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Aquatic Toxicology 50 (2000) 231–243

AQUATIC TOXICOLOGY

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Effects of chronic sublethal exposure to waterborne Cu, Cd or Zn in rainbow trout. 1: Iono-regulatory disturbance and metabolic costs

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Received 19 March 1999; received in revised form 20 October 1999; accepted 15 November 1999

Abstract

The relationships among growth, feeding behaviour, ion regulation, swimming performance and oxygen consumption in rainbow trout (*Oncorhynchus mykiss*) were compared during chronic exposure (up to 100 days) to sublethal levels of waterborne Cd (3 $\mu g \cdot l^{-1}$), Cu (75 $\mu g \cdot l^{-1}$) or Zn (250 $\mu g \cdot l^{-1}$) in moderately hard water (hardness of 140 $mg \cdot l^{-1}$, pH 8). A pattern of disturbance, recovery and stabilization was evident for all three metal exposures, although the degree of disturbance, specific response and time course of events varied. Growth was unaffected by any of the metals under a regime of satiation feeding but appetite was increased and decreased in Cu- and Cd-exposed trout respectively. Critical swimming speed was significantly lowered in fish chronically exposed to Cu, an effect associated with elevated O_2 consumption rate at higher swimming speeds. Branchial Na^+/K^+ ATPase activity was elevated in Cu-exposed fish but not in Cd-exposed trout. Disruption of carcass Na^+ and Ca^{2+} balance was evident within 2 days of exposure to either Cd, Cu or Zn, with subsequent recovery to control levels. The loss of Ca^{2+} in trout exposed to waterborne Cd persisted longest, and recovery took approximately a month. The physiological response of trout to chronic Cu exposure involves mechanisms that result in an associated metabolic cost. In comparison, Cd is neither a loading nor a limiting stress and acclimation to chronic Cd-exposure does not appear to involve a long term metabolic cost.

Keywords: Rainbow trout; Cadmium; Copper; Zinc; Metabolic rate; Swimming performance; Ion regulation

1. Introduction

The levels of waterborne Cu, Cd and Zn that induce acute toxicity in freshwater fish can vary greatly depending on water chemistry, fish species, life stage and temperature (Alabaster and Lloyd, 1982; Harrison, 1986; Hogstrand and Wood,

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0166-445X/00/$ - see front matter
PII: S0166-445X(99)00105-8

1996; Taylor et al., 1996). However, in most natural waters, heavy metals are usually only present at sublethal concentrations (Méranger et al., 1979; Ravera, 1984; Harrison, 1986) and much less is known about the effects of chronic sublethal exposure in fish. An understanding of the chronic sublethal responses to Cu and Zn is further complicated by the fact that both are required micronutrients.

Chronic exposure of fish to waterborne Cu, Cd or Zn has been shown to cause a variety of physiological and behavioural changes including loss of appetite, reduced growth, ion loss, decreased aerobic scope and mortality (Drummond et al., 1973; Chapman, 1978; Waiwood and Beamish, 1978a,b; Roch and Maly, 1979; Reid and McDonald, 1988; De Boeck et al., 1995, 1997; Hogstrand and Wood, 1996; Taylor et al., 1996; Scherer et al., 1997). However, these effects are not consistent, and some studies have shown that there can be minimal physiological disturbance even when exposure concentrations of Cd, Cu and Zn are sufficient to cause some mortalities (Kumada et al., 1980; Spry et al., 1988; Alsop et al., 1999; Hollis et al., 1999; Taylor et al., 2000). As well, chronic sublethal exposure usually results in acclimation, a physiological process that can result in an increased tolerance to acute challenges of the metal of exposure.

The general process of acclimation to metals has been characterized by a 'damage-repair' model (McDonald and Wood, 1993) comprising three phases: an initial 'shock' phase, a recovery phase, and then acclimation itself, the latter including an increased tolerance which persists indefinitely during continued exposure. The initial 'shock' phase corresponds to a period of physical damage, primarily at the gill, and assorted disturbances of internal physiological homeostasis. The damage phase is usually short-lived (a few days). Thereafter, recovery starts coincident with increased biosynthetic processes (mitosis, enhanced protein synthesis) which help repair the damage and correct the physiological disturbances. Inherent within the recovery phase is mobilization of metal binding proteins such as metallothionein (Bradley et al., 1985; Hogstrand and Wood, 1996) and an up-regulation of other pathways to counteract or compete with the deleterious effects of the metal, for example those related to ion regulation (Laurén and McDonald, 1987a,b; Hogstrand et al., 1995; Pelgrom et al., 1995). Ultimately, the internal physiology of the animal either returns to the pre-exposure condition or, a new equilibrium is established during the final period of increased tolerance.

In this and the following paper (McGeer et al., 2000), we examine the responses of rainbow trout to chronic sublethal exposure to three different metals, Cu, Cd and Zn. In the present paper, our goal was to compare and contrast some of the physiological responses and metabolic costs associated with short-term and chronic sublethal exposure to each individual metal. The levels of exposure for each metal were chosen after careful examination of the results of three recent studies on the physiological responses of rainbow trout to chronic exposure to Cd at 3 or 10 $\mu g \cdot l^{-1}$ (Hollis et al., 1999), Cu at 20 or 60 $\mu g \cdot l^{-1}$ (Taylor et al., 2000) and to Zn at 150 or 450 $\mu g \cdot l^{-1}$ (Alsop et al., 1999) in the same moderately hard Lake Ontario water. Based on these studies, exposure concentrations of 3 $\mu g \cdot l^{-1}$ Cd, 75 $\mu g \cdot l^{-1}$ Cu or 250 $\mu g \cdot l^{-1}$ Zn were selected. Although exposure concentrations were very different from each other, each metal concentration was at a level that would induce tolerance to acute challenges yet be just below the threshold for producing significant mortalities during chronic exposure to Cd (Hollis et al., 1999), Cu (Taylor et al., 2000) or Zn (Alsop et al., 1999). Therefore our work was directed at understanding how these internally consistent toxicological endpoints were reflected in physiological endpoints, specifically ionoregulation, feeding, growth, metabolic rate and swimming performance. The companion paper (McGeer et al., 2000) describes and models the tissue specific metal accumulation associated with these same exposures.

