EFFECTS OF CHRONIC CADMIUM EXPOSURE ON TROUT

EFFECTS OF CHRONIC CADMIUM EXPOSURE ON JUVENILE RAINBOW TROUT: PROTECTIVE EFFECTS OF CALCIUM AND APPLICATION OF BIOTIC LIGAND MODELLING

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Abstract

Juvenile rainbow trout were chronically exposed to cadmium in hard water, soft water, and in calcium-supplemented soft water in order to understand the effects of long term cadmium exposure in freshwater fish. A particular goal was to characterize changes in gill cadmium burden and the cadmium-binding properties of the gills during chronic sublethal exposures, so as to examine the applicability of the acute gill surface metal binding model or Biotic Ligand Model to trout chronically exposed to cadmium.

Trout were exposed for 30 days to sublethal concentrations of cadmium in: a) moderately hard, Hamilton tap water (Ca = 1000 μM), b) synthetic soft water (Ca = 130 μM), or c) calcium-supplemented soft water (Ca = 260, 470, 770, and 1200 μM Ca). For both the hard and soft water cadmium exposures, no effects were observed on growth, swimming performance, and whole body ions. Growth and whole body and plasma Ca^{2+} concentrations were similar for all treatments in the calcium-supplemented soft water experiment; however, swimming performance was significantly reduced for the 470 μM Ca + Cd exposed fish. Acclimation to cadmium occurred in the hard water and lower concentrations of calcium-supplemented exposures but not in the soft water exposure. Cadmium accumulation was greatest in kidneys and gills and

was directly related to cadmium exposure concentration. Tissue metal burdens were reduced with increased water calcium concentrations. Affinity of the gill for cadmium and the number of binding sites for cadmium decreased at higher water calcium concentrations. Affinity of the gill for Cd decreased with chronic cadmium exposure but binding site numbers increased with chronic Cd exposure. The acute gill binding model or Biotic Ligand Model, originally developed in soft water, was successfully applied to fish in both hard and soft water; however, complications arose when extending the model to fish chronically exposed to cadmium at various water calcium concentrations.

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Thesis Organization and Format

With the recommendation of my supervisory committee, this thesis has been presented in the 'open-faced' format approved by McMaster University. Therefore this thesis is organized into four Chapters and a summary. The first Chapter is a general overview of each study and pertinent background information. The second Chapter has been written as a manuscript and has been published in *Aquatic Toxicology* (1999; Vol. 46: 101-119). The third and fourth Chapters have been written as manuscripts for future submission to peer reviewed scientific journals. Abstracts have been included for Chapters 2, 3, and 4. Literature cited follows each chapter. Finally, a brief Summary of the findings concludes the thesis.

Chapter 1: General Introduction and Thesis Overview.

Chapter 2:

Title:	Cadmium Accumulation, Gill Cd Binding, Acclimation, and Physiological Effects During Long Term Sublethal Cd Exposure in Rainbow Trout.
Authors:	L. Hollis, J.C. McGeer, D.G. McDonald & C.M. Wood
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Chapter 3:

Title: Effects of Long Term Sublethal Cd Exposure in Rainbow Trout During Soft Water Exposure: Implications for Biotic Ligand Modelling.

Authors: L. Hollis, J.C. McGeer, D.G. McDonald & C.M. Wood

Comments: Data were generated and analyzed by L.H. under the guidance of J.C.M. and supervision of C.M.W. D.G.M. provided advice and logistical contributions. This study is about to be submitted for publication to *Aquatic Toxicology*.

Chapter 4:

Title: Protective Effects of Calcium Against Chronic Waterborne Cadmium Exposure in Juvenile Rainbow Trout.

Authors: L. Hollis, J.C. McGeer, D.G. McDonald & C.M. Wood

Comments: Data were generated and analyzed by L.H. under the guidance of J.C.M. and supervision of C.M.W. D.G.M. provided advice and logistical contributions. This study is about to be submitted to *Environmental Toxicology and Chemistry* for publication.

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Chapter 1

General Introduction and Thesis Overview

Cadmium in the Environment

Cadmium is a nonessential heavy metal that occurs naturally in the environment. It is used for various industrial purposes including electroplating, pigments, batteries, solders, electronic equipment, glass, and fertilizers (reviewed by C.C.M.E., 1995). Cadmium can also be found as an impurity in ores of other metals, including zinc, lead and copper and as a by-product in the refining of zinc and copper (Alabaster and Lloyd, 1982). From industrial discharges, cadmium can enter into the environment through either liquid effluent discharge or airborne emissions. An estimated 159 tonnes of anthropogenic cadmium are released annually in Canada, with approximately 90% attributable to atmospheric emissions (Porter *et al.*, 1995). In natural surface waters, cadmium occurs principally as the uncomplexed free cation (Cd^{2+}), cadmium chloride, and cadmium carbonate (reviewed by Alabaster and Lloyd, 1982 and C.C.M.E., 1995). The free metal cation, Cd^{2+}, is the bioavailable chemical species which appears to be the principal agent of toxicity to fish and other aquatic organisms (Pagenkopf, 1983; Morel and Hering, 1993).

Although Cd^{2+} is considered to be the toxic form of the metal, current Canadian water quality guidelines for the protection of freshwater life against

cadmium are based on total Cd concentrations in an unfiltered sample (Porter *et al.*, 1995). Regulations also take water hardness into consideration (Porter *et al.*, 1995) because water hardness has the greatest influence on toxicity of Cd to freshwater organisms (assessed below). Canadian Water Quality Guidelines (CWQG) for the protection of freshwater aquatic life can be modified on a site-specific basis and are derived from a single hardness-based equation for both acute and chronic Cd exposures (C.C.M.E., 1995):

$$CWQG = 10^{0.8904[\log_{10}(\text{hardness})] - 3.271}$$

Ambient Water Quality Criteria (AWQC) for cadmium in the United States are based on similar 'hardness' equations; one equation for acute criteria and another for chronic criteria, using total Cd concentrations in the water (U.S. E.P.A., 1986). In addition, this hardness correction has been incorporated into European AWQC for chronic Cd exposures (Alabaster and Lloyd, 1982).

Acute and Chronic Effects of Cadmium on Freshwater Fish

The gill makes up more than 50% of the body surface of fish and is the first site of interaction between the water and organism; therefore, the gill is the most important organ concerning metal toxicity to fish. In freshwater fish, cadmium can damage gills (Gardner and Yevich, 1970; Bilinski and Jonas, 1973; Voyer *et al.*, 1975) and result in skeletal deformities (Bengtsson *et al.*, 1975; Muramoto, 1981). Cadmium is also known to interfere with calcium uptake, the

key mechanism responsible for acute toxicity to fish (Roch and Maly, 1979; Verbost *et al.*, 1987, 1989; Reid and McDonald, 1988; Bentley, 1992; Wicklund-Glynn *et al.*, 1994). Reid and McDonald (1988) have shown that Cd inhibits the influx of Ca from the water into gills of rainbow trout with no significant effects on Ca efflux. Verbost *et al.* (1987, 1989) demonstrated that the fatal hypocalcaemia that results from acute Cd toxicity is due to the irreversible inhibition of Ca^{2+} uptake by the basolateral Ca^{2+} pump of the gill. Additional acute toxic effects of cadmium at very high concentrations include gill damage (Karlsson-Norrgren *et al.*, 1985) and impairment of oxygen transport across the gills, likely due to oedema, hypertrophy, hyperplasia, and excess mucus secretion (Majewski and Giles, 1981; Mallatt, 1985).

The phenomenon of acclimation is a well documented effect of chronic sublethal Cd exposure to fish (Pascoe and Beattie, 1979; Duncan and Klaverkamp, 1983; Benson and Birge, 1985; reviewed by McDonald and Wood, 1993). McDonald and Wood (1993) proposed a gill damage-repair hypothesis which describes the changes that occur in gills of fish chronically exposed to metals. The hypothesis states that there is an initial 'shock' phase during metal exposure which corresponds with morphological damage to the gills. With continued exposure to the metal, the fish is able to repair the damage and this compensation leads to acclimation (McDonald and Wood, 1993). Upon acclimation to sublethal concentrations of Cd, indicators of chronic exposure in fish can be subtle. For example, Kumada *et al.* (1980) and Davies *et al.* (1993)

found no significant changes in growth for juvenile rainbow trout exposed to 4 µg/L Cd for 70 to 100 days. Similarly, Farag *et al.* (1994) showed that a 21 day exposure to 2.2 µg/L Cd had no significant effects on growth of trout. When fish were exposed to 3.6 and 6.4 µg/L Cd for 178 days, no adverse effects on growth of rainbow trout were reported (Giles, 1988). In addition to growth, no subtle changes in swimming ability (Scherer *et al.*, 1997) or plasma calcium concentrations (Reid and McDonald, 1988; Giles, 1984) were observed with sublethal Cd exposures.

Water hardness has been shown to have the largest ameliorating effects for protection 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, Pärt *et al.* (1985) and Carroll *et al.* (1979) have shown that calcium, rather than magnesium (the two 'hardness' cations), is the primary cation responsible for reduced Cd toxicity and uptake in trout. Gill permeability changes and/or competition between Ca^{2+} and Cd^{2+} 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).

Biotic Ligand Modelling

Cadmium, probably as Cd^{2+}, binds to the gills of freshwater fish and disrupts the ionoregulatory functions of the gills (assessed above). Competing cations, such as Ca^{2+}, can reduce these effects by preventing cadmium from

binding to the gills. The gill membrane can therefore be considered as a complexing ligand (Playle *et al.*, 1993a,b; Playle, 1998). From this viewpoint, the gill surface can be experimentally characterized as a metal-binding ligand with a fixed number of receptor sites (B_{max}) and an average conditional Cd-gill equilibrium constant ($K_{Cd\text{-}gill}$; Playle *et al.*, 1993a,b). When these values, together with measured water chemistry, are fed into standard aqueous geochemical modelling programs such as MINEQL+ (Schecher and McAvoy, 1994), they predict the degree of saturation of the gill sites with the metal. In turn, this degree of saturation is thought to be directly predictive of acute metal toxicity in that particular water chemistry (Playle *et al.*, 1993a,b; Playle, 1998; MacCrae *et al.*, 1999). This procedure, known as the acute gill surface binding model, was developed in soft water by Playle *et al.* (1993a,b) and was derived from the original framework proposed by Pagenkopf (1983). 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 (Di Toro *et al.*, 1999). Recently, there has been great interest in using this Biotic Ligand Model as a tool for predicting metal toxicity as a function of water quality, so as to generate site-specific AWQC (Bergman and Dorward-King, 1997; Renner, 1997; Playle, 1998).

The Present Study

To date, the Biotic Ligand Model, has been applied only to acute toxicity, and the experimental data for these models have been generated using only short term exposures (2-3 h) in synthetic soft water of extremely low hardness

and alkalinity (Playle *et al.*, 1993a,b; Janes and Playle, 1995; MacCrae *et al.*, 1999). The primary objective of this thesis was to determine if the current Biotic Ligand Model for Cd, developed in soft water for acute exposures, could be extended to chronic Cd exposures in hard water, soft water, and Ca-supplemented soft water to predict gill accumulation and therefore long-term toxicity to fish. Changes in gill Cd burden, the acute Cd-binding properties of the gills, and toxic responses during acute (3 and 72 h) challenge were determined after chronic sublethal exposure of juvenile rainbow trout in hard water, soft water, and Ca-supplemented soft water. Cadmium accumulation in other compartments was measured and possible sublethal effects and costs of acclimation were examined.

Physiological and Toxicological Measurements

By measuring various physiological parameters, a more substantial assessment of the costs of chronic Cd exposure were made. During the current studies, survival over the 30 day exposures was used as a direct measure of both acute and chronic effects of Cd exposure. Growth measurements provided an indirect measure of energy expenditure, i.e. if fish exposed to Cd on a chronic basis did not grow as well as control fish, it may be inferred that the cost of homeostasis was higher for metal-exposed fish. Tissue Cd burdens (gills, liver, gall bladder, kidney, carcass, and whole body) over a series of 30 day exposures were used as direct indicators of chronic Cd exposure.

Acclimatory adjustment to chronic Cd exposure was measured by 96-h acute toxicity (LC50) tests of both metal-exposed fish and controls at the end of the 30 day exposure. For these tests fish were exposed to a range of potentially toxic levels of Cd, and mortality was monitored for 96 hours, so as to find the concentration at which 50% of the fish would survive for 96 h. The higher the concentration, the greater the degree of acclimation. These tests were used to determine whether true acclimation had occurred as a result of chronic Cd exposure and were reliable indices of acclimation (McDonald and Wood, 1993).

Whole body and plasma ions are parameters that could potentially indicate chronic Cd exposure. In the Ca-supplemented soft water study (Chapter 4), unidirectional influx of Ca^{2+} was also measured as a potentially more sensitive indicator, because Cd is a known antagonist to Ca^{2+} uptake across the gill during acute metal exposures (Verbost *et al.*, 1987, 1989). Reid and McDonald (1988) demonstrated no effects of 6.5 µg/L Cd exposure on plasma Ca^{2+} and Na^{+} during a 24 h exposure; however, influx of Ca^{2+} from the water to the fish was significantly reduced.

In-tank oxygen consumption was used as an indirect measure of metabolic rate in hard and soft water (Chapters 2 and 3). As all aerobic processes require ATP and oxygen is used in the production of ATP, it is a good measure of the integrative costs of living and like growth could potentially serve as an indicator of the costs of chronic Cd exposure.

At the end of the 30 day exposures, critical swimming speed (U_{Crit}) and/or sprint performance (stamina) were determined. Hammer (1995) has suggested that decrements of critical velocity may be successfully used as indices of sublethal toxicity. The stamina trials are complementary and give an indication of the sprint swimming capacity of the fish which is important during migration and predator-prey interactions (Scherer *et al.*, 1997).

Gill Cd Binding Assays

Gill Cd binding kinetics tests were run at the end of the 30 day exposures, initially using standard cold techniques (Playle *et al.*, 1993a,b). However, it soon became clear that the increased sensitivity provided by radiotracers (^{109}Cd) was necessary to discern small changes in 'new' gill Cd against high background levels present in the gills of chronically exposed fish. Gill metal uptake/turnover of control and Cd-exposed trout was determined by exposing the fish for 3 hours to incremental Cd concentrations labelled with ^{109}Cd. Gills, blood (hard water study only, Chapter 2), and whole bodies were sampled and analyzed for total Cd and radioactivity due to ^{109}Cd. A longer term gill Cd uptake/turnover experiment was run in Ca-supplemented soft water (Chapter 4) by exposing the fish for 72 hours, after the 30 day Cd exposure, to the Cd radioisotope ^{109}Cd at a total Cd concentration approximately three-fold higher than the chronic exposure level of 2 µg/L Cd. Gills were sampled at 12, 24, 48, and 72 h and analyzed for total Cd and radioactivity due to ^{109}Cd. A 3 h gill binding experiment using ethylenediaminetetraacetic acid (EDTA) as a complexing ligand was run in hard

water (Chapter 2) to test whether chronically accumulated gill Cd could be removed from the gill. Using the standard MINEQL+ water chemistry program (Schecher and McAvoy, 1994), gill-metal binding constants were calculated by Scatchard analyses using established techniques (Playle *et al.*, 1993a,b) to detect whether changes in affinity or receptor numbers of the gill occurred during chronic waterborne Cd exposures.

Chapter 2

Chapter 2 describes the results of chronic (30 d) sublethal Cd exposure (0, 3 or 10 µg/L Cd) in moderately hard water (140 mg/L as $CaCO_3$) on Cd accumulation in tissues, acute gill Cd binding, acclimation, and physiological effects in juvenile rainbow trout. Particular attention focused on acclimation, and on whether the Biotic Ligand Model, originally developed in dilute soft water, could be applied in this water quality to fish chronically exposed to Cd. Only the higher Cd concentration caused mortality. The costs of acclimation, if any, in the study were subtle since no significant effects of chronic Cd exposure were seen in growth rate, swimming performance, routine O_2 consumption, or whole body ion levels. Substantial acclimation (i.e. increased 96-h LC50 values) occurred in both exposure groups. Cadmium accumulated in gills and internal tissues in a time- and concentration-dependent fashion over the 30 days. Chronically accumulated gill Cd could not be removed by EDTA challenge. These gill Cd concentrations were 20- to 40-fold greater than levels predicted by the Biotic Ligand Model to cause mortality during acute exposure. In short-term gill Cd-

binding experiments, gill Cd burden increased as predicted in control fish, but was not detectable against the high background concentrations in acclimated fish. In light of these results, Cd uptake/turnover tests were performed using radioactive ^{109}Cd to improve sensitivity. With this approach, a small saturable binding component was seen, but could not be related to toxic response in acclimated fish. The affinity of the gill for Cd (log $K_{Cd\text{-}gill}$) appeared to decrease and the number of binding sites (B_{max}) appeared to increase in acclimated fish. It was concluded that gill Cd burden was not predictive of mortality in acclimated fish, and that the present gill modelling approach works in naïve (control) fish in hard water but does not work in chronically acclimated fish in hard water. The next exposure (Chapter 3) was carried out to determine if the model could be applied to fish chronically exposed to Cd in soft water.

Chapter 3

Chapter 3 describes the results of chronic (30 d) sublethal Cd exposure (0, 0.07 or 0.11 µg/L Cd) in synthetic soft water (20 mg/L as $CaCO_3$) on juvenile rainbow trout, and the implications for Biotic Ligand Modelling. Particular attention focused on acclimation, on comparison to the previous hard water study, and on whether the Biotic Ligand Model could be applied in our soft water to fish chronically exposed to Cd. Mortality was minimal for all treatments. No significant effects of chronic Cd exposure were seen in growth rate, swimming performance, 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. Cd accumulated in a time-dependent fashion in gills and liver over the 30 day exposure. No significant Cd accumulation occurred in the gall bladder or whole body during the exposure. When challenged with elevated Cd levels, Cd-exposed trout accumulated less 'new' Cd in their gills compared to controls and they internalized less than control fish. This effect of lowered Cd uptake by the gills of acclimated trout was similar to responses of fish acclimated to 10 μg l^{-1} Cd in hard water (Chapter 2). The affinity of the gill for Cd was greater in hard water (log $K_{Cd\text{-}gill}$ = 7.6; Chapter 2) than in soft water (log $K_{Cd\text{-}gill}$ = 7.3; Chapter 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 concluded that the present Biotic Ligand Model does work in soft and hard water exposures for non-exposed fish but there were complications when applying the model to fish chronically exposed to cadmium. To further understand the protective role of calcium during chronic Cd exposures in juvenile rainbow trout, a final 30 day exposure to Cd at various water calcium concentrations was run (Chapter 4).

Chapter 4

Chapter 4 describes the results of chronic (30 d) Cd exposure of juvenile rainbow trout exposed to 0 or 2 μg/L Cd with four different calcium concentrations (260, 470, 770, or 1200 μM Ca) in synthetic soft water. Mortality was highest in the low Ca (260 μM Ca) + 2 μg/L Cd treatment. No growth effects were seen for

any of the exposures. Kidneys accumulated the greatest concentration of Cd over the 30 d, followed by gills and livers in all Cd-exposed fish. Cadmium accumulation in gills, kidney, and liver decreased at higher water Ca concentrations. No differences in whole body or plasma calcium concentrations were found. Swimming performance was impaired in the 470 μM Ca + 2 μg/L Cd exposed fish. Influx of Ca^{2+} into whole bodies decreased as water Ca concentrations increased; influx of Ca^{2+} into 260 μM Ca + 2 μg/L Cd treated fish was significantly reduced compared to controls (260 μM Ca). Experiments that measured uptake of 'new' Cd into gills showed that affinity of the gills for Cd (i.e. log $K_{Cd\text{-}gill}$) decreased as water Ca concentrations increased. The number of binding sites for Cd decreased with increased water Ca concentrations. Acute accumulation of 'new' Cd into gills and the number of gill Cd binding sites (B_{max}) increased with chronic Cd exposure, while affinity of the gills for Cd (log $K_{Cd\text{-}gill}$) decreased with chronic Cd exposure. There were complications in applying the Biotic Ligand Model to fish chronically exposed to Cd because of the discrepancies in the maximum number of gill Cd binding sites (i.e. B_{max} values) amongst different studies. Furthermore, adaptive changes that occur in gills and acclimatory responses of fish chronically exposed to Cd cannot be easily incorporated into the model.

Significance of Thesis

This thesis showed that the effects of chronic Cd exposure are often subtle. There appear to be few metabolic costs associated with chronic Cd

exposure in hard and soft water. Tissue Cd burdens (gills, kidney, liver, and whole body) were the most sensitive indicators of chronic Cd exposure. The Biotic Ligand Model worked well for the unexposed fish in hard and soft water exposures; however, fish that were chronically exposed to Cd did not fit the model well due to changes in gill binding characteristics and acclimatory responses. In addition, there were discrepancies in the maximum number of binding sites (i.e. B_{max} values) amongst studies. These are the first steps in understanding how the Biotic Ligand Model can be made to work. The information contained within this thesis increased our understanding of the chronic effects of waterborne cadmium on juvenile rainbow trout and will provide useful information for the development of Water Quality Guidelines in Canada, the United States and Europe, so as to generate site-specific criteria.

References

Alabaster, J.S. and Lloyd, R. (Editors), 1982. Water Quality Criteria for Freshwater Fish, 2. Food and Agriculture Organization of the United Nations. Butterworths, London, pp. 221-251.

Bengtsson, B.E., Carlin, C.H., Larsson, A. and Svanberg, O., 1975. Vertebral damage in minnows, *Phoxinus phoxinus* L., exposed to cadmium. AMBIO, 4: 166-168.

Benson, W.H. and Birge, W.J., 1985. Heavy metal tolerance and metallothionein induction in fathead minnows: results from field and laboratory investigations. Environ. Toxicol. Chem., 4: 209-217.

Bentley, P.J., 1992. Influx of Zn by channel catfish (*Ictalurus punctatus*): Uptake from external environmental solutions. Comp. Biochem. Physiol., 101C: 215-217.

Bergman, H.L. and Dorward-King, E.J. (Editors), 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. Pensacola FL: SETAC Pr. 114 p.

Bilinski, E. and Jonas, R.E.E., 1973. Effects of cadmium and copper on the oxidation of lactate by rainbow trout (*Salmo gairdneri*) gills. J. Fish. Res. Board Can., 30: 1553-1558.

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.

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

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

Di Toro, D., Allen, H., Bergman, H., Meyer, J., Paquin, P., and Santore, R., 1999. Biotic ligand model of the acute toxicity of metals. I. Technical basis. Environ. Toxicol. Chem. Submitted.

Duncan, D.A. and Klaverkamp, J.F., 1983. Tolerance and resistance to cadmium in white suckers (*Catostomus commersoni*) previously exposed to cadmium, mercury, zinc, or selenium. Can. J. Fish. Aquat. Sci., 40: 128-138.

Farag, A.M., Boese, C.J., Woodward, D.R., and 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.

Gardner, G.R. and Yevich, P.P., 1970. Histological and hematological responses of an estuarine teleost to cadmium. J. Fish. Res. Board Can., 27: 2185-2196.

Giles, M.A., 1984. Electrolyte and water balance in plasma and urine of rainbow trout (*Salmo gairdneri*) during chronic exposure to cadmium. Can. J. Fish. Aquat. Sci., 41: 1678-1685.

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

Hammer, C., 1995. Fatigue and exercise tests with fish. Comp. Biochem. Physiol., 112: 1-20.

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

Janes, N. and Playle, R.C., 1995. Modeling silver binding to gills of rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem., 14: 1847-1858.

Karlsson-Norrgren, L., Runn, P., Haux, C., and Förlin, L., 1985. Cadmium-induced changes in gill morphology of zebrafish, *Brachydanio rerio* (Hamilton-Buchanan), and rainbow trout, *Salmo gairdneri* (Richardson). J. Fish. Biol., 27: 81-95.

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

MacCrae, R.K., Smith, D.E., Swoboda-Colberg, N., Meyer, J.S., Bergman, H.L.,

1998. Copper binding affinity of rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) gills. Environ. Toxicol. Chem., 18: 1180-1189.

Majewski, H.S. and Giles, M.A., 1981. Cardiovascular-respiratory responses of rainbow trout (*Salmo gairdneri*) during chronic exposure to sublethal concentrations of cadmium. Water Res., 15: 1211-1217.

Mallatt, J., 1985. Fish gill structural changes induced by toxicants and other irritants: a statistical review. Can. J. Fish. Aquat. Sci., 42: 630-648.

McDonald, D.G. and Wood, C.M., 1993. Branchial mechanisms of acclimation to metals in freshwater fish. In: J.C. Rankin and F.B. Jensen (Editors), 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.

Morel, F.M.M. and Hering, J.G., 1993. Principles and applications of aquatic chemistry. John Wiley and Sons, New York, N.Y., p. 588.

Muramoto, S., 1981. Vertebral column damage and decrease of calcium concentration of fish exposed experimentally to cadmium. Environ. Pollut. (Series A), 24: 125-133.

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., and Kiessling, A., 1985. The availability of cadmium to perfused rainbow trout gills in different water qualities. Water Res., 19: 427-434.

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., 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. and 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.

Porter, E.L., Kent, R.A., Andersen, D.E., Keenleyside, K.A., Milne, D., Cureton, P., Smith, S.L., Drouillard, K.G., and MacDonald, D.D., 1995. Development of proposed Canadian Environmental Quality Guidelines for cadmium. J. Geochem. Expl., 52: 205-219.

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.

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

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.

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

Scherer, E., McNicol, R.E. and 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. Washington DC: USEPA Office of Water Regulations and Standards. EPA 440/5-85-001.

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 freshwater trout

branchial epithelium and its interference with calcium transport. J. Exp. Biol., 145: 185-197.

Voyer, R.A., Yevich, P.P. and Barszcz, C.A., 1975. Histological and toxicological responses of the mummichog, *Fundulus heteroclitus* (L.) to combinations of levels of cadmium and dissolved oxygen in a freshwater. Water Res., 9: 1069-1074.

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

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

Chapter 2

Cadmium Accumulation, Gill Cd Binding, Acclimation, and Physiological Effects During Long Term Sublethal Cd Exposure in Rainbow Trout

Abstract

Juvenile rainbow trout, on 3% of body weight daily ration, were exposed to 0 (control), 3, and 10 μg l^{-1} Cd (as $Cd(NO_3)_2 \cdot 4H_2O$) in moderately hard (140 mg l^{-1} as $CaCO_3$), alkaline (95 mg l^{-1} as $CaCO_3$, pH 8.0) water for 30 days. Particular attention focused on acclimation, 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. Only the higher Cd concentration caused mortality (30%, in the first few days). The costs of acclimation, if any, in our study were subtle since no significant effects of chronic Cd exposure were seen in growth rate, swimming performance (stamina and U_{Crit}), routine O_2 consumption, or whole body ion levels. Substantial acclimation occurred in both exposure groups, manifested as 11- to 13-fold increases in 96-h LC_{50} values. In water quality regulations, which are based on toxicity tests with non-acclimated fish only, this remarkable protective effect of acclimation is not taken into account. Cd accumulated in a time- and concentration-dependent fashion to 60-120 X (gills), 8-20 X (liver), 2-7 X (carcass), and 5-12 X (whole bodies) control levels by 30 days. Chronically accumulated gill Cd could not be removed by EDTA challenge. These gill Cd concentrations were 20- to 40-fold greater than

levels predicted by the gill-binding model to cause mortality during acute exposure. In short-term gill Cd-binding experiments (up to 70 $\mu g\ l^{-1}$ exposures for 3 h), gill Cd burden increased as predicted in control fish, but was not detectable against the high background concentrations in acclimated fish. In light of these results, Cd uptake/turnover tests were performed using radioactive ^{109}Cd to improve sensitivity. With this approach, a small saturable binding component was seen, but could not be related to toxic response in acclimated fish. Acclimated trout internalized less ^{109}Cd than control fish, but interpretation was complicated by the possibility of radioisotopic exchange and specific activity dilution in the large 'cold' Cd pool on the gills. We conclude that gill Cd burden is not predictive of mortality in acclimated fish, that the present gill modelling approach does not work in acclimated fish, and that longer term ^{109}Cd turnover studies are needed for this purpose.

Introduction

Cadmium is a non-essential element that can have severe toxic effects on aquatic organisms when present in excessive amounts (reviews: Alabaster and Lloyd, 1982; Sorensen, 1991). In fish, Cd can damage gills (Gardner and Yevich, 1970; Bilinski and Jonas, 1973; Voyer *et al.*, 1975), result in skeletal deformities (Bengtsson *et al.*; 1975, Muramoto, 1981), and disturb calcium balance (Roch and Maly, 1979; Reid and McDonald, 1988; Bentley, 1992; Wicklund-Glynn *et al.*, 1994). The latter effect represents the key mechanism of acute toxicity: fatal hypocalcaemia occurs because Ca^{2+} uptake across the gills is irreversibly blocked, apparently by non-competitive inhibition of an essential transport enzyme, high affinity Ca^{2+} ATPase (Verbost *et al.*, 1987, 1989).

