ELSEVIER

Aquatic Toxicology 51 (2000) 93–105

AQUATIC TOXICOLOGY

www.elsevier.com/locate/aquatox

Effects of long term sublethal Cd exposure in rainbow trout during soft water exposure: implications for biotic ligand modelling

Lydia Hollis *, James C. McGeer, D. Gordon McDonald, Chris M. Wood

Department of Biology, McMaster University, Hamilton, ON, L8S 4K1, Canada

Received 16 August 1999; received in revised form 15 February 2000; accepted 17 February 2000

Abstract

The objectives of the study were to determine the physiological and toxicological effects of chronic cadmium exposure on juvenile rainbow trout in soft water. Particular attention focused on acclimation, on comparison to an earlier hard water study, and on whether a gill surface binding model, originally developed in dilute soft water, could be applied in this water quality to fish chronically exposed to Cd. Juvenile rainbow trout, on 3% of body weight daily ration, were exposed to 0 (control), 0.07, and 0.11 μg l^{-1} Cd [as $Cd(NO_3)_2 \cdot 4H_2O$] in synthetic soft water (hardness = 20 mg l^{-1} as $CaCO_3$, alkalinity = 15 mg l^{-1} as $CaCO_3$, pH 7.2) for 30 days. Mortality was minimal for all treatments (up to 14% for 0.11 μg l^{-1} Cd). No significant effects of chronic Cd exposure were seen in growth rate, swimming performance (stamina), routine O_2 consumption, or whole body/plasma ion levels. In contrast to the hard water study, no acclimation occurred in either exposure group in soft water, with no significant increases in 96-h LC_{50} values. Cadmium accumulated in a time-dependent fashion to twice the control levels in the gills and only marginally in the liver by 30 days. No significant Cd accumulation occurred in the gall bladder or whole body. Cadmium uptake/turnover tests were run using radioactive ^{109}Cd for acute (3 h) exposures. Saturation of the gills occurred for control fish but not for Cd-exposed fish when exposed to up to 36 μg l^{-1} Cd for 3 h. Cd-exposed trout accumulated less 'new' Cd in their gills compared to controls and they internalized less ^{109}Cd than control fish. This effect of lowered Cd uptake by the gills of acclimated trout was earlier seen for the fish acclimated to 10 μg l^{-1} Cd in hard water. The affinity of the gill for Cd was greater in hard water ($\log K_{Cd\text{-}gill} = 7.6$) than in soft water ($\log K_{Cd\text{-}gill} = 7.3$) but the number of binding sites ($B_{max} = 0.20$ μg g^{-1} gill) was similar in both media. In addition, there was a shift in affinity of the gill for Cd (i.e. lowered $\log K_{Cd\text{-}gill}$) and increased B_{max} with chronic Cd exposure in both soft water and hard water. We conclude that the present gill modelling approach (i.e. acute gill surface binding model or Biotic Ligand Model) does work for soft and hard water exposures but there are complications when applying the model to fish chronically exposed to cadmium. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Cadmium; Soft water; ^{109}Cd; Acclimation; Gill binding; Modelling; Rainbow trout

* Corresponding author. Present address: Department of Biological Sciences, University of Calgary, Calgary, AB, T2N 1N4, Canada. Tel.: +1-403-2203561; fax: +1-403-2899311.

E-mail address: lmhollis@ucalgary.ca (L. Hollis).

0166-445X/00/$ - see front matter © 2000 Elsevier Science B.V. All rights reserved.
PII: S0166-445X(00)00099-0

1. Introduction

Acute cadmium toxicity to fish has been demonstrated in many water qualities (e.g. McCarty et al., 1978; Carrol et al., 1979; Calamari et al., 1980). However, water hardness has been shown to have the largest ameliorating effects, protecting against Cd uptake and toxicity in rainbow trout (Calamari et al., 1980; Pärt et al., 1985; Pascoe et al., 1986; Davies et al., 1993). In particular, Carrol et al. (1979) and Pärt et al. (1985) demonstrated that calcium, rather than magnesium (the two 'hardness' cations), is the primary cation responsible for reduced cadmium toxicity to brook trout and reduced uptake of cadmium by rainbow trout gills, respectively. Gill permeability changes and/or competition between calcium and cadmium for binding sites on the gill have been proposed as possible mechanisms of protection by calcium (Calamari et al., 1980; Wright, 1980; Pagenkopf, 1983; Hunn, 1985; Pärt et al., 1985; Meyer, 1999; Meyer et al., 1999).

In our previous study (Hollis et al., 1999), we investigated the effects of chronic Cd exposure on juvenile rainbow trout in hard water (Ca = 40 mg l^{-1}, hardness = 140 mg l^{-1} as $CaCO_3$). We found no effect of a 30 day exposure to 3 or 10 μg l^{-1} Cd on growth, metabolic rate, and swimming performance, even though Cd-exposed fish exhibited significant acclimation (11–13 fold higher LC_{50} values compared to control fish). However, the situation may be different in low calcium (soft) water because Cd is a potent inhibitor of active Ca^{2+} uptake at the gills (Verbost et al., 1987, 1989)

Hollis et al. (1999) also tested the current gill binding model (or Biotic Ligand Model; Playle et al., 1993a,b; Bergman and Dorward-King, 1997; Renner, 1997; Playle, 1998; Meyer, 1999; Meyer et al., 1999) on juvenile rainbow trout chronically exposed to cadmium in hard water. This model, which is based on applying geochemical principles to gill metal binding to predict metal toxicity, was developed in soft water for acute metal exposures. We found that the present formulation of the model works for control fish in hard water, but cannot be extended to metal-acclimated fish due to the large, apparently non-toxic burden of Cd that accumulates during chronic exposure. Nevertheless, using a new approach with radiolabelled ^{109}Cd, we were able to distinguish between newly accumulated Cd in the gills and that which was previously bound to the gills during the chronic exposure. However this new Cd accumulation did not appear to be directly predictive of a toxic response.

The primary objectives of the present study were (a) to determine the physiological and toxicological effects of chronic cadmium exposure on juvenile rainbow trout in soft water (hardness: 20 mg l^{-1} as $CaCO_3$) which is low in Ca (5 mg l^{-1}), the most protective cation and (b) to test the acute gill surface binding model on rainbow trout that were exposed chronically to cadmium in soft water. Changes in gill Cd burden, the acute Cd-binding properties of the gills, and toxic responses during acute challenge were determined during chronic sublethal exposure of juvenile rainbow trout to 0.07 and 0.11 μg Cd l^{-1} for 30 days in synthetic soft water. Cadmium accumulation in other compartments (liver, gall bladder, whole body) were measured and possible sublethal effects (i.e. Cd influence on growth, routine metabolism, ionoregulation, and exercise performance) and costs of acclimation were examined. A largely parallel design was chosen to facilitate direct comparison with our earlier similar study (Hollis et al., 1999) conducted in hard water (eight fold higher Ca levels), as this comparison was also a major objective of the present investigation.

