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Influence of acclimation and cross-acclimation of metals on acute Cd toxicity and Cd uptake and distribution in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

The development of chronic metal toxicity models for fresh water fish is complicated by the physiological adjustments made by the animal during exposure which results in acclimation. This study examines the influence of a pre-exposure to a chronic sublethal waterborne metal on acclimation responses as well as the uptake and distribution of new metal into juvenile rainbow trout. In one series of tests, trout were exposed to either 20 or 60 μg/L Cu, or 150 μg/L Zn for a month in moderately hard water and then cross-acclimation responses to Cd were measured in 96 h LC_{50} tests. Cu exposed trout showed a cross-acclimation response but Zn exposed trout did not. Using these results, a detailed examination of Cd uptake and tissue distribution in metal-acclimated trout was done. Trout were exposed to either 75 μg/L Cu or 3 μg/L Cd for 1 month to induce acclimation and subsequently, the uptake and distribution of new Cd was assessed in both Cd- and Cu-acclimated fish using ^{109}Cd. The pattern of accumulation of new metal was dramatically altered in acclimated fish. For example, in 3 h gill Cd binding experiments, Cd- and Cu-acclimated trout both had a higher capacity to accumulate new Cd but only Cu-acclimated fish showed a higher affinity for Cd compared to unexposed controls. Experiments measuring Cd uptake over 72 h at 3 μg Cd/L showed that the Cd uptake rate was lower for Cd-acclimated fish compared to both Cu-acclimated fish and unexposed controls. The results demonstrate the phenomenon of cross-acclimation to Cd and that chronic sublethal exposure to one metal can alter the uptake and tissue distribution of another. Understanding how acclimation influences toxicity and bioaccumulation is important in the context of risk assessment. This study illustrates that knowledge of previous exposure conditions is essential, not only for the metal of concern, but also for other metals as well.

Keywords: Chronic; Copper; Cadmium; Zinc; Acclimation; Rainbow trout

1. Introduction

The majority of metals exert their acute toxic action at the gills of freshwater fish and this knowledge has been used to build robust, mechanistically based, site specific toxicity prediction models (reviewed Niyogi and Wood, 2004). The development and validation of models to predict chronic toxicity are not as advanced as those for acute toxicity. Predicting chronic toxicity is complex as responses of fish to long-term metal exposure can be variable. Understanding the toxicokinetics of accumulation of a metal during chronic sublethal exposure is a critical element in establishing the link between toxicity and exposure in risk assessment (McCarty and Mackay, 1993). Some progress has been made in understanding the accumulation–response relationship for waterborne metal exposure (see, for example, Alsop et al., 1999; Hollis et al., 1999; Taylor et al., 2000; McGeer et al., 2000a,b; Chowdhury et al., 2005a,b). These and other studies have also illustrated that accumulation patterns are complex, particularly for essential metals such as Cu and Zn (Cousins, 1985; Vallee and Falchuk, 1993) and that

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responses can be subtle and temporary (e.g. McGeer et al., 2000a).

The general physiological response to chronic metal exposure follows the damage-repair model of McDonald and Wood (1993), with an initial disruption affecting, for example, appetite, feeding and ion regulation (McGeer et al., 2000a) as well as behaviour (Scott et al., 2003). This initial disruption is followed by recovery and re-establishment of homeostasis. The initial site of impact for waterborne metals is the gills and therefore, the initial disruption is thought to be linked directly to metal accumulation on the gill. The recovery phase features an increase in biosynthetic processes that help repair the damage and correct physiological disturbances. For metal exposure, part of this process is the mobilization of binding proteins such as metallothionein (Mason and Jenkins, 1995; Hogstrand and Wood, 1996; Chowdhury et al., 2005a). In terms of physiology of the gill, much of the response is related to ionic regulation as discussed by McGeer et al. (2000a, 2002). Over time tissue metal concentrations increase and then stabilize (McGeer et al., 2000b) and ultimately, the internal physiology of the animal either returns to the pre-exposure condition or a new equilibrium is established.

A variety of physiological changes can be induced in rainbow trout through chronic exposure and these vary depending on the metal of exposure. Physiological changes that occur during chronic exposure range from the initial binding at the gill surface through to uptake into the blood, clearance from blood to tissues, mobilization of binding proteins, accumulation in tissues and finally excretion via the gills, liver or kidney. For example, the number and affinity of cation transport (uptake) proteins such as Na^+/K^+ ATPase (Lauren and McDonald, 1987) is altered while plasma clearance and biliary excretion of Cu are enhanced (Grosell et al., 1997, 2001) during chronic sublethal exposure to Cu. Chronic sublethal exposure to Cd also results in faster plasma clearance (Chowdhury et al., 2005b) but instead of enhanced elimination, gill uptake of Cd is reduced (Hollis et al., 1999). The physiological changes that occur during chronic exposure also result in acclimation, which takes the form of increased tolerance to acute challenges.