Our exposure concentrations were environmentally relevant and within the context of regulatory guidelines for the protection of aquatic life, which are derived not just from data on trout alone but rather from algae, invertebrates and teleost fish in general. The US EPA guidelines for example, give maximum allowable acute and chronic values (respectively) for Cd of 5.7 and 1.3 $\mu g \cdot l^{-1}$ (US EPA, 1986), for Cu of 24.3 and 15.8 $\mu g \cdot l^{-1}$ (US EPA,

1985) and for Zn of 374 and 30 $\mu g \cdot l^{-1}$ (US EPA, 1982) in our exposure water (moderately hard, dechlorinated Hamilton tap water from Lake Ontario). Our exposure concentrations are between acute and chronic guidelines for Cd and Zn but above both guidelines for Cu because trout are not a sensitive to Cu as are some other freshwater organisms.

2. Methods

2.1. General

Rainbow trout (1–2 g) were obtained from a local commercial supplier (Humber Springs, Orangeville, Ont.) and maintained in dechlorinated Hamilton tap water ('hard water' — ionic composition: Ca^{2+}, 1.0 mM; Mg^{2+}, 0.2 mM; Na^{+}, 0.6 mM; K^{+}, 0.2 mM; Cl^{-}, 0.7 mM; hardness, 140 $mg \cdot l^{-1}$ as $CaCO_3$; and pH 8) for at least 1 month prior to experimentation. Trout were exposed to waterborne metals at nominal concentrations of either 3 $\mu g \cdot l^{-1}$ Cd (as $Cd(NO_3)_2 \cdot 4H_2O$) or 75 $\mu g \cdot l^{-1}$ Cu (as $Cu(SO_4) \cdot 5H_2O$) or 250 $\mu g \cdot l^{-1}$ Zn (as $Zn(SO_4)_2 \cdot 7H_2O$) along with a control group, similarly treated but not exposed to metal. Measured concentrations of metals in untreated water were 0.057 ± 0.005 μg $Cd \cdot l^{-1}$ (mean $\pm$ 1 S.E.M., $n = 34$), 1.6 ± 0.3 μg $Cu \cdot l^{-1}$ ($n = 35$) and 2.4 ± 0.5 μg $Zn \cdot l^{-1}$ ($n = 11$).

Two series of exposures were conducted. In series 1, fish were exposed to Cd, Cu or Zn for up to 65 days while in series 2, trout were exposed to either Cd or Cu for 100 days. Exposure to either Cd, Cu or Zn was initiated by adding sufficient concentrated metal solution directly to the tank to achieve the target level. Exposure conditions were then maintained throughout the exposure by metering acidified (0.05%) concentrated metal solution (from a Marriot bottle) into flowing water (from a constant flow head tank) and mixing with vigorous aeration prior to delivery to fish tanks. The addition of acidified solutions had no effect on water pH values. Each tank (200 l) received a flow of 1.5 $l \cdot min^{-1}$ and was supplied with aeration. Photoperiod was set to a light:dark cycle which was similar to the natural photoperiod for Hamilton.

2.2. Exposure series 1

2.2.1. Exposure protocol

Rainbow trout were exposed to 3 $\mu g \cdot l^{-1}$ Cd, 75 $\mu g \cdot l^{-1}$ Cu or 250 $\mu g \cdot l^{-1}$ Zn (nominal concentrations) and fed to satiation so that maximum growth, appetite and feed conversion could be measured. Mean fish weight at the start of the experiment was 3.2 ± 0.1 g ($\pm$ S.E.M., $n = 600$), temperature was 14 ± 1°C (mean and range) and measured exposure concentrations were either 73.7 ± 2.1 μg $Cu \cdot l^{-1}$ (mean $\pm$ 1 S.E.M., $n = 12$) or 2.91 ± 0.10 μg $Cd \cdot l^{-1}$ ($n = 12$) or 234 ± 5.0 μg $Zn \cdot l^{-1}$ ($n = 5$). Two days prior to the start, 150 fish were non-selectively distributed to each of the four experimental tanks (Cd, Cu, Zn and control). The experimental period was divided into three segments: an initial period of 11 days during which time no metals were administered but fish were fed to satiation, a period of 35 days (days 11–46) during which fish were fed to satiation and exposed to either Cd, Cu or Zn and then, a 30 day period (day 46–76) during which the metal exposures were continued but feed was given at a fixed ration of 4% per day (which in fact, was very close to a satiation ration). At day 24 (day 13 of exposure) the Zn exposure was terminated prematurely due to mechanical failure.

2.2.2. Daily maintenance

Trout were fed by hand to satiation twice a day (morning and late afternoon) with a commercial dry ration (Martin's Feed Mill Ltd. Elmira ON; see Table 1 for composition) for an 11 day pre-exposure period and a following 35 day exposure period. Feeding consisted of adding a small amount of food (approximately 5% of amount expected to be fed) at 0.5–1 min intervals until feeding activity ceased. Feeding activity was deemed to have ceased when food particles began to collect on the bottom of the tank and to aid in this visual determination, a small white disk was fixed to the bottom. After the 46 day period of satiation feeding, fish were fed a ration of 2% of tank biomass twice a day (i.e. 4%·per day) for the remainder of the experiment. Tanks were siphoned clean and checked for mortalities daily.

2.2.3. Sampling protocol

On days 0, 3, 8, 11 (before metal exposure), 14, 17, 21, 27 and 46 (corresponding to day 3, 6, 10, 16 and 35 of metal exposure) all of the fish in each tank were individually weighed to the nearest 0.01 g. For each tank, the weighing procedure was accomplished in about 30 min or less and maximum time from handling to return to tank was about 3 min. The relatively small size of the fish meant that anesthetic administration was not required and no mortalities occurred as a result of weighing procedures. Weighing was done before the first feeding of the day and fish were fed about 1 h after return to the tank and then as normal later in the day.