2. Materials and methods

2.1. Fish holding conditions

Juvenile rainbow trout [*Oncorhynchus mykiss* (Walbaum)] were obtained from Rainbow Springs Hatchery in Thamesford, Ontario and held in flowing dechlorinated Hamilton tap water [Lake Ontario water: Ca = 40 mg l^{-1} or 1 mmol l^{-1}, Na = 14 mg l^{-1} or 0.6 mmol l^{-1}, Cl = 25 mg l^{-1} or 0.7 mmol l^{-1}, Mg = 5 mg l^{-1} or 200 μmol l^{-1}, dissolved organic matter (DOM) = 3 mg l^{-1} or 0.25 mmol l^{-1}, hardness = 140 mg l^{-1} as $CaCO_3$, alkalinity = 95 mg l^{-1} as $CaCO_3$, pH 8.0, 14°C].

Trout were held in 600 l aerated polyethylene tanks for 1 week and then slowly introduced to synthetic soft water over the course of 1 week. The synthetic soft water (Ca = 5 mg l^{-1} or 130 μmol l^{-1}, Na = 3 mg l^{-1} or 130 μmol l^{-1}, Cl = 4 mg l^{-1} or 100 μmol l^{-1}, Mg = 1 mg l^{-1} or 40 μmol l^{-1}, DOM = 0.4 mg l^{-1} or 0.03 mmol l^{-1}, hardness = 20 mg l^{-1} as $CaCO_3$, alkalinity = 15 mg l^{-1} as $CaCO_3$, pH 7.2, 17°C) was produced by reverse osmosis (Anderson Water Systems) and consisted of six parts reverse osmosis water supplemented with one part dechlorinated Hamilton tap water. Fish were held in soft water for at least three weeks before experimentation. Fish were fed 3% body weight per day (as three 1% meals per day) with Martin's Starter Food [Martin Feed Mills, Elmira, Ontario; Cd content = 1.06 ± 0.04 ($N = 6$) μg Cd g^{-1} wet weight].

2.2. Exposure system

After 3 weeks in holding tanks, 225 fish (mean fish weight = 5.6 ± 0.1 g) were randomly transferred to six 200-l polyethylene exposure tanks which were flow-through systems (flow = 1.5 l min^{-1}) with continuous aeration. Fish were fed 3% body weight per day (see above). An acidified Cd stock solution, with Cd added as $Cd(NO_3)_2 \cdot 4H_20$ (Fisher Scientific, Nepean, Ontario), was delivered to a mixing head-tank via mariotte bottles (Mount and Brungs, 1967) to achieve the desired Cd concentrations in the exposure tanks. Exposure tanks were spiked on the first day of Cd exposure to immediately reach the desired Cd concentration. Water chemistry was measured weekly throughout the exposure. Fish were exposed to (i) control = nominally zero cadmium [actual measured 'in-tank' value = mean ± 1 S.E.M. (N = number of H_2O samples taken) 0.02 ± 0.01 μg l^{-1} or 0.0002 ± 0.0001 (13) μmol l^{-1} Cd], (ii) low cadmium at nominally 0.05 μg l^{-1} Cd [measured: 0.07 ± 0.01 μg l^{-1} or 0.0006 ± 0.0001 (27) μmol l^{-1} Cd], or (iii) high cadmium at nominally 0.12 μg l^{-1} Cd [measured: 0.11 ± 0.01 μg l^{-1} or 0.0010 ± 0.0001 (31) μmol l^{-1} Cd] for 30 days in synthetic soft water (hardness = 20 mg l^{-1} as $CaCO_3$). Measured in-tank concentrations of Ca and Na were 169 ± 9 (30) μmol l^{-1} and 116 ± 4 (30) μmol l^{-1}. The three treatment conditions each had two replicates so that $N = 450$ fish per treatment. The two sublethal exposure concentrations were chosen based on an initial 96-h Cd LC_{50} measurement of approximately 1 μg l^{-1} in synthetic soft water.

2.3. Sampling

During the 30 day Cd exposure, 16 ml water samples were taken throughout the exposure, acidified with 50 μl of HNO_3, and analyzed to check Na (Na = 3 mg l^{-1} or 130 μmol l^{-1}), Ca (Ca = 5 mg l^{-1} or 130 μmol l^{-1}), and Cd content. Fish from each treatment tank were bulk weighed every 5 days. Detailed descriptions of bulk weighing procedures are given in Hollis et al. (1999).

Specific growth rates (SGR) were determined from bulk weights from individual treatment tanks taken seven times over the 30 day exposure. The best fit of these data to time was an exponential curve. SGR, as percent per day, was calculated by linear regression of ln weight versus time, using SPSS (SPSS Inc., Version 8.0 for Windows, Chicago, IL) which provides mean ± 1 S.E. for growth.

Six fish from each tank were subsampled at day 0, 2, 10, 20, and 30; gills, livers, and gall bladders were assayed for cadmium. Six additional fish from each tank were sampled for whole body Cd and ion content. Fish were sacrificed and both sets of gills, the liver, and gall bladder were excised; gills were rinsed for 10 s in 100 ml of synthetic soft water. All tissues and whole bodies were frozen in liquid nitrogen for later analysis of Cd and ion content.

Six fish from each tank were sampled at day 30 for plasma Cd and Ca concentrations. Fish were sacrificed and blood samples were taken (40–285 μl) by caudal puncture with 1 cm^3 syringes. Blood samples were centrifuged for 2 min, plasma was removed, and stored at −70°C for later analysis of Cd and Ca content.

2.4. Testing

2.4.1. Exercise performance

Fish were not fed the day of swimming tests. Swimming procedures, using the protocol of (Mc-

Donald et al., 1998) as a stamina test, are described in detail in Hollis et al. (1999). Fish were swum in a flume in groups of ten against a constant current of 63 cm s^{-1} ($\sim$six body lengths per second) until exhaustion occurred. Sprint times were corrected to a reference body length of 10 cm, and the time to 50% fatigue (± 1 S.E.) was calculated, from twenty fish from each treatment, by linear regression in SPSS (SPSS Inc., Chicago, IL.) of probit fatigue versus log time.

