$ concentrations were converted to absolute values (new Cd) following the method described earlier.

The affinity (log $K_{Cd}$) and capacity ($B_{max}$) of Cd-binding sites in the gills of both species were determined by the construction of Langmuir plots of Cd adsorption isotherms (Richards and Playle 1998). Free Cd ($Cd^{2+}$) concentrations were divided by new Cd concentrations in the gills and plotted against free $Cd^{2+}$. Free $Cd^{2+}$ concentrations were estimated using the known water chemistry and the MINEQL+ aquatic chemistry program (Schecher and McAvoy 2001). The inverse of the slopes and log of the inverse of the intercepts of the respective regression lines provided the $B_{max}$ and log $K_{Cd}$, respectively, for each species.

### *Short-term (3-h) gill Ca binding characteristics*

Interspecific differences in the nature of short-term Ca binding in gills were also characterized using the same methodology as described above. Four polyethylene bags, each equipped with an airline, were filled with 3 L of synthetic Ca-free water (0.6 mmol $Na^{+}{\cdot}L^{-1}$ and 0.6 mmol $Cl^{-}{\cdot}L^{-1}$ added as NaCl, $KHCO_3$ = 1.9 mmol·L$^{-1}$, pH 8.0) and placed in a water bath (14 °C). Five minutes before the introduction of fish (28 of each species, seven per bag), each bag was spiked first with $Ca(NO_3)_2{\cdot}4H_2O$ (Fisher Scientific, Canada) and then with 7 µCi $^{45}Ca{\cdot}L^{-1}$ (as $CaCl_2$, specific activity = 12.26 mCi·mg$^{-1}$ (Perkin-Elmer, USA) to achieve approximate total Ca concentrations of 100, 250, 500, and 1000 µmol·L$^{-1}$, respectively. After 3 h of exposure, the fish were sacrificed as described before and gills were dissected and rinsed in Nanopure water for 20 s, blotted dry, and stored at –20 °C. Water samples (5 mL) were taken in duplicate at the beginning as well as at the end of the exposure. One of each duplicate water sample was diluted in 10 mL of

scintillation fluor (ACS, Amersham) and counted for $^{45}Ca$ in a beta counter (LKB Wallac 1217 Rackbeta liquid scintillation counter). The remaining water samples were analyzed for total Ca and Na, respectively, as described earlier.

Frozen gills were later transferred to liquid nitrogen and ground to fine powder with a mortar and pestle. Powdered gills (0.2 g) were placed in glass scintillation vials and were digested in 2 mL of liquid tissue solubilizer (NCS, Amersham) for 48 h at 50 °C. Samples were then neutralized with 20 μL of glacial acetic acid, diluted in 10 mL of scintillation fluor (OCS, Amersham), and counted for $^{45}Ca$ on the scintillation counter. Counting efficiencies were determined by internal standardization, i.e., by addition/recovery tests of known amounts of $^{45}Ca$ to individual samples. New Ca in the gill and the affinity (log $K_{Ca}$) and capacity ($B_{max}$) of gill Ca binding in both species were determined as described earlier in the case of gill Cd binding. Free $Ca^{2+}$ concentrations were estimated using the known water chemistry and the MINEQL+ aquatic chemistry program (Schecher and McAvoy 2001).

## Studies with field-collected YP

Fish were sampled during July 2002 in three lakes around the Sudbury region of northern Ontario, Geneva, Hannah, and Whitson lakes. Geneva Lake (46°45′N, 81°33′W), situated at a distance of more than 100 km from Sudbury's smelters, has not been affected by mining and smelting activities in Sudbury and is a clean or uncontaminated lake. In contrast, both Hannah Lake (46°26′N, 81°02′W) and Whitson Lake (46°28′N, 80°58′W) are located within 20 km of Sudbury's smelters and represent highly metal-contaminated lakes (Couture and Kumar 2003).

### *Fish and water sampling*

Wild YP were captured live using seine net and minnow traps baited with styrofoam beads and placed in littoral zones in study lakes. Approximately 70–80 YP were sampled from each lake and returned to the laboratory live at Laurentian University in Sudbury in 40-L Rubbermaid containers with a portable aerator unit. On arrival, fish were allowed to settle down at a stable temperature of 14–15 °C in their respective lake water for 2 h before being used for experiments.

Water samples (150 mL) were collected with 2-L Van Dorn bottles from each study lake at three depths, 1, 3, and 5 m, from wherever the most fish were captured in the lake. Water pH levels were recorded immediately on site with a portable pH meter (Thermo Orion; Fisher Scientific, Canada). The pH meter was calibrated in the laboratory on each sampling day and calibration was verified using pH 4, 7, and 10 buffers (Fisher Scientific, Canada) in the field. Finally, labeled sampling containers were rinsed with lake water three times, filled to capacity (i.e., no head space), and placed on ice for transport back to the laboratory at Laurentian University. Immediately after reaching the laboratory, the water samples were acidified to pH 2 with trace metal grade $HNO_3$ for metal determination and then stored at 4 °C until analysis.

### *Short-term (3-h) gill Cd and Ca binding*

Short-term (3-h in vivo) gill Cd- and gill Ca-binding properties were evaluated in wild YP collected from three different lakes following the same methodology as described earlier for laboratory-reared YP. Synthetic water of identical water chemistry and similar range of waterborne Cd and Ca exposure concentrations, used for laboratory-reared YP, was used. The binding affinity (log $K$) and binding capacity ($B_{max}$) of both Cd and Ca binding in the gill were also determined by the construction of Langmuir plots as described earlier. Free $Cd^{2+}$ and $Ca^{2+}$ concentrations were estimated with the MINEQL+ aquatic chemistry program (Schecher and McAvoy 2001).

### *Determination of Cd burden in the gills of wild YP by inductively coupled plasma (ICP) analysis*

Approximately 10 YP from each lake were sacrificed by a blow to the head. The gills were dissected out and rinsed for 20 s in double-distilled water and blotted dry. The gills were weighed and dried at 60 °C to a constant weight (>24 h) and then microwave-digested in 3 mL of analytical-grade $HNO_3$ (Fisher Scientific, Canada) using Teflon vials. The efficacy of the digestion method for Cd recovery was monitored using National Research Council certified standards TORT-2, DORM-2, and DOLT-2 as well as method blanks of only $HNO_3$. Samples were allowed to cool overnight and then transferred to labeled, precleaned, acid-washed 50-mL Fisher brand specimen containers with 12 mL of double-distilled water. Cadmium concentrations in fish gills were determined by ICP analysis using Cd standards of known concentrations at Elliot Lake Research Field Station (Elliot Lake, Ont.).

### *Determination of metal concentrations in water via ICP analysis*

Acidified water samples stored at 4 °C were vacuum-filtered through a prebaked Whatman 934-AH glass microfibre filter to remove suspended solids and analyzed for total dissolved Cd, Ca, and Mg by ICP analysis at Testmark Laboratories Ltd. (Sudbury, Ont.). Quality assurance samples were run at the beginning of each run that included blanks and 10 and 100 $\mu g \cdot L^{-1}$ of Canadian Association of Environmental Analytical Laboratories (CAEAL) certified standards of each metal measured. All samples passed the Canadian Association of Environmental Analytical Laboratories accredited quality control standards of Testmark Laboratories Ltd.

## Statistical analyses

All data are presented as means ± SE of the mean except 96-h LC50 and 3- and 24-h LA50 data, which are expressed as means and 95% confidence intervals. Nonlinear regressions were performed using Sigma Plot 2000. 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 values were evaluated using a standard Student's $t$ test. Significance of 96-h LC50 and 3- and 24-h LA50 values between YP and RBT was also tested using a Student's $t$ test. Cadmium levels in YP gills and in water between clean and two contaminated lakes were analyzed for statistical significance by using a one-way analysis of variance followed by Bonferroni multiple comparisons tests. In the last two analyses, the data were again checked for normality and homogeneity of variances using Shapiro–Wilk's test and Levene's

test, respectively (both at $\alpha = 0.05$). Data not meeting the assumptions were adjusted with log transformation before proceeding with significance tests. The level of significance was set to $p < 0.05$ in all comparisons.