Sampling for determination of carcass Na^+, Ca^{2+} and Cl^- was incorporated with the procedures for tissue metal burden analysis (gills, liver, kidney) which are described by McGeer et al. (2000). These samples were collected after 1, 2.5, 4, 5.5, 10, 16 and 65 days of metal exposure. At least six fish were removed from each tank and the number sampled was varied to ensure the stocking densities remained constant when mortality occurred. Additionally, on day 65 of metal exposure a second sample of gill tissue was collected from each fish, immediately freeze-clamped in liquid N_2 and stored at -70°C prior to measurements of Na^+/K^+ ATPase activity.

The maximum aerobic swimming speed (Ucrit; Brett, 1964) of fish exposed to either Cd or Cu or not exposed (controls) was measured between days 40 and 46 of exposure using methods described by Alsop et al. (1999). In brief, fish were starved for a day, then in groups of 5 or 6, were introduced to the Beamish style swim chamber. After a 1 h orientation period, water velocity was incremented by 7.5 $cm \cdot s^{-1}$ at intervals of 45 min. Time, weight and fork length were measured at exhaustion, deemed to have occurred when a fish would no longer swim after two forced returns. These swim trials were done in normal water with no metal added.

Table 1
Proximate composition and metal content of food

Component	Content (%)
Crude Protein	52
Crude Fat	17
Carbohydrate	16.5
Crude Fibre	2.5
Water	12
Na^+	0.4
Ca^{2+}	1.4
Cadmium	1.1 $\mu g \cdot g^{-1}$
Copper	11.5 $\mu g \cdot g^{-1}$
Zinc	173 $\mu g \cdot g^{-1}$

2.3. Exposure series 2

2.3.1. Experimental animals, exposure and maintenance

Series 2 further explored ion regulation, swimming performance and the metabolic costs associated with exposure to chronic sublethal waterborne Cd (3 $\mu g \cdot l^{-1}$) or Cu (75 $\mu g \cdot l^{-1}$; nominal concentrations). The exposures were performed as in series 1 but with some changes in protocol. Feeding rate was much lower, there were 300 fish per treatment, temperature was higher at 17 ± 1.5°C (mean and range), and the exposures lasted 100 days. Mean weight of trout at the start of the experiment was 3.3 ± 0.1 g (mean $\pm$ 1 S.E.M. of $n = 50$) and measured levels of metal during exposure were either 75.8 ± 1.8 μg $Cu \cdot l^{-1}$ (mean $\pm$ 1 S.E.M., $n = 23$) or 3.07 ± 0.19 μg $Cd \cdot l^{-1}$ ($n = 21$). Fish were fed at a rate of 1.5% of tank biomass per day with half of the ration being fed at each of the two daily feedings. An unexposed control group of fish were subjected to the same sampling and feeding protocol with no added metal. Exposures were not simultaneous (as they had been in series 1) and the initiation of the Cd and Cu exposures were offset by 2 weeks. As a result, swimming and oxygen consumption testing could be done for Cu and Cd exposed fish after similar durations of exposure. However, the non-concurrent nature of the exposures also meant that weights could not be compared across treatments. Therefore, growth was not quantified in this experimental series.

2.3.2. Experimental protocol and sampling

On days 0, 1, 3, 7, 10, 14, 20, 30, 52, 70, 80 and 100 of exposure, six fish were sampled from each

tank and following tissue sampling for metal burden determination (see McGeer et al., 2000) the remaining carcass was saved for measurement of Na^+ and Ca^{2+}. The Ucrit of individual fish exposed to either Cd or Cu or not exposed (controls) was measured between days 46 and 51 of exposure using the same methods as described in Series 1 with the difference that sufficient metal (either Cu, Cd or none for controls) was added to the water in the swim chamber to match the exposure concentration in the rearing tank.

Following 2.5 months of exposure the rate of oxygen consumption (MO_2) of individual fish during swimming was measured in 3.2 l Blazka-style respirometers using methods described by Lauff and Wood (1996). Fish were starved for 24 h and then individuals were netted from their rearing tank, quickly measured for weight and length and transferred to a respirometer with sufficient metal added to match the exposure level. A flow-through system was used except when oxygen consumption measurements were being conducted (water flow suspended). Respirometers were covered to exclude light in the forward end of the swimming compartment and minimize disturbance. All respirometers were submerged within a water bath to ensure that a constant temperature (17°C) was maintained.

Measurements of MO_2 were done at three swimming speeds (1, 2 and 4 body lengths s^{-1}) which corresponded to approximately 20, 40 and 80% of Ucrit. Initially, water velocity was set to 1 body length s^{-1} (sub-threshold for swimming) and the fish was left for a 1 h period at this velocity to orient and adjust. Water inflow was then stopped, the respirometer sealed and sampling begun. Water samples (3 ml) were drawn at approximately 5–7 min intervals via a sampling port. The partial pressure of O_2 (P_{O_2}) in water samples was measured using a thermostatted oxygen electrode (E101, Cameron Instruments, TX) connected to an O_2 meter (OM-200, Cameron Instruments, TX). The duration of the sampling period was approximately 20 min during which water P_{O_2} never fell below 115 Torr. At the end of the sampling period, water flow was re-established and velocity in the respirometer increased from 1 to 2 body length s^{-1} over a 2 min period. After a 1 h period at 2 body length s^{-1}, the respirometer was sealed and water samples collected as before. Following this, velocity was increased to 4 body length s^{-1} and after another hour, a series of water samples was taken again for MO_2 measurements. Individuals who did not complete the full swimming regime were omitted from the analysis.

2.4. Water and tissue sample analysis

Cd and Cu content of acidified (Trace Metals Grade, Fisher Sci., Nepean ON) water samples was measured by graphite furnace atomic absorption spectrophotometry (AA1275 and GTA-95, Varian, Austr.). Zn concentration in acidified water samples was measured by atomic absorption spectrophotometry (AA1275 Varian).