2.4.2. Routine metabolism

Routine oxygen consumption, representing 'in-tank' metabolic rate, was measured 2 and 6 h after feeding after the 30 day exposure period, using procedures identical to those described by (Hollis et al., 1999). The data were weight-corrected using the weight exponent of 0.824, taken from Cho (1990).

2.4.3. Acclimation

A 96-h LC_{50} trial was run after 30 days exposure to assess possible acclimation (i.e. increased acute Cd tolerance) of metal-exposed fish. Each test cell consisted of ten fish placed, at random, into 15 l green plastic buckets having aeration and flow-through (100 ml min^{-1}) of synthetic soft water at the appropriate Cd level, as added by a mariotte bottle. Ten fish from each treatment were exposed for 96 h to Cd concentrations of 0.03 ± 0.01 (12) μg l^{-1} (control with no Cd added), 0.10 ± 0.02 (12) μg l^{-1}, 0.20 ± 0.02 (12) μg l^{-1}, 0.60 ± 0.01 (12) μg l^{-1}, and 2.60 ± 0.20 (12) μg l^{-1}. Dead fish were removed when movement ceased, and times of mortality were recorded. LC_{50} values (± 1 S.E.) were determined by linear regression in SPSS of probit mortality versus log Cd concentration.

2.4.4. Acute gill-Cd binding

Fish were not fed the day of gill binding experiments. A gill Cd uptake/turnover experiment was run using the Cd radioisotope ^{109}Cd. Five fish from each treatment (control, 0.07 μg l^{-1}, and 0.11 μg l^{-1} Cd) were placed into 15 clear plastic bags containing 3 l of aerated, synthetic soft water, placed in a water bath to maintain temperature. Each treatment group was exposed to 1 ± 0.2 (6) μg l^{-1}, 5 ± 1 (6) μg l^{-1}, 9 ± 1 (6) μg l^{-1}, 22 ± 1 (6) μg l^{-1}, or 36 ± 3 (6) μg l^{-1} total Cd added as $Cd(NO_3)_2 \cdot 4\ H_20$ (Fisher Scientific, Nepean, Ontario) and labeled with 3 μCi l^{-1} (0.11 mBq l^{-1})^{109}Cd added as $CdCl_2$ (specific activity = 2.75 mCi mg^{-1}; from New England Nuclear, Boston, MA). Water samples (5 ml) were taken at the beginning and end of the 3 h static exposure. Gills and whole bodies (remaining carcass) of five fish from each treatment were sampled at 3 h. Gills were removed, rinsed, acid digested, and later analyzed for total Cd and radioactivity due to ^{109}Cd (see Section 2.5). Remaining carcasses were placed in 20 ml polyethylene vials and analyzed for ^{109}Cd.

2.5. Tissue and water analyses

The concentrations of all measured parameters in tissues were expressed on a per gram wet tissue basis.

Gills, livers, gall bladders, and whole bodies were thawed, weighed, and then digested in 1 to 70 times their weight of 1 N HNO_3, as appropriate (TraceMetal Grade HNO_3; Fisher Scientific, Nepean, Ontario), for 3 h at about 80°C. Digests were shaken, left to settle for 10 min, then the supernatant was diluted ten times with deionized water (18 mgohm; Nanopure II; Sybron/Barstead, Boston, MA). Plasma samples were thawed and diluted ten times with 0.1 N HNO_3 for Cd analysis. Gill, liver, gall bladder, whole body, and plasma Cd concentrations were measured on a graphite furnace atomic absorption spectrophotometer (Varian AA-1275 with GTA-95 atomizer) against Fisher certified standards, as outlined by Hollis et al. (1996), using 10 μl injection volumes and N_2 gas. Operating conditions were as those described by Varian with 30 s drying time at 90°C, 12 s at 120°C, and 4 s at 1800°C during which Cd was read.

Tissue ^{109}Cd concentrations were measured on a Minaxi Auto-Gamma 5000 Series Gamma Counter (Canberra Packard Instrument Company, Meriden, CT). Tissue samples were typi-

cally counted for 5 min; typical radioactivity levels were greater than 50 times background. Tissue ^{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 cpm per gram of tissue (wet weight), b is ^{109}Cd counts in the water (cpm l^{-1}), and c is the total Cd concentration in the water (μg Cd l^{-1}).

Concentrations of different cadmium species in the water were calculated using the MINEQL + aquatic geochemical program (Schecher and McAvoy, 1994) and measured water chemistry. Gill Cd dissociation constants and capacity were calculated by Scatchard analysis as outlined by Reid and McDonald (1991). The amount of Cd bound by the gill was divided by the free Cd concentration in the water and was plotted against the amount of Cd bound by the gill. The total B_{max} (binding capacity at saturation = number of Cd binding sites) and K_D (Cd concentration at 50% saturation) of the gill were then determined from the x-intercept and slope of the Scatchard plot, respectively.

Water Na and Ca and plasma Ca (diluted 50 times with 0.02% $LaCl_3$ in deionized water) concentrations were measured using the Varian AA-1275 operated in standard flame absorption mode. Water Cd concentrations were measured using the methods described for tissues. Whole body Ca and Na levels were measured in the same way, using dilutions from the acid digests (above). Whole body Cl was measured on the acid digests using a CMT10 Chloride Titrator (Radiometer, Copenhagen). For all ion analyses the matrices for standards were the same as for unknowns and samples were always read above the lowest standard on the calibration curve. Water pH was measured using a Radiometer PHM71b meter with GK2401C combination electrode. Dissolved organic matter (DOM) was measured on the Rosemount Analytical DC-180 automated total organic carbon analyzer (Folio Instruments, Kitchener, Ontario).

For the acute gill binding model, the conditional metal-gill equilibrium constant for Cd and the number of binding sites for Cd on the gills, along with our water chemistry, were entered into MINEQL + to predict accumulation of Cd on the gills. We used log $K_{Cd\text{-}gill} = 8.6$ and $B_{max} = 2$ nmol g^{-1} (wet weight), values from Playle et al. (1993b). The volume ratio for gill modelling was five fish (each with an average gill weight of 0.6 g) in 3 l of water, the actual conditions used in the acute gill-Cd binding tests.

2.6. Statistics

Data have been expressed as means ± 1 S.E. (N). LC_{50} values, specific growth rates, and swimming times were compared by means of the Bonferroni adjustment to the independent two-tailed Student's t-test. For all other data, ANOVA followed by a Student–Newman–Keuls procedure was used for multiple comparisons of mean values. A fiducial limit of $P < 0.05$ was used throughout.

