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Cadmium accumulation, gill Cd binding, acclimation, and physiological effects during long term sublethal Cd exposure in rainbow trout

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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 softwater, 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 × (gills), 8–20 × (liver), 2–7 × (carcass), and 5–12 × (whole bodies) control levels by 30 days. Chronically accumulated gill Cd could not be removed by ethylenediaminetetraacetic acid (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 μg 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.

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1. Introduction

Cadmium is a non-essential element that can have severe toxic effects on aquatic organisms when present in excessive amounts (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 (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 (US EPA, 1986; CCME, 1995). Additional factors affecting the acute toxicity of Cd include temperature, dissolved oxygen, pH, salinity, and dissolved organic matter (Alabaster and Lloyd, 1982; Sprague, 1987). Many of these same factors also alter the uptake of Cd across the gills (Part and Wikmark, 1984; Part 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. (Playle et al., 1993a,b), Janes and Playle (1995) and, 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 softwater 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; 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).

2. Materials and methods

2.1. 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 2 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, Ont., Canada; Cd content = 1.06 ± 0.04 ($N = 6$) μg Cd g^{-1} wet weight).

2.2. Exposure system

After 2 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 $Cd(NO_3)_2 \cdot 4H_20$ (Fisher Scientific, Nepean, Ont., Canada), 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 S.E.M. (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.

2.3. Sampling

During the 30 day Cd exposure, 16 ml water samples were taken throughout the exposure, acidified with 50 μl of HNO_3, and analyzed 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, 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 s 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.

2.4. Testing

2.4.1. 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 s^{-1}) 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% confidence limits (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 hardwater 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 min, 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 min period); dV is the velocity increment (10 cm s^{-1}); t is the time swum at each velocity (40 min); 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.

2.4.2. Routine metabolism

Routine oxygen consumption, representing 'in-tank' metabolic rate, was measured 2 and 6 h after feeding after the 30 day exposure period. 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 P_{O_2} levels were monitored continuously for 1 h with an oxygen electrode (Cameron E101) connected to an oxygen meter (Cameron OM-200). Oxygen consumption readings were completed before the P_{O_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 P_{O_2} decline (four readings taken over 1 h) and the water oxygen solubility coefficients of Boutilier et al. (1984). The data were weight-cor-

rected using the weight exponent of 0.824, taken from Cho (1990).

2.4.3. Acclimation

A 96 h 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), 37 ± 4 (15), 71 ± 0.4 (14), 262 ± 9 (15) and 490 ± 14 (15) μg l^{-1}. Dead fish were removed when movement ceased and times of mortality were recorded. LC_{50} values ($\pm$ 95% C.L.) were determined by log probit analysis (Finney, 1971).

2.4.4. 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 h, 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), 10 ± 0.3 (3), 30 ± 1 (3), 60 ± 0.3 (3) 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 Section 2.5).

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), 50 ± 6 (6) or 100 ± 8 (6) μg l^{-1} total Cd added as $Cd(NO_3)_2 \cdot 4H_20$ (Fisher Scientific, Nepean, Ont., Canada) 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 Section 2.5). Blood samples ($\sim$ 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 day) 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).

2.5. Tissue and water analyses

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

Gills, livers, and remaining carcass were thawed, weighed, and then digested in 1–10 times their weight of 1 N HNO_3 (TraceMetal Grade HNO_3; Fisher Scientific, Nepean, Ontario) for 3 h at about 80°C. Digests were shaken, left to settle for 10 min, then the supernatant was diluted 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 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:

$$WB = [(G \times g_{wt}) + (L \times l_{wt}) + (C \times c_{wt})]/f_{wt}$$

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); g_{wt} is the weight of the gills (g); l_{wt} is the weight of the liver (g); c_{wt} is the weight of the carcass (g); and f_{wt} 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 g^{-1} 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, Ont., Canada). Concentrations of different cadmium species in the water were calculated using the MINEQL + aquatic geochemical program (Schecher and McAvoy, 1994) and measured water chemistry.