Carcasses were digested in approximately five volumes of 1 N HNO_3 and measured for Na^+ and Ca^{2+} by atomic absorption spectrophotometry (AA1275 Varian) Carcass Cl^- concentration was quantified using the mercuric thiocyanate method of Zall et al. (1956). Gill samples were assayed for Na^+/K^+ ATPase activity using the methods of Holliday (1985), described in detail by Morgan et al. (1997). Na^+/K^+ ATPase activity was expressed as the concentration of inorganic phosphate liberated per unit time and adjusted for the protein content of the sample (μmol PO_4·mg protein^{-1}·h^{-1}), the latter determined using the dye binding assay of Bradford (1976).

2.5. Calculations

Specific growth rate (SGR, % per day) was calculated for the interval between each weighing using the formula,

$$SGR = 100*[(\ln(wt_2) - \ln(wt_1))/(t)]$$

Where wt refers to the average weight of individuals at the start (wt_1) and end (wt_2) of the interval and t is the length of time of the interval in days. Carcass Na^+, Ca^{2+} and Cl^- levels were expressed as μmol·kg^{-1}.

Daily food consumption was calculated as % body weight per day using total daily food given and tank biomass. Estimates of tank biomass were calculated using the individual SGR between

each weighing interval. Food conversion efficiency (FCE) was calculated as biomass wet weight gain (g) per gram of food fed. FCE was determined on a daily basis from the daily increment in weight (using SGR) and total food fed for the day. Mean values for daily food consumption (appetite), FCE and SGR were calculated for exposure and pre-exposure periods. Because weighing procedures disrupted the normal morning feeding, data collected on days when weighing were done were excluded from the appetite and FCE analyses.

Ucrit calculations were performed as described by Brett (1964) where the water velocity and time at exhaustion were incorporated into the formula,

$$\text{Ucrit} = [V_L + (7.5 * m/45)]/l$$

where $V_{L'}$ is the velocity ($cm \cdot s^{-1}$) of the stage previous to exhaustion, 7.5 is the velocity increment ($cm \cdot s^{-1}$) between each 45 min stage, m is the minutes spent at the stage where exhaustion occurred and l is the fork length of the fish.

Calculations of MO_2 during swimming were performed as described by Alsop and Wood (1997) using the rate of decline in P_{O_2} which occurred when the respirometer was sealed, the solubility of O_2 in water at 17°C, the respirometer volume, and the weight of the individual fish. MO_2 was expressed as μmol O_2 g^{-1} h^{-1}.

2.6. Statistics

Results from rainbow trout exposed to Cd, Cu or Zn were compared to unexposed controls by ANOVA. The data for SGR, appetite and FCE data for the 11 day pre-exposure period (series 1) are presented as single pooled means as there were no significant differences between the four treatments. Pre-exposure carcass ion data were not collected in series 1 exposures, so a pooled mean of all control values has been used to represent the day 0 level. Data for MO_2 were analysed by first establishing a linear regression equation for swim velocity versus MO_2 for each individual and comparing regression variables of control and treated fish by ANOVA. In all cases, $\propto \leq 0.05$ was the accepted level of significance, and Duncan's New Multiple Range Test was used for multiple comparisons.

3. Results

3.1. Survival, growth and feeding

All unexposed (control) trout survived the duration of the exposures, 65 days for series 1 and 100 days for series 2. Fish exposed to 3 $\mu g \cdot l^{-1}$ Cd exhibited a 93% survival rate (similar in both exposures) and all of the mortalities occurred within the first 60 h of the series 1 exposure or over the first 2 weeks of the series 2 exposure. In satiation-fed fish (series 1) exposed to 75 $\mu g\ l^{-1}$ Cu, there were no mortalities but during the fixed ration trial (series 2), 6% of the fish died over the first 14 days; thereafter, all fish survived. There had been no mortalities in trout exposed to 250 $\mu g \cdot l^{-1}$ Zn up until day 12, when the experiment was prematurely terminated by mechanical failure.

In series 1, fish grew well in all treatments. Overall, there were no differences when SGR was compared before and during exposure (Fig. 1C). When mean weight was compared across treatments there were no differences at any time (Table 2).

Daily consumption of food (series 1) was significantly affected by metal exposure, particularly for Cu-exposed fish which exhibited increased appetite (Fig. 1A, B). Appetite was less than pre-exposure for trout exposed to Cd (Fig. 1A, B) and was particularly reduced during the first 3 days, corresponding to the period when mortality occurred. Trout exposed to 250 $\mu g \cdot l^{-1}$ Zn did not experience any change in appetite when compared to either control or pre-exposure values (Fig. 1B). FCE over the period of exposure was not affected by exposures to Cd (0.91 ± 0.05, mean $\pm$ S.E.M. of g wet weight gain per g fed, $n = 29$), Cu (0.86 ± 0.06, $n = 29$), or Zn (1.01 ± 0.12, $n = 9$) compared to controls (0.92 ± 0.05, $n = 29$).

3.2. Ionic regulation

3.2.1. Series 1

Ionic concentration was affected by exposure to Cd, Cu and Zn with Na^+ disturbance being somewhat less and shorter lived than Ca^{2+} disturbance (Fig. 2A and Fig. 2C). Carcass Cl^- levels did not