Salmonids are amongst the most sensitive of teleosts to waterborne Cd. In rainbow trout, acute (2-7 day) LC_{50} values vary considerably amongst studies, but most are in the low to mid $\mu g\ l^{-1}$ range (e.g. Ball, 1967; Chapman, 1978; Chapman and Stevens, 1978; Roch and Maly, 1979; Kumada *et al.*, 1980; Majewski and Giles, 1981; Pascoe and Beattie, 1979; Davies *et al.*, 1993), with occasionally higher values in waters of extreme alkalinity and hardness (Calamari *et al.*, 1980; Pascoe *et al.*, 1986). The principal variable controlling acute toxicity appears to be water hardness (i.e. calcium and magnesium levels, with the former being more important - Carrol *et al.*, 1979; Davies *et al.*, 1993). Indeed, hardness is recognized as an ameliorative factor in some regulatory criteria (e.g. U.S. E.P.A., 1986; C.C.M.E., 1995). Additional factors affecting the

acute toxicity of Cd include temperature, dissolved oxygen, pH, salinity, and dissolved organic matter (reviewed in Alabaster and Lloyd, 1982; Sprague, 1987). Many of these same factors also alter the uptake of Cd across the gills (Pärt and Wikmark, 1984; Pärt *et al.*, 1985) and the binding of Cd to low affinity (Reid and McDonald, 1991) and high affinity sites on the gill surface (Playle *et al.*,1993a,b; Hollis *et al.*, 1996, 1997).

Recently, there has been great interest in using high affinity gill surface binding models, such as those introduced by Playle *et al.* (1993a,b) and Janes and Playle (1995), as a tool for predicting metal toxicity as a function of water quality, so as to generate site-specific criteria (Bergman and Dorward-King, 1997; Renner, 1997). In brief, these models derive from the original framework proposed by Pagenkopf (1983) and involve experimental characterization of the gill surface as a metal-binding ligand with a fixed number of receptor sites (B_{max}) and an average conditional metal-gill equilibrium constant ($K_{Cd\text{-}gill}$). When these values, together with measured water chemistry, are fed into standard aqueous geochemical modelling programs such as MINEQL+ (Schecher and McAvoy, 1994), they predict the degree of saturation of the gill sites with the metal, which in turn is directly predictive of metal toxicity in that particular water chemistry. To date, these models have been applied only to acute toxicity, and the experimental data for these models have been generated using only short term exposures (2-3 h) in synthetic soft water of extremely low hardness and alkalinity (Playle *et al.*, 1993a,b; Janes and Playle, 1995).

The more relevant issue is whether such approaches will work in natural situations where water is often harder and more alkaline, and fish are chronically exposed to sublethal metal levels for long periods of time. For example, Hollis *et al.* (1996, 1997) found that as longer exposure times were employed, gill Cd burden gradually increased, and calcium (hardness) became less effective in keeping Cd off the gills. Indeed, in even longer exposures of trout to sublethal Cd in harder, more alkaline water, Giles (1988) and Farag *et al.* (1994) reported gill Cd burdens which increased to well above the maximum binding site number used in the models of Playle *et al.* (1993b) and Hollis *et al.* (1997). In addition, the phenomenon of acclimation during chronic exposure to Cd is well documented (Pascoe and Beattie, 1979; Duncan and Klaverkamp, 1983; Benson and Birge, 1985; reviewed by McDonald and Wood, 1993), which suggests a change in the metal binding properties of the gills.

The primary objective of the present study was to determine if the current gill-binding model, developed in soft water for acute exposures, could be extended to chronic metal exposures in hard water to predict gill accumulation and therefore long-term toxicity to fish. We examined changes in gill Cd burden, and the acute Cd-binding properties of the gills, during chronic sublethal exposure of juvenile rainbow trout in moderately hard, moderately alkaline Lake Ontario water. Chronic exposure levels of 3 and 10 μg Cd l^{-1} for 30 days were selected, in the hope that at least one of these would induce acclimation, as detected by changes in LC_{50} values. Additional goals were to characterize Cd

accumulation in other compartments (liver, whole body) and possible sublethal effects and costs of acclimation. We hypothesized that these costs would be reflected as changes in whole body ion content, as well as impairments in growth, routine metabolism, and exercise performance. The latter were examined in light of reports that sublethal Cd exposure alters routine locomotor activity levels (Benoit *et al.*, 1976; Ellgard *et al.*, 1978;), cardio-respiratory and hematological variables (Majewski and Giles, 1981), and foraging success (Scherer *et al.*, 1997).

Materials and Methods

Fish Holding Conditions

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}, dissolved organic matter (DOM) = 3 mg l^{-1} or 0.06 µmol 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 two 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).

Exposure System

After two weeks in holding tanks, 280 fish 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 $Cd(NO_3)_2 \cdot 4\ H_20$ (Fisher Scientific, Nepean, Ontario), was delivered to a mixing head-tank *via* mariotte bottles (Mount and Brungs, 1967) to achieve desired Cd concentrations in exposure tanks. Exposure tanks were spiked on the first day of Cd exposure to

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 = 0.7 ± 0.5 μg l^{-1} or 0.006 ± 0.004 (12) μmol l^{-1} Cd; mean ± 1 SEM (*N* = number of H_2O samples taken)], (ii) low cadmium [3.0 ± 0.7 μg l^{-1} or 0.03 ± 0.006 (10) μmol l^{-1} Cd], or (iii) high cadmium [10.0 ± 0.6 μg l^{-1} or 0.10 ± 0.005 (10) μmol l^{-1} Cd] for 30 days in dechlorinated Hamilton tap water. The three treatment conditions each had two replicates so that *N* = 560 fish per treatment. The sublethal exposure concentrations of 3 and 10 μg l^{-1} Cd were chosen based on an initial 24-h Cd LC_{50} measurement of approximately 50 μg l^{-1} in our water quality.

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 for Na, Ca, and Cd content. Fish from each treatment tank were bulk weighed every 10 days. All of the fish from the tank (one tank at a time) were removed and put in a tared sieve placed inside a bucket containing water from the exposure tank. The bucket was weighed, fish were briefly removed using the sieve, and the bucket reweighed. The mass of the fish was calculated from the difference between the mass of the bucket plus sieve with and without fish.

Specific growth rates (SGR) were determined from bulk weights from individual treatment tanks taken 4-5 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 SAS JMP (SAS Institute Inc., Version 2.0.5) which provides 95% confidence limits for growth.

Six fish from each tank were subsampled at day 0, 2, 10, 20, and 30 and gills, liver, and remaining carcass assayed for cadmium and ion content. Fish were sacrificed and both sets of gills and the liver were excised; gills were rinsed for 10 seconds in 100 ml of dechlorinated Hamilton tap water. All tissues plus remaining carcass were frozen in liquid nitrogen for later analysis of Cd and ion content.

Testing

Exercise performance

Fish were not fed the day of swimming tests. The protocol of McDonald *et al.* (1998) was used as a stamina test; the method employs a fixed velocity and exhaustion as the end point. Fish were swum in a flume in groups of 10 against a current of 57 cm s^{-1} (~ 7 body lengths per second) until exhaustion occurred. Fish were considered exhausted when they were impinged against the rear screen of the flume and would not swim after prodding. Fatigued fish were removed from the swimming flume and fork length and weight were recorded. Because of size differences amongst individuals, sprint times were corrected to a reference body length of 7 cm and the time to 50% fatigue ($\pm$ 95% C.L.) was calculated, from 20 fish from each treatment, by linear regression in SAS JMP of

probit fatigue versus log time.

Critical swimming speed (U_{Crit}; Brett, 1964) was also determined for control and Cd-acclimated fish using a modified 100 l Beamish-style swimming tunnel (Beamish *et al.*, 1989). Control fish were swum in the control hard water while separate groups of 3 and then 10 μg l^{-1} Cd-acclimated fish were swum in the presence and absence of 3 and 10 μg l^{-1} Cd, respectively (N = 8 for each swim test). Fish were allowed to adjust to the swimming tunnel 1 h before the test, with the water current set to 10 cm s^{-1}. The water velocity was then increased by increments of 10 cm s^{-1} every 40 minutes, until the fish became exhausted. The fish were considered exhausted when they became impinged on the rear screen of the tunnel and would not swim after manual prodding. Fish were blotted dry and fork length and weight were determined once exhaustion occurred. U_{Crit} (critical swimming speed) was determined for each fish using the equation (Brett, 1964):

$$U_{Crit} = V_f + [(T/t) \times dV]$$

where "U_{Crit}" is in cm s^{-1}, "V_f" is the velocity prior to the velocity at which exhaustion occurred (the last velocity which was swum for the entire 40 minute period), "dV" is the velocity increment (10 cm s^{-1}), "t" is the time swum at each velocity (40 minutes), and "T" is the time swum at the final velocity before exhaustion. Critical swimming speed was then converted to body lengths s^{-1} by

dividing U_{Crit} by the fork length of the fish.

Routine metabolism

Routine oxygen consumption, representing 'in-tank' metabolic rate, was measured 2 and 6 h after feeding after the 30 day exposure period. The surface of each tank was sealed with a tight-fitting, transparent lid of heavy plastic, and the flow of fresh water and aeration to the tanks was stopped 2 h after the second feeding period of the day. The water was then recirculated by means of a pump (Little Giant Company; 10 l min^{-1}) which drew water from the bottom of the tank and returned it back into the upper region of the tank. In-tank Po_2 levels were monitored continuously for one hour with an oxygen electrode (Cameron E101) connected to an oxygen meter (Cameron OM-200). Oxygen consumption readings were completed before the Po_2 in any one tank had dropped below 100 torr. The tank was then unsealed, water and air circulation were restored, and the regular evening feeding was performed. This procedure was repeated 6 h after the evening feeding. Oxygen consumption rates, measured while tanks were sealed, were calculated from the mean rate of Po_2 decline (4 readings taken over an hour) and the water oxygen solubility coefficients of Boutilier *et al.* (1984). The data were weight-corrected using the weight exponent of 0.824, taken from Cho (1990).

Acclimation

A 96 hour LC_{50} trial was performed after 30 days exposure to assess possible acclimation 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 (250 ml min^{-1}) of dechlorinated Hamilton tap 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 1 ± 0.4 (15) µg l^{-1} (control with no Cd added), 17 ± 1 (15) µg l^{-1}, 37 ± 4 (15) µg l^{-1}, 71 ± 0.4 (15) µg l^{-1}, 262 ± 9 (15) µg l^{-1}, and 490 ± 14 (15) µg l^{-1}. Dead fish were removed when movement ceased and times of mortality were recorded. LC_{50} values (± 95% C.L.) were determined by log probit analysis (Finney, 1971).

Acute gill-Cd binding

Fish were not fed the day of gill binding experiments. Gill metal uptake/turnover of control and Cd-acclimated trout was determined by exposing the fish for 3 hours, after the 30 day Cd exposure, to one of four different incremental Cd concentrations plus control. Six fish from each treatment (0, 3, and 10 µg l^{-1}) were placed randomly into 15 l plastic buckets having aeration and flow-through (250 ml min^{-1}) of dechlorinated Hamilton tap water at the appropriate Cd level, as added by a mariotte bottle. Final Cd concentrations of the five treatments were 0 ± 0.1 (3) µg l^{-1}, 10 ± 0.3 (3) µg l^{-1}, 30 ± 1 (3) µg l^{-1}, 60 ± 0.3 (3) µg l^{-1}, and 70 ± 2 (3) µg l^{-1}. After the 3 h exposure, gills were excised,

rinsed for 10 s in 100 ml of dechlorinated tap water to displace any loosely bound Cd, and later analyzed for Cd concentrations (see Tissue and Water Analyses).

A second gill Cd uptake/turnover experiment was run using the Cd radioisotope ^{109}Cd. Twelve fish from each treatment (control, 3 μg l^{-1}, and 10 μg l^{-1} Cd) were placed into nine clear plastic bags, placed in a water bath to maintain temperature, containing 3 l of aerated, dechlorinated tap water. Each treatment group was exposed to 10 ± 4 (6) μg l^{-1}, 50 ± 6 (6) μg l^{-1}, or 100 ± 8 (6) μg l^{-1} total Cd added as $Cd(NO_3)_2 \cdot 4\ H_20$ (Fisher Scientific, Nepean, Ontario) with 3 μCi l^{-1} ^{109}Cd added as $CdCl_2$ (specific activity = 2.75 mCi mg^{-1}; acquired from New England Nuclear, Boston, MA). Water samples (5 ml) were taken at the beginning and end of the 3 h static exposure. Gills, blood, and whole bodies (remaining carcass) of four fish from each treatment were subsampled at 1, 2, and 3 h. Gills were removed, rinsed, acid digested, and later analyzed for total Cd and radioactivity due to ^{109}Cd (see Tissue Analysis). Blood samples (~60 μl) were collected, by caudal severance, into hematocrit capillary tubes and analyzed for radioactivity (described below). Remaining carcasses were placed in 20 ml polyethylene vials and analyzed for ^{109}Cd.

A metal-ligand complexation experiment was also run using the 3 μg l^{-1} Cd-acclimated fish. These trout were exposed to increasing concentrations of ethylenediaminetetraacetic acid disodium salt (EDTA; BDH, Toronto, Ontario) for 3 h in the continuing presence of 3.0 ± 0.1 (5) μg l^{-1} Cd . Nominal EDTA concentrations were 0, 60, 110, 220, and 450 μg l^{-1}, corresponding to molar

levels of 0.0, 0.2, 0.3, 0.6, and 1.2 μmol l^{-1}, respectively. Six fish from the chronic (30 d) exposure to 3 μg Cd l^{-1} were placed randomly into each of the five 15 l plastic buckets containing aerated, dechlorinated Hamilton tap water. After the 3 h static exposure, gills were extracted, rinsed, and later analyzed for Cd concentrations (as above).

Tissue and Water Analyses

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

Gills, livers, and remaining carcass were thawed, weighed, and then digested in 1-10 times their weight of 1 N HNO_3 (TraceMetal Grade HNO3; Fisher Scientific, Nepean, Ontario) for 3 hours at about 80°C. Digests were shaken, left to settle for 10 minutes, then the supernatant was diluted 10 times with deionized water (18 mgohm; Nanopure II; Sybron/Barstead, Boston, MA). Gill, liver, and carcass 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 second drying time at 90°C, 12 s at 120°C, and 4 s at 1800°C during which Cd was read.

Whole body Cd was calculated based on the data for individual fish at each sample time using the following equation:

$$WB = [(G \times gwt) + (L \times lwt) + (C \times cwt)]/fwt$$

where "WB" is whole body Cd accumulation (μg Cd g^{-1} wet tissue), "G" is gill Cd accumulation (μg Cd g^{-1} wet tissue), "L" is liver Cd accumulation (μg Cd g^{-1} wet tissue), "C" is carcass Cd accumulation (μg Cd g^{-1} wet tissue), "gwt" is the weight of the gills (g), "lwt" is the weight of the liver (g), "cwt" is the weight of the carcass (g), and "fwt" is the weight of the fish (g). Gills, liver, and carcass represent 4%, 1%, and 95% of the total whole body weight, respectively.

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

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 K_D and the total B_{max} of the gill were then determined from the slope and *x*-intercept of the Scatchard plot,

respectively.

Water Na and Ca concentrations were measured using the Varian AA-1275 operated in standard 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). Water pH was measured using a Radiometer PHM71b meter with GK2401C combination electrode. Dissolved organic matter (DOM) levels were measured on the Rosemount Analytical DC-180 automated total organic carbon analyzer (Folio Instruments, Kitchener, Ontario). Concentrations of different cadmium species in the water were calculated using the MINEQL+ aquatic geochemical program (Schecher and McAvoy, 1994) and measured water chemistry.

Statistics

Data have been expressed as means ± 1 S.E. (*N*) except in the case of LC_{50}, specific growth rates, and swimming times where means ± 95% C.L; for the latter, values were considered significantly different if 95% C.L. did not overlap. For all other data, ANOVA followed by a Student-Newman-Keuls procedure was used for multiple comparisons of mean values. Regression analysis was performed on the EDTA gill-binding data. A fiducial limit of $P < 0.05$ was used throughout.

Results

Effects of Exposure

Mortality over 30 days was less than 1% in both the control and 3 μg Cd l^{-1} exposures. Fish exposed to 10 μg Cd l^{-1} exhibited 30% mortality within the first three days which ceased thereafter (i.e. acute toxicity only; Fig. 1). In no instance was the growth of the fish decreased with Cd exposure (Fig. 2).

Metal accumulation in all tissues increased steadily over the 30 d in both Cd-exposed groups and was directly related to exposure concentration (Fig. 3A-D). Cadmium concentrations were greatest in gills (Fig. 3A), followed by liver (Fig. 3B) and carcass (Fig. 3C); whole body concentrations were only slightly higher than carcass concentrations (Fig. 3D). Gill Cd levels increased 60 and 120 times from initial (day 0) values (0.10 ± 0.05 (6) μg Cd g^{-1} wet tissue) for low and high Cd exposures, respectively, after 30 days of exposure (Fig. 3A). Gill concentration factors from the water were 2000 X and 1200 X for the 3 μg l^{-1} and 10 μg l^{-1} Cd exposures, respectively. Liver Cd concentrations increased 8 and 20 times from initial values (0.13 ± 0.08 (6) μg Cd g^{-1} wet tissue; Fig. 3B) with concentration factors of 300 X and 200 X for low and high Cd exposures, respectively. Remaining carcass Cd levels increased 2 and 7 times from initial values (0.10 ± 0.04 (6) μg Cd g^{-1} wet tissue; Fig. 3C) with a concentration factor of 70 X for both the 3 μg l^{-1} and 10 μg l^{-1} Cd exposures. Whole bodies increased 5 and 12 times from initial values (0.10 ± 0.04 (6) μg Cd g^{-1} wet tissue; Fig. 3D)

with concentration factors of 500 X and 1200 X for low and high Cd exposures, respectively.

Acclimation

Acclimation to Cd was observed since the 96-h LC_{50} demonstrated dramatically increased tolerance to Cd, as indicated by 11- to 13-fold increases in the LC_{50} values from the control value of 22 ± 12 µg Cd l^{-1}, a highly significant difference (Fig. 4). There were no significant differences in the extent of acclimation between the two Cd-exposed groups by the LC_{50} criteria.

Physiological Effects and Costs of Chronic Exposure

No treatment or time related effects were seen in whole body Na^+ and Cl^- concentrations which averaged 49 ± 5 (78) mmol kg^{-1} and 37 ± 3 (78) mmol kg^{-1}, respectively. Whole body Ca^{2+} was slightly depressed, though in an irregular fashion, decreasing from control levels of 105 ± 4 (6) mmol kg^{-1} to 64 ± 2 (6) mmol kg^{-1} on day 20 in the low Cd (3 µg l^{-1}) exposure, and to 94 ± 7 (6) mmol kg^{-1} on day 30 in the high Cd (10 µg l^{-1}) exposure. There were no other significant differences.

In-tank metabolic rate measurements showed no significant differences in rates of routine oxygen consumption 2 h and 6 h after feeding in Cd-exposed fish (Table 1). Swimming stamina was not significantly affected by exposure to Cd for 30 days; however, there was a trend towards improved stamina in the metal-

exposed fish (Table 2). Critical swimming speeds (U_{Crit}) were also not significantly different between control fish and Cd-acclimated fish, when Cd-exposed fish were tested in either the presence or absence of Cd (Table 3).

Cd Uptake/Turnover in Gills of Acclimated Trout

Prior to these acute Cd exposure tests, the gills contained 0.12 ± 0.02 μg Cd g^{-1} in the control group (0 μg Cd l^{-1}), 3.91 ± 0.71 μg Cd g^{-1} in the fish exposed to 3 μg Cd l^{-1} for 30 days, and 7.85 ± 1.59 μg Cd g^{-1} in the fish exposed to 10 μg Cd l^{-1} for 30 days. After 3 h acute exposure to 10, 30, 60, or 70 μg Cd l^{-1}, there was no significant increase in gill Cd concentration in any of the three groups (Fig. 5). However, inspection of the data for the control group on a finer scale (Fig. 5, inset) indicated a slight upward trend. Clearly, under the water chemistry conditions prevailing in the present experiments, such a slight increase over 3 h is at the limit of resolution, even when background gill Cd burden is low. When the background is greatly elevated (> 40-fold) as in the fish chronically exposed to Cd, such an increase would be undetectable using 'cold' techniques.

In order to test whether the background concentration of Cd in the gills of Cd-acclimated trout was easily removable, an acute test (3 h) was performed with increasing concentrations of the chelating agent EDTA on the trout which had been exposed to 3 μg Cd l^{-1} for 30 days (Fig. 6). There was, in fact, no decrease in gill Cd burden caused by EDTA exposure, but rather the opposite, a trend for increasing gill Cd concentration with increasing levels of EDTA. None

of the individual means were significantly different from the 0 μg EDTA l^{-1} treatment, but the overall regression of gill [Cd] *versus* EDTA concentration was significant (r^2 = 0.88, $P < 0.05$). Thus the gill Cd burden accumulated during chronic exposure is not easily removable, and the presence of EDTA appears to favour further Cd uptake in the continuing presence of 3 μg Cd l^{-1}.

In light of the results of Fig. 5, Cd uptake/turnover tests were performed using radioactive ^{109}Cd to achieve greater resolution, at total Cd concentrations of 10, 50, and 100 μg l^{-1}, in an attempt to differentiate Cd binding by the gills of control and Cd-acclimated fish. Samples were taken at 1, 2, and 3 h during acute exposure. This approach did yield detectable uptake values. In all groups, uptake tended to increase with increasing acute exposure concentrations of radiolabelled Cd (Fig. 7). However, control fish reached approximate equilibrium by 3 h with about 0.17 μg g^{-1} of 'new' waterborne Cd bound to the gills (Fig. 7A). This value was independent of the acute exposure concentration (Fig. 8), suggesting that saturation had occurred. Trout which had been acclimated to 3 μg Cd l^{-1} for 30 days also approached equilibrium by 3 h (Fig. 7B), with saturation at about 0.26 μg g^{-1} of 'new' Cd bound to the gills (Fig. 8). In contrast, fish which had been acclimated to 10 μg Cd l^{-1} for 30 days exhibited much higher uptake rates of 'new' Cd at the highest exposure concentration (Fig. 7C), with no evidence of equilibrium or saturation by 3 h (Fig. 7C). At this time, 'new' Cd accumulation had reached about 0.47 μg g^{-1}, approximately 3-fold the value in the control group.

Scatchard analysis of Cd uptake/turnover for the 3 h exposure to radioactive ^{109}Cd (Fig. 8) gave approximate K_D values (dissociation constants) of 6.5 μg l^{-1} (= 0.057 μmol l^{-1} or log $K_{Cd\text{-}gill}$ = 7.2), and 17.3 μg l^{-1} (= 0.154 μmol l^{-1} or log $K_{Cd\text{-}gill}$ = 6.8) for fish chronically exposed to 0 and 3 μg Cd l^{-1} respectively. Corresponding B_{max} values (capacity) were 0.18 μg Cd g^{-1} (= 1.61 nmol g^{-1}) and 0.29 μg Cd g^{-1} (= 2.59 nmol g^{-1}). Scatchard analysis could not be performed on the data from the 10 μg Cd l^{-1} exposure group because of the lack of saturation, but inspection suggests a continuing trend of higher K_D (i.e. lower log $K_{Cd\text{-}gill}$ or affinity) and higher B_{max} values at this higher exposure level.

Based on internal appearance of ^{109}Cd, significant amounts of 'new' Cd penetrated into the blood and whole body during these acute exposures. Samples taken at 1 and 2 h (data not shown) as well as 3 h (Table 4) indicated that the blood compartment had reached equilibrium by 3 h, whereas the remainder of the body was continuing to accumulate 'new' Cd at this time. In contrast to the gills, significantly less 'new' Cd was accumulated in the blood and whole body by Cd-acclimated fish than by the control fish. Based on the total distribution of ^{109}Cd at 3 h in the acute exposures to 100 μg l^{-1} of radiolabelled Cd, 27% of 'new' Cd was found on the gills in control fish, 46% in those acclimated to 3 μg Cd l^{-1}, and 58% in those acclimated to 10 μg Cd l^{-1}. The reverse trend was seen in the blood (14%, 8%, and 3% respectively) and in the carcass (59%, 46%, and 39% respectively).

Discussion

Environmental Relevance of Acclimation

Cadmium concentrations of 3 and 10 μg l^{-1} used in our experiment were within or slightly above environmentally realistic concentrations (up to 5 μg l^{-1}) measured in North American surface waters (reviewed in Spry and Wiener, 1991; C.C.M.E, 1995). Furthermore they were relevant to U.S. E.P.A. freshwater quality criteria for aquatic life of 5.7 μg l^{-1} and 1.5 μg l^{-1}, recommended as limits for acute and chronic cadmium exposures at a water hardness of 140 mg l^{-1} as $CaCO_3$ (U.S. E.P.A., 1986). For comparable hardness, the Canadian guideline is 1.3 μg l^{-1} and the European guideline (for rainbow trout) is 0.5 μg l^{-1}, both intended to protect against chronic toxicity (Alabaster and Lloyd, 1982).

At this hardness, the 96-h LC_{50} value of control trout (22 μg Cd l^{-1}) in the present study was in the expected range based on previous reports for freshwater *Oncorhynchus mykiss* at a variety of hardness levels (see Introduction for references). The large increase (12-fold) in LC_{50} values observed with acclimation to Cd after 30 days (Fig. 4) was greater than in most previous studies (reviewed by McDonald and Wood, 1993), but is not entirely unprecedented. Pascoe and Beattie (1979) reported that the 48-h LC_{50} increased from <100 μg Cd l^{-1} to 1500 μg Cd l^{-1} for rainbow trout alevins pretreated for 7 days at 10 μg Cd l^{-1}. This remarkable protective effect of acclimation is not taken into account in water quality regulations because they are based on toxicity tests with non-

acclimated fish only. In our study, the initial mortality of 30% of the trout exposed to 10 μg Cd l^{-1} (Fig. 1) means selection for 'fitter' fish may have contributed to the apparent acclimation phenomenon; however, the same degree of acclimation was seen in trout chronically exposed to 3 μg Cd l^{-1}, where mortality was not a complicating factor (Fig. 1).

Mechanisms of Acclimation

Three possible mechanisms that may explain the increased tolerance (increased LC_{50}) of metal-acclimated fish could be decreased uptake of Cd at the gills, increased storage and detoxification of Cd, or increased resistance of gill processes that are sensitive to metals, such as ion transport (reviewed in McDonald and Wood, 1993). The first possibility of decreased uptake has been ruled out in our study (assessed below). We have also demonstrated that the second mechanism of increased storage and detoxification is a possibility for acclimation to Cd (assessed below). The third mechanism of increased resistance of gill processes is a likely possibility for Cd acclimation; however, this requires further direct investigation.

Costs of Acclimation

The costs of acclimation, if any, in our study were subtle since no significant effects of chronic Cd exposure were seen in growth (Fig. 2), metabolism (Table 1), swimming performance (Tables 2 and 3), or whole body

ions, except for slight, irregular depressions in whole body calcium. This latter effect likely reflects the key toxic action of Cd, the inhibition of active Ca^{2+} uptake across the gills (see Introduction). Farag *et al.* (1994) reported similar findings for juvenile rainbow trout exposed for 21 d to 2.2 µg Cd l^{-1} (in a mixed metal solution of Cd, Cu, and Pb) with decreased survival and loss of scales (an important calcification site) but no significant effects on growth with Cd exposure. Kumada *et al.* (1980) and Davies *et al.* (1993) also found no adverse effects on growth of juvenile rainbow trout exposed for 70-100 days to 4 µg Cd l^{-1}. Similarly, in adult rainbow trout, Giles (1988) reported no growth inhibition during 178 day exposures to 3.6 and 6.4 µg Cd l^{-1}. No effects on survival, development, or reproduction of bluegill were found by Eaton (1974) in an 11 month exposure to 31 µg Cd l^{-1}. First-generation brook trout exposed to 3.4 µg Cd l^{-1} showed no growth impairment; however, growth of second- and third-generation trout offspring was significantly reduced when exposed to 3.4 µg Cd l^{-1} (Benoit *et al.*, 1976). Swimming performance, represented by foraging ability on rainbow trout fingerlings, of adult lake trout was impaired by chronic (277 days) exposure to 0.5 µg Cd l^{-1}, but no impairment of predator escape by the fingerling rainbow trout from the lake trout was observed with Cd exposure (Scherer *et al.*, 1997). Several studies have suggested that chronic sublethal Cd exposure increases spontaneous activity, ventilation, heart rate, and hematocrit (Benoit *et al.*, 1976; Ellgard *et al.*, 1978; Majewski and Giles, 1981) although the mechanisms involved are unclear. In the present study, the tendency for improved swimming

stamina with chronic Cd exposure (Table 2), though not significant, may reflect these phenomena.

Internal Cadmium Distribution

In agreement with several other studies on chronic Cd exposure to trout (Benoit *et al.*, 1976; Roberts *et al.*, 1979; Sangalang and Freeman, 1979; Kumada *et al.*, 1980; Giles, 1988; Harrison and Klaverkamp, 1989; Farag *et al.*, 1994), gills and liver accumulated more Cd (per unit weight) than did the carcass or whole body over the 30 day exposure (Fig. 3). Gill Cd burdens increased 60-fold and 120-fold and liver Cd burdens increased 8- and 20-fold for 3 μg l^{-1} and 10 μg l^{-1} Cd exposures, respectively. Reported tissue Cd levels (absolute values) vary greatly among studies, probably as a function of water hardness and other factors, but the relative increases are comparable. Absolute levels in the present juvenile trout were similar to those reported by Giles (1988) in adult rainbow trout exposed for a similar period at similar water Cd concentrations, but at 40% lower water hardness. Several of the above mentioned studies have also demonstrated equal or higher concentrations of Cd (relative to gills or liver) in kidneys of chronically exposed trout. Benoit *et al.* (1976), Kumada *et al.* (1980), and Harrison and Klaverkamp (1989) found that kidney Cd levels depurated more slowly than other compartments, remaining elevated long after the trout were returned to Cd-free water. Thus, the kidney also appears to be a sensitive organ for Cd accumulation and represents an important means of Cd storage.