Physiological changes due to chronic sublethal exposure and the associated concomitant development of tolerance has been shown for most metals including Cd (e.g. Hollis et al., 1999), Zn (e.g. Alsop et al., 1999) and Cu (e.g. Taylor et al., 2000). Depending on the mechanisms induced by a particular metal, it is feasible that the acclimation response would also confer an ability to resist other metals. For example, a metal that stimulates a general detoxification mechanism such as production of metallothionein/glutathione (or other binding proteins) might result in an ability to resist challenges with other metals. Physiological mechanisms such as the enhanced biliary elimination that is induced during Cu exposure (Grosell et al., 1997) could be specific to the particular metal of acclimation and therefore might not confer a cross-acclimation to other metals. While there are a few studies showing the cross-acclimation phenomenon, most of these are with invertebrates (e.g. Howell, 1985; Lopes et al., 2005) and few are with fish (e.g. Xie and Klerks, 2003).

This study examines the influence of a pre-exposure to chronic sublethal waterborne Cu, Cd or Zn on acclimation responses. We were particularly interested in the phenomenon of cross-acclimation, defined as an increased acute tolerance on one metal as a result of chronic sublethal exposure to another metal. In initial trials we challenged Cu-, Cd- and Zn-acclimated fish to determine their acute toxicity of Cd (96 h LC_{50} tests). In a subsequent more detailed study, we focused on Cd uptake in Cu- and Cd-acclimated rainbow trout (uptake and distribution studies). Our research is a follow-up to the studies of McGeer et al. (2000a,b) in that the same exposure concentrations, either 3 μg/L Cd or 75 μg/L Cu were used. In those earlier studies, the impact of exposure on growth, feeding, ion regulation, swimming, oxygen consumption as well as the time course of tissue total metal accumulation were characterized in detail. Here, we report on how acclimation to either Cu or Cd influences acute responses to Cd, as well as Cd uptake and tissue distribution.

2. Material and methods

Two sets of experiments were conducted. In the first set, the acute toxicity of Cd to Cu-, Cd- and Zn-acclimated trout was evaluated. In the second, Cd uptake in Cu- and Cd-acclimated trout was characterized. These two sets of experiments were conducted at different times with different groups of rainbow trout; however, culture, maintenance and general testing conditions were similar. For both experiments, juvenile rainbow trout (*Oncorhynchus mykiss*) were obtained from a local supplier (Humber Springs, Orangeville, Ont.) and reared in flowing dechlorinated Hamilton tap water (Ca^{2+} 1.0 mmol/L, Na^+ 0.6 mmol/L, Cl^- 0.7 mmol/L, hardness of 120 mg/L as $CaCO_3$, pH 8.0, 13 °C) for at least a month prior to waterborne metal exposures. Metal exposure was via flow-through at 1–1.5 L per min into 200 L polyethylene tanks with concentrated stock solutions metered (using a Mariotte bottle) in at the appropriate rate to achieve the desired concentration. Fish were fed Martin's starter feed (Martin's Feed Mill, Elmira, Ont.) at 3% body weight per day and maintained on a photoperiod of 16-h light:8-h dark.

2.1. Acclimation and cross-acclimation LC_{50} tests

In the first series of exposures rainbow trout were exposed to either control, 3 or 10 μg/L Cd, 20 or 60 μg/L Cu or 150 μg/L Zn (all from Fisher Scientific, Nepean, Ont.) for a month. Details of the Cd, Cu and Zn exposures and associated acute toxicity tests are found in Hollis et al. (1999), Taylor et al. (2000) and Alsop et al. (1999), respectively. The additional 96 h acute toxicity tests described here were to test for cross-acclimation to Cd in Cu-acclimated and in Zn-acclimated trout. LC_{50} test details were similar to those described for the metal of exposure in the three studies cited above. In short, groups of 8–10 fish were exposed to at least 6 different metal concentrations in a flow-through system for 96 h. Fish were checked daily and survival/mortality observations were collected over 4 days.