3. Results

3.1. Effects of exposure

Mortality was minimal over the 30 day exposure with 10, 0 and 14% mortality for controls, 0.07 μg l^{-1} Cd and 0.11 μg l^{-1} Cd exposures, respectively; mortality started only on day 12. No acute toxic effects (i.e. mortality occurring within the first few days) were observed at these Cd exposures in soft water and there were no significant differences in specific growth rate (3.46 ± 0.10, 3.71 ± 0.10, and 3.49 ± 0.10% d^{-1} in the control, 0.07 μg l^{-1}, and 0.11 μg l^{-1} treatments respectively) as a result of chronic Cd exposure.

Metal accumulation in gills increased significantly over the 30 d in both Cd-exposed groups, whereas levels in livers increased only marginally (Fig. 1A and B, respectively). The liver elevation was significant relative to background Cd at day zero [0.49 ± 0.01 (6) μg g^{-1} wet tissue] but not relative to the simultaneous control. Cadmium concentrations were greatest in gills (Fig. 1A), followed by liver (Fig. 1B); gall bladders and whole bodies did not accumulate significant

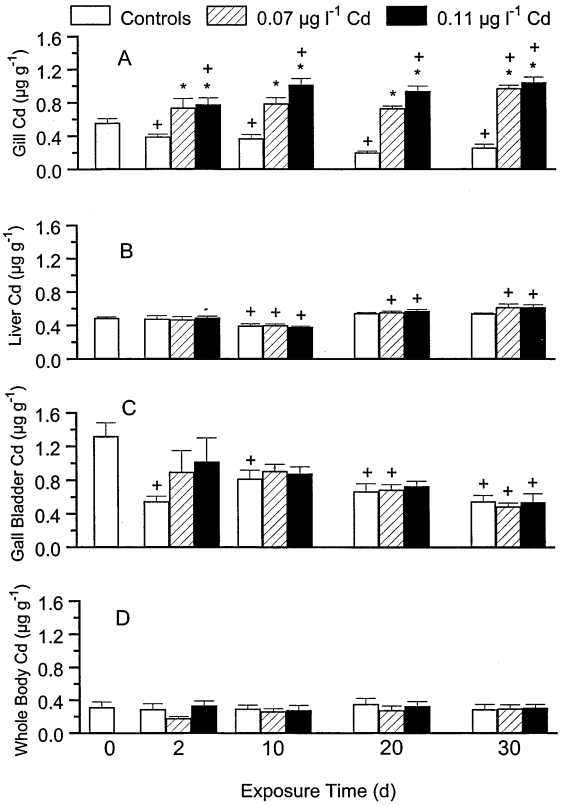

Fig. 1. Accumulation of Cd by gills (A), liver (B), gall bladder (C), and whole body (D) of juvenile rainbow trout exposed for 30 days to 0 (controls; clear bars), 0.07 (striped bars), or 0.11 µg l^{-1} Cd (solid bars) ± 1 S.E. ($N = 6$). Statistical comparisons were made against background Cd (controls) at each sampling day (*) and against background Cd at day 0 (crosses); $P < 0.05$.

Table 1
Routine oxygen consumption of juvenile rainbow trout after 30 days exposure to 0 (controls), 0.07, or 0.11 µg l^{-1} Cd, 2 and 6 h after feeding[a]

Cd exposure (µg l^{-1})	Oxygen consumption 2 h after feeding (µmol g^{-1} h^{-1})	Oxygen consumption 6 h after feeding (µmol g^{-1} h^{-1})
0	6.4 ± 0.1	5.2 ± 0.2
0.07	7.6 ± 0.2	6.2 ± 0.2
0.11	6.7 ± 0.4	6.2 ± 0.2

[a] Means ± 1 S.E. ($N = 2$ tanks of 225 fish each). There were no significant differences.

amounts of Cd over the 30 day exposure (Fig. 1C and D). Gill Cd levels increased $\sim$2-fold from initial (day 0) values of 0.56 ± 0.05 (6) µg Cd g^{-1} (wet tissue) for both low and high Cd exposures after 30 days of exposure (Fig. 1A). Gill concentration factors from the water were 14 000 times and 10 000 times for the 0.07 and 0.11 µg l^{-1} Cd exposures, respectively. Gall bladder Cd levels actually decreased from initial values of 1.32 ± 0.16 (6) µg Cd g^{-1} (wet tissue; Fig. 1C) for both the 0.07 and 0.11 µg l^{-1} Cd exposures. Whole bodies did not significantly differ from initial values (0.32 ± 0.06 (6) µg Cd g^{-1} wet tissue; Fig. 1D) for low and high Cd exposures in soft water.

3.2. Acclimation

Acclimation did not occur in soft water for fish chronically exposed to Cd concentrations of 0.07 or 0.11 µg l^{-1}. The 96-h LC_{50} values for the 0.07 µg l^{-1} Cd-exposed fish ($LC_{50} = 0.77 \pm 0.49$ µg Cd l^{-1}) and 0.11 µg l^{-1} Cd-exposed fish ($LC_{50} = 0.61 \pm 0.34$ µg Cd l^{-1}) were not significantly different from the control value of 2.07 ± 1.73 µg Cd l^{-1} (data not shown). There were no noticeable differences for time to mortality amongst the treatments.

3.3. Physiological effects and costs of chronic exposure

No treatment or time related effects in soft water were seen in whole body Ca^{2+}, Na^{+} and Cl^{-} concentrations which averaged 129 ± 6 (78), 44 ± 2 (78), and 41 ± 2 (78) mmol kg^{-1}, respectively. In-tank metabolic rate measurements showed no significant differences in rates of routine oxygen consumption 2 and 6 h after feeding in Cd-exposed fish in soft water, though the N number (two) was low (Table 1). Swimming stamina was not significantly affected by exposure to Cd for 30 days in soft water (Table 2). There were no significant differences in plasma Cd or Ca concentrations in soft water metal-exposed fish compared to controls at day 30 (Table 3). Note that on a wet weight basis, plasma Cd concentrations were only about 0.1% of those in all other tissues measured.

Table 2
Swimming performance (stamina) of juvenile rainbow trout after 30 days exposure to 0 (controls), 0.07, or 0.11 μg l^{-1} Cd[a]

Cd exposure (μg l^{-1})	Time to 50% fatigue (s)
0	100 ± 17
0.07	102 ± 14
0.11	105 ± 18

[a] Swimming times were corrected to a reference length of 10 cm (average length of fish tested). Means ± 1 S.E. ($N = 20$). There were no significant differences.