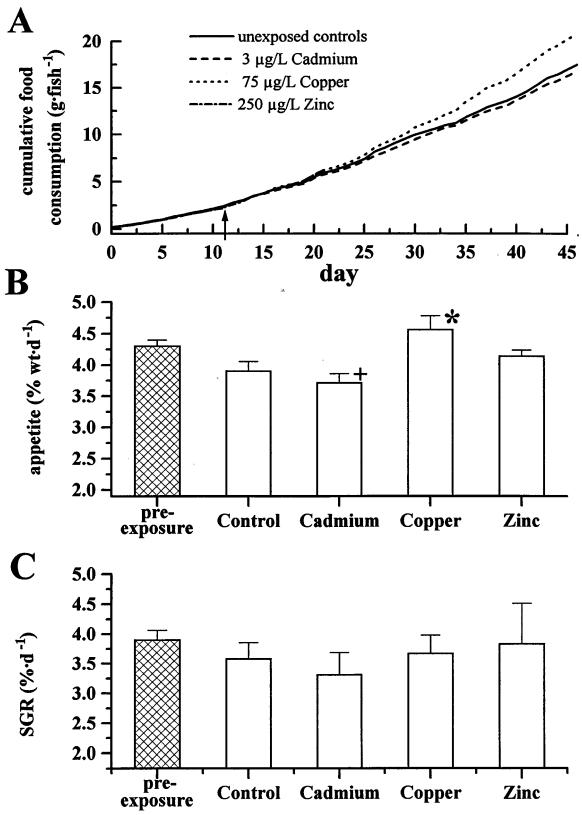

Fig. 1. Effects of Cd, Cu or Zn on feeding and growth in rainbow trout of Series 1. (A) Total accumulated food consumption; (B) Daily food consumption (appetite); (C) SGR before and during exposure to either 3 $\mu g \cdot l^{-1}$ Cd or 75 $\mu g \cdot l^{-1}$ Cu or 250 $\mu g \cdot l^{-1}$ Zn. Exposures were for 35 days, except Zn-exposed fish where the exposure ended at 13 days. An 11 day pre-exposure interval is included in each graph. An unexposed control group is also shown. For panels B and C, mean ± 1 S.E.M. ($N \geq 9$), + indicates significant difference ($P < 0.05$) from the pre-exposure mean while * indicates a significant difference from control mean.

change significantly in metal exposed fish (Fig. 2B). Carcass Ca^{2+} concentration was significantly reduced in both Cu- and Cd-exposed trout and at day 16 this reduction persisted for Cd-exposed fish (Fig. 2C). At day 65, there were no significant differences between control and exposed fish for carcass body Na^+, Cl^- and Ca^{2+} concentrations (Fig. 2), suggesting that recovery had occurred.

Gill Na^+/K^+ ATPase activity, measured at day 65, was elevated more than 2-fold in fish exposed to 75 $\mu g \cdot l^{-1}$ Cu and unchanged for trout exposed to 3 $\mu g \cdot l^{-1}$ Cd compared to controls (Fig. 3).

3.2.2. Series 2

As with series 1, exposure to 3 $\mu g \cdot l^{-1}$ Cd or 75 $\mu g \cdot l^{-1}$ Cu caused significant but short-lived reductions in carcass Na^+ levels (Fig. 4A). Carcass Ca^{2+} concentration (Fig. 4B) was also reduced but only in Cd exposed fish. A pattern of initial disturbance and subsequent recovery was evident; declines in Ca^{2+} concentration for Cd-exposed fish took the longest to return to normal.

3.3. Swimming performance

After 1.5 months of satiation feeding (series 1), rainbow trout exposed to 75 $\mu g \cdot l^{-1}$ Cu had a lower mean Ucrit value compared to control trout (Fig. 5A). There was no significant effect on Ucrit in the Cd-exposed fish. Fish exposed to Cu or Cd for 2.5 months in series 2 (fixed ration) showed no differences in Ucrit (Fig. 5B) among treatments. Note that absolute Ucrit values were significantly higher in series 2, probably reflecting the more streamline shape of these fish on lower ration, and also, that the respective metals were present during the tests of series 2 but not during the tests of series 1.

3.4. Oxygen consumption

Compared to controls, trout exposed to 75 $\mu g \cdot l^{-1}$ Cu for 2.5 months in series 2 (fixed ration) showed an increased oxygen consumption when forced to swim at four body lengths s^{-1} (Fig. 6). When swimming in low water velocities, MO_2 did not differ between groups, Cd-exposed fish were similar to controls at all speeds (Fig. 6). The slope of the MO_2 versus swimming speed regression lines were significantly different for Cu exposed fish at 5.2 ± 0.5 $\mu mol \cdot g^{-1} \cdot h^{-1} \cdot$body length$^{-1} \cdot s^{-1}$ (mean $\pm$ S.E.M., ΔMO_2 per body lengths^{-1}) while those of controls were 3.1 ± 0.7 (ΔMO_2 per body lengths^{-1}) and Cd exposed fish were 2.7 ± 0.4 (ΔMO_2 per body lengths^{-1}).

4. Discussion

4.1. Overview

The effects of chronic sublethal exposures to Cu, Cd or Zn follow a pattern of damage, recovery and acclimation as previously defined by McDonald and Wood (1993). For Cd-exposed fish, the initial damage phase was characterized by a reduced appetite, declines in Na^+ and Ca^{2+} (Figs. 1, 2 and 4) as well as metal accumulation (see Figs 2 and 5 and Tables 2 and 3 of McGeer et al., 2000). For trout exposed to Cu, ionic content declined (Figs. 2 and 4) and metal accumulated in all tissues (see Figs 1 and 4 and Tables 2 and 3 of McGeer et al., 2000). In trout exposed to Zn, there were no changes in feeding or growth (Fig. 1) but loss of ions (Fig. 2) and some metal accumulation in the gills was evident (see Fig. 3 of McGeer et al., 2000). Subsequent to the initial shock phase, ion losses were reversed. In general, this recovery period was relatively short for Cu and Zn and longer for Cd-exposed fish. For example, recovery of Ca^{2+} content was delayed in Cd-exposed trout. For Cu-exposed fish the effects of chronic exposure included increased gill Na^+/K^+ ATPase activity (Fig. 3) and elevated appetite (Fig. 1). Based on earlier studies, the recovery phase would correspond to the time when fish would be expected to show resistance to acute challenges (Dixon and Sprague, 1981a,b; Bradley et al., 1985). Recent studies with the similar-sized rainbow trout, exposed in the same water chemistry, using similar protocols and similar levels of Cd, Cu or Zn have demonstrated significant resistance as manifested by 2–14-fold increases in 96 h LC_{50} after 30 days of exposure (Alsop et al., 1999; Hollis et al., 1999; Taylor et al., 2000).