Modelling Cadmium Binding to Fish Gills and Toxicity

Recently, there has been great interest in using high affinity gill surface binding models as a tool for predicting metal toxicity as a function of water quality, so as to generate site-specific criteria (see Introduction for references). These models involve experimental characterization of the gill surface as a metal-binding ligand with a fixed number of receptor sites (B_{max}) and an average conditional metal-gill equilibrium constant ($K_{Cd\text{-}gill}$). The models are used to predict the degree of saturation of the gill sites with the metal, which in turn is directly predictive of metal toxicity in that particular water chemistry. To date, these models have been applied only to acute toxicity, and the experimental data for these models have been generated using only short term exposures (2-3 h) in synthetic soft water of extremely low hardness and alkalinity (Playle *et al.*, 1993a,b; Janes and Playle, 1995).

One of the most important conclusions of the present study is that the high affinity gill surface binding model developed by Playle *et al.* (1993a,b) actually does work for short-term (3 h) Cd binding to trout gills in the moderately hard, moderately alkaline water used in the present study. However, as elaborated subsequently, an equally important conclusion is that this modelling approach breaks down when fish have been chronically exposed to sublethal Cd levels. As a guide to further discussion Table 5 summarizes the speciation of Cd in our testwaters, as predicted by the MINEQL+ aquatic geochemical program

(Schecher and McAvoy, 1994), assuming that the dissolved solid otavite does not form due to kinetic limitations. Between 37% and 57% of the total Cd consisted of the free Cd^{2+} ion (3 and 100 μg l^{-1} exposures, respectively).

The inset of Fig. 5 illustrates the increase in gill Cd predicted by the model in the present water chemistry, using the appropriate log $K_{Cd\text{-}gill}$, $K_{Ca\text{-}gill}$, etc. values taken directly from the studies of Playle *et al.* (1993b) on fathead minnows in synthetic soft water of extremely low hardness and alkalinity. Considering the difference in species and water chemistry, together with the fact that the present measurements were close to the limit of resolution, agreement of the model prediction with measured gill values is not unreasonable. When resolution was increased by the use of ^{109}Cd, similar absolute increases in gill Cd burden were recorded (control curve of Fig. 8). Reversing the process, and applying Scatchard analysis to the control curve of Fig. 8, yielded a log $K_{Cd\text{-}gill}$ value of about 7.6 expressed as ionic Cd^{2+} (7.2 expressed as total Cd) and a B_{max} value of 0.18 μg Cd g^{-1} gill tissue (1.61 nmol g^{-1}). In reasonable agreement, Playle *et al.* (1993b) determined a log $K_{Cd\text{-}gill}$ value of 8.6 and B_{max} value of 0.26 μg g^{-1} (2.27 nmol g^{-1}) in fathead minnow exposed in very soft water where virtually all Cd (98.6%) existed as the free ion. Furthermore, in accord with theory (see Introduction), saturation of Cd-binding sites for the control fish of Fig. 8 occurred in the range which was toxic (LC_{50} = 22 μg Cd l^{-1} in the control series; Fig. 4).

However, the breakdown of the modelling approach based on gill surface binding (at least in its present format) is amply illustrated by the data in Figs. 5

and 8 for the groups chronically exposed to 3 and 10 μg Cd l^{-1}. Firstly, over 30 days these fish have accumulated 20-40 fold more Cd (4-8 μg Cd g^{-1}) than needed to saturate the acute B_{max} value (0.18 μg Cd g^{-1}), without dying (Fig. 5)! It might be argued therefore that in acclimated fish, only 'new Cd' bound to the gills during an acute challenge is relevant to toxicity, an amount that can only be determined using the ^{109}Cd technique. However, when this technique was applied, an acute 3 h challenge with 50 μg Cd g^{-1}, which was now well below the toxic range due to acclimation (i.e. LC_{50} = 250-300 μg Cd l^{-1} in acclimated fish; Fig. 4), added enough 'new Cd' to the gills to saturate the acute B_{max} value. An additional further complication is that the Scatchard analyses of the data in Fig. 8 suggest that both the log $K_{Cd\text{-}gill}$ and B_{max} values for 'new Cd' were altered as a result of acclimation, the former decreasing, and the latter increasing, a point considered further below.

The finding that gill Cd burden can increase during chronic exposure to many-fold saturation of the B_{max} value for acute toxicity is supported by several other investigations showing high, non-lethal gill Cd accumulation in moderately hard, alkaline water (Giles, 1988; Farag *et al.*, 1994). MINEQL+ modelling indicates that in the present study, the competitive action of high water Ca^{2+} (1 mmol l^{-1} = 40 mg l^{-1}) is the principal influence keeping Cd off the gills during acute exposures. A possible explanation may be related to time-dependent protection by calcium. Hollis *et al.* (1997) showed that Ca (1.13 mmol l^{-1} = 45 mg l^{-1}) initially (within 3 h) protected against Cd toxicity and gill accumulation in trout exposed to

16 μg l^{-1} Cd; however, after 1 day of exposure to Cd, Ca no longer kept Cd off of the gills - i.e. fish exposed to both Cd+Ca accumulated equal amounts of Cd on their gills compared to fish exposed to Cd only at this time. As time progressed, gill Cd accumulation continued in the presence of Ca, reaching about 8-fold saturation of the acute B_{max} value by 7 days. This observation illustrates a likely limitation of the current modelling approach for acute exposures - that it assumes equilibrium, and has no kinetic components.

The large amount of slowly accumulated Cd ('chronic pool') may well be in a different compartment than the small amount ('acute pool') bound during acute exposure. The results of the EDTA experiment (Fig. 6) support these conclusions. EDTA was ineffective in removing the large chronic Cd pool, and indeed tended to elevate total gill Cd burden. The latter result was unexpected but may reflect the fact that Ca removal from the gills by EDTA actually facilitated Cd uptake.

Interpretation of Radio-isotopic Cadmium Uptake

Two clear conclusions of the present study are (i) that total gill Cd burden is not diagnostic of toxicity in chronically exposed fish, and (ii) that any further attempts at understanding gill Cd binding in such fish must involve radiotracers, to distinguish the small 'acute pool' against the high background of the 'chronic pool'. However interpretation of radiotracer binding in metal pre-exposed fish is complicated because unlike the 'cold' net uptake approach, ^{109}Cd uptake may

involve both net uptake and isotopic displacement. - i.e. exchange of ^{109}Cd for accessible 'cold' ^{112}Cd on the gill surface. The latter, for example, is one possible explanation for the greater acute ^{109}Cd uptake, and apparent change in log $K_{Cd\text{-}gill}$ and B_{max} values, in the gills of fish chronically exposed to 3 or 10 μg l^{-1} Cd (Fig. 8). Cd-acclimated fish may appear to have more uptake or more sites or altered affinity simply because they have more 'cold' ^{112}Cd available for radioisotopic exchange.

In consequence, two interpretations are possible for the larger 'new Cd' uptake, as detected by ^{109}Cd, on the gills of Cd-acclimated trout (Fig. 8) and correspondingly lower 'new Cd' uptake into the blood and carcass (Fig. 7, Table 4). The first is that it is a real phenomenon, and that an improved 'barrier' function of the gills minimizes further internal Cd loading during challenges as part of the mechanism of acclimation. The second is that it is an artefact of the presence of more exchangeable 'cold' Cd on the gills of Cd-acclimated fish; this results in greater ^{109}Cd accumulation in gills and lower ^{109}Cd penetration into the body because of specific activity dilution during passage through the large gill 'chronic' pool. Clearly, much longer ^{109}Cd gill binding and turnover studies are required in future to differentiate the turnover kinetics of the 'acute' and 'chronic' Cd pools in the gills.

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References

Alabaster, J.S. and Lloyd, R. (Editors), 1982. Water Quality Criteria for Freshwater Fish, 2. Food and Agriculture Organization of the United Nations. Butterworths, London, pp. 221-251

Ball, I R., 1967 The toxicity of cadmium to rainbow trout (*Salmo gairdneri* Richardson). Water Res., 1 805-806.

Beamish, F W H , Howlett, J C., and Medland, T E., 1989. Impact of diet on metabolism and swimming performance in juvenile lake trout, *Salvelinus namaycush*. Can J Fish. Aquat. Sci., 46: 384-388.

Bengtsson, B.E., Carlin, C.H , Larsson, A. and Svanberg, O , 1975. Vertebral damage in minnows, *Phoxinus phoxinus* L., exposed to cadmium. AMBIO, 4. 166-168.

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

Benson, W H and Birge, W.J., 1985. Heavy metal tolerance and metallothionein induction in fathead minnows: results from field and laboratory investigations. Environ. Toxicol. Chem., 4. 209-217

Bentley, P.J , 1992. Influx of Zn by channel catfish (*Ictalurus punctatus*): Uptake from external environmental solutions. Com. Biochem. Physiol , 101C: 215-217

Bergman, H.L. and Dorward-King, E.J (Editors), 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. Pensacola FL: SETAC Pr. 114 p.

Bilinski, E. and Jonas, R.E.E., 1973. Effects of cadmium and copper on the oxidation of lactate by rainbow trout (*Salmo gairdneri*) gills. J. Fish. Res. Board Can., 30: 1553-1558.

Boutilier, R.G., Heming, T.A. and Iwama, G.K., 1984. Appendix: physicochemical parameters for use in fish respiratory physiology. In: W.S. Hoar and D.J. Randall (Editors), Fish Physiology, 10 (A). Academic Press, New York, pp. 403-430.

Brett, J.R., 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Board Can., 21: 1183-1226.

Calamari, D., Marchetti, R., and 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., 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.

Chapman, G.A., 1978. Toxicities of cadmium, copper, and zinc to four juvenile stages of chinook salmon and steelhead. Trans. Am. Fish. Soc., 107: 841-847.

Chapman, G.A. and Stevens, D.G., 1978. Acutely lethal levels of cadmium, copper, and zinc to adult male coho salmon and steelhead. Trans. Am. Fish. Soc., 107: 837-840.

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., and Brinkman, S.R., 1993. Effect of hardness on bioavailability and toxicity of cadmium to rainbow trout. Chem. Sp. Bioavail., 5: 67-77.

Duncan, D.A. and Klaverkamp, J.F., 1983. Tolerance and resistance to cadmium in white suckers (*Catostomus commersoni*) previously exposed to cadmium, mercury, zinc, or selenium. Can. J. Fish. Aquat. Sci., 40: 128-138.

Eaton, J.G., 1974. Chronic cadmium toxicity to the bluegill (*Lepomis macrochirus* Rafinesque). Trans. Am. Fish. Soc., 103: 729-735.

Ellgard, E.G., Tusa, J.E., and Malizia, A.A. Jr., 1978. Locomotor activity of the bluegill *Lepomis macrochirus*: hyperactivity induced by sublethal concentrations of cadmium, chromium and zinc. J. Fish Biol., 1: 19-23.

Farag, A.M., Boese, C.J., Woodward, D.R., and 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.

Finney, D.J., 1971. Probit Analysis, 3rd Edition. Cambridge University Press, New York.

Gardner, G.R. and Yevich, P.P., 1970. Histological and hematological responses of an estuarine teleost to cadmium. J. Fish. Res. Board Can., 27: 2185-2196.

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

Harrison, S.E. and Klaverkamp, J.F., 1989. Uptake, elimination and tissue distribution of dietary and aqueous cadmium by rainbow trout (*Salmo gairdneri* Richardson) and lake whitefish (*Coregonus clupeaformis* Mitchill). Environ. Toxicol. Chem., 8: 87-97.

Hollis, L., Burnison, K. and 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., 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.

Janes, N. and Playle, R.C., 1995. Modeling silver binding to gills of rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem., 14: 1847-1858.

Kumada, H., Kimura, S., and Yokote, M., 1980. Accumulation and biological

effects of cadmium in rainbow trout. Bull. Japan. Soc. Sci. Fish., 46: 97-103.

Majewski, H.S. and Giles, M.A., 1981. Cardiovascular-respiratory responses of rainbow trout (*Salmo gairdneri*) during chronic exposure to sublethal concentrations of cadmium. Water Res., 15: 1211-1217.

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

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

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

Muramoto, S., 1981. Vertebral column damage and decrease of calcium concentration of fish exposed experimentally to cadmium. Environ. Pollut. (Series A), 24: 125-133.

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., and Kiessling, A., 1985. The availability of cadmium to perfused rainbow trout gills in different water qualities. Water Res., 19:

427-434.

Pärt, P. and Wikmark, G., 1984. The influence of certain complexing agents (EDTA and citrate) on the uptake of cadmium in perfused rainbow trout gills. Aquat. Toxicol., 5: 277-289.

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., Dixon, D.G. and 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. and 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. 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 and 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 current regulatory limits. Environ. Sci. Technol., 31: 466-468.

Roberts, K. S., Cryer, A., Kay, J., Solbe, J.F. de L.G., Wharfe, J.R., and Simpson, W.R., 1979. The effects of exposure to sub-lethal concentrations of cadmium on enzyme activities and accumulation of the metal in tissues and organs of rainbow and brown trout (*Salmo gairdneri*, Richardson and *Salmo trutta fario* L.). Comp. Biochem. Physiol., 62C: 135-140.

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.

Sangalang, G.B. and Freeman, H.C., 1979. Tissue uptake of cadmium in brook trout during chronic sublethal exposure. Arch. Environ. Contam. Toxicol., 8: 77-84.

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

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

Sorensen, E.M., 1991. Metal Poisoning in Fish. Chapt. VI. Cadmium. CRC

Press, Boca Raton, FL, pp. 175-234.

Sprague, J.B., 1987. Effects of cadmium of freshwater fish. In: J.O. Nriagu and J.B. Sprague (Editors), Cadmium in the Environment. John Wiley, New York, pp. 139-169.

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.

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

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 freshwater trout branchial epithelium and its interference with calcium transport. J. Exp. Biol., 145: 185-197.

Voyer, R.A., Yevich, P.P. and Barszcz, C.A., 1975. Histological and toxicological responses of the mummichog, *Fundulus heteroclitus* (L.) to combinations of levels of cadmium and dissolved oxygen in a freshwater. Water Res., 9: 1069-1074.

Wicklund-Glynn, A., Norrgren, L. and Müssener, Å., 1994. Differences in uptake of inorganic mercury and cadmium in the gills of the zebrafish,

Brachydanio rerio. Aquat. Toxicol., 30: 13-26.

Table 2-1 Routine oxygen consumption of juvenile rainbow trout after 30 days exposure to 0 μg l^{-1} Cd (controls), 3 μg l^{-1} Cd, or 10 μg l^{-1} Cd 2 h and 6 h after feeding. Means ± S.E. (*N* = 2 tanks of 280 fish each). There were no significant differences.

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	4.27 ± 0.10	3.94 ± 0.34
3	3.91 ± 0.14	3.28 ± 0.14
10	4.30 ± 0.54	3.44 ± 0.44

Table 2-2 Swimming performance (stamina) of juvenile rainbow trout after 30 days exposure to 0 μg l^{-1} Cd (controls), 3 μg l^{-1} Cd, or 10 μg l^{-1} Cd. Swimming times were corrected to a reference length of 7 cm (average length of fish tested). Means ± 95% C.L. (*N* = 20). There were no significant differences.

Cd Exposure (μg l^{-1})	Time to 50% Fatigue (s)
0	277 ± 37
3	343 ± 48
10	533 ± 137

Table 2-3 Critical swimming speed (U_{Crit}) of juvenile rainbow trout after 30 days exposure to 0 μg l^{-1} Cd (controls), 3 μg l^{-1} Cd, or 10 μg l^{-1} Cd. Three and 10 μg l^{-1} Cd-acclimated fish were tested in either the presence or absence of Cd (3 and 10 μg l^{-1}, respectively). Means ± S.E. (N = 8 fish per swimming test). There were no significant differences.

Cd Exposure (μg l^{-1})	U_{Crit} in the Absence of Cd (body lengths s^{-1})	U_{Crit} in the Presence of Cd (body lengths s^{-1})
0	4.70 ± 0.09	4.58 ± 0.12[†]
3	4.33 ± 0.12	4.65 ± 0.12
10	4.49 ± 0.15	4.24 ± 0.13

[†] no Cd present

Table 2-4 Accumulation of 'new Cd' by blood and whole body of juvenile rainbow trout exposed for 3 hours to 10, 50, or 100 μg l^{-1} ^{109}Cd after 30 day exposure to 0 μg l^{-1} Cd (controls), 3 μg l^{-1} Cd, or 10 μg l^{-1} Cd. Means ± S.E. (N = 4). Statistical comparisons indicated were made for 3 and 10 μg l^{-1} chronic exposures against controls (0 μg l^{-1}) for each radioactive Cd exposure (* $P < 0.05$).

	Blood			Whole Body		
Chronic Cd Exposure (μg l^{-1})	10 μg l^{-1} Exposure 'New [Cd]' (μg g^{-1} wet tissue)	50 μg l^{-1} Exposure 'New [Cd]' (μg g^{-1} wet tissue)	100 μg l^{-1} Exposure 'New [Cd]' (μg g^{-1} wet tissue)	10 μg l^{-1} Exposure 'New [Cd]' (μg g^{-1} wet tissue)	50 μg l^{-1} Exposure 'New [Cd]' (μg g^{-1} wet tissue)	100 μg l^{-1} Exposure 'New [Cd]' (μg g^{-1} wet tissue)
0	0.017 ± 0.004	0.033 ± 0.005	0.073 ± 0.013	0.004 ± 0.001	0.007 ± 0.001	0.018 ± 0.002
3	0.009 ± 0.002	0.023 ± 0.007	0.041 ± 0.007*	0.004 ± 0.001	0.006 ± 0.001	0.012 ± 0.003
10	0.003 ± 0.001*	0.027 ± 0.011	0.016 ± 0.003*	0.001 ± 0.001*	0.007 ± 0.001	0.014 ± 0.003

Table 2-5 Calculated concentrations of Cd species in the water for various Cd exposures, using MINEQL+ (Schecher and McAvoy, 1994) aquatic chemistry program. 3 and 10 μg l^{-1} represent the chronic (30 d) exposures to Cd; 10, 50, and 100 μg l^{-1} represent the 3-h ^{109}Cd gill-uptake experiment. (DOM = dissolved organic matter)

	Species Concentration ($\mu g\ l^{-1}$)			
Cd Species	3 μg l^{-1} Cd	10 μg l^{-1} Cd	50 μg l^{-1} Cd	100 μg l^{-1} Cd
Cd^{2+}	1.10	4.32	27.00	57.00
Cd-DOM	1.19	2.86	5.40	6.00
$CdHCO_3^+$	0.06	0.23	1.50	3.00
$CdCl^+$	0.07	0.29	1.80	4.00
$CdCO_3$ Aq	0.58	2.30	14.30	30.00

Fig. 2-1

Survival of juvenile rainbow trout exposed to 0 μg l^{-1} Cd (controls; solid line), 3 μg l^{-1} Cd (dashed line), or 10 μg l^{-1} Cd (dotted line) for 30 days. Values are the averages between two replicate tanks (*N* = 280 fish per tank) for each treatment.

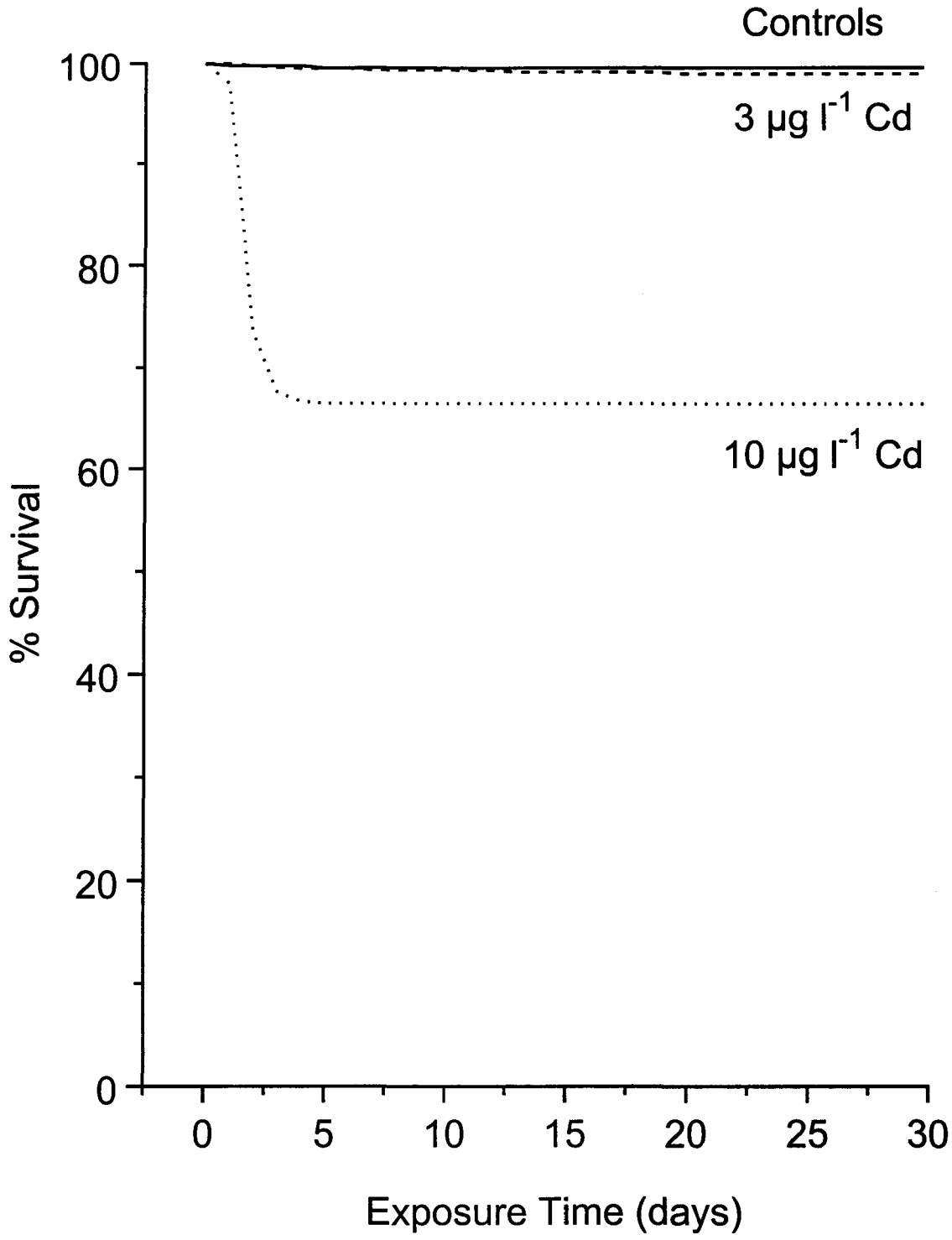

Controls
3 µg l-1 Cd
10 µg l-1 Cd
% Survival
100
80
60
40
20
0
0
5
10
15
20
25
30
Exposure Time (days)

Fig. 2-2

Body weight of juvenile rainbow trout (mean ± S.E., N = 2 tanks of 280 fish each) over 30 d exposure to 0 μg l^{-1} Cd (controls; solid line or clear bar), 3 μg l^{-1} Cd (dashed line or striped bar), or 10 μg l^{-1} Cd (dotted line or solid bar). Specific growth rate is depicted in the inset (mean ± 95% C.L., *N* = 2 tanks of 280 fish each).

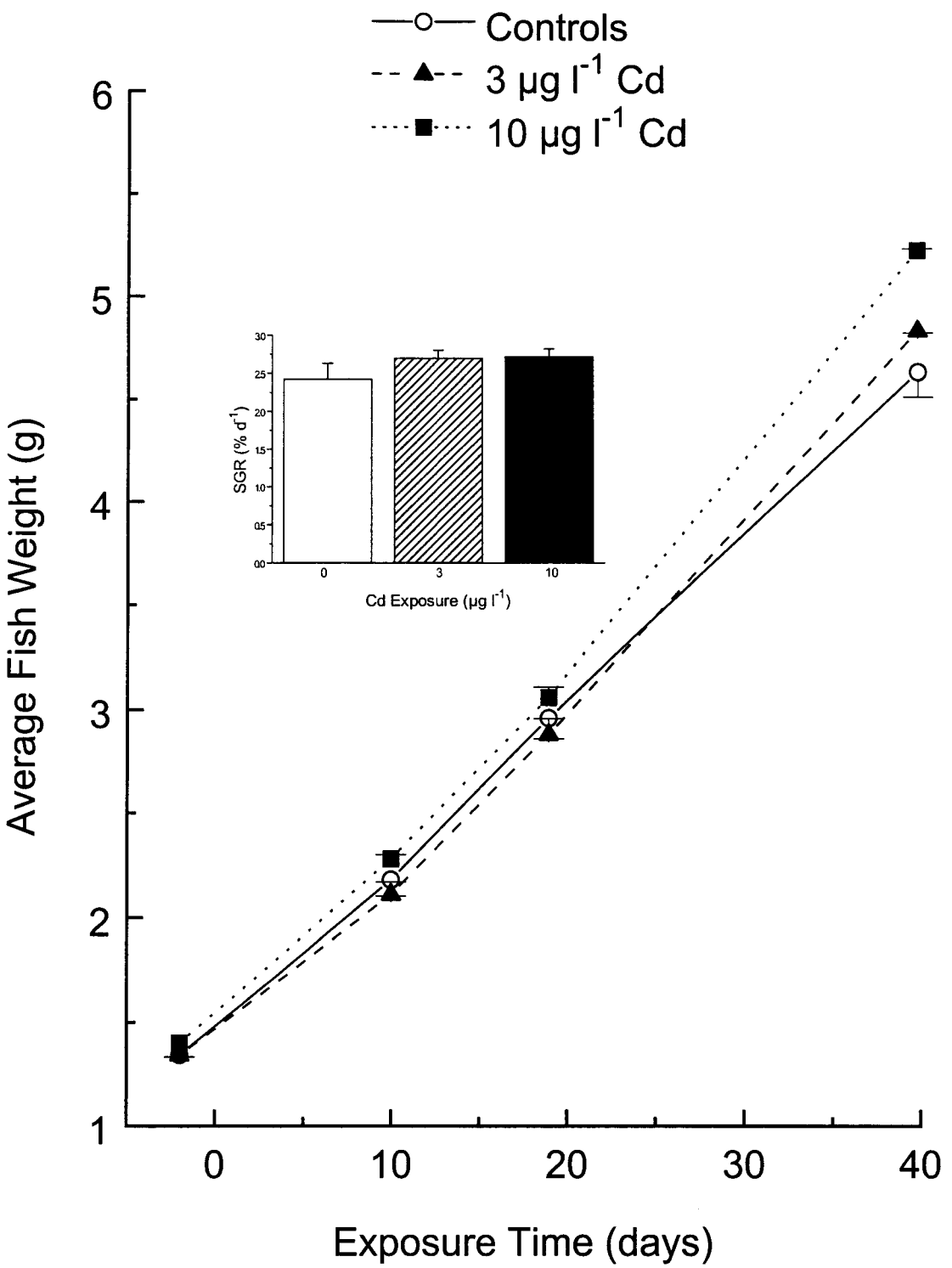

Controls
3 µg l-1 Cd
10 µg l-1 Cd
Average Fish Weight (g)
Exposure Time (days)
SGR (% d-1)
Cd Exposure (µg l-1)

Fig. 2-3

Accumulation of Cd by gills (A), liver (B), carcass (C), and whole body (D) of juvenile rainbow trout exposed for 30 d to 0 μg l^{-1} Cd (controls; clear bars), 3 μg l^{-1} Cd (striped bars), or 10 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); $P < 0.05$.