2.6. 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 thoughout.

3. Results

3.1. 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 3 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 days 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

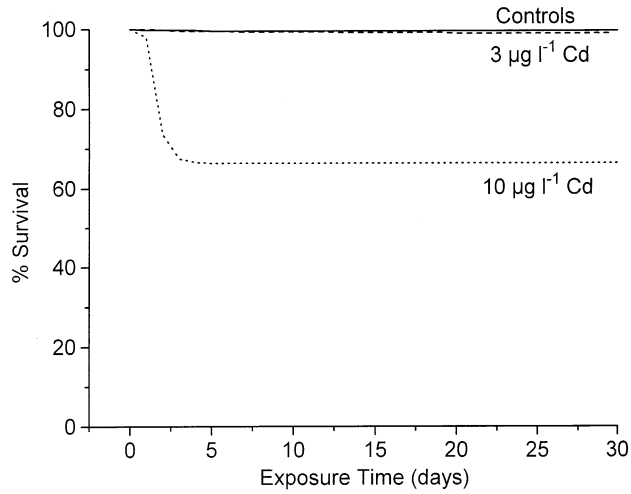

Fig. 1. Survival of juvenile rainbow trout exposed to 0 μg l^{-1} Cd (controls; solid line), 3 μg l^{-1} Cd (dashed line), or 10 μg l^{-1} Cd (dotted line) for 30 days. Values are the averages between two replicate tanks ($N = 280$ fish per tank) for each treatment.

(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 × and 1200 × for the 3 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 × and 200 × 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 × for both the 3 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 × and 1200 × for low and high Cd exposures, respectively.

3.2. 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.

3.3. 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) 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) 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 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).

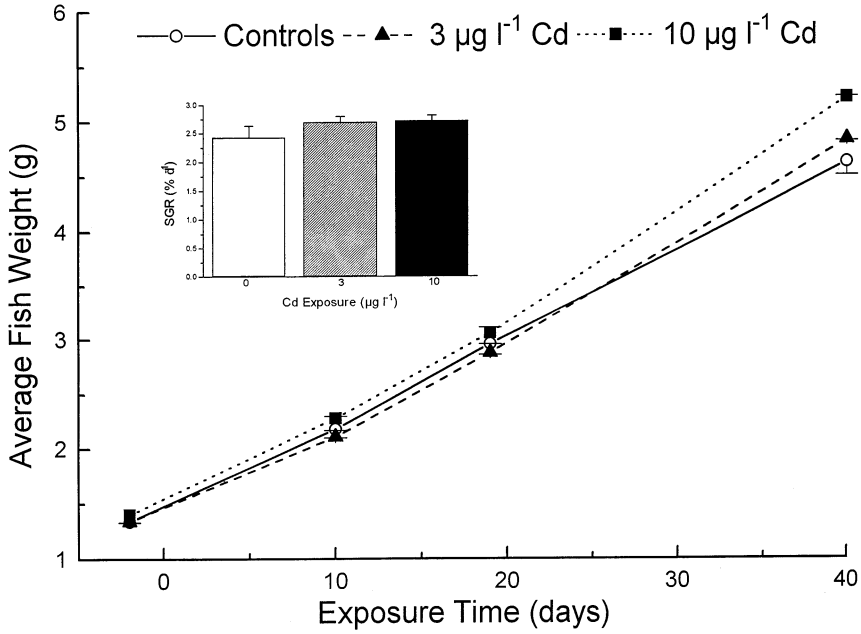

Fig. 2. Body weight of juvenile rainbow trout (mean ± S.E., $N = 2$ tanks of 280 fish each) over 30 days exposure to 0 μg l^{-1} Cd (controls; solid line or clear bar), 3 μg l^{-1} Cd (dashed line or striped bar), or 10 μg l^{-1} Cd (dotted line or solid bar). Specific growth rate is depicted in the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