4.2. Survival, growth and feeding

Exposure of trout to Cd, Cu or Zn resulted in very few mortalities. The SGR was not affected by exposure to metal (Fig. 1C) and there were never any differences in mean weight among treatments in spite of the exhaustive and extensive weighing protocol in series 1 (Table 2). A number of studies have now shown that chronic sublethal exposure of fish to either Cd (Kumada et al., 1980; Hollis et al., 1999), Cu (Seim et al., 1984; Taylor et al., 2000) or Zn (Chapman, 1978; Alsop et al., 1999) may have no effect on growth, especially when ration is high or unlimited as in this study. Nevertheless, effects on appetite provided indirect evidence that metabolism was altered during Cu and Cd exposure. Trout exposed to 75 $\mu g \cdot l^{-1}$ Cu ate significantly more for the same weight gain compared to controls (Fig. 1, Table

Table 2
Weights of rainbow trout exposed to either 3 $\mu g \cdot l^{-1}$ Cd, 75 $\mu g \cdot l^{-1}$ Cu, 250 $\mu g \cdot l^{-1}$ Zn or control conditions for 46 days in Series 1[a]

	Day	Days of exposure	Control	Treatment		
				Cd 3 $\mu g\ l^{-1}$	Cu 75 $\mu g\ l^{-1}$	Zn 250 $\mu g\ l^{-1}$
Pre Exposure	0		3.2 ± 0.1	3.3 ± 0.1	3.2 ± 0.1	3.2 ± 0.1
	3		3.8 ± 0.1	3.8 ± 0.2	3.8 ± 0.1	3.8 ± 0.1
	8		4.6 ± 0.2	4.7 ± 0.2	4.7 ± 0.2	4.7 ± 0.2
	11	0	5.2 ± 0.2	5.4 ± 0.2	5.2 ± 0.2	5.2 ± 0.2
Exposure	14	3	5.9 ± 0.2	5.7 ± 0.2	5.8 ± 0.2	6.1 ± 0.2
	17	6	6.5 ± 0.2	6.4 ± 0.3	6.4 ± 0.2	6.7 ± 0.2
	21	10	7.3 ± 0.3	7.3 ± 0.3	7.3 ± 0.2	7.6 ± 0.3
	27	16	9.4 ± 0.4	9.5 ± 0.4	9.8 ± 0.3	
	46	35	17.6 ± 0.7	16.9 ± 0.7	18.4 ± 0.6	

[a] The experimental period was split into an 11 day pre-exposure interval during which growth was monitored but no metals were administered followed by a 35 day exposure interval. Mean $\pm$ S.E.M. are presented with n of at least 100 for each, and there were no significant differences between treatments at any given day.

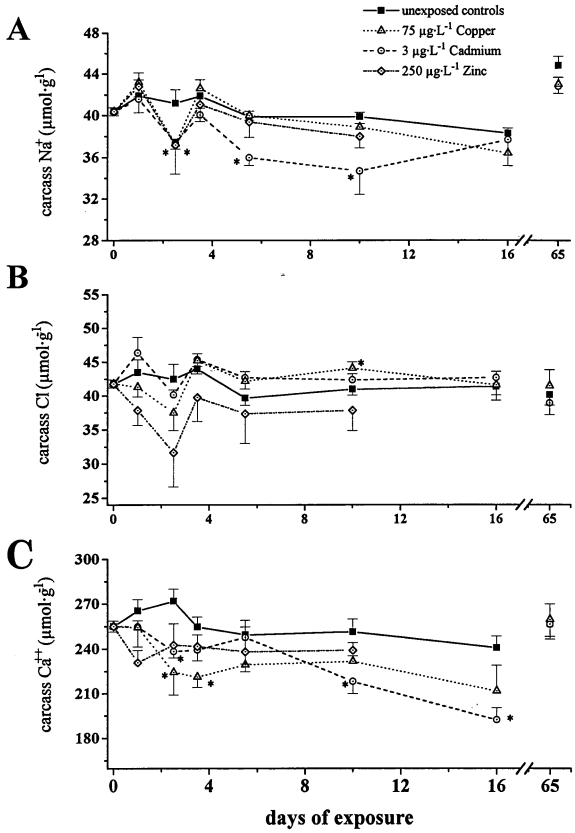

Fig. 2. Carcass concentrations of (A) Na^+, (B) Cl^- and (C) Ca^{2+} in rainbow trout exposed to either 75 $\mu g \cdot l^{-1}$ Cu (open triangles), or 3 $\mu g \cdot l^{-1}$ Cd (open circles) or 250 $\mu g \cdot l^{-1}$ Zn (open diamonds) for up to 65 days (Series 1). An unexposed control group are also shown (filled squares). Mean $\pm$ S.E.M. ($N \geq 6$) and * indicates significant difference ($P < 0.005$) from the control mean at that time.

2), suggesting an increased metabolic load which could be offset by increased input. This phenomenon has been observed previously in Cu exposed fish (De Boeck et al., 1997) and in this study was confirmed by the higher MO_2 during swimming (Fig. 6), and by one of the Ucrit tests where maximum sustained swim speed was reduced (Fig. 5A). As gill Na^+/K^+ ATPase activity was elevated in Cu-exposed trout (Fig. 3), higher energetic costs of osmoregulation may have been one of the factors contributing to the increased metabolic load, though further studies would be required to confirm this.

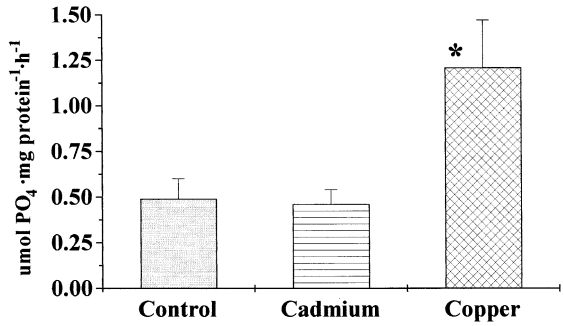

Fig. 3. Relative Na^+/K^+ ATPase activity of gill tissue sampled from rainbow trout of series 1 exposed to either 75 $\mu g \cdot l^{-1}$ Cu (hatched bar) or 3 $\mu g \cdot l^{-1}$ Cd (striped bar) for 2 months. A group of unexposed control fish is also shown (shaded bar). Mean $\pm$ 1 S.E.M. ($N \geq 6$) and * indicates a significant difference ($P < 0.05$) from control value.