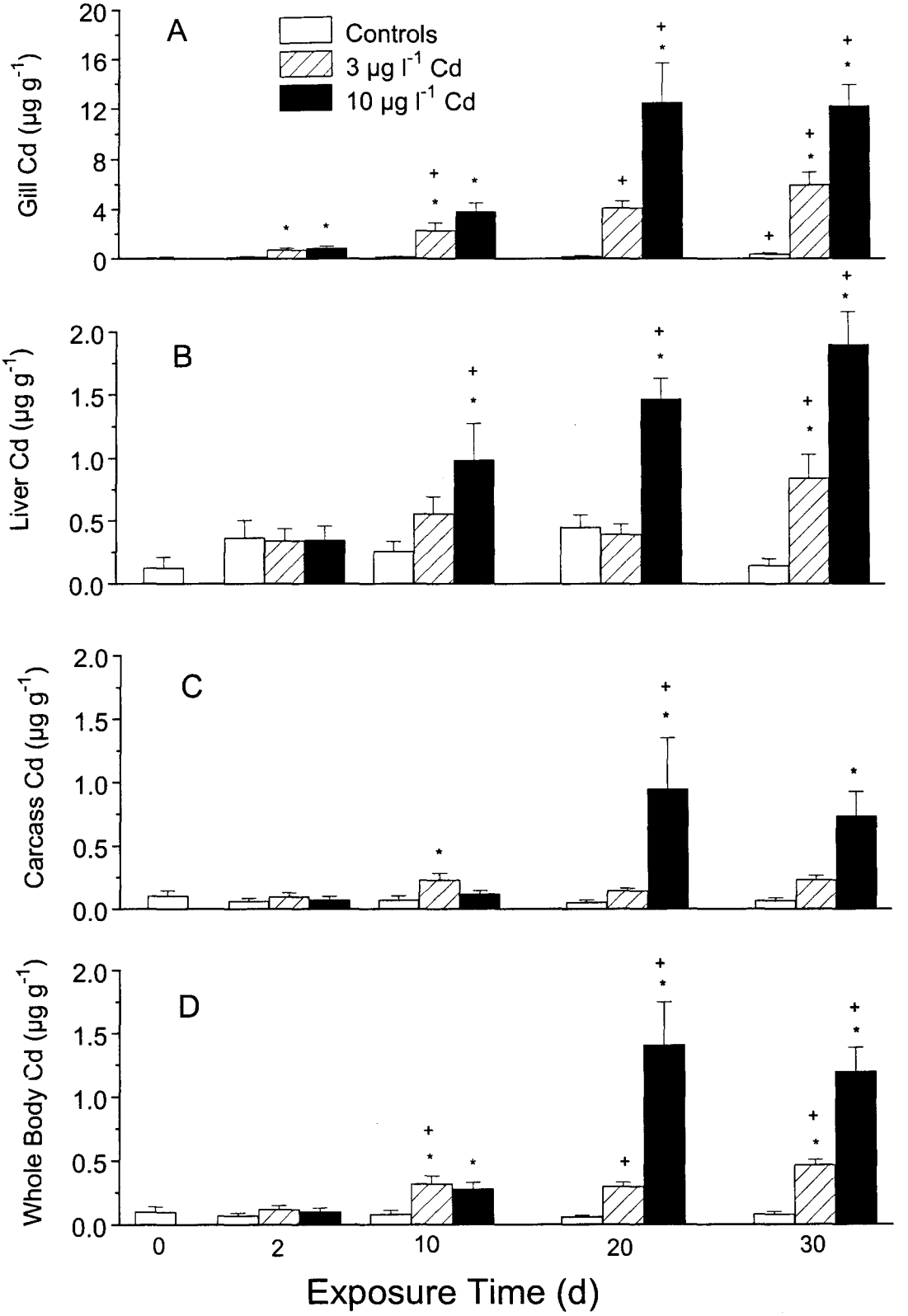

A
Controls
3 µg l-1 Cd
10 µg l-1 Cd
Gill Cd (µg g-1)
20
16
12
8
4
0
B
Liver Cd (µg g-1)
2.0
1.5
1.0
0.5
0.0
C
Carcass Cd (µg g-1)
2.0
1.5
1.0
0.5
0.0
D
Whole Body Cd (µg g-1)
2.0
1.5
1.0
0.5
0.0
0
2
10
20
30
Exposure Time (d)

Fig. 2-4

96-h LC_{50} values for Cd of juvenile rainbow trout after 30 days exposure to 0 µg l^{-1} Cd (controls; clear bar), 3 µg l^{-1} Cd (striped bar), or 10 µg l^{-1} Cd (solid bar). Means ± 95% C.L. (N = 60). * $P < 0.05$.

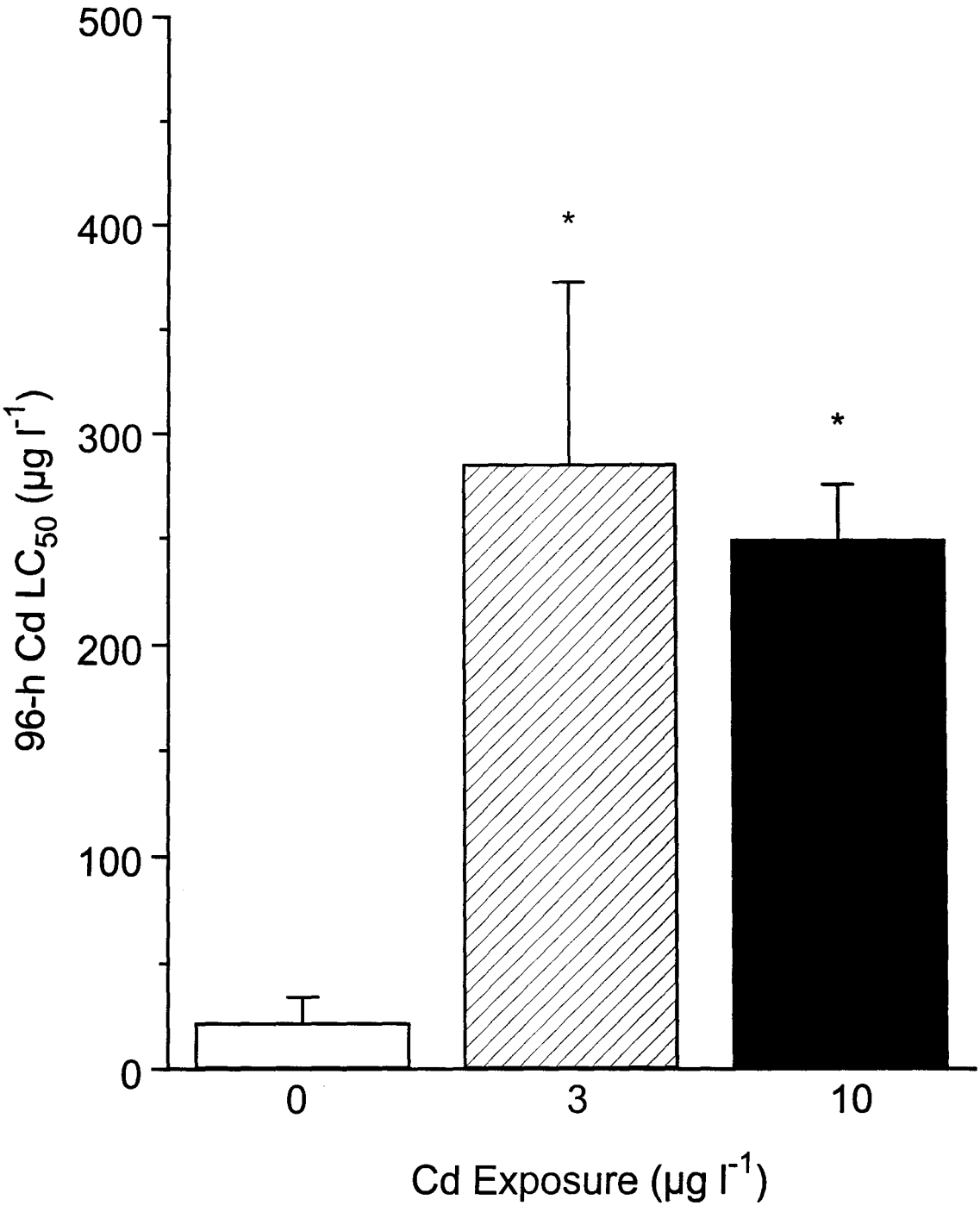

500
400
300
200
100
0
96-h Cd LC_{50} (µg l^{-1})
*
*
0
3
10
Cd Exposure (µg l^{-1})

Fig. 2-5

Accumulation of Cd by gills of juvenile rainbow trout exposed to Cd concentrations ranging from 0 to 70 μg l^{-1} for 3 h after 30 d exposure to 0 μg l^{-1} Cd (controls; clear bars), 3 μg l^{-1} Cd (striped bars), or 10 μg l^{-1} Cd (solid bars). Inset shows the data for the controls on a finer scale; the points and line indicates the modelled response (see text for details). Means ± S.E. (N = 6). Statistical comparisons were made against background gill Cd (0 μg l^{-1}) for each Cd exposure; $P < 0.05$. There were no significant differences within each exposure group.

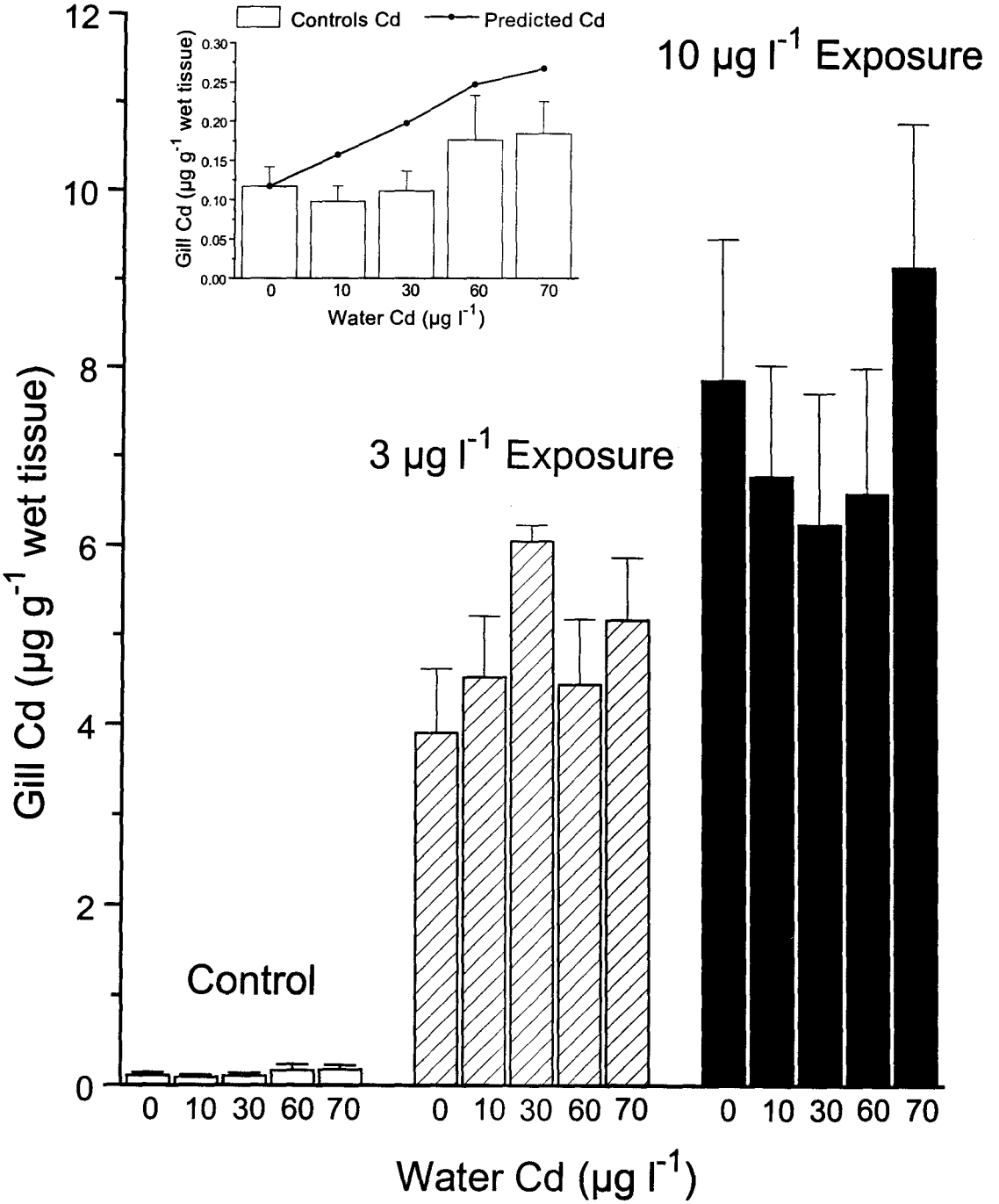

Controls Cd
Predicted Cd
Gill Cd (µg g-1 wet tissue)
Water Cd (µg l-1)
10 µg l-1 Exposure
3 µg l-1 Exposure
Control
Gill Cd (µg g-1 wet tissue)
Water Cd (µg l-1)
0 10 30 60 70

Fig. 2-6

Accumulation of Cd by gills of juvenile rainbow trout exposed for 3 h to 3 μg l^{-1} Cd and ethylenediaminetetraacetic acid (EDTA; 0-450 μg l^{-1}) after 30 d exposure to 3 μg l^{-1} Cd. Means ± S.E. (N = 5). Statistical comparisons were made against background gill Cd with no added EDTA (0 μg l^{-1} EDTA; 3 μg l^{-1} Cd); there were no significant differences ($P < 0.05$). However the overall relationship between gill Cd and water EDTA concentrations was significant (r^2 = 0.88, $P < 0.05$).

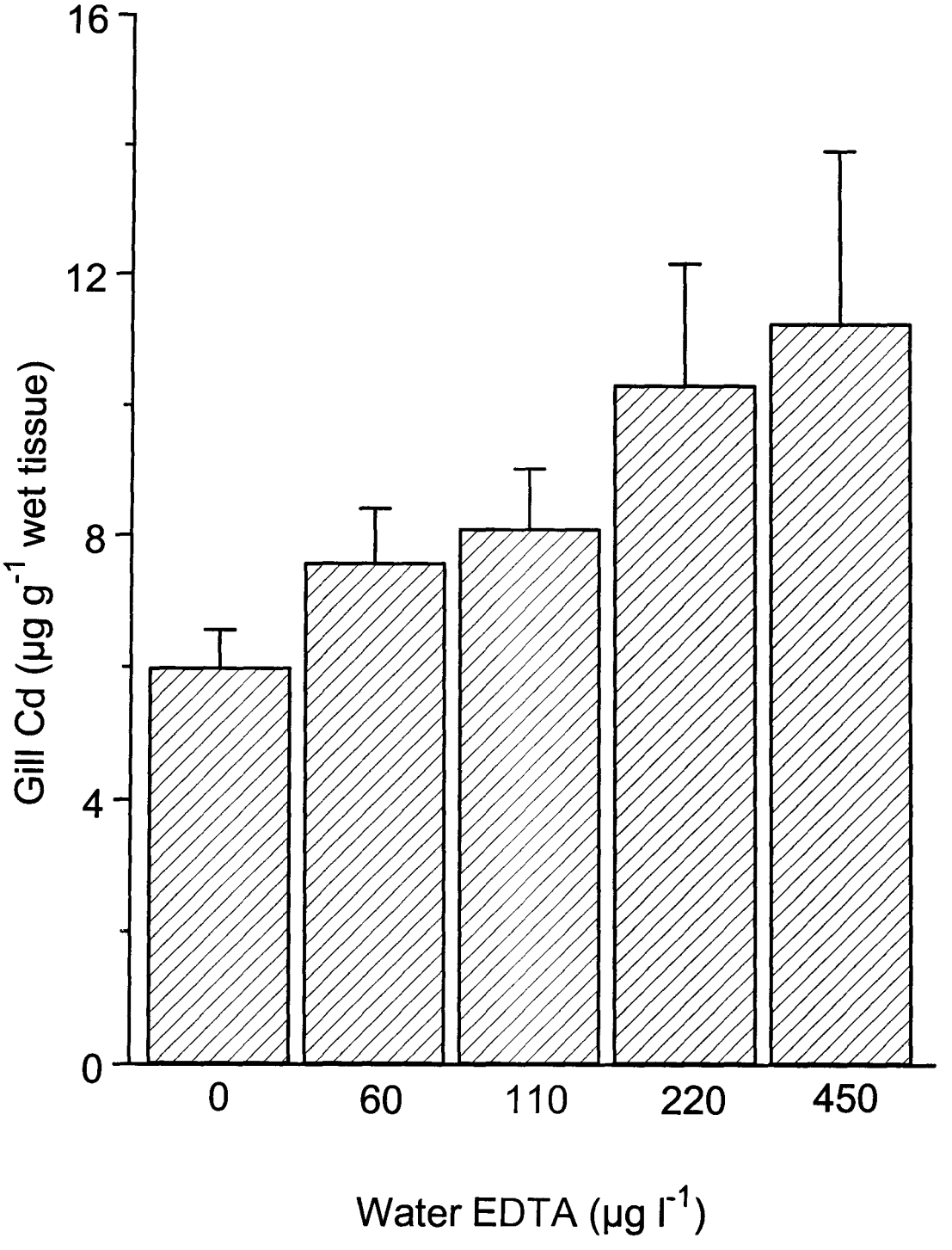

16
12
8
4
0
Gill Cd (µg g-1 wet tissue)
0
60
110
220
450
Water EDTA (µg l-1)

Fig. 2-7

Accumulation of 'new Cd' by gills of control (non-acclimated; A) and Cd-acclimated trout [exposed to 3 μg Cd l^{-1} (B) and 10 μg Cd l^{-1} (C) for 30 d] exposed for 3 h to ^{109}Cd with total Cd concentrations of 10 μg l^{-1} Cd (solid lines), 50 μg l^{-1} Cd (dashed lines), or 100 μg l^{-1} Cd (dotted lines). Means ± S.E. (N = 4). Statistical comparisons were made against 10 μg l^{-1} radioactive Cd exposure at each sampling time; * $P < 0.05$.

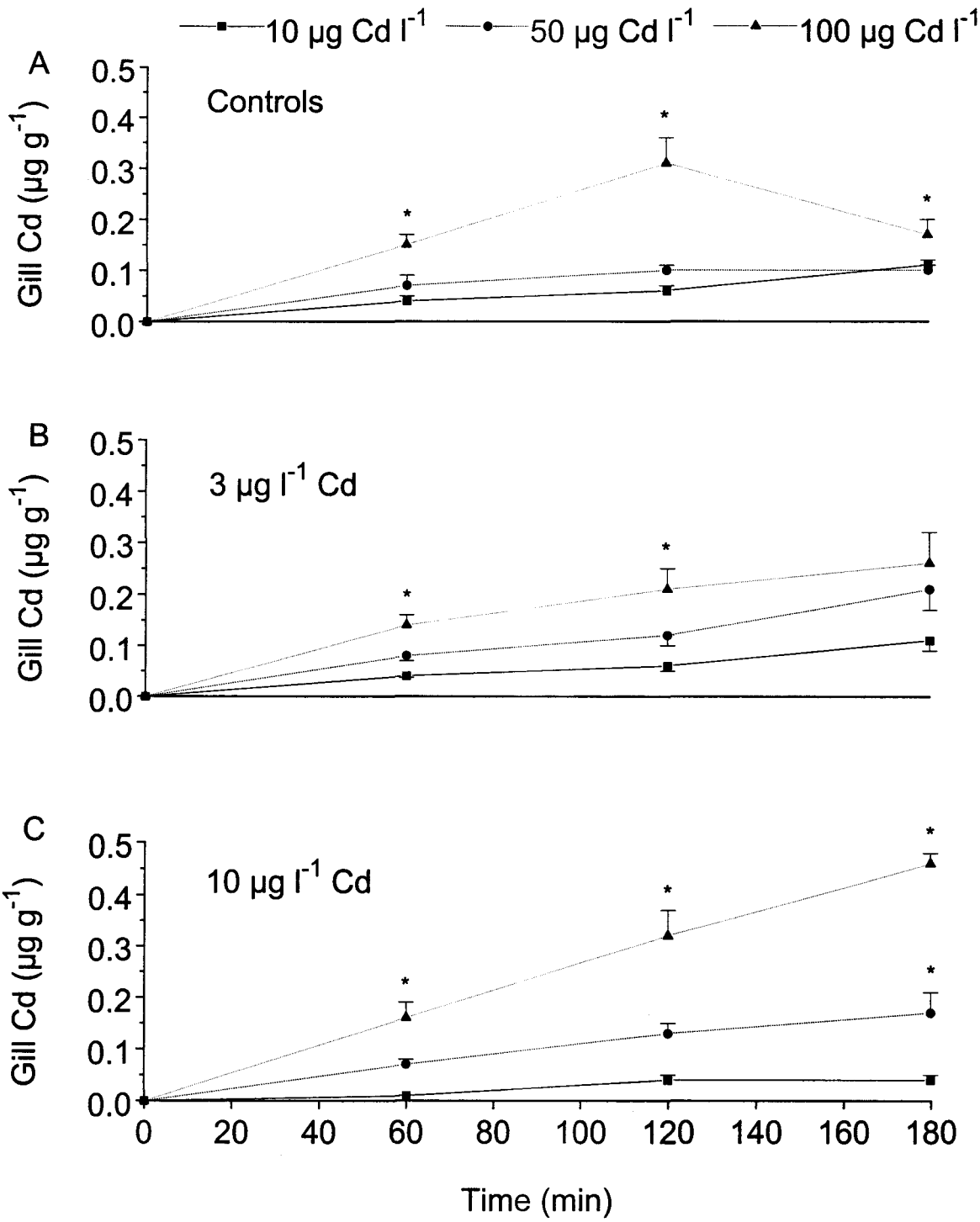
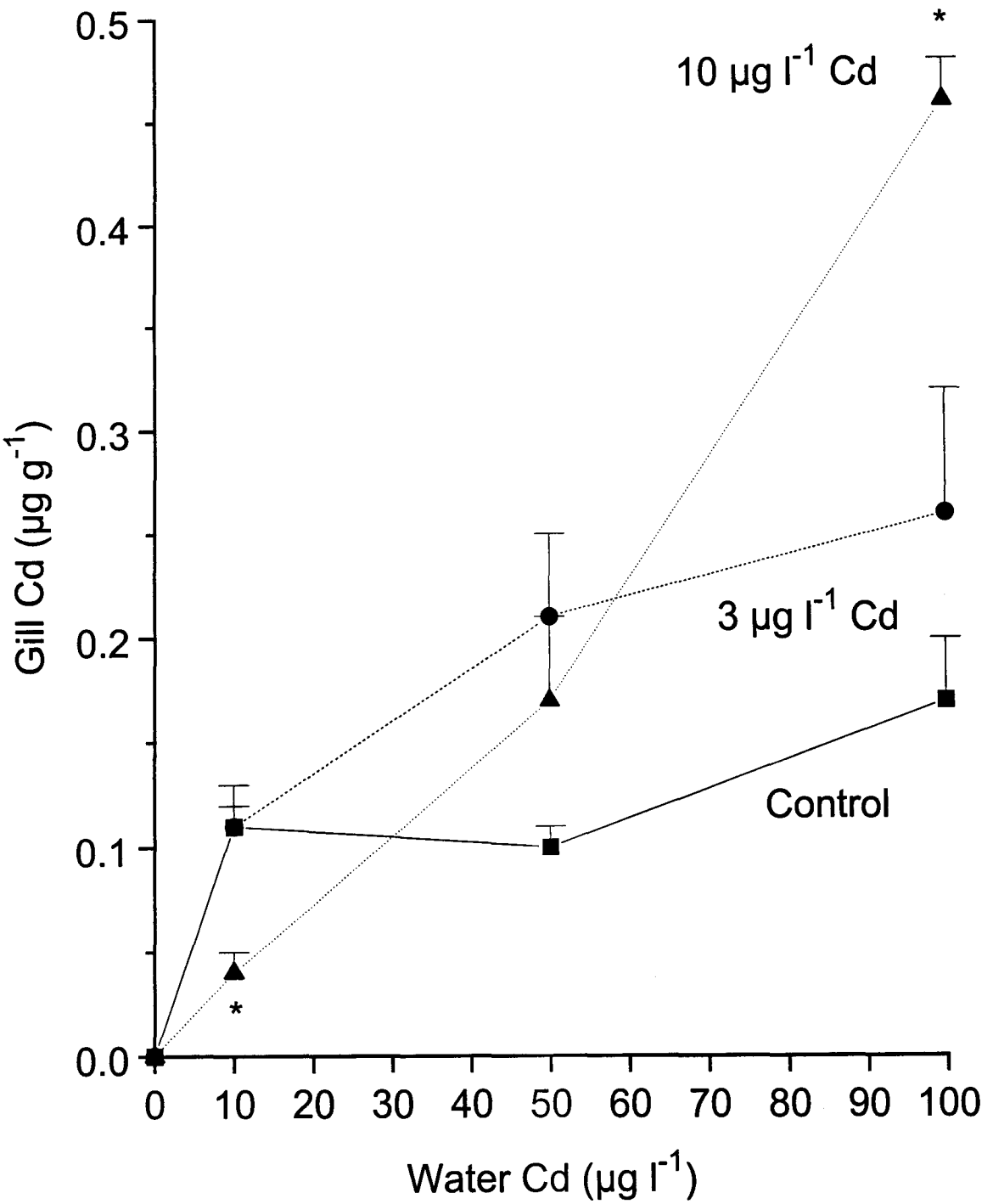

10 µg Cd l-1
50 µg Cd l-1
100 µg Cd l-1
A
Controls
B
3 µg l-1 Cd
C
10 µg l-1 Cd
Gill Cd (µg g-1)
0.0
0.1
0.2
0.3
0.4
0.5
0
20
40
60
80
100
120
140
160
180
Time (min)
*

Fig. 2-8

Accumulation of 'new Cd' by gills of rainbow trout exposed for 3 h to ^{109}Cd, with total Cd concentrations as 10, 50, or 100 μg l^{-1}, after 30 d exposure to 0 μg l^{-1} Cd (controls; solid line), 3 μg l^{-1} Cd (dashed line), or 10 μg l^{-1} Cd (dotted line). Means ± S.E. (N = 4). Statistical comparisons were made against control series for each water Cd treatment; * $P < 0.05$.


0.5
0.4
0.3
0.2
0.1
0.0
Gill Cd (µg g-1)
0
10
20
30
40
50
60
70
80
90
100
Water Cd (µg l-1)
10 µg l-1 Cd
3 µg l-1 Cd
Control
*
*

Chapter 3

Effects of Long Term Sublethal Cd Exposure in Rainbow Trout During Soft Water Exposure: Implications for Biotic Ligand Modelling

Abstract

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. 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. 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 2 X (gills) and 1.2 X (liver) control levels 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.

Introduction

Acute cadmium toxicity to fish has been demonstrated in many water qualities (e.g. McCarty *et al.*, 1978; Carroll *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, Carroll *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).

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).

We also tested the current gill binding model (or Biotic Ligand Model;

Playle *et al.*, 1993a,b; Bergman and Doward-King, 1997; Renner, 1997; Playle, 1998; Meyer, 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 facilitated direct comparison with our earlier similar study (Hollis *et al.*, 1999) conducted in hard water (eight fold higher Ca levels).

Materials and Methods

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 one week and then slowly introduced to synthetic soft water over the course of one 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).

Exposure System

After three 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 $Cd(NO_3)_2 \cdot 4\ H_20$ (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 = 0.02 ± 0.01 µg l^{-1} or 0.0002 ± 0.0001 (13) µmol l^{-1} Cd; mean ± 1 SEM (*N* = number of H_2O samples taken)], (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. 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.

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 five 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 seconds 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.

Testing

Exercise performance

Fish were not fed the day of swimming tests. Swimming procedures, using the protocol of McDonald *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} (~ 6 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.

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).

Acclimation

A 96 hour 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.

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} ^{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 Tissue Analysis). Remaining carcasses were placed in 20 ml

polyethylene vials and analyzed for ^{109}Cd.

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 HNO3; 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 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 ^{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 g 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 K_D and the total B_{max} of the gill were then determined from the slope and *x*-intercept of the Scatchard plot, respectively.

Water Na and Ca and plasma Ca (diluted 50 times) 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). 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.

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.

Results

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 (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 as a result of chronic Cd exposure (Table 1).

Metal accumulation in gills increased significantly over the 30 d in both Cd-exposed groups, whereas levels in livers increased only very slightly (Fig. 1A and B, respectively). Cadmium concentrations were greatest in gills (Fig. 1A), followed by liver (Fig. 1B); gall bladders and whole bodies did not accumulate significant amounts of Cd over the 30 day exposure (Fig. 1C and D). Gill Cd levels increased ~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 14000 times and 10000 times for the 0.07 μg l^{-1} and 0.11 μg l^{-1} Cd exposures, respectively. In contrast, fish exposed to 3 or 10 μg l^{-1} Cd in hard water had 60 and 120 fold increases from lower initial values (0.10 μg Cd g^{-1} wet tissue), respectively (Hollis *et al.*, 1999). In soft water, liver Cd concentrations increased 1.2 times from initial values of 0.49 ± 0.01 (6) μg Cd g^{-1} (wet tissue; Fig. 1B), with concentration factors of 9000

times and 6000 times for low and high Cd exposures, respectively. Hard water exposed fish showed 8 and 20 fold increases from lower initial values in liver Cd (0.13 μg Cd g^{-1} wet tissue) for low (3 μg Cd l^{-1}) and high (10 μg Cd l^{-1}) Cd exposures, respectively (Hollis *et al.*, 1999). 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 μg l^{-1} and 0.11 μg l^{-1} Cd exposures in soft water. 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.

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 Cd-exposed fish were not significantly different from the control value of 2.07 ± 1.73 μg Cd l^{-1} (Fig. 2). By way of contrast, in hard water (Hollis *et al.*, 1999) the 96-h LC_{50} increased 11-13 fold from a control value of 22 ± 12 μg Cd l^{-1} in trout chronically exposed to 3 and 10 μg Cd l^{-1}.

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) mmol kg^{-1} and 41 ± 2 (78) mmol kg^{-1}, respectively, similar to those seen previously in hard water acclimated fish (Hollis *et al.*, 1999). In-tank metabolic rate

measurements showed no significant differences in rates of routine oxygen consumption 2 h and 6 h after feeding in Cd-exposed fish in soft water (Table 2), again similar to hard water results. Swimming stamina was not significantly affected by exposure to Cd for 30 days in soft water, again in accord with the hard water study (Table 3). Interestingly, sprint times were shorter (by about 60%) and routine metabolic rates were higher (by about 50%) than those recorded earlier in hard water acclimated fish (Hollis *et al.*, 1999). There were no significant differences in plasma Cd or Ca concentrations in soft water metal-exposed fish compared to controls at day 30 (Table 4). Note that on a wet weight basis, plasma Cd concentrations were only about 0.1% of those in all other tissues measured.