2.2. Acclimation and cross-acclimation metal uptake and distribution

In the second set of exposures, juvenile rainbow trout (age approximately 6 months) were exposed to nominal concentrations of either 3 μg/L Cd ($Cd(NO_3)_2{\cdot}4H_2O$, Fisher Sci, Nepean) or 75 μg/L Cu ($CuSO_4{\cdot}5H_2O$, Fisher Sci, Nepean) for a month as previously described in McGeer et al. (2000a). A control group of fish were treated similarly but not exposed to metal. Following 1 month of exposure, trout were tested for Cd uptake and accumulation using the isotope ^{109}Cd. Average fish weight for these tests was 54.3 g. Groups of six fish were sampled directly from each exposure (and controls), euthanized (see below) and gills, liver, kidney, bile, plasma (whole blood centrifuged at approximately 12,000 rpm for 3 min) and muscle samples were collected. These samples were digested in five volumes of 1N nitric acid (Trace Metals Grade, Fisher Scientific) as described in McGeer et al. (2000b).

In one series of tests each of the three groups (control, Cd-acclimated and Cu-acclimated) were exposed in 3 h gill binding experiments to either 2, 10, 25, 50, 100 or 200 μg/L Cd with added ^{109}Cd (activity of 2 μCi/L as $CdCl_2$, New England Nuclear, Boston, MA). The 3 h tests were done as static exposures with continuous aeration and six fish in each of the 10 L container, with continuous aeration. After 3 h fish were removed from the isotope test solutions, placed in clean water for 5 min (to remove surface bound radiolabelled metal) and then euthanized by transfer to a solution of 200 mg/L MS222 (buffered with bicarbonate) and a blow to the head. A gill sample was collected, vigorously rinsed in deionized water for 10 s, blotted dry, weighed and saved for isotope counting.

In another series of tests groups of 36 fish from each exposure (Cd-acclimated, Cu-acclimated or controls) were exposed to 3 μg/L Cd with 1 μCi/L ^{109}Cd for up to 72 h. This longer exposure was done within a 140 L closed (recirculating) system with vigorous aeration and a constant water temperature of 12 °C (±1 °C). Samples of six fish were collected at 3, 6, 10, 24, 48 and 72 h. Following rinsing and euthanasia as described above, a gill sample was collected (as described above). Liver, kidney and muscle samples were collected, weighed and saved. A sample of bile was also collected with a 20-gauge needle attached to a 1 mL syringe. At the final sampling time (72 h), a blood sample was collected, centrifuged and 100 μL of plasma was saved. At this sampling time, the remaining carcass was also saved and analyzed for ^{109}Cd radioactivity.

2.3. Characterization and data modeling

In all experiments, water samples were collected before, during and after testing. Total Cd content in water (acidified), tissue, plasma and bile samples was measured by graphite furnace atomic absorption spectrophotometry (Varian Inc.) with appropriate standards (Fisher Scientific, Nepean, Ont.) checked every 12–15 samples. Water, tissue, plasma, bile and remaining carcass samples were analyzed for ^{109}Cd activity using a Minaxi Auto-Gamma 5000 Series Gamma Counter (Canberra Packard Instrument Company, Meriden, CT). Tissues counts per minute were converted to absolute metal values ("new Cd") using the measured specific activity of the water as detailed by Hollis et al. (1999).

Newly accumulated Cd was described using one of three models:

Michaelis-Menten model

$$[\mathrm{nB}] = \frac{(J_{\max} X)}{(K_\mathrm{m} + X)} \quad (1)$$

Exponential rise to maximum model:

$$[\mathrm{nC}] = \mathrm{Sat}(1 - \mathrm{e}^{-bt}) \quad (2)$$

Linear accumulation model:

$$[\mathrm{nC}] = \mathrm{Int} + \mathrm{RA}t \quad (3)$$

where [nB] is the rate of uptake of new Cd (ng/g wet weight/h), [nC] the concentration of new Cd (ng/g wet weight), $J_{\max}$ the maximum tissue uptake rate (ng/g wet weight/h), X the waterborne exposure concentration (μg/L), K_m the affinity, or waterborne concentration at half $J_{\max}$, Sat the maximum tissue loading or saturation concentration (ng/g wet weight), t the hours of exposure to 3 μg/L Cd, b a constant related to the rate of loading to saturation, RA the linear rate of accumulation into tissues (ng/g wet weight/) and Int is the intercept of the linear regression.