## Results

### Acute toxicity and lethal gill accumulation of Cd

When the 96-h LC50 values were compared between the two fish species, Cd was approximately 420 times more toxic to juvenile RBT than to YP in moderately hard water (Table 1). The Cd accumulation in the gill at 24 h increased with increasing waterborne Cd concentrations in both YP and RBT and approached saturation within the different ranges of waterborne concentrations used (Figs. 1*a* and 2*a*). The different scales between Figs. 1*a* and 2*a* reflect the very different sensitivities of the two species to Cd. Regression analyses of 96-h mortality logit versus the 24-h log gill concentrations yielded straight lines from which 24-h LA50 values, which are predictive of 96-h mortality, were calculated (Figs. 1*b* and 2*b*). The 24-h LA50 value of YP was almost 60 times higher than that of RBT (Table 1). Similarly, the 3-h LA50 value, measured by exposure of the two species to their respective 96-h LC50 levels of Cd, was more than 52 times greater in YP relative to RBT (Table 1).

### Short-term (3-h) gill Cd binding characteristics

In both species, the short term (3-h) binding of Cd to the gills increased with increasing waterborne Cd concentrations and reached an apparent "plateau" at waterborne Cd concentrations between 223 and 446 nmol·L$^{-1}$ (Fig. 3*a*). Approximately 91–96% of the total Cd was calculated to be present as free $Cd^{2+}$ by the MINEQL+ aquatic chemistry program. The Langmuir plots of Cd adsorption isotherms (Fig. 3*b*) provided the affinity (log $K_{Cd}$) and capacity ($B_{max}$) of Cd binding in the gills of the respective species. The log $K_{Cd}$ values were 7.34 and 7.20 and the $B_{max}$ values were 0.85 and 0.67 nmol·g wet gill tissue$^{-1}$ in RBT and YP, respectively. No significant differences were observed in either affinity or capacity of Cd binding in the gills between the two species. Interestingly, 3-h LA50 values corresponded to about 40% saturation of the short-term (3-h) gill Cd-binding sites in RBT yet more than 2600% saturation of the sites in YP (i.e., 26 times higher than $B_{max}$).

### Short-term (3-h) gill Ca binding characteristics

Like Cd, the short term (3-h) binding of Ca to the gills also increased with increasing waterborne Ca concentrations and reached an apparent "plateau" at waterborne Ca concentrations between 500 and 1000 µmol·L$^{-1}$ (Fig. 4*a*). Approximately 99% of the total Ca was calculated to be present as free $Ca^{2+}$ by the MINEQL+ aquatic chemistry program. The Langmuir plots of Ca adsorption isotherms (Fig. 4*b*) provided the affinity (log $K_{Ca}$) and capacity ($B_{max}$) of Ca binding in the gills of the respective species. The log $K_{Ca}$ values were 3.69 and 3.71 and the $B_{max}$ values were 0.53 and 0.38 µmol·g wet gill tissue$^{-1}$ in RBT and YP, respectively. Once again, no significant differences were observed in either affinity or capacity of Ca binding in the gills between the two species.

### Water quality and Cd burden in the gills of wild YP

Among the three lakes, Hannah Lake had the highest Ca and Mg concentrations and pH levels in water followed by Whitson and Geneva lakes (Table 2). However, the Cd concentration in the water of Hannah and Whitson lakes was significantly higher (>2.5-fold) relative to the water of Geneva Lake (clean) (Table 2). Similarly, the Cd burdens in the gills of YP collected from metal-contaminated Hannah and Whitson lakes were also significantly higher (more than 10-fold) than that in the gills of YP from uncontaminated Geneva Lake (Table 2). Average fish weight was highest in uncontaminated Geneva Lake followed by contaminated Hannah Lake and then Whitson Lake (Table 2).

### Short-term (3-h) gill Cd binding in wild YP

Short-term (3-h) in vivo gill Cd binding exhibited concentration-dependent increases against waterborne Cd in YP from all three lakes (Fig. 5*a*). At the lower waterborne Cd concentrations, values were similar in YP from the three lakes. However, the saturation in binding was observed only in YP collected from uncontaminated Geneva Lake, whereas the YP collected from contaminated Hannah and Whitson lakes exhibited linear increases and no saturation in binding within the concentration range of 44.6–446.0 nmol waterborne Cd·L$^{-1}$. The lack of saturation in binding did not allow the determination of Cd binding affinity (log $K_{Cd}$) and capacity ($B_{max}$) in YP from Hannah and Whitson lakes. The Langmuir plots of Cd adsorption isotherms (Fig. 5*b*) in YP from uncontaminated Geneva Lake yielded log $K_{Cd}$ and $B_{max}$ values of 7.19 and 2.01 nmol·g wet gill tissue$^{-1}$, respectively. Interestingly, the log $K_{Cd}$ values in laboratory-reared YP and Geneva Lake populations of YP were very similar, although the $B_{max}$ value was threefold higher (significant, $p < 0.05$) in the latter.

### Short-term (3-h) gill Ca binding in wild YP

In contrast with Cd binding, the short-term (3-h) in vivo gill Ca binding exhibited saturation binding within the range of 100–1000 µmol waterborne Ca·L$^{-1}$ in YP collected from all three lakes (Fig. 6*a*). The Langmuir plots of Ca adsorption isotherms (Fig. 6*b*) provided affinity (log $K_{Ca}$) values of 3.39, 3.63, and 3.28 and capacity ($B_{max}$) values of 1.59, 1.64, and 1.69 µmol·g wet tissue$^{-1}$ for Geneva, Hannah, and Whitson lakes, respectively. No significant difference was observed in either affinity or capacity among YP collected from any of the lakes. Interestingly, Ca binding in gills of YP collected from all three natural lakes exhibited reduced affinity, i.e., lower log $K$ (although not statistically significant) relative to that of laboratory-reared YP. However, the $B_{max}$ of Ca binding was more than four times greater (significant, $p < 0.05$) in all three wild populations of YP compared with that in laboratory-reared YP.

## Discussion

We addressed three questions through this study. First, did the difference in short-term Cd and Ca binding in the gills between YP and RBT reflect the difference in tolerance to acute Cd challenge? Second, were there any qualitative and (or) quantitative differences in short-term gill binding characteristics for Cd and Ca among wild YP collected from

**Table 1.** Acute toxicity (96-h LC50) and lethal gill accumulations (24- and 3-h LA50s) of Cd in YP and RBT in moderately hard dechlorinated Hamilton tap water.

| Species | 96-h LC50 (nmol·L$^{-1}$) ($n$ = 60) | 24-h LA50 (nmol·g wet tissue$^{-1}$) ($n$ = 60) | 3-h LA50 (nmol·g wet tissue$^{-1}$) ($n$ = 10) |
|---|---|---|---|
| YP | 72 420 (55 427 – 100 445) | 31.67 (24.46–42.26) | 17.81 (22.96–12.66) |
| RBT | 169 (130–2 29)* | 0.55 (0.45–0.64)* | 0.34 (0.39–0.29)* |

**Note:** Fish were acclimated to moderately hard water for a minimum of 4 weeks before experiments. Data are expressed as means (95% confidence interval in parentheses). Significant differences ($p < 0.05$) in values between two species are indicated by an asterisk.