3.4. 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

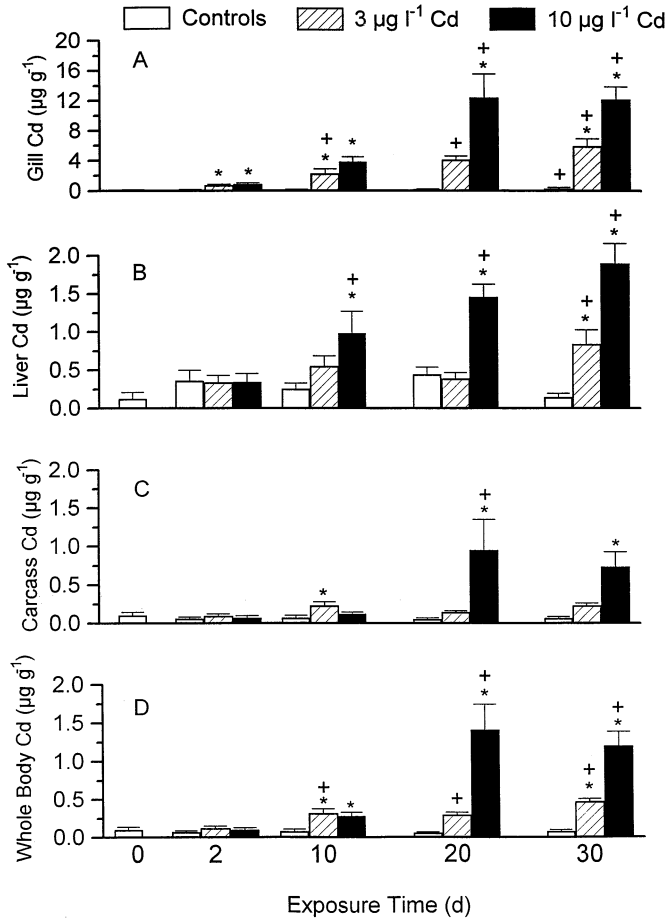

Fig. 3. Accumulation of Cd by gills (A), liver (B), carcass (C), and whole body (D) of juvenile rainbow trout exposed for 30 d to 0 µg l^{-1} Cd (controls; clear bars), 3 µg l^{-1} Cd (striped bars), or 10 µg l^{-1} Cd (solid bars). ±1 S.E. ($N = 6$). Statistical comparisons were made against background Cd (controls) at each sampling day (*) and against background Cd at day 0 (crosses); $P < 0.05$.