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. 3), 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. 3A). 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 Methods) 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. 3A).

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. 3B). At the highest exposure concentration, 'new' Cd bound to the gills was about 0.22 μg g^{-1} (Fig. 3B), 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. 3) 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 5). In addition, K_D and B_{max} both increased in fish chronically exposed to Cd in hard water (data of Hollis *et al.*, 1999). Scatchard analysis could not be done on the data from the Cd-exposed fish in soft water and the 10 μg Cd l^{-1} exposure group in hard 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 in both situations.

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. 4) 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. 4), less than 10% of the concentrations in gill tissue (Fig. 3).

Discussion

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 10 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 6). In comparison, the soft water exposure also resulted in significant accumulation of Cd in the gills, but this did not lead to acclimation of the fish (Table 6). 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 6). 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 6).

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 U.S. E.P.A. freshwater quality criteria for aquatic life of 0.64 μg l^{-1} 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 $CaCO_3$ (U.S. E.P.A., 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}; C.C.M.E., 1995).

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 (Tables 1, 2, and 6). In general, these findings are in accord with earlier reports. Kumada *et al.* (1980) and Davies *et al.* (1993) reported no significant changes in growth for juvenile rainbow trout exposed to 4 μg Cd l^{-1} for 70 to 100 days. Farag *et al.* (1994) also showed that a 21 d exposure to 2.2 μg Cd l^{-1} (in a mixed metal solution of Pb, Cu, and Cd) had no significant effects on growth for juvenile rainbow trout. No adverse effects on growth of adult rainbow trout were demonstrated by Giles (1988) when the fish were exposed for 178 days to 3.6 and 6.4 μg Cd l^{-1}. In addition, Benoit *et al.*

(1976) reported no significant impairment of growth for first-generation brook trout exposed to 3.4 μg Cd l^{-1}.

We found no differences in swimming performance (stamina) between controls and fish chronically exposed to Cd in either soft water (Table 3) or hard water (Table 6; Hollis *et al.*, 1999). Scherer *et al.* (1997) reported similar findings on swimming performance of fingerling rainbow trout: predator escape from lake trout was unimpaired by a 227 d exposure to 0.5 μg Cd l^{-1}.

Whole body and plasma ions were not changed with chronic Cd exposure in soft water (Table 4) and hard water (Table 6; 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. Giles (1984) reported similar results with larger rainbow trout (375 g) that were exposed to 3.6 μg Cd l^{-1}, where plasma calcium and magnesium levels were not significantly altered. However, in that study, a 6.4 μg Cd l^{-1} exposure resulted in significantly lower plasma Na, K, Ca, and Cl, and elevated plasma Mg concentrations.

Acclimation

Toxicological acclimation to chronic Cd exposure did not occur in our soft water exposure, because the subsequent challenge to Cd after the 30 day exposure did not yield significantly higher 96-h LC_{50} values of metal-exposed fish (0.07 or 0.11 μg Cd l^{-1}) compared to controls (Fig. 2). If anything, they

decreased. Therefore these low Cd levels were below the threshold for acclimation. 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 exposure concentrations 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 6), Hollis *et al.* (1999) showed an 11 to 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 $CaCO_3$). The principal variable controlling toxicity appears to be water hardness, specifically Ca (Carrol *et al.*, 1979; Davies *et al.*, 1993).

Internal Cadmium Distribution

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. A small, but significant, amount of Cd (~ 1.2 X) accumulated in the livers of Cd-exposed fish (Fig. 1B), whereas no Cd accumulated in gall bladders or whole bodies of Cd-exposed fish (Fig. 1C and 1D, respectively). In contrast, fish exposed to 3 and 10 μg l^{-1} Cd in hard water for 30 days had 60 to 120-fold increases in gill Cd burden, and 8 to 20-fold increases in liver Cd burden (Hollis *et al.*, 1999).

Implications for Biotic Ligand Modelling

The acute gill surface binding 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; Meyer, 1999; Meyer *et al.*, 1999). 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 (Di Toro *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). The acute gill binding model, or Biotic Ligand Model, was originally developed in soft water for acute exposures (Playle *et al.*, 1993a,b). We investigated whether this model could be applied to fish that had been chronically exposed to Cd in soft water.

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 the present study, the model successfully calculated 'new' Cd accumulation in gills of control fish (Fig. 3A) when the conditional stability constant for Cd 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 to 88% of the total Cd existed as the free ionic species, Cd^{2+}, for exposures ranging from 1 to 36 µg l^{-1} Cd (Table 7). When Scatchard analysis was applied to the saturation curve for control fish (Fig. 3A), the conditional stability constant (log $K_{Cd\text{-}gill}$ = 7.3; Table 5) was lower than that of Playle *et al.* (1993a, b) for fathead minnows (log $K_{Cd\text{-}gill}$ = 8.6). The difference in log $K_{Cd\text{-}gill}$ values translates to a twenty-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. 3A).

With regards to Cd-exposed trout, the fish exposed to 0.07 and 0.11 μg l^{-1} Cd accumulated significantly less 'new' Cd in their gills compared to controls when exposed acutely to total Cd concentrations below 25 μg Cd l^{-1}. Whole body accumulation of 'new' Cd (Fig. 4) mirrored that of 'new' Cd accumulation by the gills (Fig. 3B) 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}; Fig. 2), gill binding characteristics did change (Table 5). This reduction in gill binding and uptake of Cd for trout chronically exposed to Cd was also observed for fish acutely exposed to 10 μg l^{-1} Cd in hard water after they had been acclimated to 10 μg l^{-1} Cd for 30 days (Hollis *et al.*, 1999).

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 appear to have developed a protective mechanism (i.e. higher K_D and B_{max}; Table 5) for reduced Cd uptake. Alsop *et al.* (1999) and Alsop and Wood (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 longer exposures we note that there appear to be difficulties with applying the acute gill binding model as described by Playle *et al.* (1993b) using cold techniques. We found it necessary to use a radiotracer technique with ^{109}Cd in our hard water exposure due to the very high concentrations of accumulated cold Cd on the gills (Hollis *et al.*, 1999). We also resorted to the use of radiotracers for the present soft water study, since there was only a very small increase in uptake of 'new' Cd (~0.17 μg Cd g^{-1}) by control fish which could not easily be detected against the background of ~0.56 μg Cd g^{-1} wet weight using cold techniques. In contrast, Playle *et al.* (1993a,b) had lower gill Cd background concentrations (~0.2 μg g^{-1} wet tissue) and higher accumulation of Cd in gills (~0.6 μg g^{-1} wet tissue) at 6 μg l^{-1} Cd exposure, so that significant decreases in gill Cd uptake using complexing ligands were detectable using cold techniques. In addition, there appear to be difficulties with 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.

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References

Alsop, D.H., McGeer, J.C., McDonald, D.G., and 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.

Alsop, D.H. and Wood, C.M., 1999. A kinetic analysis of zinc accumulation in the gills of juvenile rainbow trout: the effects of zinc acclimation and implications for biotic ligand modelling. Environ. Toxicol. Chem. Submitted.

Benoit, D.A., Leonard, E.N., Christensen, G.M., and 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. and Dorward-King, E.J. (Editors), 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. Pensacola FL: SETAC Pr. 114 p.

Calamari, D., Marchetti, R., and 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., 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.

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., and Brinkman, S.R., 1993. Effect of hardness on bioavailability and toxicity of cadmium to rainbow trout. Chem. Sp. Bioavail., 5: 67-77.

Di Toro, D., Allen, H., Bergman, H., Meyer, J., Paquin, P., and Santore, R., 1999. Biotic ligand model of the acute toxicity of metals. I. Technical basis. Environ. Toxicol. Chem. Submitted.

Farag, A.M., Boese, C.J., Woodward, D.R., and 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., 1984. Electrolyte and water balance in plasma and urine of rainbow trout (*Salmo gairdneri*) during chronic exposure to cadmium. Can. J. Fish. Aquat. Sci., 41: 1678-1685.

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. and 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., 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.

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

Kumada, H., Kimura, S., and 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., and 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., and Milligan, C.L., 1998. Anaerobic capacity and swim performance of juvenile salmonids. Can. J. Fish. Aquat. Sci., 55: 1198-1207.

McDonald, D.G. and Wood, C.M., 1993. Branchial mechanisms of acclimation to metals in freshwater fish. In: J.C. Rankin and F.B. Jensen (Editors), 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., and 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. and 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., and 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., 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., 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. and 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. 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 and 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 current regulatory limits. Environ. Sci. Technol., 31: 466-468.

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

Scherer, E., McNicol, R.E. and 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. Washington DC: USEPA Office of Water Regulations and Standards. EPA 440/5-85-001.

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 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.

Table 3-1 Specific growth rate of juvenile rainbow trout over 30 days exposure to 0 μg l^{-1} Cd (controls), 0.07 μg l^{-1} Cd, or 0.11 μg l^{-1} Cd. Means ± 1 S.E. (*N* = 2 tanks of 225 fish each). There were no significant differences.

Cd Exposure (μg l^{-1})	Specific Growth Rate (% d^{-1})
0	3.5 ± 0.1
0.07	3.7 ± 0.1
0.11	3.5 ± 0.1

Table 3-2 Routine oxygen consumption of juvenile rainbow trout after 30 days exposure to 0 μg l^{-1} Cd (controls), 0.07 μg l^{-1} Cd, or 0.11 μg l^{-1} Cd 2 h and 6 h after feeding. Means ± S.E. (*N* = 2 tanks of 225 fish each). There were no significant differences.

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

Table 3-3 Swimming performance (stamina) of juvenile rainbow trout after 30 days exposure to 0 μg l^{-1} Cd (controls), 0.07 μg l^{-1} Cd, or 0.11 μg l^{-1} Cd. 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.

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

Table 3-4 Plasma Cd and Ca of juvenile rainbow trout after 30 days exposure to 0 μg l^{-1} Cd (controls), 0.07 μg l^{-1} Cd, or 0.11 μg l^{-1} Cd. Means ± S.E. (N = 6). There were no significant differences.

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

Table 3-5 Gill Cd dissociation constants (K_D) and capacity (B_{max}) for juvenile rainbow trout exposed to 0 µg l^{-1} Cd (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. Corresponding log $K_{Cd\text{-}gill}$ values are in brackets below gill Cd dissociation constants (K_D).

	Hard Water		Soft Water	
Cd Exposure	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 (3 µg l^{-1})	6.9 (7.2)	0.29	----	----

Table 3-6 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}

Table 3-7 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 ($\mu g\ l^{-1}$)	$[Cd^{2+}]\ \mu g\ l^{-1}$	$[Cd\text{-}DOM]\ \mu g\ l^{-1}$	$[CdCO_{3\ Aq}]\ \mu g\ l^{-1}$
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

Fig. 3-1

Accumulation of Cd by gills (A), liver (B), gall bladder (C), and whole body (D) of juvenile rainbow trout exposed for 30 d to 0 μg l^{-1} Cd (controls; clear bars), 0.07 μg l^{-1} Cd (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); $P < 0.05$.

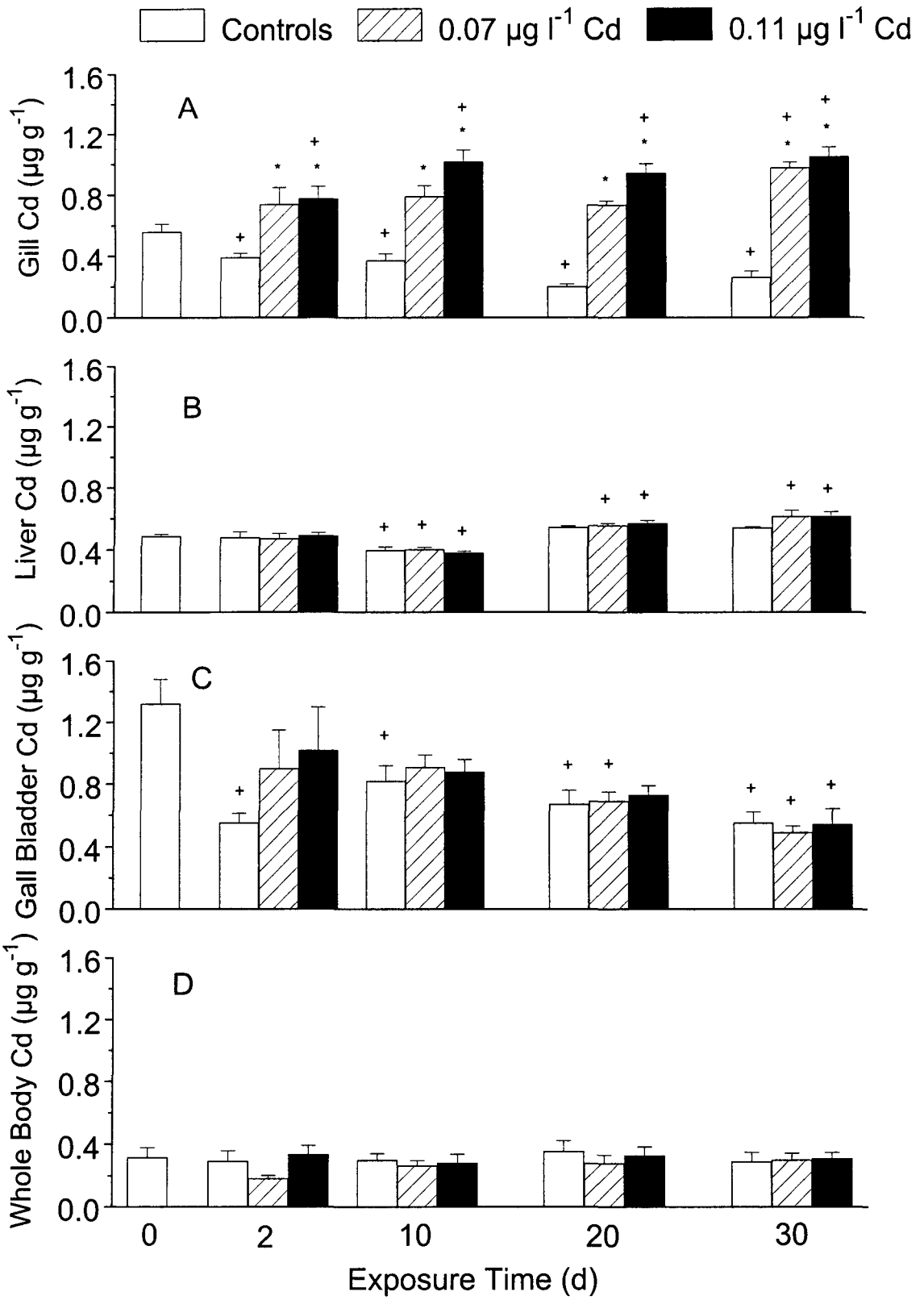

Controls
0.07 µg l-1 Cd
0.11 µg l-1 Cd
A
B
C
D
Gill Cd (µg g-1)
Liver Cd (µg g-1)
Gall Bladder Cd (µg g-1)
Whole Body Cd (µg g-1)
0.0
0.4
0.8
1.2
1.6
0
2
10
20
30
Exposure Time (d)

Fig. 3-2

96-h LC_{50} values for Cd of juvenile rainbow trout after 30 days exposure to 0 µg l^{-1} Cd (controls; clear bar), 0.07 µg l^{-1} Cd (striped bar), or 0.11 µg l^{-1} Cd (solid bar). Means ± 1 S.E. (N = 50). There were no significant differences.

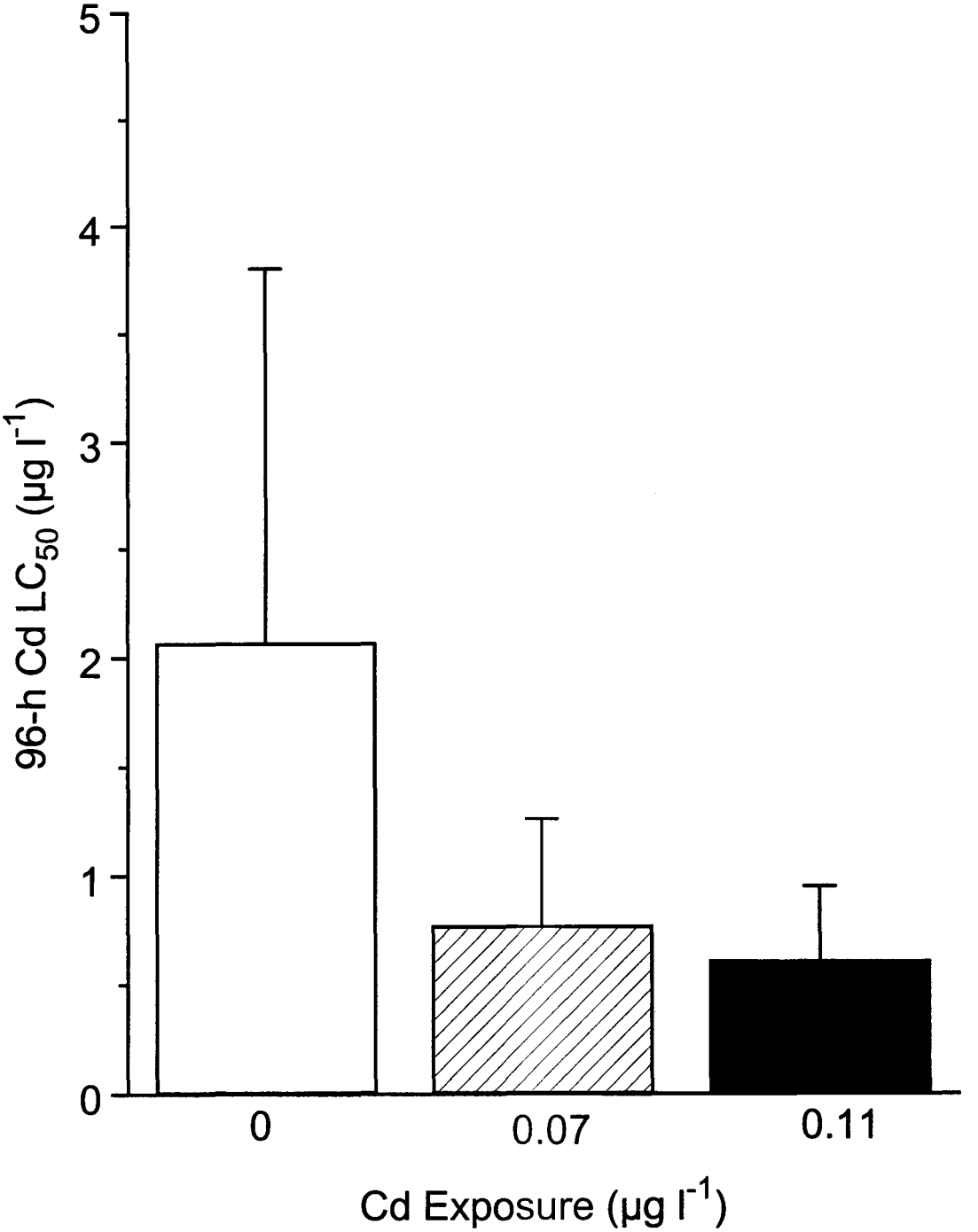

5
4
3
2
1
0
96-h Cd LC50 (µg l-1)
0
0.07
0.11
Cd Exposure (µg l-1)

Fig. 3-3

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 d 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 μg l^{-1} Cd; solid line with closed circles), 0.07 μg l^{-1} Cd (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; * $P < 0.05$.

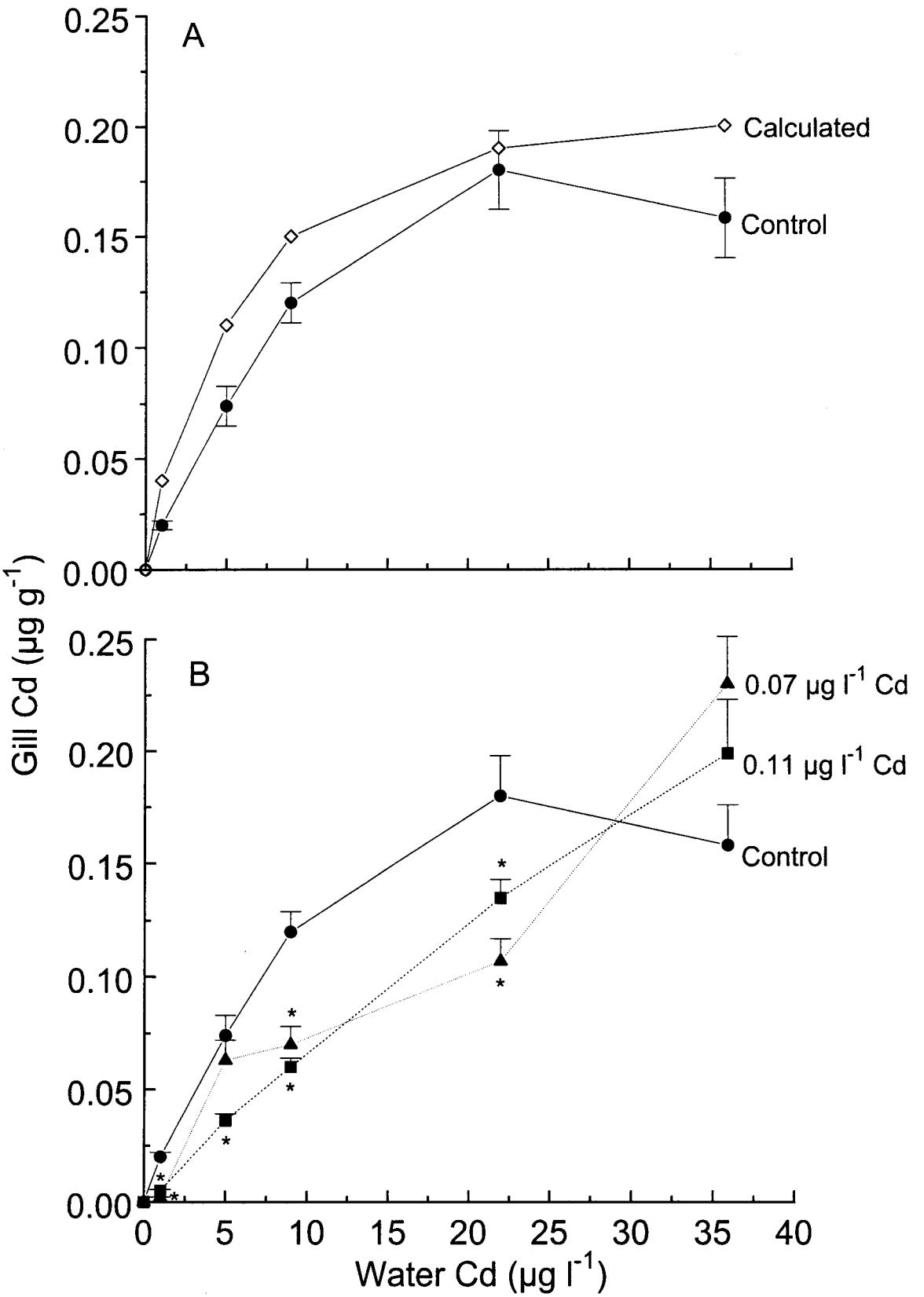
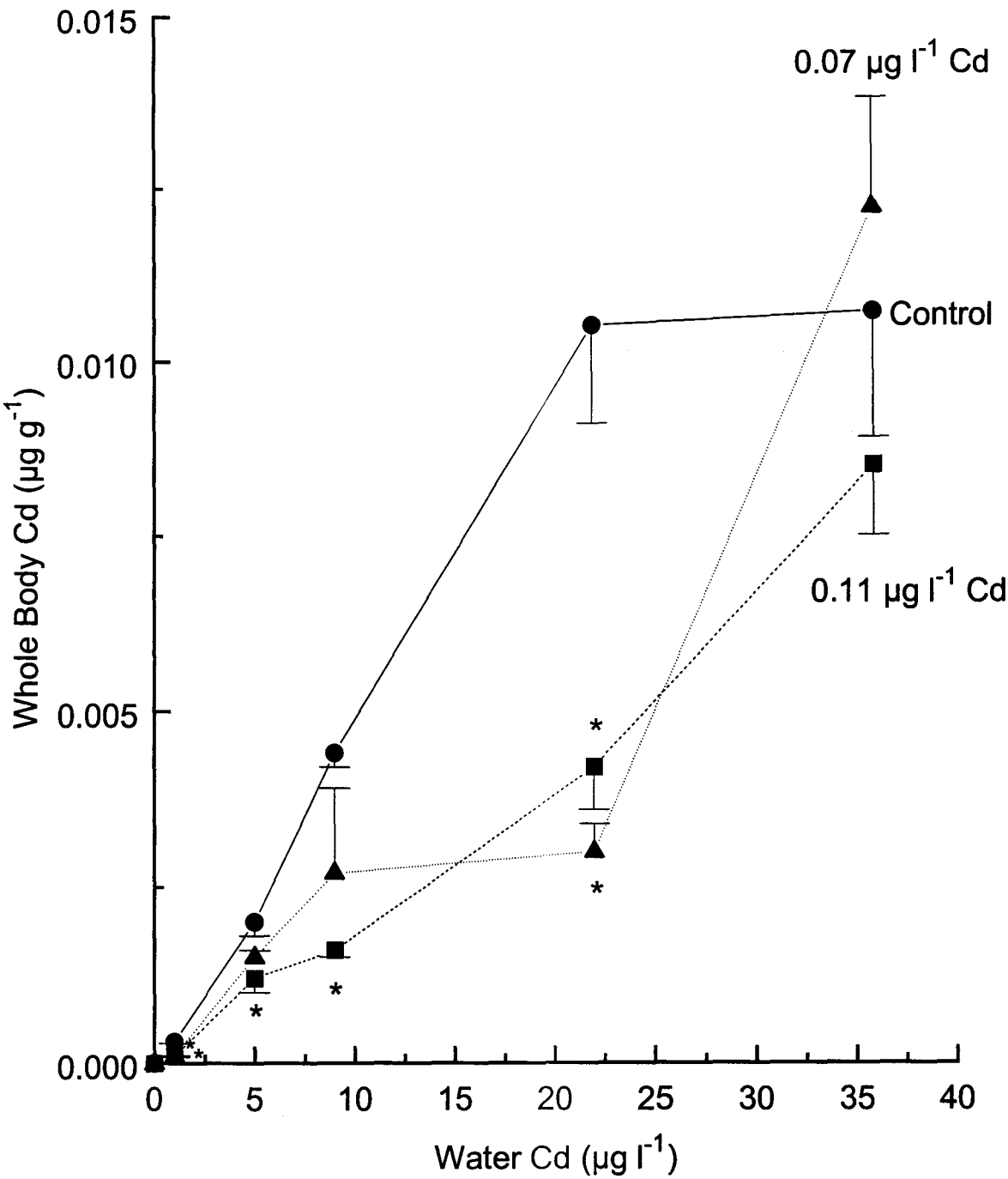

A
B
Calculated
Control
0.07 µg l-1 Cd
0.11 µg l-1 Cd
Control
Gill Cd (µg g-1)
Water Cd (µg l-1)
0.00
0.05
0.10
0.15
0.20
0.25
0
5
10
15
20
25
30
35
40
*

Fig. 3-4

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 d exposures to 0 μg l^{-1} Cd (controls; solid line), 0.07 μg l^{-1} Cd (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; * $P < 0.05$.


0.015
0.010
0.005
0.000
Whole Body Cd (µg g-1)
0
5
10
15
20
25
30
35
40
Water Cd (µg l-1)
0.07 µg l-1 Cd
Control
0.11 µg l-1 Cd
*

Chapter 4

Protective Effects of Calcium Against Chronic Waterborne Cadmium Exposure in Juvenile Rainbow Trout

Abstract

Juvenile rainbow trout, on 1% daily ration, were exposed to 0 (control) or 2 µg/L Cd [as $Cd(NO_3)_2 \cdot 4\ H_2O$] and four different calcium concentrations [260 (background), 470, 770, or 1200 µM Ca added as $Ca(NO_3)_2 \cdot 4\ H_2O$] in synthetic soft water for 30 days. Mortality was highest (~80%) in the lowest Ca plus 2 µg/L Cd treatment. Approximately 40% mortality was observed in the 470 µM Ca plus 2 µg/L Cd exposure; mortality was ≤10% for all other treatments. No growth effects were seen for any of the exposures. Kidneys accumulated the greatest concentration of Cd over 30 d, followed by gills and livers. Cadmium accumulation in gills, kidney, and liver decreased at higher water Ca concentrations. No differences in whole body or plasma Ca concentrations were found. Swimming performance was impaired in the 470 µM Ca plus 2 µg/L Cd exposed fish. Influx of Ca^{2+} into whole bodies decreased as water Ca concentrations increased; influx of Ca^{2+} into 260 µM Ca + 2 µg/L Cd treated fish was significantly reduced compared to control fish. Experiments that measured uptake of 'new' Cd into gills showed that affinity of the gills for Cd (i.e. log $K_{Cd\text{-}gill}$) decreased as water Ca concentrations increased, and the number of binding sites for Cd decreased

with increased water Ca concentrations. Acute accumulation of 'new' Cd into gills and the number of gill Cd binding sites (B_{max}) increased with chronic Cd exposure, while affinity of the gills for Cd decreased with chronic Cd exposure. Longer term gill binding (72 h) showed reduced uptake of 'new' Cd at higher water Ca levels and increased uptake with chronic Cd exposure. There were complications in applying the Biotic Ligand Model to fish chronically exposed to Cd because of the discrepancies in the maximum number of gill Cd binding sites (i.e. B_{max} values) amongst different studies. Furthermore, adaptive changes that occur in gills and the acclimatory responses of fish chronically exposed to Cd cannot be easily incorporated into the model.