Table 3
Plasma Cd and Ca of juvenile rainbow trout after 30 days exposure to 0 (controls), 0.07, or 0.11 μg l^{-1} Cd[a]

Cd exposure (μg l^{-1})	Plasma Cd (μg l^{-1})	Plasma Ca (mmol l^{-1})
0	0.32 ± 0.02	1.92 ± 0.15
0.07	0.30 ± 0.02	1.90 ± 0.06
0.11	0.25 ± 0.04	1.94 ± 0.12

[a] Means ± S.E. ($N = 6$). There were no significant differences.

3.4. Cd uptake/turnover in gills of acclimated trout

Cd uptake/turnover tests were run using radioactive ^{109}Cd, at total Cd concentrations of 1, 5, 9, 22, and 36 μg l^{-1} for the soft water exposed fish (Fig. 2), to distinguish newly accumulated Cd from the native Cd already present in the gills, and to determine 'new' Cd binding by the gills and whole bodies of control and Cd-acclimated fish. Gill and remaining carcass samples were taken at the end of the 3 h acute exposure. Control fish reached approximate equilibrium by 3 h with about 0.17 μg g^{-1} of 'new' waterborne Cd bound to the gills (Fig. 2A). The acute gill surface binding model, using fathead minnow values for log $K_{Cd\text{-}gill}$ and B_{max} (Playle et al., 1993a,b; see Section 2) and measured water chemistry, predicted uptake of Cd by the gill similar to 'new' gill Cd concentrations actually observed in the control fish with a maximum accumulation of 0.20 μg Cd g^{-1} wet tissue (Fig. 2A).

In general, Cd-exposed fish accumulated similar amounts of Cd in their gills compared to controls by the end of the 3 h exposure (Fig. 2B). At the highest exposure concentration, 'new' Cd bound to the gills was about 0.22 μg g^{-1} (Fig. 2B), not significantly different from the control group. However, at acute exposure concentrations of radiolabelled Cd below 25 μg l^{-1}, uptake was significantly lower in Cd-exposed fish than in controls. More importantly, trout which had been exposed to 0.07 and 0.11 μg Cd l^{-1} for 30 days did not appear to approach equilibrium over the concentration range tested.

Scatchard analysis of gill Cd uptake/turnover for the 3 h exposures to radioactive ^{109}Cd (e.g. Fig. 2) showed that the K_D value (dissociation constant) was greater in soft water than in hard water but capacity (i.e. B_{max}) was very similar for trout in hard and soft water (Table 4). Scatchard analysis could not be done on the data from the Cd-exposed fish in soft water because of the lack of saturation, but inspection suggests a continuing trend of higher K_D (i.e. lower log $K_{Cd\text{-}gill}$, lower affinity) and higher B_{max} values for metal-exposed fish compared to controls.

Based on the internal appearance of ^{109}Cd, 'new' Cd accumulation into the whole body of soft water exposed fish during the acute ^{109}Cd exposures showed similar patterns to gill Cd accumulation. Samples taken at 3 h (Fig. 3) indicated that the whole body of controls had reached saturation, whereas the relationships in the Cd-exposed fish had not reached saturation at the highest concentration tested. As seen in the gills, significantly less 'new' Cd was accumulated in the whole body by Cd-exposed fish when exposed to Cd concentrations below 25 μg l^{-1}. At the end of the 3 h exposure, whole body 'new' Cd accumulation at the highest exposure concentration was approximately 0.010 μg Cd g^{-1} wet tissue for both the controls and metal-exposed fish (Fig. 3), less than 10% of the concentrations in gill tissue (Fig. 2).

4. Discussion

4.1. Overview

The purpose of the current study was to determine the physiological and toxicological effects of

chronic Cd exposure on juvenile rainbow trout in soft water. Particular attention focused on acclimation, on comparison to the previous hard water study (Hollis et al., 1999), and on whether the Biotic Ligand Model could be applied in soft water to fish chronically exposed to Cd. In soft water (present study), cadmium was approximately ten times more toxic than the study run in hard water (Hollis et al., 1999). The Cd concentrations to which the fish were chronically exposed in the hard water study (3 and 10 μg Cd l^{-1}) were high enough for the fish to acclimate (i.e. increased LC_{50} values for Cd-exposed fish), and significant amounts of Cd accumulated in all tissues, with the highest Cd levels in the gills (Table 5). In comparison, the soft water exposure also resulted in significant accumulation of Cd in the gills, but this did not lead to acclimation of

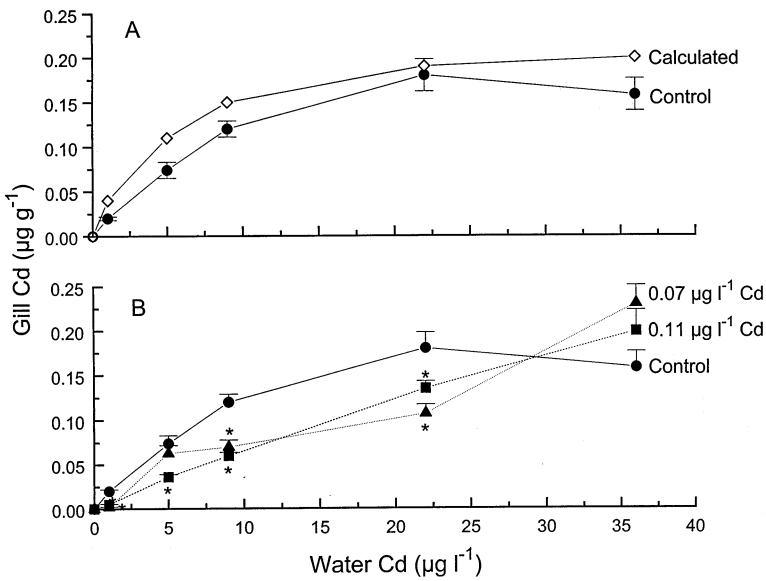

Fig. 2. Accumulation of 'new Cd' by gills of rainbow trout exposed for 3 h to ^{109}Cd, with total Cd concentrations of 1, 5, 9, 22, or 36 μg l^{-1}, after 30 days exposure. (A) Gill Cd accumulation by controls (0 μg l^{-1} Cd; closed circles) and gill Cd accumulation as calculated by the Biotic Ligand Model (acute gill surface binding model) using conditional equilibrium constants and number of binding sites for fathead minnows from Playle et al. (1993a,b); open diamonds). (B) Gill Cd accumulation by controls 0 (solid line with closed circles), 0.07 (dashed line with solid triangles), or 0.11 μg l^{-1} Cd (dotted line with solid squares). Means ± S.E. ($N = 5$). Statistical comparisons were made against control series for each water Cd treatment; * $P < 0.05$.