**Fig. 1.** (*a*) Cadmium accumulation in the gills of YP after 24 h of exposure to a waterborne Cd concentration range of 0.98 (control) to 113 879 nmol·L$^{-1}$ in moderately hard water (120 mg·L$^{-1}$ as $CaCO_3$) at 14 °C. Data are presented as means ± SE of the mean ($n$ = 10) and corrected for background Cd concentration (1.51 ± 0.43 nmol·g wet tissue$^{-1}$). (*b*) Determination of lethal accumulation at 24 h (24-h LA50) yielding 50% mortality in YP at 96 h. The YP 96-h mortality logit is plotted as a function of log gill Cd concentration from Fig. 1*a* determined on live fish at 24 h. The regression equation was log[$M$/(1 – $M$)] = log gill Cd × 1.88 – 2.79 ($r^2$ = 0.83). LA50 = 31.67 nmol·g wet tissue$^{-1}$ (95% confidence interval: 24.46–42.26).

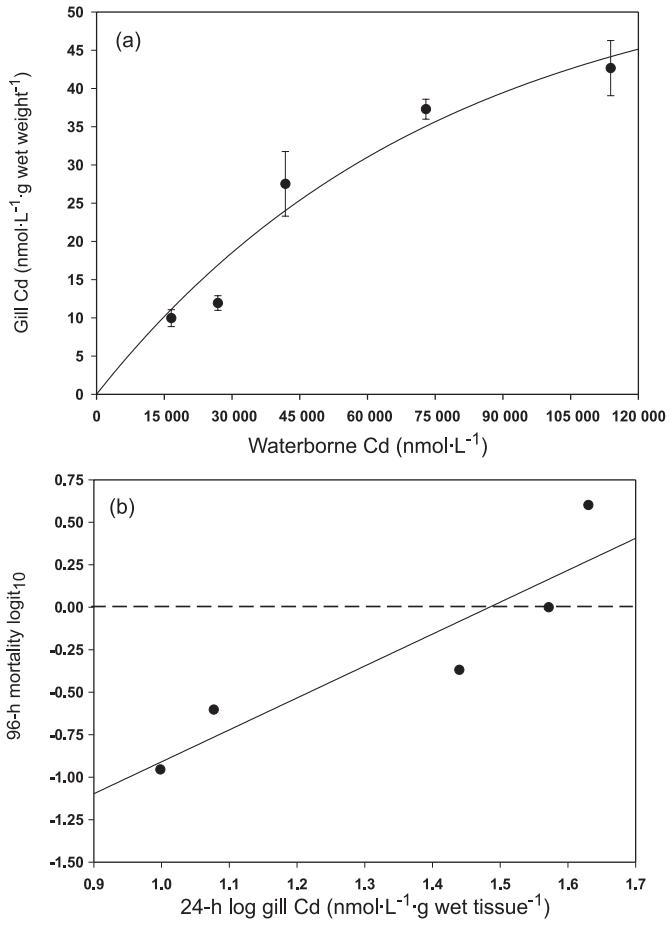
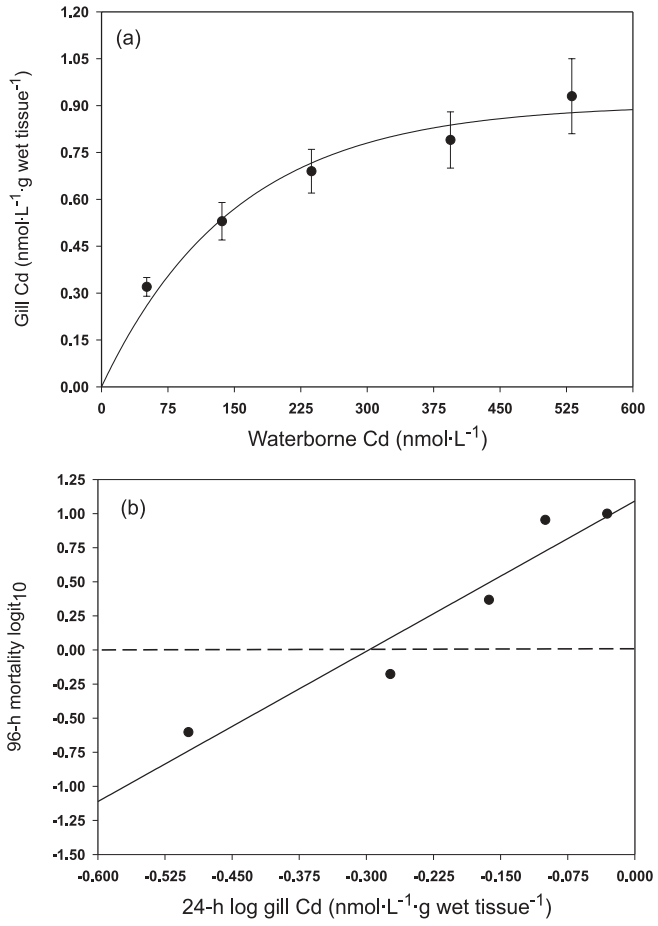



**Fig. 2.** (*a*) Cadmium accumulation in the gills of RBT after 24 h of exposure to a waterborne Cd concentration range of 0.98 (control) to 531 nmol·L$^{-1}$ in moderately hard water (120 mg·L$^{-1}$ as $caCO_3$) at 14 °C. Data are presented as means ± SE of the mean ($n$ = 10) and corrected for background Cd concentration (1.07 ± 0.34 nmol·g wet tissue$^{-1}$). (*b*) Determination of lethal accumulation at 24 h (24-h LA50) yielding 50% mortality in RBT at 96 h. The RBT 96-h mortality logit is plotted as a function of log gill Cd concentration from Fig. 2*a* determined on live fish at 24 h. The regression equation was log[$M$/(1 – $M$)] = log gill Cd × 3.67 + 1.09 ($r^2$ = 0.92). LA50 = 0.55 nmol·g wet tissue$^{-1}$ (95% confidence interval: 0.45–0.64).




metal-contaminated as well as uncontaminated natural soft-water lakes and YP reared in moderately hard water in the laboratory? Third, what are the implications of these factors for the acute Cd BLM?

The short-term 3-h in vivo Cd and Ca binding characteristics in gills of the two fish species were qualitatively similar. The quantitative differences in Cd binding in the gill between YP and RBT were small and nonsignificant and did not reflect the actual magnitude of difference in toxicity.

**Fig. 3.** (*a*) New Cd (bound Cd) in the gills of YP and RBT after 3 h of exposure to radioactive $^{109}Cd$ in synthetic water at 14 °C plotted against free $Cd^{2+}$ in the water as calculated by MINEQL+ (Schecher and McAvoy 2001). Data are expressed as means ± SE of the mean ($n$ = 7). (*b*) Langmuir plots of gill Cd binding results from Fig. 3*a* for both YP and RBT. The regression equation for YP was $y = 1.49x + 93.67$ ($r^2 = 0.98$) (log $K_{Cd} = 7.20$, $B_{max} = 0.67$ nmol·g wet tissue$^{-1}$) and the equation for RBT was $y = 1.18x + 53.37$ ($r^2 = 0.99$) (log $K_{Cd} = 7.34$, $B_{max} = 0.85$ nmol·g wet tissue$^{-1}$). Regressions are not significantly different from one another. Solid and open circles represent YP and RBT, respectively, in both panels.