Introduction

Waterborne cadmium can cause severe acute toxicological and physiological effects to aquatic organisms. However, these effects can be altered by water hardness (McCarty *et al.*, 1978; Pärt *et al.*, 1985; Pascoe *et al.*, 1986; Wicklund and Runn; 1988; Spry and Wiener, 1991; Davies *et al.*, 1993). Carroll *et al.* (1979), Pärt *et al.* (1985), and McDonald *et al.* (1989) have shown that protection against Cd uptake and acute toxicity in freshwater fish is related to water calcium concentration, rather than magnesium, illustrating that Ca is the primary cation responsible for the protective action of hard water. This protective action of calcium has been attributed to changes in gill permeability and/or competition between Cd and Ca for gill binding sites (Calamari *et al.*, 1980; Wright, 1980; Pagenkopf, 1983; Hunn, 1985, Pärt *et al.*, 1985; Meyer, 1999).

While the great majority of research has focussed on acute Cd toxicity, a number of studies have shown that Cd may cause sublethal deleterious effects during low level chronic exposures (Benoit *et al.*, 1976; Giles, 1984; Pratap *et al.*, 1989; Fu *et al.*, 1990; Pratap and Wendelaar Bonga, 1993; Iger *et al.*, 1994; Hollis *et al.*, 1999; Chapter 3). Water hardness is recognized to be protective against chronic as well as acute Cd toxicity in freshwater fish (Sauter *et al.*, 1976), and a hardness correction has been incorporated into ambient water quality criteria for chronic Cd exposures in Europe (Alabaster and Lloyd, 1982), the United States (U.S. EPA, 1985), and Canada (C.C.M.E., 1995). The primary

objective of the present study was to determine the mechanistic basis of the protective effects of Ca against chronic Cd exposure in juvenile rainbow trout. We examined changes in gill Cd burden, and the acute Cd-binding properties of the gills, accompanying 30 days exposure of trout in synthetic soft water supplemented with various calcium concentrations (260, 470, 770, or 1200 μM Ca) in the presence or absence of 2 μg/L Cd. A particular focus was to place the gill-binding results into a Biotic Ligand Modelling framework which has recently been advocated for the generation of site-specific water quality criteria (Renner, 1997; Bergman and Dorward-King, 1997; Playle, 1998; Meyer *et al.*, 1999; Di Toro *et al.*, 1999). Additional goals were to characterize Cd accumulation in other compartments (liver, kidney, and whole body), and document possible sublethal effects and costs of chronic Cd exposure, as expressed in growth on submaximal ration, whole body and plasma ion content, gill Ca^{2+} influx, and exercise performance.

Materials and Methods

Fish Holding Conditions

Rainbow trout [*Oncorhynchus mykiss* (Walbaum)] were obtained from Humber Springs Trout Farm in Orangeville, Ontario and held in flowing dechlorinated Hamilton tap water [Lake Ontario water: Ca = 40 mg/L or 1 mM, Na = 14 mg/L or 0.60 mM, Cl = 25 mg/L or 0.70 mM, dissolved organic matter (DOM) = 3 mg/L or 0.25 mM, hardness = 140 mg/L as $CaCO_3$, alkalinity = 95 mg/L as $CaCO_3$, pH 8.0, 14°C]. Trout were held in 600 L aerated polyethylene tanks for two months and then slowly introduced to synthetic soft water over the course of one week. The synthetic soft water [Ca = 10 mg/L or 0.26 mM, Na = 3 mg/L or 0.14 mM, Cl = 4 mg/L or 0.10 mM, DOM = 0.40 mg/L or 0.03 mM, hardness = 20 mg/L as $CaCO_3$, alkalinity = 15 mg/L as $CaCO_3$, pH 7.2, 13°C] was produced by reverse osmosis (Anderson Water Systems) and consisted of one part dechlorinated Hamilton tap water added to six parts ion-reduced water produced by reverse osmosis. Fish were held in soft water for at least three weeks before experimentation. Fish were fed 1% body weight per day (as one meal per day) with Martin's Starter Food (Martin Feed Mills, Elmira, Ontario; Cd content = 1.06 ± 0.04 (n = 6) µg/g Cd wet weight).

Exposure System

After three weeks in holding tanks, 85 fish were randomly transferred to each of sixteen 200 L polyethylene exposure tanks which were flow-through

systems (flow = 500 ml/min) with continuous aeration. Fish were fed a submaximal ration of 1% body weight per day (see above) in the hope of revealing metabolic costs associated with chronic Cd exposure. An acidified Cd stock solution, with Cd added as $Cd(NO_3)_2 \cdot 4\ H_20$ (Fisher Scientific, Nepean, Ontario), and a Ca stock solution, with Ca added as $Ca(NO_3)_2 \cdot 4\ H_20$ (Fisher Scientific, Nepean, Ontario), was delivered to a mixing head-tank *via* mariotte bottles (Mount and Brungs, 1967) to achieve desired Cd and Ca concentrations in the exposure tanks. Tanks were spiked on the first day of exposure to instantly reach the desired Cd and Ca concentrations. Water chemistry was measured weekly throughout the exposure.

Fish were exposed to four concentrations of Ca in either the absence or presence of Cd at a nominal concentration of ~2 µg/L. This concentration was chosen based on an initial 96-h Cd LC50 measurement of approximately 2 µg/L in the background Ca concentration of 260 µM. The eight treatment conditions each had two replicates so that n = 170 fish per treatment. The eight exposures were: (i) control = background Ca of 260 µM with nominally zero cadmium, (ii) 2 µg/L Cd with background Ca (260 µM Ca), (iii) 470 µM Ca with no added Cd, iv) 470 µM Ca + 2 µg/L Cd, v) 770 µM Ca with no added Cd, vi) 770 µM Ca + 2 µg/L Cd, vii) 1200 µM Ca with no added Cd, and viii) 1200 µM Ca + 2 µg/L Cd for 30 days in synthetic soft water. Actual measured water Ca and Cd concentrations are presented in Table 1.

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 for Na, Ca, Cl, and total Cd content (Table 1). Fish from each treatment tank were bulk weighed every 10 days. All of the fish from the tank (one tank at a time) were removed and put in a tared sieve placed inside a bucket containing water from the exposure tank. The bucket was weighed, fish were briefly removed using the sieve, and the bucket reweighed. The mass of the fish was calculated from the difference between the mass of the bucket plus sieve with and without fish.

Specific growth rates (SGR) were determined from bulk weights from individual treatment tanks taken 4 or 5 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, and gills, liver, kidney, and the remaining carcass were assayed for Cd content. Remaining carcass was also assayed for whole body ion content. Fish were sacrificed and both sets of gills and the liver were excised; gills were rinsed for 10 seconds in 100 ml of dechlorinated Hamilton tap water. All tissues plus remaining carcass were frozen for later analysis of Cd and ion content.

Six fish from each tank were also sampled at day 0, 2, 10, 20, and 30 for plasma 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 Ca content.

Testing

Exercise performance

Fish were not fed the day of swimming tests. Swimming performance was determined using the protocol of McDonald *et al.* (1998), a stamina test which uses a fixed velocity and exhaustion as the end point. Fish were swum in a flume with the appropriate treatment water in groups of ten against a current of 60 cm/s (~ 5 body lengths per s) until exhaustion occurred. Fish were considered exhausted when they were impinged against the rear screen of the flume and would not swim after prodding. Fatigued fish were removed from the swimming flume and fork length and weight were recorded. Sprint times were corrected to a reference body length of 11 cm and the time to 50% fatigue (± 1 S.E.) was calculated, from ten fish from each treatment, by linear regression in SPSS of probit fatigue versus log time.

Ca^{2+} Influx

Unidirectional Ca^{2+} uptake into trout was determined by exposing the fish for 4 h, after the 30 day Cd exposure, to radioactive ^{45}Ca, as described by Hogstrand *et al.* (1994). Eight fish from each treatment were placed randomly

into eight clear plastic bags containing 3 L of the appropriate treatment water, placed in a water bath to maintain temperature (13°C). Five minutes before the fish were added to the flux-bags, 7 μCi/L ^{45}Ca was added as $CaCl_2$ (specific activity = 10 mCi/mM; New England Nuclear, Boston, MA). Quadruplicate water samples of 5 ml were taken at 0, 2, and 4 h for later analysis of ^{45}Ca, Ca^{2+}, and Cd. After 4 h in the flux-bags, the fish were removed and rinsed for 1 min in 10 mM $Ca(NO_3)_2$ and an overdose of anaesthetic (MS 222, 0.33 g into 1 L of rinse water) to displace surface-bound ^{45}Ca, blotted dry, weighed, and individually wrapped in aluminum foil. The fish and water samples were frozen immediately after the experiment and kept at -20°C until analysis.

Acclimation

A 96 hour LC50 trial was run after 30 days exposure to assess possible acclimation of metal-exposed fish. Each test cell consisted of eight fish placed randomly into 15 L green plastic buckets having aeration and flow-through (200 ml/min) of dechlorinated Hamilton tap water at the appropriate Ca and Cd level, as added by a mariotte bottle. Eight fish from the low Ca (260 and 470 μM) treatments were exposed for 96 h to Cd concentrations of 0.07 ± 0.03 (4), 0.28 ± 0.05 (4), 0.95 ± 0.17 (4), 3.28 ± 0.30 (4), 14.58 ± 3.35 (4), or 16.95 ± 2.85 (4) μg/L Cd with the appropriate Ca concentration (250 or 470 μM Ca). Eight fish from the high Ca (770 and 1200 μM) treatments were exposed for 96 h to Cd concentrations of 0.20 ± 0.05 (4), 4.78 ± 1.69 (4), 15.60 ± 0.30 (4), 41.80 ± 4.82

(4), 139.39 ± 27.45 (4), or 247.77 ± 61.76 (4) µg/L Cd with the appropriate Ca concentration (770 or 1200 µM Ca). Dead fish were removed when movement ceased, and times of mortality were recorded. LC_{50} values (± 95% C.L.) were determined by log probit analysis (Litchfield, 1949).

Acute and longer term gill-Cd binding

Previously, we showed that the use of radiolabelled ^{109}Cd is essential to distinguish 'new' gill Cd uptake relative to the high background 'cold' concentrations already present in the gills of chronically exposed fish (Hollis *et al.*, 1999; Chapter 3). Acute gill metal uptake/turnover of Cd was determined by exposing the fish for 3 h, after the 30 day Cd exposure, to various concentrations of Cd labelled with the radioisotope ^{109}Cd. Fish were not fed the day of gill binding experiments. Five fish from each treatment (except for the 260 µM + 2 µg/L Cd exposed fish due to low number of surviving fish available) were placed into 15 clear plastic bags containing 10 L of aerated, soft water (260 µM Ca), placed in a water bath to maintain temperature (13°C). Each treatment group was exposed to the appropriate acclimation Ca concentration [260, 470, 770, or 1200 µM Ca added as $Ca(NO_3)_2 \cdot 4\ H_20$ (Fisher Scientific, Nepean, Ontario)] plus 6 ± 1 (14) µg/L, 14 ± 1 (14) µg/L, 27 ± 1 (14) µg/L, 63 ± 2 (14) µg/L, or 115 ± 2 (14) µg/L total Cd added as $Cd(NO_3)_2 \cdot 4\ H_20$ (Fisher Scientific, Nepean, Ontario) with 1 µCi/L ^{109}Cd added as $CdCl_2$ (specific activity = 1.97 mCi/mg; New England Nuclear, Boston, MA). Water samples (5 ml) were taken at the beginning and

end of the 3 h static exposure. Gills were sampled at 3 h. Gills were removed, rinsed, acid digested, and later analyzed for total Cd and ^{109}Cd radioactivity (see Tissue Analysis).

A longer term gill Cd uptake/turnover experiment was run after the 30 day Cd exposure by exposing the fish for 72 h to the Cd radioisotope ^{109}Cd at a total Cd concentration approximately three-fold higher than the nominal chronic exposure concentration. Twenty fish from each treatment (or fewer depending upon availability) were placed into twelve clear plastic bags containing 15 L of aerated, soft water (260 µM Ca), placed in a water bath to maintain temperature (13°C). Each treatment group was exposed to the appropriate Ca concentration [260, 470, 770, or 1200 µM Ca added as $Ca(NO_3)_2 \cdot 4\ H_20$ (Fisher Scientific, Nepean, Ontario)] plus 6 ± 1 (5) µg/L total Cd added as $Cd(NO_3)_2 \cdot 4\ H_20$ (Fisher Scientific, Nepean, Ontario) with 1 µCi/L ^{109}Cd added as $CdCl_2$ (specific activity = 1.97 mCi/mg; acquired from New England Nuclear, Boston, MA). Water samples (5 ml) were taken daily during the 72 h static exposure. Gills of five fish were sampled at 12, 24, 48, and 72 h. Gills were removed, rinsed, acid digested, and later analyzed for total Cd and radioactivity from ^{109}Cd (see Tissue Analysis).

Tissue and Water Analyses

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

Gills, livers, kidneys, and remaining carcass were thawed, weighed, and

then digested in 1 to 15 times their weight of 1 N HNO_3 (TraceMetal Grade HNO_3; Fisher Scientific, Nepean, Ontario) for 15 h at about 60°C. Digests were shaken, left to settle for 10 min, then the supernatant was diluted 20 to 200 times with deionized water, as appropriate (18 mgohm; Nanopure II; Sybron/Barnstead, Boston, MA). Gill, liver, kidney, and carcass 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 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.

Whole body Cd was calculated based on the data for individual fish at each sample time using the following equation:

$$WB = [(G \times gwt) + (L \times lwt) + (K \times kwt) + (C \times cwt)]/fwt$$

where "WB" is whole body Cd accumulation (µg/g Cd wet tissue), "G" is gill Cd accumulation (µg/g Cd wet tissue), "L" is liver Cd accumulation (µg/g Cd wet tissue), "K" is kidney Cd accumulation (µg/g Cd wet tissue), "C" is carcass Cd accumulation (µg/g Cd wet tissue), "gwt" is the weight of the gills (g), "lwt" is the weight of the liver (g), "kwt" is the weight of the kidney (g), "cwt" is the weight of the carcass (g), and "fwt" is the weight of the fish (g). Gills, liver, kidney, and carcass represent 3.0%, 1.5%, 0.5%, and 95.0% of the total whole body weight,

respectively.

The frozen fish from the Ca influx experiment were transferred to liquid nitrogen and ground to a fine powder with a tissue grinder (Janke and Kunkel GMBH and Co., IKA-Laboratories, Markham, Ontario). The powder was weighed out in triplicate samples of 0.5 g in glass scintillation vials. Each tissue sample was digested with 2.0 ml of liquid tissue solubilizer (Soluene-350, Canberra-Packard Can. Ltd., Mississauga, Ontario) for 48 h at 45°C. The samples were then diluted with 10 ml of scintillation fluor (Hionic Fluor, Canberra-Packard Can. Ltd., Mississauga, Ontario) and counted in a scintillation counter (1217 Rackbeta Liquid Scintillation Counter, LKB-Wallac, Turku, Finland), with quench correction by internal standardization relative to the counting efficiency of water samples (see below).

Duplicate water samples were acidified and analyzed for calcium by atomic absorption spectroscopy (Varian AA-1275 operated in standard absorption mode) and for Cd by graphite furnace (Varian AA-1275 with GTA-95 atomizer, Varian Techtron Pty. Limited, Springvale, Australia). The remaining duplicate water samples (5 ml each) were diluted with 10 ml scintillation fluor (Hionic Fluor, Canberra-Packard Can. Ltd., Mississauga, Ontario) and counted for ^{45}Ca.

The inward flux (J_{in}) for Ca^{2+} (in μM/kg/h) was calculated according to the formula from Hogstrand *et al.* (1994):

J_{in} = ACT / (SA X CE X t)

where "ACT" is the average counts in the tissue samples (cts/min/kg), "SA" is the measured mean specific activity of ^{45}Ca in the water (cts/min/µmol), "CE" is the relative counting efficiency of the 'tissue-solubilizer-fluor system', compared to the 'water-fluor system', and "*t*" is time (h).

Tissue ^{109}Cd concentrations were measured on a Minaxi Auto-Gamma 5000 Series Gamma Counter (Canberra Packard Instrument Company, Meriden, CT). Tissue ^{109}Cd concentrations were converted to absolute values ('new Cd') using the measured specific activity (b/c) of the water:

a/(b/c)

where "a" is ^{109}Cd cpm per g of tissue (wet weight), "b" is ^{109}Cd counts in the water (cpm/L), and "c" is the total Cd concentration in the water (µg/L Cd).

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 ionic Cd^{2+} concentration in the water, and was plotted against the amount of Cd bound by the gill. The K_D and the total B_{max} of the gill were then determined from the slope and *x*-intercept of the Scatchard plot, respectively. Concentrations of Cd^{2+} and other cadmium species in the water were calculated using the MINEQL+ aquatic geochemical program

(Schecher and McAvoy, 1994) and measured water chemistry.

Water and plasma Na and Ca 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). Water and whole body Cl concentrations were measured on the acid digests using the colorimetric assay of Zall *et al.* (1956) and read with an MRX microplate reader (Dynatech Laboratories Inc., Chantilly, VA). Water pH was measured using a Radiometer PHM71b meter with GK2401C combination electrode (Radiometer, Copenhagen, Denmark). Dissolved organic matter (DOM) was measured on a Rosemount Analytical DC-180 automated total organic carbon analyzer (Folio Instruments, Kitchener, Ontario).

Statistics

Data have been expressed as means ± 1 S.E. (*n*) except in the case of LC50 values where means ± 95% C.L. have been reported. 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.

Results

Effects of Exposure

Fish mortality over 30 days was greatest (~80% in total) in the 2 µg/L Cd treatment at the lowest (background) Ca = 260 µM, with acute toxicity (i.e. ~50% mortality) occurring within the first five days (Fig. 1). Approximately 40% mortality occurred for the 470 µM Ca + 2 µg/L Cd treated fish over the 30 d exposure and ~10% mortality was observed for all other treatments (Fig. 1). Despite the submaximal diet, in no instance was the growth of the surviving fish decreased with Ca and/or Cd exposure (Table 2).

Cadmium accumulation in all tissues decreased as water Ca concentration increased (Fig. 2A-D). Cadmium concentrations were greatest in kidney (Fig. 2C), followed by gills (Fig. 2A) and liver (Fig. 2B). Gill Cd levels were significantly increased ($P < 0.05$) 14, 19, 12, and 8 fold from initial (day 0) values [0.03 ± 0.02 (6) µg/g Cd wet tissue] for 2 µg/L Cd + 260, 470, 770, or 1200 µM Ca exposures, respectively, after 30 days of exposure (Fig. 2A; Table 3). Liver Cd concentrations were significantly increased 9, 7, 4, and 2 fold from initial values [0.34 ± 0.03 (6) µg/g Cd wet tissue] for 2 µg/L Cd + 260, 470, 770, or 1200 µM Ca exposures, respectively (Fig. 2B; Table 3).

Kidney Cd levels were significantly increased 16, 15, 8, and 5 fold from initial values [0.47 ± 0.02 (6) µg/g Cd wet tissue] for 2 µg/L Cd + 260, 470, 770, or 1200 µM Ca exposures, respectively (Fig. 2C; Table 3). Remaining carcass

Cd levels were not significantly different ($P > 0.05$) from initial values for any of the treatments, and average carcass Cd concentrations were 0.38 ± 0.03 (33) µg/g Cd wet tissue (data not shown). Whole body Cd concentrations were significantly increased ($P < 0.05$) 1.5 and 1.4 fold from initial values (Fig. 2D; Table 3) for 260 µM Ca + 2 µg/L Cd and 470 µM Ca + 2 µg/L Cd, respectively. The exposures of 770 µM Ca + 2 µg/L Cd and 1200 µM Ca + 2 µg/L Cd were not significantly different ($P > 0.05$) from initial whole body Cd values (Fig. 2D, Table 3). Approximate saturation of the tissues (gills, liver, kidney, and whole body) was reached for 2 µg/L Cd + Ca (260, 470, 770, or 1200 µM Ca) exposed fish by the end of the 30 day exposure (Fig. 2A-D; Table 3), as shown by the general similarity of the day 20 and day 30 measurements.

Acclimation

Toxicological acclimation to Cd was indicated only for the low Ca + Cd treated fish (Table 4). The fish exposed to 2 µg/L Cd + 260 or 470 µM Ca showed a trend of increased tolerance to Cd; however, as there was less than 50% mortality in the highest concentration tested, 96-h LC50 values could not be calculated for these two groups. At the two higher calcium concentrations, there were no significant differences in the 96-h LC50 values.

Physiological Effects and Costs of Chronic Exposure

No consistent treatment or time-related effects were seen in carcass and

plasma Ca, which averaged 111 ± 5 (198) mM/kg and 2.0 ± 0.1 (198) mM, respectively (Fig. 3). Carcass Na^+ and Cl^- concentrations averaged 37 ± 5 (198) mM/kg and 40 ± 3 (198) mM/kg, respectively, also with no consistent treatment or time effects.

Swimming stamina was not significantly altered by exposure to different water Ca concentrations in the absence of Cd. However in the presence of chronic Cd exposure (30 days), there was a tendency for swimming impairment at low water Ca concentrations (Fig. 4). Swimming performance, as determined by time to 50% fatigue, was significantly decreased ($P < 0.05$) with chronic exposure to 2 µg/L Cd + 470 µM Ca (Fig. 4).

Unidirectional influx of Ca, as assessed by ^{45}Ca appearance in whole bodies, decreased significantly ($P < 0.05$) compared to background (260 µM Ca) at water Ca concentrations of 770 and 1200 µM Ca, in the absence of Cd (Fig. 5). Unidirectional calcium influx was decreased by 50% with chronic exposure to 2 µg/L Cd at low water Ca (background; 260 µM Ca) compared to controls, but was not affected at higher water Ca levels.

Cd Uptake/Turnover in Gills of Acclimated Trout

An acute (3 h) Cd uptake/turnover test was run using radioactive ^{109}Cd at total Cd concentrations of 6, 14, 27, 63, and 115 µg/L, in an attempt to differentiate Cd binding by the gills of control and Cd-exposed fish acclimated to different water Ca concentrations. In all groups, uptake increased with

increasing acute exposure concentrations of radiolabelled Cd, with uptake tending towards saturation at higher concentrations (Fig. 6). Control fish (260 μM Ca exposed) reached approximate saturation when exposed to 115 μg/L Cd for 3 h, with about 1.15 μg/g of 'new' waterborne Cd bound to the gills (Fig. 6A).

With increased water Ca concentrations, uptake of 'new' Cd by gills decreased. Trout which had been exposed to higher water Ca concentrations (470, 770, and 1200 μM Ca) for 30 days had 37, 50, and 64% decreases in 'new' gill Cd uptake compared to controls at the highest Cd concentration tested (115 μg/L; $P < 0.05$; Fig. 6A). Similar trends with increasing water Ca levels (470, 770, and 1200 μM Ca) were seen in fish which had been exposed to 2 μg/L Cd for 30 days (Fig. 6B).

At each Ca concentration, 'new' Cd accumulation in the gills increased significantly ($P < 0.05$) with chronic Cd exposure (Fig. 6B). At the highest test concentration (115 μg/L Cd), uptake of 'new' Cd by gills increased 3.8, 1.7, and 1.6 fold for the 470 μM Ca + 2 μg/L Cd, 770 μM Ca + 2 μg/L Cd, and 1200 μM Ca + 2 μg/L Cd groups (Fig. 6B) compared to their respective control groups at the same Ca levels (260, 470, 770, or 1200 μM Ca) without Cd (Fig. 6A).

Cadmium speciation analyses from MINEQL+ indicated that 60 - 94% of the total Cd existing as the free ionic species, Cd^{2+}, for exposures ranging from 6 to 115 μg/L, respectively. Calcium concentrations of the test waters (i.e. 260, 470, 770, and 1200 μM Ca) did not affect Cd^{2+} speciation to a large extent. There was a <1% decrease in free ionic Cd^{2+} concentration as water Ca levels

increased from 260 to 1200 μM (i.e. 54.9% to 54.7% of total Cd as Cd^{2+} for 260 and 1200 μM Ca, respectively, with the remainder bound to dissolved organic matter). Scatchard analysis of Cd uptake/turnover for the 3 h exposure to radioactive ^{109}Cd (Fig. 6A) was done using ionic Ca^{2+}. This analysis indicated a trend for decreasing conditional stability constants (log $K_{Cd\text{-}gill}$; i.e. decreasing affinity) and decreasing B_{max} values (number of gill Cd binding sites) as the chronic Ca exposure level increased (Table 5). Furthermore, at each calcium concentration, conditional stability constants decreased with chronic exposure to 2 μg/L Cd, while the number of gill Cd binding sites (B_{max} values) increased with chronic Cd exposure (Table 5).

The data set for the longer term gill binding exposure (72 h) is incomplete due to mortality during the 72 h exposure (Fig. 7A,B), as well as mortality that occurred during the 30 day chronic exposure to cadmium (Fig. 1) that limited the number of fish which could be tested. The longer term gill binding exposure to 6 μg/L Cd for 72 h again revealed pronounced influences of both water Ca concentration and the effects of chronic Cd exposure. Gill uptake of 'new' Cd decreased as water Ca increased, but gill accumulation of 'new' Cd increased with chronic exposure to 2 μg/L Cd (Fig. 7A, B). The total Cd concentrations in gills remained constant over the 72 h exposure (data not shown).

Discussion

Environmental Relevance

The U.S. E.P.A. freshwater quality criteria for aquatic life for water hardness ranging from 20 mg/L as $CaCO_3$ (close to our low Ca exposure) to 120 mg/L as $CaCO_3$ (close to our high Ca exposure) recommend a limit of 0.6 to 4.8 µg/L Cd, respectively, for acute Cd exposures (U.S. E.P.A., 1986). Limits set by the U.S. E.P.A. for chronic Cd exposures are 0.3 and 1.3 µg/L Cd for water hardness ranging from 20 to 120 mg/L as $CaCO_3$, respectively (U.S. E.P.A., 1986). We used 2.0 µg/L Cd for our experiment, which is also relevant to Canadian water quality guidelines of 0.2 and 1.3 µg/L Cd for protection of freshwater life exposed chronically to cadmium in soft and hard water, respectively (C.C.M.E., 1995).

Costs of Cd Exposure

Despite the restricted ration, no significant effects on growth (Table 2) were observed during the 30 day exposure to Cd plus increasing water Ca concentrations. Therefore growth was not a sensitive indicator of ongoing sublethal Cd exposure. Other reports on chronic Cd exposure, many run at higher ration levels, support these findings with no adverse effects on growth. Hollis *et al.* (1999) showed similar results for trout chronically exposed to 3 and 10 µg/L Cd in hard water (140 mg/L as $CaCO_3$). Specific growth rates were not

altered in fish fed 3% body weight per day, and whole body Ca concentrations were not consistently affected. Giles (1988) also demonstrated that chronic (178 day) exposures to 3.6 and 6.4 µg/L Cd resulted in no significant growth effects. Farag *et al.* (1994) showed that mixed metal solutions of 2.2 µg/L Cd with 6.4 µg/L Pb and 24 µg/L Cu had no adverse effects on growth of juvenile rainbow trout during a 21 day exposure. Benoit *et al.* (1976) showed that growth of first-generation brook trout was not significantly impaired with chronic (38 weeks) exposure to 3.4 µg/L Cd. In addition, Kumada *et al.* (1980) and Davies *et al.* (1993) showed that growth of juvenile rainbow trout was not significantly different from controls when exposed to 4 µg/L Cd for 70 to 100 days. The observations covered a range of water hardness from 44 to 400 mg/L (as $CaCO_3$), and a range of daily ration levels from 0.7 to 3.6% body weight per day.

Whole body and plasma Ca concentrations were not consistently changed with chronic Cd exposure and increasing water Ca concentrations (Fig. 3). Hollis *et al.* (1999) and Chapter 3 also showed no significant differences in whole body and plasma ions with chronic Cd exposures in either hard (140 mg/L as $CaCO_3$) or soft (20 mg/L as $CaCO_3$) water, respectively. Giles (1984) showed that plasma calcium and magnesium concentrations were not significantly changed at 3.6 µg/L Cd exposures at water hardness 82 mg/L as $CaCO_3$, but were decreased at 6.4 µg/L Cd exposures. Reid and McDonald (1988) demonstrated no adverse effects of 6.5 µg/L Cd exposure on plasma Ca and Na during a 24 h exposure in both hard (Ca = 1 mM) and soft (Ca = 0.04 mM) water; however,

influx of Ca^{2+} into whole bodies was significantly reduced.

In contrast, at the lower Ca exposures swimming performance did respond to chronic Cd exposure: sprint performance was significantly reduced for the 470 µM Ca + 2 µg/L Cd exposed fish (Fig. 4). Scherer *et al.* (1997) reported impaired swimming performance, represented by foraging ability of adult lake trout exposed to 0.5 µg/L Cd in water hardness 90 mg/L as $CaCO_3$; however, escape of the prey (rainbow trout fingerlings) from the lake trout was not impaired by the Cd exposure.