Table 4
Cd dissociation constants (K_D) and capacity (B_{max}) for juvenile rainbow trout exposed to 0 (controls) or 3 μg l^{-1} Cd (low), in hard water (Hollis et al., 1999) and 0 μg l^{-1} Cd (controls) in soft water[a]

Cd exposure	Hard water		Soft water	
	K_D (μg Cd l^{-1})	B_{max} (μg g^{-1})	K_D (μg Cd l^{-1})	B_{max} (μg g^{-1})
Controls	2.7 (7.6)	0.18	5.2 (7.3)	0.21
Low Cd	6.9 (7.2)	0.29	–	–

[a] Corresponding log $K_{Cd\text{-}gill}$ values are in brackets beside gill Cd dissociation constants (K_D). Scatchard analysis could not be performed on the data from the Cd-exposed fish in soft water because of the lack of saturation.

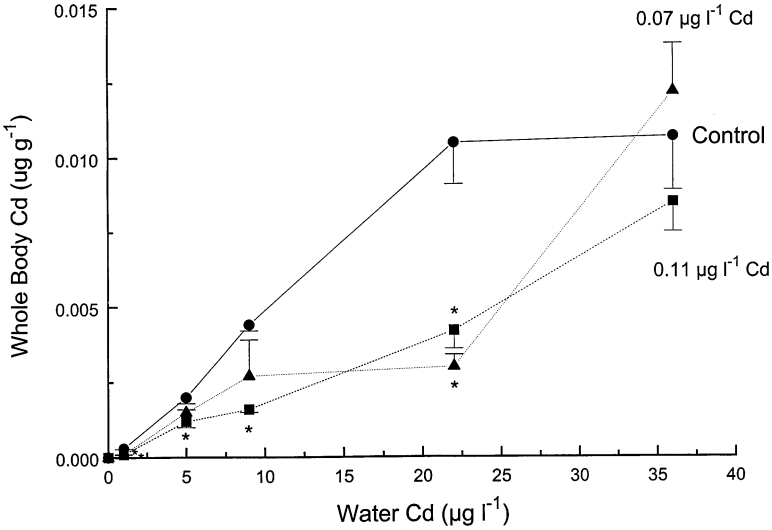

Fig. 3. Accumulation of 'new Cd' by whole bodies of rainbow trout exposed for 3 h to ^{109}Cd, with total Cd concentrations of 1, 5, 9, 22, or 36 µg l^{-1}, after 30 days exposures to 0 (controls; solid line), 0.07 (dashed line), or 0.11 µg l^{-1} Cd (dotted line). Means ± S.E. ($N = 5$). Statistical comparisons were made against control series for each water Cd treatment; * $P < 0.05$.

the fish (Table 5). No changes in growth, whole body and plasma ions, routine oxygen consumption, or swimming performance occurred for either the soft water or hard water exposure to Cd for 30 days (Table 5). Gill uptake and binding capacity (i.e. B_{max}) were similar in soft water versus hard water, and were consistently modified with chronic Cd exposure in both media (Table 5).

4.2. Environmental relevance

The levels of cadmium (0.07 and 0.11 µg l^{-1} Cd) used in our experiment were within an environmentally realistic range for soft water exposure. These values were relevant to USEPA freshwater quality criteria for aquatic life of 0.64 and 0.32 µg l^{-1}, recommended as limits for acute and chronic cadmium exposures, respectively, at water hardness of 20 mg l^{-1} as $CaCO_3$ (USEPA, 1986). Furthermore, Cd concentrations in Canadian surface waters are generally < 0.1 µg l^{-1}, and Canadian water quality guidelines for the protection of freshwater life in soft water are set at 0.2 µg Cd l^{-1} (i.e. hardness < 60 mg l^{-1}; CCME, 1995).

Table 5
Comparison of observed effects of Cd exposure in hard water (Hollis et al., 1999) versus soft water exposure for juvenile rainbow trout exposed for 30 days

Measured parameter	Hard water exposure to Cd (3 or 10 µg l^{-1})	Soft water exposure to Cd (0.07 or 0.11 µg l^{-1})
Mortality (acute toxicity)	At high Cd (10 µg l^{-1} Cd) exposure only	No acute mortality
Growth	No effects	No effects
Tissue Cd burdens	Increase in all tissues	Increase in gills only
Acclimation	Acclimation occurred	No acclimation
Whole body/plasma ions	No effects	No effects
O_2 consumption	No effects	No effects
Swimming performance	No effects	No effects
Gill binding characteristics	Trend of increased K_D and B_{max}	Trend of increased K_D and B_{max}

4.3. Indicators of chronic Cd exposure

Growth, oxygen consumption, whole body/plasma ions, and swimming performance were not sensitive indicators of ongoing sublethal Cd exposure in either hard or soft water (Table 5). In general, these findings on growth are in accord with earlier reports (Benoit et al., 1976; Kumada et al., 1980; Giles, 1988; Davies et al., 1993; Farag et al., 1994). We found no differences in swimming performance (stamina) between controls and fish chronically exposed to Cd in either soft water (Table 2) or hard water (Table 5; Hollis et al., 1999). Scherer et al. (1997) reported a similar lack of effect of chronic Cd (0.5 μg l^{-1}) on escape performance of fingerling rainbow trout. Whole body and plasma ions were not changed with chronic Cd exposure in soft water (Table 3) or hard water (Table 5; Hollis et al., 1999). Reid and McDonald (1988) also reported no effects of a 24 h exposure to 6.5 μg Cd l^{-1} on plasma electrolytes (Ca^{2+} and Na^{+}) but whole body Ca^{2+} influx was significantly inhibited.

4.4. Acclimation

Toxicological acclimation did not occur in our chronic Cd soft water exposure. The damage-repair hypothesis of McDonald and Wood (1993) states that there is an initial 'shock' phase involved with exposure to metals which corresponds with morphological damage to the gills, followed by compensation and repair of the gills with continued exposure to the metal, ultimately leading to acclimation of the fish. There was significant accumulation of Cd in the gills of the metal-exposed fish (Fig. 1); however, the low exposure concentrations (0.07 or 0.11 μg l^{-1}) may not have been high enough to induce morphological damage to the gills, and thereby acclimation.