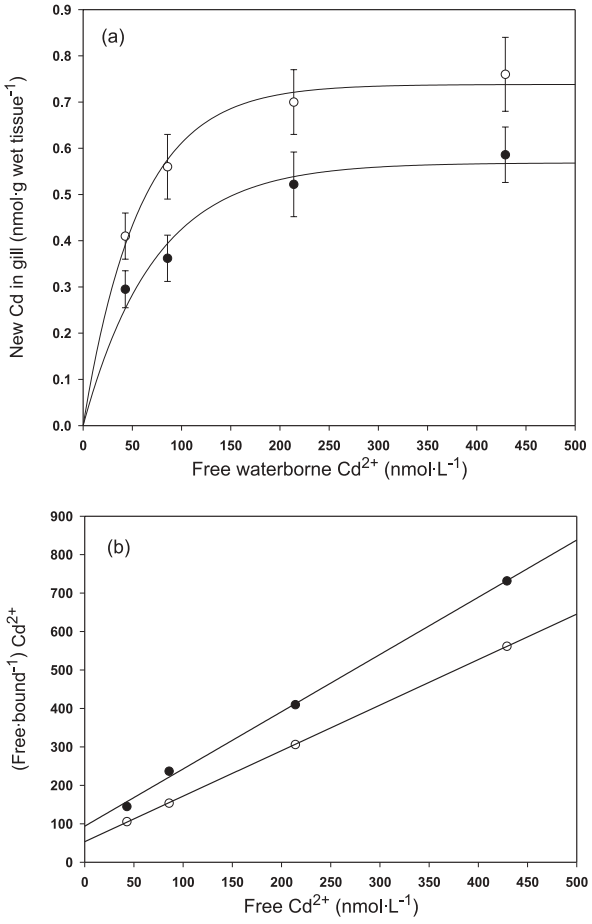
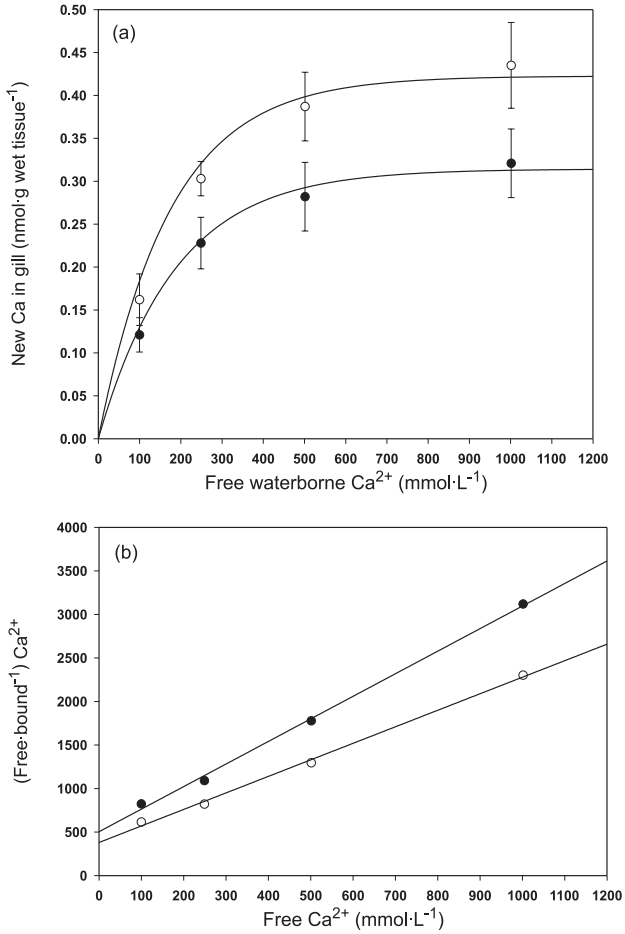



**Fig. 4.** (*a*) New Ca (bound Ca) in the gills of RBT after 3 h of exposure to radioactive $^{45}Ca$ in synthetic water at 14 °C plotted against free $Ca^{2+}$ in the water as calculated by MINEQL+ (Schecher and McAvoy 2001). Data are expressed as means ± SE of the mean ($n$ = 7). (*b*) Langmuir plots of gill Ca binding results from Fig. 4*a* for both YP and RBT. The regression equation for YP was $y = 2.59x + 503.74$ ($r^2 = 0.99$) (log $K_{Ca} = 3.71$, $B_{max} = 0.38$ μmol·g wet tissue$^{-1}$) and the equation for RBT was $y = 1.89x + 380.06$ ($r^2 = 0.98$) (log $K_{Ca} = 3.69$, $B_{max} = 0.53$ μmol·g wet tissue$^{-1}$). Regressions are not significantly different from one another. Solid and open circles represent YP and RBT, respectively, in both panels.




However, the large difference in Cd tolerance corresponded to the large differences in lethal accumulation of Cd in the gills (24-h or 3-h LA50) between the two species. The gill binding characteristics for both Cd and Ca were also qualitatively similar in nature between laboratory-reared and wild YP from the uncontaminated lake (Geneva), although there were quantitative differences in $B_{max}$ (higher capacity in wild fish). However, the gill binding characteristics for Cd, but not for Ca, in wild YP collected from the metal-contaminated lakes (Hannah and Whitson) were both qualitatively and quantitatively different relative to those in YP of Geneva Lake. Overall, these findings indicate that the present acute Cd BLM approach developed in RBT has to be modified to make it applicable to the metal-tolerant species, YP. Furthermore, these findings also demonstrate that the chronic history of fish must be taken into consideration for future refinement of the present acute Cd BLM.

## Acute toxicity and lethal accumulation (LA50)

Acute toxicity (2–7 days) or LC50 values for Cd in RBT vary considerably from study to study, but a majority of them are in the 150–850 nmol·L$^{-1}$ range (Pascoe and Beattie 1979; Davies et al. 1993; Hollis et al. 1999), with occasionally further higher values in waters with very high alkalinity and hardness (Calamari et al. 1980; Pascoe et al. 1986). The 96-h LC50 value in RBT determined in the present study (169 nmol·L$^{-1}$) was similar to the value (196 nmol·L$^{-1}$) reported earlier from our laboratory (Hollis et al. 1999) in similar water chemistry. However, the extremely high tolerance to acute Cd challenge in YP relative to RBT (96-h LC50: 72 420 (YP) and 169 nmol·L$^{-1}$ (RBT)) observed in the cur-

**Table 2.** Water quality, average fish weight, and gill Cd concentrations of YP populations from clean and metal-contaminated lakes of the Sudbury region, northern Ontario.

| Lake | Water pH ($n$ = 3) | Water Ca (mmol·L$^{-1}$) ($n$ = 3) | Water Mg (mmol·L$^{-1}$) ($n$ = 3) | Water Cd (nmol·L$^{-1}$) ($n$ = 3) | Fish weight (g) ($n$ = 70) | Gill Cd (nmol·g wet tissue$^{-1}$) ($n$ = 8) |
|---|---|---|---|---|---|---|
| Geneva | 6.61±0.05 | 0.07±0.003 | 0.03±0.001 | ND | 6.42±1.78 | 1.96±0.59 |
| Hannah | 7.39±0.14 | 0.25±0.002 | 0.14±0.003 | 2.67±0.18* | 4.08±0.82 | 20.04±3.03* |
| Whitson | 6.90±0.07 | 0.15±0.003 | 0.07±0.001 | 2.76±0.19* | 2.96±2.19 | 21.10±1.79* |

**Note:** Data are expressed as means ± SE of the mean. Significant differences ($p < 0.05$) in water Cd levels and gill Cd burden of YP between the clean lake (Geneva) and metal-contaminated lakes (Whitson and Hannah) are indicated by an asterisk. ND, not detectable; the detection limit for Cd was 0.45 nmol·L$^{-1}$.

**Fig. 5.** (*a*) New Cd (bound Cd) in the gills of YP collected from Geneva (clean) and Hannah and Whitson lakes (metal contaminated) after 3 h of exposure to radioactive $^{109}$Cd in synthetic water at 14 °C plotted against free $Cd^{2+}$ in the water as calculated by MINEQL+ (Schecher and McAvoy 2001). Data are expressed as means ± SE of the mean ($n$ = 7). Geneva, Hannah, and Whitson lakes are represented by solid circles and solid line, solid circles and thin broken line, and triangles and thick broken line, respectively. (*b*) Langmuir plots of gill Cd binding results from Fig. 5*a* for YP from Geneva Lake. The regression equation was $y = 0.50x + 32.09$ ($r^2 = 0.98$) (log $K_{Cd} = 7.19$, $B_{max} = 2.01$ nmol·g wet tissue$^{-1}$). Langmuir plots could not be constructed for YP from Hannah and Whitson lakes because of the lack of saturation.