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

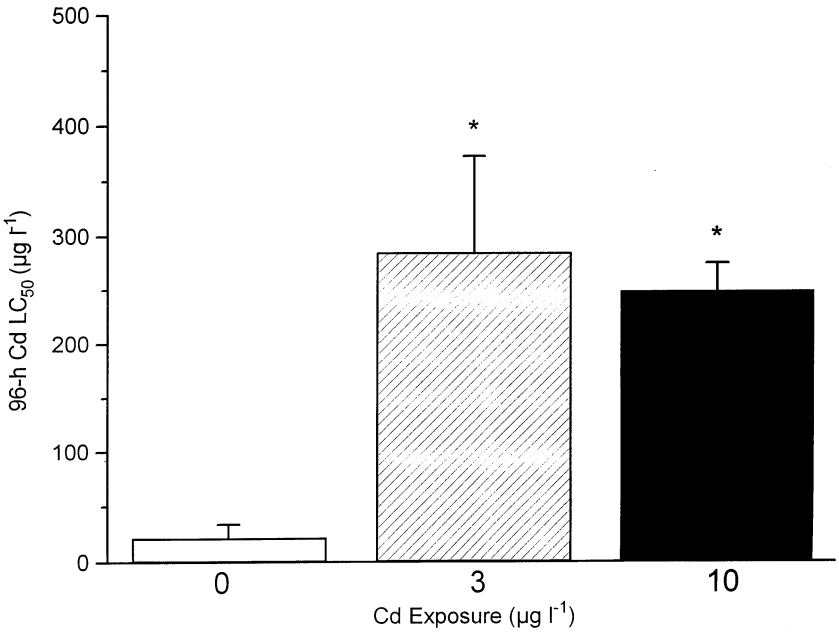

Fig. 4. 96-h LC_{50} values for Cd of juvenile rainbow trout after 30 days exposure to 0 μg l^{-1} Cd (controls; clear bar), 3 μg l^{-1} Cd (striped bar), or 10 μg l^{-1} Cd (solid bar). Means ± 95% C.L. ($N = 60$). * $P < 0.05$.

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).

4. Discussion

4.1. 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 (Spry and Wiener, 1991; CCME, 1995). Furthermore they were relevant to US EPA freshwater quality criteria for aquatic life of 5.7 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 $CaCO_3$ (US EPA, 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).

Table 1
Routine oxygen consumption of juvenile rainbow trout after 30 days exposure to 0 (controls), 3 or 10 μg l^{-1} Cd at 2 h and 6 h after feeding

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

Means ± S.E. ($N = 2$ tanks of 280 fish each).
There were no significant differences.

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 Section 1 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 (McDonald and Wood, 1993), but is not entirely unprecedented. Pascoe and Beattie (1979) reported that the 48-h LC_{50} increased from < 100 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).

Table 2
Swimming performance (stamina) of juvenile rainbow trout after 30 days exposure to 0 (controls), 3 or 10 μg l^{-1} Cd

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

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.

4.2. 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 (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.

4.3. 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 Section 1). Farag et al. (1994) reported similar findings for juvenile rainbow trout exposed for 21 days 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

Table 3
Critical swimming speed (U_{Crit}) of juvenile rainbow trout after 30 days exposure to 0 (controls), 3 or 10 μg l^{-1} Cd

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[a]
3	4.33 ± 0.12	4.65 ± 0.12
10	4.49 ± 0.15	4.24 ± 0.13

3 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.
[a] No Cd present.

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.

4.4. 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 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.

4.5. 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 Section 1 for references). These models

Table 4
Accumulation of 'new Cd' by blood and whole body of juvenile rainbow trout exposed for 3 h to 10, 50 or 100 μg l^{-1} ^{109}Cd after 30 days exposure to 0 (controls), 3 or 10 μg l^{-1} Cd

Chronic Cd exposure (μg l^{-1})	Blood			Whole body		
	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

Means $\pm$ 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 (* $P < 0.05$).