Results from the first experimental series (i.e. 3 h Cd gill and whole body loading at various waterborne Cd concentrations), were modeled using Eq. (1) according to previous studies (e.g. Hollis et al., 1999; Alsop et al., 1999). In the second experimental series (72 h exposure to 3 μg/L Cd), new Cd accumulations to the liver, kidney and muscle were modeled using Eq. (3). Gill accumulation in these experiments was characterized into a rapid uptake saturable component (termed the fast pool) and a slow uptake but large capacity component (slow pool) as previously discussed by Alsop et al. (1999) and Hollis et al. (1999). Modeling was accomplished via a two step procedure which was based on the linear nature of accumulation after 10 h. This linear section of the accumulation curve represented continuous uptake into the slow loading pool and data from 10 to 72 h was characterized using Eq. (3) (linear regression). The slope of the regression (RA in Eq. (3)) provided an estimate of the rate of loading of Cd into the slow pool. The y-axis intercept (i.e. $t = 0$ h) of the slow pool regression line, which in all cases was positive, provided evidence that the gill had initially experienced a rapid increase in Cd, associated with uptake into the saturable fast pool (Hollis et al., 1999). Based on previous studies (Alsop et al., 1999; Hollis et al., 1999) as well as high degree of linearity of accumulation trends after 10 h, we assumed that the rapidly loading pool was fully saturated by or before 10 h. The y-axis intercept of the slow accumulation regression line provided an estimate of this saturation concentration. Loading of new Cd into the slow pool at each time was subtracted from the total amount of new Cd to generate fast pool uptake. Fast pool uptake was modeled via the exponential model (Eq. (2)).

2.4. Statistics

LC_{50} values were calculated applying measured Cd concentrations and mortality data to Probit analysis within the SPSS statistical software. Significant differences were assumed when the 95% confidence intervals did not overlap. Where appropriate Cd concentration of tissues samples were compared using ANOVA. When the ANOVA indicated a significant difference ($P < 0.05$) Dunnetts test was used to compare means from unexposed controls to those of Cd- and Cu-acclimated trout.

3. Results

3.1. Cd cross-acclimation LC_{50} tests

The 96 h LC_{50} for Cd in trout pre-exposed to 20 μg/L Cu for a month was 60 (95% CI 47–85) μg Cd/L, approximately a three-fold increase over the LC_{50} for controls. The response of trout pre-exposed to 60 μg Cu/L produced a 96 h LC_{50} concentration of 126 μg Cd/L (six-fold higher than controls) but the mortality pattern was such that it was not possible to calculate a valid 95% confidence interval (no concentrations with intermediate mortality, only none or complete). When trout chronically exposed to 150 μg/L Zn were challenged with Cd, the 96 h LC_{50} value was 48 μg/L (95% CI 34–58) which was about two times higher than the LC_{50} for previously unexposed controls (96 h LC_{50} of 22 μg/L, 95% CI 10–34; Hollis et al., 1999).

3.2. Acclimation and cross-acclimation metal uptake and distribution

One month of sublethal metal exposure to either 3 μg/L Cd or 75 μg/L Cu produced no significant mortalities and all fish appeared healthy. Prior to experiments to measure new Cd uptake, Cd-acclimated fish had accumulated significant Cd concentrations in the gill, kidney and liver (Table 1) while levels in the muscle, bile and plasma were low and similar to controls. Controls and Cu-acclimated fish had similar levels of Cd (Table 1).

The 3 h gill Cd uptake kinetics experiment illustrated that, compared to controls, Cu- and Cd-acclimated fish took up dramatically increased amounts of Cd (Fig. 1). Fitting the data to the Michaelis-Menten model showed a dramatic increase in gill J_{max} (i.e. increase gill uptake rate) with increased affinity (i.e. decreased K_m) for Cd in Cu-acclimated fish and an increase in J_{max} with no change in affinity for Cd in those acclimated to Cd (Table 2). Uptake kinetics on a whole body basis were similar to the gill data in that there was an increased J_{max} of Cd-acclimated fish while Cu-acclimated fish showed an increased J_{max} and decreased K_m.

Table 1
Mean Cd concentration of tissue samples from rainbow trout following 1-month exposure to either Cd or Cu

	Control	Cd-acclimated	Cu-acclimated
Gill	0.12 (0.004)	2.47[a] (0.17)	0.22 (0.034)
Liver	0.24 (0.007)	1.11[a] (0.06)	0.26 (0.011)
Kidney	0.15 (0.008)	4.27[a] (0.74)	0.18 (0.024)
Muscle	0.17 (0.020)	0.12 (0.001)	0.16 (0.019)
Bile	0.41 (0.043)	0.48 (0.075)	0.45 (0.048)
Plasma	0.14 (0.011)	0.14 (0.007)	0.16 (0.037)

An unexposed control group is also shown, $n = 6$ except for Cd-acclimated means where $n = 5$. The standard error of the mean is shown in parentheses, units are μg/g except for bile and plasma which are μg/mL.
[a] Indicates a significant difference compared to controls for that tissue.