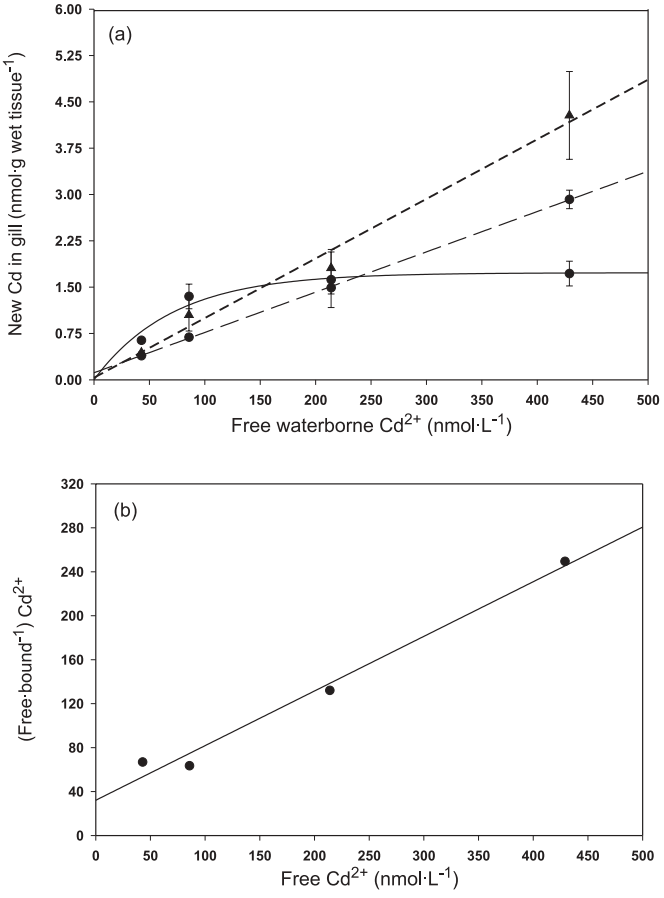
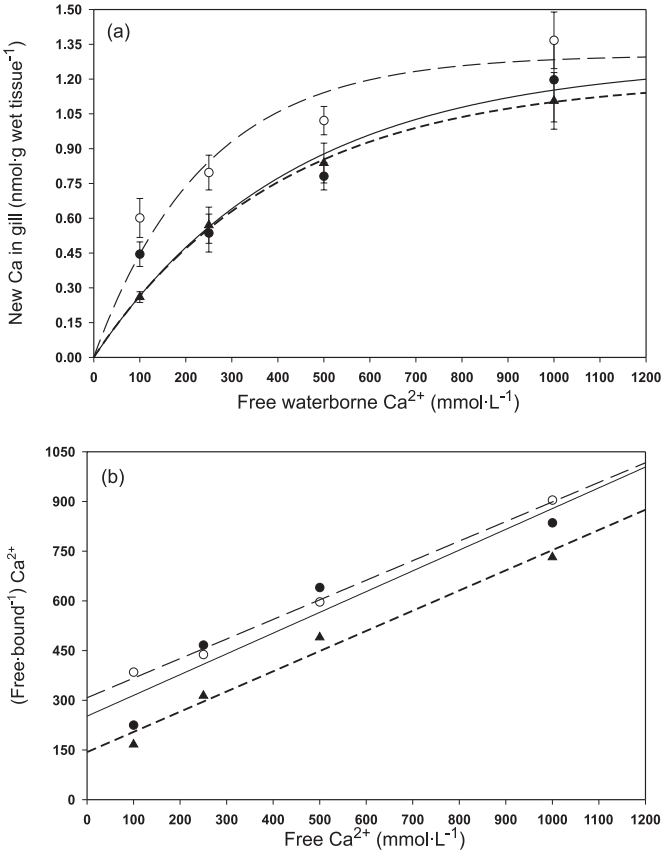



**Fig. 6.** (*a*) New Ca (bound Ca) in the gills of YP from Geneva (clean) and Hannah and Whitson lakes (metal contaminated) after 3 h of exposure to radioactive $^{45}$Ca in synthetic water at 14 °C plotted against free $Ca^{2+}$ in the water as calculated by MINEQL+ (Schecher and McAvoy 2001). Data are expressed as means ± SE of the mean ($n$ = 7). (*b*) Langmuir plots of gill Ca binding results from Fig. 6*a* for YP from three lakes. The regression equations for YP from Geneva, Hannah, and Whitson lakes were $y = 0.63x + 251.96$ ($r^2 = 0.86$) (log $K_{Ca} = 3.39$, $B_{max} = 1.59$ µmol·g wet tissue$^{-1}$), $y = 0.61x + 143.41$ ($r^2 = 0.96$) (log $K_{Ca} = 3.63$, $B_{max} = 1.64$ µmol·g wet tissue$^{-1}$, and $y = 0.59x + 307.51$ ($r^2 = 0.97$) (log $K_{Ca} = 3.28$, $B_{max} = 1.69$ µmol·g wet tissue$^{-1}$), respectively. Regressions are not significantly different from one another. Geneva, Hannah, and Whitson lakes are represented by solid circles and solid line, open circles and thin broken line, and triangles and thick broken line, respectively, in both panels.




rent study could not be compared with any other published reports, since we evaluated this fish species for the first time.

The principal objective of the BLM is the prediction of toxicity of any metal at any given water chemistry. To predict toxicity, it is necessary to determine the amount of

short-term accumulation (3 or 24 h) of metal in the gill (the biotic ligand) that is associated with 50% mortality at 96 h (3- or 24-h LA50). The reason for considering short-term gill metal accumulation for this aspect is that the short-term rapid increase of gill metal accumulation is believed to play the main role in the toxic actions of metals, as it reflects binding to physiologically active receptor sites at the gill surface that control ion homeostasis in fish (DiToro et al. 2001; Paquin et al. 2002; Niyogi and Wood 2003). The 24-h Cd LA50 values, the amount of "newly accumulated Cd" in the gill at 24 h that could be predictive of 50% mortality at 96 h, were markedly different between the two species (YP: 31.67 nmol·g wet gill tissue$^{-1}$; RBT: 0.55 nmol·g wet gill tissue$^{-1}$). The 3-h LA50 values, or the "newly accumulated Cd" in the gill at 3-h, were also significantly higher in YP than in RBT (YP: 17.80 nmol·g wet gill tissue$^{-1}$; RBT: 0.34 nmol·g wet tissue$^{-1}$) and reflected a similar magnitude of difference as at 24 h. It should be noted that both 24- and 3-h Cd LA50s for RBT occurred well within the characterized set of saturable sites at the gill ($B_{max}$ = 0.85 nmol·g wet gill tissue$^{-1}$). In contrast, both 24- and 3-h Cd LA50s in YP occurred far beyond the characterized saturable sites ($B_{max}$ = 0.67 nmol·g wet gill tissue$^{-1}$), which indicated in addition to 100% filling of high-affinity, low-capacity sites that an unknown percentage of the low-affinity, high-capacity sites were filled by Cd, thereby suggesting their role in the toxic actions of Cd in YP.

Similar but less extreme differences have been reported by Taylor et al. (2003) in relation to differences in acute Cu toxicity between the two fish species. They evaluated the 3-h Cu LA50 values for both YP and RBT by exposing the fish at their respective 96-h LC50 concentrations of Cu in hard water and reported a ninefold greater LA50 value for YP relative to RBT. This higher value correlated with a fourfold higher 96-h LC50 value for Cu in YP than in RBT in the same water chemistry. Moreover, the 3-h Cu LA50 (3.1 nmol·g wet tissue$^{-1}$) was lower than the $B_{max}$ (3.6 nmol·g wet tissue$^{-1}$) in RBT, whereas in YP, the 3-h Cu LA50 (27.8 nmol·g wet tissue$^{-1}$) was about threefold greater than the $B_{max}$ (9.0 nmol·g wet tissue$^{-1}$).