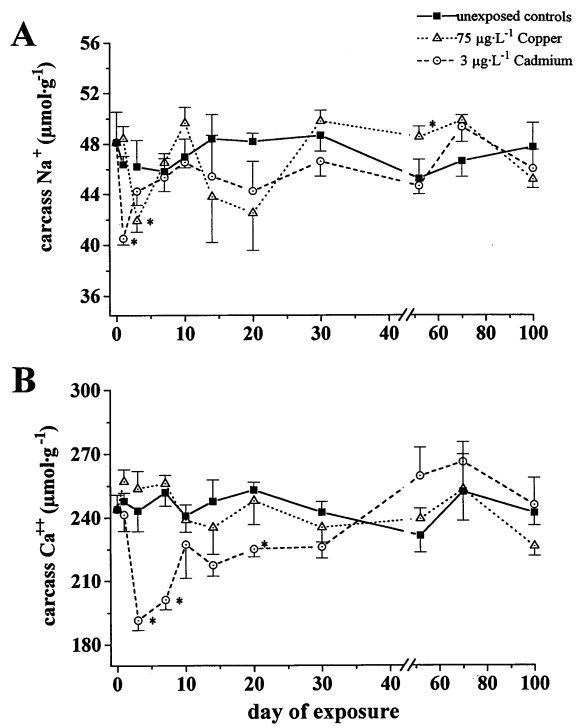

Fig. 4. Carcass (A) Na^+ and (B) Ca^{+2} in rainbow trout exposed to either 75 $\mu g \cdot l^{-1}$ Cu (open triangles), or 3 $\mu g \cdot l^{-1}$ Cd (open circles) for up to 100 day (Series 2). An unexposed control group is also shown (filled squares). Mean $\pm$ 1 S.E.M. ($N \geq 6$) and * indicates significant difference ($P = 0.05$) from the control mean at that time.

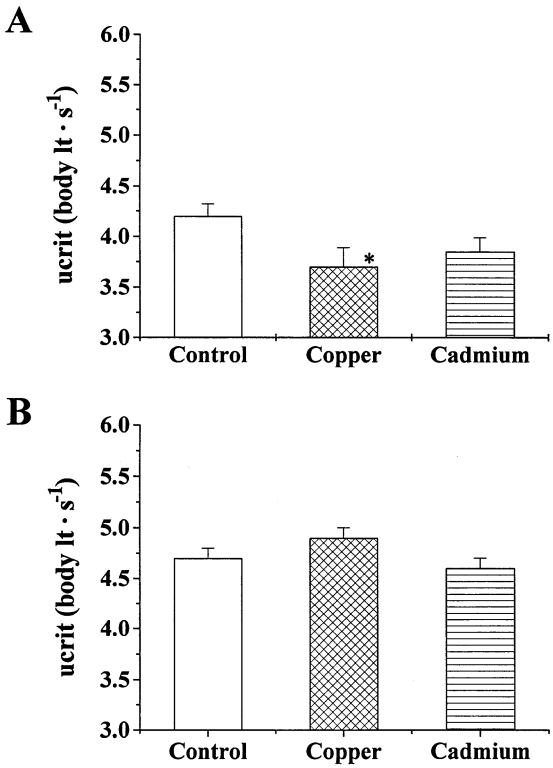

Fig. 5. Critical swimming speed (Ucrit) of rainbow trout exposed to either 75 μg·l^{-1} Cu (hatched bar) or 3 μg·l^{-1} Cd (striped bar) for either 1.5 months with satiation feeding (A; series 1 exposures) or 2.5 months with fixed ration feeding (B, series 2 exposures). In each case, Ucrit of a group of unexposed control fish is also shown (open bar). Mean ± 1 S.E.M. ($N \geq 8$) and * indicates a significant difference ($P < 0.05$) from control value.

Compared to pre-exposure conditions, fish exposed to Cd consumed less food to achieve the same SGR (Fig. 1B, C and Table 2) suggesting a lowering of metabolism or reduced activity. While we did not quantify activity in this experiment, Cd-exposed trout appeared to be less active. Hypoactivity has been documented in Al-exposed fish by Wilson et al. (1994) who suggested that reduced activity permitted a greater proportion of consumed energy to be directed towards growth. Measurements of behaviour, particularly spontaneous and basal activity levels may provide a sensitive measure of chronic sublethal effects.

4.3. Ion regulation

The results of our experiments clearly show that the initial effect of sublethal exposure to Cu, Cd and Zn is a temporary loss of Na^+ and Ca^{2+} which is followed by a recovery and return to control levels (Figs. 2 and 4). For each metal, the pattern of ionic loss and recovery is generally similar to other studies, and in accord with the accepted mechanisms of osmoregulatory disruption. Thus Cd induced a loss of ions, particularly Ca^{2+} (2C4B) but also Na^+ (2A4A) with a subsequent recovery (Giles, 1984). The loss of carcass Na^+ content (2A4A) is typical for Cu-exposed trout (Laurén and McDonald, 1987a; Beaumont et al., 1995). Loss of Ca^{2+} (Fig. 2C) has been less commonly reported in Cu exposed fish (Laurén and McDonald, 1987a). The temporary nature of the Cu-induced osmoregulatory disturbance (Figs. 2 and 4) with return to control levels has also been shown by Laurén and McDonald (1987a). It is probable that the recovery of ionic balance in metal exposed fish was aided by dietary uptake of Na^+ and Ca^{2+} as shown recently for acid-ex-