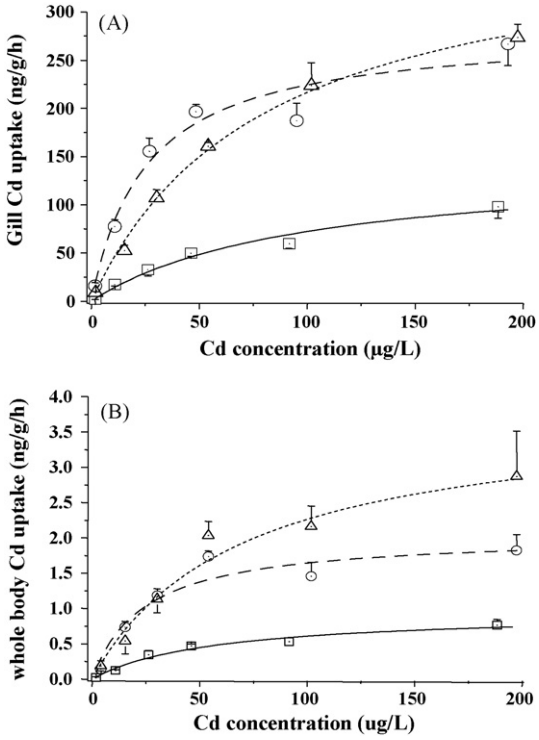

Fig. 1. Short-term (3 h) gill (panel A) and whole body (panel B) uptake kinetics of Cd for juvenile rainbow trout exposed to either 3 μg Cd/L (triangles and dotted line) or 75 μg Cu/L (circles with dashed line) for a month. An unexposed control group is also shown (squares and solid line). Groups of six fish were exposed to each of six different concentrations of Cd with added ^{109}Cd to measure new accumulation on the gill and whole body. Error bars show the standard error of the mean and $n = 6$ for each mean.

The tests to measure new Cd accumulation at 3 μg/L Cd (measured concentration was 2.7 ± 0.1 μg/L) over 72 h illustrated the effect of acclimation on uptake, and showed clear differences between Cu- and Cd-acclimated fish. Overall gill Cd uptake (Fig. 2) was much lower for Cd-acclimated fish compared to controls and Cu-acclimated fish. Gill Cd accumulation was separated into two pools, a rapidly saturable pool and a slower loading high capacity pool. The parameters derived to describe these pools, using Eqs. (2) and (3), are given in Table 3. At 3 μg/L Cd, both Cu- and Cd-acclimated trout had a rapid pool saturation concentration that was higher than control fish. The rate of accumulation into the gill slow loading pool was lowest for Cd-acclimated fish and highest for controls (Table 3). As the gills are the first site of uptake, new Cd accumulation into the gill was much higher than into other organs (Fig. 2 compared to Fig. 3).

New accumulation of Cd into the kidney was approximately 10-fold less than into the gills but the uptake trends were

Table 2
Short-term (3 h) kinetic parameters for Cd uptake into the gills and whole body of unexposed controls, Cu- or Cd-acclimated rainbow trout

	Gill kinetic parameters		Whole body kinetic parameters	
	J_{max} (ng/g/h)	K_m (μg Cd/L)	J_{max} (ng/g/h)	K_m (μg Cd/L)
Control	142.6 (20.7)	97.2 (29.0)	0.95 (0.10)	54.6 (14.2)
Cu-acclimated	281.3 (28.2)	25.4 (8.4)	2.04 (0.25)	23.2 (9.6)
Cd-acclimated	390.3 (17.9)	80.2 (8.2)	3.82 (0.47)	66.1 (19.4)

The standard error is shown in parentheses. See Fig. 1 for more details

Table 3
Kinetics of Cd uptake to the gills of rainbow trout exposed to 3 μg/L Cd for 72 h

	Slow pool accumulation			Fast pool kinetics	
	Rate (ng Cd/g/h)	Intercept	r^2	[Sat] ng/Cd (S.E.)	Time to 1/2 [Sat] (h)
Control	4.49	27.0	0.99	27.8 (4.6)	0.51
Cu-acclimated	2.18	47.3	1.00	47.8 (0.9)	0.84
Cd-acclimated	0.44	39.7	0.97	40.7 (5.9)	1.41

Slow pool accumulation was modeled by linear regression and model parameters were used to derive fast pool concentrations which were subsequently modeled by the exponential model.