MacRae et al. (1999) and later DiToro et al. (2001) compared the 24-h Cu LA50 for brook trout (*Salvelinus fontinalis*) and RBT acclimated to artificial soft water (0.125 mmol Ca·L$^{-1}$) and proposed that one critical gill accumulation value (~10 nmol·g wet gill tissue$^{-1}$ (background corrected)) could be used to predict acute Cu toxicity irrespective of fish species and water chemistry. However, this concept appears to be ineffective in comparing Cd toxicity between YP and RBT in moderately hard water, as observed also by Taylor et al. (2003) in the case of Cu toxicity. Certainly from the perspective of developing an acute Cd BLM for YP, short-term gill binding for Cd has to be characterized at a much higher range of waterborne Cd concentrations relative to RBT to incorporate low-affinity, high-capacity Cd binding sites in the gills, which are presumably involved in acute Cd toxicity, in addition to high-affinity, low-capacity binding sites.

### Short-term (3-h) gill Cd and Ca binding in laboratory-reared YP and RBT

The affinity (log $K_{Cd}$) and capacity ($B_{max}$) of high-affinity, low-capacity Cd binding sites in the gills determined by Langmuir plots were similar in YP and RBT. Taylor et al. (2003) also reported no significant differences in log $K_{Cu}$ in hard water as well as in soft water between the same two species, although they observed significantly greater binding capacity for Cu in YP relative to RBT in both hard and soft water. The log $K_{Cd}$ value in RBT of the present study (7.34) was in reasonable agreement with those (7.60 and 7.05) reported earlier by Hollis et al. (1999) and Szebedinszky et al. (2001), respectively, in RBT in moderately hard water. However, the value in this study is much less than the value (8.60) reported by Playle et al. (1993*a*, 1993*b*) in fathead minnow, which translates to a more than 20-fold greater affinity of fathead minnow gills for Cd compared with RBT. The use of synthetic, moderately hard water (without any synthetic or natural organic ligands) in the present study produced a very high yield of free $Cd^{2+}$ (calculated by MINEQL+) and created a very simplified condition where the presence of other free ions like $Ca^{2+}$, $Na^{+}$, $H^{+}$, etc. likely exerted competitive effects, thereby reducing the apparent affinity or log $K_{Cd}$. In contrast, Playle et al. (1993*a*, 1993*b*) used synthetic soft water containing very low levels of cations as competitors to $Cd^{2+}$ for the sites at fish gills; reduced $Ca^{2+}$ competition would yield greater Cd accumulation and therefore greater log $K_{Cd}$. Moreover, the use of radioactive $^{109}$Cd in comparison with cold Cd enabled us to calculate the newly accumulated Cd in fish gills with greater resolution, separate from the background levels.

The $B_{max}$ value in RBT observed in our study (0.85 nmol·g wet gill tissue$^{-1}$) was lower than the value of 2.27 nmol·g wet gill tissue$^{-1}$ reported in fathead minnow acclimated to synthetic soft water (0.05 mmol Ca·L$^{-1}$) by Playle et al. (1993*a*, 1993*b*). It was also lower than the values of 1.61 and 3.12 nmol·g wet gill tissue$^{-1}$ reported in RBT, acclimated to the same water chemistry as that used in the present study, by Hollis et al. (1999) and Szebedinszky et al. (2001), respectively. This discrepancy in the number of gill binding sites ($B_{max}$) among studies was probably due to the size or batch differences in fish and also differences in feeding regime (Hollis et al. 2000*b*) and is certainly an issue that has to be given greater attention for further development of the present acute Cd BLM.

Short-term Ca binding properties of the gills were also qualitatively quite similar between the two species. The Ca binding affinities in gills (log $K_{Ca}$) in both species were almost identical (YP: 3.69; RBT: 3.71), while the maximum number of Ca binding sites ($B_{max}$) was lower, although not statistically significant, in YP (0.38 μmol·g wet gill tissue$^{-1}$) compared with that in RBT (0.53 μmol·g wet gill tissue$^{-1}$). The log $K_{Ca}$ values of the present study were also lower than that (5.0) proposed by Playle et al. (1993*a*, 1993*b*). They calculated the log $K_{Ca}$ value, following an indirect approach, from the competitive effect of two waterborne Ca concentrations (95 and 1050 μmol·L$^{-1}$) on the accumulation of Cd in the gill at 3 h using cold Cd and Ca. However, in the present study, the log $K_{Ca}$ value was evaluated directly from the 3-h in vivo gill Ca binding assay using radioactive $^{45}$Ca. This is certainly a method more sensitive than that used by Playle et al. (1993*a*, 1993*b*) and additionally may measure a different population of sites (i.e., not necessarily just those associated with Cd binding).

Overall, these findings suggest that similar affinity for short-term Cd and Ca binding regardless of the species is a

reflection of fundamental gill membrane characteristics when the fish are acclimated to the same water chemistry. This is not surprising, since the binding properties of the gill are probably modulated by basic physiological requirements for binding and uptake of essential osmoregulatory ions such as $Ca^{2+}$ and $Na^{+}$.

**Short-term (3-h) gill Cd and Ca binding in wild YP**

The log $K_{Cd}$ of the high-affinity, low-capacity Cd-binding sites in the gills of YP from uncontaminated Geneva Lake was 7.19, similar to that (7.20) found in our laboratory YP population acclimated to moderately hard water. However, it is also important to note here that the affinity for Cd binding in the gills of YP from Geneva Lake might be greater in the ambient water of Geneva Lake because we used synthetic water of approximately 14-fold greater waterborne Ca level for the binding assay, thus producing a greater competitive effect of Ca on Cd binding. In contrast, a threefold greater $B_{max}$ was observed in YP of Geneva Lake compared to laboratory-reared YP, which was most likely due to the extremely soft nature (low waterborne Ca) of the water in Geneva Lake. A similar increase in $B_{max}$ values from hardwater acclimation to softwater acclimation was also reported by Hollis et al. (1999, 2000*a*, 2000*b*) in RBT. They also reported a decrease in affinity (log $K_{Cd}$) in RBT acclimated to soft water; however, we did not observe any decrease in affinity in the YP population of Geneva Lake.

In contrast, the properties of the high-affinity, low-capacity Cd-binding sites of wild YP from Hannah and Whitson lakes showed large differences compared with those in laboratory-reared as well as Geneva Lake YP populations. The binding pattern changed from saturable to almost linear in fish from Hannah and Whitson lakes, preventing the determination of log $K_{Cd}$ and $B_{max}$ in those YP populations. The greater Cd concentration in the water as well as in the gills of YP from Hannah and Whitson lakes compared with those of Geneva Lake indicated that the former two YP populations are chronically preexposed to waterborne Cd. This chronic Cd exposure was likely the factor responsible for the observed changes in gill Cd binding pattern. Hollis et al. (1999, 2000*a*) also reported a similar loss of saturation in gill Cd binding in RBT upon laboratory acclimation to chronic waterborne Cd in both hard water (89 nmol $Cd{\cdot}L^{-1}$) and soft water (0.62 and 0.98 nmol $Cd{\cdot}L^{-1}$). Therefore, our findings in chronically Cd-preexposed wild YP validated the phenomenon previously observed in our laboratory with RBT. Likely, the phenomenon relates to a large loss of affinity (i.e., decreased log $K_{Cd}$), which tends to linearize the relationship, a phenomenon also seen by Szebedinszky et al. (2001) in RBT subjected to chronic dietary Cd exposure.