In comparison to the present soft water exposure (Table 5), Hollis et al. (1999) showed an 11–13 fold increase in 96-h LC_{50} values for Cd-acclimated fish in hard water ($LC_{50} = 250–300$ μg Cd l^{-1}) compared to controls ($LC_{50} = 22$ μg Cd l^{-1}). Note that Cd was approximately 10 times more toxic in soft water (20 mg l^{-1} as $CaCO_3$; $LC_{50} = 2$ μg Cd l^{-1}) than in the hard water exposure (140 mg l^{-1} as $CaCO_3$). Calamari et al. (1980) showed a similar trend with forty-fold greater 48-h LC_{50} values for Cd in soft water (20 mg l^{-1} as $CaCO_3$) compared to hard water (320 mg l^{-1} as $CaCO_3$).

4.5. Implications for Biotic Ligand Modelling

Cold gill Cd concentration appears to be the most sensitive indicator of prior Cd exposure. Gills accumulated the greatest concentration of Cd over the 30 day exposure to Cd in soft water (Fig. 1A), which represented a two-fold increase relative to control levels. In this regard, we investigated whether the acute gill binding model, or Biotic Ligand Model, could be applied to fish that had been chronically exposed to Cd in soft water. This model was originally developed in soft water for acute exposures (Playle et al., 1993a,b).

The Biotic Ligand Model considers the gill membrane as a complexing ligand. Conditional equilibrium stability constants for affinity of metals binding to the gill membrane are inserted, together with relevant water chemistry, into aquatic geochemistry programs (e.g. MINEQL+) to predict metal binding to the gills and ultimately toxicity to the fish (Playle et al., 1993a,b; Playle, 1998). This approach has been expanded so that the fish gill is considered as a generalized 'biotic ligand' which is the primary site of toxic action (Meyer, 1999; Meyer et al., 1999). This model is now being considered by regulatory agencies as a tool to predict metal accumulation and toxicity to aquatic life (Bergman and Dorward-King, 1997; Renner, 1997).

In our previous study, we showed that the Biotic Ligand Model could be successfully applied to fish in a hard water environment, although complications arose when we tried to extend the model to fish that had been exposed to Cd on a chronic basis (Hollis et al., 1999). In particular, there was a large, apparently non-toxic burden of Cd that accumulated during chronic exposure and new Cd accumulation did not appear to be directly predictive of a toxic response. In the present study, the model successfully calculated 'new' Cd accumulation in gills of control fish (Fig. 2A) when the conditional stability constant for Cd

Table 6
Calculated concentrations of Cd species in the water for various Cd exposures, using MINEQL+ (Schecher and McAvoy, 1994) aquatic chemistry program (DOM = dissolved organic matter)

Cd exposure ($\mu g\ l^{-1}$)	$[Cd^{2+}]$ ($\mu g\ l^{-1}$)	[Cd-DOM] ($\mu g\ l^{-1}$)	$[CdCO_{3\ Aq}]$ ($\mu g\ l^{-1}$)
1	0.5	0.5	0.0
5	3.0	2.0	0.0
9	6.0	3.0	0.0
22	18.0	3.6	0.4
36	31.5	4.0	0.5

binding to the gill ($\log K_{Cd\text{-}gill} = 8.6$) and the number of Cd binding sites on the gill (0.2 nmol $fish^{-1}$ or 2 nmol g^{-1} of gill) from Playle et al. (1993a,b) were used along with water Cd^{2+} concentrations. In these simulations, 50–88% of the total Cd existed as the free ionic species, Cd^{2+}, for exposures ranging from 1 to 36 $\mu g\ l^{-1}$ Cd (Table 6). When Scatchard analysis was applied to the saturation curve for control fish (Fig. 2A), the conditional stability constant ($\log K_{Cd\text{-}gill} = 7.3$; Table 4) was approximately two times lower than that of Hollis et al. (1999) in hard water ($\log K_{Cd\text{-}gill} = 7.6$; Table 4). In contrast, Playle et al. (1993a,b) calculated a $\log K_{Cd\text{-}gill}$ value of 8.6 for fathead minnows. The difference in the $\log K_{Cd\text{-}gill}$ value determined in the present study (7.3) and that (8.6) calculated by Playle et al. (1993a,b) translates to a 20-fold difference in affinity. This difference is due to the difference in methods for calculating these conditional equilibrium constants. We calculated these values from Cd loading into the gills of fish where the presence of Ca^{2+}, H^+, Na^+, etc. in the water would likely exert a competitive effect, thereby reducing the apparent affinity and thus $\log K_{Cd\text{-}gill}$. In contrast, the method used by Playle et al. (1993a,b) involved the use of competitive ligands to reduce Cd accumulation on gills in very soft water, a method which is likely less sensitive to the competitive effects of Ca^{2+} and H^+. Playle et al. (1993a,b) in fact independently determined $\log K$ values for these competitive cations as part of the Biotic Ligand Model so that these influences are included when the predicted Cd accumulation is calculated by the model (e.g. Fig. 2A).

With regards to Cd-exposed trout, the fish exposed to 0.07 and 0.11 $\mu g\ l^{-1}$ Cd accumulated significantly less 'new' Cd in their gills compared to controls when exposed acutely to total Cd concentrations below 25 $\mu g\ Cd\ l^{-1}$. Whole body accumulation of 'new' Cd (Fig. 3) mirrored that of 'new' Cd accumulation by the gills (Fig. 2B) with significantly lower 'new' Cd accumulation in fish chronically exposed to Cd. Even though resistance did not change with prior exposure to Cd (e.g. changes in LC_{50}), gill binding characteristics did change in a manner consistent with the previous hard water exposure where LC_{50} increased dramatically (Hollis et al., 1999; Table 4). Thus changes in gill Cd binding are not necessarily predictive of changes in acute toxicity for fish which have been chronically exposed to the metal.

There were increased cold Cd concentrations on the gills from prior Cd exposure in hard water; therefore, changes in gill binding characteristics are probably related to increased pools of Cd in gills of Cd-exposed fish. These fish that had been chronically exposed to Cd in either hard water or soft water appear to have developed a protective mechanism (i.e. higher K_D and B_{max}; Table 4) for reduced Cd uptake. Alsop et al. (1999) have shown that juvenile rainbow trout chronically exposed to zinc, an essential metal, had significantly increased gill Zn pool sizes which were related to increased detoxification or temporary storage of Zn, in mucus for example. These workers also showed that the affinity of the gill for Zn was consistently reduced by chronic acclimation to sublethal zinc, therefore there seems to be some consistency in the responses to a non-essential metal (Cd) and an essential metal (Zn).