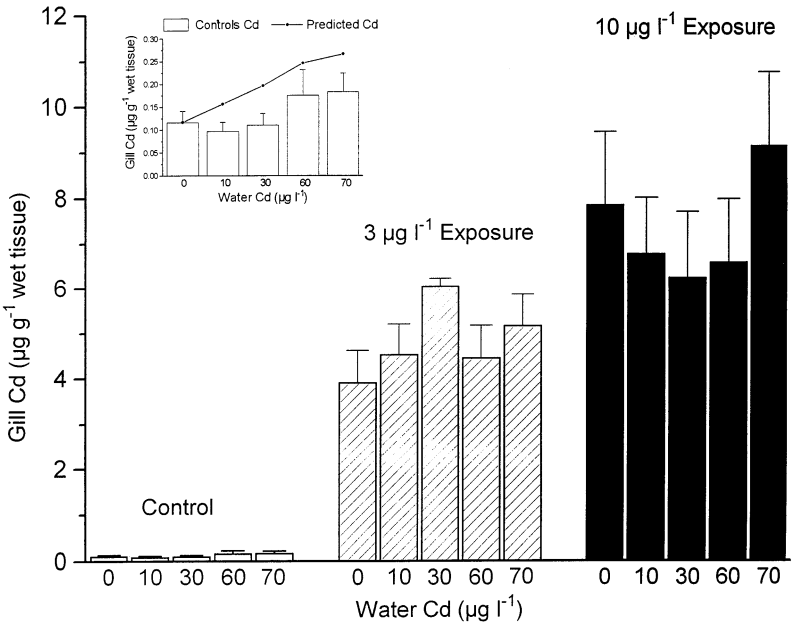

Fig. 5. Accumulation of Cd by gills of juvenile rainbow trout exposed to Cd concentrations ranging from 0 to 70 µg l^{-1} for 3 h after 30 days exposure to 0 µg l^{-1} Cd (controls; clear bars), 3 µg l^{-1} Cd (striped bars), or 10 µg l^{-1} Cd (solid bars). Inset shows the data for the controls on a finer scale; the points and line indicates the modeled 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.

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 softwater 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

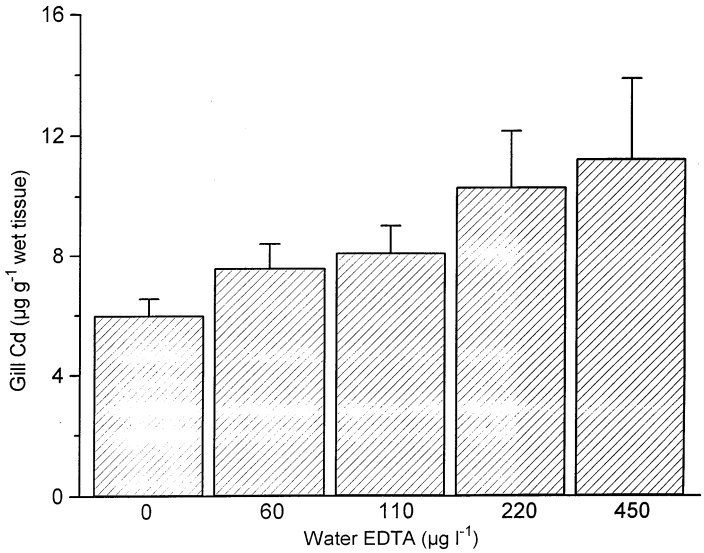

Fig. 6. Accumulation of Cd by gills of juvenile rainbow trout exposed for 3 h to 3 μg l^{-1} Cd and ethylenediaminetetraacetic acid (EDTA; 0–450 μg l^{-1}) after 30 days exposure to 3 μg l^{-1} Cd. Means ± S.E. ($N = 5$). Statistical comparisons were made against background gill Cd with no added EDTA (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 ($r^2 = 0.88$, $P < 0.05$).

^{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 $K_{\text{Cd-gill}}$ 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 $K_{\text{Cd-gill}}$ 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 Section 1), 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- to 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 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 $K_{\text{Cd-gill}}$ 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.