The Cd accumulation levels in laboratory-reared YP exposed to a waterborne Cd concentration range of 16 548 – 113 879 nmol$\cdot L^{-1}$ (i.e., concentration range for 96-h LC50 determination of YP) in moderately hard water for 24 h varied between 10 and 40 nmol·g wet $tissue^{-1}$ and were associated with 10–80% lethality at 96 h. Interestingly, the background gill Cd concentrations in YP from contaminated Hannah and Whitson lakes were 20.04 and 21.15 nmol·g wet $tissue^{-1}$, respectively (about 10-fold higher relative to that in YP from clean Geneva Lake). Hollis et al. (2000*a*) reported an approximately sixfold increase in background gill Cd concentration in RBT following 30 days of exposure to waterborne Cd concentrations of 0.62 and 0.98 nmol$\cdot L^{-1}$, which were two- to four-fold lower than the waterborne Cd concentrations of Hannah and Whitson lakes (2.67 and 2.76 nmol$\cdot L^{-1}$, respectively) in water of similar hardness (0.13 mmol $Ca{\cdot}L^{-1}$) to that of Hannah and Whitson lakes (0.25 and 0.15 mmol $Ca{\cdot}L^{-1}$, respectively). Therefore, it appears that chronic waterborne Cd exposure results in an increased Cd storage pool in the gills of fish, which probably leads to the alteration of gill Cd binding properties.

Contrary to Cd binding, there were no significant differences in the binding affinity (log $K_{Ca}$) of Ca in the gills of Cd-preexposed YP (Hannah Lake: 3.63; Whitson Lake: 3.28) as well as nonexposed YP populations (Geneva Lake: 3.39; laboratory reared: 3.71). However, about fourfold greater Ca binding capacity $B_{max}$ was observed in all of the wild YP populations (Geneva Lake: 1.60 μmol·g wet gill $tissue^{-1}$; Hannah Lake: 1.69 μmol·g wet gill $tissue^{-1}$; Whitson Lake: 1.64 μmol·g wet gill $tissue^{-1}$) relative to that (0.39 μmol·g wet gill $tissue^{-1}$) of the moderately hardwater acclimated YP population of the laboratory. This phenomenon in wild YP is likely due to the upregulation of branchial Ca uptake processes as a mechanism to survive in ion-poor soft water of the lakes. Cadmium shares the same uptake pathway with $Ca^{2+}$ in fish gills (Verbost et al. 1987) and Cd-binding sites in gills are probably part of the branchial $Ca^{2+}$ transport system (Wood 2001). Thus, the higher $B_{max}$ value for Cd in the gills of YP from Geneva Lake relative to that of laboratory-reared YP, as discussed before, was also probably indicative of an upregulation of the branchial $Ca^{2+}$ transport process. Reid et al. (1991) reported a twofold greater value of the Ca binding capacity ($B_{max}$: 0.95 μmol·g wet tissue) in softwater (hardness: 20 mg$\cdot L^{-1}$ as $CaCO_3$) acclimated RBT relative to that ($B_{max}$: 0.53 μmol·g wet $tissue^{-1}$) found in RBT acclimated to moderately hard water in this study. Therefore, it appeared that the significantly higher $B_{max}$ values in wild YP collected from extremely softwater lakes compared with hardwater-acclimated YP of our laboratory are in reasonable agreement with previous findings.

Overall, these findings suggest that acclimation to different water hardnesses has considerable effects on Cd as well as Ca binding characteristics in the gills of freshwater fish, manifested as alterations of the binding capacity ($B_{max}$) for both Cd and Ca. Furthermore, chronic preexposure to waterborne Cd may also have a major influence on the Cd binding properties of the gills and thus could have an effect on gill binding constants for Cd (log $K_{Cd}$ and $B_{max}$) as well as the acute toxicity of waterborne Cd. Hollis et al. (1999) reported an 11- to 13-fold increase in acute Cd tolerance (increased 96-h $LC_{50}$) in RBT acclimated to chronic sublethal Cd (26.7 and 88.9 nmol$\cdot L^{-1}$) in moderately hard water (1 mmol $Ca{\cdot}L^{-1}$).

Finally, although the majority of the work related to the acute BLM was carried out in soft water, our findings were in reasonable agreement with previous observations. The species difference in acute waterborne Cd toxicity was not reflected in the differences in short-term Cd and Ca gill binding characteristics (log $K$ and $B_{max}$) but rather in the differences of lethal accumulation of Cd in the gills (LA50). However, it appeared that the acute Cd BLM approach developed in RBT (involving high-affinity, low-capacity binding sites) cannot be extended to YP by simple adjustments

of LA50 values because of the probable involvement of a different set of binding sites (low-affinity, high-capacity binding sites) in the gills for the toxic actions of Cd in YP.

In the present versions of BLMs (DiToro et al. 2001; De Schamphelaere and Janssen 2002; Santore et al. 2002), the binding affinity (log $K$) and binding capacity ($B_{max}$) values used for a certain metal are fixed for a particular aquatic organism, regardless of its background or chronic history. However, the present findings demonstrated that gill Cd binding characteristics can change under the influence of chronic waterborne factors like hardness and Cd preexposure, which vary greatly from place to place in the real environment, and thus can potentially influence the acute toxicity of waterborne Cd, since metal binding to the gill has a direct bearing on toxicity. Therefore, a more integrative approach, taking these factors into account, is needed for future refinement of the acute Cd BLM for fish to make it applicable in the real environment.

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## References

Alberts, J.J., and Giesy, J.P. 1983. Conditional stability constants of trace metals and naturally occurring humic materials: application in equilibrium models and verification with field data. *In* Aquatic and terrestrial humic materials. *Edited by* R.F. Christman and E.T. Gjessing. Ann Arbor Science Publishers, Ann Arbor, Mich. pp. 333–348.

Block, M., and Pärt, P. 1986. Increased availability of cadmium to perfused rainbow trout (*Salmo gairdneri* Rich.) gills in the presence of the complexing agents diethyl dithiocarbamate, ethyl xanthate and isopropyl xanthate. Aquat. Toxicol. **8**: 295–302.

Calamari, D., Marchetti, R., and Vailati, G. 1980. Influence of water hardness on cadmium toxicity to *Salmo gairdneri* Rich. Water Res. **14**: 1421–1426.

Carrol, J.J., Ellis, S.J., and Oliver, W.S. 1979. Influences of hardness constituents on the acute toxicity of cadmium to brook trout (*Salvelinus fontinalis*). Bull. Environ. Contam. Toxicol. **22**: 575–581.

Couture, P., and Kumar, P.R. 2003. Impairment of metabolic capacities in copper and cadmium contaminated wild yellow perch (*Perca flavescens*). Aquat. Toxicol. **64**: 107–120.

Davies, P.H., Gorman, W.C., Carlson, C.A., and Brinkman, S.F. 1993. Effects of hardness on bioavailability and toxicity of cadmium to rainbow trout. Chem. Speciation Bioavailability, **5**: 67–77.

De Schamphelaere, K.A.C., and Janssen, C.R. 2002. A biotic ligand model predicting acute copper toxicity for *Daphnia magna*: the effect of calcium, magnesium, sodium, potassium, and pH. Environ. Sci. Technol. **36**: 48–54.

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

Erickson, R.J., Benoit, D.A., Mattson, V.R., Nelson, H.P., Jr., and Leonard, E.N. 1996. The effects of water chemistry on the toxicity of copper to fathead minnows. Environ. Toxicol. Chem. **15**: 181–193.

Giesy, J.P., Jr., Leversee, G.J., and Williams, D.R. 1977. Effects of naturally occurring aquatic organic fractions on cadmium toxicity to *Simocephalus serrulatus* (Daphnidae) and *Gambusia affinis* (Poecillidae). Water Res. **11**: 1013–1020.

Hollis, L., Muench, L., and Playle, R.C. 1997. Influence of dissolved organic matter on copper binding, and calcium on cadmium binding, by gills of rainbow trout. J. Fish Biol. **50**: 703–720.