The primary mechanism of acute Cd toxicity is fatal hypocalcaemia resulting from irreversible blockage of Ca^{2+} uptake across the gills, apparently by non-competitive inhibition of an essential transport enzyme, high affinity Ca^{2+} ATPase (Verbost *et al.*, 1987, 1989). Uptake of Ca^{2+} did not recover within a 12 h period once the Cd (a high level of 6.5 µg/L Cd) was removed, suggesting that Cd causes permanent damage to the Ca^{2+} ATPase (Reid and McDonald, 1988). In the present study, unidirectional influx of Ca^{2+} into trout was significantly reduced with chronic Cd exposure (2 µg/L Cd) at the lowest Ca concentration (260 µM Ca; Fig. 5). Unidirectional influx of Ca^{2+} into trout was also significantly reduced at higher water calcium concentrations with or without Cd (770 and 1200 µM Ca; Fig. 5) while whole body and plasma Ca^{2+} concentrations were not significantly different at the various water Ca concentrations (Fig. 3). Hogstrand *et al.* (1994) demonstrated that unidirectional Ca^{2+} influx was significantly reduced in rainbow trout chronically exposed to 150

µg/L Zn in hard water (1 mM Ca) for 60 days. Reid and McDonald (1988) reported similar results with significant inhibition of Ca^{2+} influx in rainbow trout exposed for 24 h to 6.5 µg/L Cd. In addition, the degree of inhibition of Ca^{2+} influx due to Cd exposure relative to control fish was greater in soft water (Ca^{2+} = 0.04 mM) than hard water (Ca^{2+} = 1 mM) acclimated trout.

Since both water Ca and Cd effects on unidirectional Ca^{2+} influx occurred in the present study without effects on whole body Ca^{2+} concentrations or growth, compensating changes in Ca^{2+} efflux are likely to have occurred. Verbost *et al.* (1987) demonstrated that transepithelial Ca^{2+} efflux in gills of rainbow trout is less sensitive to Cd^{2+} than the Ca^{2+} influx. Efflux of Ca^{2+} did not occur in gills of trout exposed to 0.1 µM Cd (or 11 µg/L Cd), however, Ca^{2+} efflux was significantly stimulated at a Cd concentration of 1 µM (112 µg/L). In contrast, Reid and McDonald (1988) found no significant effects on Ca^{2+} efflux in whole bodies of rainbow trout during a 24 h exposure to 6.5 µg/L Cd in both hard (Ca = 1 mM) and soft (Ca = 0.04 mM) water.

Acclimation

Toxicological acclimation to cadmium may have occurred at low chronic Ca exposures (260 and 470 µM Ca) + 2 µg/L Cd; however, acclimation did not occur at the higher Ca concentrations, because subsequent challenge to Cd after the 30 day exposure did not yield significantly higher 96-h LC50 values compared to controls (Table 3). The apparent 'acclimation' at the low Ca levels

may have been due to 'selection' for fitter fish since there was ~ 80% and ~40% total mortality for the 260 μM Ca + 2 μg/L Cd and 470 μM Ca + 2 μg/L Cd exposures, respectively, after the 30 day exposure to cadmium, but mortality was minimal (<10%) for the higher Ca treated fish (Fig. 1). The chronic Cd exposure concentration of 2 μg/L was therefore below the threshold for acclimation at the higher calcium levels. There was significant accumulation of Cd in the gills of these fish (Fig. 2); however, the exposure concentration of 2 μg/L Cd may not have been high enough to induce morphological damage to the gills, and thereby acclimation. McDonald and Wood (1993) proposed a damage-repair hypothesis which describes an initial 'shock' phase involved with metal exposure which results in morphological damage to the gills, followed by changes and repair of the gills with continued exposure to the metal, leading to acclimation of the fish. Lack of this damage-repair may explain the observed acclimation of trout at the lower Ca exposures and not at the higher chronic Ca exposures to Cd.

Internal Cadmium Distribution

The most sensitive indicator of chronic Cd exposure appears to be tissue accumulation of the metal. Kidneys accumulated the greatest concentration of Cd over the 30 day exposure to Cd, followed by gills and liver (Fig. 2). Several other studies have demonstrated equal or higher concentrations of Cd (relative to gills or liver) in kidneys of chronically exposed trout. Benoit *et al.* (1976), Kumada *et al.* (1980), and Harrison and Klaverkamp (1989) showed that kidney

Cd concentrations remained high once the fish were returned to Cd-free water, indicating the importance of the kidney as a storage organ for Cd.

The protective effects of calcium against Cd uptake into the organs was observed for gills, liver and kidney, with decreased Cd accumulation at higher water Ca concentrations (Fig. 2). Wicklund and Runn (1988) also showed the protective effects of Ca against Cd uptake with slower uptake of Cd into the gills with increasing water Ca levels (0.2 – 5 mM Ca), causing lower Cd accumulation in the liver and kidney. Verbost *et al.* (1987, 1989) demonstrated that Cd^{2+} and Ca^{2+} compete for the same apical channel in the initial uptake step into the gills, whereas the basolateral transports appear to differ, though Cd^{2+} non-competitively inhibits the high affinity Ca^{2+} ATPase which moves Ca^{2+} across the basolateral membrane into the bloodstream. Pärt *et al.* (1985) reported a strong inverse relationship between water Ca^{2+} concentrations up to 3 mM and Cd uptake into perfused rainbow trout gills; however, Cd retention in gill tissue was not sensitive to water Ca and therefore was different from Cd transfer.

Implications for Biotic Ligand Modelling

The Biotic Ligand Model involves the use of the fish gill, the primary site of toxic action, as a generalized 'biotic ligand' for complexing metals (Di Toro *et al.*, 1999). Conditional equilibrium stability constants for the affinity of this biotic ligand for a particular metal, along with relevant water chemistry, are entered into aquatic geochemistry programs (e.g. MINEQL+) to predict metal binding to the

gills (Playle *et al.*, 1993a,b). The predicted accumulation by the gills has been shown to be correlated with the toxicity of the metal to fish (MacCrae *et al.*, 1999).

In our previous studies, we demonstrated that the Biotic Ligand Model could be successfully applied to fish in both hard and soft water (Hollis *et al.*, 1999; Chapter 3). In the present study however, control fish (260 μM Ca) had much higher 'new' gill Cd concentrations (i.e. higher B_{max} values; Table 5) compared to the earlier studies in hard and soft water (Hollis *et al.*, 1999; Chapter 3). This discrepancy in the number of gill Cd binding sites between studies may be due to size or batch differences in fish or differences in feeding regime, which was 1% daily ration *versus* 3% in the previous studies. Whatever the explanation, this creates problems in trying to apply the Biotic Ligand Model to different sets of experiments. In our previous studies, the maximum number of binding sites was similar to the fathead minnow value of 0.2 nmol/fish or 2 nmol/g of gill from Playle *et al.* (1993a,b). The present study resulted in gill Cd binding site numbers closer to that reported by Hollis *et al.* (1997) for rainbow trout (1 nmol/fish or 10 nmol/g of gill). Hence, the Biotic Ligand Model would only be successful at predicting 'new' Cd accumulation in the gills of the Ca exposed fish from the present study (Fig. 6A) when the conditional stability constant for Cd binding to the gill (log $K_{Cd\text{-}gill}$ = 8.6) from Playle *et al.* (1993a,b) and the number of Cd binding sites (B_{max}) on the gill (1 nmol/fish or 10 nmol/g of gill) from a different source, Hollis *et al.* (1997), were used, along with the water Cd^{2+} concentrations.

Interestingly, while the log $K_{Cd\text{-}gill}$ value was derived for fathead minnow, the high B_{max} value of 10 nmol/g, similar to the present data, was derived from work on rainbow trout (Hollis *et al.*, 1997). In that study, the fish had been fed once per day on a limited ration (personal communication), as in the present study, pointing to feeding as the cause of the variation for B_{max}. Possibly, fish on restricted ration may 'up-regulate' Ca^{2+} transport from the water to augment limited dietary calcium, thereby increasing the B_{max} for Cd^{2+} binding on the gills.

The model would be able to predict Cd accumulation in control fish because it incorporates water chemistry (i.e. water Ca concentrations). However, for several reasons the model cannot be used to predict gill Cd loading and toxicity in trout chronically exposed to Cd. Both the log $K_{Cd\text{-}gill}$ and B_{max} values for 'new Cd' were altered as a result of chronic Cd exposure; the affinity decreasing, and the site number increasing (Table 5). Furthermore, total 'cold' Cd burden was increased during chronic exposure by approximately four-fold (4.2 – 2.5 μg Cd/g for 260 – 1200 μM Ca, respectively; Table 3) saturation of the B_{max} values (1.1 – 0.6 μg Cd/g for 260 – 1200 μM Ca, respectively; Table 5) for acute toxicity. The acute toxicity threshold itself appeared to change as well (Table 4), but not in proportion to changes in gill Cd binding kinetics. In addition, it was necessary to use a radiotracer technique, rather than the cold technique described by Playle *et al.* (1993a,b), to detect the small increases in 'new' gill accumulation against the high background Cd pools accumulated in the gills during the chronic 30 d exposure to Cd (Fig. 2; Table 3).

Scatchard analysis was applied to the kinetic binding curves for calcium and Ca plus Cd-exposed fish (Fig. 6A,B), which resulted in conditional stability constants of log $K_{Cd\text{-}gill}$ = 5.78 to 6.98 (Table 5) much lower than that of Playle *et al.* (1993a,b) for fathead minnows (log $K_{Cd\text{-}gill}$ = 8.6) but reasonably similar to controls from our previous studies for hard and soft water exposed fish (log $K_{Cd\text{-}gill}$ = 7.3 to 7.6; Hollis *et al.*, 1999; Chapter 3). At least in part, the difference in our log $K_{Cd\text{-}gill}$ values compared to Playle *et al.* (1993a,b) is due to the difference in methods for calculating conditional equilibrium stability constants. We calculated these values from Cd loading in gills of the fish, whereas Playle *et al.* (1993a,b) used competitive ligands to reduced Cd accumulation of gills, a method which is less sensitive to the competitive effects of Ca^{2+} and H^{+}. In the present study, as the water Ca^{2+} concentrations increased, the affinity of Cd for the gill decreased (Table 5). Using the log $K_{Ca\text{-}gill}$ value of 5.0 from Playle *et al.* (1993a,b), the log $K_{Cd\text{-}gill}$ value should theoretically move 0.61 log units (i.e. log $K_{Cd\text{-}gill}$ of 7.4 decreases to 6.8) as water Ca increases from 260 to 1200 µM Ca. This reduction is identical to the shift seen in the present study (7.0 to 6.3; Table 5), though the absolute values are slightly lower.

Trout which had been chronically exposed to 2 µg/L Cd accumulated more 'new' Cd in their gills compared to their respective Ca controls when exposed acutely to higher total Cd concentrations ranging from 6 to 115 µg/L (Fig. 6B). This influence of chronic Cd exposure (i.e. increased uptake of 'new' Cd by gills during high Cd exposure of trout which had previously been chronically exposed

to low levels of Cd) was also evident in the longer term (72 h) gill binding exposure to radioactive ^{109}Cd (Fig. 7). These results are similar to our hard water study (Hollis *et al.*, 1999) where fish chronically exposed to 3 and 10 µg/L Cd had higher gill concentrations of 'new' Cd with an acute exposure to 100 µg/L Cd (but lower if the acute exposure was only 10 µg/L Cd). However, fish exposed for 30 days to 0.07 or 0.11 µg/L Cd in soft water accumulated less 'new' Cd in their gills compared to controls, and internalized less ^{109}Cd than control fish, when acutely exposed to concentrations of Cd up to 22 µg/L (Chapter 3).

The affinity of the gills for Cd was reduced by chronic exposure to 2 µg/L Cd, while the number of binding sites increased (Table 5). These changes in gill binding characteristics are in good agreement with Hollis *et al.* (1999) and Chapter 3 where the affinity of the gill for Cd (i.e. log $K_{Cd\text{-}gill}$) decreased, and the number of gill Cd binding sites (B_{max}) increased, with chronic sublethal Cd exposures in hard and soft water. Alsop *et al.* (1999) and Alsop and Wood (1999) have similarly shown that the affinity of the gill for Zn was consistently reduced by chronic acclimation to sublethal zinc, and B_{max} was greater for Zn-exposed fish compared to controls. Gill Zn pool size was also much larger in soft (20 mg/L as $CaCO_3$) than in hard water (120 mg/L as $CaCO_3$; Alsop *et al.*, 1999). Thus two metals, one essential (Zn) and one nonessential (Cd), appear to be handled in similar ways by the gill during chronic exposure.

In conclusion, there are discrepancies in the maximum number of gill Cd binding sites for this study compared to earlier gill binding studies (Playle *et al*,

1993a,b; Hollis *et al.*, 1999; Chapter 3) but are in agreement with those of Hollis *et al.* (1997). Therefore, there are difficulties in using the Biotic Ligand Model as a tool for predicting toxic effects of Cd to fish for various water Ca concentrations. In addition, the acute toxicological threshold appears to change in an unpredictable manner as a result of acclimation in some instances but not in others. Furthermore, the adaptive changes that occur in gills of fish chronically exposed to Cd cannot be easily incorporated into the model. Cold Cd concentrations in the gills increased over the 30 d exposure to Cd, changes which were accompanied by decreases in the affinity of the gill for Cd, as well as increases in the number of Cd gill binding sites. These adaptive changes are currently not accounted for by the Biotic Ligand Model. Until the discrepancies in B_{max} values between studies, and changes in toxic threshold and in gill binding characteristics with chronic Cd exposure can be resolved for the Biotic Ligand Model, current chronic ambient water quality criteria for Cd at different Ca concentrations are reasonable limits for protecting aquatic life because they are based on water hardness – the most influential factor on Cd uptake and toxicity. Recommended Canadian Water Quality Guidelines for cadmium (C.C.M.E, 1995; Table 6) are currently set at Cd concentrations well below the chronic level (2 µg/L Cd) causing substantial fish mortality at the various water Ca concentrations from the present study.

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References

Alabaster, J.S. and Lloyd, R. (Editors), 1982. Water Quality Criteria for Freshwater Fish, 2. Food and Agriculture Organization of the United Nations. Butterworths, London, pp. 221-251.

Alsop, D.H., McGeer, J.C., McDonald, D.G., and 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.

Alsop, D.H. and Wood, C.M., 1999. A kinetic analysis of zinc accumulation in the gills of juvenile rainbow trout: the effects of zinc acclimation and implications for biotic ligand modelling. Environ. Toxicol. Chem. Submitted.

Benoit, D.A., Leonard, E.N., Christensen, G.M., and 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. and Dorward-King, E.J. (Editors), 1996. 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. Pensacola FL: SETAC Pr. 114 p.

Calamari, D., Marchetti, R., and 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., 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.

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

Di Toro, D., Allen, H., Bergman, H., Meyer, J., Paquin, PI, and Santore, R., 1999. Biotic ligand model of the acute toxicity of metals. I. Technical basis. Environ. Toxicol. Chem. Submitted.

Farag, A.M., Boese, C.J., Woodward, D.R., and 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.

Fu, H., Steinebach, O.M., van den Hamer, C.J.A., Balm, P.H.M., and Lock, R.A.C., 1990. Involvement of cortisol and metallothionein-like proteins in the physiological responses of tilapia (*Oreochromis mossambicus*) to sublethal cadmium stress. Aquat. Toxicol., 16: 257-270.

Giles, M.A., 1984. Electrolyte and water balance in plasma and urine of rainbow trout (*Salmo gairdneri*) during chronic exposure to cadmium. Can. J. Fish. Aquat. Sci., 41: 1678-1685.

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

Harrison, S.E. and Klaverkamp, J.F., 1989. Uptake, elimination and tissue distribution of dietary and aqueous cadmium by rainbow trout (*Salmo gairdneri* Richardson) and lake whitefish (*Coregonus clupeaformis* Mitchill). Environ. Toxicol. Chem., 8: 87-97.

Hogstrand, C., Wilson, R.W., Polgar, D., and Wood, C.M., 1994. Effects of zinc on the kinetics of branchial calcium uptake in freshwater rainbow trout during adaptation to waterborne zinc. J. Exp. Biol., 186: 55-73.

Hollis, L., Burnison, K. and 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., 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.

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

Iger, Y., Lock, R.A.C., van der Meij, J.C.A., and Wendelaar Bonga, S.E., 1994. Effects of water-borne cadmium on the skin of the common carp (*Cyprinus carpio*). Arch. Environ. Contam. Toxicol., 26: 342-350.

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

Litchfield, J.T., 1949. A method for rapid graphic solution of time-per cent effect curves. J. Pharmacol. Exp. Therapeut., 97: 399-408.

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

McCarty, L.S., Henry, J.A.C., and 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., and Milligan, C.L., 1998. Anaerobic capacity and swim performance of juvenile salmonids. Can. J. Fish. Aquat. Sci., 55: 1198-1207..

McDonald, D.G., Reader, J.P., and Dalziel, T.R.K., 1989. The combined effects of pH and trace metals on fish ionoregulation. In Morris, R., Taylor, E. &

Brown, D., eds, *Acid Toxicity and Aquatic Animal.* Cambridge University Press, London, pp. 221-242.

McDonald, D.G. and Wood, C.M., 1993. Branchial mechanisms of acclimation to metals in freshwater fish. In: J.C. Rankin and F.B. Jensen (Editors), 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.

Mount, D.I. and 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., and 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., 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., 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. and 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.

Pratap, H.B., Fu, H., Lock, R.A.C., and Wendelaar Bonga, S.E., 1989. Effect of waterborne and dietary cadmium on plasma ions of the teleost *Oreochromis mossambicus* in relation to water calcium levels. Arch. Environ. Contam. Toxicol., 18: 568-575.

Pratap, H.B., and Wendelaar Bonga, S.E., 1993. Effect of ambient and dietary cadmium on pavement cells, chloride cells, and Na^+/K^+-ATPase activity in the gills of the freshwater teleost *Oreochromis mossambicus* at normal and high calcium levels in the ambient water. Aquat. Toxicol., 26: 133 -150.

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 and 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 current regulatory limits. Environ. Sci. Technol., 31: 466-468.

Sauter, S., Buxton, K.S., Macek, K.J., and Petrocelli, S.R., 1976. Effects of exposure to heavy metals on selected freshwater fish: toxicity of copper, cadmium, chromium and lead to eggs and fry of seven fish species. Duluth, Minnesota: USEPA EPA 440/5-85-001.

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

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

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.

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

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 freshwater trout branchial epithelium and its interference with calcium transport. J.

Exp. Biol., 145: 185-197.

Wicklund, A., and Runn, P., 1988. Calcium effects on cadmium uptake, redistribution, and elimination in minnow, *Phoxinus phoxinus*, acclimated to different calcium concentrations. Aquat. Toxicol., 13: 109-122.

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

Zall, D.M., Fisher, M.D., and Garner, Q.M., 1956. Photometric determination of chlorides in water. Anal. Chem., 28: 1665-1678.

Table 4-1 Measured water chemistry parameters for the eight exposure treatments. Means ±1 S.E. (*n* = 16; number of water samples taken).

Treatment	Ca (µM)	Cd (µg/L)	Na (µM)	Cl (µM)
260 µM Ca (background)	257 ± 19	0.08 ± 0.06	141 ± 48	111 ± 52
260 µM Ca + 2 µg/L Cd	270 ± 22	3.00 ± 0.20	143 ± 49	112 ± 55
470 µM Ca	445 ± 20	0.02 ± 0.02	140 ± 47	109 ± 50
470 µM Ca + 2 µg/L Cd	502 ± 16	3.20 ± 0.20	140 ± 49	111 ± 51
770 µM Ca	938 ± 21	0.09 ± 0.09	101 ± 27	66 ± 33
770 µM Ca + 2 µg/L Cd	602 ± 108	1.60 ± 0.30	104 ± 28	68 ± 34
1200 µM Ca	1235 ± 73	0.04 ± 0.04	103 ± 28	67 ± 35
1200 µM Ca + 2 µg/L Cd	1218 ± 128	1.60 ± 0.20	96 ± 22	55 ± 31

Table 4-2 Specific growth rate of juvenile rainbow trout (mean ± 1 S.E., *n* = 2 tanks of 85 fish each) over 30 d exposures to 0 µg/L Cd (controls) or 2 µg/L Cd and 260, 470, 770, or 1200 µM Ca. There were no significant differences.

	Specific Growth Rate (%/day)	
Ca Conc. (µM)	0 µg/L Cd Added	2 µg/L Cd Added
260	0.90 ± 0.10	0.67 ± 0.30
470	1.11 ± 0.40	0.82 ± 0.20
770	0.77 ± 0.20	0.99 ± 0.20
1200	0.94 ± 0.10	1.00 ± 0.10

Table 4-3 Day 30 accumulation of Cd by gills (A), liver (B), kidney (C), and whole body (D) of juvenile rainbow trout exposed for 30 d to 0 µg/L Cd or 2 µg/L Cd with 260, 470, 770, or 1200 µM Ca. Means ± 1 S.E. (n = 6). Statistical comparisons were made for 2 µg/L Cd + Ca exposures against respective Ca concentrations (260, 470, 770, and 1200 µM Ca) at day 30 (*) and against background Ca (260 µM Ca) at day 0 (crosses); $P < 0.05$. Concentration factors from the water for the 2 µg/L Cd exposures are in brackets.

		[Cd] (µg/g wet tissue)	
Tissue	Water [Ca] (µM)	0 µg/L Cd Exposure	2 µg/L Cd Exposure
Gills	260	0.30 ± 0.05	4.21 ± 0.60 *+ (2100X)
	470	1.00 ± 0.11	5.74 ± 0.31 *+ (2900X)
	770	1.46 ± 0.12	3.52 ± 0.42 *+ (1800X)
	1200	0.65 ± 0.13	2.47 ± 0.35 *+ (1200X)
Liver	260	0.41 ± 0.02	3.01 ± 0.26 *+ (1500X)
	470	0.50 ± 0.01 +	2.44 ± 0.17 *+ (1200X)
	770	0.54 ± 0.03 +	1.29 ± 0.08 *+ (600X)
	1200	0.59 ± 0.04 +	0.75 ± 0.03 + (400X)
Kidney	260	0.32 ± 0.04	7.54 ± 0.62 *+ (3800X)
	470	0.49 ± 0.07	6.87 ± 0.46 *+ (3400X)
	770	0.48 ± 0.03	3.96 ± 0.33 *+ (2000X)
	1200	0.52 ± 0.04	2.20 ± 0.22 *+ (1100X)
Whole Body	260	0.46 ± 0.08	0.62 ± 0.02 *+ (300X)
	470	0.38 ± 0.02	0.59 ± 0.02 *+ (300X)
	770	0.35 ± 0.02	0.48 ± 0.01 (200X)
	1200	0.32 ± 0.02	0.44 ± 0.02 (200X)

Table 4-4 96-h LC50 values for Cd of juvenile rainbow trout after 30 days exposure to 0 µg/L Cd or 2 µg/L Cd with 260, 470, 770, or 1200 µM Ca. Means ± 95% C.L. There were no significant differences at the two higher concentrations of Ca. Significance at the lower concentrations could not be assessed.

	96-h LC50 (µg Cd/L)	
Ca Conc. (µM)	0 µg/L Cd Added	2 µg/L Cd Added
260	2.35 ± 1.91	> 24.33
470	2.35 ± 1.16	> 17.00
770	2.15 ± 2.50	1.90 ± 1.88
1200	1.15 ± 0.58	6.90 ± 6.84

Table 4-5 Log $K_{Cd\text{-}gill}$ (conditional stability constants) and B_{max} (number of gill Cd binding sites) of juvenile rainbow trout, calculated against ionic Cd^{2+}, after 30 days exposure to 0 µg/L Cd or 2 µg/L Cd and 260, 470, 770, or 1200 µM Ca.

	0 µg/L Cd Added		2 µg/L Cd Added	
Ca Conc. (µM)	Log $K_{Cd\text{-}gill}$	B_{max} (µmol/g)	Log $K_{Cd\text{-}gill}$	B_{max} (µmol/g)
260	7.0	0.010	----	-----
470	6.9	0.007	6.2	0.042
770	6.6	0.006	6.3	0.012
1200	6.3	0.005	5.8	0.015

Table 4-6 Mortality of juvenile rainbow trout exposed to 2 µg/L Cd and 260, 470, 770, or 1200 µM Ca for 30 days (from Fig. 1) and recommended Canadian Water Quality Guidelines (CWQG) for total Cd in test waters studied (C.C.M.E., 1995).

Water Hardness as $CaCO_3$ (mg/L)	Mortality Over 30 Day Exposure to 2 µg/L Cd	Recommended CWQG for Cd (µg/L)
20	80%	0.2
50	40%	0.2
80	7%	0.8
120	10%	1.3

Fig. 4-1

Survival of juvenile rainbow trout exposed to 0 or 2 µg/L Cd and 260 (background), 470, 770, or 1200 µM Ca for 30 days. Values are the averages between two replicate tanks (n = 85 fish per tank at day 0; corrected for sampling over 30 d exposure) for each treatment.

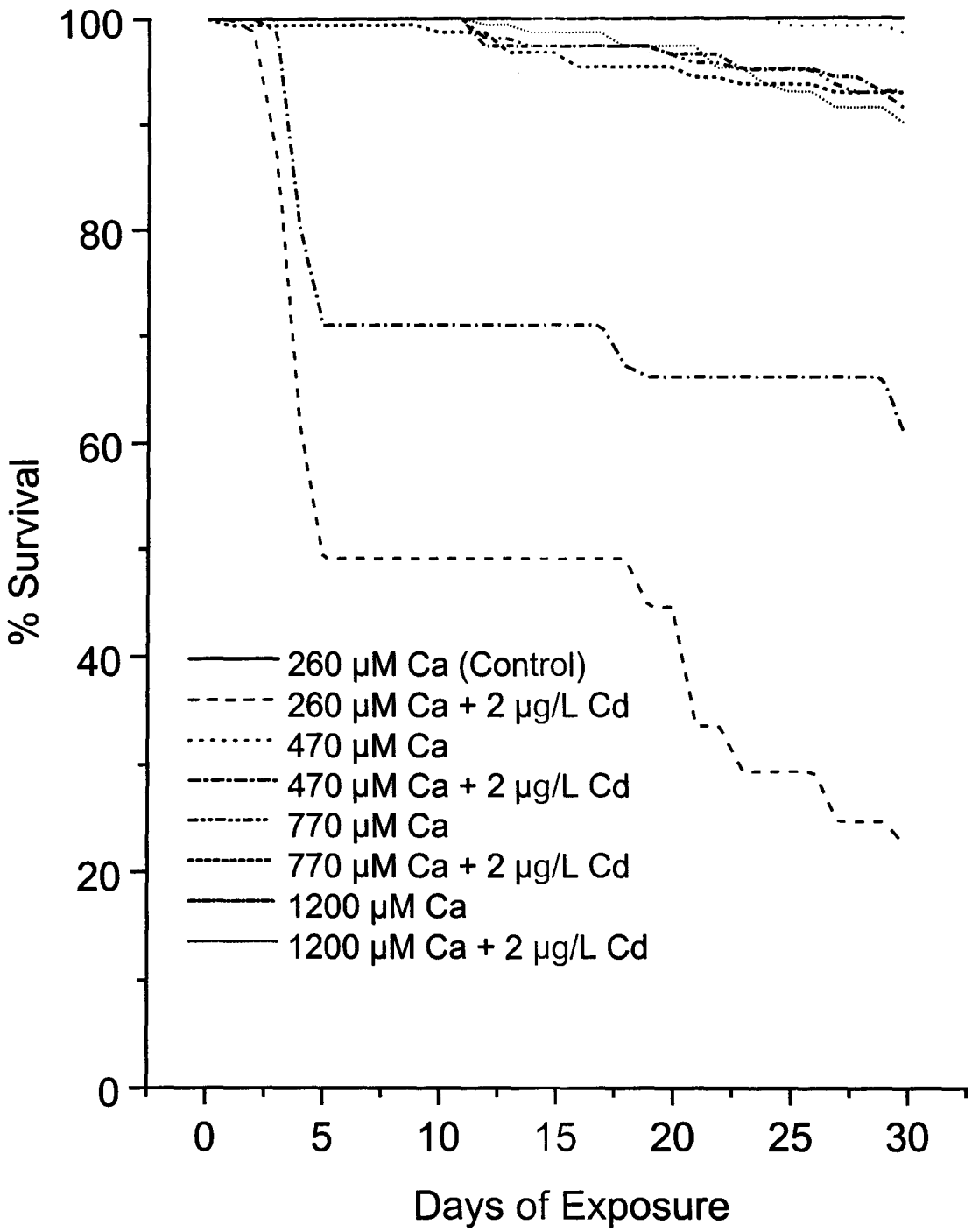

100
80
60
40
20
0
% Survival
0
5
10
15
20
25
30
Days of Exposure
260 µM Ca (Control)
260 µM Ca + 2 µg/L Cd
470 µM Ca
470 µM Ca + 2 µg/L Cd
770 µM Ca
770 µM Ca + 2 µg/L Cd
1200 µM Ca
1200 µM Ca + 2 µg/L Cd

Fig. 4-2

Accumulation of Cd by gills (A), liver (B), kidney (C), and whole body (D) of juvenile rainbow trout exposed for 30 d to 0 µg/L Cd (shaded bars) or 2 µg/L Cd (patterned bars) with 260, 470, 770, or 1200 µM Ca. Means ± 1 S.E. (n = 6). Statistical comparisons were made for 2 µg/L Cd + Ca exposures against respective Ca concentrations (260, 470, 770, and 1200 µM Ca) at each sampling day (*) and against background Ca (260 µM Ca) at day 0 (crosses); $P < 0.05$.