Finally, in our chronic exposures we note that there appear to be difficulties with the acute gill binding model as described by Playle et al. (1993b) using cold techniques. Firstly, as in our previous hard water study Hollis et al. (1999), we found it necessary to use ^{109}Cd since there was only a very small increase in uptake of 'new' Cd ($\sim 0.17\ \mu g\ Cd\ g^{-1}$) by control fish which could not easily be detected against the background of $\sim 0.56\ \mu g\ Cd\ g^{-1}$ wet weight using the cold technique employed by Playle et al. (1993b). This problem was much greater in the fish acclimated

to Cd. Secondly, there were difficulties in actually applying the Biotic Ligand Model to fish chronically exposed to Cd in both soft water (this study) and in hard water (Hollis et al., 1999). Saturation of the gills did not occur over the concentration range tested for our soft water exposed fish which had been chronically exposed to Cd. Clearly, higher concentrations of Cd exposure are required for gill binding experiments with trout chronically exposed to Cd.

Acknowledgements

We thank Lisa Taylor and Derek Alsop for their many contributions to this study, Jeff Richards and Dr Richard Playle for helpful input, and the NSERC Strategic Grants Program, the International Copper Association, Falconbridge Ltd., the International Lead Zinc Research Organization (ILZRO), and Cominco Ltd. for their financial support of this research. Dr Peter Chapman provided helpful comments on the manuscript.

References

Alsop, D.H., McGeer, J.C., McDonald, D.G., Wood, C.M, 1999. Assessing the costs and consequences of chronic waterborne zinc exposure to juvenile rainbow trout in hard and soft water. Environ. Toxicol. Chem. 18, 1014–1025.

Benoit, D.A., Leonard, E.N., Christensen, G.M., Fiandt, J.T, 1976. Toxic effects of cadmium on three generations of brook trout (*Salvelinus fontinalis*). Trans. Am. Fish. Soc. 105, 550–560.

Bergman, H.L., Dorward-King, E.J. (Eds), 1997. Reassessment of metals criteria for aquatic life protection: priorities for research and implementation. SETAC Pellston Workshop on Reassessment of Metals Criteria for Aquatic Life Protection, 1996 Feb 10–14, Pensacola FL. SETAC Pr, Pensacola FL, 114 p.

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

Canadian Council of Ministers of the Environment (CCME), 1995. Canadian Water Quality Guidelines. In: CCREM 1995, Appendix XVIII. Winnipeg, Manitoba.

Carrol, J.J., Ellis, S.J., 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.

Cho, C.Y., 1990. Fish nutrition, feeds, and feeding: with special emphasis on salmonid aquaculture. Food Rev. Intl. 6, 333–357.

Davies, P.H., Gorman, W.C., Carlson, C.A., Brinkman, S.R., 1993. Effect of hardness on bioavailability and toxicity of cadmium to rainbow trout. Chem. Sp. Bioavail. 5, 67–77.

Farag, A.M., Boese, C.J., Woodward, D.R., Bergman, H.L., 1994. Physiological changes and tissue metal accumulation in rainbow trout exposed to foodborne and waterborne metals. Environ. Toxicol. Chem. 13, 2021–2029.

Giles, M.A, 1988. Accumulation of cadmium by rainbow trout, Salmo gairdneri, during extended exposure. Can. J. Fish. Aquat. Sci. 45, 1045–1053.

Hollis, L., Burnison, K., Playle, R.C, 1996. Does the age of metal-dissolved organic carbon complexes influence binding of metals to fish gills? Aquat. Toxicol. 35, 253–264.

Hollis, L., McGeer, J.C., McDonald, D.G., 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.

Hunn, J.B., 1985. Role of calcium in gill function in freshwater fishes. Comp. Biochem. Physiol. 82, 543–547.

Kumada, H., Kimura, S., Yokote, M., 1980. Accumulation and biological effects of cadmium in rainbow trout. Bull. Japan. Soc. Sci. Fish. 46, 97–103.

McCarty, L.S., Henry, J.A.C., Houston, A.H., 1978. Toxicity of cadmium to goldfish, *Carassius auratus*, in hard and soft water. J. Fish. Res. Board Can. 35, 35–42.

McDonald, D.G., McFarlane, W.J., Milligan, C.L, 1998. Anaerobic capacity and swim performance of juvenile salmonids. Can. J. Fish. Aquat. Sci. 55, 1198–1207.

McDonald, D.G., Wood, C.M., 1993. Branchial mechanisms of acclimation to metals in freshwater fish. In: Rankin, J.C., Jensen, F.B. (Eds.), Fish Ecophysiology. Chapman and Hall, London, pp. 297–321.

Meyer, J.S., 1999. A mechanistic explanation for the ln(LC50) vs ln(hardness) adjustment equation for metals. Environ. Sci. Technol. 33, 908–912.

Meyer, J.S., Santore, R.C., Bobbitt, J.P., Debrey, L.D., Boese, C.J., Paquin, P.R., Allen, H.E., Bergman, H.L., Di Toro, D.M., 1999. Binding of nickel and copper to fish gills predicts toxicity when water hardness varies, but free-ion activity does not. Environ. Sci. Technol. 33, 913–916.

Mount, D.I., Brungs, W.A., 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1, 21–29.

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.

Pärt, P., Svanberg, O., Kiessling, A, 1985. The availability of cadmium to perfused rainbow trout gills in different water qualities. Water Res. 19, 427–434.

Pascoe, D., Evans, S.A., 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., Burnison, K, 1993a. Copper and cadmium binding to fish gills: modification by dissolved organic carbon and synthetic ligands. Can. J. Fish. Aquat. Sci. 50, 2667–2677.

Playle, R.C., Dixon, D.G., Burnison, K, 1993b. 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, 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, 1991. Metal binding activity of the gills of rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 48, 1061–1068.

Renner, R., 1997. A better understanding of the how natural waters inhibit metal uptake may change regulatory limits. Environ. Sci. Technol. 31, 466–468.

Schecher, W.D., McAvoy, D.C., 1994. MINEQL+, User's Manual. Environmental Research Software, Hallowell, ME.

Scherer, E., McNicol, R.E., Evans, R.E., 1997. Impairment of lake trout foraging by chronic exposure to cadmium: a black-box experiment. Aquat. Toxicol. 37, 1–7.

United States Environmental Protection Agency (USEPA), 1986. Quality criteria for water. USEPA Office of Water Regulations and Standards, Washington DC, USEPA 440/5-85-001.

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

Wright, D.A., 1980. Cadmium and calcium interactions in the freshwater amphipod *Gammarus pulex*. Freshwater Biol. 10, 123–133.