Hollis, L., McGeer, J.C., McDonald, D.G., and Wood, C.M. 1999. Cadmium accumulation, gill Cd binding, acclimation, and physiological effects during long term sublethal Cd exposure in rainbow trout. Aquat. Toxicol. **46**: 101–119.

Hollis, L., McGeer, J.C., McDonald, D.G., and Wood, C.M. 2000*a*. Effects of long term sublethal Cd exposure in rainbow trout during soft water exposure: implications for biotic ligand modelling. Aquat. Toxicol. **51**: 93–105.

Hollis, L., McGeer, J.C., McDonald, D.G., and Wood, C.M. 2000*b*. Protective effects of calcium against waterborne cadmium exposure to juvenile rainbow trout. Environ. Toxicol. Chem. **19**: 2725–2734.

MacRae, R.K., Smith, D.E., Swoboda-Colberg, N., Meyer, J.S., and Bergman, H.L. 1999. Copper binding affinity of rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) gills: implications for assessing bioavailable metal. Environ. Toxicol. Chem. **18**: 1180–1189.

McGeer, J.C., Playle, R.C., Wood, C.M., and Galvez, F. 2000. A physiologically based biotic ligand model for predicting the acute toxicity of waterborne silver to rainbow trout in freshwater. Environ. Sci. Technol. **34**: 4199–4207.

Morel, F.M.M., and Herring, J.G. 1993. Principles and applications of aquatic chemistry. John Wiley, Toronto, Ont.

Niyogi, S., and Wood, C.M. 2003. Effects of chronic waterborne and dietary metal exposures on gill metal-binding: implications for the biotic ligand model. Hum. Ecol. Risk Assess. **9**: 813–846.

Pagenkopf, G.K. 1983. Gill surface interaction model for trace-metal toxicity to fishes: role of complexation, pH, and water hardness. Environ. Sci. Technol. **17**: 342–347.

Paquin, P.R., DiToro, D.M., Santore, R.C., Trivedi, D., and Wu, K.B. 1999. A biotic ligand model of the acute toxicity of metals. III. Application to fish and *Daphnia* exposure to silver. integrated approach to assessing the bioavailability and toxicity of metals in surface waters and sediments. Section 3. A submission to the EPA Science Advisory Board, Office of Water. USEPA-822-E-99-001. Office of Research and Development, Washington, D.C. pp. 3-59–3-102.

Paquin, P.R., Gorsuch, J.W., Apte, S., Batley, G.E., Bowles, K.C., Campbell, P.G.C., Delos, C.G., DiToro, D.M., Dwyer, R.L., Galvez, F., Gensemer, R.W., Goss, G.G., Hogstrand, C., Janssen, C.R., McGeer, J.C., Naddy, R.B., Playle, R.C., Santore, R.C., Schneider, U., Stubblefield, W.A., Wood, C.M., and Wu, K.B. 2002. The biotic ligand model: a historical overview. Comp. Biochem. Physiol. C Comp. Pharmacol. Toxicol. **133**: 3–35.

Pascoe, D., and Beattie, J.H. 1979. Resistance to cadmium by pretreated rainbow trout alevins. J. Fish Biol. **14**: 303–308.

Pascoe, D., Evans, S.A., and Woodworth, J. 1986. Heavy metal toxicity to fish and the influence of water hardness. Arch. Environ. Contam. Toxicol. **15**: 481–487.

Playle, R.C. 1998. Modelling metal interactions at fish gills. Sci. Total Environ. **219**: 147–163.

Playle, R.C., Dixon, D.G., and Burnison, K. 1993*a*. Copper and cadmium binding to fish gills: modifications by dissolved organic carbon and synthetic ligands. Can. J. Fish. Aquat. Sci. **50**: 2667–2677.

Playle, R.C., Dixon, D.G., and Burnison, K. 1993*b*. Copper and cadmium binding to fish gills: estimates of metal–gill stability constants and modelling of metal accumulation. Can. J. Fish. Aquat. Sci. **50**: 2678–2687.

Reid, S.D., and McDonald, D.G. 1988. Effects of cadmium, copper, and low pH on ion fluxes in the rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci. **45**: 244–253.

Reid, S.D., McDonald, D.G., and Rhem, R.R. 1991. Acclimation to sublethal aluminium: modifications of metal – gill surface interactions of juvenile rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. **48**: 1996–2005.

Richards, J.G., and Playle, R.C. 1998. Cobalt binding to gills of rainbow trout (*Oncorhynchus mykiss*): an equilibrium model. Comp. Biochem. Physiol. C Comp. Pharmacol. Toxicol. **119**: 185–197.

Roch, M., and Maly, E.J. 1979. Relationship of cadmium-induced hypocalcemia with mortality in rainbow trout (*Salmo gairdneri*) and the influence of temperature on toxicity. J. Fish. Res. Board Can. **36**: 1297–1303.

Santore, R.C., DiToro, D.M., Paquin, P., Allen, H.E., and Meyer, J.S. 2001. Biotic ligand model of the acute toxicity of metals. 2. Application to acute copper toxicity in freshwater fish and daphnia. Environ. Toxicol. Chem. **20**: 2397–2402.

Santore, R.C., Mathew, R., Paquin, P.R., and DiToro, D.M. 2002. Application of the biotic ligand model to predicting zinc toxicity to rainbow trout, fathead minnow, and *Daphnia magna*. Comp. Biochem. Physiol. C Comp. Pharmacol. Toxicol. **133**: 271–285.

Schecher, W.D., and McAvoy, D.C. 2001. MINEQL+, user's manual. Environmental Research Software, Edgewater, Md.

Sherwood, G.D., Rasmussen, J.B., Rowan, D.J., Brodeur, J., and Hontela, A. 2000. Bioenergetic costs of heavy metal exposure in yellow perch (*Perca flavescens*): in situ estimates with a radiotracer ($^{137}$Cs) technique. Can. J. Fish. Aquat. Sci. **57**: 441–450.

Spry, D.J., and Wiener, J.G. 1991. Metal bioavailability and toxicity to fish in low-alkalinity lakes: a critical review. Environ. Pollut. **71**: 243–304.

Szebedinszky, C., McGeer, J.C., McDonald, D.G., and Wood, C.M. 2001. Effects of chronic Cd exposure via the diet or water on internal organ-specific distribution and subsequent gill Cd uptake kinetics in juvenile rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. **20**: 597–607.

Taylor, L.N., Wood, C.M., and McDonald, D.G. 2003. An evaluation of sodium loss and gill metal binding properties to explain species differences in copper tolerance. Environ. Toxicol. Chem. **22**: 2159–2166.

Verbost, P.M., Flik, G., Lock, R.A.C., and Wendelaar Bonga, S.E. 1987. Cadmium inhibition of $Ca^{2+}$ uptake in rainbow trout gills. Am. J. Physiol. **253**: R216–R221.

Verbost, P.M., Van Rooij, J., Flik, G., Lock, R.A.C., and Wendelaar Bonga, S.E. 1989. The movement of cadmium through fresh water trout branchial epithelium and its interference with calcium transport. J. Exp. Biol. **145**: 185–197.

Wicklund-Glynn, A., Norrgren, L., and Műssener, Å. 1994. Differences in uptake or inorganic mercury and cadmium in the gills of zebrafish, *Brachydanio rerio*. Aquat. Toxicol. **30**: 13–26.

Winner, R.W. 1984. The toxicity and bioaccumulation of cadmium and copper as affected by humic acid. Aquat Toxicol. **5**: 267–274.

Wood, C.M. 2001. Toxic responses of the gill. *In* Target organ toxicity in marine and freshwater teleosts. Vol. 1. Organs. *Edited by* D. Schlenk and W.H. Benson. Taylor and Francis, London, U.K. pp. 1–89.