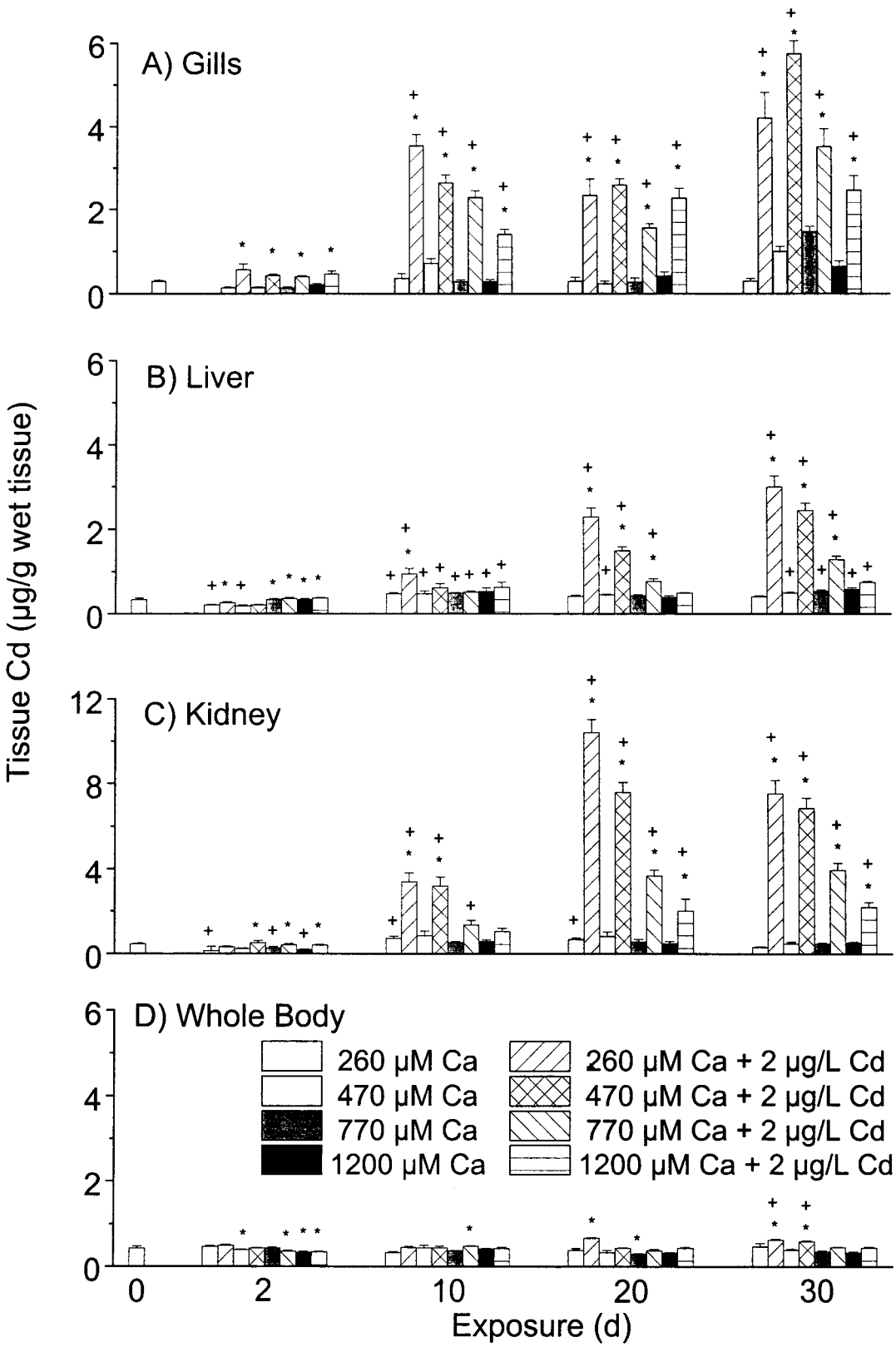

A) Gills
B) Liver
C) Kidney
D) Whole Body
Tissue Cd (µg/g wet tissue)
Exposure (d)
0
2
10
20
30
260 µM Ca
470 µM Ca
770 µM Ca
1200 µM Ca
260 µM Ca + 2 µg/L Cd
470 µM Ca + 2 µg/L Cd
770 µM Ca + 2 µg/L Cd
1200 µM Ca + 2 µg/L Cd

Fig. 4-3

(A) Whole body (remaining carcass) and (B) plasma calcium concentrations of juvenile rainbow trout exposed for 30 d to 0 µg/L Cd (shaded bars) or 2 µg/L Cd (patterned bars) with 260, 470, 770, or 1200 µM Ca. Error bars represent ± 1 S.E. (n = 6). Statistical comparisons were made for 2 µg/L Cd + Ca exposures against respective Ca concentrations (260, 470, 770, and 1200 µM Ca) at each sampling day (*) and against background Ca (260 µM Ca) at day 0 (crosses); $P < 0.05$.

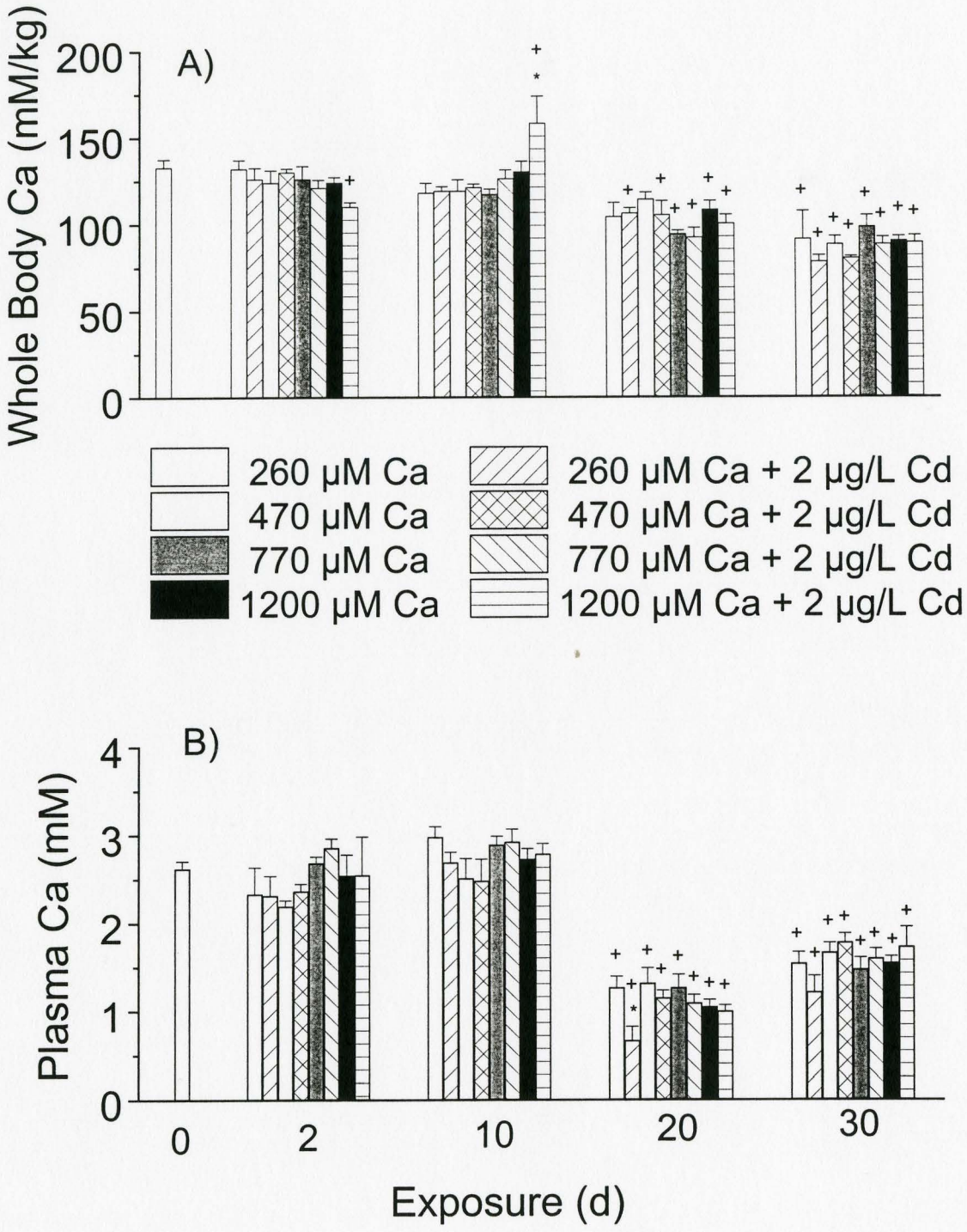

A)
Whole Body Ca (mM/kg)
200
150
100
50
0
260 µM Ca
470 µM Ca
770 µM Ca
1200 µM Ca
260 µM Ca + 2 µg/L Cd
470 µM Ca + 2 µg/L Cd
770 µM Ca + 2 µg/L Cd
1200 µM Ca + 2 µg/L Cd
B)
Plasma Ca (mM)
4
3
2
1
0
0
2
10
20
30
Exposure (d)

Fig. 4-4

Swimming performance (stamina) of juvenile rainbow trout after 30 days exposure to 0 µg/L Cd (clear bars) or 2 µg/L Cd (striped bars) with 260, 470, 770, or 1200 µM Ca. Swimming times were corrected to a reference length of 11 cm (average length of fish tested; cf. McDonald *et al.*, 1998). Means ± 1 S.E. (n = 10). Statistical comparisons were made for 2 µg/L Cd + Ca exposures against respective Ca concentrations (260, 470, 770, and 1200 µM Ca); * $P < 0.05$.

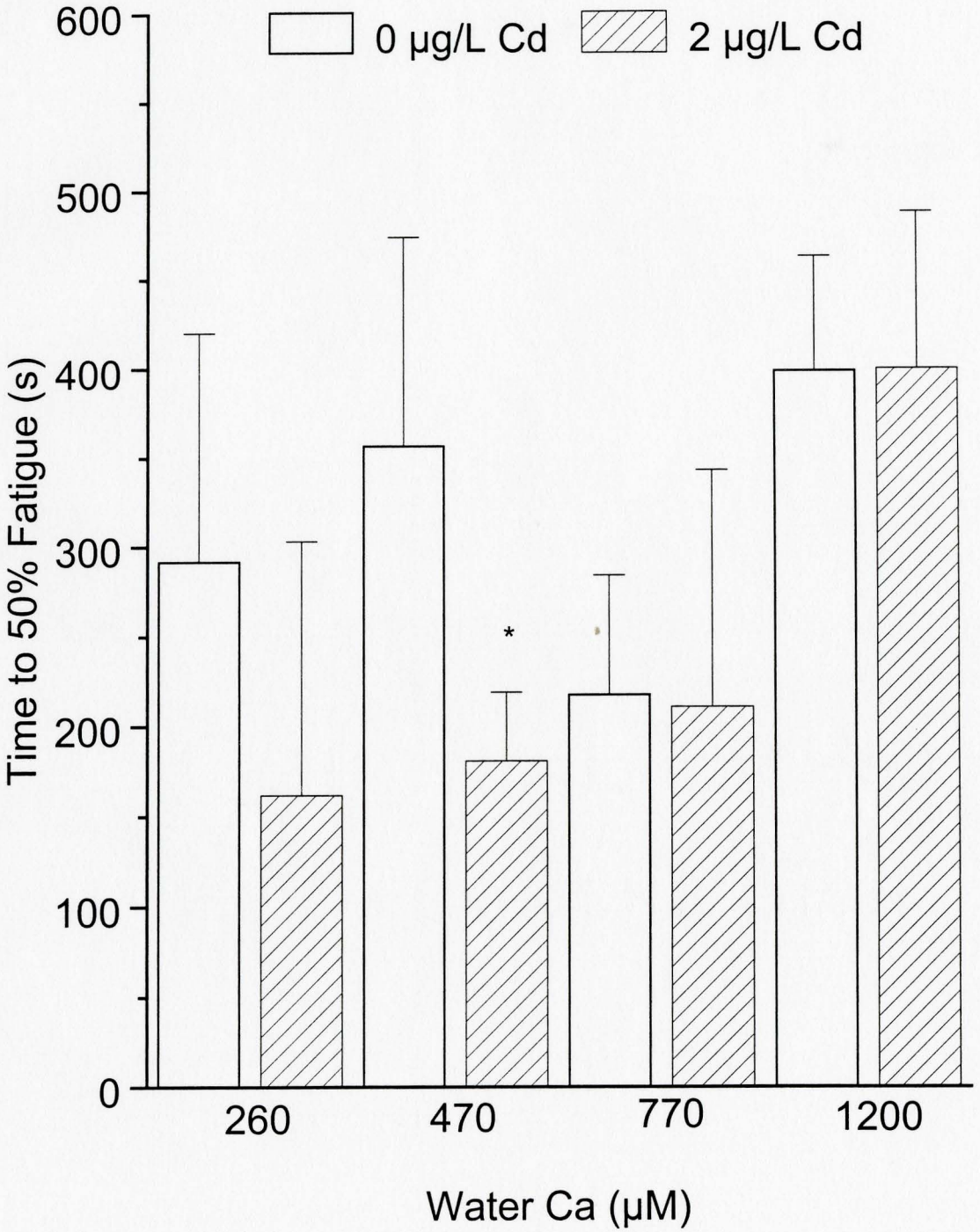

0 µg/L Cd
2 µg/L Cd
Time to 50% Fatigue (s)
600
500
400
300
200
100
0
*
260
470
770
1200
Water Ca (µM)

Fig. 4-5

Unidirectional influx of Ca, measured with ^{45}Ca, into juvenile rainbow trout after 30 d exposures to 0 µg/L Cd (controls; clear bars) or 2 µg/L Cd (striped bars) and 260, 470, 770, or 1200 µM Ca. Error bars represent ± 1 S.E. (n = 8). Statistical comparisons were made for 2 µg/L Cd + Ca exposures against respective Ca concentrations (260, 470, 770, and 1200 µM Ca) at each sampling day (*) and against background Ca (260 µM Ca) at day 0 (crosses); $P < 0.05$.

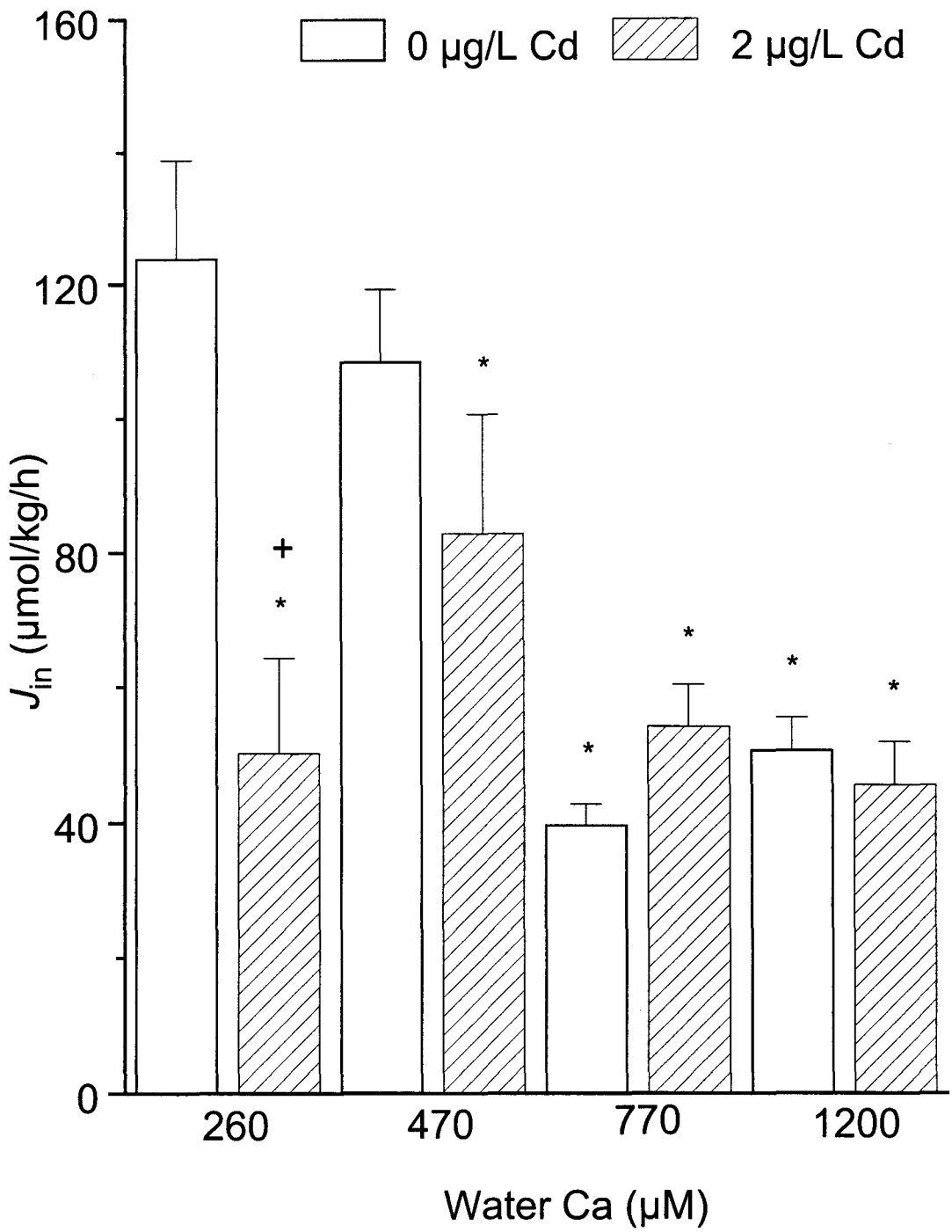

160
120
80
40
0
0 µg/L Cd
2 µg/L Cd
J_{in} (µmol/kg/h)
+
*
*
*
*
*
*
*
260
470
770
1200
Water Ca (µM)

Fig. 4-6

Accumulation of 'new Cd' by gills of (A) Ca exposed and (B) 2 μg/L Cd + Ca (260, 470, 770, or 1200 μM Ca) exposed trout acutely exposed (3 h) to ^{109}Cd with total Cd concentrations of 6, 14, 27, 63, and 115 μg/L Cd. Means ± S.E. (n = 5). Statistical comparisons were made against 260 μM Ca exposure at each sampling concentration for A and against respective Ca concentration from A at each sampling concentration for B; * $P < 0.05$.

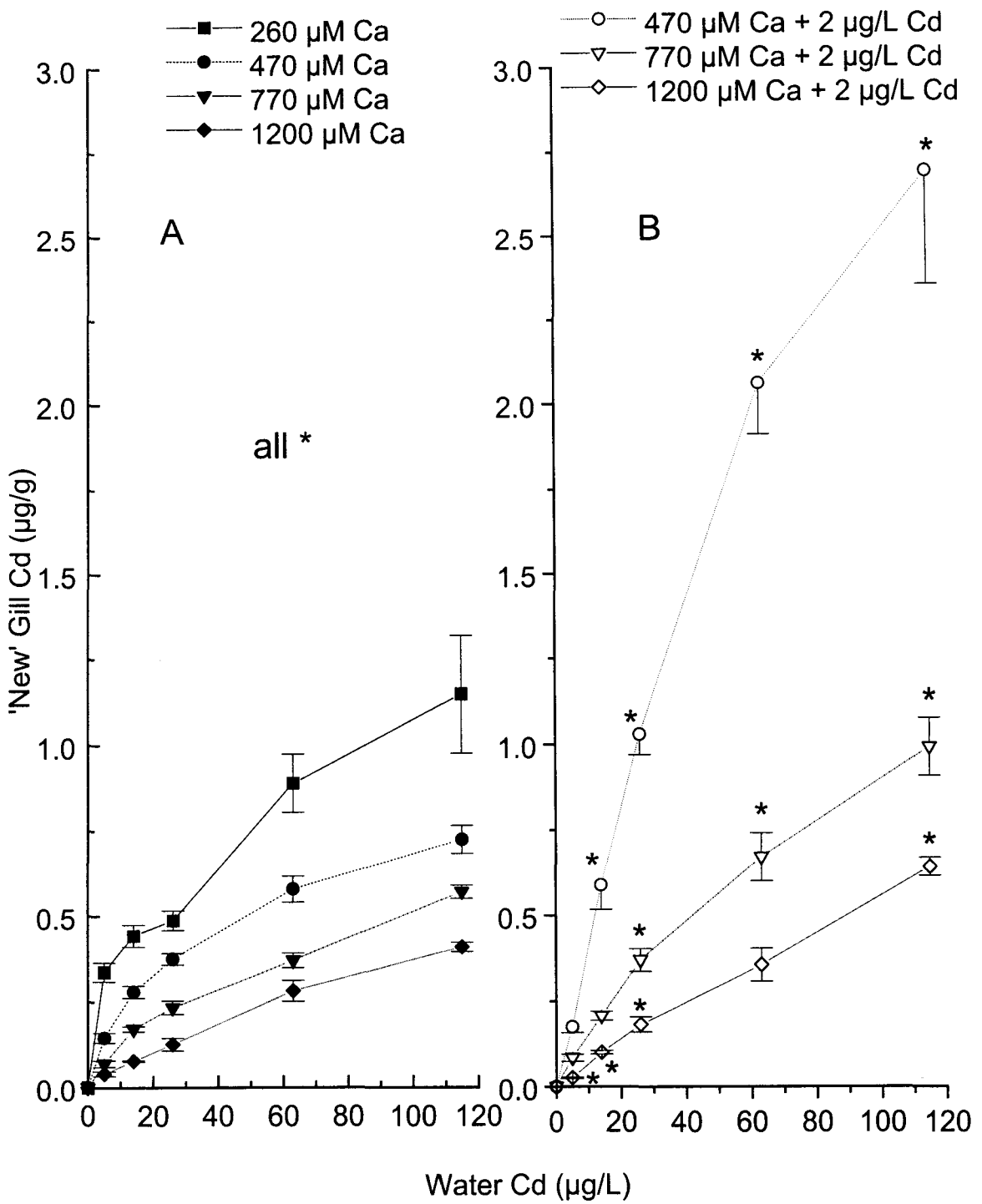
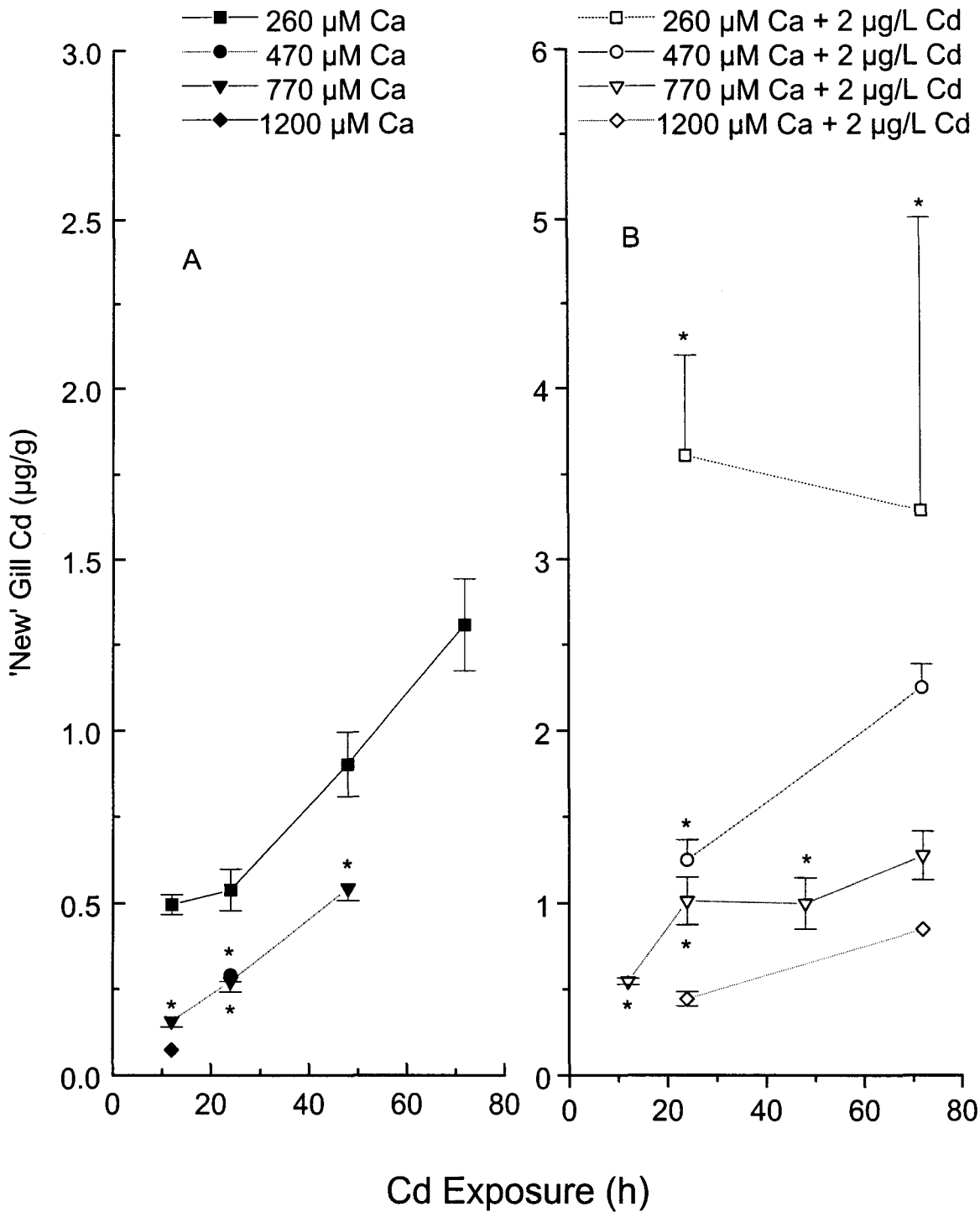

260 µM Ca
470 µM Ca
770 µM Ca
1200 µM Ca
A
all *
470 µM Ca + 2 µg/L Cd
770 µM Ca + 2 µg/L Cd
1200 µM Ca + 2 µg/L Cd
B
'New' Gill Cd (µg/g)
Water Cd (µg/L)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
20
40
60
80
100
120

Fig. 4-7

Accumulation of 'new Cd' by gills of rainbow trout exposed for 72 h to ^{109}Cd, with total Cd concentration of 6 µg/L, after 30 d exposures to 0 µg/L Cd (A) or 2 µg/L Cd (B) with 260, 470, 770, or 1200 µM Ca. Means ± S.E. (n = 1-5). Statistical comparisons were made against 260 µM Ca exposure at each sampling concentration for A, and against the respective Ca concentration from A at each sampling concentration for B; * $P < 0.05$. Note the scale difference in B.


260 µM Ca
470 µM Ca
770 µM Ca
1200 µM Ca
260 µM Ca + 2 µg/L Cd
470 µM Ca + 2 µg/L Cd
770 µM Ca + 2 µg/L Cd
1200 µM Ca + 2 µg/L Cd
A
B
'New' Gill Cd (µg/g)
Cd Exposure (h)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
1
2
3
4
5
6
0
20
40
60
80

Summary

Acute mortality occurred with chronic exposure to 10 µg/L Cd in hard water and with low Ca (260 and 470 µM Ca) plus 2 µg/L exposures; however, no acute mortality was observed with 0.07 or 0.11 µg/L Cd exposures in soft water. There were no significant effects on growth with chronic Cd exposure in hard water, soft water, or Ca-supplemented soft water. For all water qualities tested, tissue accumulations of Cd were the most sensitive indicators of chronic Cd exposure. In addition, the protective effects of Ca were demonstrated since tissue Cd accumulations decreased, and mortality decreased as water Ca concentrations increased.

Acclimation occurred with the chronic Cd exposure (3 and 10 µg/L Cd) in hard water but did not occur with the 0.07 and 0.11 µg/L Cd exposures in soft water. For the Ca-supplemented soft water exposure, there was a trend towards increased 96-h LC50 values with chronic Cd exposure (2 µg/L Cd) at low water Ca concentrations only (260 and 470 µM Ca).

There were no significant effects on whole body or plasma ions with chronic Cd exposure in hard water, soft water, and Ca-supplemented soft water. Furthermore, there were no significant effects on routine oxygen consumption or swimming performance with chronic Cd exposure in hard or soft water. However, with the Ca-supplemented soft water exposure swimming performance (stamina) was significantly impaired in the 470 µM Ca plus 2 µg/L Cd exposed fish.

Gill binding characteristics were altered with chronic Cd exposure in hard water, soft water, and Ca-supplemented soft water. There were trends of increased K_D (decreased log $K_{Cd\text{-}gill}$) and increased B_{max} with chronic Cd exposure. Furthermore, K_D increased (log $K_{Cd\text{-}gill}$ decreased) and B_{max} decreased with higher water Ca concentrations (up to 1200 μM Ca).

Finally, Biotic Ligand Modelling successfully predicted 'new' gill Cd accumulation with control fish in hard and soft water; however, there were discrepancies in the number of gill Cd binding sites between the studies and there were changes in gill binding characteristics with chronic exposure to Cd.