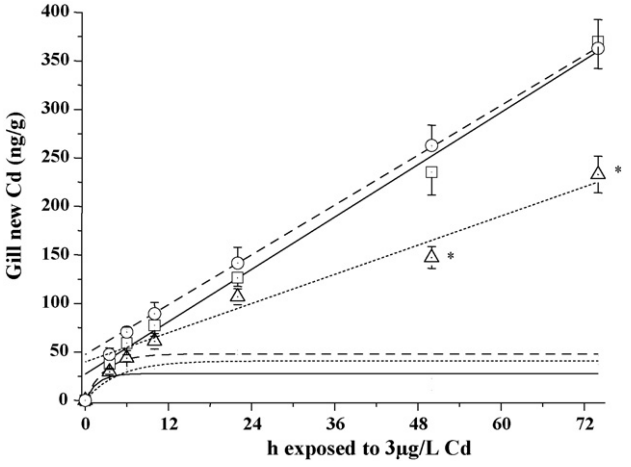

Fig. 2. Time course of gill Cd uptake in rainbow trout over 72 h of exposure to 3 μg/L waterborne Cd. Mean (with S.E.) of measured values ($n = 6$) are shown for previously unexposed fish (squares and solid line), as well as fish exposed to either 3 μg/L Cd (triangles and dotted lines) or 75 μg/L Cu (circles and dashed lines) for a month. The linear regressions for accumulation into the slow pool (upper set of lines) are shown along with the fast pool uptake (lower set of lines), which was modeled via an exponential model (see text for details) and an asterisk indicates a significant difference ($P < 0.05$) from the control mean at that time.

Table 5
Means of whole body new Cd following 72 h of exposure to 3 μg/L Cd in Cu-acclimated, Cd-acclimated and control trout

	Control	Cd-acclimated	Cu-acclimated
Whole body	2.4 (0.8)	1.5 (0.9)	6.5* (0.2)
Bile	0.117 (0.075)	0.083 (0.048)	0.097 (0.088)
Plasma	0.97 (0.11)	1.16 (0.17)	0.79 (0.12)

Standard error is shown in parentheses, $n = 6$ and asterisk indicates a significant difference from control ($P < 0.05$).

generally similar in that all fish showed accumulation. Linear modeling of new Cd accumulation into the kidney showed that unexposed controls had the highest rate of accumulation (Fig. 3A; Table 4). New accumulation of Cd into the liver was approximately 50% of the uptake into the kidney (on a concentration basis). As with the gills, uptake in the liver was highest for unexposed controls and lowest for Cd-acclimated trout (Fig. 3B; Table 5). Over the course of 3 days the uptake of new Cd into the muscle was negligible (Fig. 3C; Table 5) although there were some significant differences recorded (Cu-acclimated fish at 10 and 20 h, Fig. 3C). Uptake into the bile was also low and there were no significant differences among exposures (data not shown except for 72 h, see Table 5). Measurements of new Cd accumulation at the end of the 3-day experiment shows that plasma and bile were similar across all treatment groups, but

Table 4
Linear regression modeling parameters for new Cd uptake during 72 h exposures to 3 μg/L Cd for 72 h

	Liver Cd uptake		Kidney Cd uptake		Muscle Cd uptake	
	Rate (ng Cd/g/h)	r^2	Rate (ng Cd/g/h)	r^2	Rate (ng Cd/g/h)	r^2
Control	0.18 (0.01)	0.98	0.43 (0.03)	0.97	0.001 (0.002)	0.62
Cu-acclimated	0.16 (0.01)	0.98	0.23 (0.03)	0.94	0.001 (0.001)	0.16
Cd-acclimated	0.12 (0.02)	0.90	0.29 (0.01)	0.99	0.001 (0.001)	0.42

Cu-acclimated, Cd-acclimated and control trout are shown, more details are shown in Fig. 3 and the standard error associated with the slope (rate of accumulation) is in parentheses.