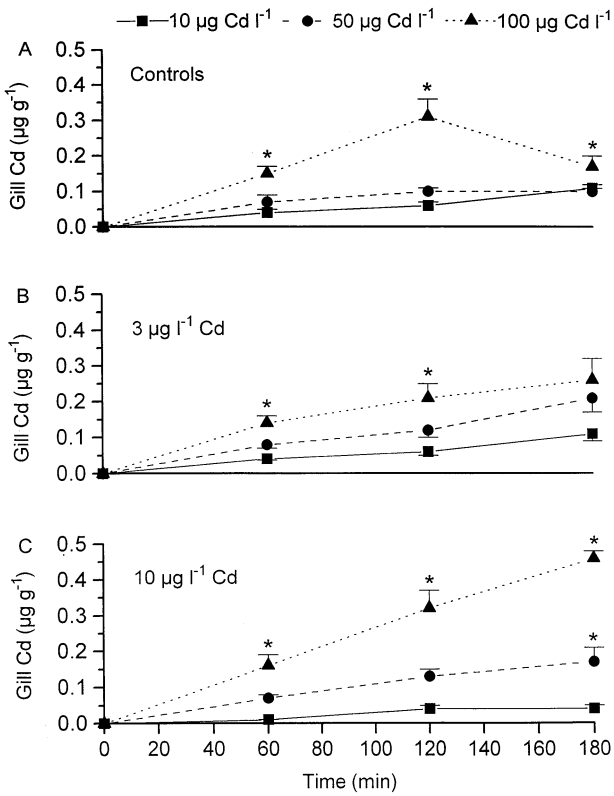

Fig. 7. Accumulation of 'new Cd' by gills of control (non-acclimated; A) and Cd-acclimated trout [exposed to 3 μg Cd l^{-1} (B) and 10 μg Cd l^{-1} (C) for 30 days] exposed for 3 h to ^{109}Cd with total Cd concentrations as 10 μg l^{-1} Cd (solid lines), 50 μg l^{-1} Cd (dashed lines), or 100 μg l^{-1} Cd (dotted lines). Means ± S.E. ($N = 4$). Statistical comparisons were made against 10 μg l^{-1} radioactive Cd exposure (i.e. lowest points in each panel) at each sampling time; * $P < 0.05$.

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.

4.6. 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

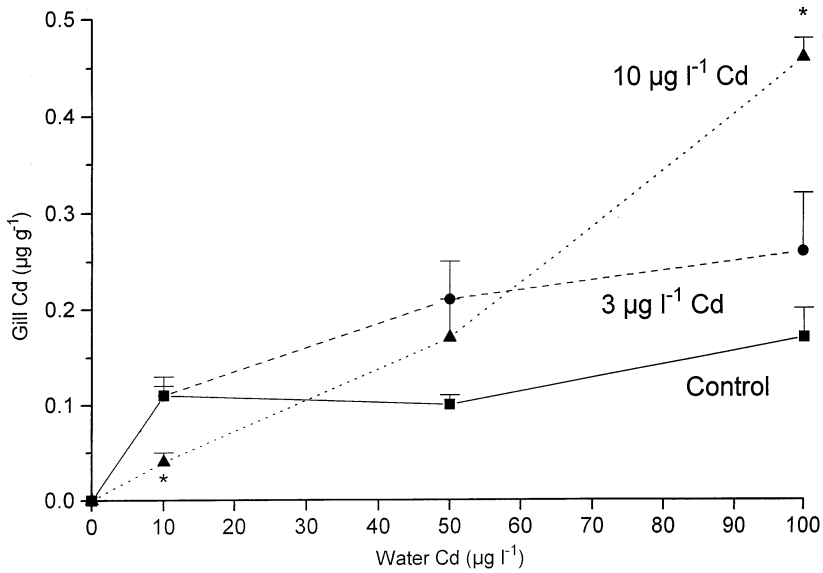

Fig. 8. Accumulation of 'new Cd' by gills of rainbow trout exposed for 3 h to ^{109}Cd, with total Cd concentrations as 10, 50, or 100 μg l^{-1}, after 30 days exposure to 0 μg l^{-1} Cd (controls; solid line), 3 μg l^{-1} Cd (dashed line), or 10 μg l^{-1} Cd (dotted line). Means ± S.E. ($N = 4$). Statistical comparisons were made against control series for each water Cd treatment; * $P < 0.05$.

Table 5
Calculated concentrations of Cd species in the water for various Cd exposures, using MINEQL+(Schecher and McAvoy, 1994) aquatic chemistry program

Species concentration (μg 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
$CdCO_{3Aq}$	0.58	2.30	14.30	30.00

3 and 10 μg l^{-1} represent the chronic (30 day) exposures to Cd; 10, 50, and 100 μg l^{-1} represent the 3-h ^{109}Cd gill-uptake experiment (DOM = dissolved organic matter).

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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