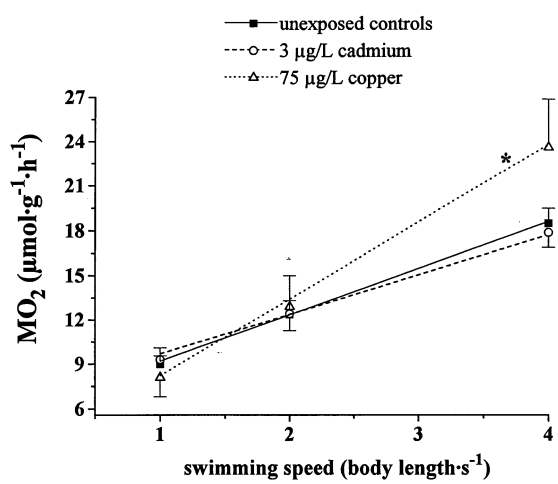

Fig. 6. Oxygen consumption rate (MO_2) at various swimming speeds in rainbow trout of series 2 exposed to either 75 μg·l^{-1} Cu (open triangle) or 3 μg·l^{-1} Cd (open circles). A group of unexposed control fish is also shown (filled squares). Best fit regression lines are shown. Fish were exposed for 2.5 months and fed a fixed ration of 1.5% body weight per day. Mean ± 1 S.E.M. ($N \geq 8$) and * indicates that the slope of the regression line is significantly different ($P < 0.05$) than that of controls.

posed trout by D'Cruz and Wood (1998). Exposure to sublethal Zn caused a disruption of carcass Ca^{2+} and Na^{+} (2A, C) but these ionic losses were not significant and short-lived. Spry and Wood (1984), Hogstrand et al. (1995) have shown Zn-induced depression of plasma ions, particularly Ca^{2+} and the latter authors have also demonstrated that recovery of normal Ca^{+} levels in the blood is linked to changes in Ca^{2+} uptake kinetics at the gill.

The initial osmoregulatory disturbance and subsequent recovery observed for Cd, Cu or Zn-exposed fish in these experiments undoubtedly reflects alterations in gill transport kinetics although this study did not address this directly. Inhibition of specific gill ion transporters by Cu has been shown for Na^{+}/K^{+} ATPase (Laurén and McDonald, 1987b; Pelgrom et al., 1995) as well as Ca^{2+} ATPase (Shephard and Simkiss, 1978). A Cd-induced inhibition of Ca^{2+} influx (Reid and McDonald, 1988) has been linked to competition at the apical uptake channel (Verbost et al., 1989). In addition, Cu and Cd are known to inhibit carbonic anhydrase (Meldrum and Roughton, 1933; Christensen and Tucker, 1976) an enzyme with high activity in the gill and linked indirectly to ion regulatory processes. Ionic disturbances associated with Zn exposure have been shown to result from changes in Ca^{2+} influx kinetics (Hogstrand et al., 1995), competition with Ca^{2+} for apical uptake channels (Hogstrand and Wood, 1996) and inhibition of Ca^{2+} ATPase (Shephard and Simkiss, 1978). Metal accumulation in the kidney may also have influenced ionoregulatory responses, particularly for Cd as this was a site of elevated accumulation (see Figs 2C and 5C of McGeer et al., 2000).

Ion loss during chronic metal exposure is known to result in a mobilization of mechanisms for recovery of gill ion transport (McDonald and Wood, 1993; Hogstrand et al., 1995). Our results showing a 2.5-fold increase in the gill Na^{+}/K^{+} ATPase activity in Cu-exposed trout after 2 months of exposure (Fig. 3) illustrates one of these mechanisms and agrees with previous studies (Laurén and McDonald, 1987a,b; Shephard and Simkiss, 1978; Pelgrom et al., 1995). The mechanisms for recovery from Cd-induced ionic losses are less well studied, but Wendelaar Bonga and Locke (1992) attributed recovery of ionoregulatory capacity to the increased number of chloride cells observed during Cd exposure. Our results show that acclimation to Cd does not involve long term changes in gill Na^{+}/K^{+} ATPase activity (Fig. 3).

4.4. Swimming and MO_2

In trout fed to satiation and exposed for 1.5 months (series 1), Ucrit was significantly decreased for Cu-exposed fish (Fig. 5A) but for those fed a fixed ration and exposed for 2.5 months (series 2), there were no differences in Ucrit (Fig. 5B). Cd-exposure had no effect on Ucrit in either Series, in accord with the findings of Hollis et al. (1999). Previously, waterborne Cu-exposure has been shown to cause a reduction in Ucrit, but only during the initial week (Waiwood and Beamish, 1978b). Our swim testing was done on chronically exposed fish and well after the damage or shock phase, so similar Ucrit performance would be expected. The differences in Ucrit for Cu-exposed trout in series 1 (Fig. 5A) but not in series 2 (Fig. 5B) may be related to differences in food consumption. Increased feeding is known to be associated with decreased Ucrit performance (Alsop and Wood, 1997). This reduction in swim performance appears to be a result of an increased metabolic demand for O_2, similar to the present situation with Cu-exposed trout (Fig. 6). This higher O_2 consumption during forced swimming of Cu exposed fish has been repeated in separate trials with a slightly different swimming regime (C. Szebedinszky and J.C. McGeer, unpubl. observations). Higher metabolic rates have also been seen in Cu-exposed brown trout (Beaumont et al., 1995) and rainbow trout (Waiwood and Beamish, 1978b). As well, De Boeck et al., (1995) has shown that chronic Cu exposure results in a reduced ability of carp to regulate oxygen consumption, as measured by critical oxygen concentration.

In conclusion, chronic sublethal exposure to waterborne Cu (an essential metal) is a loading stress as the physiological response of trout involved mechanisms that resulted in an associated

metabolic cost. In comparison, Cd (a non-essential metal) is neither a loading nor a limiting stress and acclimation to chronic Cd-exposure appeared to induce mechanisms that do not have a long term metabolic cost.

Acknowledgements

Supported by an NSERC Strategic Research Grant to CMW & DGM, an NSERC PDF to JCM and by grants from The International Copper Association, The International Lead Zinc Research Organization, Cominco Ltd. and Falconbridge Ltd. Special thanks to Erin Fitzgerald for her technical expertise and perseverance as well as to Lydia Hollis, Derek Alsop and Lisa Taylor. Thanks also to Dr P.M. Chapman, EVS Environment Consultants for his very helpful comments on the manuscript.

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