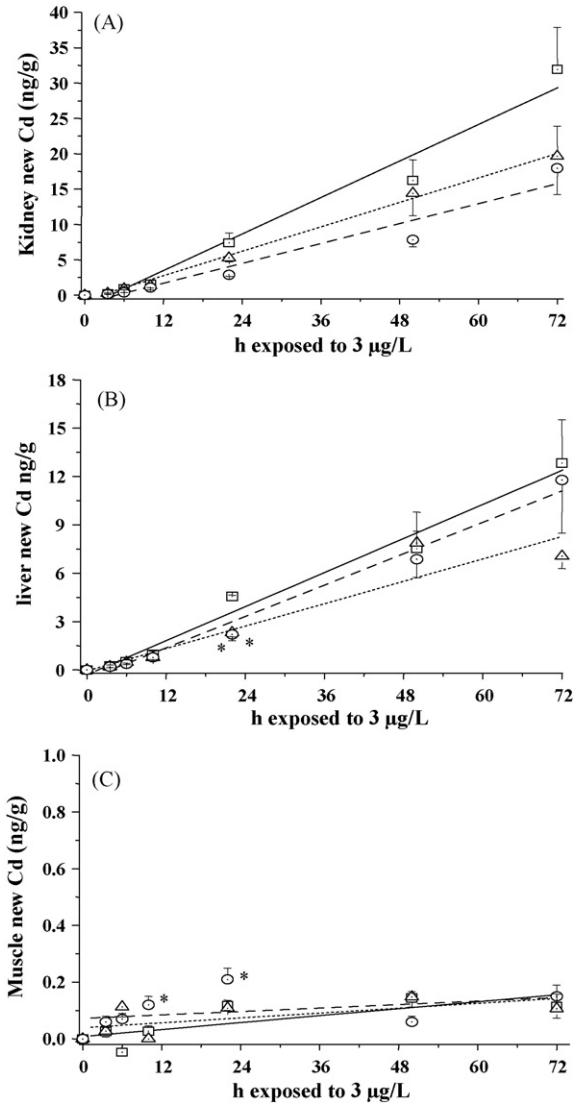

Fig. 3. New Cd uptake to the kidney (panel A) liver (panel B) and muscle (panel C) of rainbow trout exposed to 3 μg/L Cd for 72 h. Three groups of trout are shown, unexposed controls (squares and solid line), Cu-acclimated (circles and dashed line) and Cd-acclimated (triangles and dotted line). An exponential model was used to describe accumulation into the gill while linear regression was used to describe liver and bile accumulation. Symbols show means of $n = 6$ with standard errors and an asterisk indicates a significant difference ($P < 0.05$) from the control mean at that time.

whole body new Cd accumulation was dramatically higher for Cu-acclimated fish and lowest for Cd-acclimated trout (Table 5).

4. Discussion

4.1. Cd cross-acclimation LC_{50} tests

Pre-exposure to 20 or 60 μg/L Cu but not 150 μg/L Zn resulted in cross-acclimation for acute Cd exposures. The trout chronically exposed to 20 and 60 μg/L Cu were shown to be acclimated to Cu (Taylor et al., 2000). The LC_{50} for Cd in these Cu-acclimated fish was significantly higher than the unexposed control group that was reported by Hollis et al. (1999) at 22 μg/L (95% CI 10–34). The Cd pre-exposed trout showed a strong acclimation to Cd. The LC_{50} values were 285 (95% CI 215–318) and 242 (173–349) μg Cd/L as a result of chronic exposure to 3 and 10 μg/L, respectively (Hollis et al., 1999). Although, the Cd LC_{50} value for Zn-acclimated fish was higher than that of unexposed controls, the 95% confidence intervals overlapped and therefore Zn-acclimation (as shown in Alsop et al., 1999) did not result in cross-acclimation to Cd.

This is one of a very few studies with fish that has illustrated the phenomenon of cross-acclimation to metals. One other study has demonstrated that Cd exposed killifish had an increased tolerance to Cu (Xie and Klerks, 2003). A variety of hypotheses are possible as to which physiological mechanism could be influential in the cross-acclimation responses. A strong metallothionein response, as might be expected from fish chronically exposed to Cd (Chowdhury et al., 2005a), would explain the ability to resist Cd challenges. The mechanism through which Cu-acclimated trout can resist Cd challenges is unknown. Why Zn-acclimated fish were not cross-acclimated to Cd is unclear but clearly Zn-acclimation is specific enough that it does not involve mechanisms related to Cd. A previous study (Hogstrand et al., 1994) with rainbow trout chronically exposed to 150 μg Zn/L demonstrated that this level of exposure was insufficient to induce a metallothionein response.

The results of the challenges that illustrated cross-acclimation led to the planning of subsequent experiments focusing on Cd uptake and distribution in Cd exposed and Cu exposed trout. Tissue Cd concentrations, prior to subsequent new Cd exposure, were measured following a month of exposure to Cd and results (Table 1) were similar to those shown in McGeer et al. (2000b). As expected, the Cd burden in trout chronically exposed to 3 μg/L Cd was primarily in the gill, liver and kidney, while tissue burdens in controls and Cu-acclimated fish were much lower and similar to each other.

4.2. Acclimation and cross-acclimation metal uptake and distribution

Short-term gill loading experiments done at increasing Cd concentrations showed that metal-acclimated fish had a higher capacity to accumulate Cd when challenged (Fig. 1). Derivation of Michaelis-Menten kinetic variables illustrated that for Cd-acclimated fish, J_{max} was increased but affinity was the same as for previously unexposed controls. This result (i.e. an increased J_{max} with no change in affinity) is very similar to that of Hollis et al. (1999) for rainbow trout acclimated to 3 μg Cd/L. Interestingly, the gill changes that occurred during Cu exposure resulted in much higher levels of Cd being taken up compared to controls. In this case, J_{max} was increased and affinity was increased (i.e. K_m was decreased). This combination provides for significant and rapid accumulation of Cd in Cu-acclimated fish. The reasons for the stimulation of Cd uptake in Cu-acclimated trout remain to be studied. One possibility is that the increased gill ionoregulatory activity that occurs during Cu exposure, for example, the increase in Na^+/K^+ ATPase activity to counteract Cu^{2+} induced Na^+ loss (Lauren and McDonald, 1987; McGeer et al., 2002), in some way creates circumstances to promote Cd uptake. This is speculative and the physiological mechanisms involved are

unknown. When measured on a whole body basis, the Cd J_{max} of Cu- and Cd-acclimated fish was much higher than controls suggesting that the ability to transfer Cd from the gills to blood and/or organs and tissues is stimulated during acclimation to both Cu and Cd (Fig. 1; Table 2).

The results of the exposures to 3 μg Cd/L for 72 h also revealed significant differences as a result of metal-acclimation. The goal in this experiment was to understand how uptake and distribution had changed over the month of exposure. As such the exposure represented days 1–3 of exposure for controls and Cu-acclimated fish, while for Cd-acclimated fish it represented days 31–33. Overall, Cd-acclimated fish had a lower intake of new Cd compared to controls and Cu-acclimated fish which were similar (Fig. 2). The modeling process allowed us to separate out a relatively small fast Cd pool and a much larger slower loading Cd pool. A similar approach was used by Alsop et al. (1999) for Zn uptake where the fast pool was interpreted as a dynamic pool bound to high affinity sites and the slow pool was described as representing incorporation into long-term detoxification storage and structural components of the gill. The results for fast pool loading are generally consistent with the results for short-term gill loading in that there is an increase in high affinity sites (i.e. higher saturation concentration) for metal-acclimated fish compared to controls. In terms of the rate of transfer into the slow pool, Cd-acclimated fish were dramatically lower than controls or Cu-acclimated trout. In other words, in spite of a much higher capacity for short-term loading of Cd-acclimated trout, the longer term uptake rate into the gills at the acclimation concentration was reduced compared to controls. This suggests that at low concentrations the gill may be able to eliminate Cd more effectively.

Accumulation of new Cd in internal tissues over the 72 h of exposure illustrated that the kidney concentrations reach levels much higher than other tissues such as the liver or muscle (Fig. 3). This is consistent with other studies showing preferential renal uptake (Hollis et al., 1999; McGeer et al., 2000b). Previously, unexposed fish showed high rates of accumulation relative to Cd-acclimated fish (Table 4). One explanation for the lower rate of uptake into kidney and liver for Cd-acclimated trout is the lower rate of uptake into the gills. In other words, lower accumulations of Cd into the gill means less to transfer into internal organs. Interestingly, Cu-acclimated trout showed a trend towards lower Cd uptake into the kidney suggesting the chronic Cu exposure resulted in alterations in renal metal handling. That transfer of new Cd to the gills was high for Cu-acclimated fish (and similar to controls) but that the transfer rate to the kidney was much lower suggests an excess of Cd somewhere in Cu-acclimated fish. This excess was not in the liver (Fig. 3B) or muscle (Fig. 3C) or bile (Table 5) compartments. It is possible that Cu-acclimated fish had an enhanced ability to excrete/eliminate Cd and this and other possible explanations are deserving of further study.

In these studies, we have examined Cd uptake and distribution to learn more about acclimation and cross-acclimation. The results clearly show that chronic sublethal exposure to Cu results in cross-acclimation to Cd. However, chronic exposure to Zn does not produce enhanced resistance to Cd. When exposed to Cd, trout acclimated to Cu experienced elevated uptake of Cd. Because there is increased uptake of Cd, the heightened resistance to Cd in Cu-acclimated fish must be as a result of an enhanced capacity for detoxifying or otherwise eliminating Cd. Although, the Cd-acclimated fish showed a high capacity to take up new Cd into the gill during challenges, the overall strategy at low and environmentally relevant concentrations was to reduce Cd uptake.

Acclimation changes not only how fish handle the metal of exposure but also, how they respond to other metals. Understanding how acclimation influences toxicity is important for risk assessment and site-specific toxicological investigations/evaluations. Our results illustrate that the influence of cross-acclimation should also be considered. Similarly, bioaccumulation and tissue distribution of Cd are dramatically altered by acclimation not just for fish chronically exposed to Cd but also for those acclimated to Cu as well. These results illustrate that understanding the previous exposure conditions is important not only for the metal of concern but also other metals.

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